Immune Recruitment and Therapeutic Synergy: Keys to Optimizing Oncolytic Viral Therapy?

Jay D. Naik1, Christopher J. Twelves1, Peter J. Selby1, Richard G. Vile1,2,3, and John D. Chester1

Abstract

Oncolytic viruses consist of a diverse range of DNA and RNA viruses traditionally thought to mediate their effects by exploiting aberrations in tumor pathways, allowing preferential viral replication in, and killing of, tumor cells. Clinical development has progressed to late-phase trials, potentially heralding their introduction into clinical practice. However, despite this promise, the activity of oncolytic viruses has yet to achieve the potential suggested in preclinical models. To address this disparity, we need to recognize the complex interaction among oncolytic viruses, tumor, chemotherapy, and host immune system, and appreciate that direct oncolysis may not be the only factor to play an important role in oncolytic virus-mediated antitumor efficacy. Although key in inactivating viruses, the host immune system can also act as an ally against tumors, interacting with oncolytic viruses under the right conditions to generate useful and long-lasting antitumor immunity. Preclinical data also suggest that oncolytic viruses show synergy with standard therapies, which may offer improved clinical response rates. Here, we explore clinical and preclinical data on clinically relevant oncolytic viruses, highlighting areas of progress, uncertainty, and translational opportunity, with respect to immune recruitment and therapeutic synergy. Clin Cancer Res; 17(13); 4214–24. ©2011 AACR.

Introduction

The notion of using replicating viruses as potential anticancer agents goes back more than a century, with occasionally dramatic regressions of cancers following viral infections (1–6). Clinical responses were observed in preliminary studies using replicating wild-type viruses such as adenovirus (1) and mumps (5). However, progress faltered for a number of reasons, including fears over safety, the lack of objective response criteria, lack of randomized trials, and the absence of good manufacturing practice standards (1, 5, 6).

Despite these reservations, oncolytic viruses (OV) remain exciting prospective anticancer agents, because of reports of selective killing of tumor cells (7, 8). There has been a recent resurgence of interest in OVs, based not only on fundamental advances in tumor and viral biology, but also on the ability to scale-up manufacture of clinical grade viruses and improved clinical trial designs (9, 10).

Clinical Development of Oncolytic Viruses

Modern trials commenced in the mid-1990s, administering OVs by a variety of routes, including intratumoral (IT), locoregional, and, more recently, i.v. routes (Table 1).

Concerns over the safety of replicating OVs have eased, given the satisfactory treatment of several hundred patients within multiple early-phase trials of RNA [reovirus, Newcastle disease virus (NDV), and measles] and DNA [adenovirus, vaccinia, and herpes simplex virus (HSV)] OVs (11–25). Typical local response rates observed after IT administration range from ~10% to 60% (14, 16, 17, 20, 23), with the best objective radiologic response rates lower, at just under 30%, at best (20, 23). Single-agent i.v. treatment offers even lower objective response rates, at <10% (12, 19, 21, 25).

Commonly observed side effects include local reactions within injected tumor masses following IT administration and flu-like syndromes following i.v. infusion. Edema, precipitating biliary tract obstruction and jaundice (22), or bronchial obstruction and respiratory compromise (21) represent serious adverse events and have led to trial protocols excluding patients in whom disease has the potential to cause critical obstruction (Table 1; ref. 23).

A closer look at the reasons behind the difference between preclinical studies and the clinical experience may be the first step in realizing the full antitumor potential of OVs. The clinical development of ONYX-015 (Onyx Pharmaceuticals; ref. 26), a well-characterized oncolytic adenovirus, which was first used more than a decade ago, illustrates some of the challenges in developing OVs clinically.
Multiple clinical trials were completed in multiple tumor types and using various routes of administration (Table 1). Objective local response rates were improved to $>$50% by combining ONYX-015 with chemotherapy in squamous cell cancer of the head and neck (SCCHN), hinting at synergy (15). However, an unreported, incomplete phase III trial halted ONYX-015 clinical development (27).

H-101, a closely related virus, has since found use as a licensed cancer therapy in China for SCCHN in combination with radiotherapy. Unfortunately, H-101 approval is based on limited controlled trial evidence (27), and a corruption scandal over the drug approval process in China (involving unrelated agents) seems to have discouraged widespread use (28).

