Review

Selectively replicating viral vectors

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Standard methods of cancer treatment (surgery, radiation therapy, and chemotherapy) offer no specificity to malignant tissue and have reached a plateau in effectiveness. Development of new therapeutic modalities, which target malignant cells directly based on their unique molecular makeup, is evolving as a novel trend in cancer treatment (i.e. Herceptin, Rituximab, and so forth). Within this context, the concept of conditional replicating viruses, which specifically replicate within malignant tissue, provides a unique opportunity not only as a cancer therapeutic in itself, but also as a tool to deliver cancer toxic genes in a selective manner following systemic administration.^{1–4}

Initial evidence of oncolytic activity attributed to replicating viruses was actually provided by published case reports over time, which identified dramatic responses in cancer patients recovering from viral syndromes.^{5–19} Based on these observations, viruses with low pathogenicity for normal tissue and high oncolytic capacity were selected for investigation.^{19–26} This review will focus on a discussion of replicating viruses, which have cancer-specific cytotoxic effect.

Replicating adenoviral vectors: rationale

A great deal of data have been accumulated, with non-replicating adenoviral vectors suggesting a reasonable safety profile in humans.^{27–37} Eighty percent of adults have existing antibodies to adenovirus serotype 5 (the most commonly used vector). As a consequence, less than 15% of exposed patients become clinically symptomatic. Rarely, severe immune-compromised patients have developed adenoviral infection (pneumonia, viraemia) and mortality has been observed, although some patients have responded to Riboviran.²⁸ Live wild-type adenoviral vaccines have been administered orally to military recruits without adverse events²⁹ and wild-type adenoviruses have been administered intratumorally and intra-arterially to patients with cervical cancer with minimal toxicity (transient fever, fatigue).

Despite rare hepatotoxicity and case reportable mortality following doses $>2 \times 10^{13}$ particles, use of nonreplicating adenoviruses has been demonstrated to be a safe vehicle for gene delivery in normal volunteers, cancer patients, and patients with a variety of other medical disorders. Unfortunately, promising results in preclinical models have yet to be proven as efficacious in patients. As a consequence, adenoviruses genetically modified to replicate within malignant tissues are being developed.

The targets and rationale for corrective gene replacement are sound, however. It appears that a significant limitation of the gene replacement approach is the ability to adequately deliver a sufficient quantity of corrective genes into a minimum number of target cells in order to impact clinical outcome. Optimal antitumor activity using replicating viruses involves rapid replication of the virus in malignant cells with the ability to efficiently lyse the malignant cells for optimal release of viral progeny in order to infect additional adjacent malignant cells. This is balanced by the rate of growth of malignant cells not infected and the rate with which released viral progeny is cleared from the site of disease by immunologic rejection.⁴⁰

Initial conditional replicating adenoviruses were genetically constructed to inactivate tumor-suppressor genes, thereby enabling viral survival within cancer cells.⁴¹ Specifically, mutations or deletions in the *p53* or retinoblastoma (*Rb*) genes, which are present in the majority of cancer cells, interact with and modulate release of adenoviral gene products as an essential step in virus propagation. Unfortunately, the adenoviral reproductive cycle is a highly orchestrated process, and gene modification strategies, which enable selectivity often, have reduced replication capacity of the modified virus. At the molecular level, completion of the infectious cycle within malignant cells relies on timely expression of a set of regulatory proteins that interact with essential endogenous cellular pathways that determine cell viability. Moreover, viral regulatory proteins guide viral DNA replication, expression of adenoviral genes, disruption of the cell metabolism, and release of new viral particles.⁴² The adenoviral genome basically contains eight transcriptional units that are activated at different phases of infection. They are referred to as immediate early (E1a), early (E1b, E2, E3, E4), intermediate (IX and IVa2), and late genes, which encode structural proteins for the capsid and viral internal core. The early genes are mainly regulatory

