Replicating viral vectors for cancer therapy: strategies to synergize with host immune responses

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Summary

Tumour-specific replicating (oncolytic) viruses are novel anticancer agents, currently under intense investigation in preclinical studies and phase I–III clinical trials. Until recently, most studies have focused on the direct antitumour properties of these viruses. There is now an increasing body of evidence indicating that host immune responses may be critical to the efficacy of oncolytic virotherapy. Although the immune response to oncolytic viruses can rapidly restrict viral replication, thereby limiting the efficacy of therapy, oncolytic virotherapy also has the potential to induce potent antitumoural immune effectors that destroy those cancer cells, which are not directly lysed by virus. In this review, we discuss the role of the immune system in terms of antiviral and antitumoural responses, as well as strategies to evade or promote these responses in favour of improved therapeutic potentials.

Introduction

Oncolytic viruses (OVs) represent a novel class of biological cancer therapeutics and are under intense investigation for the development of clinical agents for the treatment of malignancy (Table 1). Oncolytic virotherapy involves the use of replication-competent viruses that have the intrinsic capacity to propagate selectively in tumour cells while sparing normal tissues (Kirn *et al.*, 2001). Despite encouraging progress in the field over the past two decades, including the initiation of numerous clinical trials, several barriers continue to limit the success of oncolytic virotherapy in immune-competent hosts, such as inefficient viral replication and spread throughout the entire tumour mass and tumour-specific resistance to virus-mediated cell killing (Smith *et al.*, 2011).

While earlier efforts focused largely on viral engineering to optimize direct virus-mediated tumour cell oncolysis, it has become apparent that successful viral therapies must take the host immune responses into account in order to reach their full potential (Prestwich *et al.*, 2008). The role of the immune system in oncolytic viral therapy is prominent and can be considered both inhibitory, in terms of its ability to rapidly restrict viral replication, and complementary, with respect to its capacity to produce potent antitumour responses even in those cells which are not directly infected by virus. Although the overall benefit or detriment of immune responses to the success of oncolytic virotherapies has been somewhat debated, it is ubiquitously agreed upon that a thorough understanding of these responses is crucial in order to develop optimized treatment strategies which promote synergism between virus and host to result in the most favourable outcome (Prestwich *et al.*, 2009). This review summarizes the current body of knowledge regarding immune interactions between oncolytic virus and host, as well as the strategies employed in efforts to promote synergy between the two. Since interactions between the host immune system and OVs can be categorized either as antiviral or as antitumoural, we address each of these issues separately and then discuss the potential interplay between the two.

Host antiviral immune responses

Interactions between OVs, the tumour microenvironment and the immune system are complex, yet critical in determining the outcome of antitumour therapy. By design, the host innate immune system is rapidly activated in response to the detection of viral nucleic acids in order to clear invading pathogens before a successful infection can occur. It is not surprising, therefore, that this same response also interferes with the spread and replication of therapeutically administered viruses. The ability of the innate immune response to limit viral replication has clearly been demonstrated in several animal models (Fulci *et al.*, 2006; Breitbach *et al.*, 2007) and clinical studies (Pecora *et al.*, 2002; Chiocca *et al.*, 2004). The type I interferon (IFN) response is a major component of the cellular innate antiviral response. Administration of

Table 1. Selected oncolytic viruses in clinical development.

Virus product	Description	Indication	Status
OncoVEX[GM-CSF]	HSV-1 with deletions in ICP34.5 and ICP47 modified for immediate-early expression of US11 and production of GM-CSF	Metastatic melanoma, head and neck cancer	Phase III
Reolysin	Formulation of wild-type reovirus of the serotype 3 strain Dearing	Head and neck cancer	Phase III
JX-594	Vaccinia virus with deletion of thymidine kinase and expression of GM-CSF	Hepatocellular carcinoma	Phase II
PV701	Naturally attenuated (non-recombinant) MK107 vaccine strain of Newcastle disease virus	Head and neck cancer, peritoneal cavity cancer	Phase I
MV-CEA, MV-NIS	Recombinant measles virus (Edmonston vaccine strain) expressing human carcinoembryonic antigen (CEA) or the human sodium iodide transporter gene (NIS)	Ovarian cancer, peritoneal cavity cancer, multiple myeloma	Phase I
VSV-hIFNβ, VSV(MΔ51)-M3	Recombinant vesicular stomatitis virus (Indiana strain) expressing human interferon-β (hIFNβ) or recombinant M-mutant VSV expressing murine gammaherpesvirus M3 [VSV(MΔ51)-M3]	Hepatocellular carcinoma	Phase I
Telomelysin	Recombinant oncolytic adenovirus driven by a human telomerase reverse transcriptase (hTERT) promoter	Solid tumours	Phase I

