Review

Oncolytic viral therapies

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Molecular research has vastly advanced our understanding of the mechanism of cancer growth and spread. Targeted approaches utilizing molecular science have yielded provocative results in the treatment of cancer. Oncolytic viruses genetically programmed to replicate within cancer cells and directly induce toxic effect via cell lysis or apoptosis are currently being explored in the clinic. Safety has been confirmed and despite variable efficacy results several dramatic responses have been observed with some oncolytic viruses. This review summarizes results of clinical trials with oncolytic viruses in cancer.

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Oncolytic viruses, which have been tested as cancer therapeutics, have either been naturally selected or have been genetically engineered to grow specifically in and kill tumor cells. Specificity to cancer is derived by exploiting cell surface or intracellular aberrations in gene expression that rise in malignancies during tumor evolution.¹ Adenoviruses are the most widely studied engineered oncolytic viruses in the clinic. These adenoviral constructs include Onyx 015,^{2,3} CG7060,⁴ CG7870,⁵ dl922-947,⁶ Ad5-CD/tk-rep,⁷ Ad-delta24,⁸ Ad DF3-E1,⁹ Onyx 411,¹⁰ OAV001,¹¹ KD3,¹² and 01/PEME.¹³

Historically, evidence of oncolytic activity attributed to replicating viruses was provided in published case reports as early as 1912. These reports described rare but dramatic responses in cancer patients recovering from viral syndromes.^{14–28} Based on these observations, viruses with low pathogenicity (minimal toxicity to normal tissue) and high oncolytic capacity (toxic to cancer cells) were selected for initial clinical investigation.^{28–34} Studies in the 1950s with Egypt 101 virus revealed anticancer activity in the form of transient tumor necrosis in patients. However, further studies were discontinued when immune compromised patients developed encephalitis.^{35–37} Later attenuated mumps virus was tested in cancer patients and more impressive anticancer activity was observed. Responses, however, were generally transient, and they were limited to the site of injection.^{30,31,38–41} In an attempt to pursue a more systemic approach following local site injection methods of stimulating the immune system using viral “oncolysates” were developed. Researchers exposed virus to malignant cells *ex vivo*, induced oncolysis thereby

providing antigenic material that could be used as a vaccine. Influenza viral “oncolysates” induced greater antitumor activity *in vivo* than either virus alone or unmodified tumor cell extracts.^{42,43} In one trial involving patients with advanced ovarian cancer, a subset of patients demonstrated durable responses, which appeared to be enhanced following treatment with interleukin-2 (IL-2).⁴⁴ Other studies utilized a Newcastle disease viral (NDV) oncolysate and demonstrated impressive survival improvement in early melanoma patients compared to historical controls (69% disease-free survival at 10 years *versus* 15% disease-free survival). Currently, a modified Newcastle disease virus (73T) is being utilized as a vaccine oncolysate in an ongoing clinical trial involving melanoma and renal cell patients. Preliminary results appear encouraging.⁴⁵

This review will focus on the anticancer activity of oncolytic viruses when administered directly to the patient. Discussion will center on clinical trial results, future directions, and the potential use of oncolytic viruses as safe and systemic gene delivery vehicles.

Onyx 015

Onyx 015 is a replication-conditional adenovirus genetically modified by deletion of two DNA elements. Deletion of the first element, the E1B 55 kDa fragment, enables replication of Onyx 015 in cells with a defective p53 pathway (cancer cells)^{2,46,47} and minimizes replication in cells with a functional p53 pathway (normal cells). Some controversy exists on whether or not the sole mechanism by which Onyx 015 has selective replication capacity in malignant cells is related to p53.^{48–51} However, despite controversy most preclinical results do demonstrate

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Table 1 Clinical trials involving Onyx 015

Design	No. of patients/disease	Toxicity	Response	Comments	Reference
Phase I: 5 daily consecutive injections over 3 weeks Doses up to 2×10^{12} particles	22/SCCHN	Transient fever	3 PR, 2 MR, 8 SD	Tumor necrosis induced in patients characterized with SD	Ganly et al ⁵⁹
Phase II: 2×10^{11} particles I.t. injection (every day $\times$ 5 days/3 weeks)	30/SCCHN	Transient fever, chills, asthenia, injection pain	1 CR, 3 PR, 12 SD	7/12 patients with p53 mutation developed response compared to 0/7 p53 normal patients ($P=0.017$)	Nemunaitis et al ^{58,60}
Phase II: 2×10^{11} particles $2 \times$ /d for 5 consecutive days for 2 consecutive weeks during a 21-day cycle	10/SCCHN	Fever, injection site pain	1 CR, 4 SD	Increased fever, injection pain compared to less frequent dosing	Nemunaitis et al ^{58,60}
Phase II: 2×10^{11} particles 1 injection for 5 consecutive days every 3 weeks plus Cisplatin/5FU	37/SCCHN	Fever, injection pain, expected chemo-related toxicity	8 CR, 11 PR, median survival 10.5 months	11 patients w/more than one lesion: 9/11 injected had a PR/CR versus 3/11 PR/CR noninjected lesions	Khuri et al ⁶¹
Phase I: intraperitumoral injection up to 2×10^{12} particles for 5 days every 3 weeks	16/ovarian cancer	Flu-like symptoms, emesis, abdominal pain. 1 grade III abdominal pain and diarrhea	0 PR/CR	PCR positive for circulating virus 15 days after initial dose	Vasey et al ⁶²
Phase I: 2×10^{11} particles via i.v. every week for 6 weeks and low-dose IL-2	5/solid tumor	Fever	0 PR/CR	No enhancement in cytokine-related toxicity	Nemunaitis et al ⁶³
Phase I: 2×10^{12} particles via i.v. every week for 6 weeks+CPT11, 5FU	5/colon cancer	Fever	0 PR/CR	Viral replication confirmed in metastatic liver site Day 5	Nemunaitis et al ⁶³
I.v. infusion+i.t. injection (6×10^{12} vp)	5/hepatocellular cancer	Grade I–II toxicity fever, no significant increase in liver enzyme levels	1 PR		Habib et al ⁶⁴
Phase I: 2×10^8 – 2×10^{12} vp on days 1 and 8 for two cycles. Intrahepatic artery infusion in combination w/5FU and leukovorin	9/colon cancer 2/pancreatic cancer	Mild to moderate fever, rigors, and fatigue.	1 PR (2×10^{11} vp)	Viral replication detected $>2 \times 10^{11}$ vp	Reid et al ⁶⁵
Phase I: 2×10^{10} – 2×10^{13} particles i.v. every week for 4 weeks+carboplatin and paclitaxel	10/solid tumor	Fever Mild transaminitis Transient increase in LFT at 2×10^{13} particles	0 PR/CR, 8 SD	Confirmed viral replication at lung metastatic (D5) site MTD determined to be 2×10^{13} particles	Nemunaitis et al ⁶⁶
Phase I: 2×10^9 – 2×10^{12} vp via CT-guided i.t. injection	23/pancreatic cancer	Fever	0 PR/CR, 6 MR, 10 SD	2° LFT Day 22 tumor biopsy — for viral replication	Mulvihill et al ⁶⁷
Phase II: 2×10^{12} particles on days 1 and 8 through hepatic arterial infusion	27/gastrointestinal carcinoma metastatic to the liver	Reversible grade III/IV hyperbilirubinemia in two patients; 25 fever (3 severe), 20 chills (1 life threatening), 13 nausea (1 severe)	3 PR, 4 MR, 9 SD		Reid et al ⁶⁸
Starting on day 22: combination w/ 5FU and leukovorin every 28 days					
Phase I: eight injection EUS 2×10^{10} – 2×10^{11} vp/treatment	21/pancreas	Mild transient flu-like symptoms, 1 grade III fever	2 PR, 2 MR, 6 SD		Hecht et al ⁶⁹
Phase II: trial 2×10^{12} vp q 2 week via i.v., i.t.	18/colon cancer	Flu like symptoms, nausea, emesis	7 SD		Hamid et al ⁷⁰
Phase II: unresectable i.t. injection 6×10^9 or 1×10^{10} PFU	20/hepatobiliary	3 hepatic toxicity, 1 anemia, 1 infection, 1 arterial fibrillation	1 PR	8 patients with $\geq 50\%$ decrease in tumor markers	Makower et al ⁷¹

