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REVIEW

Clinical development directions in oncolytic viral therapy

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Oncolytic virotherapy is an emerging experimental treatment platform for cancer therapy. Oncolytic viruses are replicative-competent viruses that are engineered to replicate selectively in cancer cells with specified oncogenic phenotypes. Multiple DNA and RNA viruses have been clinically tested in a variety of tumors. This review will provide a brief description of these novel anticancer biologics and will summarize the results of clinical investigation. To date oncolytic virotherapy has shown to be safe, and has generated clinical responses in tumors that are resistant to chemotherapy or radiotherapy. The major challenge for researchers is to maximize the efficacy of these viral therapeutics, and to establish stable systemic delivery mechanisms.
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Introduction

Oncolytic viruses involved in initial cancer therapeutics are non-pathogenic, naturally occurring viruses that are either wild-type or naturally occurring mutants. Specificity to cancer is determined by tumor-specific genetic mutations that result in aberrant protein expression. Adenoviruses are the most widely studied engineered oncolytic viruses clinically. Adenoviral constructs include Onyx 015,[1,2] CG7060,[3] CG7870,[4] dl922-947,[5] Ad5-CD/tk-rep,[6] Ad-delta24,[7] Ad DF3-E1,[8] Onyx 411,[9] OAV001,[10] KD3,[11] 01/PEME[12] and Telomelysin.[13,14]

Historically, evidence of viral oncolytic activity was published in case reports as early as 1912. These reports described rare but dramatic responses in cancer patients recovering from viral syndromes.[15–29] On the basis of these observations, viruses with low pathogenicity to normal tissue and high oncolytic capacity have been selected for clinical investigation.[29–35]

This review will focus on the anticancer activity of oncolytic viruses demonstrated in clinical investigation.

Onyx 015

Onyx 015 is a replication-conditional adenovirus genetically modified by deletion of two DNA elements. It was theorized that deletion of the first element, the E1B 55 kDa fragment, would facilitate replication of ONYX 015 in cells with a defective p53 pathway, which commonly occurs in cancer cells, although it has become clear that this virus is not specific for p53-null cells.[1,36,37] Clinical trials of several hundred patients have shown no evidence of nonspecific viral replication or damage to normal cells at the border of intratumoral injection sites.[38–51] When administered intravenously (i.v.), dose escalation was limited by transient liver enzyme elevation at a dose of 2×10^{13} particles.[43,46,52]

Initial phase one investigation of ONYX 015 involved intra-tumor injection in refractory cancer patients. The virus was well tolerated and evidence of activity was suggested.[53] A phase II study involving 40 squamous cell carcinoma of the head and neck patients injected intratumorally with 2×10^{11} viral particles for 5 consecutive days revealed cancer-specific viral replication in 7 of 11 patients who underwent biopsy.[40] No viral replication or toxic effects were identified in normal tissue. No particular toxic effects were observed following 533 viral injections, and nearly 20% of patients demonstrated significant, partial, or complete response (CR) of the injected lesion. In a subsequent phase II study, patients received ONYX 015 (2×10^{11} particles for 5 consecutive days/21 day cycle) in combination with cisplatin (80 mg m^{-2} once every 21 days) and 5 fluorouracil (800–1000 mg m^{-2} continuos infusion 5 days/21 days).[41] No added toxicity attributable to ONYX 015 was demonstrated in addition to the expected toxicity of cisplatin and 5 fluorouracil. Furthermore, a 63% response rate was observed, which was greater than the expected response rate of 35% from previous publications in the same patient population using similar chemotherapy regimes.[41]

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Other clinical investigations of ONYX 015 have involved advanced ovarian cancer,[42] hepatocellular carcinoma,[44] pancreatic cancer[47,49] and colorectal cancer.[48,54] In a meta-analysis[54] summarizing two intrahepatic arterial infusion trials involving colorectal cancer, survival was compared between patients receiving $<6\times10^{11}$ virus particles per infusion (7 patients) to those receiving $>6\times10^{11}$ virus particles per infusion (28 patients). A significant survival advantage was demonstrated in the high-dose group (359 days) compared with the low-dose group (155 days).[45]

