Gene Therapy

p53 Gene Transfer for Cancer

Tumor suppressor genes consist of genes whose protein products control the cell division cycle or cell death pathways and whose function is typically altered or missing in tumor cells. Through the action of these genes, cells that become damaged are blocked in the cell cycle or are destroyed outright, preventing their accumulation and progression toward cancer.

Tumor suppressor genes are typically recessive, meaning that it takes mutations or deletions of both alleles to remove the function of their protein products. The proteins themselves also may be inactivated by other mechanisms, such as the action of viral oncoproteins. Once the activity of a tumor suppressor gene is lost through mutation, deletion, or inactivation, the probability of cancer becomes much greater, as the number of damaged cells and the extent of DNA damage increase.

More than a decade has passed since *p53* was identified as a tumor suppressor gene, and thousands of studies of *p53* have contributed to the current understanding of tumorigenesis and tumor suppression. It is known that p53 is heavily involved in the recognition of DNA damage and in the cell cycle and cell death pathways that work to prevent that damage from being transmitted to progeny cells.

The *p53* gene is deleted or mutated in about half of human cancers. In others, the protein product is incorrectly localized in the cell or its activity is disrupted by viral oncoproteins or by overexpression of a p53-inactivating cellular protein.

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

Understanding the place of p53 in tumor suppression has led to therapeutic strategies to restore or augment its function in cancer cells through direct gene transfer. Introgen Therapeutics has developed a first-generation adenoviral vector in which the viral E1 region, involved in adenoviral replication, has been replaced with the *p53* gene under the control of a cytomegalovirus promoter. The vector, INGN 201, can be used to efficiently deliver the *p53* gene to both in vitro and in vivo models of human cancer.

Results of these preclinical studies have helped guide the selection of clinical indications for testing as well as the dose levels, schedule, and combination approaches that might further increase clinical activity. The vector has already been tested in more than 600 patients for safety and clinical activity in phase I, phase II, and ongoing phase III clinical trials.

The vector relies on normal adenoviral tropism for gene delivery and has no mechanism for preferential delivery to tumor cells rather than normal cells. Instead, most of the clinical studies conducted to date have utilized direct injection into tumors or into the region where tumors are located in order to maximize gene delivery to sites of disease.

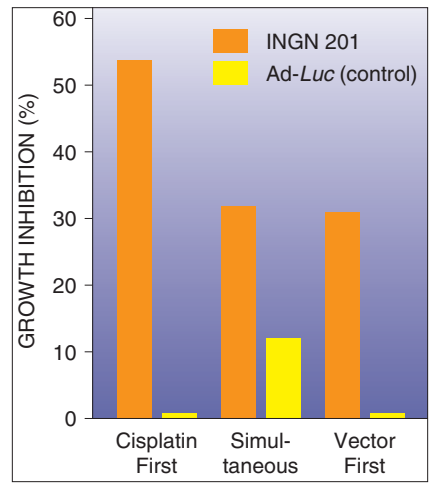
Selection of clinical targets for *p53* gene delivery therefore has focused on those cancers that would benefit from improved local or regional control of tumor growth. These include squamous cell carcinoma of the head and neck; brain, bladder, and ovarian cancers; locally advanced prostate cancer; and nonmetastatic stages of non-small cell lung cancer and breast cancer. Metastatic lesions that can be accessed for injection would also be amenable to this approach.