OVs into immune-competent hosts causes an increase in type I IFN and IFN-inducible genes within hours of infection, resulting in the induction of various antiviral pro-inflammatory cytokines such as interleukin (IL)-6, IL-12, tumour necrosis factor (TNF)-α, MIP-1α and IP-10 (Steele *et al.*, 2011). We have observed that high doses of VSV administered in immune-competent rats induced a rapid inflammatory cytokine response including IL-6, TNF-α, IFN-γ and type I IFN (Shinozaki *et al.*, 2005).

Pharmacological manipulation of antiviral cytokine responses

Due to the fact that OVs are extremely sensitive to the antiviral actions of type I IFN, the success of an OV in replicating in and destroying a tumour is dependent, in part, on the fitness of the tumour cells in IFN signalling, which can be quite variable. To address this limitation, several strategies have emerged to dampen IFN signalling in those tumours, which are intrinsically resistant to OV therapy. Histone deacetylase (HDAC) inhibitors are a new class of antineoplastic agents under clinical development, which act by inducing cell cycle arrest and apoptosis specifically in cancer cells. In addition, they have been shown to upregulate virus-mediated transgene expression and blunt type I IFN responses to viral infection. For this reason, HDAC inhibitors have been recently utilized in combination therapies with OVs and shown to enhance the spread and anticancer efficacy of VSV, vaccinia virus and HSV in multiple systems and render refractory tumours sensitive to viral oncolysis (Nguyen *et al.*, 2008; Otsuki *et al.*, 2008; Mactavish *et al.*, 2010). Importantly, the effects of HDAC inhibitors are temporary and seem to be specific to tumour cells, thereby allowing the antiviral IFN signalling to function without inhibition in normal cells. In a similar strategy, another report recently described a 'pharmacoviral'

screen of over 12 000 chemical compounds to search for pharmacological agents which maximize synergy while retaining the tumour specificity of the oncolytic virus (Diallo *et al.*, 2010). One of the compounds assessed this way, VSe1 (3,4-dichloro-5-phenyl 2,5-dihydrofuran-2-one), was shown to suppress the partially responsive type I IFN response in tumour cells resistant to VSV, thereby sensitizing the cells to VSV replication and conferring a temporary and apparently tumour-selective replication advantage *in vivo* (Diallo *et al.*, 2010).

Viral strategies to evade IFN responses

As an alternative approach to combining viruses with drug therapy, several attempts have been made to engineer viruses to inhibit type I IFN production and response. An oncolytic measles virus strain armed to express the wild-type P gene, which inhibits type I IFN production and response, demonstrated increased efficacy in myeloma xenografts (Haralambieva *et al.*, 2007). Similarly, a recombinant NDV vector expressing influenza NS1, a protein exhibiting IFN-antagonist and antiapoptotic functions, was more effective than virus not expressing NS1 in clearing aggressive malignant melanoma and resulted in higher overall long-term animal survival without signs of toxicity (Zamarin *et al.*, 2009). By combining complementary OVs, it was recently shown that vaccinia virus synergistically enhanced VSV antitumour activity, dependent in large part on the activity of the vaccinia virus B18R gene product, which locally antagonizes the innate cellular, antiviral response initiated by type I IFN (Le Boeuf *et al.*, 2010).

Antiviral inflammatory cell infiltration

Administration of OVs into immune-competent hosts results in a pro-inflammatory state marked by activation of

cellular components of the innate immune system such as natural killer (NK) cells, neutrophils, macrophages and dendritic cells (DCs) (Benencia *et al.*, 2005). These cells contribute to the antiviral response, either directly by killing infected cells or producing antiviral cytokines, or indirectly by modulating adaptive immune responses (Guidotti and Chisari, 2001). In studies applying VSV to hepatocellular carcinoma via hepatic arterial infusion in immune-competent rats, we have observed a rapid and robust virus replication peaking at approximately 24 h post treatment, followed by a logarithmic decline in titres over the course of subsequent days. Given that antiviral neutralizing antibodies are not produced in the host until several days later (Shinozaki *et al.*, 2004), we speculated that the rapid clearance of the virus is due to the actions of antiviral inflammatory cells which localize to areas of virus replication at approximately the same time point as the loss in titre. Similarly, others have reported a significant decrease in HSV transgene expression within 72 h of viral injection, which was associated with a rapid increase in intratumoural NK cells and macrophages (Fulci *et al.*, 2006). As inflammatory processes of the host impose a ubiquitous challenge to the success of OV therapies, various strategies have been employed to attempt to circumvent these responses.

