In the search for novel cancer therapies that can be used in conjunction with existing treatments, one promising area of research is the use of viral vectors and whole viruses. This review describes the underlying biological principles and current status of the field, outlines approaches for improving clinical effectiveness and discusses the unique safety and regulatory issues surrounding viral therapies.

Replication-selective virotherapy for cancer: Biological principles, risk management and future directions

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New cancer treatments with novel mechanisms of action and without cross-resistance to currently available treatments are needed. Viruses have evolved to infect, replicate in and kill human cells through diverse mechanisms. Clinicians have treated hundreds of cancer patients with a wide variety of wild-type viruses over the last century, but the approach was temporarily abandoned due to toxicity[1]. With the discovery of recombinant DNA technology, however, it became possible to genetically engineer viruses to enhance their safety and antitumoral potency (Fig. 1). Ironically, the initial approach was to make the therapeutic-gene–expressing viral vectors replication-incompetent (that is, gene therapy); this approach has yet to succeed in cancer patients. However, following the first description of a virus engineered to replicate selectively in dividing cells almost a decade ago[2], the field of viral therapy for cancer (virotherapy) has been reborn and has significantly expanded. At least 10 different viral species have entered or will soon be entering clinical trials, and one such adenovirus has entered a Phase III clinical trial (Table 1). Here we aim to review the biological principles underlying virotherapy, including both favorable attributes and potential limitations; outline approaches to improve their clinical utility; and highlight safety and regulatory issues that are unique to virotherapy. For in-depth reviews of specific viruses, we refer readers to other sources[3–7].

Ideal replication-selective oncolytic virus attributes

A number of efficacy, safety and manufacturing issues need to be assessed when considering a virus species for development as an oncolytic therapy. The virus must infect, replicate in and destroy human tumor cells, ideally including non-cycling cancer cells. The parental virus should preferably cause only mild, well-characterized human disease. Alternatively, deletion mutants that are themselves non-virulent should be considered. Non-integrating viruses have potential safety advantages in that unpredicted events caused by genomic integration are avoided. A genetically stable virus is desirable from both safety and manufacturing standpoints. Genetic approaches to prevent viral replication in essential, normal tissues is critical, and a secondary mechanism to inactivate the virus should ideally be available. Finally, the virus must be amenable to high-titer production and purification under Good Manufacturing Practices (GMP) guidelines for clinical studies.

Mechanisms of tumor-selectivity

Viruses have evolved to substantially alter the phenotype of the infected cell to maximize their replication and survival. The cellular changes induced by viral infection are often strikingly similar to the cellular changes acquired during carcinogenesis (for example, p53 tumor suppressor protein inactivation, inhibition of apoptosis). Given this genetic convergence, it is not surprising that many viruses grow preferentially in tumor cells and/or that viruses can be engineered for tumor-selectivity. Five general mechanistic approaches to tumor-selective replication have been described: 1) the use of viruses with inherent tumor selectivity (for example, Newcastle disease virus (NDV), reovirus, vesicular stomatitis virus (VSV) and autonomous parvovirus[8–11]); 2) deletion of entire genes (herpes simplex virus (HSV), adenovirus and vaccinia virus[7,12–14]) or 3) functional gene regions (adenovirus and poliovirus[15–17]) that are necessary for efficient replication and/or toxicity in normal cells but are expendable in tumor cells; 4) engineering of tumor/tissue-specific promoters into viruses to limit expression of gene(s) necessary for replication to cancer cells (adenovirus and HSV; refs. 18,19); and 5) modification of the viral coat to selectively target uptake to tumor cells (adenovirus and poliovirus[20,21]). Each of these approaches has potential advantages and disadvantages (Table 2).