Another Onyx 015 trial: Hecht et al.⁶⁹

A phase I/II trial of intratumoral endoscopic ultrasound injection of ONYX-015 with i.v. gemcitabine in unresectable pancreatic carcinoma.

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selective replication activity of Onyx 015 in malignant tissue with abnormal p53 function.^{3,4,49,52–58} Furthermore, clinical tests of over 300 patients have shown no evidence of nonspecific viral replication or normal cell damage (Table 1, unpublished data).^{58–71} When administered intravenously (i.v.), dose escalation is limited by transient liver enzyme elevation at the 2×10^{13} particle level.⁶⁶

To understand the specificity of Onyx 015 and other oncolytic adenoviruses, it is helpful to review the process of viral uptake and replication. The first encounter of the adenovirus with the target cell for most serotypes occurs through a high-affinity interaction of the adenovirus fiber protein with a cell surface receptor, the coxsackie-adenovirus receptor (CAR), followed by low-affinity interaction between the adenovirus penton base protein and cell surface integrins.^{72–78} Receptor binding leads to internalization of the virus by the cell through receptor-mediated endocytosis, lysis of the endosomal membrane, escape to the cytosol, trafficking along microtubules towards the nucleus, binding the nuclear envelope, and insertion of the viral genome into the nucleus.^{79–88} The adenoviral reproductive cycle is a highly orchestrated process, and gene modification strategies, which enable selectivity, often reduce replication capacity. At the molecular level, completion of the infectious cycle relies on timely expression of a set of viral regulatory proteins that interact with essential endogenous cellular pathways that determine cell viability.⁸⁹ The adenoviral genome contains eight transcriptional units that are activated at different phases of infection. They are referred to as early (E1A), immediate-early (E1B, E2, E3, E4), intermediate (IX and IVa2), and late genes. The early genes generate regulatory proteins that set the stage for viral DNA replication by blocking host cell antiviral mechanisms.^{90,91} E1A proteins are potent transactivators that provoke the infected cell to enter the cell cycle and progress to S phase.⁹² In S phase, the virus takes advantage of the cell's DNA replication machinery and replicates its *own* genome.¹³ To prevent apoptosis due to unscheduled DNA synthesis, the adenovirus utilizes viral E1B-19K and E1B-55K proteins as part of the early gene activation phase. The E1B-55K protein, specifically, interferes with both p53-mediated apoptosis and p53's inhibition of cellular and viral replication by binding to p53. The virus inactivates p53 and Rb expressing viral E1B and E1A proteins, respectively. Mutants that fail to accomplish these functions because of deletions of the E1B^{2,13,93} or E1A^{6,8,12,94,95} regions selectively replicate within tumor cells that lack functional p53 or Rb function, respectively. Unfortunately, E1A and E1B genes have other roles in replication, so attenuation of these genes also reduces the mutant's replication ability compared to wild-type adenovirus. E1B-55K, for instance, facilitates transport of late viral mRNAs from the nucleus to the ribosomes.⁹⁶ Impaired mRNA transport in the E1B-55K-deleted adenovirus is the probable cause of its reduced potency.⁵² The E3 region proteins maintain viral survival.^{97–102} Several E3-encoded gene products have been found that either block or activate cell death,

and the coordinated and timely expression of these factors leads to optimal conditions for generating progeny.¹⁰³ Adenoviruses lacking functional adenoviral death protein (ADP) located in the E3 region, for example, have been shown to induce host cell death and release progeny virus *more slowly* than wild-type adenovirus.¹⁰⁴ Likewise, attenuated viruses with overexpressive ADP are more efficient at eradicating tumor cells than identical viruses lacking ADP.¹² However, inappropriate timing of the expression of ADP may be counter productive depending on the therapeutic approaches being attempted. Viral constructs delivering prodrug inducible cytotoxins have been shown to be more effective when the cytotoxic gene is placed under late phase gene control.¹⁰⁵ Deletion of ADP results in longer survival of virus in infected cells leading to an increased duration of ADP (or other late phase controlled expression transgenes).¹⁰⁶ Other antiviral host defense mechanisms located on the E3 region include proteins that neutralize the effect of proinflammatory cytokine tumor necrosis factor (TNF). Adenoviruses containing deletions in many of these proteins have diminished replicative capacity or diminished ability to survive since they are less efficient at protecting the host cell from endogenous immune-mediated clearance.¹⁰⁷ Nonreplicating viral vectors provide longer transgene expression in *TNF knockout* mice compared to wild-type mice.^{108,109} Furthermore, it has also been demonstrated that viral manufactured soluble binding proteins specific to TNF and other viral toxic cytokines such as interferon are also produced by other viruses including poxviruses, cytomegalovirus, herpesviruses, and adenoviruses.^{91,110–115} Late viral genes encode structural proteins for the capsid and viral internal core.⁸⁹ A great deal of preclinical work has been carried out utilizing modified capsid components to improve cancer targeting and reduce nontarget cell uptake.