Pharmacological suppression of inflammatory cells

The utilization of immune-suppressive pharmacological agents in combination with OV therapy has generated substantial data indicating that inhibition of antiviral inflammatory responses results in improved virus replication and therapeutic efficacy. Perhaps the best-characterized drug applied for this purpose is cyclophosphamide (CPA), a DNA-alkylating agent with anticancer and immune-suppressive functions. Transient immuno-modulation with CPA resulted in the inhibition of intratumoural inflammatory cell infiltrations and improved therapeutic outcomes when administered in combination with HSV in several different tumour models (Fulci *et al.*, 2006; Currier *et al.*, 2008). Similar effects were observed when CPA was administered with adenovirus, vaccinia virus and reovirus (Qiao *et al.*, 2008b; Thomas *et al.*, 2008; Lun *et al.*, 2009), and a phase I clinical trial with CPA in combination with measles virus has been initiated for patients with multiple myeloma (Myers *et al.*, 2007). Other immunosuppressive drugs, such as cisplatin, cyclosporine and rapamycin, have been utilized with similar success in blocking antiviral inflammatory responses to oncolytic reovirus, adenovirus and vaccinia virus therapy, resulting in enhanced viral replication and improved tumour responses (Smakman *et al.*, 2006; Cheong *et al.*, 2008; Pandha *et al.*, 2009). Due to the fact that immune cells use the tumour vasculature to

traffic to tumours, strategies to inhibit vascular permeability associated with an oncolytic virus therapy has been explored. Pre-treatment with an inhibitor of angiogenesis, cyclic RGD peptide, was found to reduce vascular permeability, inflammation, and leucocyte infiltration following HSV-1 treatment of rat glioma (Kurozumi *et al.*, 2007). This strategy was found to enhance viral propagation and, hence, the antitumour efficacy of the therapy.

Virus-mediated suppression of inflammatory responses

Although systemic suppression of immune responses has been successful in promoting enhanced oncolytic virus replication and intratumoural spread, there have been concerns associated with the safety of such approaches (Prestwich *et al.*, 2009). By incorporating genes encoding anti-inflammatory proteins directly into the virus, it was speculated that the suppression of immune responses would be limited to the local area of virus replication within the tumour. Due to the fact that the success of invading viruses to propagate within their hosts is dependent upon their ability to evade detection and subsequent destruction by antiviral immune responses, many viruses have naturally evolved intricate mechanisms to counteract these responses (Alcami, 2003). One such mechanism involves the production of viral chemokine-binding proteins (vCKBPs). These vCKBPs are secreted proteins, which function to competitively bind to and/or inhibit the interactions of immunomodulatory chemokines with their cognate receptors, thereby blocking the chemotaxis of inflammatory cells (Seet and McFadden, 2002). With this knowledge in mind, coupled with our own observations that host inflammatory responses to VSV infection plays a detrimental role in suppression of intratumoural viral replication, we have recently exploited several heterologously expressed vCKBPs in order to enhance the oncolytic potency of VSV for the treatment of hepatocellular carcinoma. To this end, we engineered recombinant VSV vectors encoding for the equine herpes virus-1 glycoprotein G or the M3 gene from murine gammaherpesvirus-68, both of which are vCKBPs, which bind to a broad range of chemokines with high affinity (Altomonte *et al.*, 2008; Wu *et al.*, 2008). In these studies, we demonstrated vector-mediated suppression of antiviral inflammatory cell infiltration, namely NK cells and neutrophils, which translated to prolonged kinetics of intratumoural VSV replication and significant survival prolongation in immune-competent, orthotopic liver tumour-bearing rats. In an additional study, we incorporated the UL141 gene from human cytomegalovirus into VSV, which specifically inhibits the NK cell-activating ligand CD155, resulting in enhanced virus propagation and tumour responses corresponding to inhibition of NK and

NKT cell migration to infected tumour sites (Altomonte *et al.*, 2009). Importantly, none of these recombinant vectors resulted in any observable signs of toxicity to the host.

