Cancer Virotherapy Guided by Imaging Analysis

The use of replicative viruses for cancer therapy, or virotherapy, was pursued to address the problem of limited tumor transduction seen in earlier experiments in cancer gene therapy. Virotherapy exploits the lytic property of virus replication to kill tumor cells. Because this approach relies on viral replication, the virus can self-amplify and spread in the tumor from an initial infection of only a few cells.

Although attempted in the past and abandoned because of toxicity and inefficacy, this "virotherapy" approach has reemerged with great promise, due in a large part to a better understanding of virus biology and the ability to genetically modify viruses. Researchers can now design viruses to replicate in and kill tumor cells specifically.

An expanded knowledge of the viral life cycle has allowed researchers to identify RNA viruses, such as Newcastle disease virus, vesicular stomatitis virus, and reovirus, that preferentially replicate in tumor cells bearing defects in the double-stranded RNA-dependent protein kinase (PKR)/ interferon response pathways or with an activated Ras pathway.

In contrast, adenovirus does not have a natural predilection to replicate in tumor cells, but it can be rendered to do so by several ways. One scheme involves deleting the essential adenoviral *E1B-55kD* gene, whose protein normally binds to and inactivates p53. Such a modification was hypothesized to make ONYX-015 virus replicate only in p53-defective cells (the case in 50% of human tumors); however, this principle has been questioned. Furthermore, the replication of this virus was severely hampered compared to wildtype virus, probably due to the late virus mRNA transcription function of the missing E1B-55kD protein.

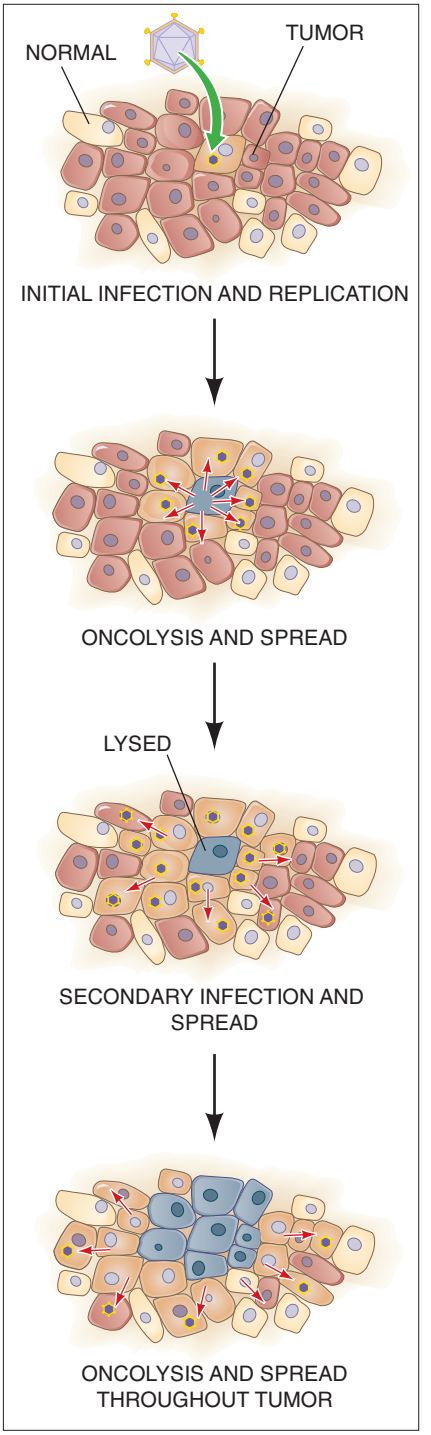
Despite these questions, the potential of such conditionally replicating adenoviruses (CRAds) to be the next cancer therapy breakthrough has been substantiated by their rapid translation into human clinical trials. ONYX-015 targeted to p53-deficient tumors has been tested in a variety of tumors, including recurrent head and neck, pancreatic, colorectal, ovarian, and hepatobiliary cancers. Variable clinical responses were observed in these trials, although the combination of this agent with chemotherapy showed clear benefits for recurrent head and neck cancer.

Several other oncolytic adenoviruses have been tested in phase I clinical trials for recurrent prostate cancer. One of these is Ad5-CD/TKrep, which is similar to ONYX-015 but contains a cytosine deaminase/herpes simplex virus–thymidine kinase (HSV-tk) fusion gene for suicide therapy. The replication of this virus, combined with expression of the therapeutic gene and external beam radiation therapy, reduced PSA levels by ≥25% in 7 of 16 patients, with 2 patients experiencing clearance of adenocarcinoma at 1-year followup.

CV706, designed to replicate in PSA-positive tumor cells, is another replicative adenovirus that has shown some positive results in patients with recurrent prostate cancer.

