

RECENT PROGRESS IN THE BATTLE BETWEEN ONCOLYTIC VIRUSES AND TUMOURS

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Abstract | In the past 5 years, the field of oncolytic virus research has matured significantly and is moving past the stage of being a laboratory novelty into a new era of preclinical and clinical trials. What have recent anticancer trials of oncolytic viruses taught us about this exciting new line of therapeutics?

Oncolytic viruses are replicating microorganisms that have been selected or engineered to grow inside tumour cells. It is well known that as a tumour evolves, mutations in multiple genes contribute to the malignant phenotype. Oncolytic viruses specifically target cancer cells because they are able to exploit the very same cellular defects that promote tumour growth. Some oncolytic viruses have been selected or designed to take advantage of frequent tumour-specific mutations in antiviral defence programmes. Others have been engineered to be dependent on signalling pathways or transcriptional programmes that are constitutively activated in tumours. A third approach is to restrict virus entry into cells based on the expression of antigens that are unique or overexpressed on the tumour cell surface. In any case, the tumour cell is killed by the oncolytic virus as it takes over the cellular translational and transcriptional machinery, ultimately leading to an induction of cell necrosis or apoptosis (FIG. 1). Over the past century, a series of case studies and anecdotal reports have indicated that viruses could indeed be used as anticancer therapeutics^{1–3}. However, it has only been in the last decade, as we have gained a more comprehensive understanding of the molecular basis of tumorigenesis, that it has been possible to develop the oncolytic virus therapeutic platform.

In principle, oncolytic viral therapy offers several advantages over conventional anticancer drugs. Viruses can be rapidly modified by recombinant DNA technology, allowing for the rational creation of 'designer viruses'. Addition of genes that code for toxic payloads

or immune-stimulatory products, as well as the natural propensity of the virus to promote tumour-specific inflammation⁴, makes oncolytic viruses multi-modal therapeutics. Owing to their ability to self-replicate within the cancer cell, oncolytic viruses have unique pharmacokinetic properties that are distinct from conventional therapeutics. In a sense, oncolytic viruses can be thought of as miniature biological machines that we can program to specifically target, replicate in and ultimately kill tumour cells. The arsenal of oncolytic viruses that are under development is ever expanding, as are the creative strategies for achieving tumour-specific replication. Owing to the complexities of tumour biology and the heterogeneity of human tissues, it seems unlikely that one kind of virus or a single targeting strategy will be sufficient to treat all cancers.

Clinical trials

Although the number of different types of oncolytic viruses that have been tested in preclinical trials is increasing, only a few have made the transition into the clinic. Only 8 years ago, the first patients were treated in the first *bona fide* clinical trials of oncolytic virus therapy. Although more than 50 phase I or II clinical trials have been subsequently conducted, there is only a single published phase III trial⁵. So, our clinical experience is limited — we are still studying these new therapeutics and developing ways to optimize their efficacy. TABLE 1 summarizes the different oncolytic viruses that have been tested in preclinical and clinical trials, and their developmental status.

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Summary

- Clinical trials have indicated that oncolytic viruses might be developed as safe and effective anticancer agents.
- The translation of oncolytic viruses from the culture dish to preclinical tumour models to studies involving patients has revealed new hurdles to cancer therapy that can be overcome using multidisciplinary approaches.
- Novel strategies can be used to facilitate viral evasion of the immune system, the prevention of viral uptake by the liver, and an increased specificity for tumour cells, either at the cell surface or through intracellular restriction.
- Oncolytic viruses can be engineered to target the same genetic mutations that provide tumour cells with a proliferative or survival advantage in patients.
- The intravenous delivery of viruses must be perfected if oncolytic virus-based therapeutics are to be used to treat patients with metastatic tumours.

Oncolytic viruses are given to patients through different approaches. For safety reasons, viral vectors based on **vaccinia virus**, adenovirus, reovirus, **newcastle-disease virus** (NDV), coxsackievirus and herpes simplex virus (HSV) (BOX 1) were administered by intratumoural injection in phase I trials^{6–9}. Typically, intratumoural delivery caused only local transient

swelling without significant side effects — the same minimal side effects were observed in trials in which HSV or adenovirus was given directly into the brain of patients with gliomas^{8,9}. In fact, oncolytic viruses were found to have such low levels of toxicity that the conventional endpoint of phase I trials — uncovering the maximum tolerated dose (MTD) — is usually not reached^{8–10}.

What does this failure to reach MTD mean? Does it mean that we are not producing or delivering enough virus to reach maximum efficacy? Recent technical advances in virus preparation will allow higher amounts of virus to be delivered to patients to answer these questions. In many trials that involved intratumoural injections there have been some signs of efficacy, such as occasional complete responses, partial responses and stable disease, observed in patients with melanoma or glioma^{6–8}. However, one common hallmark of these trials is that there was little or no spread of the virus from the primary site of injection, meaning that intratumoural administration is probably not effective against disseminated disease¹¹.

One well-studied oncolytic virus, the modified adenovirus Onyx-015, was developed based on its ability to only replicate in cells that lack **p53** — a common feature of cancer cells. Onyx-015 lacks the *E1B-55K* gene product, which is normally required for degrading the cellular p53 protein during viral infections, allowing it to only replicate in and destroy cells that lack p53, such as tumour cells¹². However, it has recently been shown that an mRNA export function of *E1B-55K*, rather than its mediation of p53 destruction, is a cellular process that is redundant in tumour cells and thereby gives Onyx-015 its oncolytic activity¹³. A modification of this vector is the only oncolytic virus to be tested in a phase III study to date⁵. In this trial, an *E1B-55K*-deleted adeno-virus, H101, was given by intratumoural injection to patients who received cisplatin-based chemotherapy. In studies of patients with squamous cell cancer of the head and neck or of the oesophagus, the response rate was significantly higher (78%) in patients who received the combination of viral therapy and chemotherapy than in patients who were treated with chemotherapy alone (39%)^{5,127}. A small number of patients who were treated with the oncolytic virus also had some regression of metastases, although it was unclear whether this was because of direct viral infection or a secondary immunological effect.

