A Phase I Clinical Trial of Ad5.SSTR/TK.RGD, a Novel Infectivity-Enhanced Bicistronic Adenovirus, in Patients with Recurrent Gynecologic Cancer


Abstract

Purpose: Ad5.SSTR/TK.RGD is an infectivity-enhanced adenovirus expressing a therapeutic thymidine kinase suicide gene and a somatostatin receptor (SSTR) that allows for noninvasive gene transfer imaging. The purpose of this study was to identify the maximum tolerated dose (MTD), toxicities, clinical efficacy, and biologic effects of Ad5.SSTR/TK.RGD in patients with recurrent gynecologic cancer.

Experimental Design: Eligible patients were treated intraperitoneally for 3 days with a range of doses of Ad5.SSTR/TK.RGD followed by intravenous ganciclovir for 14 days. Toxicity and clinical efficacy were assessed using Common Toxicity Criteria (CTC) Adverse Events grading and Response Evaluation Criteria in Solid Tumors (RECIST) criteria. Imaging using In-111 pentetreotide was obtained before and after treatment. Tissue samples were obtained to evaluate for gene transfer, generation of wild-type virus, viral shedding, and antibody response.

Results: Twelve patients were treated in three cohorts. The most common vector-related clinical toxicities were grade I/II constitutional or pain symptoms, experienced most often in patients treated at the highest dose. MTD was not identified. Five patients showed stable disease; all others experienced progressive disease. One patient with stable disease experienced complete resolution of disease and normalization of CA125 on further follow-up. Imaging detected increased In-111 pentetreotide retention in patients treated at the highest dose. Ancillary studies showed presence of Ad5.SSTR/TK.RGD virus and HSV1-tk expression in ascites samples collected at various time points in most patients treated within the higher dose cohorts.

Conclusions: This study shows the safety, potential efficacy, and possible gene transfer imaging capacity of Ad5.SSTR/TK.RGD in patients with recurrent gynecologic cancer. Further development of this novel gene therapeutic appears to be warranted.

Introduction

Nearly 22,000 women are diagnosed annually with ovarian cancer (1). Because of a lack of effective screening strategies and the nonspecific nature of presenting signs and symptoms, most patients with epithelial ovarian cancer will be diagnosed with advanced stage disease. Advances in surgical debulking and chemotherapy have clearly led to improvements in median survival for these patients (2–5). However, most patients diagnosed with ovarian cancer will ultimately develop recurrent disease, become resistant to further therapy, and eventually succumb to their disease. Thus, there has been a clear need for the development of new therapeutic approaches for those patients affected by ovarian cancer as well as other selected advanced stage or recurrent gynecologic cancers such as primary peritoneal cancer, fallopian tube cancer, and endometrial cancer.

Over the past 2 decades, various gene therapeutic approaches for ovarian cancer have been investigated (6, 7). One such approach is suicide gene therapy, alternatively known as molecular chemotherapy. This strategy is based upon delivery of gene that will express an enzyme capable of converting a prodrug into an active toxic metabolite. The most commonly investigated suicide gene therapy based gene therapeutic has been the thymidine kinase (TK) gene from the herpes simplex virus (HSV) given in combination with the nucleoside analogue ganciclovir. Expression of the HSV-TK enzyme allows for tumor cells to...
ultimately convert ganciclovir into a triphosphate configuration, which will inhibit DNA synthesis and mediate tumor cell apoptosis. The therapeutic effect of this approach is augmented by a “bystander effect,” whereby triphosphorylated ganciclovir is transferred to other nontransduced cells via gap junctions that, in turn, mediate additional tumor cell cytotoxicity (8).

Prior studies of ours using an adenoviral vector-mediated HSV-TK suicide gene therapy approach confirmed the use of this approach in preclinical models of ovarian cancer (9, 10). A subsequent phase I trial of ours showed the safety of intraperitoneal administration of an HSV-TK–expressing adenovirus in combination with intravenous ganciclovir in a cohort of 14 patients with persistent/recurrent ovarian cancer (11). Others had shown the feasibility of using a HSV-TK–mediated suicide gene approach using retroviral-based vectors (12, 13). While toxicity with this approach was limited, no significant clinical responses were noted in our initial clinical trial or in those conducted by others. In addition, while gene transfer was noted in ascites samples obtained from patients in these trials, the copy number of HSV-TK genes may have been too low to produce a meaningful clinical effect. Moreover, the manner in which tissue is retrieved for gene transfer evaluation was invasive, cumbersome, and associated with a high degree of sampling variability.

To address the limitations of adenoviral mediated cancer cell transfection and clinical activity noted in our previous trial, we have investigated various strategies to enhance adenoviral infectivity. For example, we have modified the adenoviral fiber knob of the type V adenovirus to incorporate an arginine-glycine-aspartate (RGD-4C) motif in the HI loop of the knob (14, 15). This modification directs adenoviral transfection via cell surface integrins rather than its normal receptor, the coxsackie adenoviral receptor (CAR). Proof-of-principle studies have shown that RGD-modified adenoviruses mediate enhanced gene transfer to established and primary ovarian cancer cells (16). In addition, we have also developed various novel noninvasive strategies to assess gene transfer in vivo (17, 18). One such method relies upon incorporation of the SSTR-expressing construct, which allows for noninvasive gene transfer imaging using conventional nuclear medicine imaging. The use of this approach in preclinical models has also been previously validated (19–21). Advantages of SSTR include the availability of a human-approved imaging agent (In-111 pentetreotide) for gamma camera and single-photon emission computed tomographic (SPECT) imaging, as well as excellent positron emission tomographic (PET) tracers targeting SSTR for imaging and therapy. Imaging SSTR was more sensitive than imaging TK for detection of low doses of adenoviral-mediated gene transfer, and SSTR showed a linear relationship between adenoviral dose and retention of the imaging agent targeting the SSTR, whereas a linear relationship was not found between the adenoviral dose and trapping of iodinated FIAU that was specific for TK (21). Detailed comparisons between SSTR and TK were published previously (21).

