Cancer treatment involving the *p53* gene

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Over the past decade, investigators have increased their understanding of the genetic alteration that transforms a normal cell into one that is cancerous. One gene found to play a critical role in preventing the cancerous transformation of a cell is a tumor suppressor gene known as *p53*. If this gene is deactivated, a cell will become cancerous. Therefore, methods for reintroducing this gene back into cancer cells are being developed so that the cancer cell literally can be “switched off.”

In this paper, Dr. Nemunaitis and colleagues present some findings from their latest research collaboration, which focuses on developing improved delivery systems for treating cancer by transferring *p53* genes into malignant cells. Intact *p53* function is essential for maintaining the nontumorigenic phenotype of cells. Inactivation of the *p53* protein—one of the most common alterations observed in human cancer cells—may be enhanced by some viral infections, such as the human papillomavirus, thus allowing malignant transformations to take place. The introduction of an exogenous *p53* gene into cancer cells may suppress cancer activity, prevent cell growth and proliferation, and induce cancer cell death.

This article presents the different approaches this team has developed to introduce the *p53* gene into different cancer cells and reviews the various clinical targets in which *p53* gene therapy is having the most promising results. The researchers, applying the knowledge that a viral infection can enter the human cell and deactivate *p53*, are using an innocuous adenovirus as a vector to enter cancerous cells and reintroduce the *p53* gene. In preliminary trials, reduced tumor size and improved survival have been demonstrated in lung, head and neck, ovarian, breast, and prostate cancers.

The application of laboratory research technology to human cancer therapy is now in progress. Human gene therapy has become a reality with the development of these effective techniques for delivering the gene to the target cancer cell. Although this research is still in its infancy, the encouraging results provide the promise of new treatments in the future for cancers that have been considered untreatable.

—Michael A. E. Ramsay, MD
The *p53* gene plays a critical role in the regulation of cell growth. Mutations of this gene are associated with transformation to a malignant phenotype. Correcting the gene defect through transfer of a wild-type *p53* gene into malignant cells and targeting malignant cells with oncolytic viruses (ONYX-015) genetically engineered to proliferate in cells containing mutant *p53* genes have been identified as therapeutic approaches in previous animal studies. Initial clinical trials have confirmed functional activity and expression of the transgene product in tumors injected with a replication-deficient adenoviral vector containing the wild-type *p53* gene sequence, and tumor-specific viral proliferation has been observed in patients receiving intratumoral injection of ONYX-015.

A normal cell evolves into a malignant cell as a result of several mutations involving genes critical for cellular growth. Specifically, oncogenes associated with cell proliferation (*ras*, *raf*, *PKC*-α, etc.), suppressor genes which control DNA repair and apoptosis (*p53*, *bcl-2*, etc.), and genes controlling immunologic recognition of abnormal cells (tumor antigen complexes) must mutate or lose function for malignant transformation to occur. Correcting any one of these defects through genetic manipulation has been shown to arrest malignant transformation, induce cell death of malignant cells, or both (1).

The most common genetic abnormality identified in malignant tissue involves the *p53* suppressor gene. At initial diagnosis, 60% of all cancers contain cells expressing *p53* mutant genes. Recurrent malignancy following chemotherapy or radiation therapy has been associated with an even higher incidence of *p53* mutations. Several cancers also produce factors that inhibit normal *p53* function by binding to the *p53* protein, enhancing degradation of the protein or disruption of protein-binding sites. For example, the expression of murine double-minute–2 protein acts as a false binding site in multiple myeloma (2), and human papillomavirus, which infects most patients with cervical cancer, produces factors that enhance the degradation of the *p53* protein, thereby predisposing such cells to malignant transformation (3).

The *p53* gene regulates growth, and these functions are disrupted in malignant cells. The treatment objective of this study was to supply the wild-type *p53* gene to cancer patients through direct injection of a viral vector, which should induce arrest at the G1 to S phase of growth, thereby enabling repair of DNA abnormalities. If this was unsuccessful, apoptosis (a form of control death) would be induced in the malignant cells (4).