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proteins that set the stage for viral DNA replication, by blocking host cell antiviral mechanisms.^{43,44} Adenovirus proteins control various cellular processes by interacting with multiple host cellular proteins; many of these interactions are not completely understood. The first encounter of the adenovirus with cells occurs through a high-affinity interaction of the adenovirus fiber protein with its cell surface receptor, the coxsackie-adenovirus receptor (CAR), followed by low-affinity interaction between the adenovirus penton base protein and cell surface integrins.^{45–51} Receptor binding leads to internalization of the virus into the cell by 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.^{52–61} A major function of the adenoviral early genes is to provoke the infected cell to enter the cell cycle and progress to S phase.⁶² In S phase, the virus can take advantage of the cellular DNA replication machinery and replicate its *own* genome efficiently. The viral E1A proteins are potent transactivators that relieve cellular growth suppression and induce quiescent cells to enter S phase by binding members of the Rb family, thereby releasing E2F.⁶² Expression of E2F-dependent cellular S phase genes and viral DNA replication proteins in turn leads to induction of S phase and synthesis of cellular and viral DNA.⁴ To prevent apoptosis due to unscheduled DNA synthesis, the adenovirus utilizes viral E1b-19 kDa and E1b-55 kDa proteins as part of the early gene activation phase. E1b-55 kDa specifically interferes with p53-mediated apoptosis and inhibits cellular and viral replication by binding to p53. Expression of E1 proteins, therefore, creates a state in the cell that is required for viral replication. Because inactivation of Rb and p53 is accomplished by viral E1a and E1b proteins, respectively, mutants that fail to accomplish these functions because of either deletion of E1b-55 kDa^{1,4,63} or partial or complete deletion of E1a^{64–68} have been utilized to enable selective replication within tumor cells that lack functional p53 or Rb. Unfortunately, *E1a* and *E1b* genes also have roles that affect replication such that attenuation of these genes also reduces replication ability in comparison to wild-type adenovirus. E1b-55 kDa, for instance, facilitates transport of late viral mRNA from the nucleus to the ribosomes.⁶⁹ Impaired mRNA transport in the E1b-55 kDa-deleted adenovirus is probably the cause of reduced potency when compared to wild-type adenovirus.⁷⁰

Conditionally replicating adenoviruses have also been generated, in which essential early phase adenoviral genes are driven by tumor-specific promoters.^{71–73} To drive E1a expression, multiple promoters have been used for specific cancers, such as the prostate-specific antigen (PSA) promoter for prostate cancer and the α -fetoprotein (AFP) promoter for hepatocellular cancer.^{74,75} Several adenovirus-encoded gene products have been found to either block or activate cell death, and the coordinated and timely expression of these factors leads to optimal conditions for generating progeny viruses.⁷⁶ Adenoviruses lacking functional ADP have been shown to kill cells and release progeny virus *more slowly* than wild-type adenovirus.⁷⁷ Attenuated viruses with overexpressive ADP are also more efficient at eradicating tumor cells than identical viruses lacking ADP.⁷⁸

However, inappropriate timing of expression of ADP may be counterproductive depending on the therapeutic approaches being attempted. For instance, efficacy with the 5-FC/cytosine deaminase approach has greater significance at low multiplicities of infection (MOIs) compared to at higher MOIs.⁷⁹ As a consequence, viral constructs delivering prodrug-inducible cytotoxins have been shown to be more effective when placing the cytotoxic gene under late phase gene control. However, deletion of ADP results in longer survival of virus in infected cells, leading to increased duration of expression of the inserted gene.⁸⁰

Combined therapy with other anticancer agents may also affect efficacy of conditional replicating of adenoviruses. For instance, it has been demonstrated that adenoviral binding is optimal during M phase of the cell cycle. Paclitaxel stabilizes microtubules, which halts the cell cycle at M phase and increases adenoviral binding and transgene expression.^{81,82} Agents such as paclitaxel, vinblastine, and vincristine may potentially contribute to increased efficacy when combined with adenoviral therapies.^{83–85} Immune response against infected tumor cells also appears to limit virus propagation within the tumor through premature killing of the infected cells. This has been demonstrated with oncolytic herpes simplex virus (HSV)⁸⁶ and likely affects adenovirus persistence.