ONYX 015 has been administered safely to >40 patients intravenously. Results show a slightly higher frequency of febrile response with systemic administration than intratumoral administration of ONYX 015, although the frequency was similar to what was observed with intra-arterial infusion. At doses of $<1\times10^{13}$ particles per infusion, no significant safety concerns were identified, including situations in which virus was administered in combination with low-dose IL-2 or chemotherapy (paclitaxel, CPT11 and 5 fluorouracil). The presence of ONYX 015 within metastatic malignant disease sites following i.v. infusion was demonstrated; however, evidence of significant tumor regression was not identified.[43,46,53]

Shanghai Sunway Biotech presently owns the rights to ONYX 015. They also have the rights to a nearly identical virus called H101 (trade name Oncorine), the first oncolytic virus to be commercialized, which is currently on the market in China following demonstration of improved response and time to disease progression of nasopharyngeal carcinoma in combination with cisplatin-based chemotherapy compared with chemotherapy alone.[55] Two follow-up products have been introduced, H102, currently in pre-clinical testing, and H103, an oncolytic type 2 adenovirus overexpressing the heat shock protein HSP_{70}, recently tested as a intratumoral vaccination in a completed phase I clinical trial in patients with advanced solid tumors.[56] Transient and partial regression of distant, un-injected tumors was observed in three patients during this study, and because of promising clinical antitumor activity and positive safety outcome further studies are being pursued.

Telomelysin

Telomelysin is a novel, replication-competent Ad5-based adenoviral construct that incorporates a *human telomerase reverse transcriptase* gene (*hTERT*) promoter. *hTERT* encodes for the catalytic protein subunit of telomerase, a polymerase that acts to stabilize telomere lengths and is highly expressed in tumors but not in normal, differentiated adult cells.[57] Earlier studies have shown that hTERT promoter can control the expression of exogenous genes in telomerase-positive cancer cells, and can serve as an excellent candidate for cancer-specific control of oncolytic adenoviral replication.[58]

Additional modifications of Telomelysin include the replacement of the normal transcriptional element of viral E1B gene by an IRES (Internal Ribosomal Entry Site) sequence. Furthermore, Telomelysin is the first replication-competent adenovirus that retains a fully functional viral E3 region. E3 proteins prevent Ad-infected cells from being cleared by cytotoxoic T lymphocyte, tumor necrosis factor, Fas ligand and tumor necrosis factor-related apoptosis-inducing ligand and act to decrease systemic viral clearance.[59] Other adenoviral therapeutics have dysfunctional or deleted E3 regions for safety considerations. However, removal of the E3 region and rapid clearance of viral therapeutics may also cripple the antitumor effect. Thus, retention of a functional E3 region has the theoretical advantage of optimizing antitumor activity within the constraints of clinical safety by enhanced viral pharmacokinetics and biodistribution.

In vitro studies have validated the selective infectivity and direct cytolysis of Telomelysin in cancer cells but not nonmalignant cells.[58] In animal experiments, intratumoral injection of Telomelysin demonstrated antitumor activity without significant toxicity to normal organs. Further, distant viral uptake was observed following intratumoral injection when the presence of adenoviral protein was identified in the noninjected tumor following intratumoral treatment of the contralateral tumor.[58]

A phase I study evaluating the tolerability of a single intratumoral injection of Telomelysin was recently completed.[60] Sixteen patients were entered into three dose escalation cohorts without defining a maximum tolerated dose. There were no clinically significant grade 3 or 4 treatment-related adverse events (AEs). However, multiple grade 1 and 2 AEs were reported, with the most common being fever, chills, fatigue and injection site pain. Nine evaluable patients (one neuroendocrine, three squamous cell carcinomas, three melanomas, one leiomyosarcoma and one salivary cancer) satisfied RECIST criteria for stable disease (SD) at day 28, and seven of these patients had SD at day 56. Six patients had progressive disease at day 28 assessment. Post injection biopsies performed at day 28 on four of the patients with SD revealed intratumoral necrosis. Three of these patients had melanoma.