Despite these setbacks, anticipation remains high, with recently reported phase II trials with HSV and reovirus OVs underpinning current randomized phase III trials in melanoma (23, 29) and head and neck cancers (30). Clinical observations with these OVs, as outlined below, suggest that recruitment of a host antitumor immune response or synergy with other anticancer agents may represent important factors in optimizing OV efficacy.

The DNA HSV OV, JS1/34.5-/47-/granulocyte macrophage colony-stimulating factor (OncoVEXGM-CSF, Amgen) represents a clinically advanced OV candidate designed to invoke an antitumor immune response by oncolytic release of tumor antigens, which, if presented appropriately to immune cells, may provoke an antitumor immune response. This aim is enhanced by deletion of the ICP 47 gene, promoting greater presentation of tumor antigen on the infected cell surface. Further to this, expression of GM-CSF, a protein that stimulates antigen-presenting dendritic cell (DC) activity, increases the likelihood of successful "recognition" of tumor antigen and a therapeutic antitumor immune response (31).

In a phase II trial, intratumoral JS1/34.5-/47-/GM-CSF elicited 13 (8 complete and 5 partial) objective responses based on Response Evaluation Criteria In Solid Tumors (RECIST) in 50 patients with unresectable metastatic melanoma (23). Treated patients had a 58% 1-year survival rate (23), comparing favorably with reported phase II survival rates of 25.5% (32). Data from a phase III trial in metastatic malignant melanoma patients are keenly awaited (33). The double-stranded RNA (dsRNA) reovirus (type 3 Dearing) was shown to be safe and effective in early-phase clinical trials employing IT (14, 34), i.v. (25, 35), and combination (30, 36) approaches (Table 1). An ongoing, randomized phase III trial of reovirus, in combination with chemotherapy, in refractory SCCHN followed a recent phase I and II study in which 8 of 19 SCCHN patients (involving unrelated agents) seems to have discouraged widespread use (28).

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### Translational Relevance

Oncolytic viruses (OV) are biologically targeted agents with the ability to potently, and selectively, replicate in and kill tumor cells. There is undoubted promise with late-phase trials using both DNA and RNA viruses underway; however, their clinical efficacy remains to be proven.

Increasingly, there is recognition of a potentially productive, rather than inhibitory, relationship between OVs and the host immune response, with fresh approaches encouraging host antitumor immunity showing clinical promise.

Preclinical synergy with chemotherapy is reflected in increased clinical response rates and may represent another way to optimize OV therapy. However, preclinical evidence also suggests chemotherapy may have an impact on the host immune response, with uncertain effects on long-term outcome. Clinical data corresponding to the complex interactions among OVs, tumor, chemotherapies, and the host immune system are lacking. Therefore, high-quality translational studies are required to enhance our understanding of the biology of OVs in order to improve outcomes further.

### Table 1. Oncolytic viruses in clinical development

<table>
<thead>
<tr>
<th>Virus (clinical example)</th>
<th>Tumor type</th>
<th>Status</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1B deleted adenovirus (ONYX-015, H-101)</td>
<td>SCCHN</td>
<td>H-101 licensed as combination therapy for SCCHN (China only)</td>
<td>27, 107</td>
</tr>
<tr>
<td>HSV (OncoVEXGM-CSF)</td>
<td>Melanoma</td>
<td>Phase III registration trial in melanoma</td>
<td>23, 33, 46</td>
</tr>
<tr>
<td>Reovirus</td>
<td>SCCHN</td>
<td>Phase I, phase II melanoma, lung, sarcoma</td>
<td>14, 25, 30, 34, 35</td>
</tr>
<tr>
<td>Vaccinia (JX-594)</td>
<td>HCC</td>
<td>Phase I and II HCC, SCCHN</td>
<td>22</td>
</tr>
<tr>
<td>NDV (PV-701)</td>
<td>CRC</td>
<td>Phase I and II</td>
<td>12, 21</td>
</tr>
<tr>
<td>Measles (MV-CEA)</td>
<td>Ovary</td>
<td>Phase I in ovarian cancer</td>
<td>13</td>
</tr>
<tr>
<td>VSV (VSV-hIFNbeta)</td>
<td>HCC</td>
<td>Phase I HCC</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Abbreviations: CRC, colorectal cancer; HCC, hepatocellular cancer; N/A, not available.
achieved an objective partial response (42%; ref. 30). Reo-
virus, therefore, represents another advanced contender in
the race to enter the clinic (Fig. 1; refs. 30, 36).

