

Recent Advances in Esophageal Cancer Gene Therapy

Hideaki Shimada, MD, FACS,¹ Kazuyuki Matsushita, MD,¹ and Masatoshi Tagawa, MD²

In recent years, the development of gene therapy systems as new treatment or prevention strategies for various malignant diseases has been explored. Based on the genetic background of esophageal cancer, several molecular therapies have been developed. In this article, we review p53 genetic alterations and angiogenesis in esophageal cancer. Our recent clinical results from a phase I/II study of adenoviral-mediated p53 gene therapy for patients with un-resectable esophageal cancer are reviewed. Lastly we review the available experimental therapeutic models from the literature and our experimental work over the past few years are reviewed. (Ann Thorac Cardiovasc Surg 2008; 14: 3–8)

Key words: p53, angiogenesis, gene therapy, esophageal cancer, clinical study

Introduction

Because surgical resection alone rarely results in long-term survival for advanced esophageal cancer,¹⁾ much effort is now focused on combined multi-modality treatments in an attempt to improve local control of tumor growth and to eliminate micro-metastasis present at the time of surgery. Recently, the use of neoadjuvant chemoradiotherapy followed by esophagectomy has become a widespread approach due to several favorable reports.^{2,3)} However, the prognosis remains extremely poor in un-resectable patients.⁴⁾ In this context, it will be necessary to enhance the anti-tumor efficacy of chemotherapy and radiotherapy in order to improve the outcome of the multidisciplinary therapy.

Molecular Targets in Esophageal Cancer Treatment

p53 abnormalities are observed in 40–60% of patients with esophageal cancer, even in an early precancerous

lesion.^{5,6)} Indeed, p53 genetic alteration is a good predictor for treatment responses and survival in esophageal cancer.^{7–10)} Genetic analysis can be used as a prognostic marker for esophageal carcinoma. p53 genotyping may define a subset of patients who respond to chemotherapy/radiotherapy and may predict who potentially benefit from multimodality therapy.⁷⁾

Angiogenic and growth factors also play an essential role in the process of growth, metastasis and treatment resistance of esophageal carcinoma.¹¹⁾ Among several angiogenic factors, vascular endothelial growth factor (VEGF) has been shown to be vital for resistance to treatment.¹²⁾ Immunohistochemical analyses of esophageal carcinomas have revealed that VEGF expression, dThdPase expression, and angiogenesis are good predictors for treatment responses and survival in esophageal cancer.^{13–16)} Based on reports that indicate a significant association between angiogenesis and p53 expression, it is anticipated that wild type p53 gene transfer into cancer cells may down-regulate angiogenesis. Hepatocyte growth factor (HGF) also contributes to invasion and metastasis of esophageal carcinoma cells (unpublished data).

Clinical Trials of p53 Gene Therapy

In preclinical tumor models, p53 gene transfer as mediated by retroviral or adenoviral expression vectors restored drug and radiation sensitivity or directly induced

From ¹Department of Frontier Surgery, Chiba University Graduate School of Medicine, Chiba; and ²Division of Pathology, Chiba Cancer Center Research Institute, Chiba, Japan

Address reprint requests to Hideaki Shimada, MD, FACS: Department of Frontier Surgery, Chiba University Graduate School of Medicine, Chiba 260–8670, Japan.

apoptosis.¹⁷⁻¹⁹ On the basis of its preclinical efficacy, adenovirus-mediated wild-type (wt) *p53* gene transfer or wt *p53* gene transfer in combination with cisplatin chemotherapy were studied in phase I trials in patients with advanced lung cancer.²⁰⁻²² These studies established the safety and feasibility of injections of adenoviral *p53* expression vectors into tumor lesions. They also provided evidence for in vivo transgene expression as well as for efficacy in terms of surrogate markers of biologic activity and some tumor remissions.

