Identifying the Safety Profile of Ad5.SSTR/TK.RGD, a Novel Infectivity-Enhanced Bicistronic Adenovirus, in Anticipation of a Phase I Clinical Trial in Patients with Recurrent Ovarian Cancer

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Abstract

Purpose: The purpose of this study was to evaluate the biodistribution and toxicity of Ad5.SSTR/TK.RGD, an infectivity-enhanced adenovirus expressing a therapeutic suicide gene and somatostatin receptor type 2 (for noninvasive assessment of gene transfer with nuclear imaging) in advance of a planned phase I clinical trial for recurrent ovarian carcinoma.

Experimental Design: Cohorts of Syrian hamsters were treated i.p. for 3 consecutive days with Ad5.SSTR/TK.RGD or control buffer with or without the prodrug ganciclovir (GCV) and euthanized on day 4, 19, or 56. Tissue and serum samples were evaluated for the presence of virus using qPCR analysis and were assessed for vector-related tissue or laboratory effects.

Results: Levels of Ad5.SSTR/TK.RGD in blood and tissues outside of the abdominal cavity were low, indicating minimal systemic absorption. GCV did not affect Ad5.SSTR/TK.RGD biodistribution. The mean Ad5.SSTR/TK.RGD viral level was 100-fold lower on day 19 than day 4, suggesting vector elimination over time. Animals in the Ad5.SSTR/TK.RGD ± GCV cohort had clinical laboratory parameters and microscopic lesions in the abdominal organs indicative of an inflammatory response. Toxicity in this dose cohort seemed to be reversible over time.

Conclusions: These studies provide justification for planned dosing of Ad5.SSTR/TK.RGD for a planned phase I clinical trial and insights regarding anticipated toxicity.

Despite advances in surgical debulking and chemotherapy, most patients diagnosed with ovarian cancer will develop chemoresistance and ultimately succumb to their disease (1). Thus, there has been a clear need for the development of new therapeutic approaches for ovarian cancer patients, particularly those affected by advance stage or recurrent disease.

Over the past decade, a variety of adenoviral mediated gene therapeutic approaches for ovarian cancer have been investigated (2, 3). Although these trials have shown the feasibility of gene therapy in this disease context, clinical responses have been limited in these first generation trials. Of note, the ability to determine tumor transfection was largely dependent on molecular and histopathologic assessment of excised tumor tissue samples.

Ovarian cancer of epithelial derivation has specifically been noted to be deficient in CAR, the receptor that mediates adenoviral vector binding and internalization (4). Thus, investigators have altered the tropism of adenoviruses in a variety of ways to enhance their transduction capacity and the therapeutic index of adenoviral-mediated gene therapy (5). For example, the fiber knob of adenoviruses has been genetically modified to incorporate an arginine-glycine-asparate (RGD-4C) motif in the HI loop of the knob (6, 7). The RDG-4C modification allows the virus to use cellular integrins, which are frequently expressed in ovarian cancer, and circumvent dependence on CAR for cellular infection and internalization. Early proof of principle studies showed that RGD modified adenoviruses mediate enhanced gene transfer to established ovarian cancer cells and freshly cultured primary human ovarian cancer cells, exhibiting preferential gene transfer to these cells when compared with human mesothelial tissue (thus, indicating a degree of specificity for tumor cells), and further showed enhanced infectivity of primary ovarian tumor cells in the presence of human ascites (8, 9).

Developing a noninvasive means to assess transduction would enhance the ability of investigators to determine whether adenoviral-mediated gene therapeutics were infecting

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Translational Relevance

The authors present the findings of a preclinical safety study using the bicistronic adenoviral vector, Ad5.SSTR/TK.RGD, in anticipation of a planned phase I clinical trial. Gene therapy using adenoviral vectors has shown efficacy in the treatment of recurrent ovarian cancer. This adenoviral vector encodes both a Herpes Simplex Virus thymidine kinase (*TK*) suicide gene and the somatostatin receptor type 2 (SSTR). This will enhance not only the efficacy of the adenovirus reagent but will provide a noninvasive means of assessing transfection. The findings of this study provide justification to proceed with a phase I clinical trial to investigate Ad5.SSTR/TK.RGD in patients with recurrent ovarian cancer.

intended tumor targets. Various approaches have been developed to accomplish this goal (10, 11). For example, incorporation of a somatostatin type 2 receptor expressing construct has been shown in preclinical experiments to allow for high-sensitivity imaging with positron emission tomography and single-photon emission computed tomography of adenoviral-infected tumor cells in an *in vivo* model of ovarian cancer (12). The basis of this imaging capacity is the induction of target cell binding sensitivity to octreotide.

Ad5.SSTR/TK.RGD is an RGD-4C–modified infectivity-enhanced bicistronic type 5 adenovirus that encodes a Herpes Simplex Virus (HSV) thymidine kinase (TK) suicide gene and the somatostatin receptor type 2 (SSTR; ref. 13). The purpose of this study was to determine the biodistribution and toxicology associated with i.p. administration of this novel reagent in the presence or absence of ganciclovir in an animal model in anticipation of a phase I trial in patients with recurrent ovarian cancer.