Use of inherently-selective viruses

Inherent tumor-selectivity is a characteristic of viruses as diverse as reovirus (non-enveloped, double-stranded (ds)RNA), VSV (enveloped, single-stranded RNA), NDV (negative-stranded, non-segmented RNA) and autonomous parvoviruses (non-enveloped, single-stranded DNA). By definition, naturally-occurring infections with these viruses are either asymptomatic (for example, reovirus) or cause relatively mild disease (for example, NDV). Reovirus and VSV both appear to take advantage of tumor-associated defects in the interferon response pathway involving the dsRNA-dependent protein kinase-R (PKR) (refs. 10,22), and NDV might do the same (R. Lorence, pers. comm.). For example, reovirus infection leads to activation of dsRNA-activated protein kinase, PKR, which phosphorylates the α-subunit of eIF-2, resulting in termination in the initiation of translation of viral transcripts in normal cells. However, in cells with an activated Ras signaling pathway, PKR kinase activity is impaired, allowing reovirus replication to proceed. Ras-mediated signal transduction is activated in most human cancers due to either mutated Ras or mutated/overexpressed epidermal growth factor receptor. VSV also replicates selectively in tumors with interferon resistance[10]. The precise genetic phenotypes targeted by autonomous parvoviruses are unknown, although transformation leads to increased sensitivity to killing by the non-structural proteins of H-1 (ref. 23). Although reovirus was well tolerated in immunocompetent and athymic mice, toxicity was demonstrated (for example, hind-limb necrosis and small foci of myocarditis) in SCID mice[9]. VSV was also associated with toxicity in some strains of immunodeficient mice[10]. Reovirus efficacy was relatively decreased in immunocompetent mice[9], and this might be the case with other viral agents. Although the safety profile of these agents in immunocompetent humans is attractive, their safety in immunosuppressed cancer patients must be carefully studied. In addition, the antitumoral potency of these viruses might be lim-

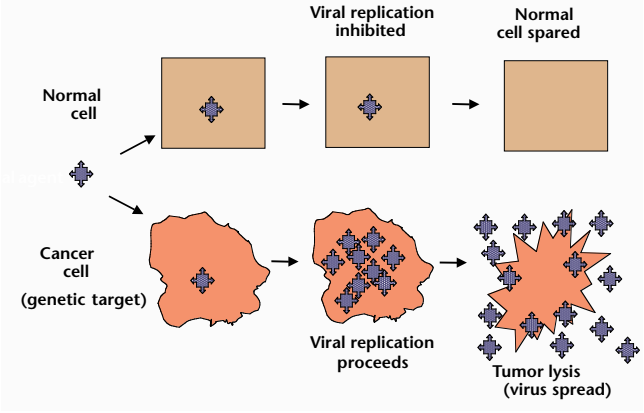

Fig. 1 Schematic representation of tumor-selective viral replication and oncolysis.

ited by their relative avirulence in human tissues—although this feature is obviously a major potential safety advantage.

Use of viral gene-deletion mutants

The utility of the gene-deletion approach was first demonstrated with HSV (ref. 12). HSV-1 is an enveloped, dsDNA virus of approximately 150 kb (ref. 5). Thymidine kinase (U_L23)-negative deletion mutants such as *dl*sptk replicated inefficiently in normal cells but were able to replicate within and kill malignant glioma cells, spread from cell to cell and dose-dependently prolong survival of animals with brain tumors[12]. However, because TK gene deletion led to anti-herpetic agent resistance, other deletion mutants were studied. Mutations in either the γ-34.5 (neurovirulence) gene[24] or ICP6 (ribonucleotide reductase) genes led to tumor-selectivity[25]. For safety reasons, a multimutated HSV-1 mutant was constructed. G207 contains deletions of both γ-34.5 genes and has a *lacZ* insertion inactivating the gene encoding ribonucleotide reductase[26]. G207 is also hypersensitive to ganci-

Table 1 Examples of replication-selective viruses in clinical trials for cancer patients

