Oncolytic viruses and immunity
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Initially, direct oncolysis was thought to be the sole mechanism through which oncolytic viruses (OVs) exert their anti-tumor effect, and the immune system was perceived as the major obstacle in oncolytic virotherapy. Over the last decade, there has been a lot of debate on whether the immune system is a friend or foe of OVs. However, we are now at a stage where the initial thinking has been reversed as a result of compelling evidence that the immune system plays a critical role in the success of oncolytic virotherapy. In this review we discuss the importance of the involvement of innate and adaptive immunity for therapeutic efficacy of OVs, and the rational combination of OVs with other immunotherapies for further enhancement of overall therapeutic outcome.

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Introduction—oncolytic viruses
Oncolytic virotherapy is a novel therapeutic approach that utilizes replication-competent viruses, which selectively replicate in and lyse cancer cells while leaving normal cells unharmed. During the course of cancer evolution, cancer cells accrue multiple mutations that allow them to grow in an uncontrolled manner [1]. The very same mutations that help cancer cells to thrive are targeted by naturally occurring OVs (e.g. reovirus [2,3] and vesicular stomatitis virus (VSV) [4]) or genetically engineered OVs (e.g. adenovirus [5,6] and herpesvirus [7]). After infecting cancer cells, OVs hijack the cell death machinery allowing death to occur only after cellular resources have been fully exploited for maximum production of progeny viruses [8]. As such, the complex cell death caused by oncolysis may not always fit into conventional cell death classifications: apoptosis, necrosis and autophagy [8,9]. Furthermore, unlike chemo-therapy and radiation-therapy, OVs are self-amplifying therapeutics, whose therapeutic outcome is determined by a three-way race among tumor growth, virus replication, and immune activation [10].

Immunity and cancer therapy
The immune system’s role in cancer therapy has, historically, been neglected, which is evident from the fact that National Cancer Institute, USA, has used human xenografts in immune-deficient mice for testing oncologic drugs since 1976 [11,12]. Only recently the importance of immune system in cancer therapy is being appreciated. Indeed, recent studies suggest that the efficacy of chemotherapy and radiation-therapy, previously thought to exert their anti-tumor effect purely by direct cytotoxicity, depends on immune system involvement [13]. With the realization of the potent anti-tumor effect of appropriately activated immune system, the last two decades have seen a surge of interest in the field of cancer immunotherapy. Here we discuss the interactions among OVs, immune system and cancer, and the therapeutic outcome thereof.

OVs and immune system
The innate immunity serves as a first line of defense against viruses, which limits the amplification and spread of viruses, whereas the adaptive immunity plays a major role against the virus during re-infection [14]. Antibodies could potentially neutralize OVs, greatly reducing the virus dose at the tumor site. This is a concern especially when delivering OVs systemically. Nevertheless, levels of neutralizing antibodies do not appear to correlate with efficacies of OVs in clinical studies [15,16]. Kim et al. reported ‘antibody-mediated complement-dependent cancer cell lysis’ as an important mechanism for therapeutic efficacy of the oncolytic virus XJ-549 both in an animal model as well as in humans [17*].

In the context of cellular innate immunity, natural killer (NK) cells are considered to have potent anti-tumor as well as anti-viral effect. Virus-infected cancer cells tend to down-regulate their class I major histocompatibility complex (MHC) making themselves a good target for NK cells [18,19]. Although NK cells may kill infected cancer cells and limit the amplification of OVs, studies have found that NK cells often have positive effects on therapeutic outcomes of OVs [18–24]. Furthermore, NK cells may play a role in the maturation of dendritic cells (DCs) and they can also induce differentiation of cancer stem
cells as well as poorly differentiated cancer cells, through secretion of IFN-γ and TNF-α [25,26]. In this regard, one would expect the combination of NK cells with OVs to result in greater anti-tumor effect. Indeed, several studies have shown that the combination of NK cells with OVs can result into additive or synergistic anti-tumor effect [27,28].