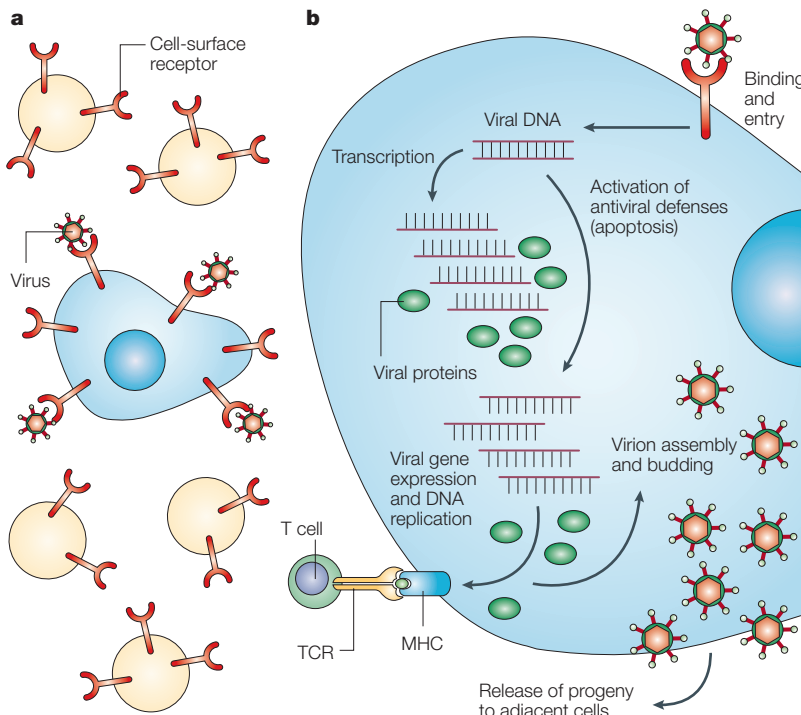


Figure 1 | Infection and killing of tumour cells by an oncolytic virus. a | Viruses interact with specific cell-surface receptors. As these proteins are overexpressed by tumour cells (blue) compared with normal cells (yellow), the virus will probably infect the tumour cell. **b** | Following binding to the cell surface receptor, the virus is internalized by endocytosis or membrane fusion, and its genome is released into the cell. Depending on the type of virus, replication and viral gene expression can take place entirely in the cell cytoplasm (such as for vesicular stomatitis virus), or in the nucleus and cytoplasm (such as for adenovirus). In either case, the virus is largely dependent on cellular machinery for viral gene expression and synthesis of viral proteins. Viral gene expression and replication leads to the activation of cellular antiviral defences, such as apoptosis, that are operational in normal cells but are often inactivated in tumour cells. Expression of viral proteins will eventually lead to immune-mediated lysis of infected cells by CD8⁺ T cells, which recognize viral peptide epitopes that are presented by major histocompatibility complex (MHC) class I molecules on the surface of the infected cell. Alternatively, cells might be lysed owing to an overwhelming amount of budding and release of progeny virions from the cell surface, or by the activation of apoptosis during the course of viral replication and gene expression. TCR, T-cell receptor.

Table 1 | **Clinical development of oncolytic viral vectors**

Virus	Mechanism of tumour targeting	Phase of development	Results	References
Adenovirus	Targets to tumour antigens; conditionally replicating	Phase III conducted	H101 — E1B-deleted vector that has been tested in combination with cisplatin by intratumoural injection in patients with squamous head and neck carcinoma; greater response in patients who received a combination of virus therapy and chemotherapy than in patients who received chemotherapy alone	5,127
Reovirus	Selectively infects RAS-transformed cells	Phase I conducted	Currently in trials to compare intratumoural administration with cutaneous administration in patients with melanoma; also in trials for patients with malignant glioma and intravenous administration	NA
Herpes simplex virus 1 (HSV1)	Only replicates in tumour cells	Phase I conducted, additional trials planned	G207 and HSV1716 vectors were found to be well-tolerated when given by intratumoural injection in patients with glioma	6
Newcastle-disease virus	Selectively replicates in interferon-defective cells	Phase I conducted	PV701 vector was found to be well-tolerated when given intravenously; some patients had anti-tumour responses	16
Vaccinia virus	Gains access to tumour by vascular leakiness	Phase I conducted, phase II planned	JX-594 vector was found to be well-tolerated in phase I clinical trial when given by intratumoural injection into melanomas; trials are being planned to test intravenous delivery	137
Coxsackievirus	Selectively infects tumour cells that overexpress DAF	Phase I conducted	Coxsackievirus A21 vector was found to be well-tolerated when administered by intratumoural injection in patients with melanoma	NA
Measles virus	Virus re-targeting to tumour antigens; overexpression of virus receptor (CD46) on some tumour cells.	Phase I ongoing	Was found to be well-tolerated when administered via intraperitoneal injection in patients with ovarian cancer	NA
Vesicular stomatitis virus	Selective replication in interferon-defective cells	Tested in preclinical (mouse) models	Shown to have anti-tumour effects in xenografts and metastatic tumours	60,61
Influenza virus	Non-structural protein 1-deleted virus specifically replicates in interferon-defective cells	Tested in preclinical (mouse) models	Shown to selectively replicate in tumour cells	68
Retroviruses	Tumour-specific promoter allows expression only in cancer cells	Tested in preclinical (mouse) models	Replicates specifically in tumour cells; potential to include suicide genes in vector	NA
Myxoma virus	Replicates selectively in signal transducer and activator of transcription 1 (STAT1) ^{-/-} or interferon-deficient cells	<i>In vitro</i> studies only	Replicates specifically in tumour cells	129