Parental Strain	Agent	Clinical phase	Tumor targets in clinical trials	Genetic alterations	Cell phenotype allowing selective replication
Engineered					
Adenovirus (2/5 chimera)	*dl*1520[a]	I–III	SCCHN Colorectal Ovarian Pancreatic	E1B-55-kD gene deletion E3-10.4/14.5 deletion	Controversial cells lacking p53 function (for example, deletion, mutation), other?
Adenovirus (serotype 5)	CN706 CN787	I I	 Prostate	E1A expression driven by PSE element E1A driven by rat probasin promoter/ E1B by PSE/promoter/enhancer	Prostate cells (malignant, normal)
Adenovirus (2/5 chimera)	Ad5-CD/tk-rep	I	Prostate	E1B-55-kD gene deletion Insertion of HSV-*tk/CD* fusion gene	Controversial cells lacking p53 function (for example, deletion, mutation), other?
Herpes simplex virus-1	G207	I–II	GBM	ribonucleotide reductase disruption (*lacZ* insertion into ICP6 gene) neuropathogenesis gene mutation (γ-34.5 gene)—both copies	Proliferating cells
Herpes simplex virus-1	NV1020	I	Colorectal	neuropathogenesis gene mutation (γ-34.5 gene)—single copy	Proliferating cells
Vaccinia virus	Wild-type ± GM-CSF	I	Melanoma	For selectivity: none or *tk* deletion Immunostimulatory gene (GM-CSF) insertion	Unknown
Non-engineered					
Newcastle Disease virus	73-T	I	Bladder SCCHN Ovarian	Unknown (serial passage on tumor cells)	Loss of IFN response in tumor cells
Autonomous parvoviruses	H-1	I		None	Transformed cells ↑ proliferation ↓ differentiation ras, p53 mutation
Reovirus	Reolysin	I	SCCHN	None	Ras-pathway activation (for example, ras mutation, EGFR signaling)

Abbreviations: SCCHN, squamous-cell carcinoma of head and neck; GBM, glioblastoma multiforme; HPV, human papillomavirus; PSE, prostate-specific enhancer; LPS, lipopolysaccharide; EGFR, epidermal growth factor receptor; tk, thymidine kinase; IFN, interferon. [a]also called Onyx-015.

Table 2 Mechanisms of tumor-specific viral replication

Approach to selectivity	Agent example(s)	Genetic alterations within virus resulting in selectivity	Genetic/phenotypic target within tumors
Deletion of entire viral gene that is:		Deletion of:	
•*necessary* for replication in normal cells, but	•G207 (HSV-1)	•Ribonucleotide reductase subunit disruption (ICP6 gene)	•Proliferation
	•1716 (HSV)	•γ-34.5 deletion	•Loss of neurovirulence
•*expendable* in tumor cells	•*dl*1520 (Ad)	•E1B-55-kD deletion	•Loss of p53 function; proliferation; other?
Deletion of functional region within viral gene that is:		Deletion of:	
•*necessary* for replication in normal cells, but	•*dl*922–947 (Ad)	•E1A CR2 (pRB family binding site	•Loss of G1-S phase checkpoint control; loss of pRB function
•*expendable* in tumor cells	•Ad-Δ24 (Ad)	•Same	
	•KD1, KD3 (Ad)	•E1A CR1 and CR2 (p300, pRB binding regions)	•Same
	•PV1 (RIPO) (PV)	•5′-IRES—replace with IRES from HRV2	•Loss of neurovirulence
Engineer tumor/tissue-specific promoter/enhancer elements to drive expression of early viral gene(s)	•CN706 (Ad)	•E1A under PSE element	•Prostate tissue
	•CN787 (Ad)	•E1A under rat probasin promoter, E1B under PSA promoter/enhancer	•Same
	•AvE1a041 (Ad)	•E1A under AFP promoter	•HCC, testicular carcinomas
	•Ad.Df3-E1 (Ad)	•E1A under DF3/MUC-1 promoter	•Breast, ovarian carcinomas
	•G92A (HSV-1)	•ICP4 under albumin enhancer/promoter	•Liver tissue, HCC
Engineer ligand for tumor-selective receptor into virus coat	•Adenovirus	•Delete CAR/integrin-binding, replace with tumor-targeting ligand	•Tumor-specific receptor
Use of inherently tumor-selective viruses	•NDV (73-T)	•Unknown (serial passage- tumor cells)	•IFN resistance of tumors
	•Reovirus	•None	•Ras pathway activation
	•VSV	•None	•IFN resistance of tumors
	•Autonomous parvovirus (H1)	•None	•Unknown; requires NS-1 and NS-2 viral proteins