Materials and Methods

Vectors and controls. Vials of GMP quality Ad5.SSTR/TK.RGD were provided by National Cancer Institute-Frederick and stored at or below -70°C until used. Ganciclovir (Cytovene; Hoffman-La Roche, Inc.) was provided by the Health Services Foundation's Clinic Pharmacy at the University of Alabama at Birmingham and kept refrigerated until used. Bottles of glutathione *S*-transferase (GST) buffer [20 mmol/L Tris, 25 mmol/L NaCl, 2.5% glycerol (pH 8.0)] were also received from National Cancer Institute-Frederick and stored at 4°C until used.

Animal test system and group assignment. Female Golden Syrian hamsters were used to evaluate the vectors in this study. Syrian hamster models are both immunocompetent and permissive to adenoviral replication similar to human cancer patients. Compared with murine and cotton rat models, Syrian hamsters support adenoviral replication at several higher orders of magnitude (14).

Animals, age 9 to 10 wk, were given an unique identification number for this study by ear punch and provided Teklad Certified Rodent Diet and tap water ad libitum during the quarantine and study period. Hamsters were individually housed in cages in a room maintained at a temperature of 65°F to 80°F. Cage size and animal care conformed to the guidelines of the Guide For Care and Use of Laboratory Animals, the Department of Agriculture through the Animal Welfare Act, and to the applicable Standard Operating Procedures of Southern Research Institution.

Animals were assigned to their respective dose groups using a computerized randomization procedure designed to yield comparable group mean body weights at randomization. The dose and schedule of Ad5.SSTR/TK.RGD, ganciclovir, and/or control GST buffer by study group for the biodistribution and toxicity studies are detailed in Tables 1 and 2, respectively. GST buffer or Ad5./TK.RGD was administered at a dose volume of 1 mL/animal/dose and ganciclovir or saline were administered at a dose volume of 3 mL/kg/dose.

Five animals per dose group in the biodistribution study were euthanized on days 4, 19, and 56. Blood samples for PCR analysis were obtained before euthanasia. Immediately after euthanasia, tissues from 13 key organs were obtained from all fanimals in all dose groups, aliquoted in 50 to 150 mg specimens, and snap frozen.

Ad5.SSTR/TK.RGD viral DNA (vDNA) was isolated from the various blood and tissue samples using the Qiagen DNeasy Tissue kit (Qiagen, Inc.). The amount of DNA was quantified by a fluorometric assay using PicoGreen (Molecular Probes). PCR amplification was carried out from vDNA obtained from each specimen in triplicate in a 20 μL reaction volume, which contained 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 8 mmol/L MgCl2, 0.25 mmol/L each of the four deoxynucleotide triphosphates, 0.5 μmol/L each of the probe and primers listed in Table 3, 2 μg of bovine serum albumin", 2 units of Taq DNA polymerase (Roche Biochemicals), and 4 μL of each DNA sample. Data were collected from the PCR assay and saved using LightCycler software, and the mean values from the triplicate analysis done for each tissue was obtained.

Evaluation of general condition, body weight, and food consumption for toxicity study. All hamsters were observed twice daily throughout the ~1 wk quarantine and subsequent study periods for signs of moribundity and mortality. Body weights were determined before treatment and thereafter up to day 56 and before the moribund euthanasia of any animals. Quantitative food consumption was measured weekly for each hamster and reported as an average daily consumption (grams/animal/day).

Analysis of clinical laboratory based toxicity. Blood from each surviving hamster was collected on days 8 and 35 and before euthanasia of those animals necropsied on days 4, 19, and 56. Samples were analyzed for general hematologic and clinical chemistry parameters.

Table 1. Animal group dosing assignments for biodistribution study

Group no.	AdRGDTKSSTR		Ganciclovir		No. of hamsters euthanized on study day			Total no. of animals
	Dose level (vp/dose)	Dose regimen (i.p.)	Dose level (mg/kg/dose)	Dose regimen (i.p.)	Day 4	Day 19	Day 56	
1	0 (GST)	Days 1-3	0 (saline)	Days 5-18	5	5	5	15
2	10^{11}	Days 1-3	0 (saline)	Days 5-18	5	5	5*	15
3	10^{11}	Days 1-3	30	Days 5-18	5	5	5	15

*One hamster (2F26) in this dose group was euthanized moribund on day 28.