NA, not available

As many patients have metastatic disease, the preferred route of delivery for oncolytic viruses is through intravenous administration. In contrast to intratumoural delivery, intravenous administration of viruses commonly causes significant systemic side effects, as well as swelling at the site of the tumour. These side effects are caused by the acute release of cytokines, resulting in severe flu-like symptoms. Researchers have shown that these symptoms can be effectively minimized by pre-medication with drugs such as acetaminophen (paracetamol in the United Kingdom), ibuprofen and loperamide, or by giving low doses of the virus before the therapeutic dose is delivered (a phenomenon called ‘desensitization’)^{14–17}. The effects of intravenous delivery of NDV have been extensively studied, showing minimal side effects and evidence of efficacy — some patients with solid tumours who were

treated with NDV underwent a complete response, and several patients experienced partial responses. In these patients, tumour growth was stabilized for months to years, and in one case histological evidence of tumour regression was accompanied by immune infiltration^{15–17}. Furthermore, when Onyx-015 was given to patients intravenously or intra-arterially, it was well tolerated and was associated with tumour shrinkage and/or disease stabilization in some patients^{14,18}.

So, oncolytic viruses seem to have limited side effects that are less debilitating than those that are associated with many standard cancer therapies, and are beginning to show some signs of efficacy. Further studies of intravenous application are planned or underway with oncolytic viruses based on reovirus, vaccinia virus and NDV vectors — these will provide a better picture of the clinical potential of these therapeutics (BOX 2).

Box 1 | **Oncolytic viruses****Adenovirus**

A non-enveloped virus with a double-stranded, linear DNA genome that forms particles that are 70–90 nm in size. There are multiple engineered versions in clinical trials, including Onyx-015 and H101.

Reovirus

A non-enveloped virus with a double-stranded, segmented RNA genome that forms particles that are 60–90 nm in size. The type III dearing strain is in clinical trials for the treatment of patients with cancer.

Newcastle-disease virus

An enveloped virus with a single-stranded, negative-sense RNA genome that forms pleiomorphic particles ranging from 150–300 nm in size. Naturally attenuated versions, such as PV701, are in clinical development.

Poxviruses

A family of enveloped viruses that contain a double-stranded, linear DNA genome and form particles that are 200 nm in diameter and 300 nm in length. Myxoma and vaccinia viruses are family members that are under therapeutic development.

Herpes simplex virus

An enveloped virus with a double-stranded, linear DNA genome that forms particles that are 150–200 nm in size. Many engineered versions are in clinical trials for treatment of patients with cancer, such as G207, 1716 and NV 1020.

Picornaviruses

A family of non-enveloped viruses with single-stranded, positive-sense RNA genomes that form particles that range from 18–30 nm in size. Members of this family that are being tested as oncolytic therapeutics include coxsackieviruses and engineered versions of poliovirus.

Vesicular stomatitis virus

An enveloped virus with a single-stranded, negative-sense RNA genome that forms 65–185 nm bullet-shaped particles.

One issue that will be important to address in future studies will be ways to further improve safety and efficacy by directing these oncolytic viruses specifically to tumour tissues. What are the recent approaches that have been developed to improve oncolytic virus delivery (TABLE 2)?

Targeting the tumour cell surface

One concern that has emerged from clinical trials is that the oncolytic viruses that have been developed for therapeutic use have been so attenuated, for safety reasons, that they lose their oncolytic ‘punch’¹⁹. How can we increase the virulence of these viruses without causing damage to normal tissues? During tumour evolution, various genetic and epigenetic events lead to the unique display or overexpression of so-called ‘tumour antigens’ on the surface of malignant cells (FIG. 1a). As cell surface recognition and virus entry is the key first step to a productive viral infection, engineering a virus to only recognize the tumour cell surface would restrict replication of a potent oncolytic virus to malignant cells.

This has recently been achieved with a modified version of **measles virus** — normally a highly contagious human pathogen that causes 1–2 million deaths per year, globally. During the course of a normal infection, measles virus binds by its haemagglutinin (H) attachment protein to one

of two cellular receptors: **CD46**, a member of the complement-regulatory protein family^{20,21}, and **SLAM** (signalling lymphocytic-activation molecule)^{22,23}. Initial targeting strategies modified the measles H-protein with carboxy-terminal extensions that included domains from growth factors such as epidermal growth factor (EGF) and insulin-like growth factor 1 (IGF1)²⁴, as well as from single-chain antibodies (scFvs) against tumour antigens such as carcinoembryonic antigen (CEA)²⁵, CD20 (REF. 26) and CD38 (REF. 27). These attachments provided the virus with some level of tumour targeting in cell culture and xenograft models, but a large amount of virus was still found to bind to the normal-cell antigens CD46 and SLAM. This raises problems in using measles virus as a therapeutic vector, as infection of normal cells through SLAM is known to mediate transient immunosuppression²⁸.

Extensive structure–function analysis of the measles virus H-protein has shown mutations in SLAM- and CD46-binding domains that created a ‘blind’ measles virus that no longer recognizes its normal cellular docking partners^{29,30}. When these blind viruses are engineered to express H–scFv fusion proteins, they exclusively infect and kill tumour cells that express the appropriate tumour antigen. These effects have been shown *in vitro*. Also, when these viruses are given intravenously to mice, they induce tumour regression. Importantly, these viruses are genetically stable — they maintain their specific targeting even after multiple rounds of infection and can be grown to high titres. One limitation to using measles virus as an oncolytic virus vector, however, is the possibility that tumours might escape viral detection through mutations that eliminate or downregulate the expression of tumour antigens. As an alternative strategy, researchers are now creating measles viruses that recognize normal antigens that are specifically expressed by the tumour vasculature, creating a vector that can disrupt tumour blood flow and might increase virus delivery to the tumour site³¹.

