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ORIGINAL REPORT

Phase I Study of Adenovirus p53 Administered by Bronchoalveolar Lavage in Patients With Bronchioloalveolar Cell Lung Carcinoma: ECOG 6597

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ABSTRACT

Purpose
This pilot phase I trial evaluated the safety and maximum-tolerated dose of *p53* gene transfer using an adenovirus vector (Ad-p53) delivered via bronchoalveolar lavage (BAL) to patients with bronchioloalveolar lung carcinoma (BAC).

Patients and Methods
Patients were initially administered two treatments of Ad-p53 to a single involved lobe, beginning at 2×10^9 viral particles (vp) per dose and escalated to a maximum of 2×10^{12} vp. If a clinical benefit was seen and the treatment was well tolerated, additional doses could be administered to additional lobes.

Results
Twenty-five patients were treated at doses between 2×10^9 and 2×10^{12} vp. At 2×10^{12} vp, one patient experienced grade 4 pulmonary toxicity, and one patient died 25 days after his second cycle; therefore, a cohort of 10 patients was treated at the recommended phase II dose of 5×10^{11} vp, with no grade 4 toxicity observed. The most frequent toxicities included low-grade fever, hypoxia, and dyspnea. Of the 23 assessable patients, 16 had stable disease as their best response. Subjective improvement in breathing was noted in eight patients. Limited distribution of vector was observed, with transient detection in patient sputum for 1 to 2 days after administration.

Conclusion
Ad-p53 can be administered safely by BAL at 5×10^{11} vp with repeated dosing. Stabilization of disease and symptomatic improvement may warrant further studies of Ad-p53 or other adenoviruses administered by BAL in patients with BAC.

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INTRODUCTION

Bronchioloalveolar carcinoma (BAC), a subtype of non–small-cell lung cancer (NSCLC), is characterized by thin sheets of tumor cells growing along the pulmonary airways and is often refractory to surgery, chemotherapy, and radiation. The biology of BAC, however, may make it amenable to local therapies such as gene therapy that typically do not penetrate easily into large solid tumor masses. Pure BAC accounts for approximately 5% of NSCLC, whereas adenocarcinomas with BAC features likely constitute a much higher percentage.[1] Current treatment options are limited and often associated with substantial morbidity.

Advances in the cellular and molecular biology of lung cancer have identified genetic alterations that hold promise as therapeutic targets for lung cancer. The *p53* gene encodes a 53-kd phosphoprotein that is involved in the regulation of the cell cycle and apoptosis and is an important mediator of chemotherapy resistance by preventing chemotherapy-induced apoptosis.[2] Expression of the *p53* gene leads to G_1 arrest or, in most cases, induction of the apoptotic pathway.[3] Mutations in the *p53* tumor suppressor gene, which occur in 40% to 70% of NSCLCs, often cause loss of p53 function and lead to uncontrolled cell division.[4-6] BAC tumors with invasion more than 5 mm showed significantly increased *p53* expression than tumors with less than 5 mm of invasion.[7]

Ad5CMV-p53 (INGN201, ADVEXIN; Introgen Therapeutics Inc, Houston, TX) is a replication-defective type 5 adenovirus that contains the human wild-type *p53* gene under the direction of the cytomegalovirus promoter. Reintroduction of the wild-type *p53* gene into a BAC cell line (H358, p53 null) via a recombinant adenovirus resulted in induction

of apoptosis and growth inhibition.[8,9] Clinical studies have been conducted with Ad-p53 in patients with advanced NSCLC. In these studies, Ad-p53 was used as monotherapy,[10] in combination with chemotherapy,[11] and in combination with external-beam radiation,[12] with evidence of responses in the Ad-p53–treated lesions compared with untreated lesions. In all of these studies, the drug was administered intratumorally by direct injection of the primary lesion using either computed tomography (CT) – guided percutaneous needle or a flexible bronchoscope.

We hypothesized that the BAC growth pattern may enable gene therapy by bronchoalveolar lavage (BAL) with vectors that do not penetrate into solid masses well. In this pilot phase I study in BAC, the principal objectives were to evaluate the safety of multiple endobronchial treatments with Ad-p53 administered by BAL in a dose-escalation scheme and to evaluate the expression of the *p53* transgene in tumor tissue exposed to the drug. The secondary objective was to evaluate the efficacy of this therapy in terms of improved local tumor control.