Clinically, initial phase I investigation of Onyx 015 involved intratumor (i.t.) injection in refractory cancer patients. The virus was well tolerated and evidence of activity was suggested.¹¹⁶ Consequently, Onyx 015 was tested in a phase II study in patients with recurrent squamous cell cancer of the head and neck region (SCCHN). In one trial involving 40 patients injected i.t. with 2×10^{11} viral particles for 5 consecutive days, cancer-specific viral replication was demonstrated in seven of 11 patients who underwent biopsy. No viral replication or toxic effect was identified in normal tissue. Interestingly, seven of 12 patients with p53 mutant cancers had responses, while zero of seven patients with cancer containing wild-type p53 genotype achieved a response.⁶⁰ In a subsequent evaluation, 10 patients received a four-fold increase in dose but demonstrated no increase in response rate, although vector-related toxicity (fever, injection site pain) increased.⁶⁰ Despite a relatively low response rate (nearly 20%), safety was maintained (533 viral injections were administered), justifying further investigation in earlier stage chemotherapy naive recurrent SCCHN patients. In the next phase II study, patients received Onyx 015 (2×10^{11} particles for 5 consecutive days/21 days) in combination with cisplatin (80 mg/m²

once every 21 days) and 5-fluorouracil (5FU, 800–1000 mg/m² CI × 5d/21d).⁶¹ The results were encouraging in that no added toxicity to Onyx 015 was demonstrated in addition to the expected toxicity of cisplatin and 5FU. Furthermore, a 63% response rate was observed, greater than the expected response rate of 35% from previous publications in the same patient population.⁶¹ Results of the SCCHN trials and other studies with Onyx 015 are summarized in Table 1.

Other clinical investigation of Onyx 015 utilizing intraperitoneal injection in advanced ovarian cancer⁶² revealed a similar toxicity as in SCCHN,⁵⁹ although no responses were observed in ovarian cancer patients. Evidence of viral replication, however, was suggested based on continued detection of viral elements (via PCR) 15 days after the first injection. Other trials include patients with hepatocellular carcinoma. In one small pilot trial, one out of five patients achieved a partial response (PR) following a combination of i.v. administration and i.t. injection of Onyx 015.⁶⁴ Two trials have been preformed in advanced pancreatic cancer patients using both CT-guided and ultrasound- (EUS) guided injection approaches. In the first involving 22 patients, six minor responses (MRs), but no PR or complete responses (CRs), were observed.⁶⁷ Two PRs and two MRs were observed in the second trial with 21 patients.⁶⁹ Two other trials involving colorectal cancer patients with liver metastasis receiving intrahepatic artery infusion of Onyx 015 demonstrated good tolerability and identified a small number of patient responses.^{68,65} In a meta-analysis⁶⁵ summarizing the two intrahepatic arterial infusion trials, survival was compared between patients receiving less than 6×10^{11} virus particles per infusion (seven patients) to those receiving greater than 6×10^{11} virus particles per infusion (28 patients). A significant survival advantage was demonstrated in the high-dose group (359 days) over the low-dose group (155 days) (Fig 1).¹¹⁷

Onyx 015 has also been administered safely to more than 40 patients i.v. (Table 1). Results demonstrate a slightly higher frequency of febrile response than what is observed with i.t. administration of Onyx 015, although the frequency was similar to what was observed with intra-arterial infusion. At doses of less than 1×10^{13} particles per infusion, no significant safety concerns were raised including situations in which virus was administered in combination with low-dose IL-2 or chemotherapy (paclitaxel, CPT11, and 5FU). The presence of Onyx 015

within metastatic malignant disease sites following i.v. infusion was demonstrated; however, evidence of significant tumor regression was not.^{63,66,116}

Future directions of clinical exploration with Onyx 015 are still being considered. Measures, which enhance Onyx 015 activity, are being explored and new generation adenoviruses are being developed. In conclusion, utilization of Onyx 015 via i.t. injection appears to be associated with modest activity, although clinical utility of local regional therapy is limited. Further exploration via intrahepatic arterial infusion or i.v. infusion awaits discovery of methods to improve Onyx 015 target specificity and activity.

Enhancement of oncolytic viral therapeutic potency

Therapeutic limitations to the efficient and selective use of replication-conditional adenoviruses as a systemic therapeutics include clearance, target cell specificity, uptake, replication rate, and oncolytic potency. Natural immune mechanisms are responsible for the clearance of viral particles and attempts to modulate viral clearance are currently being studied. Some measures suggest activity, including those that modulate neutralizing antibodies such as plasma apheresis, B-cell function inhibitors, immune suppressive therapy, and/or desensitization.^{118–134} Combination therapy with other anticancer agents that may also affect the activity of conditional replicating adenoviruses is also being explored. For instance, it has been demonstrated that adenoviral binding is optimal during the M phase of the cell cycle. Paclitaxel stabilizes microtubules that halts the cell cycle at M phase and increases adenoviral binding and transgene expression.^{135,136} Agents such as paclitaxel, vinblastine, and docetaxel may potentially contribute to increased efficacy when combined with adenoviral therapies.^{137–139}

Immune response against infected tumor cells also appears to limit virus propagation via premature killing of the infected cells. This is a problem with oncolytic herpes simplex virus (HSV)¹⁴⁰ and likely affects the persistence of adenovirus.

Targeting is a critical issue in the effective use of oncolytic viruses as systemic therapeutics. Tumor antigens on the cell surface expressed in high concentration may be utilized to facilitate viral targeting to malignant tissue. CAR expression, for instance, is variable in human cancer.^{141–154} In fact, there is some suggestion that CAR expression is downmodulated in advanced cancers.^{155,156} Retargeting to receptors other than CAR allows oncolytic viruses to bind to alternative cellular receptors resulting in “CAR-independent infection”. Targeting complexes have already been developed to bind to receptors for folate,¹⁵⁷ EGF,^{142,158–161} EpCAM,¹⁶² and FGF2.^{163–165} Activity has also been described in preclinical trials using an antifiber-Fab FGF2 retargeting molecule that links adenovirus components to the FGF2 receptor on the malignant cell.^{164–168} In some cases, retargeting has been shown to be more efficient than the native adenovirus pathway.¹⁶⁹ Retargeting with specificity to malignant

Figure 1 Survival of Onyx 015 by dose.

tissue may enable viruses to be administered at higher doses with reduced toxicity.^{166,167} In addition, CAR surface expression is strongly expressed on normal cells, which may allow it to serve as a sink and bind the therapeutic dose, limiting viral exposure to malignant tissue. For example, Kupffer cells in the liver are highly active in clearing viral particles.^{170,171} Transient inhibition of viral uptake by Kupffer cells has been shown to improve uptake beyond the liver following i.v. administration of adenoviruses.¹⁷⁰ Several studies attempting to uncouple the adenovirus from its normal cellular receptor (CAR) and retarget tumor cell surfaces with single chain antibodies, growth vectors, or peptides are ongoing.¹⁷² A mutant measles virus in fact has already had its natural cellular receptor modified to target-specific tumor cell antigens.^{173–175} Another strategy for facilitating selectivity of oncolytic viruses is “intracellular targeting.” Cancer cells, for instance, generally lack an interferon response, which is toxic to infecting viral organisms in normal cells. Oncolytic viruses dependent on interferon for clearance (vesicular stomatitis virus, Newcastle virus, myxoma virus, PV701) have significant growth advantage in these cancer cells.¹⁷⁶ Likewise, reovirus growth has shown a selectivity to cells with an activated ras signaling pathway presumably through the downregulation of interferon-induced expression of PKR.¹ Intracellular targeting can also be facilitated by transcriptionally programming viruses to replicate within malignant tissue by substituting viral promoters with cellular promoter elements.^{177–183} For example, poliovirus has been detargeted from nondividing neuronal cells and retargeted to replicate in malignant glioma cells by exchanging internal ribosomal entry site (IRES) elements from rhinovirus to polio.¹⁸⁴ The combination of a 3′ polio UTR sequence with a 5′ rhinovirus IRES element creates a chimeric virus that is incapable of efficient translation in neuronal cells, but is well translated in malignant cells.¹ Other studies have explored cancer and/or tissue-specific promoters that