Viral pharmacokinetic analysis demonstrated the transient presence of systemic Telomelysin dissemination following intratumoral injection early after injection. Evidence of viral replication was demonstrated with detection of late ($\geqslant$day 7) viral DNA in three patients; one with elevated malignant tissue hTERT expression demonstrated significant clinical response. Immunohistochemical analysis of viral E1A and hexon was negative 28 days after injection suggesting rapid viral clearance. It is of interest that two patients demonstrated response distant from the injected lesion, which was consistent with animal experience and not previously demonstrated in other oncolytic adenoviral studies.

As a result of the unique modifications built into this adenoviral construct both activity and safety of a single injection approach has been demonstrated. However, despite significant activity in a subset of patients limited clinical relevant responses were observed. As only single-dose intratumoral injection was attempted in this trial it remains unclear what the therapeutic potential is for this agent.

Newcastle virus

Newcastle virus is a paramyxovirus with infectivity normally restricted to fowl. It is an enveloped negative-stranded RNA virus, which selectively replicates in human cancer cells that have developed defects in the interferon signaling pathway.[61] Most early studies used Newcastle virus as an oncolysate tumor vaccine. These vaccines were injected into patients to generate an immune response.[62] In addition, the virus has been given intravenously, intraperitoneally and intratumorally in athymic mice implanted with human cancers, including lung cancer. These preclinical trials have shown few systemic side effects, and have demonstrated evidence of oncolytic activity.[63–66]

The first report of antitumor activity of the Newcastle virus involved one patient with cervical cancer. Cassel and Garrett injected virus (2.4×10^{12} virus particles) directly into the tumor and demonstrated intratumoral regression of the cancer both at the injection site and at a distant malignant lymph node.[67] In the mid-1970s, viral-induced oncolysates were studied as vaccines in melanoma, breast, ovarian and colon cancer. Safety and modest evidence of activity were observed.[68–75]

I.v. infusion of Newcastle virus has been well tolerated. In one placebo-controlled phase II study, 33 patients with advanced cancer received virus and 26 control patients were given placebo treatment. Of the patients treated with virus, seven patients achieved a complete or partial response and one patient had a minor response: these eight patients survived >1 year after treatment. In comparison, none of the control patients had responses. In all, 22 patients receiving virus survived longer than 1 year, whereas only 4 patients in the control group survived 1 year. Eight viral-treated patients survived for >2 years versus none of the control patients.[76]

In another trial of an attenuated Newcastle virus strain, PV701 virus was administered intravenously (5.9×10^9 p.f.u. m^{-2} to 24×10^9 p.f.u. m^{-2} every 28 days) to 79 patients with solid tumors. Side effects were mild and were limited to fever, flu-like symptoms and hypotension. Seven grade 3 AEs were observed, but toxicity decreased with subsequent doses. A maximum tolerated dose following a single infusion was established at 12×10^9 p.f.u. m^{-2}, and subsequent infusions were tolerated up to 120×10^9 p.f.u. m^{-2}. Further dose escalation was limited by hypotension. In all, 14 of 62 patients eligible for response assessment maintained SD from 4 months to >30 months. One patient with squamous cell cancer of the tonsil achieved a CR. Another patient with metastatic colon cancer achieved a partial response. Seven patients achieved minor responses of $<50\%$ reduction in tumor size. The presence of viral particles in malignant tissue was confirmed following treatment.