PATIENTS AND METHODS

Patient Selection

Patients age $\geq$ 18 years with histologically proven unresectable NSCLC with a growth pattern allowing access to the majority of tumor cells via the airway, including pure BAC, adenocarcinoma with BAC features, and papillary adenocarcinomas, were eligible. Patients were required to have a life expectancy of at least 12 weeks, have an Eastern Cooperative Oncology Group performance status of $\leq$ 1, and be more than 4 weeks from surgical resection of lung tissue and more than 2 weeks from systemic cancer therapy. Adequate cardiac, pulmonary (oxygen pressure $>$ 90% on room air), and end organ function were required. *p53* mutation status was not an entry criterion. All patients provided written informed consent for all study procedures according to federal and institutional guidelines. The clinical study was approved by the appropriate institutional review boards of the participating institutions and the National Institutes of Health Recombinant Advisory Committee.

Drug Administration

Each patient received a constant dose of Ad-p53 via BAL, repeated every other week for two doses. Standard practices for Biosafety Level 2 agents were followed. BAL was performed by fiberoptic bronchoscopy by wedging the bronchoscope in the subsegmental bronchi of the lobe to be treated. The total dose to be delivered was divided by the number of subsegments to be treated, diluted to a total volume of 50 mL with Dulbecco's phosphate-buffered saline solution, and delivered to each segment (maximum of four segments per session). The wedge was maintained for 5 to 10 minutes, followed by aspiration of any residual fluid. Because this was a pilot study primarily to determine safety, only a single lobe of the lung was initially treated. Dosing began at 2×10^9 virus particles (vp) per dose and increased in 10-fold increments for each cohort up to 2×10^{12} vp per dose. Each patient received a constant dose repeated every other week to the same lobe for two doses. If the patient perceived clinical benefit or objective response was noted without significant toxicity, the patient could go on to receive further treatments at the same dose to both the initially treated lobe and other involved lobes.

Patients were enrolled in groups of three, and each cohort was observed for a minimum of 2 weeks after the last patient completed a dose level before initiating the next higher dose level. Dose-limiting toxicity (DLT) was defined in this study as grade 3 or greater pulmonary toxicity related to treatment or grade 4 dyspnea (dyspnea at rest) or other toxicity lasting longer than 2 weeks (many of the patients had grade 2 or 3 pulmonary toxicity at study entry). If one of the first three patients in a dose level experienced a DLT, two additional patients were added to that dose level. If more than one of the five patients in a dose level cohort experienced a DLT, then the previous dose level would be considered the maximum-tolerated dose (MTD). An additional 10 patients were to be treated at the MTD to increase the likelihood of detecting serious toxicities and give a better assessment of potential treatment effects.

Toxicity and Response Criteria

Toxicity assessments were performed using the National Cancer Institute Common Toxicity Criteria version 1.0. Response assessment was performed by CT at the end of cycle 2 (day 15) and continued every 3 months afterward until disease progression was noted in the treated lobe. For patients who went on to receive further treatment, CT was performed after every two doses. Because of the nature of the disease, radiographic responses were difficult to assess and distinguish from inflammatory changes caused by therapy; however, standard WHO response criteria for solid tumors were applied to the treated lobe.[13] Investigators also documented changes in performance status, and pulmonary function testing was performed on day 1 of each cycle and day 15 of cycle 2.

Analysis of Tumor Biopsy Specimens

Transbronchial biopsies were obtained at baseline, day 3 of cycle 1, and at the end of cycle 2. Samples in paraffin blocks were analyzed for the presence of *p53* gene expression by Althea Laboratories (San Diego, CA) using a quantitative reverse transcriptase polymerase chain reaction (PCR) assay. Total RNA from biopsy material was extracted, and a random hexamer-primed cDNA reaction was performed using up to 100 ng of total RNA. Quantitative PCR amplification of Ad-p53 and the reference gene *GADPH* was performed in separate reactions.[12] PCR amplification and fluorescence detection were performed using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA). Each quantitative PCR run contained one set of control standards, Ad5CMV-spiked positive control, and the PCR reagent control (negative control).