Table 2. Animal group dosing assignments for toxicity study

Group no.	AdRGDTKSSTR		Ganciclovir		No. of hamsters euthanized on study day			Total no. of animals
	Dose level (vp/dose)	Dose regimen (i.p.)	Dose level (mg/kg/dose)	Dose regimen (i.p.)	Day 4	Day 19	Day 56	
1	0 (GST)	Days 1-3	0 (saline)	Days 5-18	5	5	5	15
2	0 (GST)	Days 1-3	30	Days 5-18	5	5	5	15
3	10^{11}	Days 1-3	0 (saline)	Days 5-18	5	5	5	15
4	10^{8}	Days 1-3	30	Days 5-18	5	5	5	15
5	10^{11}	Days 1-3	30	Days 5-18	5	5	5	15

Determination of end organ histopathologic toxicity. Five animals per dose group were euthanized on days 4, 19, and 56. Complete gross necropsy examination was done on each hamster and tissue samples from over 35 organs were harvested from each animal and fixed in 10% neutral buffered formalin. Sections of fixed tissue were subjected to H&E staining and examined by a veterinary pathologist. Records of gross findings for a specimen from postmortem observations were available to the pathologist when examining that specimen microscopically. All lesions were listed and coded by the most specific topographical and morphologic diagnoses, severity, and distribution using standardized nomenclature. All lesions were also categorized as either "drug related" or "nondrug related."

Statistical analysis. For the biodistribution study, group means and SDs were calculated for PCR data. For the toxicity study, group means and SDs were calculated for body weights, food consumption, and clinical pathology laboratory parameters, and these data were subjected to ANOVA and to Dunnett's test, where applicable.

Results

Biodistribution associated with i.p. administration of Ad5.SSTR/TK.RGD. The biodistribution of Ad5.SSTR/TK.RGD and the effect of ganciclovir on its biodistribution were evaluated in Syrian hamsters given i.p. doses of GST buffer on days 1 to 3 and saline on days 5 to 18 (group 1) or 10^{11} vp/dose Ad5.SSTR/TK.RGD on days 1 to 3 and saline on days 5 to 18 (group 2) or 10^{11} vp/dose Ad5.SSTR/TK.RGD on days 1 to 3 and 30 mg/kg ganciclovir on days 5 to 18 (group 3). Samples of spleen, pancreas, kidney, diaphragm, liver, ovary, mesenteric lymph node, jejunum, biceps femoris, bone marrow, heart, lung, brain, and blood were collected on days 4, 19, and 56. Ad5.SSTR/TK.RGD vDNA was assayed in the samples using real-time quantitative PCR, as previously described.

The results of biodistribution studies are depicted in Table 4. No vDNA was detected in 206 of 209 samples from the GST buffer–treated animals (group 1), demonstrating that the assay was target specific and that the contamination of samples was extremely low. Ad5.SSTR/TK.RGD vDNA was detected on day 4 in all samples from both vector-treated groups (groups 2 and 3), with the exception of brain samples. More vDNA was detected in intraabdominal organs (spleen, diaphragm, liver, pancreas, ovary, jejunum, mesenteric lymph node, and kidney) and blood than in organs located outside of the abdominal cavity (lung, heart, bone marrow, and biceps femoris).

Although the treatment of animals in groups 2 and 3 was different, by day 19 the pattern of distribution and the elimination of Ad5.SSTR/TK.RGD vDNA for animals in groups 2 and 3 seemed to be similar. vDNA in the brain remained undetectable and fell to undetectable or nearly undetectable in blood, kidney, heart, and biceps femoris. Ad5.SSTR/TK.RGD vDNA fell in all samples by an average of 99% in terms of copies/mg tissue or copies/mL blood from day 4 to 19.

vDNA continuously decreased in all samples, except bone marrow and heart, on day 56 in Ad5.SSTR/TK.RGD and saline-treated animals (group 2) by an average of 83%. In contrast, vDNA in animals treated with Ad5.SSTR/TK.RGD and ganciclovir (group 3) decreased only by an average of 11% and was detectable in some tissue samples (kidney, brain, heart, biceps femoris, and blood), which had undetectable vDNA on day 4, and/or 19.

Effect of Ad5.SSTR/TK.RGD + ganciclovir on general condition, body weights, and food consumption. The toxicity of Ad5.SSTR/TK.RGD and the effect of ganciclovir on its toxicity were evaluated in Syrian hamsters given i.p. doses of GST buffer on days 1 to 3 and saline on days 5 to 18 (group 1), or GST buffer on days 1 to 3 and 30 mg/kg ganciclovir on days 5 to 18 (group 2) or 10^{11} vp/dose Ad5.SSTR/TK.RGD on days 1 to 3 and saline on days 5 to 18 (group 3) or 10^{8} vp/dose Ad5.SSTR/TK.RGD on days 1 to 3 and 30 mg/kg ganciclovir on days 5 to 18 (group 4) or 10^{11} vp/dose Ad5.SSTR/TK.RGD on days 1 to 3 and 30 mg/kg ganciclovir on days 5 to 18 (group 5). No mortality occurred in mice treated in the toxicology portion of this study before scheduled euthanasia and no adverse clinical signs with respect to their general condition were noted.