Moreover, adenovirus-mediated *p53* gene transfer through intrahepatic arterial administration or intravenous injection were revealed to be safe and to efficiently induce biological activities. Only limited and transient inflammatory responses to the drug occurred, but pre-existing immunity to adenoviruses did not preclude adenovirus-mediated *p53* gene delivery.²³ Repeated intravenous administration also demonstrated no severe toxicities, the presence of circulating adenovirus 24 h after administration, and detectable *p53* transgene within tumor tissue distant from the site of administration.²⁴

A Phase I/II Study of *p53* Esophageal Cancer Gene Therapy

Based on the preclinical studies of adenoviral-mediated *p53* gene (Ad5CMV-*p53*) delivery on esophageal cancer cells,²⁵ a Phase I/II study was approved by the Chiba University Graduate School of Medicine Institutional Review Board and the Recombinant DNA Advisory Committee of the National Institutes of Health Office of Biotechnology Activities.²⁶ Eligible patients were selected among those who were not subjected to radical surgery. Individual doses were 10 to 25×10¹¹ viral particles (VP) depending on tumor size. On a 28-day cycle, intra-tumoral injections of Ad5CMV-*p53* were administered on days 1 and 3, and the patients were treated for up to five cycles.

A total of 10 patients were enrolled in this trial (Table 1). On commencement of gene therapy, five patients had tumor invasion into adjacent organs (Patient 3, 4, 7, 9, and 10) and four had high risk factors for surgery (Patients 1, 2, 5, and 8). The remaining patient had a T3 tumor with multiple lymph node metastases (Patient 6). Eight patients had tumors with *p53* mutations among exons 5 to 8. Treatment cycles ranged from one to five cycles, with two being the median number of cycles. Patient 6 did not undergo a second cycle of the treatment because he showed rapid progression of distant metastases after

the first cycle. The most common adverse events were fever and local pain (NCI grade 1 or 2). Fever was observed in all patients and pain in 30%. Overall, drug administration was feasible and well tolerated.

Nine of the 10 eligible patients have died of the disease. One patient had local tumor progression and four patients had systemic progression during the initial 56 days. Six of 10 patients were alive for more than one year (Table 1). Three patients showed no evidence of tumor on multiple biopsies after the treatment (Patients 2, 3, and 5). Patient 2 had a tumor with a deep ulcer before the treatment. After three or four cycles, the ulcer had changed to a mild shape. No viable cancer cells were observed in biopsy specimens. Stable local tumor and no residual squamous cell carcinoma (SCC) were found on esophageal biopsies during the five cycles of the treatment. Patient 3 had a large 50 mm mass and was unable to swallow at the initiation of gene therapy. After two injections of Ad5CMV-*p53*, he was able to swallow liquid and meals. Although the endoscope could not pass through the stenotic portion of the lesion before the treatment, it passed through after the treatment. Patient 5 did not show tumor progression for 24 months after initial gene therapy. Although a few viable cancer cells were observed in biopsy specimens at 24 months, the tumor was well controlled by argon-plasma coagulation. He currently remains progression-free and alive 65 months after completion of the treatment. The overall survival rate was 60% at one year. Four patients have developed clinically diagnosed distant metastases (two cases of bone metastasis, one of liver and lymph node metastasis, and one of lung metastasis). Three of these four patients with development of distant metastases died of the disease within 3 months after initial viral treatment. The median time to local as well as distant progression was 6 months (Fig. 1).

Our clinical results demonstrate that administration of Ad5CMV-*p53* via intratumoral injection is feasible, safe and well-tolerated in a population of patients with chemoradiation resistant esophageal SCC. Acute NCI grade 1 or 2 toxicities, including local pain and fever, were acceptable and provided further evidence of biological activity from the treatment. Transduction of the vector-*p53* gene into tumor tissue was confirmed by vector-specific DNA-PCR analysis in all patients' biopsy specimens. Although 80% of patients showed increased *p53* mRNA after treatment and 75% of these showed elevated *p21* and *MDM2* downstream markers, the increase of RT-PCR signals was more minor than we expected. This could partly be explained by the differences in bi-

Table 1. Characteristics of the patients with esophageal squamous cell carcinoma treated with Ad.CMV-p53

