Anti-Cancer Drugs 1999, **10** (suppl 1):S39–S42

New horizons: gene therapy for cancer

José Baselga

Hopital General Universtari Vall d'Hebron, Servico de Oncologia Medica, Barcelona, Spain.

p53 gene mutations appear in numerous human cancers and are associated with anumber of cellular mechanism changes including a lack of apoptosis. Repeated intratumoral injection of the adenoviral p53 vector (Ad5CMV-p53) in patients with non-small cell lung cancer and head and neck cancer is feasible and well tolerated. Treatment results in expression of the p53 transgene and evidence of increased apoptosis. Dose-related antitumor activity has been seen in phase I trials in both lung and head and neck cancer. Transgene expression appears to occur even in patients who mount an immune response to the adenoviral vector. The evidence to date indicates that gene transfer can occur without contamination of health care workers by the vector. There is preliminary clinical evidence suggesting that the *in vivo* synergy seen between Ad5CMV-p53 and cisplatin may also occur in patients. Phase II trials are justified and have been started.

Key words: Ad5CMV-p53, gene therapy, head and neck cancer, lung cancer, p53.

Introduction

The p53 gene is important in regulating (or dysregulating) response to damage in both normal and cancer cells.[1] When the gene is mutated, deleted or the gene product is non-functional, cells do not respond appropriately to DNA damage: they continue to grow instead of dying by apoptosis or shifting to growth arrest (Figure 1). In laboratory cell lines, the re-establishment of normal p53 function by transfection with wild-type p53 results either in apoptosis or in cells moving into G_1 arrest, with inhibition of DNA synthesis and growth.[2–5]

Dr. J Baselga has received honoraria from Rhône-Poulenc Rorer.

Correspondence to José Baselga, Hopital General Universtari Vall d'Hebron, Servico de Oncologia Medica, Passeig de la Vall d'Hebron, 119, 08035 Barcelona, Spain.
Tel: (+34) 93 274 60 77; Fax: (+34) 93 274 60 59;
E-mail: baselg@hg.vhebron.es

p53 is found to be mutated in a large proportion of cancers.[6] According to American Cancer Society estimates in 1995, 56% of the 170 000 annual new cases of lung cancer had p53 mutations. The corresponding figures were 30% for cancer of the prostate, 49% for colorectal cancer and 24% for cancer of the breast. In certain tumors a dysfunctional p53 has been associated with poor survival rates, more aggressive tumor growth, and enhanced tendency for tumor metastases.

Transfer of the p53 gene to cancer cells can be achieved in a variety of ways, arguably the most effective and convenient being the use of an adenoviral vector in which the A1 portion is replaced by a p53 gene expression cassette under the control of a cytomegalovirus (CMV) promoter (Figure 2).

Preclinical work with the vector Ad5CMV-p53 demonstrated that transfection reduced growth in murine tumor models expressing either wild-type or mutant p53.[7] The p53 vector proved capable of inducing apoptosis in tumor cells, including those which already express p53, while the growth and histomorphology of non-malignant fibroblasts was unaffected.[7–9] Data in nude mice demonstrate that local therapy with Ad5CMV-p53 induces tumor regression.[10] Preclinical studies have also evaluated the effect of combining cisplatin with Ad5CMV-p53 on the growth of lung tumor cell lines, results showed this combination to have a profound inhibition of tumor growth in comparison to tumors treated with cisplatin alone.[10]

Clinical experience of p53 replacement

Non-small cell lung cancer (NSCLC)

Sufficient data are now available from clinical trials to demonstrate that p53 replacement may be a worthwhile approach. The first p53 gene therapy clinical trial, conducted at the University of Texas,

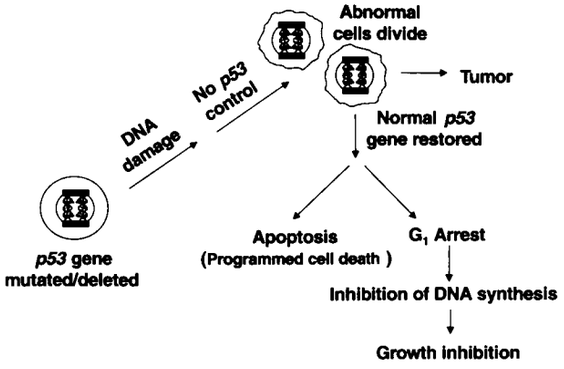

Figure 1. Mechanism of action of p53.