Other antiviral host defense mechanisms affecting the survival of viruses include proinflammatory cytokine tumor necrosis factor (TNF). Wild-type adenovirus can actively combat these host defense mechanisms. In the case of gene-attenuated adenoviruses containing partial E3 region deletions, lack of expression of immunoinhibitory genes essential for viral immune defense and survival may also affect efficacy. There are several critical viral proteins contained within the E3 region,^{87–92} which play a role in assuring survival of the adenovirus. GP19K inhibits MHC class I expression and adenoviral-specific cytotoxic T-lymphocyte killing. Protein 11.6 induces lysis of the infected cell to release progeny of the infected cell, enabling infection of an adjacent cell. Proteins 10.4 and 14.5 complex and interact with a variety of cell surface receptors, specifically Fas, which, if not present, would predispose the host cell to eradication. Finally, protein 14.7 inhibits TNF α and Fas-mediated cell death. Adenoviruses containing deletions in any one of these proteins may have diminished replicative capacity or diminished ability to survive because they are less efficient at protecting the host cell from endogenous immune-mediated clearance. Non-replicating viral vectors provide longer transgene expression in TNF knockout mice compared to wild-type mice.^{93,94} Furthermore, it has also been demonstrated that viral-manufactured soluble binding proteins specific to TNF and other viral toxic cytokines such as interferon varieties are produced by a variety of viruses including poxviruses, cytomegaloviruses, herpes viruses, and adenoviruses.^{95–101} Other immune factors influencing viral replication, survival and spread include induction of neutralizing antibodies to adenoviral components. Measures that modulate neutralizing antibodies such as plasma apheresis, B-cell function inhibitors, immune suppressive therapy, and/or desensitization are being studied.^{102–119}

In conclusion, given our detailed knowledge of adenoviral gene function — specifically factors that affect adenoviral replication, survival, and transgene expression — combined with demonstration of safety and evidence of efficacy in animal models, a variety of gene-attenuated conditional replicating adenoviral constructs have been manufactured and are being explored in clinical trial.

ONYX 015

ONYX 015 is a replication-conditional adenovirus in which a⁶³ wild-type adenovirus was genetically modified by deletion of the E1B–55 kDa DNA fragment and a partial deletion of the E3 region, which spares production of GP19K and ADP proteins. Deletion of the E1B–55 kDa fragment enables replication of ONYX 015 within malignant cells with abnormal p53 function^{63,120–121} and minimizes replication capacity within cells containing normal p53 function. The protein produced from the E1B–55 kDa fragment binds p53, thereby blocking its inhibitory affect on viral replication and host cell survival. Normal cells with functioning p53 protein more easily inhibit viral replication because the gene-modified adenovirus can no longer block p53-mediated inhibition. Controversy exists as to whether or not this is the sole mechanism by which ONYX 015 has selective replication capacity with malignant cells.^{122–125} Nevertheless, despite controversy on the mechanism by which ONYX appears to demonstrate selective activity with the malignant tissue, most have been able to identify selective replicative activity of ONYX 015 in malignant tissue^{126–135} and clinical results demonstrate selective replication capacity within malignant tissue without significant adverse toxicity suggestive of nonmalignant cell replication.^{136–144}

Initial phase I investigation of ONYX 015 involved intratumor injection in refractory cancer patients. Results revealed that ONYX 015 was well tolerated and evidence of activity was suggested.¹⁴² Results of these trials led to further exploration of ONYX 015 in patients with recurrent squamous case cancer of the head and neck region (SCCHN). Phase II testing involving 37 patients who received a dose of 2×10^{11} viral particles through intratumor injection for five consecutive days revealed high quantities of viral particles in 7 of 11 patients who underwent biopsy postinjection of ONYX 015. No viral particles or toxic effect was identified in normal tissue. More than 50% shrinkage of the injected lesion occurred in 21% of the tumors injected. Interestingly 7 of 12 patients with p53 mutant cancers achieved a response compared to none of seven responses in patients with cancer containing wild-type p53 genotype.¹³⁶ The overall partial or complete response rate was 14%, stable disease rate was 41%, and tumor progression rate was 45%.¹³⁶ Increasing the number of injections 4-fold demonstrated no increase in response rate but increased vector-related toxicity (fever, injection site pain) was observed.¹³⁶ Despite a relatively low response rate, safety was adequately demonstrated (533 viral injections were administered), justifying subsequent investigation in earlier stage recurrent SCCHN patients who have not previously received chemo-

therapy. In this next phase II study, patients received ONYX 015 (2×10^{11} particles for five consecutive days/21 days) in combination with cisplatin (80 mg/m² 1×/21 days) and 5-fluorouracil (5-FU; 800–1000 mg m² CI×5 days/21 days).¹³⁸ The results were encouraging in that no added toxicity to ONYX 015 was demonstrated above and beyond the expected toxicity of cisplatin and 5-FU. Furthermore, a 63% response rate was observed, which was greater than the expected response rate of 35% from previous publications in the same patient population.¹³⁸ These results have led to ongoing phase III investigation.