**Adp53 VECTOR DESIGN**

A replication-deficient adenovirus containing a wild-type *p53* gene sequence was constructed from a serotype 5 adenovirus. The outer coat of the vector maximizes the ability of the viral genome, which contains the wild-type *p53* gene, to bind and pass through the malignant cell wall. The specific vector utilized for clinical trials contains a cytomegalovirus promoter to drive the production of the wild-type *p53* gene product and deletions within the E1 and E3 components of the viral genome. These later modifications inhibit the replication capacity of the virus and reduce, to some degree, viral antigen expression, thereby attempting to limit immunologic rejection of the virus (5).
SAFETY PROFILE OF ADENOVIRAL VECTORS

Eighty percent of adults have existing antibodies to adenovirus serotype 5, but less than 15% of exposed patients become clinically symptomatic (6). The most common symptoms of an adenovirus serotype 5 infection are flulike in nature, similar to the common cold. Serious infections in immune-compromised patients related to adenovirus serotype 5 are rare. Replication-competent adenoviral vaccines administered to hundreds of military recruits in the 1960s were associated with no evidence of adverse clinical sequelae. Live adenovirus inoculum was also given intratumorally and intra-arterially to patients with cervical cancer at the National Cancer Institute in the 1950s (7). No significant toxicities other than transient fever and malaise were observed, including subsets of patients who received steroids prior to introduction of the virus. Toxicity (involving pulmonary and hepatic dysfunction) related to high doses of wild-type adenovirus serotype 5 has been observed in animal models. However, antitumor activity was observed at lower doses, similar to those administered to patients.

When various normal cell types were incubated with the Adp53 vector, no evidence of malignant transformation was observed. In addition, when malignant tissue was explored genetically, no evidence of malignant transformation was associated with adenoviral components. Thus, oncogenicity of the vector or adenovirus serotype 2 or 5 is unlikely.

Additionally, exhaustive trials have been performed in animals using a variety of doses and schedules of administration of the Adp53 vector as a single agent and in combination with radiation therapy and chemotherapy. Antitumor activity and evidence of transgene expression with functional transgene product using adenoviral vectors and other viral vectors have been confirmed without evidence of significant toxicity (5, 8–11).

Studies done in humans with the β-GAL adenoviral vector injection revealed no evidence of replication-competent adenovirus or contamination to patient caretaker staff (12). Staff members provided blood, urine, and stool samples for testing, and no replication-competent virus or elevated antibody formation was detected.

Adenoviral vectors with E1 and E3 deletions containing the cytosine deaminase gene have also been administered to normal individuals to study immune response (Harvey BG, et al. American Society of Gene Therapy, abstract 167, 1998). Six volunteers received an intradermal injection of $10^6$, $10^7$, or $10^8$ plaque-forming units (PFU) (2 patients per group). Five of the 6 volunteers showed a rapid increase in anti-Ad5 neutralizing antibody titers above baseline. The peak antibody response occurred 2 weeks after vector injection. Erythema occurred at the site of injection, with maximum induration of approximately 7 mm by day 3 and complete disappearance of induration by day 10. Skin biopsies of the erythema revealed T-cell, B-cell, and macrophage infiltrate. Vector DNA was detected in biopsies of patients who received the $10^8$ dose on day 3, but no evidence of vector DNA was detected on day 28. No systemic toxicity was observed in any of the normal volunteers.

Safety with the use of adenoviral vectors, then, appears to be well tested. However, there has been cause for concern. Viral replication was observed in patients with cystic fibrosis receiving E1-deleted (E3-competent) vector (13), which is why further vector modification involving the E3
component has been pursued, and trials in oncology patients require intense monitoring in the appropriate clinical setting.

PRECLINICAL STUDIES WITH Adp53

Preclinical studies with Adp53 have explored the activity of this vector via direct intratumor injection and systemic infusion in xenograft models in human lung, head and neck, ovarian, breast, and prostate cancer. Following Adp53 injection, tumor volume was significantly reduced in a dose-related manner, and survival was improved. Furthermore, results were significantly enhanced when the use of the Adp53 vector was combined with radiation therapy, chemotherapy, or both (9, 11, 14–16).

PRECLINICAL STUDIES WITH ONYX-015

ONYX-015 is a replicating serotype 5 adenovirus on the outer coat, which contains a serotype 2 adenoviral genome (16). It has been attenuated by removing the E1B gene. The E1B gene product binds to the wild-type p53 gene product, thereby inhibiting its function. Removal of the E1B gene within the adenovirus limits the capacity of that virus to proliferate within cells containing normal p53 function. Thus, at a low multiplicity of infection (MOI) which would be clinically relevant, the ONYX-015 virus can proliferate in p53 mutant cells but proliferates poorly in normal cells. Proliferation of a virus within malignant cells ultimately leading to cell lysis is termed oncolysis.

In animal-human xenograft studies, intratumor injection of ONYX-015 virus has been tested in cervical cancer (C33 cervical carcinoma cells) and head and neck cancer (HLaC laryngeal carcinoma cells), both of which have a p53 functional deficiency (17). Significant tumor growth inhibition was observed compared with controls following viral injections. Mice achieving a complete response remained disease free for 4 to 6 months before sacrifice. U87 glioblastoma tumors, which do not have a p53 mutation, were not affected by injection with the ONYX-015 virus. Evidence of viral proliferation based on histochemical staining for adenovirus exon protein was confirmed in the sensitive tumors but not in the U87 tumors. Additional studies evaluating ONYX-015 plus chemotherapy (fluorouracil or cisplatin) revealed further improvement in median survival (17).