Patient No.	Age & gender	Time to tumor	Site of p53 progression ^a	Viral particles mutation	Treatment cycles	Tumor size	Local & overall response ^d	Prognosis after GT ^e
1	64M	7M	Exon 5	15 × 10 ¹¹	2	43 mm	SD & SD	19M dead
2	71M	7M	Exon 7	10 × 10 ¹¹	5	25 mm	SD & SD	15M dead
3	62M	5M	Negative ^b	15 × 10 ¹¹	3	50 mm	SD & PD	3M dead
4	78M	2M	Exon 8	10 × 10 ¹¹	2	30 mm	PD & PD	6M dead
5	66M	2M	Exon 7	10 × 10 ¹¹	3	40 mm	SD & SD	65M alive
6	60M	1M	Exon 7	20 × 10 ¹¹	1	70 mm	SD & PD	2M dead
7	67F	4F	Exon 5	10 × 10 ¹¹	2	38 mm ^c	SD & SD	13M dead
8	58M	8M	Exon 6	10 × 10 ¹¹	2	40 mm	SD & SD	15M dead
9	48M	2M	Exon 7	25 × 10 ¹¹	4	100 mm	SD & SD	12M dead
10	77M	12M	Negative ^b	20 × 10 ¹¹	2	68 mm	SD & PD	2M dead

^aTime to tumor progression after completion of chemo-radiation therapy. ^bNo mutation among exons 5, 6, 7, and 8. ^cTumor size was sum of esophageal tumor and lymph node. ^dTreatment response was determined 4 weeks after completion of therapy by external review board. ^eSurvival months after first injection of AdCMV-p53. SD, stable disease; PD, progressive disease.

opsy sites. All patients were evaluated for evidence of antitumor activity and clinical benefit. Six patients the disease was established both the local site as well as systemically and survived more than one year after starting treatment. Among these six patients, one is alive as of today, more than 5 years after completion of gene therapy.

Future Clinical Trial of p53 Gene Therapy Combined with Chemo-Radiotherapy or Immunotherapy

According to recent reports, adenovirus-mediated p53 gene transfer is frequently used, together with *cis*-dichloro-diammineplatinum(II) administration or ionizing radiation.^{22,27,28} A phase II trial of adenovirus-mediated p53 gene transfer in conjunction with radiation therapy or chemotherapy for lung cancer patients was safe and effective.^{27,28} Because treatment response is strongly associated with survival, a combination treatment with standard chemoradiation and Ad5CMV-p53 may be an attractive modality. Further preclinical studies are required to develop a new combination therapy with CDDP, 5-FU, and Ad5CMV-p53, and also a combination with concurrent chemoradiotherapy (Fig. 2).

Another promising combination treatment with p53 gene transfer is dendritic cell (DC) therapy. Recently, the immunologic and clinical effects of a cancer vaccine consisting of DC transduced with the full-length wild-type p53 gene delivered via an adenoviral vector in patients with extensive stage small cell lung cancer were reported.²⁹ They reported that p53-specific T cell responses

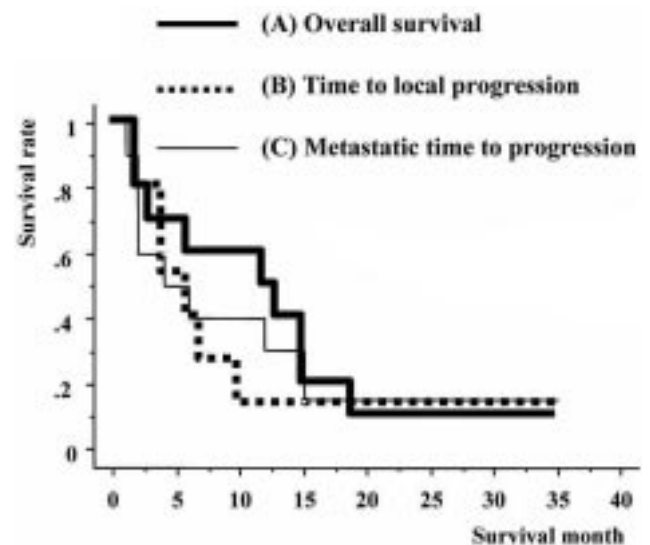


Fig. 1. Overall survival (A), time to local progression (B), and time to metastatic progression of all patients (C).

to vaccination were observed in 57.1% of patients. They observed a high rate of objective clinical responses to chemotherapy (61.9%) that immediately followed vaccination. They supposed that optimal use of vaccination might be more effective, not as a separate modality, but in direct combination with chemotherapy.

