Live Viruses in Cancer Treatment

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Although antitumor activity and a low toxicity profile have been demonstrated for several oncolytic viruses, the development of viral therapy in cancer treatment has been limited by a lack of definitive phase III trials. The use

Most people think of "viral therapy" as an obscure, experimental approach to the treatment of disease. However, replicating viruses have been used as an effective therapeutic modality for more than 200 years. One of the greatest clinical advances in medical history—the eradication of smallpox—was made with a replicating virus. In 1796, Edward Jenner discovered that pus from the wounds of infected patients contained live cowpox virus, which could be used as an effective vaccine against smallpox.[1] This discovery lead to the vaccination of several million people and world clearance of the disease.[2]

Interestingly, rare reports of complete remission induced in cancer patients in association with smallpox vaccination were sporadically reported.[3] Observations of tumor regression in association with other viral infections have also been described in cancer patients infected with herpes zoster,[4,5] hepatitis virus,[6,7] influenza,[8] varicella,[9] measles,[10-12] and other viruses.[13-15] The first published report of tumor destruction related to replication competent viruses occurred in 1912 when a woman with cervical cancer developed significant tumor necrosis following administration of an attenuated rabies virus for prophylactic treatment after a dog bite.[16,17] Early in vitro demonstration of viral oncolysis was first shown in 1922 with vaccinia virus, which was shown to propagate in several malignant tumor lines.

Following these observations, extensive work was performed investigating the potential use of viral therapy to treat cancer. In 1950, a strain of encephalitis virus was shown to induce a dose-related oncolytic effect in vivo with a mouse sarcoma tumor.[18] Viral replication was shown to correlate with tumor cell lysis; however, viral encephalitis developed in several mice. It was later found that serial passaging of the encephalitis virus in vitro prior to tumor injection in vivo would reduce the proliferative capacity in normal tissues, thereby minimizing the occurrence of encephalitis and enhancing oncolytic capacity. Clinical investigation was stimulated following additional work with Newcastle disease virus (NDV), influenza virus, and other viruses in animal models that showed cessation of ascites tumor (Ehrlich cells) growth and eradication of the malignancy in some animals.[19-23]

Clinical trials were carried out in advanced cancer patients between 1950 and the early 1970s, investigating administration of replication-proficient viruses.[17,24-29] Transient responses were seen. Several mechanisms of action were described, involving direct tumor lysis related to viral proliferation, tumor antigen induced immune activation, modulation of cancer oncogene expression (ie, c-fos, protein kinase C [PKC] modulation with measles injection), apoptosis related to expression of unique viral proteins (ie, E1A),[30,31] release of immunostimulatory cytokines,[32,33] and activation of other antitumor immune responses (ie, natural killer [NK] cell activation).[34] Viruses with low pathogenicity for normal tissue and high oncolytic capacity were investigated. Such viruses include NDV, mumps virus, herpes simplex virus (HSV), Egypt 101 virus, influenza virus, adenovirus serotype 5, vaccinia, and ONYX-015. Historical and current studies will be discussed.

Viral Uptake and Release

Most oncolytic viruses require proliferation in the same species or cell lineage and depend on host factors for successful evolution through life-cycle stages (binding, entry, intracellular transport, genome replication, viral gene expression, assembly and release of progeny). To initiate this process, the virus requires a suitable receptor at the surface of the cell for uptake[35] and transcription factors to bind to the promoter/enhancer elements in the viral genome, to induce expression of viral DNA. Manipulations of the viral coat protein genes and tumor-specific viral promoters have not adversely
affected replication of oncolytic viruses in malignant tissue, but have limited replication capacity in normal tissue. Additional specificity to malignant tissue has been shown following modification of the viral coat protein thereby enabling specific binding to tumor antigens not expressed on normal cell surfaces.[36] and engineering of tumor specific promoter and enhancer regions with the viral genome to generate viruses with selective malignant cell replication capacity.[37,38] The release of oncolytic virus progeny (up to 10^4 viruses per cell) coincides with the death of the host tumor cell. The first "burst" of replicating viruses[39] generally occurs less than 24 hours after treatment and may continue as long as conditions are favorable for replication and immune destruction of released virus is limited. Malignant cells are capable of evading immune defenses, and this effect may facilitate local spread of released virus.[40]