Various strategies have been used to create adenoviruses that not only specifically bind tumour cells, but that also preferentially replicate in tumour cells. These viruses make use of the cellular transcription machinery and promoter elements that are specifically active in tumour cells. For example, by creating a virus whose replication depends on the expression of prostate-specific antigen (PSA)-regulated genes, it is possible to develop an oncolytic virus that replicates specifically in prostate cancer cells³². Alternatively, deletion of the adenovirus *E1B* gene creates a virus that can replicate exclusively in tumour cells, because of aberrations in nuclear mRNA export that are commonly found in tumour cells^{15,33}.

These types of conditionally replicating adenoviruses (CRADs) are restricted to tumour cells by intracellular mechanisms. They are limited in their efficacy, however, as their natural cellular receptor, the coxsackievirus and adenovirus receptor (**CAR**), is widely expressed on normal cells, but has variable or low levels of expression on tumour cells³⁴. It is therefore

Box 2 | **Optimizing oncolytic viruses**

What are the features that are required for oncolytic viruses to be used effectively to treat patients with cancer?

- Not a human pathogen, but will infect human cells. This will minimize the chance that pre-existing immunity against the vector will hinder its therapeutic utility.
- Limited side-effects or toxicity to normal tissues.
- Recombinant technology is available. This technology will facilitate the introduction of transgenes that can be used to monitor viral spread, and to arm vectors with therapeutic or suicide genes.
- Viral life cycle should include rapid replication, cytolysis and spread. This will facilitate amplification of each viral therapeutic dose, allowing the virus to spread more rapidly than the vector-specific immune response. This will also maximize the efficacy of each therapeutic application — a virus capable of cell–cell transmission would have the added benefit of spreading with minimal systemic exposure and restricted or delayed immune involvement. This will allow the virus to rapidly kill the tumour cell in which it replicates, rather than establishing a chronic infection, to minimize cell destruction.
- Can be given systemically.
- Is a potent adjuvant, enabling the virus to also act as an anticancer vaccine. This serves not only to eradicate the tumour, but also to establish anti-tumour immunity and to contain metastases.
- Does not recombine with the host cell genome, or even enter the nucleus. This will minimize the risk of virus–host genetic recombination events.
- Selective replication in tumour cells to limit the infection of normal cells. Selectivity can be based on genetic defects of tumour cells, overexpression of certain cell-surface proteins or receptors that also bind viral particles, or factors in the tumour microenvironment that facilitate viral replication and spread.

necessary to develop a ‘de-targeting/re-targeting’ strategy to optimize the delivery of CRAds to tumours. The adenovirus ‘fibre–knob’ protein facilitates binding of viruses to the CAR on the cell surface. Initial strategies to redirect adenovirus infection employed various bi-specific molecules that blocked the interaction of adenovirus fibre–knob with CAR and re-directed the virus to a different receptor³⁵. Recently, genetic manipulation of the adenovirus fibre–knob protein has provided further insights into the biology of adenovirus infections and provided some interesting new targeted therapeutic candidates. For instance, studies have shown that the fibre–knob protein facilitates liver infection, at least in part, by interacting with certain blood components³⁶. Mutation of the knob domain created a virus with significantly reduced liver uptake and lower levels of toxicity when administered systemically to mice.

There are more than 50 different serotypes of adenovirus, but the strain that is most commonly used as an oncolytic virus is **adenovirus type 5** (Ad5)³⁷. Other serotypes of adenovirus have distinct cellular receptors from CAR, so it might be possible to create chimeric viruses that are mostly comprised of Ad5, genetically, but express different pieces of the fibre–knob protein from other adenovirus serotypes to modify receptor specificity. For instance, the Ad3 receptor is often expressed at high levels on ovarian cancer cells, so an Ad5 virus that expresses an Ad3 fibre–knob protein is able to efficiently infect ovarian cancer cells, even if they do not express the Ad5 receptor, CAR³⁸. In a similar manner, engineered chimeric Ad5/Ad35 fibre–knob proteins have a propensity to infect primary chronic myelogenous leukaemia (CML) and chronic lymphocytic leukaemia (CLL) cells³⁹. To further increase adenovirus binding, the H1-loop of the fibre–knob

domain can be modified to include an arginine–glycine aspartic acid (RGD-4C) peptide sequence that results in increased binding to $\alpha_v\beta$ integrins^{40,41}.

Exploiting the tumour microenvironment

Given that virally encoded receptors are highly evolved proteins, why completely re-engineer an already efficient system? An alternative approach is to use the *in vivo* tumour environment to augment selectivity. For example, subtle alterations in the fusion (F)-protein of measles virus allow it to be processed to an active form only in the protease-rich tumour microenvironment⁴². The F-protein of measles virus facilitates viral entry into cells by mediating fusion of the viral and cellular membranes, and is normally produced as an inactive precursor that is naturally cleaved by furin to expose a fusion peptide. As many cancer cells secrete high levels of proteases, such as matrix metalloproteinase 2 (MMP2), it was reasoned that a virus that produced an F-protein with the furin site replaced by an MMP2 cleavage site (F^{MMP2}) would be preferentially activated in the vicinity of tumour cells. Indeed, when F^{MMP2} is transfected into HT1080 cells (which are known to secrete MMP2), multinucleated syncytia are formed, indicating that the fusion protein was activated by MMP2 (REF. 43). The advantage of using viruses that can be activated by components of the tumour environment is that they can be easily tailored to infect specific types of cancer cells, depending on the types of MMPs that they secrete. Further studies are required to determine whether these minor alterations, such as the insertion of protease cleavage sites, have any effects on virus replication and the infection process.

