Effect of Recombinant Adenovirus-p53 Combined With Radiotherapy on Long-Term Prognosis of Advanced Nasopharyngeal Carcinoma

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ABSTRACT

Purpose

To centrally assess the safety, efficacy, and 6-year follow-up of recombinant adenovirus-p53 (rAd-p53) combined with radiotherapy (RT) for patients with nasopharyngeal carcinoma (NPC).

Patients and Methods

A randomized controlled clinical study on rAd-p53 combined with RT in 42 patients with NPC was compared with a control group of 40 patients with NPC treated with RT alone. In the group receiving rAd-p53 combined with RT, rAd-p53 was intratumorally injected once a week for 8 weeks. Concurrent RT (70 Gy in 35 fractions) was given to the nasopharyngeal tumor and neck lymph node. Patients and tumors were monitored for adverse events and responses.

Results

rAd-p53–specific p53 mRNA was detected in postinjection of rAd-p53 biopsies from 16 (94.1%) of 17 patients. Upregulation of p21/WAF1 and Bax and downregulation of vascular endothelial growth factor were observed in postinjection tumor biopsy. Complete response rate in the group receiving rAd-p53 combined with RT was observed at 2.73 times that of the group receiving RT alone (66.7% v 24.4%). Six-year follow-up data showed that rAd-p53 significantly increased the 5-year locoregional tumor control rate by 25.3% for patients with NPC treated with irradiation (P= 002). The 5-year overall survival rate and 5-year disease-free survival rate of the group receiving rAd-p53 combined with RT were 7.5% (P= .34) and 11.7% (P= .21) higher than those of the group receiving RT alone. No dose-limiting toxicity or adverse events appeared, except fortransient fever after rAd-p53 administration.

Conclusion

In patients with NPC, rAd-p53 was safe and biologically active. Our results indicated that rAd-p53 improves radiotherapeutic tumor control and survival rate in patients with NPC.

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a cancer unique in the world, and occurs predominantly in southern China.Theincidence rate ofNPChas been as high as 25 to 50 per 100,000 persons in Guangdong Province, a southern area of China.[1-3] Radiotherapy (RT) has been the gold standard of therapy for all stages of NPC for many decades in China, because of anatomic constraints and an intermediate degree of radiosensitivity.A large number of trials in patients with locally advanced NPC have compared concurrent chemotherapy plus RT versus RT alone, but there is uncertainty about the magnitude of survival benefits. Some trials of concomitant chemoradio- therapy failed to detect any improvement on distant failure and survival in Chinese patients with advanced NPC; survival in patients with stages III and IV (M0) disease remained poor.[4] The 10-year overall survival rate for patients with NPC was 40% to 50%. Patients with stages I and II disease exhibited 10-year overall survival rates of 70% to 80% and 50% to 60%, respectively. Unfortunately, the diagnosis of NPC in most patients occurred at stages III and IV (M0) advanced disease. Current treatments for advanced NPC have been disappointing. The 10-year overall survival rates for patients with stages III and IV (M0) were 30% to 40% and 10% to 20%, respectively. More than 50% of these patients with advanced disease experienced relapse or developed metastasis within 3 years after treatment.[1-3]

The P53 gene, widely regarded as the genome guardian of cells, plays a key role in cell cycle control, apoptosis, and inhibition of tumor cell proliferation. Wild-type P53 promotes cell cycle arrest and apoptosis of tumor cells after irradiation, but mutated P53 abrogates this response, and induces resistance to radiation.[5,6] A mutation in the P53 gene occurs in 30% to 70% of patients with NPC, which contributes to recurrence, metastasis, and incurability with regard to irradiation. In patients with NPC, the local recurrence rate is 20% to 50%, and the distant metastasis rate is 20% to 30%.[7-10] Foundational studies have demonstrated that recombinant adenovirus-p53 (rAd-p53) gene transduction restores P53 function in cancer cells, and increases radiosensitivity of NPC cells in vitro and in vivo. Administration of rAd-p53 could be used in adjuvant therapy with RT.[11,12] Numerous clinical trials of rAd-p53 gene therapy have initially targeted head and neck squamous cell carcinoma (HNSCC), and rAd-p53 has been demonstrated to be safe, feasible, and effective in HNSCC.[13,14] Pretreatment experiments showed that expressions of p53 start 6 hours after infection of rAdp53, increase 24hours later, reach a pinnacle after 48 to 72 hours, and decrease to disappearance 120 hours later in vitro and in vivo. Therefore, in the clinical trial, 48 to 72 hours after intratumoral injection of rAd-p53 (Gendicine; China Shenzhen SiBiono GeneTech Co Ltd, Shenzhen, People's Republic of China), RT was

performed. Injections of rAd-p53 once a week were suitably combined with five fractions of irradiation per week.15