Abbreviations: HSV, herpes simplex virus; Ad, adenovirus; CR, conserved region; PV, poliovirus; IRES, internal ribosomal entry site; HRV, human rhinovirus; PSE, prostate-specific enhancer; PSA, prostate-specific antigen; AFP, α-fetoprotein; HCC, hepatocellular carcinoma; NDV, Newcastle disease virus; VSV, vesicular stomatitis virus; IFN, interferon; NS, non-structural.

clovir and its construction minimizes the possibility of reversions or mutations that could simultaneously affect both loci. The safety of G207 has been demonstrated following direct inoculation of up to 1×10^7 plaque-forming units (p.f.u.) into HSV-sensitive mice by multiple routes and into exquisitely HSV-sensitive primates (*Aotus*) at doses up to 1×10^9 p.f.u. (ref. 5).

Early gene-region–deletion mutants of human adenovirus (non-enveloped, dsDNA viruses ~38 kb) have also been studied. Examples include deletions of the E1A-CR2 and the E1B-55-kD gene regions which are responsible for binding/inactivating pRB-family members and p53, respectively[27,28]. These viruses should therefore target cancer cells with genetic defects in these pathways: pRB and p53 pathway functions are lost in most human tumors through diverse mechanisms including gene mutation or overexpression of inhibitors. The E1A-CR2 mutant *dl*922–947 replicated at or above wild-type adenovirus levels in all carcinoma cells tested, whereas replication was reduced by several logs in quiescent normal cells[15]. The Δ-24 E1A-CR2 mutant was significantly inhibited by pRB expression in RB^- tumor cells[16]. The tumor-selectivity of the E1B-55-kD gene-deletion mutant *dl*1520 (also known as Onyx-015) has been demonstrated in patients[29] (see below) and with normal cells *in vitro*[14], but *in vitro* data on the role of p53 has been conflicting[4]. Dominant-negative (inactivating) p53 expression in $p53^+$ tumor cells can lead to modestly enhanced replication of *dl*1520 in some[13,30] but not all[31] cases. Other cellular factors such as S-phase fraction[32] and $p14^{ARF}$ (ref. 33) also play roles. Finally, expression of an E1B-55kD-resistant but functional p53 in normal cells did not inhibit adenovirus replication, arguing that ongoing p53 activity did not impair adenovirus replication (M. Dobblestein, pers. comm.). The role of p53 during adenovirus infection and replication remains unclear.

The antitumoral potency of these deletion mutants differed greatly. *dl*922–947 demonstrated significantly greater potency than *dl*1520 both *in vitro* and *in vivo*[15,34], and in a nude-mouse–human tumor xenograft model, intravenously administered *dl*922–947 had significantly superior efficacy to even wild-type adenovirus[15]. The reduced potency of *dl*1520 might be due to the loss of p53-independent E1B-55-kD functions (for example, viral mRNA transport)[30]. In contrast, the E1A mutations in *dl*922–947 and Δ-24 are targeted to a single conserved region; other critical functions of the gene product are thereby left intact. Therefore, because many viral proteins are multifunctional, targeted deletions might be preferable to complete gene deletions.

