CT Evaluation of Non-small Cell Lung Cancer Treated with Adenoviral p53 Gene Therapy

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Citation

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Abstract

This report describes the CT findings of lung cancers that underwent treatment utilizing Adenoviral mediated transfer of wild type human p53 gene (gene therapy) and correlates these findings with biopsy results. CT examinations of tumors treated with Adenoviral p53 gene therapy that demonstrated an increase in size indicated tumor progression. A decrease in size indicated a response to therapy in 25% of the lesions. Assessment of tumor size by CT did not always correlate with biopsy findings. While it is useful for assessing tumor progression, CT may have limited use in assessing tumor activity.

WORK PERFORMED AT:

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DISCLOSURES

J. A. Roth is a scientific advisor to Introgen Therapeutics, Inc. of Austin, TX and he holds stock in the company. J. A. Merritt, an employee of Introgen Therapeutics, Inc., holds options on the company's stock.

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INTRODUCTION

As knowledge of the mechanisms of oncogenesis has increased, there has been increased focus on targeting treatment to disease at the genetic level. Of primary interest in this pursuit are oncogenes and tumor-suppressor genes (or anti-oncogenes), which appear to be important in cancer growth. The normally occurring oncogene, sometimes called a proto-oncogene, is important for signal induction and transcription of cell division. Mutation of an oncogene results in uncontrolled cell growth as seen in cancers. Tumor-suppressor genes, or anti-oncogenes, encode proteins that are involved in controlling the growth of cells. When cell division has taken place a predetermined number of times, tumor-suppressor genes are involved in arresting the process. The p53 gene, found on the short arm of chromosome 17, is a tumor-suppressor gene that binds to DNA and functions to arrest cell division. If a cell's DNA is damaged, the p53 gene encodes a protein that activates the p21 gene, which serves to halt the cell division until the defective DNA is repaired or until the cell undergoes apoptosis (programmed cell death) [1]. The p53 gene is mutated in approximately 50% of common human tumors. Mutations of the p53 gene also have been shown to be involved in tumor progression and in development of resistance to chemotherapy and radiation therapy [2,3,4]. Introducing wild-type (normal copy) genes into cancerous cells can inhibit or even reverse the oncogenic process and is being investigated as an alternative to conventional cancer therapies of limited effectiveness [5, 6].

In cases of lung cancer treated with investigational therapy, tumor size assessment with CT examinations of the chest remains the dominant imaging method for evaluation [7]. In this study, the CT appearance of tumors treated with Ad-p53 gene therapy was evaluated in 16 patients.

MATERIALS AND METHODS

Patients with histologically proven non-small cell lung

cancer and who had unresectable tumors were enrolled in a trial of Adenovirus-mediated p53 gene transfer into advanced lung tumors. Patients were eligible if they were unable to receive primary external beam therapy, had unsuccessful external beam therapy, or had unsuccessful chemotherapy. A total of 28 patients were enrolled, and their tumors included unresectable lung tumors, endobronchial tumors, and isolated metastatic lesions. For our evaluation, only the tumors within the lung parenchyma that were accessible by transthoracic percutaneous needle biopsy were included. Thus, a total of 16 patients were included in this evaluation (Table 1).

Figure 1

Table 1: CT findings in patients with non-small cell lung cancer treated with Ad-p53 therapy

Patient*	Age/sex	Loc-ation	*Size in cm (% change)	Histology & biopsy results	#Best Overall Response	Chemo-therapy	Metast-asis
1	45/F	RUL	4 x 6	Squamous		No	
			No change	+	SD		
			Increased 6 x 6 (33%)	+			New lung
2	61/M	LUL	10 x 9	Squamous		No	Lung
			Increased 12 x 9 (16%)	+	SD		↑ lung
3	71/F	LLL	5 x 5.5	Squamous		No	-
			Increased 6 x 6 (30%)	+	PD		
4	72/F	LUL	4 x 5	Large cell		No	-
			Decreased 3 x 5 (25%)	+			
			Decreased 1.5 x 5 (63%)	-	PR		
			No change	-			
			No change	-			
			No change	-			
5	68/F	RUL	3.5 x 5	Squamous		No	-
			Decreased 4.5 x 3.5 (10%)	+			
			Decreased 3.5 x 4 (20%)	+			
			No change	+	SD		
			Increased 4.5x 5 (29%)	+			