We have incorporated these infectivity and noninvasive gene transfer assessment enhancements into our adenoviral-mediated suicide gene therapy approach. Specifically, Ad5.SSTR/TK.RGD, a bicistronic RGD-modified adenovirus encoding both a HSV-TK and a somatostatin receptor (SSTR)-expressing construct, has been developed (22). Preclinical studies have confirmed the antitumor activity and gene transfer imaging capacity of this reagent in preclinical modes of ovarian cancer (23, 24). Safety studies have also identified toxicity and biodistribution of Ad5.SSTR/TK.RGD in Syrian hamsters (25). The primary purpose of this study was to determine the maximum tolerated dose (MTD) and spectrum of toxicities associated with intraperitoneal administration of Ad5.SSTR/TK.RGD given in combination with intravenous ganciclovir to patients with recurrent ovarian and other selected gynecologic cancers. The ability to image gene transfer, the potential clinical efficacy, and the biologic effects of this novel therapeutic strategy were also assessed.

Materials and Methods

General study design

This study was a phase I dose-escalating trial evaluating intraperitoneal administration of Ad5.SSTR/TK.RGD in combination with intravenous ganciclovir in cohorts of eligible patients. With the standard 3 + 3 design, this study was approved by the University of Alabama at Birmingham (UAB, Birmingham, AL) Institutional Review Board (IRB) and Institutional Biosafety Committee. Approvals were also obtained from the NIH Recombinant DNA Advisory Committee and the U.S. Food and Drug Administration (FDA).

Patient eligibility

Eligible patients included women aged 19 years or older with histologically proven recurrent epithelial ovarian, primary peritoneal, fallopian tube, or endometrial cancer, who had been previously treated with conventional surgical
procedures and standard adjuvant therapies. Patients were required to have adequate organ laboratory function as defined by a white blood cell (WBC) count >3,000 μL, granulocyte count >1,500 μL, platelets >100,000, creatinine clearance >80 mg/dL, creatinine <2.0, aspartate aminotransferase or alanine aminotransferase <2.5× the upper limit of the normal reference range, bilirubin <2.0, and a prothrombin time/international normalized ratio/partial thromboplastin time (PT/INR/PTT) <1.5× the upper limit of normal reference range. Patients were also required to have an ejection fraction of >55% on echocardiogram, oxygen saturation >92% on room air, a Gynecologic Oncology Group (GOG) performance status 0 to 2 and a life expectancy >3 months. Those patients found to have tumors of low malignant potential, germ cell or sex cord/stromal tumors, active cardiac or pulmonary disease, or coagulation disorders were excluded. All patients were required to sign the provided informed consent.

**Ad5.SSTR/TK.RGD**

Ad5.SSTR/TK.RGD is a tropism-modified recombinant adenoviral vector developed in the Gene Therapy Center at UAB (22). The virus is a replication-defective Ad5 derivative carrying deletions in the E1 and E3 genomic regions with a modified fiber gene. A dual expression transgene cassette has been inserted into the E1 region that results in expression of SSTR under the control of the immediate-early cytomegalovirus (CMV) promoter and TK under the control of the SV40 promoter. The fiber gene carries an insertion of an RGD motif.

Ad5.SSTR/TK.RGD was formulated in 20 mmol/L Tris, 25 mmol/L NaCl, 2.5% (w/v) glycerol, pH 8.0 (glutathione S-transferase) and was provided in sterile, single use containers that were stored at –80 degrees and kept refrigerated until administration. The daily dose of Ad5.SSTR/TK.RGD was diluted in 250 mL of 0.9% NaCl, USP bag. Ad5.SSTR/TK.RGD was manufactured with support from the NCI RAID Program at the Cell and Gene Therapy Center at Baylor College of Medicine and at the Biopharmaceutical Development Program/SAIC-Frederick, Inc. at NCI-Frederick (Frederick, MD). Stability testing of the reagent continued throughout the duration of the clinical study.

**Ganciclovir**

Ganciclovir (Cytovene) was obtained in vials of sterile powder from Syntex Laboratories, Inc. and reconstituted by adding 10 mL of sterile water for injection to provide a final solution at 50 mg/mL. The appropriate dose was then diluted in 100 mL of normal saline.

**Treatment plan, Ad5.SSTR/TK.RGD dose cohorts, and ganciclovir dosing**

Before treatment, patients underwent evaluation which included: history and physical examination, toxicity grading, performance status assignment, complete blood count (CBC), chemistry panel, hepatic function panel, coagulation studies, CA-125, echocardiogram, oxygen saturation, and computed tomography (CT) of the abdomen and pelvis. Those patients who completed pretreatment evaluation and met eligibility criteria were enrolled onto study and subsequently had an intraperitoneal Quinton Curl, 22.4-inch, double-cuffed, Tenckhoff catheter (Tyco Healthcare) placed by an interventional radiologist at least 1 week before use.