Two additional viral species have been targeted through the gene-deletion approach. Vaccinia virus is an enveloped, dsDNA virus of approximately 200 kb (ref. 35). Its safety record has been well established with its use as a smallpox vaccine. Most vaccinia recombinants have transgenes inserted into the thymidine kinase gene region of vaccinia virus (ref. 36), potentially enhanc-

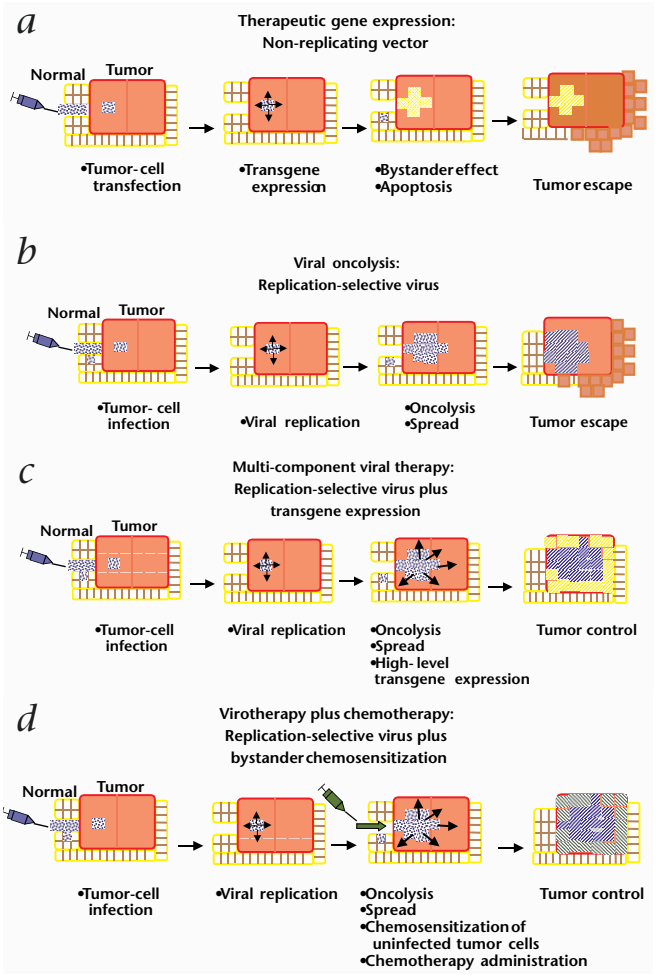

Fig. 2 Schematic representation of mechanisms of tumor destruction with viral agents. ***a***, Limited tumor destruction with non-replicating gene-therapy vector. ***b***, Intratumoral replication, spread and necrosis induction by virotherapy agent within tumor mass. ***c***, Intratumoral replication, spread, necrosis induction and additional bystander effect with virotherapy agent 'armed' with an exogenous therapeutic gene. ***d***, Intratumoral replication, spread, necrosis induction and concomitant bystander chemosensitization.

ing its selectivity for dividing cells. Vaccinia viruses have been used both as a tool to induce an anti-tumoral immune response and as a means of lysing tumor cells directly after virus replication[7,37]. Poliovirus is a non-enveloped single stranded RNA virus. Translation of the non-capped RNA is dependent on a cell-type–specific internal ribosomal entry site (IRES) element. Substitution of the poliovirus IRES with that of human rhinovirus type 2 (PV1(RIPO)) eliminated neurovirulence in non-human primates at the administered doses, but replication within human glioblastoma cell lines was retained[17]. Unlike the other viruses described above, however, this virus has exogenous genetic material inserted into the deleted region. The host range of this virus might therefore have been significantly altered by insertion of the IRES of another viral species, and this safety issue needs to be addressed.

Use of specific promoters to control viral replication

Both adenovirus and HSV have been engineered to put the expression of regulatory genes under the control of tumor/tissue-specific promoters. For example, in HSV the albumin promoter/enhancer elements have been used to target hepatocellular carcinomas[19], and for adenovirus the promoter/enhancer elements for prostate-specific antigen, MUC-1 and α-fetoprotein have been used to target prostate, breast and hepatocellular carcinomas respectively[18,38,39]. In contrast, control of E1A expression by the E2F promoter/enhancer seems to target a wide range of tumor types (P. Hallenbeck, pers. comm.). The clinical efficacy of the tumor/tissue-specific promoter approach will be dependent on other factors including the promoter activity in target tumors and in various normal tissues, as well as the overall efficiency of viral replication.