Oncolytic Viruses and Selective Replication

Appreciating the rationale for the action of OVs may help
to put the role of the immune response and synergy into
perspective. Preclinical evidence on oncolytic efficacy con-
centrates on the exploitation of dysregulated signaling
pathways in tumor cells, which may attenuate antiviral
responses or support viral replication (Fig. 1).

RNA viruses benefit from disruption of antiviral immune
responses. Reovirus benefits from attenuation of the dsRNA-sensing, protein kinase receptor (PKR), via RAS
activation (37, 38). Deficiencies in cellular IFN responses
in tumors allow NDV and vesicular stomatitis virus (VSV)
to replicate selectively (Fig. 1; refs. 39, 40).

Other viruses have been engineered to enhance antitu-
mor activity or improve safety (Fig. 1). The function of
selectively inactivated, replicative genes may be redundant
because of abnormalities in tumor cells, but equally can
enhance safety, by preventing OV replication in normal
cells. Figure 1 outlines mechanisms, including the follow-
ing: inactivation of the ICP 34.5 gene controlling neuro-
virulence and late protein synthesis in HSV OVs, for
example, JS1/34.5-/47-/OncoVEXGM-CSF used in mela-
noma (31); deletion of thymidine kinase (TK), required
for vaccinia replication, for example, JX-594 used in mel-
anoma and liver tumors (41); and deletion of the adeno-
viral E1B gene, the product of which normally binds to
and inactivates p53, for example, ONYX-015/H101 used
in SCCHN (26).

Strategies for Maximizing the Efficacy of
Oncolytic Virus Therapy: Exploiting the Host
Immune System

A comprehensive review of the immune system in the
context of OV therapy is beyond the scope of this article
and has been summarized elsewhere (42, 43). Briefly,
the 2 arms of the immune system are as follows: adaptive

Figure 1. Tumor selective replication of oncolytic viruses. Aberrant tumor pathways that offer redundancy to oncolytic viral genes contributing to
tumor selectivity: 1, activation of epidermal growth factor receptor (EGFR) abrogates vaccinia growth factor (vgf), which normally stimulates EGFR, in
readiness for vaccinia infection; 2, activation of RAS induces an inhibitor of protein kinase receptor (PKR), which would normally prevent translation of RNA viral
(reovirus or NDV) genes, to control infection; 3, aberrantly activated protein expression compensates for ICP-34 absence (ICP-34 normally induces protein
expression), restricting replication to dividing (tumor) cells; 4, tumor suppressor inactivation can compensate for absent viral proteins; for example, E1B is
normally required to inactivate p53; 5, upregulated cellular thymidine kinase (TK) in tumor compensates for absent TK in tk-deleted vaccinia virus (tkdeleted VV);
6, IFN responses are powerful mediators of antiviral responses and often impaired in tumors, especially benefiting RNA viruses NDV and VSV. Downstream
attenuation of PKR also benefits the replication of reovirus and ICP-34-deleted (ICP-34deleted) HSV. Clinically relevant OV examples are given in
parentheses.
Potential interactions of the host immune system with OVs and tumors are summarized in Tables 2 and 3. These interactions are complex and illustrate how the host immune response can be focused on the virus (antiviral immunity), or the tumor (antitumor immunity). The development of antitumor immunity depends on the interplay between tumor and immune system, and it is well recognized that tumors employ multiple mechanisms to avoid antitumor immunity, including decreased immunogenicity, resistance to immune-mediated killing, and immune subversion (Table 3).

Theories of immune activation suggest that effective immunity requires an appropriate danger signal indicating cellular or tissue distress (44) or stimulation of pattern recognition receptors (PRR) on immune-activating cells (45). The immune premise of OVs is the provision of these activating functions by oncolytic killing of tumor cells (danger) and release of tumor antigens (stimulation of PRR), thereby engaging effective antitumor immunity.