Another clinical investigation of ONYX 015 has involved advanced ovarian cancer patients. Tolerability of ONYX 015 was similar to what was observed in the SCCHN patients, although no evidence of efficacy was demonstrated.¹⁴⁰ Further work has also involved exploration in hepatocellular carcinoma patients. One out of five patients achieved a partial response.¹⁴¹ These patients received ONYX 015 (6×10^{12} particles) through intrahepatic arterial infusion. In another trial involving colorectal cancer patients with liver metastasis, there was a suggestion of survival advantage in patients receiving higher doses of ONYX 015 compared to lower dose.¹⁴² ONYX 015 has also been administered safely to more than 20 patients through intravenous administration. Results demonstrate a slightly higher frequency of febrile response than what is observed with intratumoral administration of ONYX 015, although similar to what was observed with intra-arterial infusion. Otherwise, at doses 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 and chemotherapy (paclitaxel, CPT11, 5-FU). The presence of ONYX 015 within metastatic malignant disease sites following intravenous infusion was demonstrated; however, evidence of significant tumor regression was not.^{139–143}

Future directions of clinical exploration with ONYX 015 are ongoing. Ultimately, completion and evaluation of the phase III ONYX 015 trial in SCCHN patients are needed to understand the role of this novel therapeutic in oncology.

Egypt 101 virus

Egypt 101 virus is a strain of West Nile virus, which is an adenovirus subtype. Preclinical testing in the early 1950s showed oncolytic activity in a uterine/cervix cancer cell line (HeLa).¹⁴⁵ In a trial involving 34 cancer patients, intravenous and intratumoral routes of injection were studied.^{145,146} Low-grade fever was observed as the primary toxic effect to the virus, and in the majority of patients, transit tumor necrosis was observed.^{145,146} However, a small number of patients with hematologic malignancies developed encephalitis.¹⁴⁷ Most responses lasted less than 3 months and no durable complete responses were achieved. It was interesting to note that distal tumor necrosis correlating with viral presence was also observed in patients receiving intravenous infusion of the virus. Nevertheless, clinical investigation with Egypt 101 virus was not pursued due to the potential of encephalitis in immune-compromised patients and limited durability of response.

Mumps virus

The oncolytic effects of mumps virus were initially shown in a rat sarcoma model.¹⁴⁸ One trial involving 90 cancer patients receiving a crude mumps virus explored a variety of routes of administration (oral, rectal, intratumor, inhalation, and intravenous).¹⁴⁹ Toxicity was manageable and was primarily limited to transient low-grade fever. Thirty-seven patients (41%) achieved a partial response and 79 patients maintained stable disease or better for a period of at least 2 months.^{149,150} Patients receiving multiple intratumoral injections over a prolonged period of time appeared to achieve a higher response rate and longer duration of response than patients receiving one or two injections.^{151,152} Fifteen patients received only intravenous infusion of mumps virus and six of these achieved a partial or complete response.¹⁵³ These encouraging results were followed up in a subsequent trial involving 200 refractory cancer patients. Toxicity was similar. However, transient tumor regression was only noted in 26 of 200 patients.¹⁵⁴ Due to the variability in the responses and the inability to manufacture a uniform product for further clinical testing of mumps virus, therapeutic development was not pursued.

Influenza virus

Preclinical data suggest that influenza virus has significant oncolytic activity,¹⁵⁵ but neurologic toxicity complicates the possibility of using this virus as a systemic therapeutic.¹⁵⁶ Thus, clinical investigation of influenza virus has focused on *ex vivo* use in which the virus is exposed to malignant cells in order to induce oncolysis. This material (oncolysate) is then used as a tumor vaccine. Influenza virus “oncolysates” have been shown to induce greater antitumor activity *in vivo* than virus alone or unmodified tumor cell extracts.^{157,158} In one trial, 40 patients with advanced ovarian cancer were treated with influenza virus oncolysate; one patient had resolution of a pleural effusion and tumor masses decreased in three patients. Response duration was variable, lasting between 3 and 19 months. Two responders developed recurrent pleural effusions and both responded to reinjection of the oncolysate. Treatment with IL-2 appeared to enhance the immunologic response initially achieved with the influenza oncolysate.¹⁵⁹ Future clinical investigation may be limited by capacity to manufacture a uniform product.