Patients were then assigned into one of 3 Ad5.SSTR/TK.RGD dose cohorts ranging from 1 × 10^{9} to 1 × 10^{12}vp/dose (6 × 10^{7} – 6 × 10^{10} pfu/d; Supplementary Table S1) Similar to the strategy validated in our preclinical studies, assigned Ad5.SSTR/TK.RGD doses were administrated via the intraperitoneal catheter daily for 3 consecutive days. Ganciclovir was then administered intravenously from days 5 to 18 at a dose of 5 mg/kg at a constant rate for more than 1 hour, twice daily. On days 1 to 4, 11, 18, and 29, a history and physical examination, performance status, toxicity grading, CBC, and chemistry panel was obtained on all study patients. CA-125 and CT scan of the abdomen and pelvis were repeated on day 29.

Peritoneal aspirates, as well as urine, saliva, and serum specimens were obtained immediately preceding Ad5.SSTR/TK.RGD dose administration on days 4, 11, 18, and 29 for planned ancillary biologic studies. All samples were processed and deidentified before conducting ancillary biologic studies.

**Evaluation of clinical toxicity**

Grading of toxicity was carried out on the days previously specified using the NCI Common Toxicity Criteria (CTC), version 3.0. Dose-limiting toxicity was defined as any vector-related, grade III, nonhematologic toxicity or a hematologic toxicity as defined by any admission for neutropenic fever, absolute neutrophil count <500 for >5 days, or platelet count <20,000. The MTD was defined as the dose exceeded by the dose at which at least 2 patients experienced dose-limiting toxicity.

**Evaluation of clinical efficacy**

Clinical efficacy was determined by comparing pretreatment CT findings with those noted on CT on day 29 using Response Evaluation Criteria in Solid Tumors (RECIST) criteria, version 1.1 (26). Measurable disease was defined as at least one lesion >1 cm that could be accurately measured in one dimension. In each patient, up to 5 lesions per organ or 10 lesions total were identified as target lesions. Complete response required disappearance of all target lesions and normalization of CA-125. Partial response was defined as >30% decrease in the sum total recorded dimensions of a patient’s target lesions. Progressive disease was deemed as >20% increase in the sum total recorded dimensions of a patient’s target lesions. Any condition that did not qualify for partial response or progressive disease was deemed stable disease.

**Noninvasive imaging of Ad5.SSTR/TK.RGD gene transfer**

Ad5.SSTR/TK.RGD gene transfer was assessed noninvasively in 9 patients (3 patients per each dose cohort).
Specifically, patients were imaged by a whole-body planar technique on a Philips Forte dual-headed gamma camera, at 4 hours, after intravenous dosing with In-111 pentetreotide (mean = 6.1 mCi, 222 MBq; range, 204–238.7 MBq). There were 2 separates doses and imaging sessions for each patient, separated in time by at least 1 week. The first session was obtained the week before Ad5.SSTR/TK.RGD therapy and the second imaging session was carried out 1 day after the final dose of therapy.

Radionuclide images were graded qualitatively (and blinded to Ad5.SSTR/TK.RGD dose group) as showing no uptake beyond expected biodistribution (0), mild uptake (less than expected bowel uptake; 1+), or uptake equal to or greater than bowel (2+). For each imaging session a ratio of activity in the abdominal cavity to background (mediastinum) was calculated in 2 ways. First, the average counts in 3 adjacent regions of interest (ROI) that included the entire abdomen and pelvis was determined and divided by counts in an identical sized ROI placed over the mediastinum. Second, an identical size ROI was drawn around the region in the abdomen of "apparent" increased uptake (unrelated to normal excretion) and divided by the same mediastial ROI.

Evaluation of Ad5.SSTR/TK.RGD gene transfer and wild-type adenovirus generation in ascites samples

Total DNA from cellular material obtained from ascites samples was isolated by a QiAamp DNA Mini Kit (QIAGEN) according to the manufacturer’s protocol. Real-time quantitative PCR was used to evaluate for Ad5.SSTR/TK.RGD gene transfer and for the generation of wild-type (WT) virus using primers and probe specific for the RGD4C peptide coding sequence and the early viral E1A gene, respectively. Both E1A and RGD primers were confirmed to only amplify the virus being tested. To adjust for the amount of DNA varying between samples, the primers and probe for the human β-actin housekeeping gene were used to determine the cellular DNA content present in each sample. Primers and probe sets, which were used in this study, as designed by the Primer Express 1.5 software and synthesized by Sigma-Aldrich, are detailed in Supplementary Table S2. FastStart TaqMan Probe Master mix (Roche Applied Science) was used to run duplexing RT-PCR reactions in triplicate wells with each ascites sample along with the standard dilutions containing 10³, 10⁴, 10⁵, and 10⁶ copies/μL of Ad5.SSTR/TK.RGD or wild-type Ad5 genome and 50, 5.0, 0.5, or 0.05 ng/μL of human DNA on a LightCycler 480 (Roche Molecular Biochemicals). Thermal cycling conditions were set to 8 minutes, at 95°C, followed by 45 cycles of 10 seconds at 95°C and 40 seconds at 60°C. Data were analyzed with the LightCycler 480 1.5.0 SP1 software and the resultant numbers of RGD- or E1A-containing viral genomes were normalized to the amount of human DNA detected in the same sample to allow for comparison between patients and at different time points.

