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ORIGINAL ARTICLE

Docetaxel plus trans-tracheal injection of adenoviral-mediated p53 versus docetaxel alone in patients with previously treated non-small-cell lung cancer

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This phase II clinical trial compared the efficacy and safety of two types of treatment in patients with non-small-cell lung cancer (NSCLC) who had previously been treated with chemotherapy. Patients diagnosed with NSCLC (with either measurable or evaluable lesions) and had been treated with chemotherapy were eligible for this study. They were randomly assigned to two groups. The DOCp53 group received trans-tracheal Adp53 injection (1×10^{12} vp dosage) on day1 and day8, as well as docetaxel at 75 mg m^{-2} on day 2. The DOC group only received docetaxel at 75 mg m^{-2} on day 1. Patients in each group received treatment for two cycles before reevaluation. Between February 2005 and December 2006, 40 patients were recruited for this study. In all, 19 of them were assigned to the DOCp53 group and 21 were assigned to the DOC group. After a mean follow-up of 12 months, the median survival time (MST) was 7.7 months (95% CI, 4.53 to 10.84) for patients in the DOCp53 group and 5.9 months (95% CI, 4.11 to 7.68) in the DOC group ($P = 0.44$, log-rank test). In the DOCp53 group, two patients with diffuse metastatic disease of lung had partial response (PR) in addition to eight with stable disease (SD). No PR and 13 SD were observed in the DOC group. Toxic reactions that could be attributed to the Adp53 vector were transient fever and limited hemoptysis. For patients with relapsed NSCLC, trans-tracheal injection of Adp53 in addition to docetaxel did not improve overall survival or efficacy. However, direct trans-tracheal injection of Adp53 proved to be a safe procedure, with tolerable levels of toxicity.
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Introduction

Lung cancer is a major type of cancer in many countries. Although surgery is used to treat this disease in early stages, chemotherapy is the main treatment for unresectable tumors. For patients with advanced non-small-cell lung cancer (NSCLC), the first-line chemotherapy is usually a platinum-based two-drug combination regimen for four to six cycles in duration. However, the majority of tumors will progress after treatment despite an initial favorable response. In addition, 60% of the tumors will develop resistance to the same drugs. As a result, the prognosis of NSCLC remains poor. Although second-line cytotoxic chemotherapy and small-molecule epidermal growth factor receptor (EGFR) inhibitors have been used to treat patients with relapsed advance NSCLC, the response rate has been less than 10% (refs 1, 2).

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The *p53* gene is a tumor suppressor gene on chromosome 17p13.1. It encodes a nuclear protein (p53 protein) that functions as a transcription factor, blocking progression through the cell cycle in late G1 phase.[3] Intact p53 protein activates the following two pathways in response to cellular DNA damage: the growth/arrest pathway to permit DNA repair; or the apoptotic pathway leading to programmed cell death. Non-functional p53 protein encoded by a mutated *p53* gene is often found in cancer patients, including those diagnosed with NSCLC.[4]

p53 mutations have been associated with lung cancer more often than any other genetic abnormality. Rates of detected mutations range from 20 to 60% of all cases of NSCLC[4] and they are more common in cancers associated with smoking, such as squamous cell carcinoma, compared with adenocarcinomas.[5] Loss of heterozygosity (deletion of genetic material from one 17p allele) also occurs in the bronchial tissue of 20% of smokers, possibly identifying a population at high risk for developing malignancy.[6,7] In particular, specific hotspot mutations in p53 that result in substitution of thymidine for guanine residues are commonly seen in smoking-induced cancers.[8] Faulty DNA repair capacity for

repairing tobacco carcinogen-induced DNA damage seems to increase the susceptibility to lung cancer in chronic smokers.[9] Three decades of *p53* research have led to many advances in understanding the basic biology of normal and cancer cells. A major breakthrough was the realization that mutant *p53* has a life of its own: it contributes to cancer not only through loss of activity, but also through gain of specific 'mutant functions'. New roles and functions of p53 relevant for tumor suppression were included in the regulation of microRNAs networks, the modulation of cell–stroma interactions and the induction of senescence. Research on mutant p53 is entering the clinical and translational era.