It is also possible to select for NON-ENVELOPED VIRUSES, such as reovirus, that are sensitive to the tumour microenvironment. Reovirus normally infects cells of the

ENVELOPED AND NON-ENVELOPED VIRUSES

Broadly speaking, viruses can be subdivided into two groups: those that acquire a plasma membrane-derived envelope as they bud from an infected cell; or those that have only a protein coat and do not bud from the plasma membrane, but rather escape the infected cell following plasma membrane rupture.

Table 2 | **Anti-tumour mechanisms of oncolytic viruses**

Virus	Anti-tumour mechanism(s)	References
Vectors that naturally target tumour antigens		
Echovirus (type 1)	Targets integrin $\alpha_1\beta_2$, which is overexpressed by ovarian cancer cells	47
Coxsackievirus (A21)	Targets DAF/intercellular-adhesion molecule 1, which are overexpressed by melanoma cells	115
Poliovirus (PV1)	Targets CD155, which is overexpressed by glioma cells	116
Measles virus	Targets CD46, which is overexpressed by various tumour cells	117
Engineering vectors to bind tumour antigens		
Measles virus	Engineer virus to bind CD46/signalling lymphocytic-activation molecule (SLAM) protein instead of H-protein; engineer virus to produce single-chain antibodies directed against tumour antigens	51
Adenovirus	Engineer virus to produce fibre-knob protein (Ad5/Ad3 or Ad5/Ad35 vectors) that binds to tumour cells instead of liver cells	39,41
Vesicular stomatitis virus	Engineer vesicular stomatitis virus to produce sindbis glycoprotein–single-chain antibody fusion protein that bind breast cancer cells through HER2/NEU	113,130
Targeting vectors to the tumour microenvironment		
Measles virus	Inclusion of matrix metalloproteinase 2 or furin cleavage sites in F-protein to activate only in proteolytic environments	NA
Newcastle-disease virus	Inclusion of matrix metalloproteinase 2 or furin cleavage sites in F-protein to activate only in proteolytic environment	NA
Reovirus	Protease-rich tumour environment promotes reovirus processing and intermediate sub-viral particle-based infectivity	NA
Replicates only in tumour cells		
Vesicular stomatitis virus	Replicates only in cells that are interferon-resistant	61,112,118
Myxoma virus	Replicates only in cells with activated signal transducer and activator of transcription 1 (STAT1)	NA
Herpes simplex virus	Replicates only in cells with <i>E1B-19K</i> deletion (anti-apoptotic)	NA
Adenovirus	Replicates only in cells with tumour-specific promoter-driven expression of E1A (for example, survivin, secretory leukoprotease inhibitor or cyclooxygenase 2)	93,131–133
Influenza virus	Replicates only in cells with non-structural protein 1 deletions	68
Reovirus	Replicates only in cells with activated RAS	119
Vaccinia virus	Replicates only in cells with activated epidermal growth factor receptor and E2F	95
Vectors made from viruses that are not pathogenic to humans		
Adenovirus	Non-seroprevalent adenoviruses (for example, Ad11, Ad4, Ad30)	120,121,134
Vesicular stomatitis virus	Indiana and New Jersey serotypes are non-pathogenic to humans	NA
Myxoma virus	Non-pathogenic to humans	NA
Newcastle-disease virus	Non-pathogenic to humans	NA
Cloaking strategies to evade the adaptive immune response		
Vaccinia virus	Extracellular enveloped virus form evades neutralization by antibodies or complement	100,101
Adenovirus	Coating with polyethylene glycine or other polymers, encapsulation with liposomes	102,103, 122–125
Immune suppression		
Herpes simplex virus	Co-administer virus with cyclophosphamide to abrogate innate or adaptive immunity against virus	106,127
Reovirus	Co-administer virus with cyclophosphamide to abrogate innate or adaptive immunity against virus	NA

NA, not available

gastrointestinal tract, where proteases can convert the non-infectious reovirus into an infectious form called the intermediate sub-viral particle (ISVP). When given intravenously, reovirus is not efficiently processed to the infectious form. However, it is possible to select for variants that have been converted into ISVP by the action of proteases that are overexpressed in the tumour microenvironment⁴⁴. These selected reoviruses have been shown, *in vivo*, to selectively infect and kill malignant lymphoid cells that produce a protease-rich microenvironment⁴⁵.

'Naturally smart' viruses

Viruses have evolved to gain access to the cell by binding to proteins that are displayed on the plasma membrane and that often have crucial roles in regulating normal cell proliferation, homeostasis or adhesion. It is perhaps not surprising that a subset of these virus receptors are overexpressed on tumour cells. Recognizing this, Darren Shafren's group have screened a collection of picornaviruses (BOX 1) in a search for viruses that preferentially infect tumour cells, based on their overexpression of natural virus receptors. For example, the echovirus type 1 normally infects cells through the $\alpha_2\beta_1$ integrin, which is often overexpressed on ovarian cancer cells. Coxsackievirus A21 has evolved to bind to DAF or intercellular-adhesion molecule 1 (ICAM1)^{46,47}, two proteins that are often found in abundance on the surface of melanoma cells. A recombinant poliovirus, PV1(RIPO), binds to the receptor CD155, which is often over-expressed on various tumour cell types^{48–50}.

Although these and other virus receptors are upregulated by tumour cells, they are still found on normal cells, so how can anti-tumour-cell specificity be achieved? Kah Whye Peng and colleagues have been examining the role of cellular receptor expression in mediating virus infections. Using the recombinant measles targeting system described above⁵¹ they are preparing viruses that have high or low affinity for synthetic cellular receptors. They have found, perhaps not surprisingly, that both receptor density and affinity are crucial determinants of virus infection. So, cells that express a higher density of receptor (tumour cells) will be preferred targets for virus infection⁵². This, however, cannot be the only factor and, as discussed below, additional mutations in tumour cells that cripple their innate cellular immunity will probably work in combination with receptor overexpression to make the malignant cell prone to viral oncolysis⁵³.