PATIENTS AND METHODS

P53 Gene Agent and Usage

rAd-p53, a P53 gene agent, is a recombinant replication-incompetent human serotype 5 adenovirus, in which the E1 region is replaced by a human wild-type P53 expression cassette. rAd-p53 limits the infectivity of the virus to only one cycle, and the adenovirus genome does not integrate into host genome DNA, and does not impair any normal cells. rAd-p53 was stored at -20°C in concentrations of 1_10E12 virus particles/mL. rAd-p53 solution was thawed and diluted moderately in physiologic saline according to tumor size within 0.5 hour of use.

Study Design and Patients

This was a prospectively randomized controlled clinical trial. The trial – including protocols and informed consent process – was approved by the Chinese State Food and Drug Administration and Beijing Hospital Ethics Committee on September 12, 2001. The inclusion criteria were as follows. Patients were 18 to 80 years old. They had a histologic diagnosis of nasopharyngeal squamous cell carcinoma, with measurable focus in nasopharynx and neck by CT image and no distant metastasis. Disease was clinically staged according to the fifth edition of the International Union Against CancerTNM staging system (1997).3 Initial treatment withRTwas judged to be the accepted standard of care. Patients had to have a life expectancy of at least 6 months, and a Karnofsky performance score of at least 70. Patients were required to have adequate bone marrow function (WBC count≥4.0 ×109/L; hemoglobin≥7g/L; platelet count≥70×109/L) and adequate liver and renal function (AST, ALT, blood urea nitrogen, and creatinine_1.5 times the upper limit of normal). The exclusion criteria were as follows. Pregnant or nursing women, and patients with uncontrolled serious infections or with serious heart and lung failure, were excluded. From October 2001 to May 2003, a total of 82 patients with NPC were referred to the two participating cancer centers,Beijing Cancer Hospital (Beijing, People's Republic of China) and Fujian Province Cancer Hospital (Fuzhou, People's Republic of China). Patients with NPC who met the inclusion criteria signed the informed consent forms, and were assigned to one of two groups by the randomized table: rAd-p53 combined with RT, or RT alone (control group). Only one tumor site was selected per patient and tautologically injected. The patients receiving rAd-p53 combined
with RT received an intratumoral injection of rAd-p53 at 1_10E12 virus particles/mL once a week (on Friday) for 8 weeks per tumor, which was done directly or guided by nasopharyngeal endoscope for nasopharyngeal cavity tumor, or ultrasound for neck node. In the group receiving rAd-p53 combined with RT, there were 22 injection sites in the nasopharynx, and 20 injection sites in the neck. Irradiation was started 2 days after the first injection. For both groups, the same irradiation technique was administered to either the nasopharyngeal tumor or neck lymph node, with the

conventional fractionation of 2 Gy, once a day weekly from Monday to Friday, with a total radiation dose of 70 Gy in 35 fractions by high energy (_6 MV) accelerator photons. To assess response, the two largest orthogonal diameters, A and B of nasopharyngeal tumor or neck lymph node, were measured according to computed tomography image at pretreatment and three times post-treatment: 40 Gy dose time point (end of 4th week), 70 Gy dose time point (end of 7th week), and validation time point (2 months after treatment). The tumor size and tumor shrinkage rate were calculated. Immediate tumor response rates, such as complete response (CR), partial response, stable disease, and progressive disease rates, were determined according to the WHO's evaluation standard of solid tumor treatment by tumor shrinkage rate. A comparative study was also performed on the immediate response rate at 40 Gy dose, 70 Gy dose, and validation time points and long-term survival between the two groups.