In animal tests, by introducing normal *p53* gene into tumor tissues, researchers have confirmed the expression of functional p53 protein in these cells. More importantly, they also observed tumor regression and improved survival rate in test animals without adverse effects on non-malignant tissues.[10–12] These studies suggest that direct intra-tumoral transfer of the *p53* gene using an adenoviral vector (Adp53) should be a safe procedure in human, and may potentially have clinical significance in treating patients with NSCLC.[13,14] As Adp53 (Gendicine, Shenzhen Sibiono Gene Tech, Shenzhen, China) has been approved by the State Food and Drug Administration for human clinic trial, we decided to test the safety and efficacy of trans-tracheal injection of adenoviral-mediated Adp53. To reach this goal, we compared the effects of Adp53 and docetaxel with those of standard single-agent docetaxel in patients with advanced NSCLC, whose disease had relapsed after 4–6 cycles of platinum-based doublet chemotherapy.

Patients and methods

Study population

Eligible patients were between 38 and 70 years old. They had an Eastern Cooperative Oncology Group score $\leqslant 2$, had less than 10% weight loss in the last 3 months, and a life expectancy of at least 12 weeks. All patients had histological or cytological confirmation of NSCLC. They had at least one uni-dimensional measurable target lesion $\geqslant 1$ cm by spiral CT scan. The tumors progressed after 4–6 cycles of platinum-based doublet chemotherapy. The blood test results met the following parameters: neutrophils $\geqslant 1.5 \times 10^9$ per l, platelets $\geqslant 100 \times 10^9$ per l, AST and ALT $\leqslant 2\times$ the upper limit of the institutional normal range, total bilirubin $\leqslant 1.25\times$ the upper limit of the institutional normal range and creatinine concentration $\leqslant 120\,\mu\text{mol}\,\text{l}^{-1}$. Adequate pulmonary function was required, with forced expiratory volume in 1 second $\geqslant 40\%$ of normal and partial arterial oxygen pressure $\geqslant 60$ mm Hg. Patients with symptomatic brain metastases, those who had used docetaxel before, had active uncontrolled infection, or had a fever greater than 38.3 °C were excluded from this trial. All patients were required to sign an informed consent form, and the protocol was approved by the institutional ethics committee.

Pretreatment and follow-up evaluations

Before enrollment, patients provided us their full medical histories and underwent physical examination and performance status assessment. Laboratory tests included complete and differential blood counts and assays of electrolytes, glucose, calcium, albumin, transaminases, alkaline phosphatase, total bilirubin and creatinine. An ECG was also recorded. The following examinations were carried out within 1 month before the actual study: chest and abdominal CT scan, radionuclide bone scan, brain MRI and spirometry.

Complete Blood Counts were carried out every week throughout the study. Every 28 days, patients underwent a clinical examination focusing on cancer-related symptoms and treatment toxicities. On these occasions, all laboratory tests mentioned above were repeated. Toxicity was graded based on standard WHO criteria. Responses were assessed 4 weeks after the end of the two cycles of therapy. Imaging studies were repeated whenever deemed necessary clinically. Complete and partial responses were evaluated based on RECIST criteria. Follow-up visits were conducted every 2–3 months.

Study design

This is a two-center, open-label, randomized controlled phase II trial. The central office stratified patients according to the stages and histological types of their tumors. Adp53 (Gendicine) has been approved by the State Food and Drug Administration of China for human clinic trial. This study was performed under an investigational new-drug application approved by the State Food and Drug Administration of China. Participation was voluntary and informed consent was given by every patient. The clinical protocol was approved by the ethics committee of each hospital conducting the trial. In the DOCp53 group, patients received Adp53 intra-tracheally at dose levels of 1×10^{12} viral particles (VP) per side of lung on day 1 and day 8. They were also given $75\,\text{mg}\,\text{m}^{-2}$ of IV docetaxel on day 2 and day 9. In the DOC group, patients were only given $75\,\text{mg}\,\text{m}^{-2}$ docetaxel on day 1 and day 8.

The treatment for both groups was repeated after 3 weeks. Each patient received the assigned dose throughout the study for a total of two courses of treatment. If treatments had to be interrupted because of toxicity, the patient was withdrawn from the study, but included in the survival analysis. Dosage was adjusted according to hematologic toxicity: docetaxel was administered at full dose unless the neutrophil count was $\leqslant 1.5 \times 10^9$ per l or the platelet count was $\leqslant 100 \times 10^9$ per l. Adp53 dosage was adjusted according to tumor distributing in single or both lateral lungs.