Tumour growth and innate immunity

Our immune systems can be operationally divided into innate and adaptive responses. Innate immunity is a non-specific defence mechanism that is triggered immediately following pathogen detection and does not develop IMMUNOLOGICAL MEMORY for antigens. Adaptive immunity, however, takes days to weeks to develop, involves the generation of antibodies, the activation/selection of immune cells, and immunological memory, making future immune responses more efficient.

Oncolytic viruses activate both of these systems — how can they be engineered or manipulated to deal with or exploit these systems for therapeutic gain?

Cells have evolved sophisticated networks to sense and inactivate invading viruses early in the infection process. This innate immunity of individual cells is governed in large part by an antiviral cytokine family called the type I interferons. Once these interferons bind their receptors, they trigger the transcription of many gene products^{54–57}, which are collectively called interferon-stimulated genes (ISGs). Interestingly, the biological properties of the interferons and ISGs are not only antiviral; they also have physiological consequences that are incompatible with efficient tumour evolution^{55–59}. This is probably because interferons have a crucial role in cancer immunosurveillance — a process whereby the adaptive immune system recognizes tumours as foreign entities and eliminates them⁵⁸. As tumours evolve, they become non-responsive to interferons and lose expression of key ISGs such as the major histocompatibility complex (MHC) genes, the protein products of which are required for antigen presentation to immune system cells. As a result, the tumour becomes invisible to the host immune system^{58,59}. Other ISGs promote apoptosis, halt cell growth or are anti-angiogenic, so it is not surprising to find that many different kinds of tumour cells have acquired defects in the ability to respond to interferons^{60,61}.

One strategy to develop oncolytic therapeutics is to select or design viruses that are especially sensitive to the antiviral properties of interferons (TABLE 2). Such viruses should have their replication strongly suppressed in interferon-responsive normal tissues but still be able to flourish in interferon-non-responsive tumour cells⁶¹. Most viruses carry genes whose products are dedicated to 'short-circuiting' the antiviral activity of interferons. So, tumour-selective oncolytic activity could be achieved by deleting or attenuating these anti-interferon-gene products.

For example, **vesicular stomatitis virus (VSV)** encodes a protein called matrix (M) that blocks the production of ISGs by binding to RAE1 (also known as MRNP41)⁶², an mRNA export factor. In the infected cell, the M-protein effectively blocks the production of all ISG protein products by blocking nuclear mRNA export and sequestering ISG mRNAs in the nucleus. VSV M-protein mutants that have lost this activity induce the interferon response and limit the growth of VSV in normal tissues. The M-protein mutant viruses can still replicate well in, and kill, tumour cells that do not respond to interferon^{60,61,63–66}.

Myxoma virus is a rabbit poxvirus (BOX 1) that is being developed as an oncolytic agent based, at least in part, on its inability to grow in interferon-responsive cells⁶³. Myxoma virus expresses gene products that can counteract rabbit interferons but are unable to antagonize interferons from other species, including mice and humans. So, in normal human cells, infection by myxoma virus is blocked at a very early stage. In interferon-non-responsive human tumour cells, however, the virus is able to replicate. A similar case can be

IMMUNOLOGICAL MEMORY

The maintenance of an expanded number of circulating antigen-specific T- and B-lymphocytes, such that subsequent encounters with the same antigen are met with a more rapid immunological response.

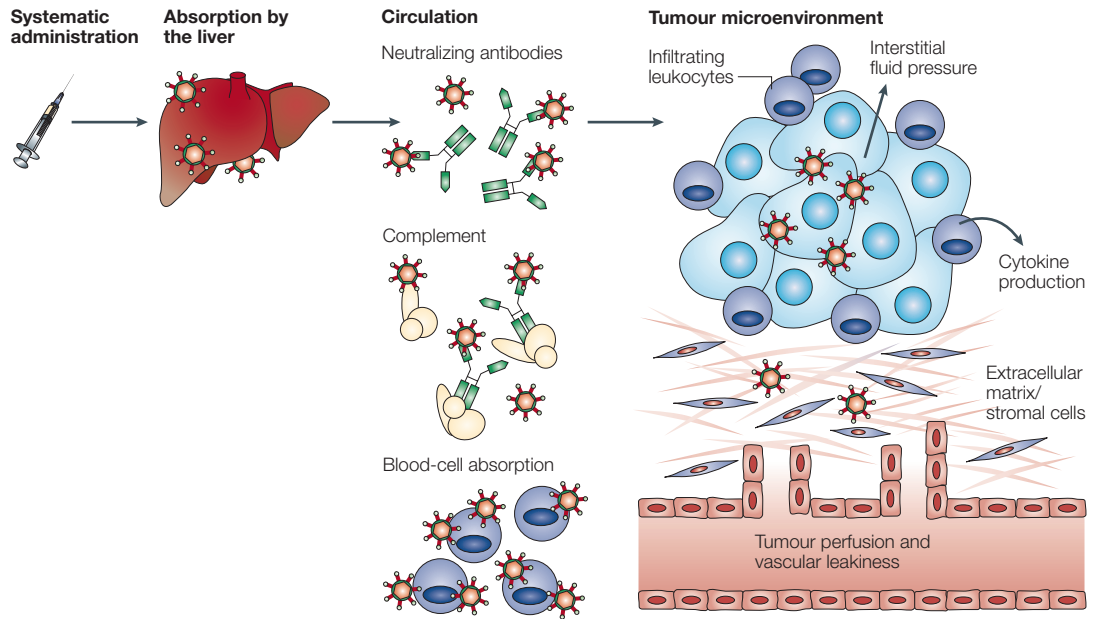


Figure 2 | **Barriers to optimal delivery of oncolytic viruses to tumours *in vivo*.** When an oncolytic virus is given systemically to a patient (for example, by intravenous injection), there are many barriers that prevent it from reaching the tumour and infecting cancer cells. Within minutes, most of the initial virus inoculum is absorbed by the liver. Virus that escapes this organ can enter the circulation, where it can be quickly neutralized through absorption by blood cells, through the complement cascade or by neutralizing antibodies, particularly in instances of pre-existing immunity. For a virion to access the tumour, it must leave the circulation, traversing or leaking through the vascular endothelium against a gradient of interstitial fluid pressure. Additionally, resident or infiltrating leukocytes limit cell–cell spread of the virus, either directly through antiviral activity or indirectly by the release of soluble inflammatory mediators, including interferons and other cytokines.