HSV

HSV is a double-stranded DNA virus. Two initial genetic modifications have been used to construct oncolytic forms of this virus, which enable selective activity within malignant tissue. The first method consists of inactivating the function of a viral gene (*ICP6*) that encodes the large subunit of ribonucleotide reductase — an enzyme required for efficient viral DNA replication.^{160–162} This enzyme is expressed abundantly in tumor cells but not in nondividing cells. As a consequence, the gene-modified HSV-1 has selective replication capacity in tumor cells. The second approach consists of deleting another viral gene (γ -34.5 gene), which functions

as a virulence factor during HSV infection.¹⁶³ Mutations in this gene also result in a block to replication in nondividing cells.^{165,180} The oncolytic HSV-1 virus, G207, which has been extensively tested in animal models and is currently in clinical trials, has been constructed using a combination of these two approaches.^{166–169} Replication-sensitive HSV-1 γ -34.5 viral mutants have been shown to be effective in treatment of both CNS^{170–172} and non-CNS^{173–180} tumors in animal models. Clinical trials involving patients with high-grade glioma and patients with melanoma^{181–184} demonstrate safety. All patients also developed an immune antibody response against HSV antigens. PCR analysis of tissue revealed the presence of HSV DNA at injection sites and at distal tumor sites.¹⁸⁵ Combination of HSV mutants with chemotherapy or radiotherapy generally reveals enhancement in antitumor activity.¹⁸⁶ Radiation enhanced anticancer activity of HSV when explored in pancreatic, glioblastoma, and cervical cancer models¹⁸⁷ but did not alter antitumor effect of oncolytic HSV in prostate cancer. However, high-dose radiation, combined with oncolytic HSV mutants, did improve efficacy in other prostate cancer models.^{188–190} Low-dose irradiation also improved efficacy of HSV mutant therapy in a cervical cancer model.¹⁹¹ In chemotherapy combination studies, it is interesting to note that both chemotherapy-resistant and sensitive tumors were equally responsive.¹⁹² A variety of chemotherapy agents (mitomycin C, cisplatin, methotrexate) have demonstrated enhancement in combined effect.¹⁹³ Use in immunocompetent animal models has also demonstrated systemic tumor regression with generation of cytotoxic T cells in response to *local regional* injection with an HSV virus mutant.^{194–197}

Attempts to enhance the immunologic affect studies, which utilize insertion of *IL-12* gene into HSV mutant G207, have been done.^{198–200} The antitumor effect was significantly greater in replication-competent IL-12-transfected virus when compared with G207 not carrying an *IL-12* gene. Induced cytotoxic T-lymphocyte activity was shown to be specific to the injected tumor. Furthermore, HSV mutant delivery of *IL-10* gene, an immunosuppressive cytokine, showed no improvement in efficacy.¹⁹⁹ Toxic effect of G207 was not altered by the gene-transfected virus.

Others have also identified use of HSV mutants to deliver genes, which convert prodrugs into cytotoxic agents. In particular, in one study, cytochrome *p450* transgene and HSV-1 thymidine kinase (*TK*) gene were delivered through an HSV (HSV-1) replication-competent virus through intratumoral injection in a hepatocellular carcinoma model.²⁰¹ Cancer regression was significantly improved when the cytochrome *p450* was activated to produce cyclophosphamide. However, when treated with ganciclovir, viral replicative activity was actually inhibited and tumor response was not demonstrated. As a consequence, antiviral effect of ganciclovir in viruses carrying the *TK* gene may be utilized as a “turn-off” safety value if viral-related toxicity develops.²⁰¹ Further clinical investigation with several HSV vectors is ongoing.

Promoter-inducible adenovirus

Adenoviral replication can be controlled by placing transcriptional response elements specific for cancer into the

early region of the viral genome. CV706 is a replication-competent adenovirus, which has been genetically modified such that the *E1A* gene is controlled by a PSA enhancer/promoter region. This modification limits replication capacity of CV706 to PSA-producing cells.²⁰² *In vitro*, viral replication and oncolysis were demonstrated in PSA-producing prostate cancer cell lines, whereas prostate cancer cell lines not productive of PSA were unaffected by CV706.²⁰² Early clinical results confirm specificity and suggest efficacy. In one study, 20 patients with locally recurrent prostate cancer that failed prior radiation therapy were treated with a single injection of CV706 (dose of 1×10^{11} – 1×10^{13} viral particles per injection) through transrectal ultrasound-guided transperineal technique.²⁰³ Viral cDNA was detected in posttreatment prostate gland biopsies and in circulation, providing evidence of intraprostate replication of CV706.²⁰⁴ Efficacy was also suggested in that all five patients who received the two highest doses of CV706 developed a greater than 50% reduction in PSA. No irreversible grade III or IV toxicity was observed. Commonly observed grades I and II toxicity involved fever, chills, injection site pain, and hematuria. Neutralizing antibody to viral antigens occurred in most patients but did not correlate with response. Preclinical models also suggest enhanced anticancer effect of CV706 when combined with radiation therapy without additional toxicity or loss of specificity.²⁰⁵ The timing of viral injection and radiation was critical. Synergistic activity was only observed if radiation was initiated 7 days after viral injection. In this situation, viral replication was increased. It is unclear exactly why CV706 replication was increased 7 days after radiation therapy; however, because of the small target size of the adenoviral genome (36 kb), it is likely that radiation-induced damage to the *viral* genome has less of an impact on function than with the human genome, which is much larger (10^6 kb) and more likely to suffer functional disruption from radiation-induced damage. Therefore, in a setting containing more active DNA repair elements (in the irradiated cells),²⁰⁶ an environment will be set up for rapid replication of preserved DNA (more likely the viral DNA).²⁰⁵