Herpes simplex virus (HSV)

HSV is a double-stranded DNA virus. Genetic modification enabled the construction of oncolytic virus selectively activity within malignant tissue. One modification involved inactivation of viral gene *ICP6*, which encodes the large subunit of ribonucleotide reductase, an enzyme required for viral DNA replication.[77–80] This enzyme is expressed abundantly in rapidly dividing tumor cells but is sparse in normal cells. As a consequence, the *ICP6* gene modified HSV-1 replicates selectively in tumor cells. The second gene modification approach consists of deleting another viral gene, the *γ-34.5* gene, which functions as the virulence factor during HSV infection.[81] Mutations in this gene also limit replication in non-dividing cells.[82,83] The oncolytic HSV-1 virus, G207, has been extensively tested in animal models and is currently in clinical trials.[84–87] Replication-sensitive HSV1 γ-34.5 viral mutants have been shown to be effective in the treatment of both central nervous system[88–90] and non-central nervous system[91–99] tumors in animal models. Clinical trials involving patients with high-grade glioma, colorectal cancer, non-small cell lung carcinoma and melanoma[100–103] have demonstrated safety. Four different herpes simplex oncolytic viruses have been tested in clinical trial. Toxicity includes fever, chills and transient liver enzyme elevation and is greater in patients who have low HSV-1 antibody titers at baseline. However, all patients developed an immune antibody response against HSV antigens within weeks following treatment; thus, significantly less toxicity occurs with continued treatment to patients with high initial HSV antibody titers. PCR analysis of tissue demonstrated the presence of HSV DNA at injection sights.[104] Preclinical results in immune competent models have also suggested immune-mediated distant responses.[93,94,105–107] Given significant lack of systemic activity of viral-induced oncolysis following local–regional treatment in clinical study, several new vectors carrying immune-stimulating transgenes have been developed (granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-2, interleukin-12, B7.1).[107–112] Additionally, combination of HSV mutants with chemotherapy or radiotherapy has demonstrated enhanced antitumor activity.[80,101,113–118] Radiation increased the anticancer activity of HSV when used in pancreatic, glioblastoma and cervical cancer models[119] but did not alter the antitumor effect of HSV in prostate cancer. However, high-dose radiation combined with oncolytic HSV virus did improve efficacy in other prostate cancer models.[102,115,116] Low-dose irradiation also improved efficacy of HSV viral therapy in a cervical cancer model.[118] In chemotherapy combination studies, interestingly, both chemotherapy-resistant and -sensitive tumors were equally responsive.[120] A variety of chemotherapy agents (mitomycin C, cisplatin, methotrexate, taxanes) have demonstrated enhanced antitumor effect when combined with HSV.[101]

Other studies have evaluated the use of HSV to deliver other genes, such as those that convert benign pro-drugs into cytotoxic agents. In one study, the cytochrome p450 gene and HSV-1 thymidine kinase (TK) gene were delivered using a HSV-1 replication-competent virus via intratumoral injection in a hepatocellular carcinoma model.[121] Cancer regression significantly improved with

the cytochrome p450 conversion of cyclophosphamide to the active metabolite phosphoramide mustard.[78] Similar results have been produced with the cytosine deaminase transgene.[122] Interestingly, HSV TK activation of acyclovir or ganciclovir in HSV-infected cells inhibits viral replication without affecting tumor growth.[95,123–125] This suggests that the *TK* gene and ganciclovir may be useful as a safety valve if persistent virus and related toxicity developed.[121]

OncoVEX$^{GM\text{-}CSF}$ (BioVax, Worcester, MA) is a replication-competent HSV (HSV-1) that has been modified with several novel genetic enhancements that make it a potent oncolytic and immunogenic vector.[126–130] The vector contains the coding sequence for human GM-CSF under the control of the human cytomegalovirus immediate early promoter, which has been shown to enhance the immune response.[131] As a safety factor, the gene for TK remains intact preserving sensitivity to clinically effective antiviral agents. This genetic arsenal aims to promote an *in situ* tumor-specific vaccine that is potentiated by viral replication.