selectively control the cell site of viral replication (Table 2). Adenoviral replication can be restricted to specific cancers and tissues by placing transcriptional elements only responsive to those cancers in the early region of the viral genome. These DNA sequences, or promoters, prevent viral replication unless the infected cell produces the protein encoded for the DNA sequence. The demonstration that injections of CV706 could destroy large PSA-producing tumors in xenograft models supported translation to clinical trials.

Promoter-driven viral therapeutics

One, the PSA promoter, is already being utilized in clinical trial. The CV706 replication-competent adenovirus, formerly known as CN706, contains a genetically modified E1A gene controlled by a PSA promoter region. Preclinical *in vitro* studies confirm increased viral replication in PSA-producing cells when compared to non-PSA-producing prostate cancer cells.¹⁹⁷ Furthermore, single injections of CV706 destroyed large PSA-producing tumors in mouse xenograft models, leading to clinical trials.¹⁹⁷ These initial phase I trials confirmed replication specificity and suggested efficacy (Table 3). In one study, 20 men with locally recurrent prostate cancer after failing radiation therapy received single injections of CV706 ranging from 1×10^{11} to 1×10^{15} viral particles through a transrectal ultrasound-guided transperitoneal technique.¹⁸⁵ PCR analysis confirmed the shedding of viral DNA in the blood, suggesting replication.¹⁸⁵ A total of 13 patients experienced more than a 30% decrease in PSA levels, and all five patients given the two highest doses had more than a 50% decrease in PSA, suggesting efficacy.¹⁸⁵ The majority (74%) of adverse events were grade I, and the most common low-grade adverse events (grade II and below) were low-grade fever, injection site pain, and hematuria.¹⁸⁵ Five serious events (grade III or higher) were recorded, but they were reversible and not related to

Table 2 Summary of inducible cancer promoters linked to viral replication

Promoter	Targeted cancer	Reference
PSA	Prostate cancer	DeWeese et al ¹⁸⁵
α-fetoprotein	Hepatocellular cancer	Hallenbeck et al ¹⁸⁶
MMTV	Breast cancer	Lee et al ¹⁸⁷
MDR1 (not to a specific cancer but to human drug resistance gene inducible by cytostatic drugs)		Walther et al ¹⁸⁸
TYR (tyrosinase)	Melanoma	Vile and Hart ¹⁸⁹
Neuronal specific	CNS malignancies	Morelli et al ¹⁹⁰
CEA	Colon cancer	Gao et al ¹⁹¹
MUCI	Breast	Kurihara et al ⁹
pS2	Breast	Hernandez-Alcoceba et al ¹⁹²
HPV16 and HPV18	SCCHN, cervix	Steele et al ¹⁹³
Rous sarcoma virus	SCCHN	Thompson et al ¹⁹⁴
Keratin gene	SCCHN	Gibson et al ¹⁹⁵
FLK1	Endothelial cells/solid tumor	Savontaus et al ¹⁸³
hTERT	Solid tumor/telomerase	Kim et al ¹⁹⁶
Kallikrein	Prostate cancer	Rodriguez et al ¹⁹⁷ and Yu et al ⁴
EF1alpha	Solid tumor	Thompson et al ¹⁹⁴
P5	Solid tumor	Kawakami et al ¹⁹⁸

Table 3 Summary of clinical trials involving prostate-specific antigen promoter-driven replicating adenoviruses

Virus	No. of patients/ disease	Design	Toxicity	Response	Comments	Reference
CV 706	20/prostate cancer	Phase I via transrectal ultrasound Single injection 1×10^{11} – 1×10^{13} particles	Fever, chills, injection pain, no irreversible grade III at 4 toxicity	>50% decrease of PSA in five patients at maximum effective dose		DeWeese et al ¹⁸⁵
CG7870*	20/prostate cancer	3 cohorts: 1×10^{12} (10), 3×10^{12} (5), 1×10^{13} particles (5)	19/20 at least one adverse event (most mild to moderate) Flu-like symptoms, local reactions at injection site, urinary tract signs/symptoms 1 severe anemia not related to study	Positive viral cDNA in post-treatment biopsies 10/12 patients w PSA > or = 5.0 ng/mL had regression, 2/10 PSA $\geq 50\%$ decrease	71% alive w/o progression after 6 months.	Yu et al ⁵
CG7870 and CG7060	37/prostate cancer	Phase I/II trials: patients w/ prostate cancer by intraprostatic and i.v. administration (37) Maximum dose 1×10^{12} vp	Viral genome detected up to 4 weeks after infusion	No dose-limiting toxicity One-quarter of patients had low levels of wild-type genome		Husak et al ¹⁹⁹

*Contains full-length E3 region.

CV706.¹⁸⁵ This trial confirmed safety and provided support for additional clinical testing and for the development of prostate-specific adenoviral vectors.

Another prostate-specific replication-competent adenovirus is CG7870. CG7870 has improved replication specificity over an older generation PSA-specific virus CV706, because both its E1A and E1B genes are under the control of prostate-specific transcriptional regulatory elements.⁵ In one preclinical trial, nude mice with PSA-positive prostate cancer xenografts were injected with CG7870 through the tail vein. Results indicated a 100-fold increase in efficacy over CV706. In addition, CG7870 effected significant responses in distant tumor sites, suggesting efficacy in administering the virus i.v.⁵ In a phase I/II trial, CG7870 was administered through intraprostatic injection to 20 patients with locally recurrent hormone naive prostate cancer with doses from 1×10^{12} to 1×10^{13} particles. In eight of 12 evaluable patients (including three of three at the highest dose level), PSA levels decreased by 25–50%. In addition, 75% of evaluable patients remained progression free after 6 months. No serious adverse events from CG7870 resulted from the study. Only one grade III toxic event occurred, an elevated d-dimer and it resolved without treatment within 29 days.²⁰⁰ In a follow-up of several trials with both CG7870 and CV706, all 37 patients studied had various levels of circulating viral genome for up to 4 weeks after treatment. No dose-limiting toxicities were encountered, indicating that the virus was well tolerated at doses of up to 10^{13} particles.¹⁹⁹ Future therapeutics are currently exploring transgene delivery using the CG7870 backbone with Diphtheria toxin A.^{201,202}