Reverse Transcriptase Polymerase Chain Reaction and Immunohistochemistry

After intratumoral injection of rAd-p53, the adenoviral particle infects tumor target cells and delivers the adenoviral genome carrying the therapeutic P53 gene to the cell nucleus for transcription. Biopsies at preinjection and 48hours after the first intratumoral injection of rAd-p53 were assessed for p53 protein and p53 target gene P21, Bax, and vascular endothelial growth factor(VEGF; the downstream p53-transactivated gene) by immunohistochemical staining (IHC) in 50 couple available samples of the 25 patients. IHC staining of paraffin-embedded tissue was performed according to standard methods. Sections in which more than75%of cells had definitive nuclear reactivity were scored 4+; 50% to 75%, 3+; 25% to 50%, 2+; 5%to 25%, 1+; and fewer than 5%, 0. Biopsies were assessed for exogenous p53 mRNA by reverse transcriptase polymerase chain reaction (RT-PCR) according to previous methods in 17 couple useful comparable samples from the 25 patients.

Adverse Events

Patients were monitored for adverse events. Toxic and adverse events were assessed as light (grade 1), mild (grade 2), serious (grade 3), and life threatening (grade 4) according to theWHO's evaluation standard for adverse events. Particular attention was paid to body temperature.

Statistical Methods

All data were statistically analyzed using SPSS11.5 statistical software (SPSS Inc, Chicago, IL). The primary endpoints of locoregional control, distant metastasis, disease-free survival, and overall survival were estimated using the Kaplan-Meier statistical method. Univariate analysis was used to identify prognostically significant variables for these endpoints. Differences between survival curves were assessed with the log-rank technique; the method was used to evaluate differences between portions. All reported P values resulted from two-sided statistical tests. All P values less than .05 were considered significant.

RESULTS

Detection of exogenous P53 gene expression in tumor biopsy.
rAdp53 – specific p53mRNAwas detected by RT-PCR analyses of tissue samples in 16 (94.1%) of 17 assessable samples taken 48 hours after intratumoral injection of rAd-p53. Preinjection biopsies were negative for rAd-p53 – specific p53 mRNA. No rAd-p53 – specific p53 mRNA sequence was found in preinjection biopsies by RT-PCR method. Furthermore, IHC-positive staining scores showed that for p53, preinjection level of 1.44 increased to 2.48 post injection (P= .050); for p21, preinjection level of 0.32 increased to 0.88 postinjection (P= .015); for Bax, preinjection level of 0.92 increased to 1.63 postinjection (P= .088); and for VEGF, preinjection level of 2.80decreased to 1.50 postinjection (P= .308). IHC analysis revealed increasing rates of p53 (48.0%; 12 of 25), p21/WAF1(44.0%; 11 of 25), and Bax (37.5%; nine of 24), but a decreasing rate of VEGF (21.0%;five of 24) in postinjection tumor biopsy compared with preinjection tumor biopsy.

Table 1. Characteristics of Patients and Tumors

Characteristic	Group Receiving rAd-p53 Combined With RT (n = 42)		Group Receiving RT Alone (n = 40)	
	No.	%	No.	%
Sex				
Male	29		31	
Female	13		9	
Age, years				
Median	47.4		50.3	
Standard deviation	12.7		11.3	
Range	19-70		18-71	
Histologic type				
Keratinizing	4		3	
Undifferentiated	38		37	
UICC stage				
I	0		0	
II	11	26.2	12	30.0
III	16	38.1	13	32.5
IV (M0)	15	35.7	15	37.5
UICC stage				
T1	0	00.0	2	5.0
T2	24	57.5	25	62.5
T3	9	21.4	4	10.0
T4	9	21.4	9	22.5
UICC stage				
N0	3	7.1	2	5.0
N1	18	42.9	21	52.5
N2	15	35.7	13	32.5
N3	6	14.3	4	10.0

Abbreviations: rAd-p53, recombinant adenovirus-p53; RT, radiotherapy; UICC, International Union Against Cancer.