As an alternative method of modulating antiviral immune responses, a highly tumour-specific oncolytic adenovirus, Ad-p53T, was constructed such that its replication would be governed by aberrant telomerase activity and dysfunctional transcription of p53 (Gurlevik *et al.*, 2010). This strategy resulted in significantly diminished antiviral CD8-specific immune responses and, consequently, a reduction of cytotoxicity *in vivo*. While a control adenovirus with non-selective viral replication resulted in death by liver failure in immune competent hosts, treatment of lung metastases with Ad-p53T resulted in significantly prolonged survival in the absence of toxicity. This study would support the fact that inhibition of host immune responses could actually be beneficial in improving the safety of OV therapy in some contexts.

Arguments against immune evasion

Although strategies aimed at evading or suppressing innate antiviral immune responses may result in improved potency of OV therapy, the prudence of inhibiting key players in host antiviral defences has been the subject of intense debate. Some fear that the manipulation of antiviral immune mechanisms will impair the ability of normal cells to detect and inhibit virus replication in normal tissues, leading to increased toxicity. In fact, high doses of CPA have been reported to result in severe reovirus toxicity (Qiao *et al.*, 2008b), and similarly, elevated doses of cisplatin resulted in a toxic combination with recombinant VSV (Park *et al.*, 2008). However, in the case of our recombinant vCKBP-expressing VSV vectors, thorough safety screening has been performed, and no additional toxicities in comparison with the wild-type vectors have been detected, presumably due to the inherent sensitivity of VSV to type I IFN responses in normal cells, which is unaltered by expression of vCKBPs. (Altomonte *et al.*, 2008; 2009).

A second contention against immune suppression argues that intact host immune responses are not only important, but they are necessary for effective oncolytic viral therapy. A compelling body of evidence has demonstrated that activation of NK and CD8+ T cells are absolutely crucial for the efficacy of VSV and HSV by mediating potent antitumour immune responses, without which the OVs were relatively inefficient in tumour cell killing (Thomas and Fraser, 2003; Diaz *et al.*, 2007). Furthermore, it was shown that OV therapy using VSV or vaccinia virus results in indirect killing of uninfected tumour cells via induction of CXCL1 and CXCL5 chemokines, which causes neutrophil infiltration and subsequent blockage of blood flow to the tumour, thereby inducing apoptosis (Breitbach *et al.*, 2007). Although neutrophil depletion resulted in enhanced virus replication and spread within the tumour, the phenomenon of reduced blood flow and bystander cell killing was lost, and the efficacy of therapy was compromised. These data support the notion that inflammatory cell infiltrations could be imperative to the success of OV therapy.

Antitumour immune responses

Traditionally, the immune system was thought to be a barrier, limiting the efficacy of oncolytic viral therapy by rapidly clearing the virus from the host before complete destruction of the tumour can be achieved. However, despite the apparent counter-productivity of innate immune responses to oncolytic virus therapy, other aspects of the host immune system are crucial for the elimination of metastatic disease. Tumour cells possess multiple mechanisms for evading immune rejection from the host. Since OVs possess an intrinsic ability to induce adaptive immune responses, they may in fact act as cancer immunotherapy to induce tumour-specific immune responses and to overcome tumour-mediated tolerance mechanisms (Prestwich *et al.*, 2008; 2009). In addition, various strategies have been employed to further exploit antitumour immune responses and augment OV therapy.

Viral engineering to enhance antitumour immunity

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine secreted by macrophages, T cells, mast cells, endothelial cells and fibroblasts, which has been shown to possess strong immunostimulatory functions. GM-CSF promotes progenitor cell differentiation into DCs and can generate tumour-reactive cytotoxic T-lymphocytes (CTL) (Dranoff *et al.*, 1993). Gene transfer of GM-CSF to tumour cells results in enhanced tumour antigen presentation by recruited DCs and macrophages to mediate protective immunity against tumours (Dranoff *et al.*, 1993; Huang *et al.*, 1994). To date, reports of recombinant vaccinia virus, adenovirus, HSV, measles virus and NDV engineered to express GM-CSF have all described improved therapeutic outcomes due to enhanced antitumour immune responses (Grote *et al.*, 2003; Liu *et al.*, 2003; Kim *et al.*, 2006; Janke *et al.*, 2007; Cerullo *et al.*, 2010). OncoVEX[GM-CSF], a replication-competent HSV-1 vector expressing GM-CSF, has now entered phase III clinical trial for metastatic melanoma, based on a 28% response rate in the phase II trial, where there was evidence of an immune-mediated antitumour oncolysis in non-injected tumours in addition to the direct

oncolytic effect observed in intratumourally injected tumours (Kaufman and Bines, 2010). In addition, a phase I study investigating JX-594, an oncolytic vaccinia virus expressing GM-CSF, for therapy of primary and secondary liver tumours has demonstrated partial responses with evidence of efficacy in non-injected tumours, indicating that viral-mediated immune stimulation played a role (Park *et al.*, 2008).

