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Thymidine kinase gene therapy with concomitant topotecan chemotherapy for recurrent ovarian cancer

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Introduction: Patients with recurrent ovarian cancer were treated with a replication-deficient recombinant adenovirus containing the herpes simplex virus thymidine kinase gene administered intraperitoneally (i.p.) followed by administration of an anti-herpetic prodrug and topotecan.

Materials and Methods: A total of 10 patients with stage IIIc epithelial ovarian cancer underwent secondary debulking to ≤0.5 cm residual tumor. Patients with normal i.p. flow received i.p. delivery of adenovirus. Two patients each were treated on dose level 1 (2×10^{10} vector particles (VP)), dose level 2 (2×10^{11} VP), and dose level 3 (2×10^{12} VP); four patients were treated on dose level 4 (2×10^{13} VP). Acyclovir and topotecan were started 24 hours after vector delivery.

Results: No patient treated at any dose level incurred unanticipated toxic effects, and all side effects resolved. The most common adverse event was myelosuppression: grade 3 or 4 thrombocytopenia with grade 2–4 anemia in three patients and grade 3 or 4 neutropenia in eight patients. Three patients developed thrombocytosis and three patients had a mild elevation of serum glutamic pyruvic transaminase/alanine aminotransferase. Temperature elevations that were not associated with detectable infection occurred in two patients.

Discussion: I.p. delivery of adenoviral vector with concomitant topotecan chemotherapy was well tolerated without significant lasting toxicities. Side effects were independent of the dose of adenoviral vector. **Cancer Gene Therapy (2000) 7, 839–852**

Key words: Ovarian cancer; thymidine kinase; gene therapy; intraperitoneal therapy; acyclovir; topotecan.

Ovarian cancer is the leading cause of death from gynecological cancer, with an overall 5-year survival rate of 28% to 35%. In the United States alone there are ~19,000 new cases and 12,000 deaths from ovarian cancer each year.[1] The estimated number of cases worldwide is ~140,000.[2]

The initial chemotherapy for ovarian cancer usually includes the drugs cisplatin or carboplatin in a regimen combined with paclitaxel or cyclophosphamide. Up to 73% of patients with stage III and IV tumors respond to this therapy.[3] However, this initial treatment response does not translate into a favorable long-term prognosis; only 4–25% of treated patients who had stage III and IV ovarian cancer are alive and disease-free at 5 years.[4] Considering these poor patient outcomes, alternative therapeutic approaches are necessary to improve the long-term survival of patients with ovarian cancer.

The phase I study described here was designed to evaluate the safety and toxicity profile of intraperitoneal (i.p.) gene therapy (GT) for patients with locally recurrent ovarian cancer after failure of combined radical surgery and chemotherapy. Patients with this profile do not have any standard treatment available to them that demonstrates a high degree of efficacy in eradicating the tumor with a reasonable degree of safety.

GT mechanism

The proposed strategy involves transduction of tumor cells by an adenoviral vector carrying the herpes simplex virus thymidine kinase (HSV-tk) gene (ADV-HSV-tk), which mediates sensitization to the antiviral drug acyclovir (ACV). The viral enzyme ADV-tk metabolizes ACV to a monophosphate, and the human enzyme completes the activation to a triphosphate. The phosphorylated ACV is incorporated into nascent DNA chains of proliferating cells and acts as

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a chain terminator, thereby resulting in cell death.[5–7] Because normal mammalian cells do not possess HSV-tk, cytotoxicity depends upon successful introduction of the HSV-tk gene, expression of the enzyme, phosphorylation of ACV, and synthesis of DNA. Nondividing cells may express HSV-tk and phosphorylate ACV but are not harmed if they do not synthesize DNA. This approach is especially suitable for the treatment of ovarian cancer in which rapidly dividing tumor cells are adjacent to tissues made up largely of nonproliferating cells. Equally important is the growth pattern of ovarian cancer, which usually spreads on the peritoneal surface of the abdominal cavity and rarely invades deeply into the adjacent tissue even in its advanced stages.

Preclinical studies

We have studied adenovirus-mediated thymidine kinase (ADV-tk) GT of ovarian cancer *in vitro* and *in vivo*.[8,9] A high cell-killing efficacy of all cell lines tested *in vitro* and improvement in the long-term survival of nude mice bearing human ovarian cancer transplants was established.[10] Treatment efficacy was dependent upon viral dose and tumor burden, with cures achieved in the presence of peritoneal carcinomatosis representing low-volume disease. This finding is consistent with the observation that the depth of viral penetration is limited.