Use of viral coat modifications for tumor-selective uptake

Finally, efforts to engineer tumor-selective uptake through viral-coat protein modifications have focused primarily on adenoviruses[21,40,41]; however, the approach should be feasible with other viruses as well. Engineering tumor-selective viral uptake will require ablation of the natural viral-uptake mechanism, identification of tumor-specific 'receptor' targets on cancer cells and engineering of new 'ligands' into the viral coat without disrupting viral integrity. None of this has so far been definitively achieved with a virotherapy agent. Viruses with newly introduced restrictions in natural host ranges should have enhanced safety, whereas those with new host tissue ranges might have serious safety concerns[40].

Clinical research results: Adenovirus

*dl*1520 (Onyx-015, now CI-1042) has been genetically engineered for replication-selectivity and was the first such virus to be used in cancer patients. A staged approach to clinical research was designed for trials using this virus[42]. The strategy was to sequentially increase systemic exposure to the virus only after safety with more localized delivery had been demonstrated. Treatment proceeded from intratumoral administration to intracavitary (for example, intraperitoneal[43]), intra-arterial (hepatic artery[44]) and eventually intravenous administration[45]. Chemotherapy combinations were studied only after single-agent safety had been demonstrated. *dl*1520 has been well-tolerated at the highest feasible doses ($2 \times 10^{12} - 2 \times 10^{13}$ particles, or $1 \times 10^{11} - 1 \times 10^{12}$ p.f.u.) by all routes of administration ($n > 230$ patients)[42]. Flu-like symptoms were the most common toxicities and were more severe in patients receiving intravascular treatment. Acute inflammatory cytokines (including IL-1, IL-6, tumor necrosis factor α and interferon-γ) increased significantly following repeated intra-arterial and intravenous infusions[44,45]. Neutralizing antibodies increased in nearly all patients. Viral replication varied depending on the tumor type and/or route of administration. Viral replication was demonstrated in head and neck and colorectal tumors following intratumoral or intra-arterial administration respectively, but not in pancreatic (intratumoral) or ovarian (intraperitoneal) tumors. CN706, a prostate-specific, promoter/enhancer–driven adenovirus was also well tolerated in Phase I testing of intratumoral injection (J. Simmons, pers. comm.), and the second-generation prostate-targeted adenovirus CN787 (ref. 46) is in intravenous trials. Even wild type adenovirus was well tolerated following intra-tumoral injection in the 1950s, albeit at very low doses[4].

Single-agent antitumoral activity with *dl*1520 was minimal (15% regression rate) in head and neck cancers despite repeated daily injections[29]. Neutralizing antibodies did not block antitu-

moral activity following intratumoral injection, but their role following intravascular administration is not yet clear. No objective responses were documented with *dl*1520 alone in Phase I or I/II trials in patients with pancreatic[47], colorectal[44] or ovarian carcinomas[43]. However, a potentially synergistic interaction with chemotherapy has been demonstrated in patients with head and neck cancers (intratumoral administration[48]) and colorectal liver metastases (hepatic arterial administration[44]). In a controlled fashion, head and neck cancer patients with at least two tumor masses had one tumor injected with *dl*1520 while the other mass was left uninjected. The *dl*1520-injected tumors were significantly more likely to respond than were non-injected tumors[48]. Refractory, advanced colon tumors that had progressed on both 5-fluorouracil-based regimens and on *dl*1520 as single therapies responded significantly to the combination[44]. The mechanism of chemosensitization is not yet known, and it does not appear to be limited to E1B-gene deletion mutant adenoviruses[49].