Along the same lines, other cytokines have been incorporated into OVs with varying successes. Recent examples include IL-12, IL-24, IL-2 and IFN-β (Kirn *et al.*, 2007; Shin *et al.*, 2007; Vigil *et al.*, 2007; Luo *et al.*, 2008; Saloura *et al.*, 2010). It has been hypothesized that virus-mediated expression of IFN-β would improve tumour specificity by inhibiting viral replication in normal tissues while permitting propagation in tumours, which possess various defects in type I IFN signalling. In addition, IFN-β can provide antiangiogenic effects (Dong *et al.*, 1999) and beneficial immune modulation via the induction of tumour-specific CTL (Brown *et al.*, 2002). A vaccinia virus expressing IFN-β was found to have superior tumour selectivity and efficacy, in association with generation of antitumour immunity, when compared with a control vector lacking IFN-β (Kirn *et al.*, 2007). Similarly, recombinant VSV expressing IFN-β enhanced inflammatory cytokine production and NK cell activation, leading to enhanced bystander killing of tumour cells (Saloura *et al.*, 2010).

Oncolytic viral vectors have also been used to express chemokines in order to sequester immune effector cells to the tumour microenvironment. The gene encoding for the chemokine RANTES was inserted into a replication-competent adenovirus, resulting in recruitment of DCs to infected tumour sites and eliciting antigen-specific CTL and NK cell responses to promote tumour regression (Lapteva *et al.*, 2009). Similarly, DC and T-cell infiltration of tumours was stimulated through expression of MIP1α and Fms-like tyrosine kinase-3 (FLT-3) ligand by an oncolytic adenovirus, which resulted in improved tumour responses despite the enhancement of both antiviral and antitumour immunity (Edukulla *et al.*, 2009).

Although tumour cells express a variety of tumour-associated antigens (TAAs), a multitude of mechanisms exist which allow tumours to evade rejection from the host immune system. OVs can function as potent agents for enhancing the immunogenicity of the tumour microenvironment via induction of tumour cell death, modulation of the cytokine balance in favour of DC recruitment, and direct interactions with immune cells. Viral oncolysis is associated with the release of TAAs into the tumour microenvironment, which can then be taken up by DCs. In addition, the release of intrinsic cell factors, such as uric acid, can be identified as danger signals to activate DCs (Matzinger, 1994). DCs are important components of the innate immune response, and play a crucial role in the generation of adaptive immune responses through antigen presentation and priming of T cells. Virus-infected cells are highly effective in delivering antigens for cross-presentation and cross-priming of adaptive immune responses (Schulz *et al.*, 2005).

In addition to the intrinsic ability of OVs to stimulate antitumour immune responses, it is possible to engineer the vector to express a TAA to efficiently prime T-cell responses. This strategy was successfully used to launch an antitumour immune response via incorporation of a TAA into an oncolytic VSV vector, resulting in an increase in antigen-specific CD8+ T cells in a murine melanoma model (Diaz *et al.*, 2007). In addition, prime-boost strategies have been proposed in which two different OVs are administered, the first one priming the immune response through expression of a TAA, followed by a boosted secondary response produced by the sequential virus, which also encodes the TAA, leading to a robust tumour-specific immunity (Irvine *et al.*, 1997; Bridle *et al.*, 2010). Furthermore, the tumour cell death resulting from direct oncolysis from the viruses, as well as TAA-specific CD8+ T cell-mediated killing, causes additional TAAs to be released and presented by DCs to T cells, resulting in further activation of tumour-specific immune responses and representing a potent arsenal against systemic metastases.

In a novel therapeutic approach, it was recently demonstrated that a cDNA library from normal tissue could be expressed by VSV, resulting in the presentation of a broad range of TAAs when injected into tumours of the same histological classification as that from which the cDNA was obtained (Kottke *et al.*, 2011). This strategy resulted in dramatic tumour regressions, and those tumours, which escaped immune selection, could be treated by second-line virus-based immunotherapy.