We also found that ADV-tk can enhance the sensitivity of ovarian cancer cells to selected chemotherapeutic agents.[11] A synergistic interaction was observed with the simultaneous application of ADV-tk GT and topotecan in our preclinical studies.[11] Ov-Ca-2774 cells, which possess a mutation in tumor suppressor *p53* gene and are resistant to cisplatin-based chemotherapy, represent the most clinically challenging tumors. With GT alone, 66% of Ov-Ca-2774 cells were killed at a specific given multiplicity of infection. Combination of the two therapies resulted in 100% cytotoxicity. This synergism was seen even when topotecan was added 72 hours after GT.[11] Similar results were obtained in the ovarian cancer cell lines Ov-Ca-1225 and SKOV3.

Topoisomerase I is an enzyme that is critical for cell growth and proliferation. It catalyzes the cutting and mending of a single DNA strand and is required for DNA replication, DNA repair, and gene expression.[12] Topotecan exerts its cytotoxic effect by stabilizing the covalent DNA-enzyme complex, thus blocking DNA repair.[13,14] When DNA replicates in the presence of the stabilized complex, double-stranded DNA breaks occur, and the resulting fragmentation of DNA causes cell death.[14]

The combination of GT and chemotherapy with topotecan interferes with DNA replication at two different steps, with the shared aspect of damage to nascent DNA chains. Based on data from our preclinical studies, we propose GT with ADV-tk and chemotherapy with topotecan in patients with recurrent ovarian cancer.[11]

Table 1. Inclusion and Exclusion Criteria

Inclusion criteria
Diagnosis of epithelial ovarian carcinoma
Stage I, II, III, or IV disease
Completed initial surgery and chemotherpay (cisplatin or carboplatin) and being off treatment for at least 6 weeks
Clinical evidence of recurrent, progressive, or residual disease
Performance status of 0 or 1 by Eastern Cooperative Oncology Group
Tumor masses left after secondary debulking surgery of ≤0.5 cm in diameter
Adequate baseline organ function
Exclusion criteria
Acute infection
HIV-positive serological tests
History of significant heart disease
Inadequate i.p. flow study
Pretreatment with topotecan

MATERIALS AND METHODS

The protocol (BB-IND 7311) for this phase I clinical trial was approved by the US Food and Drug Administration in December of 1997. The study began in January of 1998 and ended in May of 1998.

Entry criteria are listed in Table 1. A total of 10 patients with stage IIIc ovarian cancer were enrolled in the study. As a consequence of preclinical data showing optimum response with low-volume disease, most likely due to the limited depth of vector penetration, we felt it was necessary to surgically debulk patients before application of vector (largest remaining tumor nodule was ≤0.5 cm). At the time of surgery, two i.p. Tenckhoff catheters were placed.

Patient profiles are detailed in Table 2. The median age of the patients was 56.8 years (range 29–78 years). All patients had a performance status of 0 and presented with epithelial ovarian cancer. Six patients had undergone optimal tumor debulking at the time of primary surgery, and four patients had undergone suboptimal debulking. Three patients had received one chemotherapy regimen, four patients had received two chemotherapy regimens, two patients had received three chemotherapy regimens, and one patient had received four different regimens before GT.

The complete study calendar is shown in Table 3.

Histology showed eight patients with papillary serous adenocarcinoma, one patient with endometrioid adenocarcinoma, and one patient with endometrioid adenocarcinoma, clear cell.

Vectors

The recombinant adenovirus containing the HSV-tk gene (ADV-RSV-tk) has been described previously and was prepared at the Baylor College of Medicine Gene Vector Laboratory. Systemic vector toxicity was analyzed in animal models, including the cotton rat, and did not show any significant pathology.[15] Infectious titers were analyzed by limiting dilution analysis on the 293 human embryonic kidney cell line.[16]

Vector delivery

After bowel function was re-established following optimal debulking surgery, a peritoneal flow study with ^{99m}technetium sulfur colloid was performed to assure adequate i.p. distribution. The vector was diluted in 10 mL of sterile normal saline and injected *via* the i.p. catheters. After vector injection, each