No treatment-related effects on body weight were observed, with one exception. On day 4, a statistically significant 10%

Table 3. Probe and primers for PCR analysis

Name	Sequence	Location in Ad5	Location in Ad5RGDTKSSTR	Note
Ad5-vTaqman probe	TCTCTACGCCAACTCCGCCCACG	21561-21583	21988-22010	Hexon gene in Ad5 virus
Ad5-v 5′ primer	TCACAGACCTGGGCCAAAA	21539-21557	21966-21984	
Ad5-v 3′ primer	ATCCACCTCAAAAGTCATGTCTAGC	21609-21585	22012-22036	

Table 4. Biodistribution of Ad5.SSTR/TK.RGD vDNA in samples (Copies/mg tissue or mL blood) for animal groups 2 and 3

A. Group 2 (Ad5.SSTR/TK.RGD and saline-treated animals)

Time tissues	Day 4 mean ± SE	Day 19 mean ± SE	Day 56 mean ± SE	Change % day 19 v day 4	Change % day 56 v day 19
Spleen	301,257 ± 139,212	2,924 ± 661	517 ± 186	-99%	-82%
Pancreas	54,388 ± 18,144	1,081 ± 334	100 ± 50	-98%	-91%
Kidney	5,097 ± 3,878	2 ± 1	0 ± 0	-100%	-100%
Diaphragm	118287 ± 27987	2,220 ± 442	431 ± 189	-98%	-81%
Liver	114,680 ± 45,043	1,313 ± 288	59 ± 34	-99%	-95%
Ovary	48,695 ± 25,529	181 ± 95	0 ± 0	-100%	-100%
Mesenteric LN	18,116 ± 5,507	1,506 ± 810	156 ± 70	-92%	-90%
Sm Int/jejunum	18,916 ± 7,183	329 ± 95	25 ± 15	-98%	-92%
Biceps femoris	17 ± 8	0 ± 0	0 ± 0	-100%	UC
Bone marrow	140 ± 49	6 ± 3	66 ± 66	-96%	1083%
Heart	67 ± 22	0.15 ± 0	0.29 ± 0	-100%	90%
Lung	852 ± 413	45 ± 10	7 ± 3	-95%	-85%
Brain	0 ± 0	0 ± 0	0 ± 0	UC	UC
Blood	11,560 ± 2,428	0 ± 0	0 ± 0	-100%	UC
Average	49,434 ± 12,870	686 ± 125	97 ± 30	-99%	-86%

B. Group 3 (Ad5.SSTR/TK.RGD and ganciclovir-treated animals)

Time tissues	Day 4 mean ± SE	Day 19 mean ± SE	Day 56 mean ± SE	Change % day 19 v day 4	Change % day 56 v day 19
Spleen	90,979 ± 20,168	2781 ± 926	930 ± 321	-97%	-67%
Pancreas	178,719 ± 61,867	635 ± 365	399 ± 232	-100%	-37%
Kidney	5,392 ± 2,788	0 ± 0	110 ± 93	-100%	UD
Diaphragm	225,529 ± 104,045	1104 ± 438	298 ± 111	-100%	-73%
Liver	183,115 ± 43,191	1178 ± 612	249 ± 155	-99%	-79%
Ovary	25,132 ± 9,251	292 ± 218	175 ± 91	-99%	-40%
Mesenteric LN	20,861 ± 8,831	1125 ± 913	359 ± 160	-95%	-68%
Sm Int/jejunum	37,097 ± 13,437	125 ± 102	84 ± 34	-100%	-32%
Biceps femoris	96 ± 31	0 ± 0	326 ± 244	-100%	UD
Bone marrow	118 ± 92	46 ± 19	48 ± 47	-61%	3%
Heart	75 ± 60	0.2 ± 0	170 ± 130	-100%	71248%
Lung	331 ± 127	70 ± 44	36 ± 25	-79%	-49%
Brain	0 ± 0	0 ± 0	43 ± 40	UC	UD
Blood	4,544 ± 2,206	0 ± 0	3347 ± 3325	-100%	UD
Average	55,142 ± 14,176	526 ± 177	469 ± 260	-99%	-11%

NOTE: Group 2 = 10^{11} vp/dose of AdRGDTKSSTR daily on days 1 to 3 and saline daily on days 5 to 18. SE, $n = 5$ except for day 56, where $n = 4$; Change% day 19 vs day 4 = [(day 19 - day 4) / day 4]%; Change% day 56 vs day 19 = [(day 56 - day 19)/day 19]%; Group 3 = 10^{11} vp/dose of AdRGDTKSSTR daily on days 1 to 3 and Ganciclovir daily on days 5 to 18; SE, $n = 5$; Change% day 19 vs day 4 = [(day 19 - day 4)/day 4]%; Change% day 56 vs day 19 = [(day 56 - day 19)/day 19]%.
Abbreviations: UC, unchanged; UD, undetermined.

reduction ($P \leq 0.05$) in group mean body weight was noted for the 10^{11} vp/dose Ad5.SSTR/TK.RGD and ganciclovir-treated animals (group 5) when compared with the group mean body weight in GST buffer and ganciclovir-treated animals (group 2). This difference was attributable to body weight loss for all five hamsters in this group, which occurred before the administration of ganciclovir.

A 13% to 32% reduction in food consumption was noted between day 1 and day 4 or 8 was noted in 10^{11} vp/dose Ad5.SSTR/TK.RGD treated animals (groups 3 and 5) relative to food consumption noted in corresponding control groups. Thereafter, food consumption for all Ad5.SSTR/TK.RGD-treated animals was similar to that noted in control groups.