**Egypt 101 Virus**

Egypt 101 virus is a strain of the West Nile virus, which is an adenovirus subtype. Preclinical testing of Egypt 101 virus in the early 1950s showed oncolytic activity in a uterine/cervix cancer cell line (HeLa).[41] Testing of live virus administration via oral and intravenous routes in normal volunteers revealed minimal toxicity (low-grade fever), thereby justifying clinical trials in cancer patients,[42-44] although a small number of patients with hematologic malignancies did develop transient encephalitis.[45] Fever generally occurred within 48 hours after inoculation and often coincided with the detection of live virus in circulation or excretion.[42]

**Clinical Trials**

In the first such trial, involving 34 cancer patients (27 evaluable for response), tumor regression was observed in 4 patients, stabilization occurred in 5, and 18 showed no response to a single injection of live virus.[42] A subsequent trial involving 30 patients with cervical carcinoma tested several routes of inoculation (direct intratumoral injection, arterial infusion, intravenous infusion).[43,45] Toxicity was limited to low-grade fever, and regression or stabilization of disease was observed in the majority of patients. Unfortunately, most responses were transient (< 3 months), and no patients achieved a durable complete response.

Analysis of cervical tissue and vaginal smears revealed proliferating virus in 77 samples from 20 patients studied. However, with analysis of 140 samples, 10 patients showed no evidence of viral presence. Response did not necessarily correlate with recovery of virus, although patients achieving more extensive necrosis generally harbored detectable virus. Studies to explore more intensive dosing of the virus or combination with other anticancer agents were not pursued.

**Mumps Virus**

Mumps, a paramyxovirus, has a tight helical RNA inner core enclosed in an outer lipid/protein shell. Oncolytic efficacy of mumps virus was initially demonstrated in a rat sarcoma model.[46]

**Clinical Trials**

The first clinical trial investigating mumps virus involved 90 patients with advanced cancer and explored several routes of administration including oral, rectal, intratumor, inhalation, and intravenous, depending on the location of the tumor.[25] Initial hematologic response to treatment included leukocytosis followed by lymphopenia. Transient fever, which could be inhibited by prophylactic treatment with low-dose prednisone, was also observed.

The authors noted that elevated antibodies at baseline were associated with a lesser tumor response.[25] However, three of the four patients achieving an "optimal" response had elevated neutralizing antibodies to mumps virus prior to treatment. Overall, 37 (41%) patients achieved a ≥ 50% reduction in tumor size, and 79 patients with stable disease or better demonstrated clinical improvement (improved appetite, reduced pain, increased body weight).

Of the 90 patients, 65 received a combination of local intratumoral injection and/or intravenous infusion of the virus, and 24 (37%) of these 65 patients showed a partial or complete response. Most partial or complete responses occurred in patients with gastric carcinoma. However, the highest proportion of complete or partial responses occurred in cutaneous carcinoma and uterine carcinoma. Additionally, 9 of 10 patients with metastatic pulmonary carcinoma achieved clinical improvement with regression in tumor bulk.

Patients receiving multiple intratumoral injections over a prolonged period achieved a higher response rate and longer duration of response.[46,47] Fifteen patients received intravenous mumps virus alone. Six of these patients received fewer than nine intravenous treatments, and none had a positive response. In contrast, of the nine patients receiving nine or more systemic treatments, six achieved a response (P < .02).[48] This was the first study to suggest that a multitreatment
administration schedule may have a clinical advantage. Further exploration of a systemic route of administration was not performed. A follow-up study involving 200 cancer patients administered mumps virus intratumorally.[49] Toxicity was minimal. Transient tumor regression was noted in 26 patients. Responses were observed in patients with cancer of the breast, rectum, colon, thyroid gland, uterus, and skin. Due to the transient nature of the response and the difficulty in manufacturing a uniform product, further clinical testing of the mumps virus was not pursued.

NDV is an avian paramyxovirus. Initial oncolytic activity was observed in several human and murine cancer cell lines.[19-21,50,51] Preclinical data also suggested that NDV may enhance tumor-specific immunity, possibly through induction of cytokines such as tumor necrosis factor, interferon, interleukin (IL)-6, and IL-1.[52-54] Replication of NDV was also suggested to be as much as 10,000-fold increased in malignant cells compared to normal fibroblasts. Selective uptake of NDV is seen in Daudi cells compared to normal peripheral blood lymphocytes.[55,56] The mechanism for the selectivity of replication is unclear. However, some results suggest that it may be related to elevated myc oncogene expression within malignant cells. Sensitivity to NDV-induced oncolysis in B104-C6a neuroblastoma cell lines is increased following myc transfection.[51] It also appeared that antitumor activity was not related to nonviable NDV. Furthermore, no toxicity was seen in mice.[57,58]