Other selective transcriptional promoters currently being tested (Table 2) include: PSA and kallikrein for prostate cancer,^{5,197,185} α -fetoprotein for hepatocellular cancer,¹⁸⁶ TYR promoter for melanoma,¹⁸⁹ and carcinoembryonic antigen (CEA) for colon cancer.¹⁹¹ The MMTV,¹⁸⁷ MUCI,⁹ and pS2 promoters¹⁹² are also being tested for breast cancer as are a number of promoters for head and neck cancer including HPV 16 and HPV 18¹⁹³ and Rous sarcoma virus.¹⁹⁴ FLK1 promoter is being tested for endothelial cells and other solid tumors,¹⁸³ and hTERT, a telomerase promoter, is being tested in solid tumors.¹⁹⁶ Tissue-specific promoters help adenoviruses concentrate replication in specific tissues, aiding in selective replication, and provide an additional safety mechanism to prevent replication in normal cells. This technology is especially useful in systemic administration of adenoviruses, as it should prevent replication in cells not containing such molecular abnormalities (normal cells). Other promoters, which have not been tested extensively in animal trials, include a promoter for Rous sarcoma virus,¹⁹⁴ EF1alpha promoter,¹⁹⁴ promoters for keratin genes,^{194,188,203} and the p5 promoter of the AAV.¹⁹⁸

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 with defects in

the interferon signaling pathway.²⁰⁴ Most early studies used Newcastle virus as an oncolysate tumor vaccine. These vaccines would then be injected into patients to generate an immune response.¹⁷⁶ Clinical trials involving Newcastle disease virus are summarized in Table 4. In addition, the virus has been given i.v., intraperitoneally, and i.t. in athymic mice implanted with human cancers. These preclinical trials have shown few systemic side effects, and have demonstrated evidence of activity.^{134,216–218}

The first report of activity with Newcastle virus involved one patient with cervical cancer. Cassel and Garrett²⁰⁸ 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. In a phase II study in 1975, allogeneic and autologous human melanoma cells infected with Newcastle virus were used as an oncolysate postoperatively for patients with stage III melanoma. In addition to an increased number of CD8⁺ T cells, a 15 year follow-up indicated a 55% rate of survival.^{207,206} In another trial involving patients with high-risk stage II melanoma, viral oncolysate was administered following surgical excision of metastatic nodes (virus was administered weekly to week 4, then biweekly to week 52, then every 6 weeks to week 160). After 3 years of treatment, progressive disease occurred in only 12% of patients. Also, for 83 patients, survival was 68% after 5 years, significantly higher than historical controls.^{209,210,212} Autologous NDV oncolysate was also used in a phase II trial to treat colorectal cancer after the resection of metastatic liver lesions.²¹³ The oncolysate was initiated 2 weeks after surgery and administered every 2 weeks for five cycles with one booster 3 months later. Nine of 23 patients had an increase in delayed-type hypersensitivity reactions when irradiated autologous tumor cells were placed intradermally after treatment. Of the patients, 61% developed recurrent disease, lower than the 87% recurrence in historical controls. Toxicity was limited in both this trial and the melanoma trial with side effects restricted to local inflammation and mild fevers.²¹³ Newcastle virus has also been used as a surgical adjuvant vaccine in 208 patients with locally advanced renal cancer. In addition to oncolysate, these patients also received low-dose interferon-alpha and IL-2. The median disease-free survival was 21 + months, higher than similar historical controls, while toxicity was limited to fever, flu-like symptoms, and local inflammation.²¹⁴ In another trial, oncolysate was 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. In addition, they all received low-dose IL-2 (1×10^6 IU subcutaneously on days 1–19 and 42 to 60) and interferon-alpha (3×10^6 IU subcutaneously 3 days a week). Although patients with primary breast cancer had a survival benefit compared to historical controls, the other groups had no suggestion of survival advantage.²¹⁵

Newcastle virus has also been tested via i.v. infusion and 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 (Table 2). Of the patients treated with virus, seven patients achieved a complete or PR and one patient had a MR; these eight patients survived more than 1 year after treatment. In comparison, none of the control patients had responses. A total of 22 patients receiving virus survived longer than 1 year, whereas only four patients in the control group survived 1 year. Eight viral treated patients survived for more than 2 years, whereas none of the control patients survived that long.³⁹

Most recently, a phase I trial of an attenuated Newcastle virus strain, PV701, was administered i.v. (5.9×10^9 PFU/m² to 24×10^9 PFU/m² every 28 days) in 79 patients with solid tumors (Table 4). Side effects were mild and were limited to fever, flu-like symptoms, and hypotension. Seven grade III adverse events were observed, but toxicity decreased with subsequent doses. A maximum-tolerated dose (MTD) following a single infusion was established at 12×10^9 PFU/m², and subsequent infusions were tolerated up to 120×10^9 PFU/m². Further dose escalation was limited by patients developing hypotension. Of 62 patients, 14 eligible for response assessment maintained stable disease (SD) for 4 months to more than 30 months. One patient with squamous cell cancer on his tonsil achieved a CR. Another patient with metastatic colon cancer achieved a PR. Seven patients achieved MRs of less than a 50% reduction in tumor size. The presence of viral particles was confirmed in malignant tissue following treatment.

In conclusion, Newcastle disease virus approaches have demonstrated an acceptable toxicity profile. The most recent construct, PV701, has been well tolerated via i.v. infusion and warrants further investigation.

Herpes simplex virus. HSV is a double-stranded DNA virus. Initial genetic modification used to construct oncolytic forms of this virus enabled selective activity within malignant tissue. One modification involved inactivation of viral gene ICP6 that encodes the large subunit of ribonucleotide reductase, an enzyme required for viral DNA replication.^{219–222} This enzyme is expressed abundantly in rapidly dividing tumor cells, but is more sparse in normal cells. As a consequence, the ICP6 gene modified HSV-1 replicates selectively in tumor cells. The second approach consists of deleting another viral gene (the γ -34.5 gene), which functions as the virulence factor during HSV infection.²²³ Mutations in this gene also limit replication in nondividing cells.^{224,225} The oncolytic HSV-1 virus, G207, has been extensively tested in animal models and is currently in clinical trials.^{226–229} Replication-sensitive HSV1 γ -34.5 viral mutants have been shown to be effective in treating both CNS^{230–232} and non-CNS^{233–241} tumors in animal models. Clinical trials involving patients with high-grade glioma, colorectal cancer, solid tumors, and melanoma^{242–245} have demonstrated safety. Results are summarized in Table 5. Four different herpes simplex oncolytic viruses have been tested in clinical trial (Table 6). Toxicity in the form of fever, chills, and transient liver enzyme elevation has been found to be greater in patients who have low initial HSV-1