CV706 has also been administered systemically in preclinical models without significant toxicity, and efficacy has been demonstrated although — consistent with other viral approaches — neutralizing antibodies to viral antigens were induced, limiting efficacy of intravenous administered CV706. Enhanced efficacy, however, was suggested with the use of a newly developed affinity column of virus capsid proteins used to remove adenovirus antibody.

To improve efficacy of CV706, further genetic modification of the core genome involving replacement of the E3 region was constructed.²⁰⁷ This new virus is called CV787.²²⁷ Antitumor activity with CV787 appears to be several logs greater than activity with CV706 and was further enhanced when combined with chemotherapy.^{208,209} Additional replication-controlled viruses containing cancer-specific promoter/enhance regions have been constructed and showed enhanced selectivity compared to wild-type viruses.²¹⁰ The ability to control through cancer-specific transcription regulation should promote cancer-targeted specificity and facilitate safety of systemic delivery.

Replicon vaccine vectors

Replicon-based vectors are derived from positive strand RNA viruses.²¹¹ These vectors have a small genome size (7–9 kb) and exclusively replicate within the cytoplasm — thus can be delivered as either naked RNA, packaged into virus-like particles and delivered through infection, or as a DNA plasmid, which encodes replicon DNA placed under the control of a mammalian expression promoter.²¹¹

Most replicon-based vectors that have been studied *in vitro* have been derived from α -viruses.²¹² α -Virus replicon vectors appear to induce strong antibody and CD8⁺ T-cell responses to encoded immunogenes and have been shown to protect immunized animals from tumor challenge.²¹³ One self-replicating RNA (replicon) vaccine vector derived from an Australian flavivirus Kunjin was recently shown to induce a protective CD8⁺ T-cell response after a single immunization in a murine B16 melanoma model.²¹³ The degree of the CD8⁺ T-cell responses appears to be comparable to other viruses.^{214–216}

Kunjin replicons do not cause overt apoptosis or cytopathic effect of the host cell while replicating within the cytoplasm. This may have advantage over use of live viruses when delivering certain gene therapy approaches, which require prolonged duration to generate sufficient transgene products to impact cancer growth. Interestingly, despite virtually no cytopathology *in vitro*,²¹³ KUN replicons appear to efficiently induce CD8⁺ T-cell responses *in vivo*. Generally, effective CD8⁺ T-cell induction requires delivery of exogenous viral antigens to dendritic cells.²¹⁷ However, flaviviruses can directly infect, replicate in, and activate dendritic cells,^{218,222,224} suggesting that KUN replicons may also be able to replicate and produce vaccine antigens with dendritic cells. Direct infection of dendritic cells by vaccine vectors may represent a more efficient strategy for CD8⁺ T-cell induction than cross-priming.²²³ Other α -viruses have also infected dendritic cells and results demonstrate the ability to express an encoded vaccine antigen.^{224,225} KUN replicons are a novel alternative to use of oncolytic viruses as replication-selective gene delivery vehicles, particularly with immune-modulating gene antigens. However, clinical investigation awaits further preclinical safety evaluation.

Self-deleting suicide vectors

Mutations in cancer cells invariably disrupt p53's ability to bind DNA. A novel replicating vector using a retrovirus genome was recently synthesized in which "Cre" expression was placed to be dependent on transactivation by p53 so that self-deletion occurs in cells expressing functional p53.²²⁶ Cells lacking functional p53 would retain the virus and could be lysed following activation of a suicide gene. One such nonreplicating vector was recently shown to be effective *in vitro* using a p53 dysfunctional U87 cell line and a self-deleting retrovirus vector delivering HSV TK suicide gene. Cytotoxic effect was demonstrated *in vitro* and *in vivo* following administration of ganciclovir. Selectivity was increased even more following induction of p53 expression.²²⁶

Further genome modification was being planned to enable development of a replication-competent vector. The possibility of clinical tests is still in the distant future.