Table 2. Patient Profiles

Vector particles per group and patient	Age at study entry	Study entry date	Prior surgery	Chemotherapy regimens for ovarian cancer
Group 1: 2×10^{10}				
1-1	72	January 1998	May 1996	2
1-2	28	January 1996	August 1995, June 1996, February 1997	3
Group 2: 2×10^{11}				
2-1	55	February 1998	1991 February 1992	3
2-2	61	March 1998	February 1997	2
Group 3: 2×10^{12}				
3-1	53	March 1998	May 1997	1
3-2	60	March 1998	July 1996	1
Group 4: 2×10^{13}				
4-1	78	April 1998	April 1993	2
4-2	69	April 1998	December 1996	2
4-3	51	April 1998	January 1997	1
4-4	52	May 1998	December 1995	4

catheter was flushed with 125 cc of normal saline (total 250 cc). Patients changed position every 30 minutes (upright, Trendelenburg, left and right lateral, dorsal and ventral supine positions) to assure adequate i.p. distribution.

Patients 1-1 and 1-2 received dose level 1 (2×10^{10} vector particles (VP)), patients 2-1 and 2-2 received dose level 2 (2×10^{11} VP), patients 3-1 and 3-2 received dose level 3 (2×10^{12} VP), and patients 4-1 through 4-4 received dose level 4 (2×10^{13} VP). Each patient group was followed for at least 2 weeks before treating patients on the next dose level.

ACV/valacyclovir treatment

ACV (Glaxo Wellcome, Thousand Oaks, Calif) treatment at 15 mg/kg intravenously (i.v.) for over 1 hour every 8 hours was started 24 hours after vector injection for 42 doses. For each dose, the lyophilized powder was dissolved in sterile water to give a solution of 10 mg/mL. Based on the patient's weight, the appropriate calculated dose volume was removed from the vial and added to infusion fluid (typically 100 mL). The last two patients received the oral equivalent of ACV from valacyclovir at a dose of 2 g every 8 hours.[17]

Table 3. Study Calendar for Phase I Study

	Pre-operative	Post-operative day	GT day 2, 7, 14	6 weeks	Every 3 months
History	X	X	X	X	X
Physical exam	X	X	X	X	X
Blood tests	X	X	X	X	X
CA-125	X	X	X	X	X
Carcinoembryonic antigen	X			X	X
HIV	X				
Urine analysis	X			X*	X*
Urine: Virus PCR		X	X		
Chest x-ray	X				X*
Diagnostic imaging	X				X*

Topotecan treatment

Topotecan (Smith Kline Beecham Pharmaceuticals, Philadelphia, Penn) treatment began 24 hours after vector injection. Based on the phase II study performed at the MD Anderson Cancer Center (Houston, Tex), topotecan was administered as an i.v. infusion of 1.0 mg/m^2 for 30 minutes each day for 5 days.[18] At 30 minutes before each chemotherapy, patients received 8 mg of ondansetron (Glaxo Wellcome Oncology, Research Triangle Park, NC) and 10 mg of dexamethasone (Merck, West Point, Penn).

Medications

All patients received broad-spectrum oral antibiotic therapy with ciprofloxacin (Bayer Corporation Pharmaceutical Division, West Haven, Conn) the evening before and the morning of vector delivery, continuing the antibiotic regimen twice daily for 5 days. The first seven patients received granulocyte colony-stimulating factor (Amgen, Thousand Oaks, Calif) after a significant drop in white blood cells (grade 3 and 4 toxicity). The last three patients received granulocyte colony-stimulating factor prophylactically.

Polymerase chain reaction urine analysis for adenovirus

Urine specimens were collected the day before vector injection and then daily until three analyses were free of vector DNA.

Toxicities

Toxicities were evaluated according to the National Cancer Institute (NCI) common toxicity criteria.

Follow-up

Patients are seen as outpatients at 2 and 6 weeks after the procedure, every 3 months for 1 year, and then every 6 months after the treatment for 5 years (Table 3).

Table 4. Toxicities

	Dose level (n = 2)				Dose level 2 (n = 2)				Dose level 3 (n = 2)				Dose level 4 (n = 4)			
Grade	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Neutropenia			1	1			1	1			1	1	1	1	1	1
Thrombocytopenia	1					1		1					3			1
Thrombocytosis*										2					1	
Anemia		2				2					1		1	1	1	
Glutamic pyruric transaminase/alanine amino transferase elevation	1								1				1			
Fever														1		
Infection		1								2			1		1	
Nausea	1				1				1				1			
Vomiting	1				1				1				1			
Diarrhea	1								1				2			
Peripheral edema	1				1								1			
Allergy						1										

*Not included in NCI criteria.