If a patient developed progressive disease within 2 months of treatment, the treatment was considered as a failure. If a patient had an objective response or had no change after treatment, we would follow him/her up until progressive disease was confirmed. Successive treatment was allowed, if progressive disease was observed.

Adp53 vector administration

Adp53 vector (Gendicine) is a recombinant human injection supplied by Shenzhen Sibiono Gene Tech. Gendicine is a modified adenovirus-p53, it can reproduce for only 1–2 generations *in vivo*. It was administrated intra-tracheally through the cricothyroid membrane with an intravenous infusion needle. Total amount of 1×10^{12}VP Adp53 vector was diluted to 5 ml with sterile distilled water for a uni-lateral involvement of lung. During the injection, patients were positioned according to tumor's location in the lung. For example, the patient was positioned in the right lateral position, if his tumor was in the inferior lateral segment of the right bronchus. To suppress cough reflex, 5 ml of 2% lidocaine was injected intra-tracheally as local anaesthetic, using the same needle via cricothyroid membrane. The procedure was carried out safely and quickly and was well tolerated by patients.

Statistical analysis

Survival was defined as the time between the date of random assignment and the date of patient's death or that of the last follow-up evaluation. Survival curves were established with the Kaplan–Meier method and were compared with each other using the log-rank test. Usual statistical tests (χ^2 test, Fisher's exact probability test, and the Mann–Whitney U test) were used to compare variables between the two study groups. Differences were considered significant at $P<0.05$.

Results

Patient characteristics

A total of 42 patients participated in this study in two institutions between February 2005 and December 2006. There were 21 patients in each study group. One patient assigned to the DOCp53 group did not meet the criteria after the physical examination and was excluded from the study; another patient from the same group dropped out in the middle of the treatment. At the time of the last follow-up evaluation, 16 patients in the DOC group had died and 5 were alive. In the DOCp53 group, 14 patients had died and 5 were alive.

Table 1 describes the status of the 40 patients and their tumor characteristics. Six patients had stage IIIB disease, whereas the others had stage IV disease. All patients in the DOCp53 group and the DOC group were assessable for response analysis, toxicity, TTP and survival analysis. We were able to follow-up all patients after the treatment and conduct analyses. All prognostic factors were well balanced between the two groups. When this study was completed, six patients in the DOCp53 group and six in the DOC group underwent targeted chemotherapy (either gefitinib or erlotinib) for progressive disease.

Efficacy

The objective response rate was evaluated at the end of each cycle of treatment. All patients were assessable for the tumor response and no complete responses were obtained in either group. Two cases of partial response and eight cases of stable disease were observed in the DOCp53 group (Table 2). No partial responses and 13 cases of stable diseases were observed in the DOC group. The difference was not statistically significant ($P>0.05$). Two patients with partial responses in the DOCp53 group had diffused lung metastasis, one with bronchioalveolar carcinoma and another with adenocarcinoma.

Table 1 Patient characteristics

Parameter	*Subgroup*	*Integrated group*	*Docetexel group*	P-*value*
Age years		61.1 ± 10.8	63.7 ± 5.4	0.34
Gender, *n* (%)				
	Male	12 (30%)	15 (37.5%)	
	Female	7 (17.5%)	6 (15%)	
WHO Performance status, *n* (%)				0.78
	0–1	15 (37.5%)	17 (42.5%)	
	2	4 (10%)	4 (10%)	
Histology, *n* (%)				
	Squamous carcinoma	5 (12.5%)	5 (12.5%)	
	Adenosquamous carcinoma	2 (5%)	3 (7.5%)	
	Adenocarcinoma	10 (25%)	10 (25%)	
	Bronchioalveolar carcinoma	1 (2.5%)	2 (5%)	
	Spindle cell carcinoma	1 (2.5%)	1 (2.5%)	
Disease stage, *n* (%)				
	IIIb	3 (7.5%)	3 (7.5%)	
	IV	16 (40%)	18 (45%)	
Metastasis, *n* (%)				
	<3 sites	14 (35%)	16 (40%)	
	>3 sites	5 (12.5%)	5 (12.5%)	
Subsequent therapies after progress, *n* (%)				
	Gefitinib	5 (12.5%)	6 (15%)	
	Erlotinib	1 (2.5%)	0 (0%)	
	None	13 (32.5%)	15 (37.5%)	