Table 4 Clinical trials involving Newcastle disease virus

Trial/design (# patients)	Efficacy	Side effects	References
Phase I: solid tumor (80) Given via i.v. infusion	14/62 stable disease ≥ 4 months 1 CR — squamous cell cancer of the tonsil 1 PR — colon cancer	MTD 120×10^9 PFU/m ² Mild: flu-like symptoms, mild hypotension	Pecora et al ²⁰⁵
Phase II: stage III melanoma Oncolysate consisting of allogeneic and autologous human melanoma cells infected w/live Newcastle virus — administered postoperatively Case report: direct injection, cancer of the cervix (one patient)	Evidence of increased number of CD8 ⁺ T cells 15-year follow-up showed 55% overall survival I.t. regression of the cancer at the site of the injection and also regression of a distant malignant lymph node Detection of replicating virus w/in urine 11 days after injection	Transient fever	Schirmmacher et al ²⁰⁶ Batliwalla et al ²⁰⁷
Phase II: advanced cancer (33 patients) (<i>versus</i> control of 26 placebo inhalants)	7 CR/PR 1 MR No responses in control patients 22/33 survived at least 1 year <i>versus</i> 4/26 control patients 7 at least 2 years <i>versus</i> none control 7/8 w/ colorectal cancer metastatic to the liver survived 1 year <i>versus</i> 1/5 control.	Transient fever	Csatary et al ³⁹
High-risk stage II melanoma (83 patients) tested after surgical excision of metastatic nodes. Viral oncolysate administered weekly to week 4, then every 2 weeks to week 52, then every 3 weeks to week 120, then every 6 weeks to week 160. Colorectal cancer following resection of metastatic liver lesions (23 patients) Autologous NDV oncolysate used: initiated 2 weeks after surgery and repeated five times at 2-week intervals followed by one boost 3 months later	Evidence of progressive disease 6% at 1 year, 8% at 2 years, 12% at 3 years 88% of 83 patients survived 3 years and 68% survived 5 years.	Fever, local pain	Cassel et al ²⁰⁹ , Cassel and Murray ^{210,211} and Sinkovics and Horvath ²¹²
Colorectal cancer following resection of metastatic liver lesions (23 patients) Autologous NDV oncolysate used: initiated 2 weeks after surgery and repeated five times at 2-week intervals followed by one boost 3 months later	9/23 (40%) increase in delayed-type hypersensitivity reactions to irradiated autologous tumor cells placed intradermally after vaccination 61% of patients had disease recur w/in 18 months <i>versus</i> 87% recurrence in 130 control patients who underwent the same surgical procedure. Median disease-free survival was 21+ months	Fever, local pain	Schlag et al ²¹³
Locally advanced renal cell carcinoma (208) Surgical adjuvant vaccine: low-dose IL-2 and interferon-alpha in combination w/the oncolysate. Primary breast cancer (63), recurrent breast cancer (27), and metastatic ovarian cancer (31). Administered on days 1, 7, and 42 Also received low-dose IL-2 (1×10^6 IU daily subcutaneously) on days 1–19 and 42–60 Also received 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	Primary breast cancer patients appeared to have a survival benefit compared to historical controls, but no survival advantage suggested in the other disease groups	Low-grade fever, mild flu-like symptoms, local inflammation	Kirschner et al ²¹⁴ Haas et al ²¹⁵

Table 5 Summary of clinical trials with HSV

Specific virus	Trial/design	Efficacy	Side effects	References	Comments
NV1020	Phase I study in patients (12) w/adenocarcinoma of the colon w/metastasis to the liver Single intrahepatic arterial infusion (doses from 3×10^6 to 1×10^8 PFU) On day 28 and 1 month later, all patients received chemo of either 5FU (via pump) or CPT11 (via i.v.)	Decreases in tumor size noted for 2 patients up to 28 days after NV1020 administration 1 (at 3×10^7 PFU) showed overall reduction of at least 20% in tumor measurements at 28 days (prior to receiving chemo). By month 2, it further regressed to a PR of at least 50% Second patient treated w/ 1×10^8 PFU similar PR by month 2. Both PRs observed after the initiation of chemo w/ 5FU and CPT11 4 other patients MRs w/at least 25% reduction in tumor size after chemo.	Pain (75%), pyrexia (58%), headache (50%) Tachycardia (17%), nausea (17%), vomiting (17%), rigors (25%) Transient elevation in gamma glutamyl transferase, gastroenteritis No deaths No dose-limiting toxicity observed	Bennett et al ²⁴⁶	10/12 decrease in CEA from baseline to day 6 (20% below baseline). Day 14, 7/12 decrease. After chemo, majority of patients had at least 50% decline of CEA levels. No evidence of disseminated HSV infection, viral reactivation, or shedding NV1020 detected in one saliva sample and two peripheral blood samples from a single patient treated at highest dose level
G207	Phase I: Recurrent glioma (21) Intratumoral Maximum dose: 3×10^9 PFU Minimum dose: 1×10^6 PFU	MRI volume decrease in eight patients 2 patients alive over 4 years G207 DNA detected by PCR in 2/7 patients	Safety confirmed No serious adverse events	Markert et al ²⁴⁷	
HSV 1716	Phase I: Recurrent glioma (9) Intratumoral Maximum dose: 1×10^5 PFU Minimum dose: 1×10^3 PFU	4 patients alive over 14 months	Safety confirmed No adverse events exhibited	Rampling et al ²⁴⁸	
HSV 1716	Phase I: high-grade glioma (12) — all but one patient recurrent tumors. I.t. injection All 1×10^5 PFU Tumor surgically resected after 4–9 days	Viral replication demonstrated in two patients Significant amounts of viral DNA detected by PCR in 10 patients	Safety confirmed	Papanastassiou et al ²⁴⁹	
HSV 1716	Phase I: stage IV melanoma (5) Between 1 and 4 injections of 1×10^3 PFU into a single nodule	Histopathological evidence of tumor necrosis in three patients receiving multiple injections. HSV antigen detected by immunohistochemistry only in tumor cells.		Mackie et al ²⁵⁰	
Oncovex ^{GM-CSF}	Phase I: solid tumor (8) 10^6 , 10^7 PFU direct injection	3/8 detectable virus blood/urine <2 weeks 1 PR	Local tumor necrosis, pain, fever	Han et al, ²⁵¹ Liu et al ²⁵² and Hu et al ²⁵³	Ongoing trial

Table 6 Herpesvirus constructs in clinical trial

Virus	Genes mutated	Transgenes	References
NV 1020	UL24, UL56, γ 34.5	—	222,244,254–256
G207	ICP6, γ 34.5	LAC 2	222,229,235,257
1716	γ 34.5	—	222,224,234,258,259
Oncovex ^{GM-CSF}	γ 34.5, ICP47	GM-CSF	222,260