Preclinical Adaptive Immune Response Data

Preclinical work suggests that OVs may promote immune responses, which outweigh direct oncolysis in mediating antitumor efficacy (Table 2). Long-term immune control may arise from OV-infected tumor cells boosting both innate and subsequent adaptive tumor-specific immune responses. The clinical OVs JS1/34.5-/47-/GM-CSF and JX-594 express a GM-CSF transgene in order to enhance adaptive antitumor immunity (41, 46). GM-CSF improves antigen presentation through activation of DCs, consequent immune recognition of released tumor antigens, eventually stimulating an increase in tumor-specific cytotoxic T lymphocytes (TS-CTL), which have been associated with long-term tumor control in both clinical and preclinical studies (Table 2).

We have shown, in immunocompetent mice carrying B16 melanoma cells, that reovirus and VSV can enhance tumor clearance and induce specific long-term protection from tumor rechallenge via generation of melanoma antigen-specific lymphocytes (47–49).

Table 2. Key interactions among tumors, the host immune system, and oncolytic viruses, resulting in enhanced antitumor effects

<table>
<thead>
<tr>
<th>Immune effect</th>
<th>Innate mechanism</th>
<th>Adaptive mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased antitumor immunity</td>
<td>Improved lung cancer prognosis, related to NK infiltration of tumor (C; ref. 87)</td>
<td>Melanoma regression, following adoptive transfer and tumoricidal effect of TS-CTL (C; ref. 89)</td>
</tr>
<tr>
<td>OV-stimulated antitumor immunity</td>
<td>Improved tumor killing, associated with increased innate neutrophil infiltration and vascular shutdown (VV, VSV; P; ref. 61)</td>
<td>Tumor regression in non-OV–injected sites, associated with antitumor immunity (HSV, VV, measles; C; refs. 20, 23, 29; P; ref. 62)</td>
</tr>
<tr>
<td></td>
<td>Improved tumor killing, due to induction of IT antitumor cytokines (reovirus; P; ref. 54)</td>
<td>Tumor regression, associated with generation of TS-CTL, and decrease in immune-suppressive cell levels (HSV; C; ref. 29)</td>
</tr>
<tr>
<td></td>
<td>Enhanced release and presentation of tumor-associated antigens (P; ref. 91)</td>
<td>Protection from tumor rechallenge, via TS-CTL (reovirus, VSV; P; refs. 47–49, 55)</td>
</tr>
<tr>
<td>Manipulation of antiviral or antitumor immunity</td>
<td>Increased tumor killing with enhanced OV replication after attenuation of the host innate response (HSV, VSV, VV, and reovirus; P; refs. 63–65, 81)</td>
<td>Tumor regression, associated with GM-CSF–induced TS-CTL (VV, HSV; C; refs. 23, 29; P; refs. 41, 55)</td>
</tr>
</tbody>
</table>

NOTE: The complex interactions between tumor and host immune system are further altered by the introduction of OV. Understanding and exploiting the conditions that result in antitumor effects may help to maximize OV therapy.

Abbreviations: C, clinical evidence; P, preclinical evidence; TS-CTL, tumor-specific cytotoxic T lymphocyte.
will be key in mediating successful OV immunotherapy (Tables 2 and 3).

**Preclinical Innate Immune Response Data**

Data using clinically relevant OVs show an intriguing relationship with the host innate system. OVs may be inactivated, preventing direct oncolysis (Table 3), but also show a potentially productive inflammatory antitumor response (Table 2). Reovirus can boost a variety of innate antitumor functions, including NK cell recruitment, alongside activation and induction of DC maturation (54).

Cytokines such as the interleukins (IL) and IFNs are proteins that regulate the growth and function of immune cells, thereby potentially having either positive or negative effects on antitumor immunity. Although reovirus can directly influence the balance of tumor cytokines from immunosuppressive to inflammatory, increasing cytokines associated with tumor rejection (55), several attempts have been made to directly incorporate cytokine transgenes into OVs. IFNs are cytokines that enhance tumor antigen presentation and cytotoxicity. IFN-1β transgene expression from an oncolytic VSV vector enhances overall antitumor activity in a murine mesothelioma model through a T-cell–activating mechanism. In addition, severe combined immunodeficient (SCID) mice were protected from lethal oncolysis due to increased IFN-γ production, induction of antiangiogenic proteins, and an enhanced therapeutic effect when assessed in vivo (60).