Anti-Angiogenesis Gene Therapy

Based on the fact that VEGF, HGF, and other angiogenic factors play a definite role in tumor invasion and metastasis in esophageal carcinoma,¹¹⁻¹⁶ anti-angiogenic gene

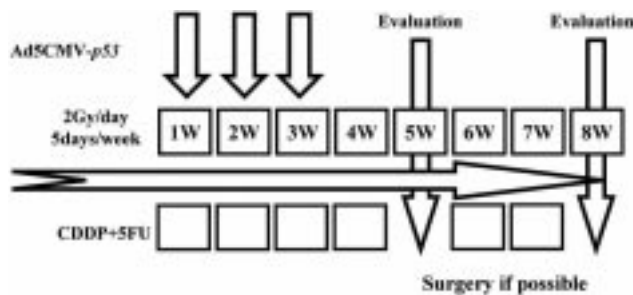


Fig. 2. Protocol of *p53* gene therapy combined with chemoradiation for clinical T4 esophageal carcinoma.

therapy might also be a favorable approach against advanced stage esophageal carcinoma. Recently, NK4, four-kringles containing an intra-molecular fragment of HGF was isolated as a competitive antagonist for the HGF-c-Met system.³⁰ Independent of its HGF-antagonist action, NK4 inhibited angiogenesis induced by VEGF and basic fibroblast growth factor, as well as HGF, indicating that NK4 is a bifunctional molecule that acts as an HGF-antagonist and angiogenesis inhibitor. In experimental models of distinct types of cancers, NK4 protein administration or NK4 gene therapy inhibited tumor invasion, metastasis, and angiogenesis-dependent tumor growth.^{31,32} Esophageal carcinoma cell treatment with NK4 also proved to induce malignant tumors to be “static” in both tumor growth and spreading.³³ Based on the preclinical studies of NK4 gene delivery, a Phase I/II study was approved by the Chiba University Graduate School of Medicine Institutional Review Board.

Double Gene Transfer to Enhance Single Gene Therapy

Some reports have shown that double gene transfer enhanced the anti-tumor activity of single gene transfer.^{34–37} They suggested that *p14*, *p16*, and *p33* genes were able to enhance apoptotic tumor cell death with *p53* gene therapy. We also suggested a role for *mdm2* in the synergistic effects observed in combined *p33* and *p53* gene transfer into esophageal carcinoma cells.³⁶ Although stable expression of both genes in the same tumor cells was supposedly achieved for double gene therapy,³⁹ this mode of gene therapy should undergo clinical study in the near future.

Oncolytic Virus Therapy

Conventional cancer gene therapy, even if considered to

temporarily suppress tumor growth, does not inhibit extended tumor growth and distant metastases. A phase II trial, using an E1B-deleted adenovirus (ONYX-015) designed to selectively lyse *p53*-deficient cancer cells, was reported in which the virus was administered to patients via a direct intratumoral injection for untreated oral squamous cell carcinoma.⁴⁰ They demonstrated that the virus replicates selectively in the tumor as opposed to normal tissue after this direct injection. No adverse effects of the viral injection were noted. Those data supported the concept that ONYX-015 is replication deficient in normal, compared with cancerous, tissues and has potential as a selective anticancer agent against tumor tissues.

Therefore, we examined the potential clinical feasibility of virus-mediated tumor destruction, which can further suppress tumor growth. We showed that human tumors were more susceptible to Ad in which the E1A expression was controlled by a putative tumor promoter rather than normal cells, and that a replication of the Ad was greater in tumor cells than in normal cells.⁴¹ The promoter region of the midkine gene was a good candidate to drive therapeutic genes in a tumor specific manner through conditionally replicative adenovirus.⁴²

Immunology Based Gene Therapy

Antitumor immune responses are initiated after the acquisition of putative tumor antigens by dendritic cells (DCs). Therefore, enhanced antigen presentation is a crucial step for the early phase of cell-mediated immunity.^{41,43} Destruction of tumors can release the tumor antigens and DCs come to recognize them thereafter. Activation of DCs induced production of a number of cytokines, and we showed that the interleukin-2 or 12 family secreted from tumors could induce systemic antitumor immunity.^{41,43}