Table 2 Antitumor activity

		DOCp53	95% CI	DOC	95% CI	P-value
PR, *n*		2		0		0.15
SD, *n*		8		13		0.34
PD, *n*		9		8		0.62
OS	MST, ms	7.7 ms	4.53 ms to 10.84 ms	5.9 ms	4.11 ms to 7.68 ms	0.496
	6 ms OS, *n*	5		4		0.56
	1-year OS, *n*	2		2		0.34

Abbreviations: ms, months; MST, mean survival time; OS, overall survival; PD, progressive disease; PR, partial response; SD, stable disease.

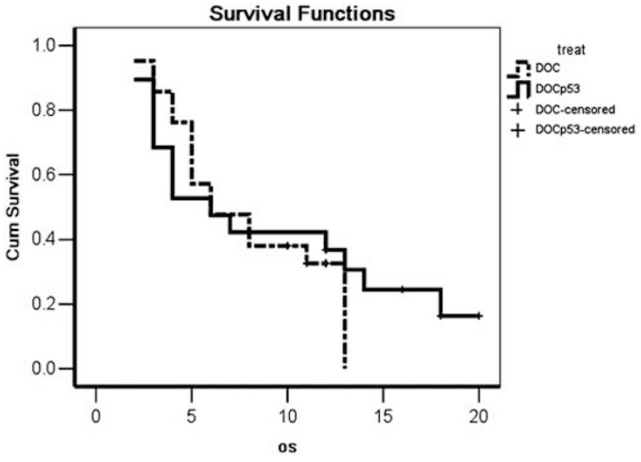

Figure 1 The overall survival of the two groups of patients. The line is DOCp53 group; the dot is DOC group.

Survival

Survival was analyzed after a median follow-up of 7.9 months. The MST was 7.7 months (95% CI, 4.53 to 10.84) in the DOCp53 group and 5.9 months (95% CI, 4.11 to 7.68) in the DOC group. The 6-month and 1-year survival rates were 26.3 and 10.5% in the DOCp53 group, and 19.1 and 9.5% in the DOC group (log-rank test, $P = 0.44$; Figure 1), respectively.

Toxicity

An apparent toxic reaction attributed to the Adp53 vector was transient fever. Grade I or grade II fever was observed in 12 patients related to therapy, although prophylactic use of paracetamol was used (0.5 g q8h for 3 days), $P < 0.001$. in all, 11 patients had mild and limited hemoptysis related to thyrocricocentesis. Other adverse events are summarized in Table 3. The number of cases of neutropenia, asthenia, nausea, anxiety and dyspnea was similar in both groups.

The number of early death (less than 2 months) after treatment outset was also similar in the two groups. There were 11 cases of early death (less than 2 months after treatments). Non-tumor related factors were the causes of two deaths in the DOCp53 group and three deaths in the DOC group. Disease progression caused four deaths in the DOC group and two deaths in the DOCp53 group (Table 4).

Table 3 Treatment-related adverse events.

Adverse events	Acute toxicity incidence of each arm		P-value
	DOCp53, n	DOC, n	
Neutropenia (WHO3/4)	3	5	0.13
Asthenia	4	2	0.55
Nausea	6	6	0.28
Hemoptysis	11	0	0.01*
Pneumonia	1	1	0.54
Anxiety	1	0	0.28
Dyspnea	1	0	0.28
Fever	12	2	0.01*

*$P < 0.05$.

Table 4 Early deaths less than 2 months after treatment outset

Events	DOCp53, n	DOC, n
Tumor progress	2	4
Others	2	3
Infection	1	2
Pulmonary embolism	1	1

Discussion

Gene therapy is a method of transferring genes into cells that are deficient for this specific gene product, using either viral (eg, retrovirus, adenovirus, adenovirus-associated or vaccinia virus) or physical means. In general, local injection is necessary as systemic administration is not practical because of the high immunogenicity of the vectors, and the high prevalence of cross-reacting antibodies.