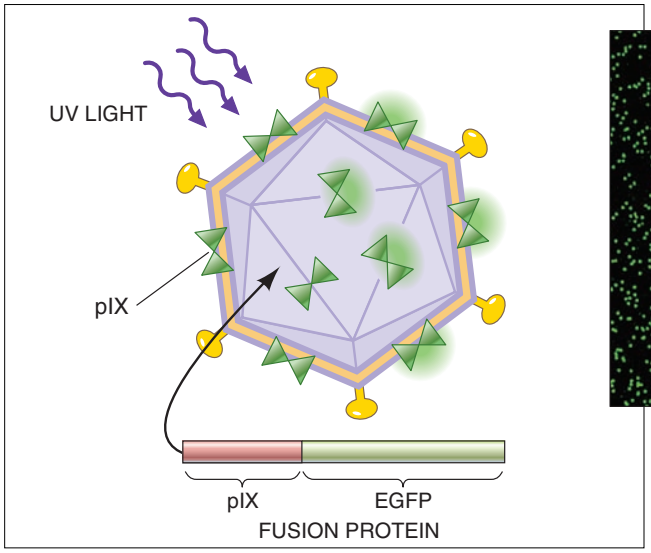
"Gene Therapy" is edited by Joanne T. Douglas and David T. Curiel of the Gene Therapy Center, University of Alabama at Birmingham.

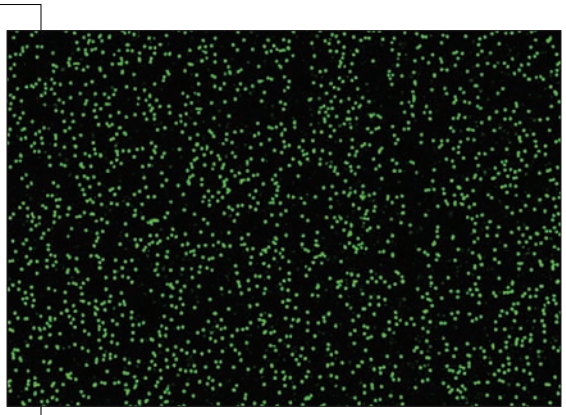
joanne.douglas@ccc.uab.edu



GREG GAMBINO

Conditionally replicating adenovirus (CRAds) infect and replicate in specific tumor cells. As the original tumor cell is lysed, the viral progeny spread to neighboring tumor cells, sparing normal cells.





Visualization of Ad-IX-EGFP. Incorporation of enhanced green fluorescent protein (EGFP) into the adenovirus capsid allows direct visualization by fluorescent microarray.

These initial CRAd clinical trials clearly have validated the safety of using oncolytic adenoviruses for cancer therapy. Yet what these trials failed to ascertain, and what remains to be rigorously determined, are the crucial functions of CRAds: (1) selective replication and killing, (2) robustness of replication, and (3) spread of the virus. A better understanding of CRAd biology will contribute greatly to the advancement and realization of these agents for cancer treatment. Given the lack of true tumor models for evaluating replicative adenoviruses and the value of clinical trials, the acquisition of this information from human patients becomes even more imperative in the development of CRAds.

Limited understanding of CRAd behavior in patients is due to the lack of a noninvasive imaging system for monitoring replicative agents. Several studies have addressed this problem, including a group who used positron emission tomography (PET) scanning to detect thymidine kinase as a reporter of oncolytic herpes simplex virus replication. Detection was restricted to infected cells expressing the reporter gene, which does not truly represent the physical distribution of the virus itself. Another group employed soluble human carcinoembryonic antigen (hCEA) and β-human chorionic gonadotropin (βhCG) peptide markers as a way to monitor oncolytic measles virus therapy in mice, which correlated with therapeutic outcome but could not show viral localization.

Other conventional imaging systems for gene therapy have been designed to detect transgene expression of such reporters as green fluorescent protein (GFP), somatostatin receptor type 2 (SSTR-2), sodium iodide symporter, luciferase, and thymidine kinase.

Despite their utility for assessing gene delivery and expression, these reporters by themselves are not suitable for monitoring CRAd activity. The essence of oncolytic virus function is to infect and kill target cells, a concept that is at odds with reporter gene expression. As well, reporter gene expression may not truly represent the underlying level of viral replication and the physical distribution of viral progeny.

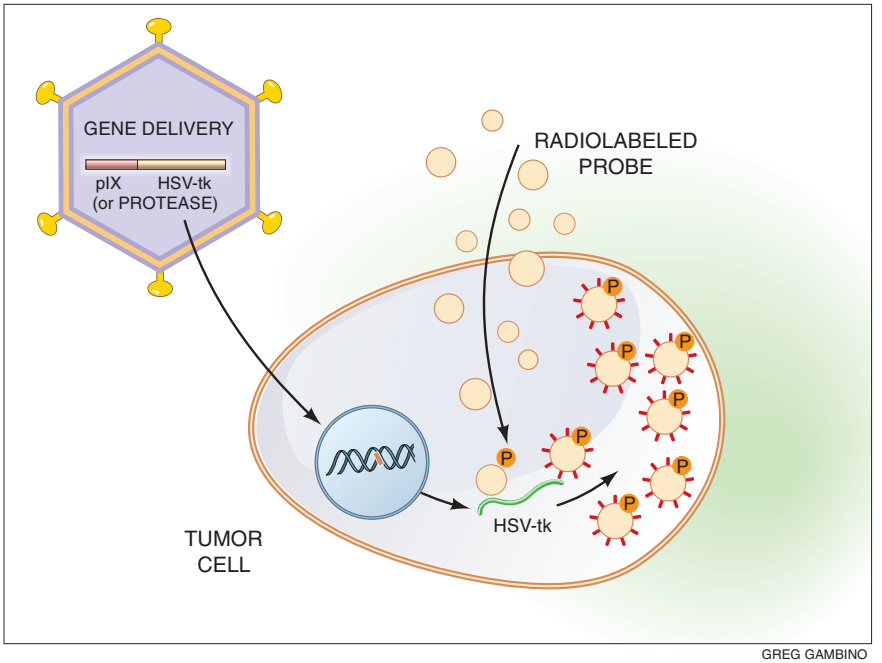
No completed clinical trials so far have incorporated a monitoring component into replicative agents, and therefore researchers have had to rely on conventional histology of biopsy specimens and analysis of body fluids for the detection of virus. Such static assessments fall short of accurately depicting the dynamic mechanism of replicative agents.