**Clinical Trials**

Clinical activity was initially noted in a patient with advanced cervical cancer following direct inoculation of the virus into the tumor.[50] Evidence of intratumor viral proliferation was suggested based on tumor necrosis and the detection of replicating virus within the urine 11 days after injection.[50] Since the initial patient, several variant strains of Newcastle disease virus have been explored in clinical trials. In 1993,[46] 33 patients with advanced cancer received NDV via inhalation twice a week and the results were compared to those in 26 patients who received placebo inhalation. Seven patients achieved a complete or partial response, and one patient achieved a minor response. All eight patients achieving a response survived 1 or more years following treatment. No responses were observed in control patients. Of 33 patients receiving virus, 22 survived at least 1 year, compared to only 4 of 26 of the control patients (P < .02), and 7 patients who received virus survived at least 2 years, compared to no patients in the control group (P < .0001).

Overall, 18 (55%) patients achieved a favorable response (regression or stabilization) vs only 2 (8%; P < .01) placebo-treated patients. Additionally, seven of eight patients with colorectal cancer metastatic to the liver survived 1 year compared to one in five control patients with similar disease involvement (P = .04). Quality of life also appeared to improve in patients treated with virus compared to the patients receiving placebo. The mechanism of antitumor activity was unclear. Direct tumor lysis was induced, but induction of antitumor immune mechanism was also suggested.[54]

- **Viral Oncolysate** Safety of NDV in clinical testing as a single agent provided justification for testing this virus as an agent for enhancing tumor vaccine potency. Clinical trials involving patients with early-stage melanoma explored the use of irradiated autologous tumor cells mixed with live NDV as a vaccine approach. The combined product following tumor lysis was termed a "viral oncolysate."

In one trial, patients with high-risk stage II melanoma were tested following surgical excision of metastatic nodes. The viral oncolysate was administered weekly to week 4, then every 2 weeks to week 52, every 3 weeks to week 120, and every 6 weeks to week 160. Evidence of progressive disease was shown to be 6% at 1 year, 8% at 2 years, and 12% at 3 years. For 83 patients, the 3-year survival rate was 88%, and 5-year survival was 68%. Similar historical control patients had a significantly lower survival rate over a similar period.[34,59-61]

In another trial, autologous NDV oncolysate was used to treat colorectal cancer following resection of metastatic liver lesions.[62] The administration schedule was initiated 2 weeks after surgery and repeated five times at 2-week intervals followed by one boost 3 months later. Nine of 23 patients (40%) showed an increase in delayed-type hypersensitivity reactions to irradiated autologous tumor cells placed intradermally following vaccination. Of the vaccinated patients, 61% developed recurrent disease within 18 months of vaccination, compared to an 87% recurrence rate in 130 control patients who underwent the same surgical procedure.[62] No significant toxicity was observed in either the melanoma trial or colorectal carcinoma trial. Side effects of the NDV-treated vaccine were limited to local inflammation and occasional febrile responses.

NDV has also been used as a surgical adjuvant vaccine in 208 patients with locally advanced renal
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In cell carcinoma. These patients received low-dose IL-2 and interferon-alpha in combination with the NDV oncolysate. Toxicity was limited to low-grade fever, mild flu-like symptoms, and local inflammation at the injection site. Median disease-free survival was 21+ months, which was greater than would be expected with similar historical patients.[63]

In yet another study, the NDV oncolysate was also administered on days 1, 7, and 42 to 63 patients with primary breast cancer, 27 patients with recurrent breast cancer, and 31 patients with metastatic ovarian cancer.[64] All patients also received low-dose IL-2 (1 × 10^6 IU daily subcutaneously) on days 1 to 19 and 42 to 60 and interferon-alpha (3 × 10^6 IU subcutaneously 3 days per week) for the first 18 days then 3 days per week between days 42 and 58. Patients with primary breast cancer appeared to have a survival benefit compared to historical controls (P = .026), but no survival advantage was suggested in the other disease groups.