The *p53* gene is a tumor suppressor gene. A mutated *p53* gene translates into non-functional p53 protein, which makes cells more susceptible to DNA damage and unregulated growth. In NSCLC, p53 mutations are present in 60%, particularly those associated with smoking (ie, squamous cell as opposed to adenocarcinoma).[15] Because of the importance of p53 to cell growth regulation and to chemotherapy-induced apoptosis, many gene therapeutic strategies have focused on restoring normal p53 function in tumor cells.[16]

In a previously conducted initial phase I trial, nine patients with relapsed NSCLC containing p53 mutations received direct local injections of a retroviral vector containing wild-type p53 (ref. 17). Although tumor regression was evident in three patients and tumor growth was stabilized in three others, low transduction efficiency associated with the retroviral vector was a problem. Subsequent studies using an adenoviral vector containing wild-type p53 have demonstrated greater transduction efficiency than the retroviral vector.[18–20] In one representative trial, 24 patients with advanced NSCLC received cisplatin ($80\,mg\,m^{-2}$, day 1) and escalating doses of adenovirus-mediated *p53* gene intratumorally for a total of up to six courses (28 days per course).[21] In all, 17 patients' condition were stabilized, and two achieved a

partial response. Despite these promising initial data, disappointing results were noted in a later multicenter nonrandomized phase II study. In this study, patients received combination chemotherapy (either carboplatin and docetaxel or cisplatin and vinorelbine) and direct intratumoral adenovirus-mediated wild-type p53 transfer.[22] Each patient served as his/her own control, with the isolated response of the treated tumor lesion being compared with the response of a comparable lesion not injected with p53. There was no added benefit with the intratumoral injection of wild-type p53 in patients with advanced NSCLC, when they were receiving effective first-line chemotherapy. In terms of toxicity, Schuler[23] reported intratumoral injection of Adp53 with a bronchoscopic approach was clinically well tolerated at dose levels up to 1×10^{12} VP. No histological evidence of adenoviral-induced infection or inflammatory response was observed, despite direct exposure of the local bronchial airway site to Adp53.

Gendicine, a recombinant human Adp53 injection, (Shenzhen Sibiono Gene Tech) obtained a drug license from the state drug administration of China in 2003 (SDA; Beijing, China). It consists of advenovirus vector and normal *p53* tumor suppressor gene. It got the license so quickly that the route-of-drug admission is not widely studied, it is often used intratumorally. Lung cancer originated from trachea or alveolar epithelial and P53 dysfunctional mutation may have presented before tumor occurrence. Gendicine given intra-trachea may transfect either trachea epithelial cell or lung cancer cell, which is meaningful to lung cancer patients. Our study compared efficacy and safety of docetaxel plus Gendicine versus standard docetaxel in second-line therapy for patients with unresectable NSCLC. Gendicine was given intratracheally through the cricothyroid membrane injection to minimize toxicity related to the potential systemic circulation of the vector. This procedure proved to be clinically well tolerated at dose levels of 1×10^{12} VP per side of lung. No evidence of adenoviral-induced infection or inflammatory response was found, despite direct exposure of the local bronchial airway site to Adp53. Toxicity attributed to the Adp53 vector was minimal. The most noticeable adverse effect was tolerable fever. It may have been related to the transient systemic dispersion of the vector. Bronchial hemorrhage caused by Adp53 injection was minimal and easily controlled. There were no treatment related deaths. The median survival time is similar in the two groups. There were 2 PR and 8 SD in docetaxel plus Adp53 therapy and 13 SD in docetaxel alone. Although statistically there was no benefit when p53 was added to docetaxel, this new treatment did improve efficacy in two patients with diffuse lung metastasis. As the population size in this trial is small and the conclusion lacks statistical power, more studies are needed to further investigate the role of Adp53 combination regimens in treating NSCLC.

In conclusion, direct intra-tracheal injection of Adp53 through the cricothyroid membrane into endobronchial NSCLC is safe, with acceptable levels of toxicity. It is a relatively simple procedure and easy to conduct. This regimen may be a choice for those lung cancer patients who have diffuse spread of lung cancer. It is our plan to do research in this specific group of patients so that we can define the subgroup of patients, who is more suitable for Adp53 treatment. We believe that DOCp53 is a practical regimen that may be considered when treating the subgroup of patients with diffuse lung metastasis.

Abbreviations

Adp53, adenoviral-mediated *p53* gene; NSCLC, non-small-cell lung cancer; VP, viral particles.

Conflict of interest

The authors declare no conflict of interest.

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Author contributions

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