ONYX 411

As described previously, the adenovirus early region has two genes, *E1a* and *E1b*, which encode a number of proteins and facilitate viral DNA replication within the host cell.^{4,227} One of the functions of the *E1a* protein product is to mediate entry of the host cell into S phase through binding of protein Rb.^{228,232} In so doing, *E1a* protein liberates the E2F transcription factor from the protein Rb–E2F complex. E2F subsequently activates the expression of multiple cellular genes required for progression into the cell cycle.^{233,234} As a consequence of E2F activation, apoptosis is induced. However, the *E1b* gene product suppresses induction of both p53-dependent and -independent induced apoptosis. ONYX 411 is a gene-modified adenovirus containing a deletion in the *E1A*–CR2 region, which has been designed to be restricted to cells having defects in the Rb pathway.^{235,236} Selectivity of the 411 virus has been further enhanced by replacing the *E1A* and *E4* promoter regions with a human E2F1 promoter.²³⁶ Rb pathway defects occur in nearly all human cancers.²³⁷

Preclinical testing reveals that the 411 virus does selectively replicate in malignant cells, not in nonmalignant cells with normal E2F/Rb expression. Furthermore, cytopathic effect appears to be significantly greater than ONYX 015 *in vitro*, in some tumor classes by more than 1000-fold.^{234,235} Intravenous infusion of ONYX 411 was shown to have an improved safety profile compared to ONYX 015 at doses up to 4×10^9 plaque-forming units, and to have significantly greater cytopathic effect in immunocompetent mice.²³⁶ ONYX 411 may be uniquely engineered to have efficient replication capacity in a broad array of malignant cells. Pursuit of this vector as a gene delivery vehicle following systemic administration also appears promising.

Vaccinia

Vaccinia is a double-stranded DNA virus and is a member of the poxvirus family. The virus is tropic for human cells and its high immunogenicity properties were exploited in making smallpox vaccine and eradicating smallpox.

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

The following are examples of preclinical and phase I trials that explore the use of vaccinia virus as a vector for gene therapy. In one study,²³⁸ recombinant vaccinia virus was constructed in an effort to enhance the immunogenicity of transfected melanoma cells. The virus expressed a mini-gene encoding a fusion product between 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*.²³⁹ In another study,²³⁹ a recombinant vaccinia virus vector was created containing the tumor-suppressor *p53* gene and demonstrated a high level of expression of p53 protein in transfected glioma cells, which resulted in a high level of apoptosis.²⁴⁰ In a phase I study of intravesical vaccinia virus infection,²⁴⁰ it was 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 effects.²⁴¹ The intense immune response was characterized by the following: (a) mucosal edema and vascular ectasia; (b) recruitment of dendritic cells to the injection site; and (c) mucosal and submucosal infiltration by lymphocytes, eosinophils, and plasma cells into tumor and normal tissue.²⁴¹ 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 have looked at the use of vaccinia virus as an immunotherapeutic agent. Poxvirus vaccines have been studied in clinical trials as a treatment for melanoma. Vaccinia melanoma oncolysate has been used as an active specific immunotherapeutic agent; this strategy exploits vaccinia virus' high level of immunogenicity and, when used as an adjuvant therapy in stage 3 melanoma patients, vaccinia virus conferred a survival advantage in certain subsets of patients but there was no difference in survival when all patients were considered together.²⁴²

Because wild-type vaccinia virus does not selectively infect cancer cells, the virus has to be made replication-conditional in order to create a specific oncolytic agent. One strategy, which has been used to create a replication-conditional virus, is to delete the viral *TK* gene. While the viral *TK* gene is necessary for infectivity in normal cells (possessing small concentrations of intracellular nucleotide pools), it is not necessary in cancer cells²⁴² (possessing relatively high concentrations of intracellular nucleotides). Thus, another novel vector was created, which replaced the viral *TK* gene with the gene for GM-CSF and created a mutant vaccinia virus capable of selectively infecting melanoma cells and inducing an antitumor immune response.²⁴² 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. Out of seven patients studied, two patients had a complete response and three patients had a partial response.²⁴² Other researchers created a different mutant vaccinia virus (deleting the viral *SPI-1* and *SPI-2* genes), which resulted in conditional viral replication in cancer cells but not in normal cells.²⁴³ The efficacy of these latter viral mutants has not yet been tested.