Assessment of Ad5.SSTR/TK.RGD-mediated expression of hSSTR2 and HSV1-tk genes

Total RNA was isolated from ascites samples by use of the QiAamp RNeasy Mini Kit (QIAGEN) according to the manufacturer instructions and used as a template for quantitative reverse transcription PCR (RT-PCR) as follows. RT-PCR was carried out using the TaqMan one step PCR master mix supplemented with RT enzyme mix (Applied Biosystems) and both the hSSTR2 target and human glyceraldehyde-3-phosphate dehydrogenase (hGAPDH) housekeeping gene-specific primers and probes (Supplementary Table S2) were added at a final concentration of 100 nmol/L and delivered into the LightCycler 480 System (Roche Molecular Biochemicals). Known amounts of Ad5.SSTR/TK.RGD genome (10⁸, 10⁷, 10⁶, or 10⁵ copies/μL) and human genomic DNA (50, 5.0, 0.5, or 0.05 ng/μL) were used to generate standard curves for quantification of cDNA copies synthesized on hSSTR2 or hGAPDH mRNA isolated from ascites samples, respectively. For this assay, each unknown and standard sample was subjected to duplexing RT-PCR reactions in triplicate wells under thermal cycling conditions set to 30 minutes at 48°C, 10 minutes at 95°C, 40 cycles of 15 seconds at 95°C, and 1 minute at 60°C. Data were analyzed with LightCycler 480 1.5.0 SP1 software, and the resultant hSSTR2 copies were normalized to the amount of housekeeping gene (hGAPDH) detected in the same sample to allow for comparison between patients and at different time points.

To determine the expression levels of the HSV1-tk gene in ascites cells infected with Ad5.SSTR/TK.RGD vector both the HSV1-tk gene and hGAPDH housekeeping gene-specific primers and probes (Supplementary Table S2) were used to run duplexing RT-PCR with RNA samples as described earlier.

Assessment for Ad5.SSTR/TK.RGD viral shedding

Total DNA from the patients’ blood and saliva specimens was isolated by the QiAamp DNA Mini Kit (QIAGEN) according to the manufacturer protocol. Urine specimens were initially concentrated with the Millipore Amicon Ultra-4 Centrifugal Filter Units and then used to isolate viral DNA with the QIAamp MinElute Virus Spin Kit (QIAGEN), according to the manufacturer’s instructions and used as a template for RT-PCR. RT-PCR was carried out using the TaqMan one step PCR master mix supplemented with RT enzyme mix (Applied Biosystems) and both the hSSTR2 target and human glyceraldehyde-3-phosphate dehydrogenase (hGAPDH) housekeeping gene-specific primers and probes (Supplementary Table S2) specific for the RGD4C coding sequence as described earlier.

Evaluation of an antiadenoviral neutralizing antibody response

To evaluate for an antiadenoviral neutralizing antibody response after treatment, a nonreplicative, luciferase-expressing virus, Ad5-RGD-Luc1, was neutralized by either serum or ascites before infection of SKOV3.ip1 cells, a cell line derived from the implantation of SKOV3 cells (American Type Culture Collection) in nude mice. After neutralization in respective samples, Ad5-RGD-Luc1 transduction efficacy was determined by a luciferase assay. Triplicates of
SKOV3.ip1 cells were plated into 96-well plates (10,000 per well) and grown overnight before infection. A 1:2 dilution of serum or ascites of each time point specimen was prepared in Opti-MEM (Media Preparation Shared Facility, UAB) in a normalized volume. Nonreplicative Ad5-RGD-Luc1 at 100 plaque-forming unit (pfu) per cell was mixed with each dilution for 30 minutes at room temperature before addition to appropriate wells. This infection was allowed to proceed for 48 hours. A luciferase assay was carried out by a luciferase assay system (Promega) on an Orion microplate luminometer (Berthold) reading Cultur-plate-96 wells (Research Parkway) according to the manufacturer’s protocols.

**Statistical analysis**

A deidentified list of patients was provided with dose level of Ad5.SSTR/TK.RGD, age, race, cancer type, treatment date, and number of prior chemotherapy regimens. Clinical and laboratory adverse events were tabulated by category and grade. Formal statistical tests were limited to a 2-way ANOVA for noninvasive imaging and in regard to detecting an adenosine neutralizing antibody response.

**Results**

**Patient demographics and treatment**

From August 2009 to November 2010, 12 patients were screened and consented to participate in this phase 1 trial. Table 1 summarizes study patient demographics. The median patient age was 61 (range, 50–68 years). Ninety-two percent of patients were Caucasian, and 75% had recurrent ovarian cancer. The median number of prior chemotherapy regimens was 4 (range, 1–11). All patients had successful placement of an intraperitoneal catheter. Patients were enrolled in escalating Ad5.SSTR/TK.RGD dose cohorts (1 × 10⁹–1 × 10¹² vp/dose) and all completed treatment with Ad5.SSTR/TK.RGD according to protocol. In-111 pentetreotide was not commercially available during treatment of the first 3 patients in the highest dose cohort. After obtaining FDA and IRB approval, 3 additional patients were enrolled in the highest dose cohort to facilitate noninvasive assessment of gene transfer in such patients.

**Toxicity associated with intraperitoneal administration of Ad5.SSTR/TK.RGD**

All reportable clinical and laboratory adverse events by grade noted in patients enrolled in this study are summarized in Table 2. One patient had a grade 1 intraperitoneal catheter-associated infection before administration of Ad5. SSTR/TK.RGD that appropriately responded to antibiotics. Of the 107 clinical adverse events noted, 94 were classified as not or unlikely to be related to Ad5.SSTR/TK.RGD treatment. Thirteen were classified as attributable to Ad5.SSTR/TK.RGD treatment, 7 as “possibly” and 6 as “probably.” The clinical adverse events attributable to Ad5/SSTR/TK.RGD occurred in 6 patients and appeared to be dose dependent (1 patient in the intermediate dose cohort and 5 patients in the highest dose cohort). These events were grade I/II in nature and included fatigue (1), fever (2), flu-like symptoms (3), headache (2), abdominal pain (4), or back pain (1). All were in general transient and managed medically. There were no grade III–IV dose-limiting clinical toxicities attributable to Ad5.SSTR/TK.RGD.