In summary, NDV oncolysate is well tolerated, and clinical efficacy has been suggested. Preclinical data indicate that further improvements in the therapeutic effect may be possible. NDV oncolysate activity can be enhanced using antibodies that facilitate viral uptake by attaching to the viral hemoglutinin neuraminidase molecule on the infected tumor cells.[65] Also, the combination of NDV oncolysate with low-dose cyclophosphamide (Cytoxan, Neosar) to reduce antiviral suppressor lymphocyte numbers,[66,67] and the enhanced induction of immune effector populations by combining NDV with immune-modulation agents (IL-2, interferon) may also improve potential efficacy.[34] Further clinical trials are ongoing.

**Influenza Virus**

Influenza viruses are grouped in the family orthomyxoviridae. They contain a single strand of RNA genome encased in a spherical or filamentous envelope. Preclinical data suggest that the influenza virus has significant oncolytic activity,[68] but, unfortunately, neurologic affinity also results in central neurologic toxicity.[69] Thus, clinical investigation of influenza virus has focused on its use as an oncolytic agent for manufacturing tumor vaccines (“influenza oncolysate”).

**Clinical Trials**

Influenza oncolysates have been shown to induce greater antitumor activity in vivo than virus alone or unmodified tumor cell extracts.[52,70] In one trial, 40 patients with advanced ovarian cancer were treated with influenza oncolysate. Administration was done biweekly, then weekly, followed by monthly injection. Intraperitoneal injections were also administered to seven patients with ascites and two patients with pleural effusions.

Three patients showed complete resolution of ascites following infusion of influenza oncolysate,[68] and one patient had resolution of his pleural effusion. Partial responses were observed in three patients. Response duration lasted from 3 to 19 months, and survival varied from 4 to 42 months. Two responders later developed pleural effusions, and both responded to reinjection with the oncolysate. Toxicity included transient fever, nausea, malaise, and abdominal pain. Treatment was stopped in two patients because of toxicity. Combination with IL-2 appeared to further enhance the immunologic response initially achieved with only the influenza oncolysate.[71] Further clinical investigation is ongoing.

**Vaccinia Virus**

Vaccinia is a naturally occurring ortopox virus with a linear double-stranded DNA genome.[72] The activity of vaccinia virus was shown to be equivalent to that of the original cowpox virus used for vaccination of smallpox. The use of live vaccinia virus to vaccinate millions of people for smallpox minimized concerns regarding the safety of the virus in human cancer investigation.

Several strains of vaccinia virus have been examined for activity as an oncolysate.[73,74] As is the case with influenza virus, neurotropism is associated with the degree of oncolytic activity.[75,76] Immune responses to viral antigens have been shown to play a role in tumor destruction following infection with vaccinia virus.[39] Animal results show that survival of mice with metastatic CC36 colon tumors was improved following vaccinia oncolysate injection.[77,78] Activity was enhanced with low doses of IL-2. Similar results with other solid tumors have also been shown in various animal models.[73,79-81] Cytotoxic T lymphocytes induced by vaccinia oncolysate vaccine appear to bind to tumor antigens as well as viral antigens.

**Clinical Trials**

Several clinical trials have been performed with vaccinia oncolysate.[82-84] In one trial, stage II melanoma patients received 13 weekly intradermal injections of the viral oncolysate for 12 months or until recurrence. Twenty-five of 39 patients had no evidence of disease at 1 year. Of patients
treated with the oncolysate, 64% developed antimelanoma antibodies.[81] There was a correlation between the development of melanoma-neutralizing IgG antibody titers and disease-free survival.[81] Preliminary toxicity with vaccinia oncolysate was limited to transient fevers and pain at the injection site.

These results led to a randomized, double-blind, multi-institutional trial of vaccinia oncolysate for patients with stage II melanoma.[85,86] A total of 250 patients were treated. One group received vaccinia oncolysate at a protein dose of 2 mg/mL, while the other group received vaccinia virus alone at a dose of 105 plaque-forming units (PFU)/mL. Treatment was given once a week for 13 weeks, then once every 2 weeks for an additional 39 weeks or until recurrence.

There were no statistical differences in survival between the two cohorts of patients, with a median disease-free survival of 38 months for patients treated with the virus oncolysate, and 37 months for patients treated with virus alone. Although a survival advantage with vaccinia oncolysate was suggested in subsets of patients (ie, males between age 44 and 57 who had one to five positive nodes), the number of patients was insufficient to achieve statistical significance. Unfortunately, the investigators did not include a control group of patients who did not receive virus.