DC-based immunotherapy has been clinically evaluated, but it still requires modification to improve the outcomes. We previously demonstrated that F-gene–deleted non-transmissible Sendai virus (SeV/dF)–activated DCs (DCs/SeV/dF) have strong antitumor effects against squamous cell carcinoma.⁴⁴ SeV/dF shows high transfection efficiency to DCs and leads them to up-regulate co-stimulatory molecules. Intratumoral injection of DCs/SeV/dF resulted in a marked and representative inhibition of the tumor, even when the tumors were well-vascularized. Results from these preclinical studies are promising for further clinical trials to evaluate the best combination of molecular approaches against esophageal carcinoma.

Acknowledgment

This work was supported, in part, by the 21st Century COE (Center of Excellence) Programs Grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

- Shimada H, Okazumi S, Matsubara H, Nabeya Y, Shiratori T, et al. Impact of the number and extent of positive lymph nodes in 200 patients with thoracic esophageal squamous cell carcinoma after three-field lymph node dissection. *World J Surg* 2006; **30**: 1441–9.
- Shinoda M, Hatooka S, Mori S, Mitsudomi T. Clinical aspects of multimodality therapy for resectable locoregional esophageal cancer. *Ann Thorac Cardiovasc Surg* 2006; **12**: 234–41.
- Nabeya Y, Ochiai T, Matsubara H, Okazumi S, Shiratori T, et al. Neoadjuvant chemoradiotherapy followed by esophagectomy for initially resectable squamous cell carcinoma of the esophagus with multiple lymph node metastasis. *Dis Esophagus* 2005; **18**: 388–97.
- Shimada H, Shiratori T, Okazumi S, Matsubara H, Nabeya Y, et al. Has surgical outcomes of pathologic T4 esophageal squamous cell carcinoma really improved? Analysis of 268 cases during 45 years of experience—. *J Am Coll Surg* 2008; **206**: 48–56.
- Hollstein MC, Metcalf RA, Welsh JA, Montesano R, Harris CC. Frequent mutation of the p53 gene in human esophageal cancer. *Proc Natl Acad Sci USA* 1990; **87**: 9958–61.
- Gao H, Wang LD, Zhou Q, Hong JY, Huang TY, et al. p53 tumor suppressor gene mutation in early esophageal precancerous lesions and carcinoma among high-risk populations in Henan, China. *Cancer Res* 1994; **54**: 4342–6.
- Ribeiro U Jr, Finkelstein SD, Safatle-Ribeiro AV, Landreneau RJ, Clarke MR, et al. p53 sequence analysis predicts treatment response and outcome of patients with esophageal carcinoma. *Cancer* 1998; **83**: 7–18.
- Shimada H, Nabeya Y, Okazumi S, Matsubara H, Funami Y, et al. Prognostic significance of serum p53 antibody in patients with esophageal squamous cell carcinoma. *Surgery* 2002; **132**: 41–7.
- Shimada H, Kitabayashi H, Nabeya Y, Okazumi S, Matsubara H, et al. Treatment response and prognosis of patients after recurrence of esophageal cancer. *Surgery* 2003; **133**: 24–31.
- Shimada H, Okazumi S, Takeda A, Nabeya Y, Matsubara H, et al. Presence of serum p53 antibodies is associated with decreased in vitro chemosensitivity in patients with esophageal cancer. *Surg Today* 2001; **31**: 591–6.