Four patients, one in the second dose cohort and 3 in the third dose cohort, experienced a total of 11 grade III and 4 grade IV clinical toxicities and 8 grade III laboratory toxicities. The patient in the second dose cohort experienced symptoms of a small bowel obstruction after placement of her intraperitoneal catheter and before treatment with Ad5 SSTR/TK.RGD; her symptoms resolved and she was treated according to protocol without further significant adverse effects. A second patient developed disease-related

<table>
<thead>
<tr>
<th>Dose</th>
<th>Patient ID</th>
<th>Age</th>
<th>Race</th>
<th>Cancer</th>
<th>Number of prior chemotherapy regimens</th>
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<tr>
<td>1 × 10⁹ vp/dose</td>
<td>101</td>
<td>66</td>
<td>C</td>
<td>Ovarian</td>
<td>8</td>
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<tr>
<td></td>
<td>102</td>
<td>64</td>
<td>C</td>
<td>Endometrial</td>
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<td>103</td>
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<td>C</td>
<td>Ovarian</td>
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<td></td>
<td>201</td>
<td>61</td>
<td>C</td>
<td>Ovarian</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>202</td>
<td>59</td>
<td>C</td>
<td>Ovarian</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>203</td>
<td>68</td>
<td>C</td>
<td>Ovarian</td>
<td>6</td>
</tr>
<tr>
<td>5 × 10¹⁰ vp/dose</td>
<td>301</td>
<td>66</td>
<td>A</td>
<td>Endometrial</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>302</td>
<td>61</td>
<td>C</td>
<td>Endometrial</td>
<td>1</td>
</tr>
<tr>
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<td>303</td>
<td>54</td>
<td>C</td>
<td>Ovarian</td>
<td>11</td>
</tr>
<tr>
<td></td>
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<td>C</td>
<td>Ovarian</td>
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<td>50</td>
<td>C</td>
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<td>61</td>
<td>C</td>
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<td>8</td>
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</table>

Abbreviations: A, African American; C, Caucasian.
of these grade III/IV clinical toxicities were deemed attributable specifically to the Ad5.SSTR/TK.RGD treatment.

The most common laboratory abnormality noted in treated patients was grade I/II anemia; 10 of 12 patients had some degree of anemia noted. There were no other grade III/IV laboratory abnormalities reported other than the 8 grade III laboratory abnormalities previously described. All laboratory abnormalities were deemed disease related and none were attributed specifically to Ad5.SSTR/TK.RGD treatment.

Clinical efficacy

All patients were assessed approximately 1 month after intraperitoneal Ad5.SSTR/TK.RGD and intravenous ganciclovir using RECIST version 1.0 criteria by comparing pretreatment abdominal pelvic CT examinations to that obtained pretreatment and pre- and posttreatment CA-125 responses (Table 3). Five patients (42%) showed RECIST-defined stable disease whereas 7 patients (58%) experienced RECIST-defined progressive disease. Three patients (25%) had a decrease in CA-125 levels and 9 (75%) had an increase in CA-125.

One patient (#302) with RECIST-defined stable disease at day 29 did not receive additional treatment and was eventually noted to have near complete resolution of disease by 5 months after her treatment (Fig. 1). She also had a >50% reduction in her CA125 at day 29 and subsequently normalized her CA-125 levels which would have characterized her as having a response to therapy according to internationally accepted criteria (27). She continues to be followed without evidence of disease progression approximately 25 months after treatment.

Three other patients were alive with disease 16 to 29 months after treatment per protocol and were being treated with other therapies. As previously stated, one patient had died of other causes (respiratory failure) soon after treatment per protocol. Seven patients had died of disease 1 to 20 months following treatment per protocol; 4 of these patients had been treated with other therapies after participating in this trial.

Noninvasive imaging of gene transfer and assessment of somatostatin expression

Patients receiving the lowest and middle Ad5.SSTR/TK.RGD doses were graded qualitatively as the same, with no significant difference in In-111 pentetreotide images before and after therapy. In contrast, all 3 evaluable patients in the highest Ad5.SSTR/TK.RGD dose group showed a qualitative increase in In-111 pentetreotide retention when comparing images before and after therapy (e.g., patient #1, 1+ to 2+; patient #2, 0 to 1+; patient #3, 1+ to 2+). By ROI analyses the average ratio (entire abdomen/pelvis) for all 9 evaluable patients before Ad5.SSTR/TK.RGD therapy was 1.07 (SE = 0.04), and there was a borderline increase ($P = 0.051$ by 2-way repeated measures ANOVA) in the ratio to 1.21 (SE = 0.06) after therapy. The sample was too small to determine whether there was a dose–response effect. However, for the 3 evaluable patients in the highest Ad5.SSTR/TK.RGD dose

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**Table 2. Clinical (A) and laboratory (B) toxicity noted in study patients by category and grade**

<table>
<thead>
<tr>
<th>Toxicity grades (NCI CTC V.3)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Total occurrences</th>
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<td><strong>A. Body system</strong></td>
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<td>0</td>
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<td>2</td>
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<td>Hyperkalemia</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>4</td>
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<tr>
<td>Elevated creatinine</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>9</td>
<td>3</td>
<td>0</td>
<td>12</td>
<td>25</td>
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<tr>
<td>Hypocalcemia</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
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<tr>
<td>Thrombocytopenia</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Elevated PTT</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Elevated INR</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>40</td>
<td>17</td>
<td>8</td>
<td>0</td>
<td>65</td>
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</table>
ANCILLARY BIOL OGIS ST UDES

Various tissue samples were obtained before and at several time points after Ad5.SSTR/TK.RGD vector administration to assess for gene transfer and expression, viral shedding, generation of wild-type virus, and generation of an antiadenovirus neutralizing antibody response using methodologies previously described. There clearly appeared to be a correlation between detectable vector and Ad5.SSTR/TK.RGD dose. Figure 3A illustrates the detection of Ad5.SSTR/TK.RGD in 2 patients from the intermediate dose cohort (5 × 10^10 vp/dose) and from all patients in the highest dose cohort (1 × 10^12 vp/dose).