made for NDV, which encodes an interferon antagonist called the viral (V)-protein. The V-protein has evolved to disrupt interferon signalling in chicken cells, but not mammalian cells, by binding to one of the signal transducer and activation of transcription (STAT) proteins, which are known mediators of interferon signalling⁶⁷.

Influenza virus encodes a protein, non-structural protein 1 (NS1), which has been shown to inhibit responses to interferon by various mechanisms⁶⁸. Not surprisingly, NS1-deletion mutants are strongly attenuated for growth in normal tissues but retain oncolytic activity in interferon-non-responsive tumour cells. Recently, the *ICP0* gene of HSV has been shown to inhibit interferon responses, and HSV mutants that lack this gene preferentially replicate in interferon-non-responsive tumour cells.

A hallmark of cancer cells is uncontrolled protein synthesis^{69–72} — this also disrupts the innate antiviral response, which depends on restricting the activity of the translational machinery. For instance, the double-stranded RNA (dsRNA)-dependent protein kinase (PKR) is a well-known ISG that has a crucial antiviral function. Following activation by viral infection, PKR phosphorylates the eukaryotic translation initiation factor 2 α -subunit (EIF2 α), causing an inhibition of viral protein synthesis^{73–76}. In tumour cells, the phosphorylation of EIF2 α is antagonized by activation of the RAS pathway⁷⁷ or aberrations in EIF2B-mediated guanine-nucleotide

exchange activity downstream of EIF2 (REF. 78). Many viruses also escape antiviral responses in normal cells by expressing PKR antagonists^{79–81}. However, as PKR activity is often reduced in tumour cells, virally encoded PKR inhibitors become redundant. So, it is possible to create a tumour-specific virus by eliminating its encoded PKR-inhibitor. For instance, the HSV gene product ICP^{34.5} is a known PKR antagonist⁸² that is deleted in the oncolytic virus G207. This creates an oncolytic virus whose replication is attenuated in normal cells (as it cannot block PKR activity) but replicates well in, and kills, tumour cells^{83–87}.

Another common defect in tumour cells that might make them susceptible to oncolytic virus activity involves the downregulation of p53 or its downstream targets. Recently, several groups have identified a role for p53 in interferon-mediated antiviral activity⁸⁸, and, indeed, mice with supernumerary copies of the normal *Trp53* gene are both more resistant to VSV infection and have a decreased incidence of tumour formation⁸⁹. The oncolytic activity of VSV in p53-deficient tumour cells has been described, indicating that at least one component of the oncolytic activity of many viruses might be ascribed to loss of innate immunity, coincident with p53 mutations⁹⁰.

Induction of apoptosis is one of the mechanisms that infected normal cells use to prevent further spread of viruses. In turn, viruses often encode gene products that block this process^{91–93}. Tumour cells accumulate defects

in apoptotic programmes, so another design strategy for oncolytic viruses would be to delete viral anti-apoptotic genes, creating mutants that only replicate in apoptosis-deficient tumour cells. For example, an adenovirus with a deletion in the anti-apoptotic *E1B-19K* gene shows superior potency and selectivity as an oncolytic vector in various tumour cell lines and animal models⁹⁴.

The EGF receptor (EGFR)–RAS signalling pathway is activated in more than 80% of tumour samples, and has become an important focus of novel small-molecule therapeutics⁹⁵. As mentioned previously, the activated pathway requires the activity of PKR to reduce some components of the innate antiviral response⁷⁷. An engineered deletion of the vaccinia growth factor gene (*VGF*) in vaccinia virus has created an oncolytic virus that can only replicate in cells with activating mutations in the EGFR–RAS pathway⁹⁶. Additional deletions of the vaccinia *E3L* gene product, which functions as a PKR antagonist and an anti-apoptotic factor, could provide cancer cell targeting at three different levels⁹⁷.

Diplomatic immunity

The mammalian adaptive immune system has evolved to restrict the replication and spread of invading pathogens (FIG. 2). For oncolytic virus-based therapeutics, this is a double-edged sword. On the one hand, these defence mechanisms pose an impediment to the delivery and/or spread of oncolytic viruses. On the other hand, viral stimulation of the adaptive immune system seems to activate anti-tumour immune surveillance systems, increasing the effectiveness of oncolytic virus therapy. The adaptive immune response, which is of serious concern for the delivery and spread of oncolytic viruses is triggered by free circulating, virion-associated or cell-associated viral gene products. These are recognized by specific immunoglobulin surface receptors on B lymphocytes, leading to the eventual activation of B cells and production of antiviral antibodies. These antibodies might be neutralizing (preventing virus binding to cellular receptors and subsequent cell entry) or could facilitate complement-mediated lysis of infected cells. In addition, T-cell-binding of viral gene products presented as short peptides in the context of MHC molecules activates antiviral immunity, including the production or upregulation of antiviral cytokines or the antigen-restricted lysis of virus-infected cells by CD8⁺ T cells.