Effect of Ad5.SSTR/TK.RGD + ganciclovir on clinical laboratory parameters. Hematology changes were observed only for hamsters given 10^{11} vp/dose of Ad5.SSTR/TK.RGD with or without ganciclovir (groups 4 and 5) in the toxicology portion of this study. These changes, relative to the values observed for animals in the corresponding vehicle/ganciclovir dose groups, included higher group mean neutrophil (109-197% higher; days 4 and 8), monocyte (210-319% higher; day 4), and platelet (13-45% higher; days 4 and 8) counts; and lower group mean lymphocyte (16-31% lower; day 4) and reticulocyte (39-45% lower; day 8) counts.

Clinical chemistry changes were observed for animals given 10^{11} vp/dose of Ad5.SSTR/TK.RGD with and without ganciclovir (groups 4 and 5) and were comparable for both groups of animals. These changes, relative to the values observed for animals in the corresponding vehicle/ganciclovir dose groups (groups 1 and 2), included lower (≤7% lower) group mean and/or individual albumin values on days 4 and 8, lower (≤25% lower) group mean albumin/globulin values on days 4 and 8 (group 5 only), and higher group mean alkaline phosphotase values on day 4 and/or day 8. In addition,

increased amylase values, relative to values for hamsters in the vehicle control group, were noted on day 4 and/or day 35 for a small number of hamsters in the 10^{11} vp/dose Ad5.SSTR/TK.RGD-treated groups, and high lipase values were noted on at least 1 day in a small number of hamsters in the 10^{11} vp/dose Ad5.SSTR/TK.RGD ($n = 3$) and in the 10^{11} vp/dose Ad5.SSTR/TK.RGD + ganciclovir ($n = 2$) dose groups; these findings were possibly related to treatment with the vector.

Effect of Ad5.SSTR/TK.RGD + ganciclovir on end organ histopathology. On day 4, with the exception of one animal in a vehicle control group, gross lesions were observed only for animals in both dose groups given 10^{11} vp/dose of Ad5.SSTR/TK.RGD (groups 3 and 5), and included lesions on the liver (adhesion, thick), small intestine (dilation of the duodenum, jejunum, and ileum), large intestine (dilation of the cecum), and mesentery (adhesion). These lesions were likely related to treatment with the vector. The lesions in the liver and, to a lesser extent the mesentery, were still present on days 19 and 56 in these two groups.

On day 4, microscopic evaluation showed vector- and/or treatment-related inflammation, fibrosis, and/or a fibrinopurulent exudate on the serosal or capsular surface of the abdominal organs (including the liver, gallbladder, spleen, stomach, small and large intestines, urinary bladder, and/or uterus), and on the peritoneum, and/or the mesentery of the animals treated with 10^{11} vp/dose of Ad5.SSTR/TK.RGD (groups 3 and 5). The lesions in the abdominal organs seemed to be directly related to treatment with the vector because these lesions were observed almost exclusively for animals treated at 10^{11} vp/dose of Ad5.SSTR/TK.RGD (with or without ganciclovir). In contrast, findings noted in the peritoneum and mesentery were likely associated with i.p. dosing because the noted lesions were also observed for animals given GST buffer with or without ganciclovir and for animals given the lower dose (10^{8} vp/dose) of vector (groups 1, 2, and 4).

On day 19, lesions (chronic inflammation, chronic active inflammation, fibrosis, and/or a fibropurlent exudate) were still present on the serosal or capsular surfaces of the abdominal organs of animals in the 10^{11}vp/dose Ad5.SSTR/TK.RGD dose groups (with or without ganciclovir), and in the peritoneum and mesentery of animals in all dose groups. Minimal to mild chronic and chronic active inflammation also was present in the peritoneal lining of animals in all dose groups; however, peritoneal fibrosis was observed only for animals given 10^{11} vp/dose of Ad5.SSTR/TK.RGD, with or without ganciclovir.

On day 56, the majority of the lesions that had been observed in the abdominal cavity of animals in all dose groups on the days before necropsy had resolved, with the exception of fibrosis and inflammation. Chronic inflammation and/or fibrosis were observed in the mesentery of animals in all dose groups. In addition, fibrosis was observed on the serosal and capsular surfaces of various abdominal organs and on the peritoneal lining of animals given 10^{11} vp/dose of Ad5.SSTR/TK.RGD, with or without ganciclovir but not for animals in the other dose groups. These latter findings suggested a stronger, more widespread inflammatory response that resulted in fibrosis was produced in animals given 10^{11} vp/dose of Ad5.SSTR/TK.RGD, compared with animals given a lower dose of vector or control GST buffer.