Patient Response to Treatment

Between October 1, 2001, andMay1, 2003, 82 patients with NPC were randomly assigned to the group receiving rAd-p53 combined with RT(n_42) or the group receiving RT alone (n_40).No patient withdrew from this study. No significant

differences in sex, age, physical status, histologic type, or clinical stage (including T and N stages) were found between the two groups (Table 1). Baseline average tumor size of the group receiving rAd-p53 combined with RT (882.5; standard deviation [SD], 565.5 mm2) was a little larger than that of the group receiving RT alone (842.7; SD, 599.9mm2), but they were not significantly different (P=.787). In both groups, all patients were in or beyond stage II. Proportion of patients in stages III and IV (M0) was 73.8% (31 of 42) for the group receiving rAd-p53 combined with RT, and 70.0% (28 of 40) for the group receiving RT alone. Average tumor shrinkage rates at 40-Gy dose, 70-Gy dose, and validation time points were 66.9%, 88.4%, and 92.0% for the group receiving rAd-p53 combined with RT, and 44.1%, 64.3%, and 75.1% for the group receiving RT alone, respectively. Response rate of the group receiving rAd-p53 combined with RT was higher than that of the group receiving RT alone at each treatment point (P= .01). At validation time point, the CR rate of the group receiving rAd-p53 combined with RT was 2.73 times that of the group receiving RT alone (66.7% v 24.4%; P=.01; Table 2).

Adverse Events

Total data showed that all the values in blood, urine, and stool examination, and liver and renal function remained within normal range before and after treatment in the group receiving rAd-p53 combined with RT. Lung and heart function were normal before and after treatment. This indicates that rAd-p53 was safe and welltolerated in the patients with NPC. Additionally, rAd-p53 administration did not appear to increase the adverse effects caused by radiation treatment.

All fever events were grade 1 (less than 38°C) and grade 2 (38°C to 40°C), and were transient and self-limited. The incidence of fever among the 42 patients receiving rAd-p53 injections was 81.0% (34 of 42), which included grade 1 fever (43.0%) and grade 2 fever (38.0%). Development of fever was observed as early as approximately 3 hours after injection, lasting about 4 hours and then disappearing spontaneously. Only slight pain and discomfort at local injection sites were felt from the repeat injections. No other adverse effects were found during the subsequent 6 years of follow-up.

6-Year Follow-Up Outcome

The date of last follow-up was December 31, 2007, with a median follow-up duration of 61.0 months (range, 6 to 74 months). No patients were lost to follow-up. Locoregional (nasopharynx and/or neck lymphnode) recurrence had occurred in one patient in the group receiving rAd-p53 combined with RT, and in 10 patients in the group receiving RT alone at the last 6-year follow-up. The median time to recurrence was 73 months (95% CI, 70 to 75 months) in the group receiving rAd-p53 combined with RT, and 59 months (95% CI, 50 to 67 months) in the group receiving RT alone (P=.002). One-, 3-, and 5-year recurrence rates were 0.0%, 2.7%, and 2.7% in the group receiving rAd-p53 combined with RT, and 12.5%, 24.3%, and 28.0% in the group receiving RT alone, respectively (P=.002; Fig 1). Distant metastases were found

in 11 patients in both the group receiving rAd-p53 combined with RT and the group receiving RT alone at the 6-year follow-up. The sites involved were bone (four patients), lung (four patients), and liver (three patients) in the group receiving rAdp53 combined with RT; and bone (four patients), lung (four patients), and liver (three patients) in the group receiving RT alone.

The median time to metastasis was 59 months (95% CI, 51 to 67 months) in the group receiving rAd-p53 combined with RT, and 56 months (95% CI, 48 to 65 months) in the group receiving RT alone (P=.760). One-, 3-, and 5-year metastatic rates were 11.9%, 19.1%, and 26.8% in the group receiving rAd-p53 combined with RT, and 15.1%, 28.8%, and 28.8% in the group receiving RT alone, respectively (P=.760; Fig 2).The median survival time was 59 months (95% CI, 52 to 66 months) in the group receiving rAd-p53 combined with RT, and 54 months (95% CI, 46 to 62 months) in the group receiving RT alone. One-, 3-, and 5-year survival rates were 97.6%, 78.6%, and 66.7% in the group receiving rAd-p53 combined with RT, and 95.0%, 65.0%,and 59.2% in the group receiving RT alone, respectively (P=.340; Fig3). The median disease-free survival time was 58 months (95% CI, 51 to 66 months) in the group receiving rAd-p53 combined with RT, and 49 months (95%CI, 40 to 58 months) in the group receiving RT alone. One-, 3-,and 5-year disease-free survival rates were 90.5%, 81.0%,and 68.7% in the group receiving rAd-p53 combined with RT, and 80.0%, 60.0%, and 57.0% in the group receiving RT alone, respectively(P=.21; Fig 4).