Determination of Vector Biodistribution

Plasma, urine, and sputum samples were collected to assay for the presence of active Ad-p53 particles using the cytopathic effect (CPE) bioassay. Samples were collected at baseline, on day 1 of cycle 1 (at 2 and 6 hours after treatment), day 3 of cycle 1, day 1 of cycle 2, and day 15 of cycle 2. Specimens were analyzed using a two-stage amplification procedure followed by PCR-based identification.[14] Briefly, patient samples were inoculated first on human 293 cells, a cell line that complements the E_1 deletion of Ad-p53 and thus allows Ad-p53 virus replication and cell death by CPE in infected cells.[15] To detect replication competent virus, supernatants from the 293 cells were then inoculated on A549 cells, which do not support E_1-deleted viral replication but are permissive for wild-type adenovirus replication and exhibit cell death when infected. Observation of CPE on A549 cells in this assay is indicative of the presence of replication competent infectious virus particles in the original sample. Supernatants from cells exhibiting CPE were assayed by real-time TaqMan PCR (Applied Biosystems) using Ad-p53 specific primers to determine whether the CPE was induced by Ad-p53 or another adventitious viral infection in the patient.

Statistical Considerations

Descriptive characteristics were used to summarize baseline patient features. Survival and progression-free survival were estimated using Kaplan-Meier curves. Exploratory analysis was performed for all laboratory correlates.

RESULTS

Safety and Determination of MTD

A total of 29 patients were enrolled between November 1998 and September 2002, with three patients never receiving treatment as a result of decreasing performance status before treatment could be initiated. Two patients later deemed ineligible as a result of abnormal coagulation parameters went on to receive treatment, and their toxicity data, but not response data, are included here. Patient characteristics are listed in Table 1. One patient had papillary carcinoma, whereas the rest of the patients were classified as having BAC, although many of

Table 1. Patient Characteristics of Eligible Treated Patients

Characteristic	No. of Patients (N = 24)	%
Age, years		
Median	55	
Range	33-76	
Sex		
Female	14	58.3
Male	10	41.7
Race		
White	22	91.7
Black	1	4.2
Performance status		
0	15	62.5
1	9	37.5
Status of disease		
Initial diagnosis	7	29.2
Recurrent	16	66.7
Other	1	4.2
No. of metastatic sites		
None	4	16.7
Single	4	16.7
Multiple*	16	66.7
Pleural effusion present	4	16.7
Disease stage		
III	1	4.2
IV	24	95.8
Prior chemotherapy		
Single agent	10	41.6
Multiagent	17	70.8
Other	2	8.3
Prior immunotherapy	1	4.2
Prior radiation therapy	1	4.2
Prior limited radiation therapy	1	4.2
Prior surgery	13	54.2

*Metastatic sites include multiple lobes.

these patients may be classified as having adenocarcinoma with BAC features by the current classification system. More than half of the patients had prior surgical resection, and 70% had previously received multiagent chemotherapy.

Table 2 lists the number of patients entered per dose cohort. Three patients were entered onto cohort 1 (dose level 1, 2×10^9 vp) with no DLT observed. Three patients were enrolled onto cohort 2 (2×10^{10} vp); however, one patient experienced a DLT of a bronchopleural fistula and dyspnea. No further DLT was seen in the additional two patients at this dose level or in the three patients enrolled onto dose level 3 (2×10^{11} vp). At dose level 4 (2×10^{12} vp), one of the three patients experienced a grade 5 pulmonary decompensation 25 days after receiving his last treatment. Although it was not clearly related to the treatment, it was considered a DLT, and two additional patients were entered onto this cohort with no subsequent DLT. Although no further DLTs were observed, it was felt that the severity of the grade 5 pulmonary toxicity warranted a dose reduction for safety reasons. Because no DLT was seen in cohort 3, the investigators chose to investigate an intermediate dose between levels 3 and 4 of 5×10^{11} vp. Twelve patients were enrolled at this dose, but two patients developed worsening clinical status before receiving the first dose, and no DLTs were observed in the 10 patients who received treatment.