Systemic infusion of ONYX-015 at a dose of $10^8$ PFU was also administered for 10 days into the tail vein of nude mice implanted with C33-a or HCT116 human xenograft tumors. Tumor growth at distal sites following infusion was significantly reduced, and survival was improved with ONYX-015 treatment compared with mice infused with vehicle solution. No significant toxicity was observed. Results suggest that both intratumor and intravenous infusions of ONYX-015 are safe and effective in inducing tumor regression and prolonging survival. Efficacy was correlated with viral proliferation and was improved when combined with chemotherapy and radiation therapy.

CLINICAL STUDIES WITH Adp53

Several studies with Adp53 have been performed. Phase I trials investigating tolerability of Adp53 in non–small cell lung cancer have been recently completed (18, 19). Fifty-two patients with advanced non–small cell lung cancer who had not responded to conventional treatment were
entered into these trials. Adp53 doses were escalated from $10^6$ to $10^{11}$ PFU and injected monthly into a single primary or metastatic tumor by bronchoscopy (12 patients) or computed tomographic guidance (40 patients). Patients were treated by direct assignment with or without cisplatin (80 mg/m²) given intravenously over 2 hours prior to Adp53 injection. Each patient received up to 6 courses of treatment, and median follow-up was 9.9 months. Vector-specific deoxyribonucleic acid (DNA) was detected by polymerase chain reaction (PCR), and $p53$ transgene expression was determined by reverse transcriptase PCR and immunohistochemistry.

In patients receiving the combination of cisplatin and Adp53 (n = 24), vector was present in plasma within 30 minutes of injection and decreased in the next 60 minutes. No replication-competent adenovirus was detected in any body fluids tested. Antibody titers increased in patients receiving at least 2 doses and remained elevated for several months after completion of injections. Patients receiving cisplatin also had an impressive increase in their tumor apoptotic index from 0.010 to 0.044 ($P = 0.011$) compared with baseline in samples harvested after the first course of Adp53 injection. The terminal deoxynucleotidyl-transferase-dUTP nick-end labeling (TUNEL) assay showed an increase in the number of apoptotic cells in 11 of the 14 evaluable patients, a decrease in 1 patient, and no change in 2 patients. Anti-adenoviral type 5 IgG antibody response (2-fold increase) above baseline was shown in 19 of 21 evaluable patients following course 1, and in 15 of 15 patients following course 2. Cytotoxic effect assays also revealed the presence of Adp53 vector in plasma within 30 minutes of intratumor injection in all 16 patients tested and decreased to nondetectable levels within 60 minutes. Tumor biopsies were collected and evaluated in 14 patients 3 days posttreatment, and results demonstrated $p53$ transgene expression by reverse transcriptase PCR in 6 of 14 (43%). All of these patients had received $3 \times 10^9$ PFU. Toxicity attributed specifically to the vector was limited to transient fever and injection site pain. Cisplatin-related toxicity was not observed in any greater frequency than would be expected when Adp53 gene vector was not combined with cisplatin.

Two patients fulfilled a definition of partial response, 17 patients experienced stable disease for a transient period (minimum 1 month), 4 patients had progressive disease, and 1 patient was not evaluable for response. Overall, median survival was 164 days. Similar results were seen in patients who received Adp53 without cisplatin (n = 28). Transgene expression was observed in 9 of 16 patients (56%) at doses >$10^9$ PFU, but only 3 of 10 patients treated with $?10^9$ PFU showed evidence of transgene expression. These results also correlated with an increase in the apoptotic index. Two patients achieved a partial response, 16 patients had stable disease, 7 had progressive disease, and 3 were not evaluable. Median survival was 141 days. Interestingly, a higher proportion of patients who received endobronchial-directed injection achieved clinical benefit compared with those who received computer tomographic-guided injections.

These trials showed that Adp53 injections at a dose of $10^{11}$ PFU in patients with non–small cell lung cancer are safe and well tolerated. The maximum tolerable dose of the vector has not been reached. This therapy can be administered monthly, alone or with cisplatin, with no increase in cisplatin-related toxicity. The immune response to the Adp53 vector does not limit continued injections, and there is evidence of objective activity and clinical benefit.