A phase I study demonstrated OncoVEX$^{GM\text{-}CSF}$ to be well tolerated with local inflammation, erythema and fever being the main AEs. Biological activity was evident by viral replication, local reactions, GM-CSF expression and HSV antigen-associated tumor necrosis was observed.[132] Tumor flattening, shrinkage and necrosis were noted in tumor types including melanoma, breast, and head and neck in both injected and on-injected tumors.

A phase II study of OncoVEX$^{GM\text{-}CSF}$ in metastatic melanoma demonstrated a 26% objective response rate including un-injected regional and distant metastatic sites. One-year overall survival rates of 61, 58 and 48% for all patients, all stage IV patients and stage IV M1c patients, respectively.[133] A recently published meta-analysis of 2100 patients with stage IV metastatic melanoma reported a 1-year overall survival rate was 25.5%. The median survival time is 16+ months for all patients treated with OncoVEX$^{GM\text{-}CSF}$ as well as for the stage IV subset; the median survival time in the meta-analysis was 6.2 months (95% confidence interval, 5.9 months to 6.5 months). Although these data are not directly comparable the results with OncoVEX$^{GM\text{-}CSF}$, they are provocative.

There were 13 objective systemic responses (8 CR, 5 partial response; 26% overall: 6 CR, 3 partial response; 22.5% stage IV), 10 of which continue for >6 months. Response onset was from 2 to 10 months following the first dose. Although local responses often occurred rapidly (after as few as two injections), maximum objective response has been observed as long as 12 months post first dose when biopsy confirmed one patient as disease free. In six patients, distant responses at un-injected sites were documented in the lung, liver, pancreas, regional and distant lymph nodes, and at other soft tissue sites. In most cases, considerably <50% of the overall disease burden was injected. Two patients achieved surgical CRs, one following excision of a newly identified brain metastasis, and one following additional treatment with interleukin-2.

Eighty-five percent of patients had AEs related to OncoVEX$^{GM\text{-}CSF}$ all of which were grade 1–2. The most common AEs were consistent with a mild influenza-like syndrome (fever (54%), chills (40%), nausea (42.5%), fatigue (32%), vomiting (20%) and headache (24%)). There were 21 serious AEs all of which were considered unrelated to OncoVEX$^{GM\text{-}CSF}$. Autoimmune vitiligo was noted in three patients; two of whom achieved CR and one in whom lesions were responding before leaving the study because of non-compliance issues. Considering the relatively benign safety profile in both the phase I and II studies, further evaluation is underway. There is currently a multi-national phase III study in metastatic melanoma in progress and also a phase III clinical study in squamous cell carcinoma of the head and neck is scheduled for the second half of 2010.

Reovirus

Research into the therapeutic potential of reovirus holds particular interest as this double-stranded RNA-containing virus is able to replicate and produce lysis in specifically transformed cells possessing an activated Ras pathway while sparing normal cells.[134] Although reovirus belongs to the *Reoviridae* family, which includes rotovirus, infection in humans is usually subclinical and limited to the upper respiratory and gastrointestinal tract.[135] Three viral serotypes have been isolated and all are commonly found in the environment as this virus possesses a highly stable unenveloped icosahedral capsid, thus it is estimated that nearly half of the population has been exposed and carries antibodies to the virus.[136–138] Importantly, reovirus type 3 Dearing strain exhibits replication in cells with an activated Ras signaling pathway, a significant finding because of the link between oncogenesis and mutations in the *ras* gene and pathway, and it has been demonstrated that this is due to inhibition of double-stranded RNA-activated protein kinase resulting in cell lysis.[139,140] Although normal mouse fibroblast cells (NIH3T3) do not normally support reovirus replication, NIH3T3 cells transformed with activated ras, epidermal growth factor receptor or V-erb B oncogene (for example, activated ras pathway elements) are lysed by uninhibited reovirus replication. It is now understood that an activated ras pathway, which is present in many ovarian, breast, colon and lung cancers, prevents viral-induced PKR activation and subsequent EIF-2 α-phosphorylation, potentiating cellular protein production and viral replication. In normal cells without ras activation, early viral replication induces EIF-2 α-phosphorylation, which inhibits cell protein synthesis. Thus, reoviruses exhibit preferential oncolytic effects in ras-activated cancer cells.