Herpesvirus

Two Phase I trials of HSV-derived mutants have been published. G207 was the first HSV vector specifically designed for cancer therapy to enter a Phase I dose-escalation trial ($1 \times 10^6 - 3 \times 10^9$ p.f.u., or approximately 3×10^{11} particles) in patients with refractory malignant gliomas ($n = 21$)[50]. No shedding of G207 was detectable and no toxicity could be definitively ascribed to viral inoculation. An extended Phase Ib study with higher doses and/or a Phase II study will be necessary to better determine true efficacy. A second Phase I trial tested the HSV ICP34.5 deletion mutant HSV1716 ($n = 9$) at much lower doses ($1 \times 10^3 - 1 \times 10^5$ p.f.u.)[51], and no toxicity was attributed to the virus. No viral replication data is available. HSV-1 mutants with properties of selective replication can therefore be safe in normal brain at the doses studied. A third Phase I trial is underway with NV1020 administered into the hepatic artery (Y. Fong, pers. comm.); this HSV mutant is deleted in only one of the two copies of ICP34.5.

Effective treatment of other sensitive tumor types (for example, breast and colon) will require vascular delivery of HSV mutants. Multifocal metastases to liver or brain have been effectively treated in animals using either targeted arterial or intravenous administration. However, serum contains both pre-existent and induced inactivators of HSV such as complement and immunoglobulins that, in some settings, might limit efficacy[52].

Vaccinia virus

Vaccinia virus has been used primarily as a cancer vaccine to date. Wild-type vaccinia virus was well tolerated following both intratumoral and intravesical treatment[7], and viral replication was demonstrated. Vaccinia viruses expressing tumor-associated antigens[53,54] or proinflammatory cytokines[37,55] were well tolerated in a number of Phase I trials using subcutaneous, intradermal or intratumoral inoculation. Not surprisingly, no objective, systemic antitumoral responses were seen in these patients with highly advanced disease in Phase I trials, although tumor infiltration by $CD4^+$ and $CD8^+$ lymphocytes was reported.

RNA viruses

In addition to the DNA viruses listed above, several RNA viruses are in clinical trials. Reovirus is being evaluated in a Phase I dose-escalation study of intralesional administration in a variety of solid tumors (D. Morris, pers. comm.). Although NDV has historically been tested as an immunostimulant in autologous or allogeneic tumor vaccines[56–59], an attenuated strain (PV701) has now been evaluated as an oncolytic agent in a Phase I dose-escalation trial of intravenous administration (approximately 70 patients). The most common adverse events were fever, chills, nausea/vomiting and fatigue; hypoxia and transient transaminasitis have been noted in patients with pulmonary or liver metastases respectively (Lorence, pers. comm.). Published data is awaited and additional studies to explore loco-regional administration are planned.

Limitations and potential hurdles to overcome

Potential limitations to this approach have been identified. First, although viruses rapidly spread in cell-culture monolayers, viral spread within a solid tumor mass is often limited[60], particularly in immunocompetent hosts. The relative inefficiency of viral spread might relate to their relatively large sizes (for example, 90 nm for adenovirus), much larger than anti-tumoral chemicals, peptides and even antibodies. Potential physical limitations to viral spread include fibrosis, intermixed normal cells (up to half of the cells within some tumors) and necrotic regions. Insufficient expression of viral receptors (for example, coxackie-adenovirus receptor) on target tumors has also been shown to limit efficacy[61]. The immune response will presumably limit ongoing viral replication and spread in immunocompetent patients eventually[42], although immune responses might also lead to enhanced antitumoral effects[62]. The route of viral administration will be a critical determining factor. Neutralizing antibodies do not appear to block efficacy following intratumoral injection in mice or patients[29,63,64], whereas replication can be inhibited following intravascular administration. Finally, although intravenous adenovirus and HSV can have antitumoral efficacy in immunodeficient mice[65], the inefficiency of delivery to distant metastatic sites is a major hurdle. Rapid clearance of viruses from the bloodstream can result from uptake of reticulo-endothelial cells, antibody binding or complement-mediated effects.