The same Adp53 vector was tested in patients with head and neck cancer (20). Patients with recurrent or refractory squamous cell carcinoma of the head and neck region with a performance
status of 0–2 were eligible for trial. This trial concluded that repeated intratumoral injections of up to \(10^{11}\) PFU were safe and well tolerated. Transgene expression occurred despite evidence of adenovirus antibody response. Peri- and postoperative Adp53 injections had no adverse effect on surgical morbidity and/or wound healing. Evidence of activity based on tumor regression following injection of Adp53 was observed (1 complete response, 2 partial responses).

Another trial utilizing Adp53 (SCH-58500) enrolled patients who had colorectal cancer with liver metastasis. In this trial, 16 patients received hepatic arterial infusion of Adp53 vector. A single dose was administered prior to laparotomy. Patients received escalating dose levels ranging from \(7.5 \times 10^{9}\) PFU to \(2.5 \times 10^{12}\) PFU. Adverse events included fever in 15 of 16 patients and headache in 3 of 16 patients. Transgene expression was confirmed in normal liver and tumor. No responses specifically attributed to the Adp53 therapy alone were observed; however, 12 patients subsequently received floxuridine and 11 achieved a 50% reduction in disease, suggesting consideration of sequential therapeutic approaches in trial designs utilizing Adp53 (21).

**CLINICAL TRIALS WITH ONYX-015 VIRUS**

Preliminary phase I studies indicated that intratumor ONYX-015 injections are well tolerated, and viral proliferation has been confirmed. The duration of tumor response appeared to be greater in patients receiving multiple injections, compared with a single injection per cycle (every 21 days). The optimal dose suggested for phase II investigation was \(1 \times 10^{10}\) PFU given for 5 days every 21 days.

Phase II studies performed in patients with recurrent head and neck cancer who had not previously been exposed to chemotherapy or radiotherapy in the recurrent tumor setting utilized a dose of \(1 \times 10^{10}\) PFU of ONYX-015 daily ? 5 days every 3 weeks via intratumor injection. Injections were given throughout the perimeter of the tumor, and the volume of the injected medium was normalized to 30% of the target tumor volume. Neutralizing antibodies were found in 10 of 20 patients prior to injection, and the \(p53\) gene sequence was mutated in 7 of 13 patients. An increased response was suggested in patients with a tumor size of \(\leq 5\) cm in diameter. The most frequent side effect observed in the phase II trial was pain at the injection site, which occurred in 32% of patients. Transient fever and chills occurred in 28% of patients. Thus far, preliminary results suggest that the ONYX-015 virus is well tolerated at a dose of \(10^{10}\) PFU given 5 consecutive days every 3 weeks. Subsequent studies exploring ONYX-015 virus (\(1 \times 10^{10}\) PFU daily ? 5 days every 3 weeks) combined with chemotherapy (cisplatin 100 mg/m\(^2\), intravenously on day 1; and 5-FU 800–1000 mg/m\(^2\) by continuous infusion on days 1–5 every 3 weeks) have also been performed.

At the time of the preliminary analysis, 26 patients had been treated, and 62% achieved a partial response or complete response (23%, complete response). Median survival has also not been reached and is at 6+ months at this time. Despite being preliminary, the data are very encouraging, particularly when compared with expected response rates in which similar patients receiving chemotherapy without ONYX-015 virus would be expected to achieve a 35% partial or complete response rate and would be expected to have a median survival of 26 months. These preliminary results suggest that ONYX-015 replicates in recurrent refractory head and neck cancer, and that ONYX-015 is well tolerated following intratumor injection alone or when combined with chemotherapy.
CONCLUSION

Results of clinical trials are encouraging. A variety of Adp53 adenoviral vectors and attenuated replication-capable adenovirus (ONYX-015) showed good tolerability. Transgene product expression from the transfected vector was confirmed; it was functional and was associated with antitumor activity in patients with advanced disease. Unfortunately, at this time therapy is limited to direct intratumor injection. Such local treatment could reduce tumor bulk prior to surgery, thereby facilitating complete surgical resection and reducing morbidity related to surgery. It may also be possible to inject surgical beds to reduce recurrence from marginal disease. If immunologic difficulties leading to vector neutralization can be overcome, systemic infusion of Adp53 vector may be well tolerated. Preclinical studies to limit immunoreactivity to the Adp53 vector through inhibition of the immune response or alteration of the vector or other gene transfer vehicles are ongoing (22) and include use of chemotherapy combination regimens or a ligand liposome complex to deliver wild-type p53 gene systemically.

Our research program investigating p53 targeted approaches with Adp53, ONYX-015, and SCH-58500 has entered well over 100 patients into clinical trials—more than any other single site worldwide. Donations from the Mary C. Crowley Foundation and the Lane Newsom Fund have recently been used to establish a high-technology clinic at Baylor, which will further allow us to treat patients using a variety of gene therapy approaches.

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References