Table 7 Summary of results with reovirus

Reovirus (Reolysin)	Phase I: i.t. injection solid tumor (18) 10^7 – 10^{10} PFU	Serum PCR and for virus at 10^9 , 10^{10} dose levels 1 CR, 1 PR, 8 SD of injection lesions	Headache, transient flu-like symptoms	Morris et al ²⁸⁸
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antibody titers at baseline. However, all patients developed an immune antibody response against HSV antigens within weeks following treatment; thus, continued treatment to patients with high initial HSV antibody titers have had significantly less toxic effect. PCR analysis of tissue has revealed the presence of HSV DNA at injection sights.²⁴⁹ Preclinical results in immune-competent models have also suggested immune-mediated distant responses,^{235,236,261–263} although a systemic immune effect has not yet been observed in clinical trial (Table 5). Given the significant lack of systemic activity of viral-induced oncolysis following local–regional treatment in the clinic, several new vectors carrying immune-stimulating transgenes have been developed (GM-CSF, IL-2, IL-12, B7.1).^{263–268} In addition, combination of HSV mutants with chemotherapy or radiotherapy has demonstrated enhancement in antitumor activity.^{222,243,269–274} Radiation enhanced anticancer activity of HSV when used in pancreatic, glioblastoma, and cervical cancer models,²⁷⁵ 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.^{244,271,272} Low-dose irradiation also improved efficacy of HSV viral therapy in a cervical cancer model.²⁷⁴ In chemotherapy combination studies, interestingly, both chemotherapy-resistant and -sensitive tumors were equally responsive.²⁷⁶ A variety of chemotherapy agents (mitomycin C, cisplatin, methotrexate) have demonstrated enhancement in antitumor effect when combined with HSV.²⁴³

Other studies have also identified the use of HSV to deliver other genes, such as those that convert benign prodrugs into cytotoxic agents. In one particular study, cytochrome *P450* gene and HSV-1 thymidine kinase (TK) gene were delivered using a HSV-1 replication-competent virus via i.t. injection in a hepatocellular carcinoma model.²⁷⁷ Cancer regression significantly improved with the cytochrome *P450* conversion of cyclophosphamide to the active metabolite phosphoramidate mustard.²²⁰ Similar results have been produced with cytosine deaminase transgene.²⁷⁸ Interestingly, HSV TK metabolic activation of acyclovir or ganciclovir in HSV-infected cells inhibits

viral replication without affecting tumor growth.^{237,279–281} This suggests that TK gene and ganciclovir may be useful as a safety valve if persistent virus and related toxicity developed.²⁷⁷

In conclusion, certain HSV vectors appear to be active and selective against malignant tissue. Safety, particularly with respect to neurovirulence, will require further clinical investigation at MTDs. Use of tissue promoters, such as albumin and calponin,^{282–284} as part of the viral core backbone combined with transgenes, which may potentiate systemic immune response (e.g. GM-CSF) may have promise for future therapeutics.

Reovirus: Reoviruses are double-stranded RNA viruses that cause mild respiratory and gastrointestinal illnesses in humans. Although normal mouse fibroblast cells (NIH3T3) do not normally support reovirus replication, NIH3T3 cells that have been transformed with activated ras, epidermal growth factor receptor, or V-erb B Oncogene (e.g. 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 alpha phosphorylation potentiating unabated cellular protein production and viral replication. As in normal cells without ras activation, early viral replication induces EIF-2 alpha phosphorylation thereby inhibiting cell protein synthesis, reoviruses exhibit preferential oncolytic effects in ras activated cancer cells.

Reoviruses have demonstrated preclinical 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.²⁸⁵ Reoviruses have also demonstrated activity against Lewis lung cancer metastasis in mice following i.v. administration.²⁸⁶ In this study, 65–80% of the mice tested showed regression in their tumors.^{286,287} Investigators have recently demonstrated oncolytic activity of reovirus against human ovarian and colon cancer cell lines carrying a high percentage of k-ras mutations implanted in mice.²⁰⁴ In

addition to demonstrating the susceptibility of human colon and ovarian 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 colon and ovarian cancers. I.t. injection of virus consistently resulted in major reductions of the ovarian and colon cancer tumor volumes. Of particular significance, the i.v. administration of virus in the immunocompromised mice consistently resulted in the regression of tumors at a remote site.

In animal model studies and in human pathogenesis, reovirus has not caused severe or life-threatening symptoms. Accordingly, reovirus looks like a promising therapy for colon cancer, ovarian cancer, breast cancer, lung cancer, and other malignancies having activation of the ras pathway, especially as a systemic treatment in combination with immunosuppressive therapy.¹⁷⁶ Clinically, 18 patients with refractory solid tumors have been treated in a phase I investigation, and a dose of up to 1×10^{10} PFU has been well tolerated (Table 7). Preliminary results also identified one patient achieving a CR and 1 a PR. Eight maintained SD for a prolonged period. Further work is being developed.

Vaccinia: Vaccinia is a double-stranded DNA virus and a member of the poxvirus family. Vaccinia virus has tropism for human cells and its high immunogenicity. The immunogenicity properties were exploited in making smallpox vaccine that lead to the eradication of smallpox.

Three properties of vaccinia virus are being used to develop the virus as a cancer therapy. These include the following: (1) Vaccinia virus has a high efficiency of infection, it replicates in the cytoplasm without chromosomal integration, and the 200 kb genome allows the insertion of a large amount of recombinant DNA without losing infectivity. (2) To develop cancer vaccines, the immunostimulatory properties of the virus are being harnessed to incite an immune response against cancer cells. (3) To develop an oncolytic virus, replication-conditional viral mutants are being constructed to target specific cancer types. We will briefly touch each of these areas.

The following are examples of preclinical and phase I trials (Table 8) that explore the use of vaccinia virus as a vector for gene therapy. In one study,²⁹⁹ 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 (ER) 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 activated specific CTL clones *in vitro*.³⁰⁰ In another study,³⁰⁰ a recombinant vaccinia virus vector was created containing the tumor suppressor p53 gene. This virus demonstrated a high level of expression of p-53 protein in transfected glioma cells, resulting in high levels of apoptosis.²⁸⁹ A phase I study of intravesical vaccinia virus infection²⁸⁹ demonstrated that vaccinia virus can be safely administered into the bladder and gave evidence that the treatment was associated with an intense immune response with few clinical side