IL-12 is another cytokine of interest that shows pleiotropic effects, including stimulation of T-helper cells, increased tumor infiltration and cytotoxicity by CTLs and NK cells, and stimulation of IFN-γ production, resulting in angiogenic effects (57–59). IL-12 expression from the clinically relevant HSV virus NV1020 led to increased IFN-γ production, induction of antiangiogenic proteins, and an enhanced therapeutic effect when assessed in vivo (60).

Innate neutrophil infiltration also enhances therapeutic efficacy of measles and vaccinia viruses, the latter by triggering endothelial collapse, angiogenic effects, and bystander apoptosis of tumor (61, 62). In this instance, interfering with the neutrophil response increased direct oncolytic killing, but decreased bystander antivascular therapy and overall antitumor efficacy (Table 3; ref. 61). However, other studies have shown the opposite effect, with inhibition of the innate response improving replication and therapeutic efficacy of HSV, vaccinia, and reovirus (Table 2; refs. 53, 63–65). These differences underline the influence of experimental conditions. Ultimately, complex OV, tumor, and immune interactions may not be adequately represented in present preclinical models, and clinical relevance may be best sought in a translational setting.

**Clinical Immune Data with Oncolytic Viruses**

Immune response data on OVs in clinical practice are limited, but give an indication of the host response to tumor

<table>
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<th>Immune effect</th>
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<th>Adaptive mechanism</th>
</tr>
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<tbody>
<tr>
<td>Decreased antitumor immunity</td>
<td>Tumor cell immunogenicity, due to caspase expression (P; ref. 93)</td>
<td>Tumor resistance to immune killing, via death receptors (P; ref. 91)</td>
</tr>
<tr>
<td>OV stimulated antiviral immunity</td>
<td>OV inactivation with potential reduced oncolysis due to:</td>
<td>OV inactivation with potential reduced oncolysis due to:</td>
</tr>
<tr>
<td></td>
<td>· IFN response (VSV; P; ref. 80)</td>
<td>· Induction of nAb response (VV, HSV, reovirus, VSV, measles; C; refs. 22, 23, 25; P; refs. 48–49)</td>
</tr>
<tr>
<td></td>
<td>· Complement mediated killing (HSV; P; ref. 95)</td>
<td>· Potent antiviral, versus TS-CTL, immune response (reovirus, VSV; P; refs. 48–49)</td>
</tr>
<tr>
<td>Manipulation of antiviral immunity</td>
<td>Decreased tumor killing following loss of bystander effect, due to attenuation of innate response (VSV; P; ref. 61)</td>
<td>Increased in vivo toxicity from OV, due to ablation of nAb response (reovirus; P; ref. 96)</td>
</tr>
</tbody>
</table>

**Table 3.** Interactions between tumor, the host immune system, and oncolytic viruses attenuating anticancer effects, or increasing toxicity

NOTE: The complex interplay among tumor, host immunity, and OV may be detrimental to antitumor therapy. Direct loss of immune control of tumors may be observed, oncolytic therapy may be attenuated by the host immune response to OVs, and OV toxicity may be increased. Understanding and avoiding these interactions may serve to enhance OV-mediated therapy.

Abbreviations: C, clinical evidence; P, preclinical evidence; NKG2D, activating receptors for NK cells; T-reg, T regulatory cell.
and to OV alike. Available data relate to the phase II trial of GM-CSF–expressing JS1/34.5-/47-/OncoVEXGM-CSF described earlier (23). This trial is notable for the clinically significant proportion of complete responses (16%).

The authors used novel immune assessment criteria, allowing a limited degree of tumor progression, prior to response (considered clinically insignificant and not requiring alternative treatment intervention), permitting the development of an immune-mediated response (66). These guidelines were developed for immune-stimulating therapies, such as the monoclonal antibody ipilimumab, which has recently shown a ground-breaking 3-month survival advantage over a peptide vaccine in metastatic melanoma (67). Six of the 13 patients showing an objective response also showed characteristics consistent with immune response criteria, with limited progression in soft tissue and visceral sites, followed by complete and 2 partial RECIST responses (23).