- Vallböhmer D, Lenz HJ. Predictive and prognostic molecular markers in outcome of esophageal cancer. *Dis Esophagus* 2006; **19**: 425–32.
- Gorski DH, Beckett MA, Jaskowiak NT, Calvin DP, Mauceri HJ, et al. Blockage of the vascular endothelial growth factor stress response increases the antitumor effects of ionizing radiation. *Cancer Res* 1999; **59**: 3374–8.
- Shah MA, Ramanathan RK, Ilson DH, Levnor A, D'Adamo D, et al. Multicenter phase II study of irinotecan, cisplatin, and bevacizumab in patients with metastatic gastric or gastroesophageal junction adenocarcinoma. *J Clin Oncol* 2006; **24**: 5201–6.
- Shimada H, Takeda A, Nabeya Y, Okazumi SI, Matsubara H, et al. Clinical significance of serum vascular endothelial growth factor in esophageal squamous cell carcinoma. *Cancer* 2001; **92**: 663–9.
- Shimada H, Takeda A, Shiratori T, Nabeya Y, Okazumi S, et al. Prognostic significance of serum thymidine phosphorylase concentration in esophageal squamous cell carcinoma. *Cancer* 2002; **94**: 1947–54.
- Shimada H, Hoshino T, Okazumi S, Matsubara H, Funami Y, et al. Expression of angiogenic factors predicts response to chemoradiotherapy and prognosis of oesophageal squamous cell carcinoma. *Br J Cancer* 2002; **86**: 552–7.
- Fujiwara T, Cai DW, Georges RN, Mukhopadhyay T, Grimm EA, 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.
- Wills KN, Maneval DC, Menzel P, Harris MP, Sutjipto S, et al. Development and characterization of recombinant adenoviruses encoding human p53 for gene therapy of cancer. *Hum Gene Ther* 1994; **5**: 1079–88.
- Spitz FR, Nguyen D, Skibber JM, Meyn RE, Cristiano RJ, et al. Adenoviral-mediated wild-type p53 gene expression sensitizes colorectal cancer cells to ionizing radiation. *Clin Cancer Res* 1996; **2**: 1665–71.
- Schuler M, Rochlitz C, Horowitz JA, Schlegel J, Perruchoud AP, et al. A phase I study of adenovirus-mediated wild-type p53 gene transfer in patients with advanced non-small cell lung cancer. *Hum Gene Ther* 1998; **9**: 2075–82.
- Swisher SG, Roth JA, Nemunaitis J, Lawrence DD, Kemp BL, et al. Adenovirus-mediated p53 gene transfer in advanced non-small-cell lung cancer. *J Natl Cancer Inst* 1999; **91**: 763–71.
- Nemunaitis J, Swisher SG, Timmons T, Connors D, Mack M, et al. Adenovirus-mediated p53 gene transfer in sequence with cisplatin to tumors of patients with non-small-cell lung cancer. *J Clin Oncol* 2000; **18**: 609–22.
- Atencio IA, Grace M, Bordens R, Fritz M, Horowitz JA, et al. Biological activities of a recombinant adenovirus p53 (SCH 58500) administered by hepatic arterial infusion in a Phase 1 colorectal cancer trial. *Cancer Gene Ther* 2006; **13**: 169–81.
- Tolcher AW, Hao D, de Bono J, Miller A, Patnaik A, et al. Phase I, pharmacokinetic, and pharmacodynamic study of intravenously administered Ad5CMV-p53, an