The ideal system for monitoring CRAds and other oncolytic viruses should meet a number of criteria.

1. The detected signal should correlate with the level of viral replication or progeny production.
2. The signal should be directly associated or packaged with the virions to allow direct physical detection of viral dispersion.
3. The monitoring system needs to be self-perpetual so that produced virions as well as their progeny would be detected, allowing imaging of the "viral mass" that accrues at multiple time-points and over multiple generations from the initial dose.
4. The approach should minimally disrupt the replication and spread efficiency of the virus.
5. Lastly, the detectability should be sufficient for noninvasive imaging of the replication events.

Such a system would provide the flexibility for dynamic detection of both viral replication and spread to yield valuable and meaningful data.

In the past, the only method to visualize viral particles was with electron microscopy. Technology has made possible the labeling of viruses with synthetic fluorophores for trafficking studies. Although practical for "one-pass" experiments, this technique is not applicable for detecting viral replication in a "multi-pass" manner.



GREG GAMBINO

Incorporation of enzymatically active agents into the viral capsid (HSV-tk or a protease) allows the virion to convert the PET tracer agent into an imageable signal.

More recently, viruses also have been labeled genetically through the fusion of green fluorescent protein (GFP) with structural proteins. Specifically for adenovirus, fluorescent labeling has also been accomplished through the binding of a fusion tetR-EGFP protein to tetO sequences incorporated into the viral genome, which subsequently gets packaged with the virions. These methods have generated fluorescent viral particles useful in tracking infection processes. The adaptation of this genetic labeling concept for CRAds would provide a means to detect progeny virions in terms of both replication and distribution.

On the basis of these considerations, we developed a monitoring system commensurate with the mandates of CRAd biology. Our strategy involved the incorporation of an "imaging motif" with the adenoviral capsid. This strategy thus theoretically provides the desired ink of imaging read-out to new viral mass, the latter representative of the CRAd ability to replicate in a therapeutic manner.

Our previous work had demonstrated that the pIX minor capsid protein was located on the exposed surface of the virus. We thus incorporated the fluorescent reporter protein enhanced green fluorescent protein (EGFP) on pIX. Of note, the rescued viruses exhibited a clear green fluorescence, indicating that the EGFP was incorporated into the viral capsid.

Our studies thus have defined a method to study CRAd replication via direct fluorescent monitoring. Of note, for a variety of cancers, currently employed endoscopic procedures are fully consistent with our system of direct monitoring of CRAd amplification and spread during therapeutic application.

For tumors not accessible to such endoscopic procedures, an alternative CRAd imaging modality would be required. In this regard, PET scanning has been coupled to reporter expression of the *HSV-tk* gene for imaging analysis of vector-delivered transgenes. We are thus pursuing a strategy of incorporating *HSV-tk* into the pIX capsid site to allow monitoring of CRAd replication via PET scanning. Because PET scanning is currently in clinical use, the means to monitor CRAd therapies would thus be available at many treatment centers.

In the aggregate, these novel imaging modalities have provided the means to monitor the utility of CRAd agents. Such monitoring is critical as scientists seek to advance the design of these agents.

DAVID T. CURIEL

Gene Therapy Center
University of Alabama at Birmingham

david.curiel@ccc.uab.edu

Pub date: 6 Feb 2005

BIBLIOGRAPHY

Fueyo J, Alemany R, Gomez-Manzano C, et al: Preclinical characterization of the antiglioma activity of a tropism-enhanced adenovirus targeted to the retinoblastoma pathway. *JNCI* 95:652-660, 7 May 2003.

Kruyt FA, Curiel DT: Toward a new generation of conditionally replicating adenoviruses: pairing tumor selectivity with maximal oncolysis. *Hum Gene Ther* 13:485-495, 1 Mar 2002.

Le LP, Everts M, Dmitriev IP, et al: Fluorescently labeled adenovirus with pIX-EGFP for vector detection. *Mol Imaging* 3:105-116, Apr 2004.

Nettelbeck DM, Curiel DT: Tumor-busting viruses. *Sci Am* 289(4):68-75, Oct 2003.