Despite an emphasis on the cancer vaccine properties of JS1/34.5-/47-/OncoVEXGM-CSF, the only immune study on tumor and blood samples reported includes just 11 of the 50 trial patients recruited in total (29). Nevertheless, indicative observations were made, with the generation of cytotoxic T cells against a melanoma-associated antigen (MART-1) found in the tumor and peripheral blood of responding patients. In addition, comparatively low levels of immune-suppressive T-regulatory (T-reg) cells linked to poorer outcomes in other clinical studies were found within injected tumors (Table 3; refs. 29, 68).

Another well-studied cytokine, IL-2, offers the potential to increase NK cell and CD8+ T-cell function, and the ability to increase vascular permeability (69). When expressed from a vaccinia virus vector in 6 patients with malignant pleural mesothelioma, IL-2 expression was detectable and associated with T-cell infiltration in half of biopsied tumors obtained from all 6 patients. No systemic toxicity was observed, but nor were any objective clinical responses seen, nor further studies reported (70).

Clinical evidence of both radiologic and immune-mediated antitumor responses has been observed in trials employing vaccinia, JS1/34.5-/47-/OncoVEXGM-CSF, JX-594, and ONYX-015. These responses were seen at sites distant from those injected, in keeping with preclinical observations of systemic, immune-mediated effects (Table 2; refs. 15, 20, 22, 23). Biopsies of noninjected tumor sites have shown immune cell infiltration consistent with this finding (20). However, another study observed vaccinia virus (JX-594) in biopsies from noninjected sites, suggesting systemic dissemination of virus and direct oncolysis as a viable alternative mediator of tumor responses (22).

Preclinical work with OVs raises questions of whether the traditional dose-escalation approach is appropriate for early-phase trials of OVs. Our own preclinical studies in murine melanoma models suggest it may actually be counterproductive to administer the maximum tolerated dose of OV, as this approach may encourage antiviral, rather than antitumor, immunity (Table 3; refs. 48, 49). It is well established that current clinical doses and modes of administration result in clinically robust, protective nAb responses to reovirus, NDV, and vaccinia virus, even in heavily pretreated patients (Table 3; refs. 21, 22, 71). Levels of nAb do not directly relate to initial clinical response, that is, preexisting antiviral immunity does not necessarily prevent direct oncolytic therapy, perhaps reflecting the immune-suppressive local tumor environment, allowing OV replication (21, 22, 71). However, it would be of interest to establish whether the nAb response occurs at the expense of an eventual adaptive antitumor immune response and, more importantly, whether this has an impact on long-term outcome.

Overall, present clinical data support, to a limited extent, preclinical observations that antitumor immune responses are important in long-term OV efficacy. It would seem desirable that primary immune endpoints are explored and validated in future trials of OV therapy.

Maximizing Oncolytic Virus Therapy: Combination Therapy for Synergy

Preclinical: Oncolytic virus combination with chemotherapy

Multiple preclinical studies indicate a highly desirable synergistic effect when combining chemotherapy with OV. Table 4 lists OVs showing synergy in combination with chemotherapy and some possible mechanisms involved. A common preclinical method for assessing synergy is the Chou Talalay combination index (CI). This commonly used analysis involves plotting dose–effect curves for each therapy and multiplying diluted combinations of the therapies, using the "median effect" equation, to obtain a CI. CI values of <1, 1, and >1 indicate synergy, additive effect, and antagonism, respectively (Table 4; ref. 72).

The taxane chemotherapies (docetaxel and paclitaxel) consistently show strong synergistic activity (CI < 1) in preclinical combination studies with a variety of OVs, including adenovirus, reovirus, and HSV (see Table 4). Various mechanisms of synergy are suggested, perhaps reflecting the complex biology of OV and broad effects of chemotherapy. The microtubule-stabilizing action of taxanes seems to be important in facilitating reoviral and adenovirus replication (73, 74). Induction of apoptosis may be a common pathway for OV synergy with taxanes. Reovirus-induced, caspase-dependent apoptosis is synergistically enhanced by the prolonged G2 to M-arrest induced by paclitaxel in lung cancer cell lines. Similarly synergistic apoptotic cell death results from the combination of HSV-induced G1-arrest and taxane G2 to M-arrest in prostate cancer cells (75).