In summary, whether using mutant vaccinia viruses having tumor-selective replication features to promote oncolysis, or using vaccinia virus tumor lysates to develop antitumor vaccines, vaccinia virus appears to offer multiple potentially effective ways to treat cancer.

Reovirus

Reoviruses are double-stranded RNA viruses that cause mild respiratory and gastrointestinal illnesses in humans. Normal mouse fibroblast cells (NIH3T3) do not support reovirus replication, but 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 α -phosphorylation, potentiating unabated cellular protein production and viral replication. Because in normal cells (lacking ras activation) early viral replication induces EIF-2 α -phosphorylation (inhibiting cell protein synthesis), reoviruses exhibit preferential oncolytic effects in (ras-activated) cancer cells.

Reovirus has demonstrated preclinical activity in mouse flank tumor models using 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.²⁴⁴ Reovirus has also demonstrated activity against Lewis lung cancer metastasis in mice following intravenous administration.²⁴⁵ In this study, 65–80% of the mice tested showed regression in their tumors.^{245,246} 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.²⁴⁶ 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 immune-compromised mice implanted with human colon and ovarian cancers. Intratumoral injection of virus consistently resulted in major reductions of the ovarian and colon cancer tumor volumes. Of particular significance, the intravenous administration of virus in the immune-compromised 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 potential promising therapy for colon cancer, ovarian cancer, breast cancer, lung cancer, and other malignancies having activation of the ras pathway. The intravenous administration of reovirus along with immunosuppressive therapy holds promise and should be tested clinically.²⁴⁵

Newcastle

Newcastle virus is paramyxovirus with normal infectivity limited to fowl. The virus is an enveloped, negative-stranded

RNA virus, which may cause conjunctivitis but not systemic disease in humans working with poultry. Newcastle virus is selectively cytolytic for human cancer cells due to a defect in the interferon signaling pathway.²⁴⁷ As a result, tumor cells lack antiviral defenses and viral replication, and oncolysis can proceed to completion. Many early studies focused on creating vaccines by infecting tumors with Newcastle virus and using the oncolysates to create an immune response.²⁴⁸ In one phase II study initiated in 1975, an oncolysate consisting of allogeneic and autologous human melanoma cells infected with live Newcastle virus was administered as a vaccine postoperatively to patients with stage 3 melanoma. Treated patients showed evidence of increased number of CD8⁺ T cells and a 15-year follow-up of treated patients showed a 55% overall survival. This was taken as evidence that Newcastle virus oncolysates were acting as an effective vaccine for melanoma.^{249,250} In addition, the virus has been given intravenously, intraperitoneally, and intratumorally in human cancers implanted in an athymic mice, resulting in few systemic side effects; and high rates of selective oncolysis, often resulting in few systemic side effects; and high rates of selective oncolysis, often resulting in clinical complete remissions.^{251–254}

Castle and Garrett injected Newcastle virus into a cancer of the cervix in a patient and demonstrated intratumoral regression of the cancer at the site of the injection and also regression of a distant malignant lymph node.²⁵⁵

Most recently, Pecora et al reported a phase I trial of the intravenous administration of PV701 (an attenuated Newcastle virus strain) into patients with solid cancers. Side effects were generally mild and included flu-like symptoms and mild hypo tension.²⁵⁶ Of 62 patients eligible for response assessment, 14 had stable disease (430+ months) and 2 had radiographic evidence of major responses. A complete response was documented in one patient with squamous cell cancer of the tonsil and a partial response was seen in a patient with colon cancer. A patient with stable mesothelioma involving the retroperitoneal lymph nodes had election microscopy performed on his tumor specimens after many months of intravenous viral therapy. Inflammatory cells largely replaced malignant tissue and edema and viral particles were seen at tumor cell membranes. This study is important because it further confirms results with ONYX 015 that intravenous administration of manufactured oncolytic viruses is safe.

Conclusion

Several varieties of conditional replicating viruses and viral constructs have entered clinical investigations. No significant concerns regarding safety have evolved, but demonstration of efficacy has been limited to local regional therapeutic approaches. Theories to improve efficacy involving enhancement in tumor targeting, replication efficiency, and gene delivery are opening doors to systemic clinical testing based on promising results in preclinical studies. Use of conditional replicating viruses holds significant promise as a future cancer therapeutic modality.

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