MD Anderson Cancer Center in Houston, examined the safety and activity of retrovirus-mediated delivery of a wild-type p53 gene to NSCLC.[11] The first evidence of activity was observed in this phase I study of nine patients with NSCLC who were given a single injection not of an adenoviral but a retroviral p53 vector.[11]

In four patients, the lesion was endobronchial and the vector was administered via a bronchoscope. In four patients with chest wall lesions, the vector was administered via a needle under computed tomography (CT) control; and in the final patient, whose sole site of metastatic disease was an adrenal mass, treatment was also locally delivered. Treatment was well tolerated, with side-effects restricted to transient pain at the injection site.

Successful gene transfer was demonstrated in eight of nine patients by using polymerase chain reaction and *in situ* hybridization to reveal retroviral DNA integration in tumor cells. (The single exception was a patient who did not complete the planned course of therapy.) Moreover, an increase in TUNEL staining, which suggests apoptosis, provided indirect evidence of p53 transgene expression in six of the seven patients analyzed.

There was also evidence of clear anti-tumor activity: among the seven evaluable patients, three showed evidence of tumor regression in the treated lesions and a further three showed disease stabilization (defined by lack of growth for 8 weeks or more) at these sites. In contrast to the situation at treated sites, untreated lesions continued to progress in all of these patients.

Infection is easier with an adenoviral vector than with a retrovirus, there is specificity for dividing cells, longer and higher levels of gene expression can be achieved, there is likely to be less toxicity to normal cells, and comfort can be drawn from the long history of adenoviral use in vaccines. For all of these reasons, subsequent clinical development concentrated on adenoviral p53 transfer.

Two phase I studies involving adenovirus-mediated transfer of the p53 gene are currently ongoing at the University of Texas, MD Anderson Cancer Center.[12–14] The first study is evaluating the safety and activity of the Ad5CMV-p53 (an adenoviral type 5 vector containing a wild-type p53 gene controlled by the CMV promoter) in head and neck cancer.[14] The second study is examining the safety of sequential administration of Ad5CMV-p53 with cisplatin for the treatment of NSCLC.[13]

In the phase I study of 50 patients with NSCLC tumors showing a p53 mutation, doses of adenoviral vector escalating from 10^6 to 10^9 pfu were injected intratumorally under the direction of CT scanning or via a bronchoscope.[13] In certain patients, Ad5CMV-p53 was administered together with cisplatin 80 mg/m^2. DNA-PCR evidence of gene transfer was found in 13 of 18 patients tested. On the TUNEL assay for evaluating

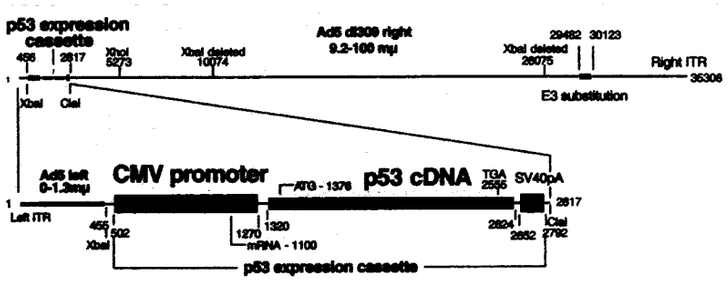

Figure 2. Components of Ad5CMV-p53.