Table 8 Summary of clinical trials with vaccinia virus

Virus	No. of patients/disease	Design	Toxicity	Response	Reference
Vaccinia	4/bladder	Phase I: 2×10^8 PFU Intravascular infusion prior to cystectomy	Mild dysuria	3 patients disease free at 4 years	Gomella et al. ²⁸⁹
Vaccinia/GM-CSF recombinant virus	7/melanoma	Phase I doses: 10^4 to 2×10^7 PFU/lesion or 10^4 to 8×10^7 PFU/session	Infrequent, dose-related, mild flu-like symptoms resolved w/in 24 hours	3 mixed responses, 1 PR, 1 CR Viral replication demonstrated, GM-CSF mRNA present at injection site to week 31 0 CR/PR i.t. IL-2 transgene detected 3 weeks after injection	Mastrangelo et al. ²⁹⁰
Vaccinia/IL-2	6/mesothelioma	Phase I: i.t. injection 10^7 PFU	No significant toxicities	25/39 disease free at 1 year	Mukherjee ²⁹¹
Vaccinia	39/melanoma (stage II)	Phase I viral oncolysate 13 injections weekly for 12 months or until recurrence	Transient fever Injection pain		Wallack et al. ²⁹²
Vaccinia oncolysate	250/melanoma (stage II)	Phase III viral oncolysate <i>versus</i> replicating virus (10^5 PFU) 1 injection/week $\times$ 13 weeks then 1 injection/2 weeks $\times$ 39 weeks or recurrence	No significant toxicity	No difference in disease-free survival or overall survival (at 2 years (47.8% <i>versus</i> 51.2%))	Wallack et al. ²⁹³ Wallack et al. ²⁹⁴ Scoggin et al. ²⁹⁵
Vaccinia MUC1-IL-2	28/prostate, NSCLC	Phase I: 5×10^5 – 5×10^7 PFU i.m. weekly	No grade III/IV toxicity	1 NSCLC SD $\times$ 7 mo 1 prostate 50% decrease PSA	van Ophoven et al. ²⁹⁶
Vaccinia/PSA/GM-CSF	33/prostate (rising PSA s/p surgery/XRT)	Phase I: 2.65×10^6 – 2.65×10^8 PFU inj 1 $\times$ /mo $\times$ 3 mo	Grade I/II fever, vesicular, rash	14 SD $\times$ 6 month 6 SD $>$ 2 years	Eder et al. ²⁹⁷
Vaccinia/PSA	42/metastatic prostate	Phase I: inj 1 $\times$ /mo $\times$ 3 mo	Grade I/II fever, vesicular, rash	Increase PSA-specific T cells	Gulley et al. ²⁹⁸

Table 9 Adenoviral gene delivery approach

Specific virus	Trial/design	Efficacy	Side effects	References
Ad5-CD/Tkrep	Phase I: 16 patients w/locally aggressive prostate cancer received single intraprostatic injection (10^{10} – 10^{12} particles) of virus and then 1–3 weeks of 5FC and vGCV and standard dose 3DCRT	7/16 had $\geq 25\%$ decrease in PSA; 3/16 $\geq 50\%$ decrease PSA Transgene expression confirmed 2 weeks after injection.	No dose-limiting toxicity 93% of adverse events grade I–II severity: no serious events	Freytag et al ³⁰⁵ Rogulski et al ³⁰⁶

effects.²⁹⁴ Of the four patients studied, three of them survived and were free of disease at 4 years. The authors felt that the data supported further study of vaccinia virus vectors in the treatment of bladder cancer.

Many studies use vaccinia virus as an immunotherapeutic agent. Vaccinia oncolysate has been studied as a vaccine in early stage melanoma (Table 8). Results suggested good tolerability and survival advantage compared to historical controls. However, a prospective controlled trial failed to validate the use of vaccinia oncolysate. The control group did not receive standard care but, instead, it 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. While 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,²⁹⁰ 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.²⁹⁰ This virus has been administered intralesionally in a phase I 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 PR.²⁹⁰ Other studies have also investigated a vaccinia virus carrying a PSA transgene in the treatment of both minimal disease and metastatic disease prostate cancer. Evidence of cancer-specific immune activation was demonstrated, and tolerability was reasonable. In minimal disease patients with rising PSA following surgery or radiation therapy, 14 of 33 maintained SD for at least 6 months and six remained disease free for greater than 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.³⁰¹ 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. Animal results are encouraging.^{302–304}

In summary, whether using mutant vaccinia viruses having tumor selective replication features or using

vaccinia virus tumor lysates as antitumor vaccines, vaccinia virus appears to offer multiple potentially effective ways to treat cancer.

Other viral approaches

A great deal has been learned with respect to use of replicating virus as a potential cancer therapeutic. Although activity has been demonstrated, FDA approvable products have not yet been generated. Clinical trials support the concept of selective and/or conditional oncolytic viral replication. Reported toxicities, even with wild-type adenovirus have not been clinically unacceptable with doses up to 10^{13} particles (adenovirus). In one study,²⁴⁰ an E1B-deleted adenovirus 5 containing a combined fusion gene of cytosine deaminase and TK⁷ was administered to 16 cancer patients (Table 9).³⁰⁵ There was no serious toxicity, although replication was inhibited early with the administration of 5FC and ganciclovir.

The E1B and E1A-deleted adenoviral vectors provide selective replication capacity and can be utilized as gene delivery vehicles to potentiate the viral oncolytic effect.³⁰⁷ Interestingly, in Freytag's work,⁷ the prodrug anticancer effect was greater than the direct viral cytolytic effect when the prodrug was administered early. Other viruses have also been explored preclinically as selective cancer therapeutics (e.g. Onyx 411,^{307,308} poliovirus,³⁰⁹ vesicular stomatitis virus,³¹⁰ influenza virus,³¹¹ and measles virus³¹²). Replicating bacteria have also been briefly tested in clinical trial.³¹³ Additional measures to modify viral activity by combining treatment with relevant immune modulation therapy (rituximab, enbrel) requires further exploration. Other studies have also shown that modification of the E3 region may minimize viral potency with respect to the durability of selective replication. Modification of the E3 region appears to potentiate viral activity with respect to replication capacity, duration of survival, and induction of apoptosis.^{106,314,315} Changing the surface components, such as the fiber and knob, to alter cancer binding, particularly when CAR expression is reduced or not displayed has also demonstrated improved anticancer activity.³¹⁶ Furthermore, contaminant of viral particles in liposomes or polymer-coated ligands may improve viral uptake particularly when administered systemically.³¹⁷

Conclusion

Clinical instigation of conditional replicating oncolytic viral therapies continues to be pursued in oncology. There is a high degree of confidence now in the safety and selectivity of a variety of oncolytic viruses. Furthermore, understanding of viral biology has led to the modification of viral genome in order to address therapeutic options and safety issues. In addition, work now being carried out in addressing optimal viral surface coat components, systemic clearance factors, potency, replication capacity, oncolytic capacity, and gene delivery will begin to cross paths, as more is understood. This will lead to more potent treatments. For the future, we are heading towards developing selective replicating viruses that can avoid immune clearance, thereby enabling systemic administration. Ultimately, statistical confirmation of efficacy will need to be demonstrated in comparison to standard of care measures. One day cancer may be targeted for treatment based on specific molecular abnormalities rather than histologic characterization, and the therapeutic effect of such selective replicating viruses will be limited to the cancer and not involve normal organs.

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