Discussion

Molecular chemotherapy or suicide gene therapy is based on the delivery and selective expression of a gene encoded toxin into cancer cells to achieve tumor eradication (2). One toxin frequently used to accomplish molecular chemotherapy is the thymidine kinase (*TK*) gene from the HSV. Expression of the viral TK gene allows tumor cells to have an enhanced sensitivity to nucleoside analogues, such as ganciclovir and results in a "bystander effect" where many more cells are killed than transduced with the gene (15). Preclinical studies have shown that adenoviral-mediated delivery of the HSV-TK gene with ganciclovir is tumoricidal *in vitro*, is associated with a "bystander effect" *in vitro*, and that administration of the adenovirus containing the HSV-TK gene with ganciclovir confers a survival advantage in severe combined immunodeficient mouse models of i.p. ovarian carcinomatosis (16, 17).

These aforementioned preclinical studies supported a phase I human clinical trial using unmodified adenoviral-mediated delivery of the HSV-TK gene followed by infusion with ganciclovir in recurrent ovarian cancer patients (18). Fourteen patients with persistent/recurrent ovarian cancer were treated per study specifications. Transient and easily ameliorated vector-associated fever was experienced by 4 of 14 (29%) of treated patients. Other possible vector-associated constitutional (fatigue, insomnia) symptoms, abdominal pain, and gastrointestinal (nausea, diarrhea) symptoms were experienced by 6 of 14 (43%) treated patients. No other vector-specific side effects were noted. Six patients experienced catheter-associated complications. Of 13 patients evaluable for response, 5 (38%) had stable disease and 8 (62%) had evidence of progressive disease. Gene transfer assessments were done using PCR and rtPCR on ascites specimens retrieved through an i.p. Tenkhoff catheter. rtPCR studies showed the presence of mRNA in 13 of 13 evaluable patient ascites specimens. Although detection of mRNA in each patient was encouraging, the copy number was low, which may have contributed to the limited clinical effect noted. Moreover, in this trial, there was no investigational mechanism to determine relative gene transfer to tumor versus mesothelial or other nontumor tissues. Although the safety observed in this initial trial was encouraging, the ability of the unmodified adenoviral vector to achieve meaningful levels of gene transfer to cancer cells in the human system was disappointing. Moreover, the manner in which tissue was retrieved for gene transfer assessment was invasive, cumbersome, and associated with a high degree of sampling variability. These results mimicked those noted in other HSV-TK–based gene therapy trials in ovarian cancer (18–23).

Recent randomized studies in glioma and hepatocellular cancer patients have confirmed the clinical potential of HSV-TK–based gene therapy combined with ganciclovir as an antitumor approach (24–26). Following promising randomized phase II data, a phase III Europe-wide randomized study in glioma patients was initiated. Patient enrollment was completed in 2007 and preliminary analysis has shown a potentially significant overall survival benefit.[10] These recent studies in

[10] A. Hemminki, unpublished data.

glioma and hepatocellular cancer differed from prior trials in ovarian cancer in that HSV-TK encoding virus was injected as an adjuvant treatment following surgical resection of primary tumor.

Ad5.SSTR/TK.RGD is an RGD-4C–modified infectivity-enhanced bicistronic type 5 adenovirus that encodes a HSV-TK suicide gene and the SSTR. *In vitro* studies showed effective cytotoxicity when SKOV3.ip1 or OvCAR3 cells were treated with Ad5.SSTR/TK.RGD and ganciclovir (27). Moreover, a survival advantage was noted in a murine animal model of ovarian cancer when animals were treated with Ad5.SSTR/TK.RGD and ganciclovir (28). In addition, the functional status of the somatostatin receptor imaging cassette was also validated both *in vitro* and *in vivo* using radiolabeled P2045, a somastotatin analogue (27, 28). These studies led to the development of a phase I clinical trial investigating this novel therapeutic in patients with recurrent ovarian cancer. In the process of producing clinical grade Ad5.SSTR/TK.RGD for this clinical trial, unacceptable recombination and deletion of the expression cassette was encountered. Further studies showed that this occurred as a result of having the same cytomegalovirus promoter and poly(A) tail flanking the TK and SSTR constructs in the RGD-modified bicistronic vector. The vector was reconfigured with different promoters and poly(A) tails flanking the TK and SSTR constructs. Additional studies validated the functional components of this reconfigured Ad5.SSTR/TK.RGD vector (29).

The current study has validated the safety of this reconfigured Ad5.SSTR/TK.RGD vector when given i.p. in combination with ganciclovir in female Syrian hamsters. Syrian hamsters have been shown to be a promising immunocompetent model that is permissive to human adenovirus replication in tumors as well as normal tissues (30). Biodistribution studies showed that the extent of systemic absorption of Ad5.SSTR/TK.RGD for female hamsters given three consecutive daily i.p. doses (days 1-3) of 10^{11} vp/dose Ad5.SSTR/TK.RGD was low. On all days of scheduled tissue collection (days 4, 19, and 56), highest levels of vDNA were observed in those tissues residing in the peritoneal cavity (spleen, liver, pancreas, and diaphragm) and lowest levels were observed in brain, muscle (biceps femoris), heart, and bone marrow. Blood and tissue levels of vDNA decreased over time after administration of Ad5.SSTR/TK.RGD. Administration of daily i.p. doses of ganciclovir (30 mg/kg/dose, days 5-18) seemed to have no discernible effect on the relative tissue distribution of vDNA derived from Ad5.SSTR/TK.RGD.