Recent clinical investigation has involved the use of a nonreplicating vaccinia/granulocyte-macrophage colony-stimulating factor (GM-CSF) gene vector.[87] Seven patients with stage IV melanoma were treated by intratumoral injection. Transfer of transgene product was confirmed using reverse transcriptase-polymerase chain reaction (RT-PCR) assay and primers specific for vaccinia thymidine kinase (TK) and GM-CSF gene. The researchers noted a correlation between gene transfer and transient local tumor regression.

HSV is a DNA virus in which a core double strand of viral DNA is generally encapsulated within an icosahedron outer envelope. HSV is a natural pathogen of the mammalian host that replicates at a primary site (skin, cornea or mucosa) and moves by axonal transport to a second site of replication (sensory ganglia). From there, HSV reestablishes infection. When dormant in the sensory ganglia, HSV is not detectable systemically.

A better understanding of the controls that restrict HSV expression and DNA replication may enable the use of HSV as a possible gene delivery vehicle in the future.[88-90] The TK-deficient HSV mutant “dlsptk” has been shown to replicate in dividing cells, but is severely impaired for replication in nondividing cells.[91-93] A dose-related survival improvement was shown in nude mice following injection of dlsptk in xenograft models with U87 tumors and 9L glioma cells.[91,94]

**HSV Modifications**

- **G207** Researchers have developed another HSV mutant, G207, which does not contain a deleted TK gene. Rather, it contains deletion of the g-34.5 gene and a lacZ gene insertion.[95] Clinically relevant characteristics of G207 include hypersensitivity to antiviral drugs (acyclovir, gancyclovir [Cytovene]), growth selectivity in nondividing cells, lack of neurologic pathogenicity, and the presence of lacZ gene under control of the HSV promoter, which facilitates the detection of replicating virus using histochemistry.[96]

G207 shows activity following intraneoplastic inoculation in subcutaneous and intracerebral U87 gliomas. Necrosis following intratumoral injection associated with viral replication occurs within 15 days. Furthermore, viral titration studies and histochemistry analysis showed that replication of the HSV virus was restricted to tumor cells and did not adversely affect surrounding brain tissue.[96,97] No toxic effects on normal rat glial cells or neurons in culture were observed, and G207 was nontoxic in vivo. Additional work with G207 has shown activity against meningioma in cell culture and in implanted nude mice.[98]

- **HSV-1716** Methods of attempting to improve the oncolytic effect of HSV have involved infection of a producer cell line, PA-1. Intraperitoneal injection of PA-1 cells infected in vitro with HSV-1716 resulted in significantly reduced tumor volume compared to administration of virus alone and produced a significant survival advantage, suggesting that the use of producer cell lines may augment efficacy of HSV-1716.[99]

Others have also shown that the combination of HSV-1716 with chemotherapy in animal models further improved the oncolytic effect. Specifically, mice implanted with non-small-cell lung cancer cells exposed to HSV-1716 human large-cell carcinoma in vitro followed by treatment with chemotherapy (mitomycin-C [Mutamycin], cisplatin, methotrexate, doxorubicin) demonstrated reduction in tumor volume.[100,101]

- **Inserted Genes** HSV (G207) with an inserted IL-12 gene has also been tested in a CT26 colon carcinoma animal model. The antitumor effect was significantly greater in the replication-competent IL-12-transfected virus, compared with IL-12-negative replication-competent virus. Cytotoxic T-lymphocyte activity specific to the inoculated tumor was also demonstrated. There was no
Preclinical Studies
In vitro studies have demonstrated that G207 efficiently replicates in the majority of malignant breast cancer cell lines tested. Furthermore, in athymic mice harboring subcutaneous or intracerebral breast cancer cells sensitive to G207 in vitro, growth inhibition and prolonged survival was identified.

• hrR3—Another strain of replication-efficient herpes simplex virus type 1, called hrR3, has been explored in a rat glioma model.[103] Direct intratumoral injection of hrR3, which contains a small lacZ transgene, was injected into subcutaneous human gliomas with subsequent excision at different time points from injection. A computer-assisted volumetric analysis of lacZ transduction showed that lacZ expression was significantly higher in tumors inoculated with hrR3, compared to tumors inoculated with a replication-defective adenoviral vector containing lacZ. This also correlated with an increased lacZ-positive viral yield from tumors inoculated with hrR3. Furthermore, tumor growth was suppressed when treated with hrR3 and occasional complete regressions were observed compared to tumor injected with the replication-defective adenoviral vector.