To evaluate the levels of Ad5.SSTR/TK.RGD-mediated transgene expression in ascites cells we analyzed total messages using the TaqMan One Step PCR assay, which includes reverse transcription (RT) cDNA synthesis. The data presented in Fig. 3B also showed a correlation between HSV1-tk gene expression and dose. Detectable levels of HSV1-tk expression were noted at day 4 following administration of Ad5.SSTR/TK.RGD in 2 patients from the intermediate dose cohort and from all patients in the highest dose cohort. This RT-PCR assay showed higher sensitivity allowing detection of expression of heterologous HSV1-tk gene up to day 29 after Ad5.SSTR/TK.RGD administration in most patients from dose cohorts 2 and 3. The detection of HSV1-tk expression at day 29 in 6 of 8 patients in the higher 2 dose cohorts leads one to consider whether these patients could have been treated with additional ganciclovir in hopes of further enhancing an antitumor effect. Three of the 5 patients with stable disease had detectable vector and HSV1-tk. In 2 of these patients, including the patient who had a significant clinical response, HSV1-tk gene expression was not detected at day 29, suggesting the possibility that all transfected cells had been eradicated after ganciclovir exposure. However, 2 of 5 patients with stable disease were treated in the lowest dose cohort and had no detectable Ad5.SSTR/TK.RGD vector or HSV1-tk expression at any time points. Thus, it is difficult to make a firm correlation between the detection of vector or gene expression and clinical outcome from the results of this study.

Somewhat similar data were obtained when RNA samples were analyzed for hSSTr2-specific mRNA (Fig. 3C), except for the fact that the endogenous level of hSSTr2 gene expression resulted in high background values that interfered with detection fidelity. Nevertheless, a significant increase of hSSTR2 gene expression on day 4 above endogenous background levels observed on day 0 was noted in the same patients from dose cohorts 2 and 3 that were positive for HSV1-tk. None of the earlier methods could detect Ad5.SSTR/TK.RGD genome presence or vector-mediated transgene expression in patients from dose cohort 1 administered at the lowest viral dose.

Viral-shedding studies did not reveal any detectable levels of Ad5.SSTR/TK.RGD virus in patient’s serum, saliva, or urine specimens (data not shown). No emergence of wild-type adenovirus was detected in any ascites samples (data not shown).

Neutralizing antiadenoviral antibody response was determined by blocking adenoviral gene transfer in the

### Table 3. Clinical efficacy of Ad5.SSTR/TK.RGD treatment as noted by RECIST and CA-125 response

<table>
<thead>
<tr>
<th>Dose</th>
<th>Patient ID</th>
<th>Cancer</th>
<th>Pretreatment</th>
<th>Posttreatment</th>
<th>CA-125</th>
<th>Subsequent treatment</th>
<th>Current status</th>
<th>Survival, mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 × 10^9 vp/dose</td>
<td>101</td>
<td>Ovarian</td>
<td>SD</td>
<td>3,006.6</td>
<td>3,918.0</td>
<td>Yes</td>
<td>DOD</td>
<td>20</td>
</tr>
<tr>
<td>102</td>
<td>Endometrial</td>
<td>SD</td>
<td>13.8</td>
<td>144.0</td>
<td>Yes</td>
<td>AWD</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>103</td>
<td>Ovarian</td>
<td>PD</td>
<td>84.9</td>
<td>123.1</td>
<td>Yes</td>
<td>AWD</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>5 × 10^10 vp/dose</td>
<td>201</td>
<td>Ovarian</td>
<td>PD</td>
<td>319.1</td>
<td>409.2</td>
<td>Yes</td>
<td>DOD</td>
<td>7</td>
</tr>
<tr>
<td>202</td>
<td>Ovarian</td>
<td>PD</td>
<td>9.6</td>
<td>14.3</td>
<td>Yes</td>
<td>DOD</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>203</td>
<td>Ovarian</td>
<td>PD</td>
<td>337.9</td>
<td>354.0</td>
<td>Yes</td>
<td>DOD</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>1 × 10^12 vp/dose</td>
<td>301</td>
<td>Endometrial</td>
<td>SD</td>
<td>25.7</td>
<td>78.1</td>
<td>No</td>
<td>DOD</td>
<td>1</td>
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<tr>
<td>302</td>
<td>Endometrial</td>
<td>SD</td>
<td>845.7</td>
<td>53.8</td>
<td>No</td>
<td>Alive, NED</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>303</td>
<td>Ovarian</td>
<td>PD</td>
<td>274.8</td>
<td>277.3</td>
<td>No</td>
<td>DOD</td>
<td>4</td>
<td></td>
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<tr>
<td>304</td>
<td>Ovarian</td>
<td>SD</td>
<td>299.0</td>
<td>255.9</td>
<td>No</td>
<td>Died of other causes</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>305</td>
<td>Ovarian</td>
<td>PD</td>
<td>533.0</td>
<td>947.5</td>
<td>Yes</td>
<td>AWD</td>
<td>16</td>
<td></td>
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<tr>
<td>306</td>
<td>Ovarian</td>
<td>SD</td>
<td>400.1</td>
<td>385.1</td>
<td>No</td>
<td>DOD</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AWD, alive with disease; DOD, dead of disease; NED, no evidence of disease; PD, progressive disease; SD, stable disease.
presence of serum or ascites samples collected from patients before and after Ad5.SSTR/TK.RGD administration. High levels of preexisting immunity against adenovirus were noted on day 0 in most treated patients (Supplementary Fig. S1). Limited immune responses (ascites only) to adenovirus were observed in 3 patients (103, 201, and 202). A significant increase in adenoviral neutralizing antibody response was noted in most ascites samples collected from patients on day 4 and 29 as compared with day 0 \((P < 0.05)\). Despite the high levels of neutralizing antibody, all patients from group 3 showed the presence of Ad5.SSTR/TK.RGD vector resulting in relatively high levels of \(HSV1-tk\) and \(hSSTr2\) gene expression in ascites cells on day 4.