	40 Gy					70 Gy					Validation				
Group	FN	CR	PR	SD	PD	FN	CR	PR	SD	PD	FN	CR	PR	SD	PD
rAd-p53 + RT	42	3	31	8	0	42	21	20	1	0	42	28	13	1	0
RT	57	0	25	32	0	60	5	36	19	0	41	10	25	6	0

Table 2. Response Rate of Group Receiving rAd-p53 Combined With RT Compared With Group Receiving RT Alone. Treatment Time Point, P < .001

Abbreviations: rAd-p53, recombinant adenovirus-p53; RT, radiotherapy; FN, focus number of tumor; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.
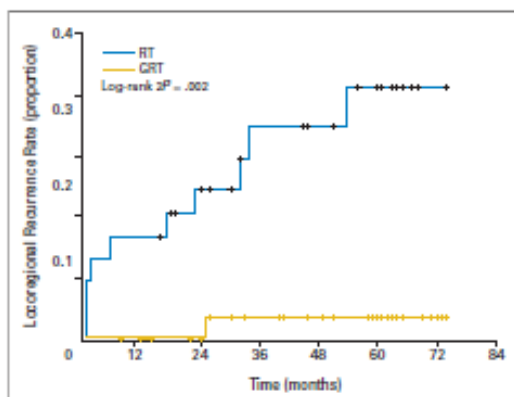

Fig 1. Locoregional recurrence rate of group receiving recombinant adenovirus-p53 (rAd-p53) combined with radiotherapy (GRT) compared with that of group receiving radiotherapy alone (RT).
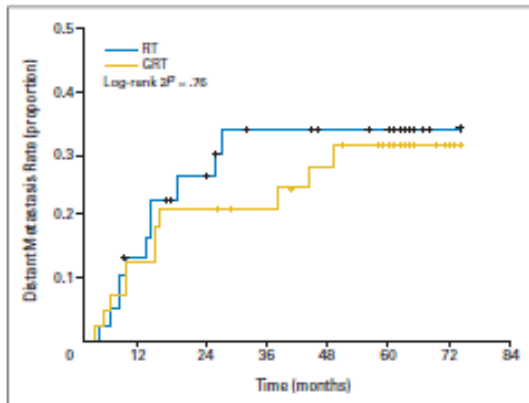
Fig 2. Distant metastasis rate of group receiving recombinant adenovirus-p53 (rAd-p53) combined with radiotherapy (GRT) compared with that of group receiving radiotherapy alone (RT).
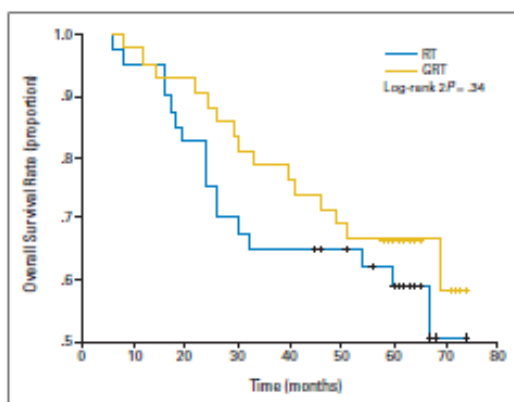

Fig 3. Overall survival rate of group receiving recombinant adenovirus-p53 (rAd-p53) combined with radiotherapy (GRT) compared with that of group receiving radiotherapy alone (RT).

Fig 4. Disease-free survival rate of group receiving recombinant adenovirus-p53 (rAd-p53) combined with radiotherapy (GRT) compared with that of group receiving radiotherapy alone (RT).
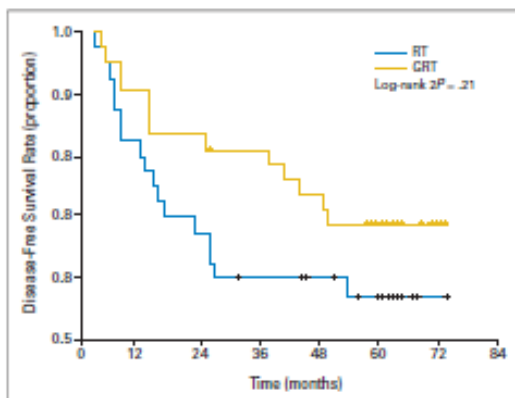
DISCUSSION