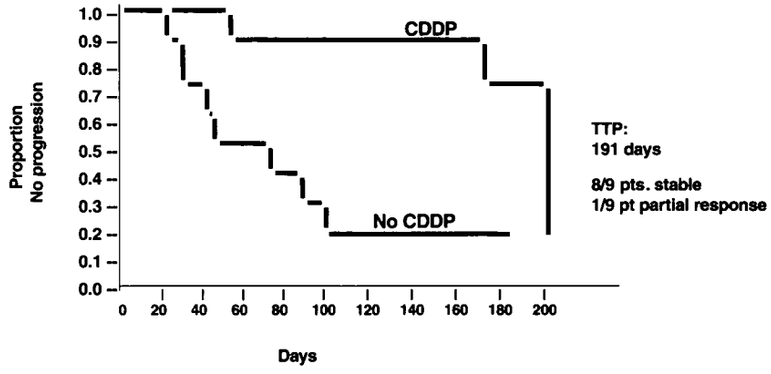

Figure 3. Time to progression (correlated with dose) of a phase I study of Ad5CMV-p53 in NSCLC.[13]

apoptosis, increased levels of staining were found, and immunohistochemistry and reverse-transcriptase PCR also suggested gene expression.

A modest level of dose-related anti-tumor activity was evident. Thus, of five patients treated with the lower doses of 10^6 or 10^7 pfu, all but one progressed. However, four of six patients treated with 10^8 or 10^9 pfu had stable disease and one a partial response. Progression-free survival was a median of 56 days in patients receiving low doses and 167 days in the higher-dose group (Figure 3).This evidence led to the selection of the higher-dose approach for phase II trial.

There was also a clear relationship between concomitant use of cisplatin and slower time to progression.

Head and neck cancer

The second phase I study was conducted in 33 patients with incurable head and neck squamous cell cancer who received between 10^6 and 10^{11} pfu of Ad5CMV-p53.[14] In this study, patients with tumors expressing wild-type p53 were also eligible. Patients who were to undergo palliative resection were treated three times a week for 2 weeks, with a seventh dose injected into the tumor margin after surgery and an eighth dose after 72 h. In non-resected patients, six doses were given over 2 weeks to one site of disease.

Importantly, transgene expression sustained over a period as long as 67 days post-dosing was detected by reverse-transcriptase PCR even in certain patients who showed a substantial increase in anti-adenoviral antibody. The development of a marked immune response to the vector therefore does not appear to prevent gene transfer and expression.

Issues of biodistribution, dissemination and safety were addressed by attempts to detect Ad5CMV-p53 in body fluids. Despite the intratumoral method of injection, Ad5CMV-p53 was evident in the blood within 30–90 min in all patients receiving a dose of 10^{10} or 10^{11} pfu. However, the virus had been cleared from the blood within 24 h in all cases. Ad5CMV-p53 was detected in the urine in 66–100% of patients who received the 10^{10} or 10^{11} pfu dose. Nevertheless, no virus was detected in the body secretions or blood of health workers caring for the patients studied, and their anti-adenoviral antibody titers did not rise over the period of treatment.

There were indications of anti-tumor activity. Among the 12 patients treated with 10^9–10^{11} pfu of the vector who were not resected, a partial response was seen in two (Table 1). Perioperative and postoperative administration of Ad5CMV-p53 had no adverse effect on surgical morbidity or wound healing.

Table 1. Anti-tumor activity in indicator lesions of Ad5CMV-p53 in advanced head and neck cancer[14]

Dose (pfu)	10^6–10^9	10^9–10^{11}
No. of patients	21	12
Non-resectable (n = 18)	SD: 5 Prog: 7 NE:1	PR: 2 SD: 1 Prog: 2
Resectable (n = 15)	CR: 1 NA: 7	NA: 7

SD, stable disease; Prog, progressive; NE, non-evaluable; partial response; CR, complete response; NA, not available

In a multinational phase II trial, 78 patients with recurrent or refractory head and neck cancer are being randomized to one of two schedules of single-agent Ad5CMV-p53. In the first arm, patients are treated on days 1, 2 and 3 every 4 weeks; in the second arm, they receive treatment on days 1, 3, 5, 8, 10 and 12 every 4 weeks. The doses administered range from 2×10^{10} to 10×10^{10}, according to tumor burden. The endpoints are response rate, time to progression and overall survival.