Discussion

Prior studies of ours and those of others have used a variety of viral vector systems to mediate suicide gene therapy for ovarian cancer. Though showed to be feasible and reasonably well tolerated, these trials showed limited clinical effects in treated patients. Indeed, this has been the experience in the context of other clinical trials where suicide gene therapy strategies using unmodified vector systems have been investigated for a variety of cancer sites (28–30). In the largest of these clinical trials, no significant benefit in median progression-free survival was noted in 248 patients with glioblastoma multiforme treated immediately after surgical resection and radiotherapy with a HSV-TK–expressing retrovirus in combination with ganciclovir compared with those that received standard surgical resection and radiation (31).

In this study, we report the safety and potential efficacy of using an infectivity-enhanced adenovirus to mediate a HSV-TK–based suicide gene therapeutic approach in patients with recurrent ovarian and other selected gynecologic cancers. Specifically, we were able to administer Ad5.SSTR/TK.RGD intraperitoneally daily for 3 days at dosages up to \(1 \times 10^{12}\) vp/dose. Our ancillary studies showed a consistent dose-related increase in detectable Ad5.SSTR/TK.RGD vector and \(HSV1-tk\) expression in ascites samples; the highest concentrations being noted within the highest dose cohort. Of note, no detectable Ad5.SSTR/TK.RGD shedding was noted. As observed in our other adenoviral mediated gene therapy trials, a robust antiviral antibody response was noted (11, 32, 33).
The most common clinical toxicities attributable to Ad5.SSTR/TK.RGD were grade I/II in nature and generally consisted of manageable constitutional or pain symptoms. There clearly was a relationship between adverse events and dosage of Ad5.SSTR/TK.RGD as most attributable toxicities were noted in the highest dose cohort. Other adverse events were in most instances attributable to the underlying cancer in treated patients and, in general, were medically managed. Of note, 2 patients experienced bowel obstructive symptoms after Ad5.SSTR/TK.RGD treatment. Though such symptoms have been noted as a result of inflammatory responses in patients treated intraperitoneally with a p53-expressing adenovirus (Ad-p53), we have not noted such in the current study or prior studies of ours evaluating other adenoviral-mediated gene therapies (34). The fact that Ad-p53 was administered at higher dosages (up to $2.5 \times 10^{13}$ vp/dose daily over 5 days) than that used in the current study or prior studies of ours may have contributed in part to the increase in reagent-mediated obstructive symptoms noted in the Ad-p53 clinical trial. No vector-specific laboratory abnormalities were noted. There were no grade III/IV adverse events.

Figure 2. Anterior whole body, planar In-111 pentreotide gamma camera images of a representative patient before (A) and after (B) Ad treatment (highest Ad dose cohort) show diffuse peritoneal activity following therapy (arrows) as well as normal distribution in liver, spleen, bowel, and urine. The image in B was 1 day after the last Ad5.SSTR/TK.RGD dose (of 3 doses given over 3 days). Therefore, the single imaging time point post-Ad5.SSTR/TK.RGD therapy was 24, 48, and 72 hours after the 3 respective Ad5.SSTR/TK.RGD doses. This time point is coincided with the highest levels of TK expression according to our ancillary studies evaluating TK expression in peritoneal aspirates.

Figure 3. A, quantification of Ad5.SSTR/TK.RGD vector in ascites samples. The Ad5.SSTR/TK.RGD genome copies were quantified in each patient’s ascites sample (in triplicate) with primers and probe specific for RGD-4C–coding sequence (RGD) and then normalized to amount of cellular DNA detected in the same sample with primers and probe for human β-actin (housekeeping gene) using duplexing quantitative PCR settings. No RGD DNA was detected pretreatment (data not depicted in figure). B, assessment of vector-mediated expression of HSV1-Tk gene in ascites samples. HSV1-Tk gene-specific mRNA was quantified using total mRNA isolated from ascites samples as template (in triplicate) and then, normalized to the amount of human GAPDH (housekeeping gene) mRNA, which was detected in the same sample using duplexing RT-PCR settings. No TK mRNA was detected pretreatment (data not depicted in figure). C, assessment of vector-mediated expression of SSTr2 gene in ascites samples. SSTr2 gene-specific mRNA was quantified using total mRNA isolated from each ascites sample as template (in triplicate) for reverse transcription reaction followed by quantitative PCR. The determined copy number of SSTr2 mRNA was normalized to the amount of human GAPDH (housekeeping gene) mRNA, which was detected in the same sample using duplexing RT-PCR settings.
dose-limiting clinical or laboratory toxicities directly attributable to Ad5.SSTR/TK.RGD; thus, the MTD was not identified. Manufacturing constraints limited our ability to deliver higher dosages of this reagent.