Toxicity studies showed adverse effects in female hamsters given i.p. doses of 10^{11} vp/dose of Ad5.SSTR/TK.RGD once daily for 3 consecutive days (days 1-3); minimal or no toxicity was observed for hamsters given a lower dose (10^{8} vp/dose) of Ad5.SSTR/TK.RGD. Using standard conversion rates, this dose corresponds to 1.7×10^{11} vp/kg in humans or 600× the human dose on a body weight basis. The toxicity of Ad5.SSTR/TK.RGD was predominantly characterized by laboratory changes (increases in neutrophils, monocytes, platelets, and alkaline phosphotase; decreases in lymphocytes, reticulocytes, albumin, albumin/globulin values) and histopathologic lesions in the abdominal organs (inflammation, fibrosis, and/or a fibrinopurulent exudate) that were indicative of an inflammatory response to the vector. The toxicity of AdRGDTKSSTR seemed to be reversible. No observable drug-related effects were noted for animals given i.p. doses of ganciclovir of 30 mg/kg/day on days 5 to 18 and ganciclovir had no effect on the toxicity of Ad5.SSTR/TK.RGD.

Infectivity-enhanced adenoviruses have only recently been investigated in the context of a clinical trial (31). The results of this preclinical study are comparable with safety studies evaluating a RGD-4C–modified conditionally replicative adenovirus (CRAd), Ad5-D24-RGD. This infectivity-enhanced CRAd was noted in these preclinical safety studies to have low systemic absorption, to clear virus rapidly, and to be associated with histopathologic findings consistent with mild peritonitis when administered i.p. (32). No vector-related dose limiting toxicity has been noted in recurrent ovarian cancer patients treated i.p. with Ad5-D24-RGD in an ongoing phase I trial.[11]

These safety studies were designed with the approval of the Food and Drug Administration in anticipation of a planned phase I clinical trial that will assess the safety, potential efficacy and biological effects of Ad5.SSTR/TK.RGD in patients with epithelial ovarian cancer and other selected malignancies. Ad5.SSTR/TK.RGD will be administered i.p. on days 1 through 3; there are plans for three treatment cohorts that will be treated with doses ranging from 10^{9} to 10^{12} vp/d. i.v. ganciclovir will be given on days 5 to 18. This clinical trial has recently been approved by the FDA and will open to patient accrual soon.

In conclusion, an acceptable safety profile has been shown in preclinical biodistribution and toxicity studies evaluating the i.p. administration of Ad5.SSTR/TK.RGD in combination with ganciclovir in an appropriate animal model. Proceeding with a planned phase I trial that will investigate these novel gene therapy advancements in patients with recurrent ovarian cancer is justified and will be pursued.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

[11] R.D. Alvarez, unpublished data.

References

1. Cannistra SA. Cancer of the ovary. N Engl J Med 2004;351:2519–29.
2. Barnes M, Deshane J, Rosenfeld M, Siegal G, Curiel D, Alvarez R. Gene therapy and ovarian cancer: a review. Obstet Gynecol 1997;89:145–55.
3. Kanerva A, Raki M, Hemminnki A. Gene therapy of gynaecological diseases. Expert Opin Biol Ther 2007;7:1347–61.
4. Douglas JT, Kim M, Sumerel LA, Carey DE, Curiel DT. Efficient oncolysis by a replicating adenovirus (Ad) *in vivo* is critically dependent on tumor expression of primary Ad receptors. Cancer Res 2001;61:813–17.
5. Raki M, Rein DT, Kanerva A, Hemminki A. Gene transfer approaches for gynecological diseases. Mol Ther 2006;14:154–63.
6. Dmitriev I, Krasnykh V, Miller CR, et al. An adenovirus vector with genetically modified fibers demonstrates expanded tropism via utilization of a coxsackievirus and adenovirus receptor- independent cell entry mechanism. Virology 1999;72:9706–13.
7. Krasnykh V, Dmitriev I, Navarro JG, et al. Advanced generation adenoviral vectors possess augmented gene transfer efficiency based upon coxsackie adenovirus receptor-independent cellular entry capacity. Cancer Res 2000;60:6784–7.
8. Vanderkwaak T, Wang M, Navarro J, et al. An advanced generation of adenoviral vectors selectively enhances gene transfer for ovarian cancer gene therapy approaches. Gynecol Oncol 1999;74:227–34.

9. Blackwell J, Li H, Navarro J, et al. Circumventing inhibitory factors in ascites fluid using a tropism modified adenoviral vector. Hum Gene Ther 2000;11:1657–69.

10. Shah K. Current advances in molecular imaging of gene and cell therapy for cancer. Cancer Biol Ther 2005;4:518–23.

11. Raty JK, Liimatainen T, Unelma Kaikkonen M, Grohn O, Airenne KJ, Yia-Hertual S. Non-invasive imaging in gene therapy. Mol Ther 2007;15:1579–86.