Combined adoptive immune cell transfer with viral delivery

Combination strategies involving the adoptive transfer of immune cells together with OVs have been proposed to increase the therapeutic effect of each monotherapy through multiple mechanisms. The combination of VSV expressing a model tumour antigen (OVA) together with adoptive T-cell therapy targeted against the same antigen resulted in potent systemic antitumour immunity, far superior to that achieved via either monotherapy (Kottke *et al.*, 2008; Wongthida *et al.*, 2011). Sequential administration of immature DCs and HSV-1 injected intratumourally resulted in significant reductions in tumour volumes (Farrell *et al.*, 2008). Furthermore, it was recently demonstrated that virus-induced tumour inflammation acts synergistically with tumour-targeted DC vaccination, resulting in potent antitumoural CD8+ T-cell responses (Woller *et al.*, 2011).

Because many immune cells naturally home to tumours, they can be used as cell carriers to provide the dual benefit of virus delivery and stimulation of antitumour immune responses. A recent report described the loading of VSV onto antigen-specific T cells to simultaneously enhance adoptive T-cell therapy, while providing a vehicle for OV delivery to the tumour site (Qiao *et al.*, 2008a). In a similar study, it was demonstrated that T cells, in addition to mature DCs, could efficiently deliver reovirus to melanoma tumours in immune-competent mice for effective viral-mediated tumour oncolysis and antitumour immune priming (Ilett *et al.*, 2009). Furthermore, the use of T cells and DCs as cell carriers effectively shielded the virus from neutralizing antibodies in pre-immunized mice. In a similar approach, cytokine-induced killer (CIK) cells, which possess natural tumour-homing abilities, were used in combination with oncolytic vaccinia virus to achieve directed targeting and subsequent regression of tumours in both immunocompetent and immune-deficient mouse models (Thorne *et al.*, 2006). By pre-infecting CIK cells with vaccinia virus, the virus remained hidden during a prolonged intracellular eclipse phase until interaction with the tumour, at which time the virus was released. An additional benefit of using cell carriers to deliver OVs to tumours is that it provides a protective shield against neutralizing antibodies and other blood components which can inactivate the virus before it reaches the tumour (Goel *et al.*, 2007). This approach would allow for systemic delivery of the OV for treatment of disseminated disease, as well as multiple administrations of the virus, even after neutralizing antibodies reach high titres in the bloodstream. Due to these benefits, various cell carrier strategies are currently under development.

Concluding remarks

In this review, we have provided an overview of the complex interactions at play among OV therapies and host immune responses. Due to the multifaceted nature of these responses, there are apparent contradictions regarding the benefits of enhancing versus inhibiting the immune response in order to synergize with OV therapy. While we and others have provided evidence in support of inhibition of host inflammatory responses in order to maximize virus replication and spread, thereby exploiting the direct cell killing aspect of oncolytic viral therapy, undeniable evidence indicates that inflammatory responses may be essential for the efficacy of OV therapy. So who is correct, and what is the reason for the apparent discrepancy? Perhaps the biggest factor at play in these contradictory reports is the limitations of available animal models to test our hypotheses. Due to technical limitations, researchers are often forced to utilize subcutaneous

tumour models, which could result in drastically different immune responses than those induced in an orthotopic setting. Furthermore, the intrinsic variations among different tumour types, such as the vascularity, degree of IFN sensitivity and immunogenicity, are immense and could play a crucial role in determining the most effective type of OV therapy for that particular tumour. Therefore, it would be necessary to directly compare the efficacy of immune evasion versus stimulation in the same tumour model in order to accurately assess the potential benefit of each strategy.

Despite the ongoing debate regarding the importance of antiviral immune responses, it is the general consensus that truly successful treatment protocols will need to harness the ability of OVs to promote adaptive immunity against the tumour in order to protect the host from residual tumour cells which evade direct virus infection. The ideal approach would involve simultaneous suppression of viral clearance mechanisms while producing potent antitumour immune responses. As oncolytic viral therapy moves further into the clinic, it is expected that host/virus interactions will become better elucidated, and strategies to exploit and synergize with host immune responses will become further developed until we have optimized vector strategies which produce significant survival benefits, without compromising safety both in preclinical models and in challenging clinical scenarios.

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