Viruses can be taken up by antigen presenting cells directly through macropinocytosis or indirectly when OV-infected cells are engulfed, leading to presentation of viral antigens to T cells and ultimately activating the adaptive arm of the immune system against viruses [29]. Despite this possibility of anti-OV effect of adaptive immunity, most studies suggest that adaptive immunity enhances the therapeutic outcome of OVs [30**,31].

Several preclinical studies have demonstrated a prime role of immune system in the therapeutic efficacies of a wide range of OVs (Table 1) [32–37]. Prestwich et al., 2009, published one of the most compelling studies demonstrating the requirement of immune system in oncolytic virotherapy [38*]. Their study reported that an oncolytic reovirus was able to purge lymph node and splenic metastases from the murine melanoma cell line B16Ova, a line that is extremely resistant to reovirus in vitro, in immune-competent C57BL/6 mice but not in severe combined immunodeficient mice. This study concluded that virus-mediated immune responses, rather than virus-mediated oncolysis, were critical for the anti-tumor efficacy of the reovirus [38*]. Similarly, Apostolidis et al. found that locoregional administration of an oncolytic Newcastle disease virus (NDV) in immune-competent mice could significantly delay growth of tumors established from the murine colon cancer cell line CT26, despite these cells being very resistant to NDV in vitro [39]. In line with this, Diaz et al. showed that an oncolytic VSV that replicates extremely aggressively in B16Ova cells in vitro has no anti-tumor effect against B16Ova tumors, in mice, in the absence of CD8+ T cells or NK cells [21]. Furthermore, incorporation of immune-stimulatory genes such GM-CSF [40], IL-12 [41], IL-2 [42], IL-15 [43] and RANTES [44] in OVs has been shown to enhance therapeutic efficacy of OVs in immune-competent animal models. Importantly, in some instances, even non-replicating, heat or UV-inactivated viruses have been shown to eradicate established tumors in immune-competent animal models, underscoring the impact of immune system on virus-mediated anti-tumor effect [45,46**].

Although there is not enough clinical data to conclude if the importance of immunity in the overall therapeutic efficacy of OVs in human patients will be similar to what has been observed in preclinical studies, there are some indications that immune system would favor oncolytic virotherapy in the clinical setting (Table 1). For example, in a phase I clinical trial, Talimogene laherparepvec or T-VEC, an oncolytic herpes simplex virus encoding human GM-CSF, was found to increase immune cell infiltration into OV-injected tumors, and 4 out of 30 patients showed extensive inflammation in uninjected tumors, suggestive of systemic anti-tumor immune responses [47*]. T-VEC also showed anti-tumor activities in both injected and uninjected distant lesions including visceral metastases in melanoma patients in phase II and III clinical trials [48,49]. Analysis of immune cells in the patients revealed that intra-lesional injection of the virus induced local and systemic antigen-specific T cell responses, and significantly reduced immune-suppressive cells (Tregs and MDSCs) [50]. Likewise, an oncolytic vaccinia virus JX-594, which also encodes hGM-CSF was shown to regress both injected and uninjected liver tumors in a phase I clinical trial [16]. Regression of the uninjected tumors was thought to be due to activation of systemic anti-tumor immunity, although there was no direct evidence to prove this. Furthermore, in a case report of an ovarian cancer patient treated with an oncolytic adenovirus encoding hGM-CSF (ONCOS-102), progressive infiltration of CD8 + T cells in the tumor and concomitant systemic induction of tumor-specific CD8+ T cells were observed [51]. Taken together, these preclinical and clinical studies make a strong case for the critical role of immunity in the success of oncolytic virotherapy.

**Mechanism of anti-tumor immune modulation by OVs**

In the last two decades the field of cancer immunotherapy has seen some major breakthroughs culminating into the FDA approval of several immunotherapeutics. While the approved immunotherapeutics, mostly immune checkpoint inhibitors (ICIs), have shown impressive and long-lasting responses in a subset of cancer patients, majority of patients fail to respond to these agents [32]. ICIs, such as anti-PD-1/PD-L-1 and anti-CTLA-4, act by restoring T cell function and rely on pre-existing tumor-specific T cells for therapeutic success [52,53]. Immunologically unresponsive or ‘cold’ tumors have one or more of the following characteristics: lack of tumor antigens, lack of T cells recognizing tumor antigens, heavy presence of immunosuppressive cells such as regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSC), M2 macrophages and immunosuppressive cytokines such as IL-10 and TGF-β [52,54*], and are very likely to be resistant to ICIs [54*].