Figure 2

6	72/F	RLL	6.5 x 7	Adeno		No	Lung
			Decreased 6 x 6.5 (14%)	+			↑ lung
			Increased 7 x 7 (7%)	+	SD		No change
7	65/F	RLL	3.5 x 3.5	Adeno		No	-
			No change	+	SD		
			Increased 4 x 4 (30%)	+			
			No change	+			
			No change	+			
			Increased 5 x 5 (104%)	+			
8	68/F	LLL	5 x 5	Large cell		No	-
			Increased 5 x 6 (20%)	+	SD		
			Increased 8 x 6 (92%)	+			
9	55/M	LLL	5 x 5	Squamous		No	-
			Increased 5 x 6 (20%)	+			
			No change	+			
			No change	+			
			No change	+			
			No change	+	SD		
10	67/F		4 x 2.5	Adeno		No	-
			No change	+			
			No change	+			
			No change	+			
			No change	+			
			No change	+	SD		

Figure 3

11	62/F		5 x 6.5	Squamous		No	-
			Increased 5 x 8 (23%)	+			
			No change	+	SD		
			Increased 5.5 x 8 (35%)	+			
12	75/M	RUL	3 x 6.5	Squamous		No	-
			No change	+			
			No change	+			
			Increased 4 x 6.5 (33%)	+			
			No change	+			
			No change	+	SD		
13	70/M	LLL	1.5 x 2	Adeno		Cisplatin	R lung
			No change	+			↑ R. lung,
			Decreased 0.5 x 1 (83%)	+	PR		new liver
14	65/M	LUL	4 x 7	Adeno		Cisplatin	Lung
			No change	+			New liver mets
			No change	+		Stopped – renal failure	No change
			Decreased 3 x 7.5 (19%)	+			↑liver and lung
			No change	+			New adrenal
			No change	+	SD		↑ all

Figure 4

15	70/F	LUL	2.5 x 3	Adeno		Cisplatin	-
			No change	+			
			No change	-			
			No change	+			
			No change	-			
			No change	-	SD		
16	48/M	RLL	7 x 9	Squamous		No	-
			Decreased 5 x 7.8 (38%)	-			
			No change	+			
			Increased 7 x 10 (11%)	+			
			Decreased 6.5 x 10 (3%)	-	SD		

+ - Sequential numbers were assigned to the patients for manuscript preparation and do not represent identifiers used during the trial.* - Size in centimeter and measured as medial-lateral (width) and anterior-posterior (depth); ( ) = Percent change from baseline.# - Best overall response is the best response achieved (compared to baseline) while on protocol. Response is based on a change in size, which is reported as the product of the diameters. CR is complete disappearance of clinical disease, PR is a 50% or greater decrease; PD is an increase of 25% or more; SD is any variation in the lesion not meeting the criteria of a complete, partial, or progressive disease. CR= complete response, PR= partial response, SD= stable disease, PD= progressive disease.

All patients had documented p53 mutations. The presence of adenovirus vector DNA was assessed with polymerase chain reaction (PCR) analysis and the vector-specific p53 messenger RNA was detected by reverse transcriptase-PCR analysis. Apoptosis (programmed cell death) was assessed by increased terminal deoxynucleotide transferase-mediated biotin uridine triphosphate nick-end labelling (TUNEL) staining in posttreatment biopsy specimens.

Each patient underwent monthly injections of adenoviral vector expressing wild-type p53 (Ad-p53) into the primary NSCLC lesion. The feasibility of this procedure in a subset of our study group has been previously reported [6]. Ad-p53 was diluted in phosphate-buffered saline and injected by percutaneous needle directly into the tumor. For lesions of 4 cm or less, the final volume given was 3 ml, and for lesions larger than 4 cm, the final volume given was 10 ml. All injections were directed to the center of the lesion. The number of treatments was between two and six. Three patients also underwent chemotherapy with Cisplatin; for one patient (# 14), chemotherapy was discontinued because of renal failure. Two patients (#2, #6) went off the protocol because their health deteriorated.