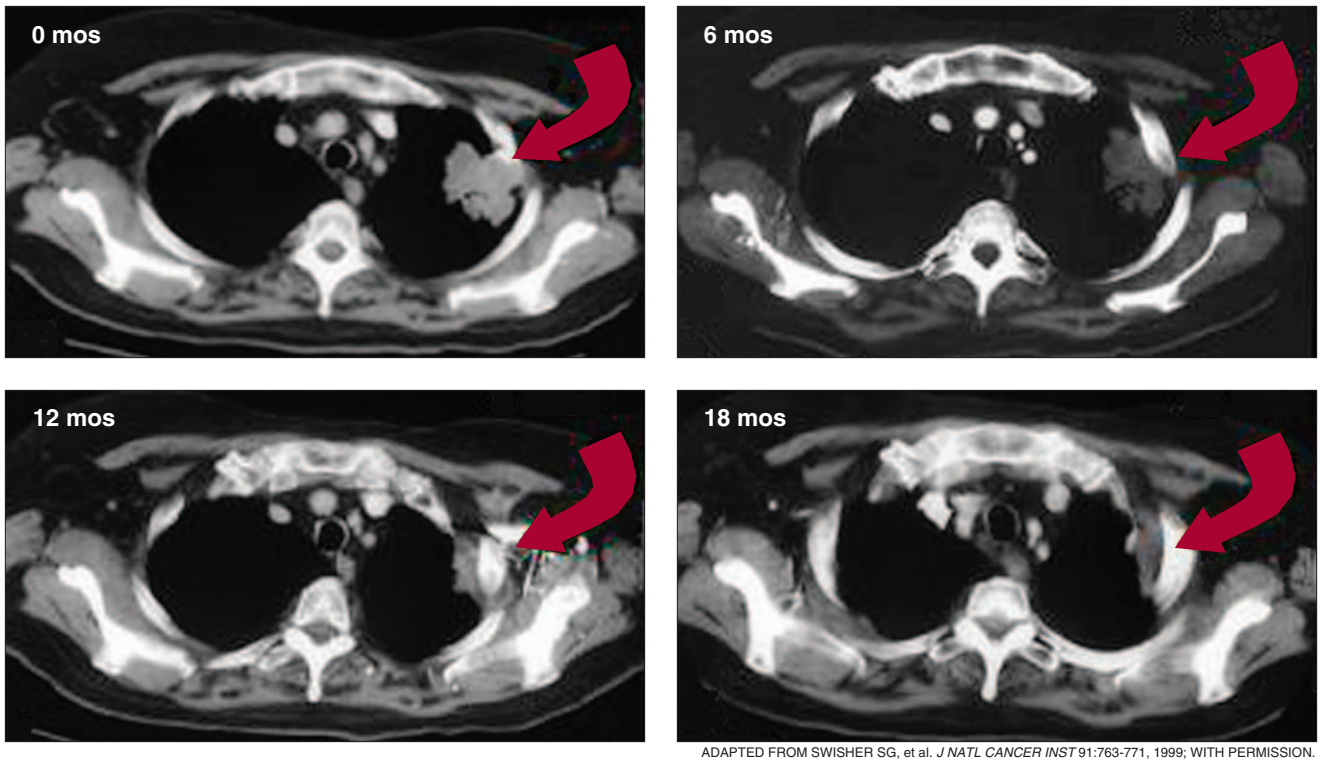
Various routes of administration are being tested, including intratumoral injection, bronchial lavage, intravesical instillation, and intraperitoneal administration. The safety of the vector and the effect on gene expression in the tumor after administration by each route have been studied in phase I trials.

Those studies also examined administration of the vector in combination with conventional treatment by surgery (head and neck, prostate) or chemotherapy (lung). The combination of gene transfer and radiotherapy has also been tested in a phase II trial in lung cancer, and the combination of INGN 201 plus chemotherapy is currently being studied in patients with refractory squamous cell tumors of the head and neck.

The adenoviral vector is well tol-

The effectiveness of INGN 201 in inhibiting tumor cell growth can be enhanced by the addition of cisplatin in a schedule-dependent manner. In an in vitro model of human squamous cell carcinoma, treatment with cisplatin 1 day before INGN 201 was better at inhibiting tumor cell growth than schedules with either simultaneous treatment or INGN 201 treatment before cisplatin. Ad-*Luc* is a control adenoviral vector.





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Serial CT scans from a patient with advanced non-small cell lung cancer show tumor shrinkage following treatment with 6 monthly intratumoral injections of INGN 201, given in a phase I trial. Although minimal changes were evident on radiographs 1 month after treatment, antitumor activity was clearly evident by 6 months and persisted at 1 year after cessation of all cancer treatments, including INGN 201 (18 months). The clinical response occurred despite an increase in antiadenoviral antibody titers seen within 1 month of the first vector administration.

erated, and no dose-limiting toxicity has been found at levels as high as 3×10^{12} viral particles per injection. The schedule of administration has varied among different studies, ranging from a single injection (in lung cancer) to six times in 2 weeks (in head and neck cancer). Many patients have received multiple monthly cycles of treatment.

In each of the completed phase I trials, vector uptake and gene expression were documented in the target tumor. Although patients generally had an increase in antiadenoviral antibody titer following treatment, the presence of these antibodies did not abrogate transduction or gene expression. The results of these studies indicate that multiple cycles of intratumoral injection are reasonable from both safety and transduction standpoints.

In the phase I studies, several tumors showed shrinkage or elimination in response. Growth of many other tumors was stabilized (< 25% increase in size from baseline) for periods of 2 to 14 months. Two of 33 patients with squamous cell carcinoma of the head and neck had a partial response (> 50% reduction in tumor size) in their treated tumors, and 1 patient had a complete response (elimination of the treated lesion).

Responses occurred in head and neck tumors possessing both normal wild-type and mutant *p53*, indicating that the vector can have activity regardless of the tumor's own *p53* status. Of 52 phase I patients with non-small cell lung cancer, 4 had a partial response in the treated lesion.

A large phase II study in patients with recurrent or refractory squamous cell carcinoma of the head and neck has also been completed. This trial tested two administration schedules, once daily for 3 days and six injections spaced over 2 weeks, each repeated monthly.

Preliminary results from 106 patients indicated that of the 167 treated lesions that were evaluable for response, 6 showed complete responses and 11 had partial responses. As in the phase I trials, responses occurred in tumors with both wild-type and mutant *p53*. An additional 82 lesions displayed disease stabilization for 2 to more than 7 months. A trend toward an improvement in survival was seen in patients receiving the greater number of injections in each cycle of administration.

As part of a pivotal phase III program, the vector is now being tested in patients with refractory squamous cell tumors of the head and neck.

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