Finally, hrR3 has also been explored following intravascular infusion in a rat glioblastoma model. Survival of rats receiving hrR3 was improved particularly when concurrently treated with RMP-7 (an analog that disrupts the blood-brain barrier).[104] Further clinical investigation with HSV is ongoing.

Adenovirus Serotype 5
Adenovirus contains a single DNA strand encased in a hexagonal envelope. Replication-deficient adenovirus serotype 5 vectors are common gene delivery vehicles for numerous gene therapy trials. A great deal of accumulated data suggest that adenovirus serotype-5 has a low toxicity profile in humans.[105] Eighty percent of adults have existing antibodies to adenovirus serotype 5, but less than 15% of exposed patients become clinically symptomatic.[106] The most common symptoms of an adenoviral serotype 5 infection are flu-like in nature and include cough, gastroenteritis, conjunctivitis, cystitis, and pneumonia. However, these symptoms are rarely seen, even in immunocompromised patients.[107]

Oral adenoviral vaccines were given to thousands of military recruits in the 1960s without adverse effects or increase in cancer incidence.[108] Both long- and short-term safety of live adenoviral injections has been shown in several animal models.[109-115] Live adenovirus inocula were also given intratumorally and intra-arterially to patients with cervical carcinoma at the National Cancer Institute in the 1950s.[43] No significant toxicities, other than transient fever and malaise, were observed in these women, including subsets of patients treated with steroids and others in whom neutralizing adenovirus antibodies were not present.

In humans, nonreplicating adenoviral b-GAL vector injection was administered to patients with endobronchial lung cancer. Evidence of replication-competent adenovirus was studied in caretaker staff samples. Specifically, 73 staff provided 78 blood samples, 272 urine samples, and 193 samples with which to study antibody formation or the presence of replication-competent adenovirus. No replication-competent adenovirus was detected, and elevated antibody formation did not inhibit gene expression with repeat injections.[116] Other trials have involved the administration of a therapeutic gene using a nonreplicating gene-modified adenovirus. This strategy has been shown to be safe in normal volunteers[117] and in cancer patients.[118,119]

ONYX-015
Wild-type adenoviruses capable of replication encode proteins that inactivate the p53 gene. This enables viral replication in infected cells.[120,121] Specifically, a 55-kD protein from the E1B region of the adenovirus genotype binds and inactivates the p53 gene.[122] ONYX-015 is an adenovirus constructed with an E1B gene-deleted region, so that it no longer produces the 55-kD protein. Inability to block p53 because of such a deletion enables normal cellular p53 protein to maintain its function, thereby inhibiting viral replication. As a result, ONYX-015 has lower replicative capacity in normal cells, but enhanced replicative capacity in tumor cells (which have abnormal p53 function).[123,124]

In animal human xenograft studies, intratumorally injected ONYX-015 had greater efficacy in p53-deficient cancers. Efficacy was further enhanced when ONYX-015 was used in combination with either fluorouracil (5-FU) or cisplatin.[124-126] Intravenous infusions of ONYX-015 in animals revealed limited toxicity and tumor regression related to intratumoral access and/or replication. Efficacy was again augmented when used in combination with 5-FU.[127]

However, since the initial report on ONYX-015,[123] controversial data have emerged, leading to
questions about the selective replication capacity of ONYX-015. Many tumor cell lines with normal p53 sequences have been found to be sensitive to the effects of ONYX-015 in vivo.[128-131] Although replication selectivity appears not to be limited to p53 mutant-containing cells, other factors that alter p53 function (HPV-E6 effect, p14ARF production, or increased MDM-2 expression) may play a role.[132-136] Despite the controversial findings in malignant tissue, cytopathic effects have not been observed in normal tissue at clinically relevant multiplicity of infection (MOI) values when tested in vivo.[128,130,137,138] Furthermore, it has been shown clinically that the presence of a p53 gene mutation within the tumor tissue does correlate with the response to intratumoral injection of ONYX-015 as a single agent.[139]