CT examinations were performed on all patients prior to initial treatment and at monthly intervals. The monthly CT examinations were done immediately prior to the CT-guided percutaneous biopsy and injection of Ad-p53. CT examinations were performed with intravenous contrast using 10 mm collimation. Prior to injection of the Ad-p53, aspiration biopsies of the lesion were performed using CT - guided transthoracic coaxial technique. Histologic findings from the needle biopsies were recorded and correlated with CT findings (Table 1).

A total of 71 CT scans including 16 baseline studies were performed utilizing intravenous contrast. The CT examinations were evaluated with emphasis on changes in the size and characteristics of the primary treated lesion. The tumors were measured in two dimensions, using the maximum diameter in the axial plane as the initial point. A second axial measurement perpendicular to the initial measurement was obtained for the second value. All measurements and evaluations were by consensus of two fellowship trained thoracic radiologists (RFM, MTT).

The clinical protocol was approved by the Biosafety and Surveillance Committee and the Institutional Review Board of the institution, by the Recombinant DNA Advisory committee of the National Institute of Health, and by the U. S. Food and Drug Administration. All patients gave informed consent to participate. Introgen Therapeutics, Inc supplied the Ad-p53 (RPR/INGN 201).

RESULTS

There were 16 patients in this study: 10 women and 6 men. The average age was 65 years old (range, 45-75). Overall, as per protocol, 13 patients had stable disease, 2 had partial disease response, and 1 had progressive disease (Table 1). All patients with progressive disease had positive biopsy results. Of the two patients with partial disease response, one (#4), had a negative biopsy sample and the other (#13), had a positive biopsy sample. Of the 13 patients with stable disease, biopsy results were positive in 11 and mixed (negative and positive) in 2 (#15and 16).

Evaluation of the CT examinations of the 16 patients revealed that there were 16 baseline CT examinations and 55 follow-up exams. Comparison of the follow-up CT examinations with the baseline examinations revealed that 33 lesions were unchanged in size, 13 lesions increased, and 9 lesions decreased in size. The histologic findings were correlated with the changes in tumor size noted on the CT images. All 13 of the tumors that increased in size had

malignant cells in the biopsy samples, 6 tumors that decreased in size had malignant cells in the biopsy samples, and 3 tumors that decreased in size did not show tumor cells histologically (Fig. 1). Of the 33 lesions that did not change in size, 27 had malignant cells in the biopsy specimen and 6 did not. When the biopsy results were correlated with the tumor size on follow-up CTs in patient #15, there were interesting findings (Fig. 2). When the tumor decreased in size, the biopsy was negative. However, the next follow-up examination showed a stable tumor size, but the biopsy was positive. Then at the next CT, there was an increase in size of the tumor and a positive biopsy. At the final follow-up, the tumor decreased in size and the biopsy was negative.

Figure 5

Fig. 1.- 72 year-old woman (Patient # 4) with large cell carcinoma:A, Pretreatment CT shows a 5 cm lobulated mass of the left upper lobe. B, CT after two treatments shows a decrease in size of the tumor that correlates with a partial response. Biopsy was negative for malignant cells throughout the course of treatment.

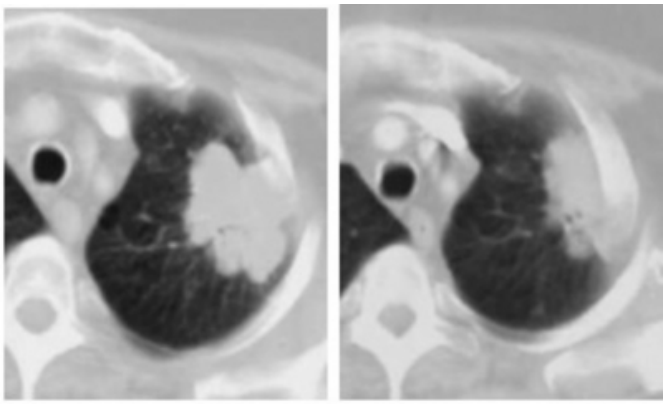
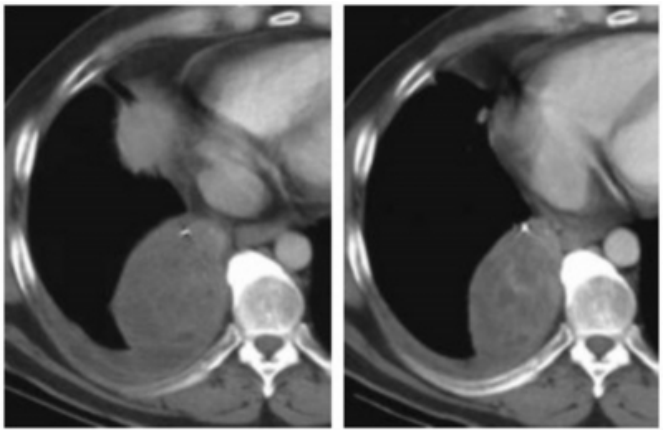