Reoviruses have demonstrated pre-clinical activity in mouse flank tumor models with cell lines that overexpress certain ras pathway elements. Examples include V-erb-transformed NIH3T3 cells, human V87 glioblastoma cells overexpressing platelet-derived growth factor receptor and ras-transformed C3H-10T1/2 cells.[140] Reoviruses have also demonstrated activity against Lewis lung cancer

metastasis in mice following i.v. administration.[141] In this study, 65–80% of the mice tested showed tumor regression.[141,142] Investigators have recently demonstrated oncolytic activity of reovirus against human cancer cell lines carrying a high percentage of k-ras mutations implanted in mice.[61] The k-ras mutation is observed in 30% of non-small cell lung carcinoma tumors. In addition to demonstrating the susceptibility of human k-ras-positive cancer cells to reovirus infection *in vitro*, this study assessed the ability of reovirus to cause tumor regression and promote survival in immunocompromised mice implanted with human k-ras-positive cancers. Intratumoral injection of virus consistently resulted in major reductions of tumor volume. Of particular significance, i.v. administration of virus to immunocompromised mice consistently resulted in the regression of tumors at remote sites.

Clinically, 18 patients with refractory solid tumors have been treated in a phase I investigation, and a dose of up to 1×10^{10} p.f.u. was well tolerated. Preliminary results identified one patient achieving a CR and one a partial response. Eight maintained SD for a prolonged period.[62,143]

Phase I clinical studies have been initiated in a range of cancer models, including a dose escalation study performed using intratumoral administration of reovirus in patients with recurrent malignant gliomas in which a maximum tolerated dose was not reached and treatment was well tolerated,[144] as was the i.v. administration of wild-type reovirus in patients with bone and soft tissue sarcomas metastatic to the lung in a phase II open label study.[145] A recent phase I open-label dose escalation study using i.v. administration of reovirus type 3 Dearing (Reolysin, Oncolytics Biotech, Calgary, Alberta, Canada) in patients with advanced cancer was well tolerated and exhibited successful intratumoral localization of reovirus after systemic delivery and confirmed the feasibility of i.v. delivery of high doses of reovirus.[146]

Several studies have recently been completed involving the combination of reovirus and radiotherapy or chemotherapy, using taxanes in particular, to achieve synergistic tumor kill. In a phase I clinical study a wild-type reovirus serotype 3 Dearing strain (Reolysin, Oncolytics Biotech) was administered intravenously in combination with a chemotherapeutic agent, gemcitabine, exhibiting disease control for the majority of patients at a well-tolerated dose.[147] Wild-type reovirus serotype 3 Dearing strain was administered in combination with docetaxel in a phase I study in patients with a range of advanced malignancies resulting in objective radiological evidence of anticancer activity and toxicity consistent with that expected from the chemotherapeutic agent alone.[148] Reovirus dose escalation has also been recently evaluated in patients with advanced solid tumors in combination with carboplatin–paclitaxel and because of the promising results in patients with head and neck cancer a phase II study has been initiated for this indication.[149] Marked responses or stabilization in the treated legions for the majority of the patients was achieved in a recently completed phase II clinical study that evaluated the biological effects of intratumoral administration of Reolysin in combination with low-dose radiotherapy in patients with advanced cancer.[150]

The early clinical results and wide scope of potential application, as well as the relatively low inherent morbidity and mortality risk because of reovirus's limited pathogenecity in humans, are promising for this oncolytic agent; however, a greater understanding of the circumvention of humoral and cellular immune responses is needed in order to improve the efficacy of this treatment.