RESULTS

Toxicity

Toxicity results for the 10 patients treated during this clinical trial are given in Table 4 using NCI common toxicity criteria. The adverse events observed were similar to those seen with topotecan alone. The most common adverse events after GT and topotecan were thrombocytopenia (in 9 patients) and neutropenia (in all 10 patients). However, no bleeding occurred that required platelet transfusion, and platelets recovered in all patients without specific treatment. Grade 2 and 3 anemia was observed in seven patients.

Three patients had thrombocytosis: patient 3-1 (752,000 on GT day 5), patient 3-2 (619,000 on GT day 8), and patient 4-2 (616,000 on GT day 4). On GT day 7, patient 4-3 had a rise in peripheral blood platelets to 400,000, which is the upper limit of the normal range. All four patients subsequently underwent thrombocytopenia (range 45,000–78,000) between GT day 12 and GT day 15.

Patient 3-1 developed a deep venous thrombosis in the common femoral vein bilaterally with extension to the inferior vena cava on GT day 10 under prophylactic treatment with low-dose aspirin and heparin subcutaneously. This patient had a history of thrombotic diathesis and had already undergone placement of an inferior vena cava filter after her first tumor debulking surgery. Because of the extent of the thrombus, the patient received a thrombolytic therapy with urokinase. This patient also experienced isciatic nerve irritation in the left leg on GT day 6.

Three patients had a mild elevation of serum glutamic pyruvic transaminase/alanine aminotransferase ($<2.5 \times$ normal/10–55 U/L) after vector administration. In two of these patients (patients 3-2 and 4-3), GOT, alkaline phosphatase, lactate dehydrogenase (LDH), and GGT remained stable or showed decreasing values. In one case (patient 1-1), GOT rose with the glutamic pyruvic transaminase (GPT) from 29 to 61 U/L

GOT and GPT serum levels returned to normal at 2 weeks after inception of GT, except for patient 3-2, whose GPT remained elevated at 88 U/L at 6 weeks.

Patient 4-2 showed an elevation of LDH of up to 1077 on GT day 4 without any elevation of other liver enzymes. She subsequently complained about right upper quadrant pain for 3 days (GT days 7–9). Ultrasound of the right upper quadrant was normal, and the liver did not show any abnormalities. On GT day 14, LDH returned to normal range.

I.p. catheters were removed as part of the treatment protocol, and the tips were cultured for any postoperative fever of unknown origin. Three patients developed fever associated with a possible infection of their i.p. catheters. In patients 1-2 and 3-1, catheters were removed on the first day after vector delivery (9 and 10 days after catheter placement) because of temperature elevation to >38°C without visible skin irritation. At the time of secondary debulking, patient 1-2 had undergone small bowel resection and patient 3-1 had extensive enterolysis and tumor resection from the surface of the right ascending colon. For both patients, cultures showed enterococci.

At 19 days after catheter placement and 10 days after the start of treatment with topotecan, patient 3-2 developed severe myelosuppression (neutropenia grade 3) combined with fever. As a matter of precaution, the i.p. catheters were removed and their tips were cultured. The culture revealed *Staphylococcus aureus*, most likely as a contamination.

All infections were successfully treated with the removal of the infected catheters and antibiotic regimens.

One patient had an infected central line and a fever of

38.5°C. One patient had neutropenic sepsis that resolved with antibiotic treatment.

Temperature elevations not associated with an identified infection occurred in two patients. Patient 4-3 developed a fever of 40°C at 10 hours after vector injection and patient 2-1 had a temperature elevation of 37.7°C at 15 hours after vector administration.

Nausea, vomiting, and diarrhea were mild and responded well to symptomatic treatment.

Three patients complained about mild peripheral edema with swelling of ankles and hands during the first week of treatment. This was most likely secondary to the dexamethasone administration with topotecan.

Polymerase chain reaction analysis of the urine specimens revealed the following results: one patient at dose level 1 presented with vector excretion for 2 days after vector injection; on dose levels 2 and 3, no vector was detected in daily urine specimens; on dose level 4, three patients showed vector excretion for 2–5 days. The last vector DNA in urine analysis was seen 12 days after vector delivery (patient 4-2), and all patients went home without vector excretion.