Twenty-three of 24 patients received at least two cycles of therapy. One patient was removed from the study as a result of other complicating disease factors not related to therapy. Eight patients went on to receive more than two cycles because of perceived clinical benefit and lack of significant toxicity, and nine patients had more than one lobe treated. Four patients received six or more cycles, and one patient received 13 treatments without significant toxicity. Toxicities considered possibly, probably, or definitely related to treatment are listed in Table 3. At a dose of 2×10^{12} vp, one patient experienced grade 5 hypoxia and pneumonitis with pulmonary infiltrates. This patient died 25 days after his second cycle, and it was uncertain whether the respiratory decline was caused by treatment toxicity, intercurrent infection, or progression of disease. One other patient at this dose level experienced temporary grade 4 dyspnea but otherwise did well with a total of 11 treatments. The most common toxicities were hypoxia (38%) and dyspnea (65%); however, many patients had grade 2 or 3 pulmonary toxicity at baseline. Common grade 1 and 2 adverse events that seem to be dose related included altered hemoglobin, fatigue, fever, and chills.

p53 *Transgene Expression*

Biopsies were performed at baseline, day 3 of cycle 1, and the end of cycle 2 (day 15) and tested by reverse transcriptase PCR for vector-specific *p53* expression. Samples from 16 patients were available for analysis. Three patients (19%) treated at dose levels of 2×10^{11} vp, 5×10^{11} vp, and 2×10^{12} vp tested positive for *p53* transgene expression on day 3 of cycle 1. No samples collected at baseline or at the end of cycle 2 were positive for *p53* transgene mRNA.

Biodistribution of Ad-p53

Plasma, urine, and sputum samples were collected from the patients treated in the first four cohorts and assayed by CPE for the

Table 2. Patients per Cohort: Intent-to-Treat Population

Patient Group	Treatment Arm (No. of patients)				
	2×10^9 vp	2×10^{10} vp	2×10^{11} vp	2×10^{12} vp	5×10^{11} vp
Entered	3	5	4	5	12
Duplicate registration	0	0	1	0	0
Ineligible	0	0	1	0	1
Never started therapy	0	0	0	0	2
Included in analysis	3	5	2	5	9

Abbreviation: vp, viral particle.

Table 3. Drug-Related* Adverse Events of Grade 3 to 5 or Any Grade Occurring in More Than 20% of Patients

Toxicity	Any Grade		Grade 3		Grade 4		Grade 5	
	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%
Cough	19	73.1	1	3.8	0	0	0	0
Dyspnea	17	65.4	3	11.5	3	11.5	0	0
Hypoxia	10	38.5	8	30.8	0	0	1†	3.8
Pneumonitis/infiltrates	7	26.9	4	15.4	0	0	1†	3.8
Hemoptysis	7	26.9	0	0	0	0	0	0
Other pulmonary	6	23.1	0	0	1	3.8	0	0
Anemia	15	57.7	0	0	0	0	0	0
Fatigue	12	46.2	2	7.7	0	0	0	0
Fever	12	46.2	0	0	0	0	0	0
Rigors/chills	11	42.3	0	0	0	0	0	0
Anorexia	6	23.1	0	0	0	0	0	0
Myalgias	6	23.1	0	0	0	0	0	0
Hyponatremia	5	19.2	1	3.8	0	0	0	0
Chest pain	5	19.2	1	3.8	0	0	0	0
Arthralgias	4	15.4	1	3.8	0	0	0	0
Lymphopenia	1	3.8	1	3.8	0	0	0	0

*Considered possibly, probably, or definitely drug related by the investigator.
†Occurred in the same patient.

presence of infectious Ad-p53 particles. Plasma samples from 15 patients and sputum samples from 11 patients were collected. Samples collected at baseline and on day 1 of cycle 1 at 2 and 6 hours after treatment were analyzed. In plasma, one of 120 samples tested positive by CPE. This sample was obtained 2 hours after dosing in a patient treated at 2×10^{12} vp. The pretreatment, 6 hour, day 2, and day 4 samples were negative by CPE. All 112 urine samples were negative. Eighty-six sputum samples were obtained from 11 patients, and except for one sample, all were obtained on the day of injection. A total of 13 sputum samples in six patients tested positive by CPE. Four patients in cohorts 3 and 4 (2×10^{11} vp and 2×10^{12} vp, respectively) were tested, and all had positive sputum samples.

Clinical Response

Twenty-three patients were assessable for clinical response. The best overall responses are listed in Table 4. Among the 23 assessable patients, 16 patients (69%) had stable disease, and seven patients (30%) experienced disease progression. One patient was reported as having a partial response; however, this response was not confirmed. Data on carbon monoxide diffusing capacity uncorrected for hemoglobin concentration demonstrated more than 20% improvement in three of the 22 patients with complete pulmonary function data. In addition, eight patients went on to receive more than two cycles as a result of subjectively improved breathing. Median progression-free survival time was 4.7 months (95% CI, 1.7 to 7.1 months), and median overall survival time was 7.1 months (95% CI, 6.3 to 16.0 months).