Seneca valley virus

A small non-pathogenic picornavirus with potential antineoplastic activity, Seneca Valley Virus-001 (also known by the trade name NTX-010, Neotropix) specifically targets and infects tumor cells with neuroendocrine characteristics, including small cell cancers and carcinoid, and replicates intracellularly resulting in cell lysis.[151] It is the representative member of a new genus, Senecavirus. The cytolytic potential of this virus was first examined in neuroendocrine and pediatric tumor cell lines.[152] After promising preclinical results, Seneca Valley Virus-001 (SVV-001) was first tested intravenously in a five-log increment dose escalation phase I study in patients with nueroendocrine cancers, 6 small cell and 24 carcinoid-type, and was found to be well tolerated and showed evidence of intratumoral viral replication in delayed kinetics in the serum viral titer, post-infusion serum titers greater than the dose administered, and positive immunohistochemistry and/or reverse transcriptase-PCR signal for viral antigens in the tumor mass although antibody production was detected.[153] A phase I single infusion multi-center study is also currently active in pediatric patients with relapsed or refractory neuroblastoma, rhabdomyosarcoma and rare tumors with neuroendocrine features (COG-ADVL0911), and finally, there is an active single infusion phase II randomized Seneca Valley Virus-001 after platinum-based chemotherapy study in patients with extensive-stage small cell lung cancer in which the primary objective is progression-free survival of treated patients compared with placebo (NCCTG-N0923). In addition to the ability of SVV-001, the first non-pathogenic picornavirus to be tested as an oncolytic viral therapy, to specifically target cancer cells, no pre-existing antibodies to the virus are found in humans. Via systemic delivery this agent could potentially be used either as a single agent or in combination with standard cytotoxic therapies.

Vaccinia

Vaccinia is a double-stranded DNA virus and a member of the poxvirus family. Vaccinia virus has tropism for human cells and is highly immunogenic. The immunogenic properties were exploited in the production of smallpox vaccine, leading to the eradication of smallpox.

Three techniques have been exploited for the development of oncolytic vaccinia viruses. These include the following: (1) Vaccinia virus has a high efficiency of infection, replicates in the cytoplasm without chromosomal integration, and its 200 kb genome allows the insertion of a large amount of recombinant DNA without loss of infectivity. (2) The immunostimulatory properties of the virus are being harnessed to incite an immune response against cancer cells. (3) Replication-conditional viral mutants are being constructed to target specific cancer types.

In one study,[154] recombinant vaccinia virus was constructed in an effort to enhance the immunogenicity of transfected melanoma cells. The virus expressed a minigene encoding a fusion product that combined an endoplasmic reticulum-targeting signal and the HLA-A201 binding 27–35 peptide. Infection of melanoma cells with this recombinant virus resulted in high levels of cytotoxicity from specific cytotoxoic T lymphocyte clones *in vitro*. In another study,[155] a recombinant vaccinia virus vector was created containing the tumor-suppressor p53 gene. This virus demonstrated a high level of p53 expression in transfected glioma cells, resulting in high levels of apoptosis. A phase 1 study of intravesical vaccinia virus infection[156] demonstrated that vaccinia virus can be safely administrated into the bladder and found that the treatment was associated with an intense immune response with few clinical side effects.[157] Of the four patients studied, three survived and were free of disease at 4-year follow-up.

Many studies use vaccinia virus as an immunotherapeutic agent. Vaccinia oncolysate has been studied as a vaccine in early stage melanoma.[156–165] Results suggested a good tolerability and survival advantage compared with historical controls. However, an unpublished prospective controlled trial failed to validate the use of vaccinia oncolysate. The control group did not receive standard care, but instead received live vaccinia virus without tumor oncolysate, which potentially could have affected patient response.