Summary

Certain patients have already demonstrated highly encouraging responses to Ad5CMV-p53 treatment. However, additional clinical data are definitely needed regarding this exciting new treatment modality. Future directions of research include the combination of Ad5CMV-p53 with radiation, which experiments involving human tumor xenografts in nude mice suggest may be effective. In terms of combination with chemotherapy, existing evidence of synergy with cisplatin is now being complemented by evidence of enhanced efficacy when Ad5CMV-p53 is combined with docetaxel.

References

1. Shimamura A, Fisher DE. p53 in life and death. *Clin Cancer Res* 1996; **2**: 435–40.
2. Takahashi T, Carbone D, Takahashi T, *et al.* Wild-type but not mutant p53 suppresses the growth of human lung cancer cells bearing multiple genetic lesions. *Cancer Research* 1992; **52**: 2340–3.
3. Georges RN, Mukhopadhyay T, Zhang J, *et al.* Prevention of orthotopic human lung cancer growth by intracheal instillation of a retroviral antisense K-*ras* construct. *Cancer Res* 1993; **53**: 1743–6.
4. Fujiwara T, Cai DW, Georges RN, *et al.* Therapeutic effect of a retroviral wild-type p53 expression vector in an orthotopic lung cancer model. *J Natl Cancer Inst* 1994; **86**: 1458–62.
5. Cai DW, Mukhopadhyay T, Liu YJ, *et al.* Stable expression of wild-type p53 gene in human lung cancer cells after retrovirus-mediated gene transfer. *Hum Gene Ther* 1993; **4**: 617–24.
6. Harris CC. Structure and function of the p53 tumor suppressor gene: clues for rational cancer therapeutic strategies. *J Natl Cancer Inst* 1996; **88**: 1442–55.
7. Clayman GL, El-Naggar AK, Roth JA, *et al. In vivo* molecular therapy with p53 adenovirus for microscopic residual head and neck squamous carcinoma. *Cancer Res* 1995; **55**: 1–6.
8. Liu T-J, Zhang W-W, Taylor DL, Roth JA, Goepfert H, Clayman GL. Growth suppression of human head and neck cancer cells by the introduction of a wild-type p53 gene via a recombinant adenovirus. *Cancer Res* 1994; **54**: 3662–7.
9. Zhang W-W, Fang W, Mazur W, French BA, Georges RN, Roth JA. High-efficiency gene transfer and high-level expression of wild-type p53 in human lung cancer cells mediated by recombinant adenovirus. *Cancer Gene Ther* 1994; **1**: 5–13.
10. Nguyen DM, Spitz FR, Yen N, *et al.* Gene therapy for lung cancer: enhancement of tumor suppression by a combination of sequential systemic cisplatin and adenovirus-mediated p53 gene transfer. *J Thorac Cardiovasc Surg* 1996; **112**: 1372–7.
11. Roth JA, Nguyen D, Lawrence DD, *et al.* Retrovirus-mediated wild-type p53 gene transfer to tumors of patients with lung cancer. *Nat Med* 1996; **2**: 985–91.
12. Roth JA, Swisher SG, Merrit JA, *et al.* Gene therapy for non-small cell lung cancer: A preliminary report of a phase I trial of adenoviral p53 gene replacement. *Semin Oncol* 1998; **25**: 33–7.
13. Swisher SG, Roth JA, Lawrence DD, *et al.* Adenoviral-mediated p53 gene transfer in patients with advanced NSCLC. *Proc Am Soc Clin Oncol* 1997; **16**: 437a (abstr 1565).
14. Clayman GL, El-Naggar AK, Merritt J, *et al.* Adenovirus-mediated p53 transfer in a phase I trial of patients with advanced recurrent head and neck squamous carcinoma. *Proc Am Soc Clin Oncol* 1997; **16**: 383a (abstr 1363).