- **Clinical Trials**: A phase I investigation documented the safety of ONYX-015 when administered at doses up to 2 × 10^{12} particles, given daily once every 3 weeks, or 2 × 10^{11} particles for 5 consecutive days once every 3 weeks to chemotherapy refractory head and neck cancer patients with accessible tumor.[140] Phase II investigation was subsequently done in patients with advanced recurrent head and neck cancer who received two treatment schedules. One group (n = 30) received intratumoral injection for 5 days (once a day) over a 21-day cycle, and in a subsequent trial, 10 patients received a "hyperfractionated" schedule, which involved 10 injections over 5 days in a 21-day cycle.[141] The most common treatment-related toxicity was fever, which occurred in 60% of the ONYX-015 injected patients. Transaminitis was not observed. The systemic distribution of ONYX-015 was documented using quantitative PCR in 12 of 29 patients 24 hours after the last ONYX-015 injection in cycle 1. Only 2 of 19 patients continued to show circulating viral genome 14 days after intratumoral injection. No patients were found to have circulating genome 22 days later. Biopsy analysis of malignant tissue by in situ hybridization for adenoviral DNA demonstrated replication of ONYX-015 within tumor cells, and not within normal tissues.[141]

Intratumoral injection of ONYX-015 in combination with cisplatin and 5-FU (2 × 10^{11} particles/injection) was administered to 30 patients with recurrent (chemotherapy-naive) squamous cell cancer of the head and neck region.[142] Among evaluable patients, 63% achieved at least a 50% decrease in two dimensions of the injected tumor, and 27% of this subgroup achieved a complete response. However, as opposed to the results of single-agent administration, no correlation was found with tumor p53 mutation status. Eleven patients had two lesions each; one lesion was injected with ONYX-015 by protocol design and another was not. Of 11 injected tumors, 9 responded, compared to only 3 of 11 responses in "same patient"-matched noninjected tumors (MacNamara's test, P = .01).[142] Other than a similar incidence of fever, chills, and injection site pain, no additional toxicity in excess of what would be expected with chemotherapy was observed.

- **Use in Refractory Cancer**: Following preclinical evidence of safety in animal models, intravenous infusion of ONYX-015 has also been explored in refractory cancer patients.[143] ONYX-015 was administered on a weekly basis at doses ranging from 2 × 10^{10} particles to 2 × 10^{13} particles. Combination chemotherapy (carboplatin [Paraplatin] and paclitaxel) was administered after completion of cycle 2 in patients receiving initial low doses of ONYX-015. When the tolerability of chemotherapy plus IV ONYX-015 was determined to be safe, the chemotherapy was moved up to day 7 of cycle 1. A total of 126 IV infusions were given in 42 cycles to 10 patients. Mild transaminitis and fever developed in all patients receiving ≥ 2 × 10^{12} particles/infusion within 48 hours of the initial infusion, but these parameters returned to baseline prior to cycle 2 with continued dosing and did not recur in cycle 2. Toxicity appeared to be correlated with elevated serum IL-6 levels. Nine patients underwent pharmacokinetic testing, and in all nine, evidence of viral genome was detected in the blood 90 minutes after the end of infusion. Similar pharmacokinetic profiles were seen in cycles 1 and 2, suggesting the lack of relationship to either circulating viral genome or induction of neutralizing antibodies. All patients showed marked elevation of neutralizing antibodies. Patients receiving ONYX-015 at a dose of ≥ 2 × 10^{12} particles appeared to have a more prolonged duration of circulating viral genome, and a higher proportion of these patients had detectable genome 6 hours after infusion. Furthermore, evidence of replication was suggested in three patients, in whom the concentration of viral particles per mL increased 0.5- to 10-fold from 6 hours to 48 hours after administration. These three patients underwent tumor biopsy, and one had sufficient tissue for immunohistochemical staining. This assay revealed extensive intranuclear presence of viral particles in malignant cells with no evidence of viral inclusions in adjacent normal cells. Unfortunately, no clinical responses were observed in this trial.

**Conclusions**

Antitumor activity and a low toxicity profile have been demonstrated for a variety of oncolytic
viruses. However, the establishment of this approach as a therapeutic option for cancer patients has been limited by a lack of definitive phase III trials. Incorporation of replicating viruses to potentiate the efficacy of standard therapeutic approaches to cancer awaits conclusive clinical testing. Nevertheless, based on preliminary results with new generations of oncolytic viruses, ongoing research in this area appears encouraging.

Today, methods of using oncolytic viruses to enhance immunity, induce oncolysis, and transfer therapeutic genes are better defined, and preliminary clinical results suggest some efficacy. Ultimately, oncolytic viruses may play a small role as a locoregional therapeutic approach, but the selective intratumor replication capacity of some of these viruses may enable their use as selective gene delivery vehicles in cancer patients.

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