Wild-type vaccinia virus does not selectively infect cancer cells. The virus requires modification to be made replication-conditional. One strategy is to delete the viral *TK* gene. Although the viral *TK* gene is necessary for infectivity in normal cells that possess small concentrations of intracellular nucleotide pools, it is not necessary in cancer cells,[158] which possess relatively high concentrations of intracellular nucleotides. Another novel vector involved replacing the viral *TK* gene with the gene for GM-CSF, creating a mutant vaccinia virus capable of selectively infecting melanoma cells and inducing an antitumor immune response.[158] This virus has been administered intralesionally in a phase one clinical trial involving patients with refractory and/or recurrent melanoma. Injected lesions contained an active inflammatory response and demonstrable viral replication. Two out of seven patients studied had a CR, and three patients had a partial response.[158] Other studies have investigated a vaccinia virus carrying a prostate-specific antigen transgene in the treatment of prostate cancer patients with both minimal disease and metastatic disease. Evidence of cancer-specific immune activation was demonstrated, and tolerability was reasonable. In minimal disease patients with rising prostate-specific antigen following surgery or radiation therapy, 14 of 33 maintained SD for at least 6 months and 6 remained disease free for >2 years. Another mutant vaccinia virus, which deleted the viral *SPI-1* and *SPI-2* genes, resulted in conditional viral replication in cancer cells but not in normal cells.[166] The efficacy of this virus has not yet been tested. Other gene combinations, such as *B7-1*, *ICAM-1* and *LFA3*, have also been added to the vaccinia core construct. Results from animal studies are encouraging.[167–169]

Conclusion

Encouraging safety profiles and local–regional activity have been demonstrated with a variety of oncolytic viral therapeutics. Unfortunately, the inability to demonstrate systemic response with currently available viral constructs limits future clinical development opportunities. The next generation of oncolytic viral products incorporates numerous modifications and strategies in an attempt to enhance activity. Specific strategies to improve viral immunogenicity and enhance potency include methods to reduce viral clearance, reduce immune inhibition of viral activity, increase intracellular viral release and replication, improve tumor cell specificity, uptake and expression, improve viral replication capacity, combination with other anticancer drugs, and addition of anticancer genes to second-generation vector design. Vector modification to 'arm' oncolytic viruses enables delivery of cancer-toxic genes. Other design modifications increase tumor delivery by the addition of cancer receptor/antigen components and enhance cancer cell expression through cancer promoter modification; these modifications have demonstrated encouraging early results.

Physical shielding to enhance delivery and reduce viral clearance is being tested[170–175] (plus Rehman 2001). Currently, liposome encapsulated, polymer coated, and cell carrier modes of oncolytic virus delivery are being developed for preclinical testing. The liposome and polymer coated methods can be coated with tumor-specific antibodies, peptides or small molecules to further enhance tumor-specific uptake and delivery.[176–184] Plasmapheresis rotation of viral serotype[170–175] and B-cell suppression have had limited testing as methods to reduce normal immune reactivity against administered viral particles. Restoration of the E3 region of the viral genome or E3 protein activity, in an effort to limit effects of tumor necrosis factor-α through combination with soluble tumor necrosis factor-α receptors, demonstrates positive effect.[13,14,46,52,185] However, enhancement in viral access and uptake by malignant cells is not the only obstacle in the creation of oncolytic viruses. Clusters of viral particles have been demonstrated to accumulate in malignant tissue following i.v. administration without further

replication or spread. Consequently, new approaches which enhance viral replication and cell to cell spread are under investigation,[174,186,187] while at the same time attempts are underway to identify more highly replicative viruses. Improving development of tumor-specific promoters to limit viral replication in malignant tissue may enable greater confidence in the utilization of replication aggressive viruses.[14,188–197] Ultimately these modifications will likely need to be built into viral constructs that deliver molecular targeted therapeutics[198–202] and can be utilized in combination with traditional therapeutics,[203–208] thereby creating a 'super' virus.

Overall, it is likely that a combination of these approaches will be required to optimally maximize oncolytic viral activity, thereby expanding potential systemic therapeutic opportunities.

Conflict of interest

The authors declare no conflict of interest.

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