OVs have the potential to convert immunologically ‘cold’ tumor into an inflamed, immunologically ‘hot’ tumor (Figure 1). There are a variety of mutually non-exclusive mechanisms through which OVs could modulate the tumor microenvironment (TME). First, oncolysis by OVs could cause the release of tumor associated/specific antigens and enhance cross-presentation of such antigens by dendritic cells (DCs), ultimately eliciting adaptive
Table 1

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<th>Oncolytic viruses as immunotherapeutics.</th>
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<td>Type of immunotherapy</td>
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<td>OVs</td>
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<td>VSV</td>
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<td>Reovirus</td>
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<td>NDV</td>
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<td>Oncolytic adenovirus (Delta24-RGD)</td>
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<td>HSV-2 (FusOn-H2)</td>
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<td>ICP34.5-deleted HSV</td>
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<td>OVs armed with immune-stimulatory genes</td>
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<td>HSV-1 encoding GM-CSF</td>
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<td>Oncovex^GM-CSF or T-VEC</td>
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<td>Oncolytic vaccinia virus JX-594 encoding GM-CSF</td>
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<td>Combination therapy</td>
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<td>Oncolytic adenovirus with PD-1 inhibitor</td>
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<td>Oncolytic VSV plus recombinant adenovirus vaccine boost</td>
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<td>Adoptive T cell therapy plus oncolytic VSV</td>
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<td>Oncolytic adenovirus encoding CCL20/IL-15 + NK cells + CD8+ T cells</td>
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<td>Oncolytic HSV-1 + bortezomib + NK cells</td>
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<td>T-VEC plus anti-PD-1 antibody</td>
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Notes: VSV, vesicular stomatitis virus; NDV, Newcastle disease virus; HSV, herpes simplex virus; CXCL10, chemokine (C-X-C) motif 10; GM-CSF, granulocyte-macrophage-colony-stimulating factor; MIP-1α, macrophage inflammatory protein-1α; NK, natural killer cells; TAMs, tumor-associated macrophages; TILs, tumor infiltrating lymphocytes.
Immunity against tumor [55–60]. Second, OV s are known to induce immunogenic cell death (ICD) [61], which plays an important role in the induction of anti-tumor adaptive immunity [62]. Discussion of the mechanisms through which OV s induce ICD is beyond the scope of this review; readers are encouraged to see an excellent review by Guo and Bartlett on this topic [63**]. Third, some OV s such as reovirus [64] and VSV [65] can directly
interact with DCs and enhance their antigen priming capability. Fourth, OVs have the ability to reduce immune-suppressive Tregs and MDSCs in the TME [50]. Lastly, OVs can modulate the cytokine milieu in the TME, for example, revirus infection in human melanoma cells has been shown to abrogate the immunosuppressive cytokine IL-10 and enhance secretion of the proinflammatory cytokines IL-6, IL-8, RANTES and MIP-1α/β [66]. Likewise, infection with oncolytic VSV in murine melanoma cells rapidly induced proinflammatory cytokines including IL-6, type I IFN and TNF-α [67]. Interestingly, the cytokines/chemokines upregulated by these OVs are not only immunostimulatory in function but they can also kill residual uninfected cancer cells [67]. Perhaps the best evidence for the therapeutic potency of simply modulating TME through virus infection comes from a recent study by Dai et al. [46**]. In this study the authors showed that repeated intra-tumoral injection of heat-inactivated modified vaccinia virus Ankara could eradicate tumors in different aggressive murine tumor models. Although the virus used in this study is not an oncolytic virus, this study provides an insight into the anti-tumor potency of virally-modulated TME. Taken together, these studies suggest that OVs have the potential to modulate TME from immunologically ‘cold’ to immunologically ‘hot’ status.