Figure 6

Fig. 2.- 48 year-old man (#16) with squamous cell carcinoma.A and B, Pretreatment CT shows a 7cm right lower lobe mass (A). After the first treatment, CT showed a decrease in the transverse diameter of the tumor (B) and the biopsy was negative. At the next treatment, the size was unchanged, but the biopsy was positive.

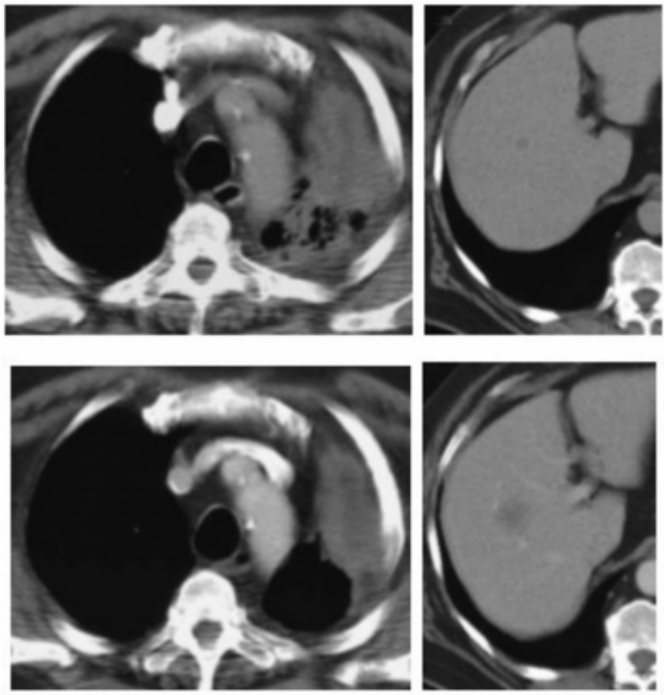


CT characteristics of the lesions and associated findings were recorded when apparent. All tumors appeared solid at baseline, and there was no decrease in attenuation on any follow-up CT that would suggest necrosis. There were two patients with CT findings of obstructive pneumonitis and bronchial narrowing. In one patient (#14), obstructive pneumonia developed after the first injection, which improved on the following CT; on both CTs, the lesion remained stable in size and positive at biopsy. Another patient (#12), who had narrowing of the right upper lobe bronchus at base line, had progressive narrowing on two of the follow-up CT examinations. On both CTs the tumor increased in size and was positive at biopsy.

New metastatic disease or disease progression during therapy occurred in five patients (patients #1, 2, 6, 13 and 14). Of these five patients, the best response of the primary tumor being treated was stable disease in four patients and partial response in one patient. On the individual CT examinations, new metastases were present when the tumor decreased in size on 1 study, (patient #13), when the tumor increased in size on 1 study (patient #1), and when disease remained stable on 2 studies (patient #14). Progressive metastatic disease was present when the tumor decreased in size on 2 studies (patients #6 and 14) (Fig. 3), when the tumor increased in size on 1 study (patient #2), and when disease remained stable on 2 studies (patient #13 and 14). The biopsy of the primary tumor remained positive in all of these cases.

Figure 7

Fig. 3.-65 year-old man (Patient # 14) with adenocarcinoma. A, B, C, D, Pretreatment CT of the chest shows a left upper lobe mass (A) and a 1cm metastatic lesion of the liver (B). CT after three treatments shows a slight decrease in size of the lung mass (C), but an increase in size of the liver lesion (D).



Negative biopsy samples were obtained in three patients (#4, 15, and 16). In patient #4, the biopsy remained negative after two courses of treatment. Patients #15 and #16 had negative biopsies at the final sampling, but positive biopsies were intermixed with negative biopsies during the treatment.