Though no partial or complete responses were noted in this trial, 5 patients (42%) had RECIST-defined stable disease 1 month after treatment and 3 (25%) had a decrease in CA-125 levels. Of note, the delayed and durable complete response noted in one patient with recurrent endometrial cancer who had stable disease 1 month following treatment was clearly impressive. Though she had the fewest number of prior therapies, it is likely that she would have had at best only a partial or transient response if treated with additional chemotherapy. Although such patients are often characterized as chemotherapy sensitive or resistant once they have recurrence of their cancer, it is unknown whether such characterization would apply in the context of gene therapies. Regardless, she remains clinically free of disease with a normalized CA-125 approximately 25 months after treatment. This delayed response is clearly promising yet the true efficacy of this approach will require further evaluation in a phase II clinical trial.

The delayed response noted in this patient raises the question as to what should be the optimal methods and timeline to assess response to gene-based therapies such as that used in this study. Recent reports suggest that gene therapies of the nature used in this trial and other approaches such as oncolytics may facilitate an antitumor immunologic response that would result in an enhanced therapeutic response (35–37). In addition, it is becoming increasingly evident that patients with ovarian cancer who appear to have evidence of an intratumoral immune response have better outcomes (38). Though not evaluated in the current trial, it would be very reasonable to evaluate antitumor immunologic endpoints such as select tumor-infiltrating lymphocytes or cytokines in future clinical trials. In addition, it may also be prudent to use recently revised RECIST criteria that have been developed to address evaluation of therapies such as this that elicit a significant immune response that may initially cause tumor swelling and be falsely interpreted as tumor progression (26).

The results of this trial mimic that noted in a prior phase I study of ours which evaluated a RGD-modified infectivity-enhanced adenoviral virotherapeutic (33). Specifically, 21 patients with recurrent ovarian and other select gynecologic cancers were treated intraperitoneally with escalating dosages of Ad5-Δ24-RGD, an RGD-modified conditionally replicative adenovirus. In this trial, patients were treated with viral dosages of up to 1 × 10^{12} VP/dose daily for 3 days with limited grade I/II constitutional or pain-type symptoms. Though no partial or complete responses were noted, 15 patients (71%) had stable disease and 7 (33%) were noted to have a decrease in CA-125 levels.

This study also evaluated a novel in vivo gene transfer imaging strategy. Ad5.SSTR/TK.RGD contains a human type II SSTR-expressing construct that allows for gene transfer imaging using In-111 pentetreotide with whole body gamma camera imaging. Imaging clearly showed statistically increased retention of the In-111 pentetreotide in patients following the highest adenoviral dose, as compared with imaging before Ad5.SSTR/TK.RGD therapy. The abdominal regions of “apparent” uptake (not including normal excretion, liver, or kidneys) had ROI ratios that averaged 1.07 (SE = 0.1) before treatment, and significantly increased (P = 0.036) to 1.42 (SE = 0.1) for the same regions after treatment. The ROI ratios refer to the ROI for abdominal region of “apparent” uptake, divided by the same-size ROI for the mediastinum (background). This result was impressive because 3-dimensional imaging studies were not conducted. Future studies with SPECT or PET (using a SSTR avid PET probe) in combination with CT will allow improved imaging of gene transfer with this approach.

Others have used various serum marker and imaging approaches to monitor gene transfer noninvasively in patients with ovarian cancer treated with various virotherapy strategies. Galanis and colleagues reported the results of a phase I trial of an Edmonston vaccine strain of measles virus engineered to express the marker peptide carcinoembryonic antigen (CEA) administered intraperitoneally to patients with recurrent ovarian cancer every 4 weeks for up to 6 cycles (39). Modest increases of CEA levels in the serum (12–16 ng/mL) were observed in all 3 patients treated at the highest dose level (10^{9} TCID_{50}). Rajec and colleagues showed the ability to conduct sodium iodide symporter SPECT imaging in a patient treated with oncolytic adenovirus Ad5/3-Δ24-hNIS (40).

New strategies are being developed to enhance suicide gene therapy for cancer. These approaches include double suicide gene therapy (41), combined suicide gene/immunotherapy (42), combined suicide gene/virotherapy (43) to name but a few. Further refinements in enhancing vector infectivity and in gene transfer imaging could certainly be incorporated into these approaches. Development of Ad5/3 vectors using dual imaging strategies is currently underway in our group.

In conclusion, this study showed the feasibility, safety, and potential clinical efficacy of using infectivity-enhanced adenoviruses intraperitoneally to mediate suicide gene therapy for ovarian and other selected gynecologic cancers. In addition, our study established the feasibility of using SSTR as a noninvasive approach to image gene transfer. Further investigation of these approaches to enhance suicide gene therapy in these cancers appears to be warranted.

Disclosure of Potential Conflicts of Interest

I. Dmitriev and D.T. Curiel are coinventors on the US patent 7,297,542 titled “Adenovirus vector containing a heterologous peptide epitope in the HI loop of the fiber knob”, which contains claim #1: A recombinant Ad5 adenovirus comprising an Ad5 fiber gene modified in the HI loop domain of an Ad5 fiber knob by introduction of a ligand comprising an Arg-Gly-Asp (RGD) peptide into said HI loop domain. The Ad5-SSTR/TK-RGD vector used in a phase I clinical trial, which is described in the article, has RGD in the fiber HI loop. The other authors disclosed no potential conflicts of interest.

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