Paclitaxel sensitivity is also synergistically enhanced by vaccinia-induced release of type I IFN following viral infection and high-mobility group protein B1 following cell lysis (76). Finally, physical effects may play a part in synergy, as shown in preclinical studies in which the combination of oncolytic HSV and taxane chemotherapy resulted in cell lysis and breakdown of tumor, with improved ingress and replication of virus in tumor cells.
Table 4. Synergy between oncolytic viruses and chemotherapy agents

<table>
<thead>
<tr>
<th>Virus</th>
<th>Agent (tumor model)</th>
<th>Putative mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV (G207)</td>
<td>Temozolamide (glioma; ref. 99)</td>
<td>Temozolamide induced increase in stress response genes, with ICP-34 homology</td>
</tr>
<tr>
<td></td>
<td>Taxane-docetaxel (prostate; ref. 75)</td>
<td>Mitotic slippage with ↑ apoptosis through combined G2 to M and G1-arrest (ref. 75)</td>
</tr>
<tr>
<td>Reovirus (Reolysin)</td>
<td>Taxane (paclitaxel), cisplatin gemcitabine,</td>
<td>Prolonged mitotic arrest, with ↑ apoptosis</td>
</tr>
<tr>
<td></td>
<td>vinblastine (lung, ref. 79)</td>
<td>(refs. 78, 79)</td>
</tr>
<tr>
<td></td>
<td>Cisplatin, paclitaxel (melanoma; ref. 78)</td>
<td>↑ Caspase-dependent apoptosis (ref. 78)</td>
</tr>
<tr>
<td>Vaccinia virus</td>
<td>Taxane–paclitaxel (ovarian, colorectal; ref. 76)</td>
<td>Chemosensitization by:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Postinfection, type I IFN release</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Postlysis, high-mobility group protein</td>
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<tr>
<td></td>
<td></td>
<td>B1 release (ref. 76)</td>
</tr>
<tr>
<td>Adenovirus (ONYX-015)</td>
<td>Taxane–paclitaxel, cisplatin (lung; ref. 97)</td>
<td>E1a-induced cell cycle activation (ref. 102)</td>
</tr>
<tr>
<td></td>
<td>Paclitaxel (ovarian; ref. 100)</td>
<td>E1a sensitization to chemotherapy (ref. 101), mitotic slippage, and apoptosis (ref. 100)</td>
</tr>
</tbody>
</table>

NOTE: Preclinical therapeutic synergy with OV is recognized across a range of tumor types and chemotherapies and is ascribed when the Chou Talalay when the combination index is less than 1 (see main text). Underlying putative mechanisms of synergy are outlined.

This study used an alternative method to combination index to attribute synergy.

(77). Other chemotherapies also show synergy via similar mechanisms, for example, cisplatin, which potentiates apoptosis in melanoma lines (78, 79); however, the wide-ranging, high-level, synergy observed between various OVs and taxanes would support such combinations being explored clinically (Table 4).

A recent report describes a systematic attempt to maximize synergy while retaining oncolytic ability (81). Diallo and colleagues describe a pharmacoviral screen in which the impact of each of more than 12,000 chemical compounds on viral oncolysis was assessed in a cell-based assay, using a high-throughput screening method. The cytotoxicity of low titers of the VSV mutant, VSV-Δ51, which is highly sensitive to the IFN response, was assessed, with and without drug, in a partially resistant cell line (81). Their approach identified a number of potential compounds showing synergy for the replication and spread of VSV-Δ51 in vitro.

One of the chemical compounds assessed in this way, 3,4-dichloro-5-phenyl 2,5-dihydrofuran-2-one (VSe1), was shown to suppress the IFN response to VSV, conferring on VSV a temporary and, apparently, tumor-selective replication advantage in vivo. The discovery by Diallo and colleagues of a specific compound complementing the known biology of the mutant VSV-Δ51 suggests this screening approach could be replicated with other viruses. Although this is an attractive prospect, it has not yet been realized in clinical practice (81).