12. Zinn K, Buchsbaum D, Chaudhuri T, Mountz J, Grizzle W, Rogers B. Noninvasive monitoring of gene transfer using a reporter receptor imaged with a high affinity peptide radiolabeled with 99mTc or 188Re. J Nucl Med 2000;41:887–95.

13. Belousova N, Harris R, Zinn K, et al. Circumventing recombination events encountered with production of a clinical-grade adenoviral vector with a double-expression cassette. Mol Pharmacol 2006;70:1488–93.

14. Thomas MA, Spencer JF, La Regina MC, et al. Syrian hamster as a permissive immunocompetent animal model for the study of oncolytic adenovirus vectors. Cancer Res 2006;66:1270–6.

15. Freeman S, Whartenby K, Koeplin D. Tumor regression when a fraction of the tumor cells contain the HSV-TK gene. J Cell Biochem 1992;16:45–8.

16. Rosenfeld M, Feng M, Michael S, Siegal G, Alvarez R, Curiel D. Adenoviral mediated delivery of the herpes simplex virus thymidine kinase gene selectively sensitizes human ovarian carcinoma cells to ganciclovir. Clin Cancer Res 1995;1:1571–80.

17. Rosenfeld M, Wang M, Siegal G, et al. Adenoviral mediated delivery of herpes simplex thymidine kinase results in tumor reduction and prolonged survival in a SCID mouse model of human ovarian carcinoma. J Mol Med 1996;74:455–62.

18. Alvarez R, Navarro J, Wang M, et al. Adenoviral mediated suicide gene therapy for ovarian cancer. Mol Ther 2000;2:524–30.

19. Robinson W, Adams J, Marrogi A, Freeman S. Vaccine therapy for ovarian cancer using herpes simplex virus thymidine kinase (HSV-TK) suicide gene transfer technique: a phase I study [abstract]. Gynecol Oncol 1998;68:88.

20. Link C, Eldeman M, Tennant L, Levy J, Moorman D, Seregina T. LTKOSN.1 murine vector producer cell (VPC) for the *in vivo* delivery of the herpes simplex thymidine kinase (HSV-TK) gene for ovarian cancer [abstr. 915]. Am Soc Gene Ther 1999.

21. Hasenburg A, Tong XW, Rojas-Martinez A, et al. Thymidine kinase gene therapy with concomitant topotecan chemotherapy for recurrent ovarian cancer. Cancer Gene Ther 2000;7:839–44.

22. Hasenburg A, Tong XW, Fischer DC, et al. Adenovirus-mediated thymidine kinase gene therapy in combination with topotecan for patients with recurrent ovarian cancer: 2.5-year follow-up. Gynecol Oncol 2001;83:549–54.

23. Hasenburg A, Fischer DC, Tong XW, et al. Histologic and immunohistochemical analysis of tissue response to adenovirus-mediated herpes simplex thymidine kinase gene therapy of ovarian cancer. Int J Gynecol Cancer 2002;12:66–73.

24. Sandmair AM, Loimas S, Puranen P, et al. Thymidine kinase gene therapy for human malignant glioma, using replication-deficient retroviruses or adenoviruses. Hum Gene Ther 2000;11:2197–205.

25. Immonen A, Vapalahti M, Tyynelä K, et al. AdvHSV-tk gene therapy with intravenous ganciclovir improves survival in human malignant glioma: a randomised, controlled study. Mol Ther 2004;10:967–72.

26. Li N, Zhou J, Weng D, et al. Adjuvant adenovirus-mediated delivery of herpes simplex virus thymidine kinase administration improves outcome of liver transplantation in patients with advanced hepatocellular carcinoma. Clin Cancer Res 2007;13:5847–54.

27. Hemminki A, Belousova N, Zinn K, et al. An adenovirus with enhanced infectivity mediates molecular chemotherapy of ovarian cancer cells and allows imaging of gene expression. Mol Ther 2001;4:223–31.

28. Hemminki A, Zinn K, Liu B, et al. *In vivo* molecular chemotherapy and non-invasive imaging with an infectivity enhanced adenovirus. J Natl Cancer Inst 2002;94:741–9.

29. Belousova N, Harris R, Zinn K, et al. Circumventing recombination events encountered with production of a clinical-grade adenoviral vector with a double-expression cassette. Mol Pharmacol 2006;70:1488–93.

30. Thomas RD, Roy D. Base sequence-specific attack of stilbene estrogen metabolite(s) on the mitochondrial DNA: implications in the induction of instability in the mitochondrial genome in the kidney of Syrian hamsters. Int J Mol Med 2001;7:389–95.

31. Kimball KJ, Numnum MT, Barnes MN, et al. A phase I study of a tropism modified conditionally replicative adenovirus (CRAd) for recurrent ovarian cancer [abstract]. Mol Ther 2008;16:S113.

32. Page JG, Tian B, Schweikart K, et al. Identifying the safety profile of a novel infectivity-enhanced conditionally replicative adenovirus, Ad5–24-RGD, in anticipation of a phase I trial for recurrent ovarian cancer. Am J Obstet Gynecol 2007;196:389e1–10.

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