Approaches to improving efficacy of oncolytic viruses

Several encouraging strategies are being explored to improve the potential utility of these agents (Fig. 2). First, because replication-selective viral treatment should not lead to cross-resistance with standard therapies, combinations with radiotherapy and chemotherapy might lead to additive or synergistic efficacy[14,48,49,66–69]. Viral replication does not appear to be significantly inhibited by these agents[49,67]. Endogenous viral gene expression can be modified to enhance antitumoral potency; examples with adenovirus include reintroduction or overexpression of the adenovirus death protein[46,70], deletion of the E1B-19-kD gene[71] or deletion of the E1A CR2 region[15]. Viral replication within tumors can lead to induction of cytokines with anti-tumoral and anti-vascular properties[44], as well as tumor-specific cytotoxic T lymphocytes[62]. Viruses can be 'armed' to express exogenous therapeutic genes including cytokines or prodrug-activating enzymes[7,39,72–77]. Although these combination gene-therapy agents hold great promise, in some cases the biology of the virus lifecycle can be adversely affected (for example, prodrug-activating enzyme therapy)[78]. Retargeting of adenoviruses through protein-coat modifications might allow improved infectibility of CAR-deficient tumors[40]. Finally, strategies to immunomodulate the host have been explored. For example, antibody clearance from the blood or complement inhibition[52] are strategies that have been used in murine tumor models with adenoviruses and HSV respectively. The lack of an immunocompetent model for

replication-competent adenoviruses has been a critical limitation for this approach[4].

Patients, patient contacts and the general public

Risk assessment for virotherapy trials must not only take into account potential risks to the treated patient but to patient contacts and the general public. Important factors include the spectrum of disease caused by the parental viral strain, the level of pre-existing immunity to the parental virus in the population, the ability of the virus to evade the immune response and the tropism of the virus. If tropism has been modified, has the spectrum of infectible cells been narrowed (to avoid infection of normal tissues) or are previously resistant tissue types now infectible (raising the risk of a new spectrum of disease)? What is the risk of reversion to the wild-type strain? Are effective antiviral agents available? Viruses expressing therapeutic transgenes raise additional questions. Has the viral vector itself been demonstrated to be safe and selective in patients in the absence of the transgene? What is the likely toxicity of transgene expression in normal tissues? For example, a prodrug-activating enzyme might have little or no toxicity in the absence of the relevant prodrug, whereas an inflammatory cytokine such as tumor necrosis factor α might lead to serious local or even systemic toxicities. If reversion to a wild-type, non-selective virus were to occur, would the transgene still be expressed? What would be the consequences of a recombination of the engineered virus with a related wild-type virus in the population?

Viral safety can be improved both by genetic engineering and by reducing exposure to the public. Prodrug-activating enzyme genes can be inserted into the virus as a safety mechanism to shut down replication in the presence of prodrug (for example, herpesvirus thymidine kinase with ganciclovir). The risk of reversion to wild-type virus can be decreased by engineering multiple selectivity mechanisms and safety features into the agent (for example, G207). Exposure of patient contacts can be reduced through patient isolation (for example, negative airflow might be considered). The first patients treated might be isolated for a predetermined number of days or until viral shedding in bodily fluids is no longer detectable.

Summary

Virotherapy holds great promise as a treatment platform for cancer. Advantages include the potential lack of cross-resistance with standard therapies and their ability to cause tumor destruction by numerous mechanisms. However, hurdles such as the immune response, systemic distribution and intratumoral spread are major potential limitations and must be addressed. These issues are both timely and important as the study of replicating agents for tumor therapy is rapidly evolving and extending even beyond engineered viruses. For example, tumor-targeting, replication-selective bacteria such as *Salmonella typhimurium* have also entered clinical trials[79]. These novel replication-selective agents raise new safety issues and require new risk management approaches for investigators and regulatory personnel to address.

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