Our results indicate that successful exogenous gene transfer of p53 can be achieved by intratumoral administration of rAd-p53. The probable clinical mechanism for increased cytotoxicity by rAd-p53 involved, in part, p53-induced cell cycle arrest and apoptosis by upregulation of p21 and Bax, and induced antiangiogenesis by

downregulation of VEGF. There is similarity of rAd-p53 to the other rAd-p53 trials in the world. In one study, after rAd5CMVp53 (Advexin, INGN 201; Introgen Therapeutics, Austin, TX) injection, p53 gene transfer and p53 specific transgene expression were detected using PCR analysis in esophageal squamous cell carcinoma tumor biopsy from all patients, and mRNA levels of p53, p21, and mdm2 increased in all but one patient.16 In two reports of lung cancer patients, as demonstrated in postinjection biopsies using RT-PCR analysis, vector-specific wild-type p53 mRNA sequences17 and p21 and Bax expression increased significantly after intratumoral delivery of rAd-p53.18 After intraperitoneal administration of rAd-p53 (SCH 58500) for recurrent ovarian cancer, successful exogenous gene transfer and expression of p53; upregulation of p21, Bax, and mdm2; and dowregulation of survivin were observed in postinjection tumor biopsy.19 Induction of mRNA and protein expression of p53 and the p53 target gene P21 were demonstrated in samples from patients with bladder cancer treated with intravesical instillation of rAd-p53 (SCH 58500).20

The same foundational study showed that VEGF expression correlated with high vascular grade. Angiogenesis is required for the growth and progression of malignancies. However, wild-type p53 expression was inversely associated with VEGF expression, s μ ggesting that wild-type p53 is involved in the suppression of the VEGF gene. rAd-p53 infection markedly inhibited the expression of an angiogenic factor (VEGF), and increased the expression of a novel antiangiogenic factor (brain-specific angiogenesis inhibitor1), resulting in reduced neovascularization in vivo. Thus, the antiangiogenic effect of adenoviral-mediated wild-type p53 overexpression may contribute to the ability of this viral vector to inhibit tumor growth.21-24 Our results demonstrated that downregulation of VEGF was observed in tumor biopsy tissue treated by intratumoral injection of rAd-p53. Obvious necrosis was found in tumor biopsy tissues of patients who received intratumoral injections of rAd-p53, which may be correlated with rAd-p53 – induced downregulation of VEGF.

In the last 10 years, adenoviral-mediated P53 gene agent has been used in numerous clinical trials for advanced HNSCC worldwide, either alone or in combination with conventional treatments, such as chemotherapy or RT. rAd-p53 seems to act synergistically with conventional treatments such as chemotherapy and/or RT. In addition, this apparent synergy still exists in patients who were resistant to chemotherapy and RT.13, 14 rAd-p53 therapy has been demonstrated to be safe, feasible, and efficient, including local transgenic expression and evidence of local tumor regression for patients with HNSCC13,14 and for patients with esophageal cancer,16 lung cancer,17,18 ovarian cancer,19 bladder cancer,20 and so on.

rAd-p53 is known to trigger a strong immune response in patients. After intratumoral injection of rAd-p53, we observed infiltration of many lymphocytes in biopsies of tumor lesions of patients enrolled onto the trial. In this study, we demonstrated that the CR rate of locoregional tumors at validation time point for the group receiving rAd-p53 combined with RT was 2.73 times that of the group

receiving RT alone (P= .01). Furthermore, locoregional tumor control was improved after treatment in the clinical CR associated with rAd-p53; at the 6-year follow-up, the group receiving rAd-p53 combined with RT and the group receiving RT alone had developed 5-year locoregional failure rates of 2.7% and 28.0%. Moreover, rAd-p53 improved radiotherapeutic locoregional tumor control by 25.3%, although the differences in survival were not statistically significant. But from a clinical view, achieving a 7.5% increase in the 5-year survival rate and an 11.7% increase in the 5-year disease-free survival rate for patients with advanced NPC should be clinically significant. The statistical insignificance is a result of the small sample size of the trial. In the future, we plan to conduct a trial with a larger sample size to prove the statistical significance of the product. In conclusion, rAd-p53, as a gene therapy agent, can be combined with RT because of its safe and radiosensitized efficacy in patients with NPC, and represents a potential therapeutic implication for patients with advanced cancer.

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