This synergy of VSV-Δ51 and VSe-1 is in keeping with other preclinical observations that suppression of the innate antiviral immune response can improve oncolysis and efficacy (Table 2; refs. 63–65). There is further preclinical evidence, in melanoma, that reoviral synergy with cisplatin accompanies ablation of the local innate inflammatory response (78), shown preclinically to boost innate antitumor immunity (54). An important question to resolve is, therefore, whether the improvement in direct oncolysis accompanying selective suppression of the innate antiviral response may be offset by the potential loss of antitumor immunity. A reasonable hypothesis would be to expect synergy to be reflected in greater immediate tumor shrinkage (compared with chemotherapy alone). In contrast, development of an antitumor response may be expected to correspond to longer duration of response, prior to subsequent progression. These translationally relevant questions could be addressed, for example, in the ongoing trial of chemotherapy ± reovirus in SCCHN.

Clinical oncolytic virus and chemotherapy combination data

None of the currently available data clearly indicate whether preclinical synergy between OVs and chemotherapy can be translated into improved clinical outcomes, but signals suggest improved response from the combination of OV and cytotoxic drugs in clinical trials. Although not developed commercially, a combination phase II trial of ONYX-015, combined with cisplatin and 5-fluorouracil chemotherapy, in patients with recurrent SCCHN showed notable complete (8/37) and partial response rates (19/37) in injected nodules (15). These results compared favorably with historical data obtained with either virus alone or chemotherapy alone (22%–33%; refs. 17, 83), and were consistent with preclinical models showing synergy with the same agents (26).
Reovirus combined with docetaxel has proven safe in a phase I trial of 16 patients, with 1 objective complete response, 3 partial responses, and 7 patients with stable disease observed (84). Reovirus was detectable in tumor biopsies, and docetaxel did not compromise the nAb response to reovirus (NARA). In contrast, a similar early-phase trial of gemcitabine combined with reovirus led to liver toxicity and reduced NARA. Only 1 objective response was seen among 16 patients treated, plus 6 patients with stable disease (85). Reovirus with taxane–platinum combination chemotherapy has also featured in a phase II study in relapsed SCCHN patients. Nineteen patients, most of whom were refractory to previous platinum-based chemotherapy, were treated with i.v. reovirus, along with carboplatin and paclitaxel chemotherapy, with partial response rates more than 40% and stable disease in a further 30% (30). However, in contrast to the JS1/34.5+/47-/GM-CSF phase II data in melanoma (23), no complete responses were observed, and though promising in terms of response rate, the small sample size and current lack of information about duration of response do not immediately predict the success of chemo-virotherapy in this setting, according to predictive algorithms (30, 86). The result of a key ongoing randomized phase III trial using the same chemotherapy combination ± reovirus in SCCHN patients is, therefore, awaited with genuine interest.

Conclusions

OVs represent a diverse group of viruses with the ability to selectively kill tumor cells and, thus, represent attractive anticancer agents. Preclinical oncolytic activity has not, thus far, been translated into routine clinical practice, which may reflect the inability of preclinical models to replicate the complexity of diverse interactions between virus tumor and intact host immune system.

It is clear that embracing existing knowledge, by encouraging antitumor immunity (JS1/34.5+/47-/GM-CSF) or exploiting synergy with chemotherapy (reovirus, ONYX-015) to enhance OV efficacy has already contributed to emerging promise, leading to late-phase OV clinical trials (23, 30, 33). Robust late-phase data are required before we can accept OVs as legitimate alternatives to current therapies; however, current approaches seem to be on the cusp of offering the genuine prospect of improved clinical outcomes.

Questions still remain. For example, is it possible, or even desirable, to overcome antiviral immunity? Is antitumor immunity really more important than direct oncolysis? If so, is it possible to quantify this and consistently to manipulate the host immune response against tumors? Synergy may offer improved response rates, but will it also lead to long-term tumor control? Is synergy also compatible with productive antitumor immunity? These questions may well be too complex to resolve using current preclinical models and further highlight the continuing need for in-depth translational studies, ideally in the context of OV trials. Deriving clear answers will help direct future approaches, offer enhanced therapy, and could ultimately lead to improved survival for patients.

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