- adenoviral vector containing the wild-type p53 gene, in patients with advanced cancer. *J Clin Oncol* 2006; **24**: 2052–8.
25. Shimada H, Shimizu T, Ochiai T, Liu TL, Sashiyama H, et al. Preclinical study of adenoviral p53 gene therapy for esophageal cancer. *Surg Today* 2001; **31**: 597–604.
 26. Shimada H, Matsubara H, Ochiai T, Shiratori T, Miyazaki S, et al. Phase I/II adenoviral p53 gene therapy for chemoradiation resistant advanced esophageal squamous cell carcinoma. *Cancer Sci* 2006; **97**: 554–61.
 27. Swisher SG, Roth JA, Komaki R, Gu J, Lee JJ, et al. Induction of p53-regulated genes and tumor regression in lung cancer patients after intratumoral delivery of adenoviral p53 (INGN 201) and radiation therapy. *Clin Cancer Res* 2003; **9**: 93–101.
 28. Schuler M, Herrmann R, De Greve JL, Stewart AK, Gatzemeier U, et al. Adenovirus-mediated wild-type p53 gene transfer in patients receiving chemotherapy for advanced non-small-cell lung cancer: results of a multicenter phase II study. *J Clin Oncol* 2001; **19**: 1750–8.
 29. Antonia SJ, Mirza N, Fricke I, Chiappori A, Thompson P, et al. Combination of p53 cancer vaccine with chemotherapy in patients with extensive stage small cell lung cancer. *Clin Cancer Res* 2006; **12** (3 Pt 1): 878–87.
 30. Matsumoto K, Nakamura T. Mechanisms and significance of bifunctional NK4 in cancer treatment. *Biochem Biophys Res Commun* 2005; **333**: 316–27.
 31. Wen J, Matsumoto K, Taniura N, Tomioka D, Nakamura T. Inhibition of colon cancer growth and metastasis by NK4 gene repetitive delivery in mice. *Biochem Biophys Res Commun* 2007; **358**: 117–23.
 32. Ogura Y, Mizumoto K, Nagai E, Murakami M, Inadome N, et al. Peritumoral injection of adenovirus vector expressing NK4 combined with gemcitabine treatment suppresses growth and metastasis of human pancreatic cancer cells implanted orthotopically in nude mice and prolongs survival. *Cancer Gene Ther* 2006; **13**: 520–9.
 33. Shimada H, Shiratori T, Matsushita K, Ochiai T. A Phase I/II study of NK4 gene therapy for digestive squamous cell carcinoma. Proceedings of 65th Annual Meeting of the Japanese Cancer Association. 2006; pp. 267.
 34. Lee YJ, Galoforo SS, Battle P, Lee H, Corry PM, et al. Replicating adenoviral vector-mediated transfer of a heat-inducible double suicide gene for gene therapy. *Cancer Gene Ther* 2001; **8**: 397–404.
 35. Freytag SO, Khil M, Stricker H, Peabody J, Menon M, et al. Phase I study of replication-competent adenovirus-mediated double suicide gene therapy for the treatment of locally recurrent prostate cancer. *Cancer Res* 2002; **62**: 4968–76.
 36. Boucher PD, Im MM, Freytag SO, Shewach DS. A novel mechanism of synergistic cytotoxicity with 5-fluorocytosine and ganciclovir in double suicide gene therapy. *Cancer Res* 2006; **66**: 3230–7.
 37. Sandig V, Brand K, Herwig S, Lukas J, Bartek J, et al. Adenovirally transferred p16INK4/CDKN2 and p53 genes cooperate to induce apoptotic tumor cell death. *Nat Med* 1997; **3**: 313–9.
 38. Shimada H, Liu TL, Ochiai T, Shimizu T, Haupt Y, et al. Facilitation of adenoviral wild-type p53-induced apoptotic cell death by overexpression of p33 (ING1) in T. Tn human esophageal carcinoma cells. *Oncogene* 2002; **21**: 1208–16.
 39. Shimizu T, Shimada H, Ochiai T, Hamada H. Enhanced growth suppression in esophageal carcinoma cells using adenovirus-mediated fusion gene transfer (uracil phosphoribosyl-transferase and herpes simplex virus thymidine kinase). *Cancer Gene Ther* 2001; **8**: 512–21.
 40. Morley S, MacDonald G, Kirn D, Kaye S, Brown R, et al. The dl1520 virus is found preferentially in tumor tissue after direct intratumoral injection in oral carcinoma. *Clin Cancer Res* 2004; **10**: 4357–62.
 41. Tagawa M, Kawamura K, Shimozato O, Ma G, Li Q, et al. Virology- and immunology-based gene therapy for cancer. *Cancer Immunol Immunother* 2006; **55**: 1420–5.
 42. Yu L, Hamada K, Namba M, Kadomatsu K, Muramatsu T, et al. Midkine promoter-driven suicide gene expression and -mediated adenovirus replication produced cytotoxic effects to immortalised and tumour cells. *Eur J Cancer* 2004; **40**: 1787–94.
 43. Iwadate Y, Inoue M, Saegusa T, Tokusumi Y, Kinoh H, et al. Recombinant Sendai virus vector induces complete remission of established brain tumors through efficient interleukin-2 gene transfer in vaccinated rats. *Clin Cancer Res* 2005; **11**: 3821–7.
 44. Yoneyama Y, Ueda Y, Akutsu Y, Matsunaga A, Shimada H, et al. Development of immunostimulatory virotherapy using non-transmissible Sendai virus-activated dendritic cells. *Biochem Biophys Res Commun* 2007; **355**: 129–35.