DISCUSSION

CT examinations of the chest are the primary method of evaluating bronchogenic carcinomas. Although many argue the accuracy of CT for staging lung cancer, it remains the most common imaging examination to evaluate the thorax [8,9,10,11]. As in clinical practice, CT also remains the predominant method of assessing tumors in the setting of investigational therapy for lung cancer. Some studies utilize Positron Emission Tomography (PET) imaging to try and assess the functional activity of lung cancers after treatment, but PET remains under investigation, and it is not universally available for investigational studies. Many efforts are underway to investigate techniques for the functional and molecular activity of tumors [12], but these also are investigational. Until methods of functional and molecular imaging become routine for investigational imaging purposes, CT of the chest remains the primary method of assessing lung carcinomas that are treated with investigational therapy.

In this study, CT was used to assess response of the primary lesion to Ad-p53 gene therapy. When a primary lesion increased in size there was histologic evidence of malignant cells. In some cases, a decrease in size correlated with a negative biopsy sample, but not always. Similarly, a lesion that was stable in size was twice as likely to be positive at biopsy than negative. The sample size of this study is too small to determine if this correlation is significant, but it appears that an increase in size indicates residual active disease.

Since the Ad-p53 is injected directly into the tumor, we investigated whether there were changes of the appearance of the lesion on CT that might indicate response to therapy. We found no changes in the appearance of the primary lesion that would suggest activity of the treatment.

Although the primary focus of this study was the CT appearance of the primary tumor, evidence of metastatic disease was also noted. Five patients had evidence of progression indicated by new or enlarged metastatic lesions. In four of the patients, the best response was stable disease and in the other patient it was partial disease response. In all of these patients, the biopsy results of the primary lesion remained positive, but interestingly, the primary lesions remained stable or decreased in size in 7 out of 9 CT examinations. Adenoviral p53 treatment is not administered as a systemic therapy, and although only a limited number of cases with these findings, the progression of metastatic disease with no change or a decrease in size of the primary lesion would suggest that the response of the primary lesion is due to the Ad-p53 therapy.

Newer methods of treating lung cancer are rapidly being developed and being tested in clinical trials. Most notable are agents that employ gene therapy and anti-angiogenesis therapy. Many of these agents are cytostatic and not cytotoxic, which means that tumor cell growth and dissemination will be halted, but not all tumor cells may be eradicated. For radiologists, these new agents may alter our method of examining these patients. CT will remain an important and probably primary method of evaluation until functional and molecular imaging methods are routinely available. However, a change in the size of the tumor may be apparent to the radiologist, but the significance of that change may not be known. If the tumor increases in size, it is consistent with progressive disease. However, if the tumor

remains stable in size or decreases in size, no assessment of the tumor viability or effectiveness of the cytostatic drug can be determined. PET is currently used in lung cancer imaging and may play a key role in evaluating lung cancers when newer treatments are employed. Since many of these therapies alter the blood supply to the lesion, examining the enhancement pattern of the tumors (tumor perfusion) using CT and MRI may also be useful in this evaluation. Further evaluation and development of methods for functional imaging are needed and are currently under investigation.

There are several limitations to our study. This is a retrospective review and there are a limited number of cases. The emphasis was on assessment of the tumor size and associated CT characteristics, and no attempts at functional imaging such as PET for correlation were performed. Also, while biopsies were performed on all patients, because of technical limitations, the biopsy site and injections may not have been at the exact location of the previous biopsy, particularly in large tumors. In addition, because cell death in tumors is not uniform, sampling large tumors can produce mixed results. One of these events may explain the mixed biopsy results in patients #15 and #16. However, it also is possible that the tumor activity varies, and the biopsy results represent the true activity. Larger trials are currently in progress and more experience to address some of these limitations will be gained from these studies.

CONCLUSION

Our initial experience of the CT appearance of non-small cell lung cancers treated with Adenoviral p53 gene therapy is reported. In this study, the follow-up evaluation of tumor size varied and did not always correlate with the biopsy results or the presence of metastatic disease. This study indicates the need for newer radiographic methods, including functional and molecular techniques, to accurately assess tumor response to newer therapies in lung cancer.

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