In both clinical trials and rodent tumour models, the presence of neutralizing antibodies against viral antigens significantly curtails the therapeutic efficacy of a number of adenoviral vectors⁹⁸. However, this might not always be the case, as neutralizing antibodies do not seem to restrict virus delivery or gene expression in some mouse tumour models. For example, when given by intratumoural injection, measles virus and HSV retain their therapeutic activity in spite of a robust neutralizing antibody response. It is not clear, however, whether these same viruses could effectively be delivered to disseminated tumours by intravenous infusion. In this regard, it is interesting to note that in phase I clinical trials, neutralizing antibodies that arise against NDV do not prevent

this virus from generating anti-tumour responses and apparently do not interfere with viral delivery to the tumour — at least in some patients. By contrast, neutralizing antibodies are a significant hurdle to systemic delivery of VSV and reovirus-based oncolytic vectors⁹⁹.

So, it seems that it is only in some settings that anti-virus immunity can interfere with oncolytic virus delivery and anti-tumour efficacy. Furthermore, blood-cell and liver-cell absorption of virus, as well as destruction of viral particles by the complement cascade, impair therapeutic utility (FIG. 2). Numerous strategies to circumvent these hurdles are under development (TABLE 2). One obvious approach is to give two or more antigenically distinct virus vectors (such as VSV followed 2 weeks later by HSV) sequentially so that the specific immunity that arises subsequent to the first virus will have no impact on the second therapeutic viral agent — this experiment needs to be performed.

A significant challenge to the use of vaccinia virus, poliovirus and measles virus vectors in oncolytic therapy is that, because of vaccination programmes, many patients already have a mature and effective adaptive immune repertoire of antibodies and cells that react against the therapeutic virus. Some properties of vaccinia virus might allow it to circumvent this problem. Vaccinia coexists as an intracellular mature form, which is effectively inhibited by neutralizing antibodies, and also as a minor species of infectious virus called extracellular enveloped virus (EEV), which escapes antibody neutralization. Studies have shown that by administering naturally occurring mutants that have a propensity to form EEV, it might be possible to evade pre-existing immunity, increasing tumour infectivity and spread of this oncolytic vector^{100–102}.

An alternative approach is to mask therapeutic viruses from antibody neutralization with chemical conjugates. For instance, coating adenovirus with multivalent co-polymers of poly *N*-(2-hydroxypropyl) methacrylamide renders the virus resistant to antibodies^{103,104}. This ‘cloaking device’ has the added benefit of preventing complement activation and reducing uptake of the virus by liver cells during systemic administration¹⁰⁴. In fact, Len Seymour and colleagues have reported that use of this conjugate resulted in a tenfold increase in circulating virus concentrations, which could have significant clinical impact¹⁰⁵. A potential disadvantage of this approach is that the masked virus loses infectivity, as its cell-attachment protein, such as the fibre–knob protein, is conjugated, and a second targeting molecule — such as poly-lysine, growth factor or antibody domains — must be linked to the polymer to facilitate virus infection¹⁰³.

If the patient’s immune system impedes oncolytic virus delivery, spread and ultimately efficacy, viral therapy might be facilitated by ablation of the patient’s immune system, which also occurs during radiation therapy and chemotherapy for cancer. In mouse tumour model studies with reovirus, HSV and adenovirus oncolytic vectors, it has been shown that anti-tumour efficacy can be increased by treatment with

ADJUVANT

Any compound that, when given simultaneously with antigen, increases the immunogenicity of that antigen, increasing the immune response.

the chemotherapeutic agent cyclophosphamide, which inhibits neutralizing antibody production^{59,99,106–110}. But immune suppression might also limit some of the effects of oncolytic viruses. An important component of the long-term therapeutic benefit of at least some oncolytic virus therapeutics actually seems to be activation of the host anti-tumour immune response. For instance, HSV oncolytic therapy is reported to be more effective in immune competent mouse tumour models than in nude mice^{86,111,112}. Systemic treatment with HSV leads to both humoral and cellular long-term anti-tumour immunity against a breast cancer cell¹¹³. Two independent groups have documented increases in long-term anti-tumour immunity following therapeutic treatment with HSV and VSV^{86,111,114,115}, and others have reported a similar phenomenon with certain vaccinia strains¹⁰⁰. As these studies have primarily involved implanted tumours, which are highly immunogenic, the contribution of the ADJUVANT activity of oncolytic virus therapy to overall anti-tumour activity in poorly immunogenic or spontaneous tumour models remains to be determined.

So, a picture that is emerging is that oncolytic viruses not only mediate direct tumour oncolysis, but could, in combination with their inherent adjuvant properties, induce or reactivate cancer immunosurveillance programmes. These observations raise two points that might affect oncolytic virus therapy. First, it might not be crucial for the oncolytic virus alone to completely eradicate a tumour to be therapeutically effective.

Rather, if the virus can quickly establish a tumour-specific infection, this will lead to a localized inflammation, *in situ* cytokine production and ultimately an anti-tumour immune response. Second, oncolytic viruses that have been engineered to produce immune stimulatory factors on infection of tumour cells are likely to be more effective therapeutics.

Future directions

Over the past two decades, a great deal of effort has been put into understanding, at the molecular level, the interplay between mammalian cells and their viral parasites. Interestingly, these studies have provided us with many insights into how cells regulate the expression of their own genetic information. We have also learned that viruses usurp many of the same signalling and regulatory pathways that are de-regulated by malignant mutations in tumour cells. As oncolytic viruses can be readily modified by genetic manipulation, it has become possible to use this platform to engineer novel therapeutics to exploit various oncogenic mutations. Combined with opportunities for developing multi-modality therapeutics and modulating the host anti-tumour response, this approach has caught the imagination of a broad spectrum of the scientific community and inspired a multidisciplinary effort to turn laboratory concepts into fully functional and effective therapeutics. In the next several years, oncolytic viruses have the potential to supplant many of today's standard cancer therapies.

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Competing interests statement

The authors declare no competing financial interests.

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