Combination of OVs with immunotherapeutics

Given the potential of OVs to modulate the immune landscape in TME, it would be logical to surmise that combination of OVs with immunotherapeutics may result in synergistic therapeutic effect. Indeed, a study by Woller et al. showed that localized tumor infection with an oncolytic adenovirus could overcome systemic tumor resistance to PD-1 inhibitor by broadening neoantigen-directed T cell responses in mice [58**]. Interestingly, the tumor cells were found to upregulate their PD-L1 expression in response to virus infection. Therefore, both the therapeutics complemented each other and the outcome was a synergistic anti-tumor effect [58**]. Very recently, Ribas et al. reported the findings of a phase Ib trial in which they studied the impact of T-VEC on therapeutic efficacy of anti-PD-1 antibody pembrolizumab in patients with metastatic melanoma [68*]. The combination treatment was well tolerated; T-VEC was found to promote T cell infiltration into tumors and improved the overall therapeutic efficacy of pembrolizumab. This clinical trial essentially confirmed the findings from animal study that OVs can enhance the therapeutic efficacies of checkpoint inhibitors by converting immunologically ‘cold’ tumors into immunologically ‘hot’ tumors [54*,58**,60]. This study provides the hope that the benefits of checkpoint inhibitors may be harnessed in combination with OVs, even in tumor types that have previously shown very poor response to checkpoint inhibitors, such as breast, prostate and colon cancer [52].

Another logical combination of OVs would be with chimeric antigen receptor (CAR)-redirected T cells. CAR-T cells can recognize whole antigens (MHC unrestricted) on tumor cell surface, minimizing the probability of cancer cell escape by MHC I downregulation [69]. Several studies have shown the feasibility of using CAR-T cells for targeting virus-encoded antigens [70–72]. The combination of an OV encoding a unique tumor antigen with a CAR-T that recognizes the virus-encoded antigen should work synergistically potentially through: (i) tumor debulking by the OV, (ii) positive modulation of immunity in TME by OV for optimal function of CAR-T cells, and (iii) CAR-T cells should be able to kill infected residual cancer cells that may be resistant to the OV.

Conclusion

The success of oncolytic virotherapy depends on its ability to mobilize the host’s immune system against tumor. The approval of T-VEC has sparked great optimism in the field of oncolytic virotherapy with several more OVs currently being evaluated in the advanced phase of clinical trials. On the other hand, immunotherapeutics such as ICIs have shown unprecedented response rates in the clinic, bringing the field of immunotherapy into the main limelight of cancer therapy. However, both of these therapeutic platforms are still far from being adequate and more work needs to be done in order to expand the therapeutic benefits to broader population of cancer patients. Given the ability of oncolytic virotherapy and immunotherapy to complement each other, it would be reasonable to expect that their combination would be more effective in the battle against cancer.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


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First study to show 'antibody-mediated complement-dependent cell lysis' as one of the many mechanisms through which oncolytic poxviruses exert their anti-tumor effect. The effect was shown both in animal model (rabbits) and in humans.
30. Melcher A, Parato K, Rooney CM, Bell JC: Thunder and lightning: immunotherapy and oncolytic viruses collide. Mol Ther 2011, 19:1006-1016. This comprehensive review discusses the fine balance between anti-virus and anti-tumor activities of the immune system and provides evidence for beneficial role of immune system in oncolytic virotherapy. It also discusses several new strategies for combination of immunotherapeutics with oncolytic viruses to mount a multifaceted biological attack against cancer.
An early study that provided evidence for the requirement of host immune system in the success of oncolytic virus.


This elegant study demonstrates robust activation of immune-system by viruses and the power of immune system to completely abrogate tumors when activated appropriately. The virus used in this study is a non-replicating poxvirus and the author showed that even heat or UV-inactivated virus can highly activate anti-tumor immunity ultimately causing abrogation of established tumors in mice.


First human trial combining an oncolytic virus with PD-1 inhibitor. This study clearly shows that the oncolytic virus T-VEC can improve efficacy of an anti-PD-1 antibody.