DISCUSSION

The major objective of this phase I clinical trial was to evaluate the toxicity associated with the combination of GT and topotecan. GT with an anti-herpetic drug and ADV-RSV-tk has been used in animal models, and several protocols have been published for application in humans.[6,19–25] To our knowledge, no prior study has been conducted to assess the combination of GT with topotecan after secondary optimal tumor debulking.

The adverse patient effects encountered in this trial (Table 4) were not different from those reported with topotecan alone; therefore, we conclude that no serious complications attributable to GT were encountered.

ACV

The most common adverse events found in previous studies with ACV were inflammation or phlebitis at the injection site, transient elevations of serum creatinine or blood-urea-nitrogen/urea nitrogen (the higher incidence usually occurring after rapid i.v. infusion), nausea or vomiting, itching, rash, hives, and elevation of transaminases.[8]

In our study, we saw one allergic reaction at the injection site, three transient elevations of liver enzymes, and four complaints of mild nausea and vomiting. Changes in creatinine or urea nitrogen were not seen.

Topotecan

Topotecan demonstrated broad antitumoral activity in preclinical studies.[26] Topotecan showed clinical activity against cisplatin-refractory ovarian cancer, with response rates of ~20%, which is one of the highest activities of any chemotherapeutic agents against this disease.[17,27,28] The most common adverse event described with topotecan was myelosuppression, with up to 92% of patients experiencing grade 3 or 4 neutropenia and 67% experiencing grade 3 or 4 thrombocytopenia.[17,27,29]

The median durations for grades 3 and 4 neutropenia and thrombocytopenia were 10 and 5 days, respectively.[18] Maculopapular pruritic exanthema occurred in 20% of patients. Gastrointestinal side effects were mild. No cardiovascular, genitourinary, or neurological toxic effects were observed. No correlation between glomerular filtration rate or bilirubin level and hematological toxicity or response was reported.[18] Similarities were observed in this study (Table 4).

Vector- and procedure-associated adverse reactions

The side effects of i.p. GT are mainly unknown. A potential complication of i.p. vector delivery was abdominal pain secondary to peritoneal inflammation. All of our patients received vector injection 1 week postoperatively, while most were still experiencing postoperative abdominal pain. No additional pain was reported after vector injection.

Although peritoneal sepsis is another possible complication of any transabdominal injection, empirically occurring in $<1\%$ of patients, none of the 10 patients in this study developed this complication. However, i.p. catheters were infected in two (and possibly three) patients. Bowel surgery may present a special risk factor for the development of catheter infections.

For future studies, we recommend removal of the catheters after vector injection to prevent infection. To avoid vector spillage, we do not suggest pulling the catheter directly after vector delivery.

Although the risk is minor, a primary safety concern is the possible development of replication-competent adenoviruses and wide dissemination of such a recombinant virus in the patient, the patient's family, and health care workers.[30] However, this problem is minimized by the production of replication-deficient vectors and by testing for the absence of replication-competent contaminants at various stages of production. (In daily urine samples, vector DNA was detected primarily at the highest dose level. The earliest vector excretion in urine was seen on the day of vector injection and the last was detected 12 days after vector delivery. No wild-type vector was detected.) Therefore, we conclude that the use of adenovirus as the parent vector poses a very limited health risk even with vector excretion in the urine.

The patient toxicities encountered in this trial mainly mirror those of reported second- and third-line chemotherapies. However, thrombocytosis, abnormalities of liver enzymes, and temperature elevation might be secondary to GT.

Gross and microscopic analysis of systemic vector toxicity in an animal model did not show any liver abnormalities. However, liver enzymes or blood samples were not examined in that study, so that we cannot compare our results with this animal study. The only significant microscopic lesions in the animal model were epicardial inflammation and splenic hemosiderosis.[15]

As the observed side effects of our trial were distrib-

uted over the four different dose levels and no higher toxicities were seen in dose levels 3 and 4 (Table 4), we did not encounter a maximum tolerated dose in this route of administration. However, the production of higher virus titers was prohibited by cost in a laboratory-driven design. Future studies may include analysis of greater vector doses. For a phase II study, we suggest using the highest vector dose tested in this trial.

In conclusion, i.p. vector delivery after optimal tumor debulking surgery with concomitant topotecan chemotherapy was well tolerated and did not result in any significant lasting toxicities.

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