DISCUSSION

Despite a priori concerns about inflammatory toxicities associated with repeated treatments with a recombinant adenovirus, we found that repeated dosing of a single lobe of the lung with Ad-p53 is well tolerated in this cohort of patients with severe lung involvement by BAC, and the recommended phase II dose is 5×10^{11} vp per treatment. One patient who developed a DLT at 2×10^{12} vp died 25 days after the second cycle as a result of pulmonary complications thought to be caused by progressive disease or intercurrent infection, but treatment toxicity could not be completely ruled out; thus, an intermediate dose was chosen. No DLT was seen in the subsequent 10 patients enrolled at this intermediate dose. The most frequently observed toxicities were transient cough and increased dyspnea; however, determining whether pulmonary symptoms were a result of treatment toxicity or the disease process was difficult.

No Ad-p53 infectious particles were identified in any urine samples, and only a single positive plasma sample was seen. Thirteen

Table 4. Best Overall Response by Treatment Arm

Response	Treatment Arm (No. of patients)					Total No. of Patients
	2×10^9 vp	2×10^{10} vp	2×10^{11} vp	2×10^{12} vp	5×10^{11} vp	
Stable	2	5	1	2	6	16
Progression	0	0	1	3	3	7
Unavailable	1	0	0	0	0	1

Abbreviation: vp, viral particle.

positive samples were obtained from the sputum, and all tested patients in cohorts 3 and 4 had positive samples suggesting, a dose-response relationship. Because both stable disease and progressive disease were observed among the patient with detectable Ad-p53 particles in the sputum, no relationship with response was observed. Similarly, no relationship with toxicity was observed. The lack of detectable systemic infectious virus particles in the urine and blood suggests that the drug in this form remains primarily localized to the area of treatment. BAL did result in successful but temporary transfection of patient tumor tissue with expression of transgene-specific mRNA in three (19%) of 16 tested patients (in cohorts 3, 4, and 5). Two of these patients had stable disease, and one patient had progressive disease. Detection of transfection in this trial is possibly limited because viral distribution is likely not uniform throughout the lobe, and we were unable to completely control the areas from which biopsies were taken. Prior Ad-p53 studies in lung cancer have been restricted by the requirement for intratumoral injection of the drug directly to the site of the tumor to achieve sufficient transfection and *p53* transgene expression. The identification of transgene expression in some patients suggests that administration of Ad-p53 or other recombinant adenoviruses by BAL may be a technique that can reach diffuse pulmonary disease, thus expanding the scope of application of this local gene therapy.

In this phase I study, 67% of patients demonstrated stable disease. One partial response was reported, but no confirmatory CT was performed. Possibly more importantly, a subjective improvement of respiratory status was reported by many patients. Eight of 23 patients went on to receive more than two treatments as a result of perceived clinical benefit, and one patient received up to 13 doses over 2 years. Administration of Ad-p53 by BAL is a safe and well-tolerated treatment, even with repeated dosing. *p53* mutational status or immunohistochemistry was not tested on these patients because this was a phase I trial. Although many trials involving *p53* incorporate these analyses, responses have been seen in head and neck cancer and breast cancer trials of Ad-p53 in patients with wild-type *p53*, and the BAC cell line initially tested with Ad-p53 had a homozygous deletion, which would result in lack of immunohistochemical staining.[14,16]

Recent trials have suggested that reintroduction of wild-type *p53* may also reverse chemotherapy resistance. Antonia et al[17] delivered adenoviral vector vaccinations to 23 patients with extensive-stage small-cell lung cancer. Only one objective response was seen with vaccination alone, but subsequent chemotherapy in these patients showed a 61.9% clinical response. This suggests that the combination of gene therapy with chemotherapy might be particularly effective, even though Schuler et al[18] found no improvement in response to either carboplatin/paclitaxel or cisplatin/vinorelbine after intralesional injection with rAd/p53 when compared with untreated lesions. Increasing evidence shows the complexity of the cell cycle and apoptotic pathways. It is likely that redundant pathways and escape mechanisms exist within the *p53* pathway, and the effect of these mechanisms on the efficacy of *p53*-related therapies needs to be elucidated.[6,19] However, this study represents a proof of concept that repeated doses of gene therapy vectors can be safely administered via the airway and supports the further use of BAL delivery of vectors expressing *p53* or other more effective payloads.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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