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Vaccine therapy for ovarian cancer using Herpes Simplex virus thymidine kinase (HSV-TK) suicide gene transfer technique: a phase I trial

William R. Robinson[1], Jan Adams[1], April O'Quinn[1], and Scott M. Freeman[2]

[1] Department of Obstetrics and Gynecology, [2] Department of Pathology, [1, 2] Tulane Cancer Center, Tulane University School of Medicine, New Orleans, Louisiana.

Corresponding author: William R. Robinson, M.D., 1430 Tulane Ave, TW-40, Tulane Medical Center, New Orleans, Louisiana 70112, Tel: (504) 584-2805; Fax: (504) 584-1805; E-mail: wrobins@tmcpop.tmc.tulane.edu

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Summary

Genetically altered tumor cells expressing the HSV-TK gene have been used as vaccine therapy for multiple cancers, based on their ability to kill adjacent native cancer cells and activate an antitumor immune response. Our in vitro studies demonstrate that transduction with the HSV-TK system confers ganciclovir (GCV) susceptibility to cultured ovarian cancer cells. A murine tumor model was developed using HSV-TK modified ovarian cancer cells to test efficacy in a preclinical setting. Mice bearing intraperitoneal tumors were injected with gene modified cells and ganciclovir (GCV). The mice were evaluated for survival and immune response by analysis of tumor samples collected post treatment. Murine HSV-TK tumors undergo hemorrhagic tumor necrosis and express a cytokine cascade including TNF-a, IL-1a, IL-6, IL-2, IFN-g and GM-CSF following GCV treatment. Tumor regression occurs much less frequently in immune deficient mice than immune competent mice. These studies led to a Phase I trial of intraperitoneal administration of the vaccine in which 18 patients with recurrent chemotherapy resistant ovarian cancer were enrolled. The mean survival of patients in the Phase I trial was 11.9 months. 4/18 patients had responses based on physical findings or CA-125. One patient died of breast cancer with no evidence of ovarian cancer at 24 months. Toxicities include all patients developing grade I or II temperature elevations without other evidence of infection, seven patients who developed grade I abdominal discomfort or nausea, and one patient with a grade III elevation of kidney function tests. We conclude that the use of an HSV-TK modified vaccine is associated with tumor regression in mice, and results in the alteration of the tumor microenvironment, which becomes less immunosuppressive. The use of the vaccine in humans is technically feasible and associated with minimal toxicity. Survival in these heavily pretreated patients is similar to that seen using standard cytotoxic chemotherapy.

I. Introduction

Ovarian cancer remains the most lethal female genital malignancy in the United States. It will occur in approximately 26,000 women and result in 14,000 deaths in 1997 (Parker, 1997). Progress in the treatment of ovarian cancer has been limited by the inability of physicians to diagnose the disease at an early stage. Signs and symptoms are vague and infrequent, and no effective screening techniques have been identified. Most cases are therefore widely metastatic at diagnosis. As a result, long term survival (20-30%-5 year) in ovarian cancer patients has improved only minimally since 1980, despite improvements in surgical techniques and new chemotherapeutic agents (Morrow, 1993, Venesmaa, 1994). Treatment of ovarian cancer with standard chemotherapy (usually cis- or carbo-platin and paclitaxel) often results in an initial response. However, in most cases the tumor will re-occur within a few months to a few years. The recurrent tumors are frequently resistant to chemotherapeutic agents, and these patients usually succumb to the disease. The cancer generally remains confined to the peritoneal cavity, and death most commonly results from acute or chronic bowel obstruction.

In recent years, interest in alternative therapies for ovarian cancer has grown in response to the slow progress associated with standard therapy. This has been accompanied by rapid increases in our understanding of the molecular etiology of the disease. As a result, investigators have begun to utilize gene transfer techniques in a variety of strategies aimed at specific molecular targets. Some of the most promising approaches include compensation/repair of mutations of the host genome, augmentation of the host immune response, and manipulation of drug sensitivity.

A. Compensation/repair of host mutations

A variety of malignant tumors have been associated with alterations of certain sequences of the host genome known as oncogenes and anti-oncogenes (or tumor suppressor genes). Ovarian and breast cancers have been associated with overexpression of oncogene Her-2/neu, and loss of function of the tumor suppressor gene p53. In addition, mutations in the tumor suppressor gene BRCA-1 have been identified in association with familial breast and ovarian cancers. Efforts to alter the function of these sequences may be directed at the level of the DNA, messenger RNA, or the protein product.

Curiel and Alvarez have developed an adenovirus vector encoding an anti-erbB-2 sFv directed at abrogating the expression of erbB-2. This strategy was effective in reducing tumor burden and increasing survival in a murine model and is currently being investigated in a human trial (Deshane, 1996).

B. Augmentation of host immune response

The ability of tumors to develop mechanisms of escape from immune surveillance appears to be an important part of malignant transformation. Immunotherapy has therefore been considered as an alternative to cytotoxic chemotherapy, but preliminary trials have yielded disappointing results (Gall, 1986, Berek, 1985). This relative immunoresistance appears to result from genetic changes in the tumor which allow neoplastic cells to escape from immune surveillance (Whartenby, 1995, Becker, 1993). Genetic manipulations have been utilized in two ways to augment the host immune response to tumors. Tumor infiltrating lymphocytes can be isolated and genetically modified to produce specific cytokines that induce a more effective antitumor response. These lymphocytes can, then, be reintroduced to the patient as passive (or adoptive) immunotherapy. In the second approach, a form of active immunotherapy, tumor cells themselves are genetically modified to express cytokines or co-stimulatory molecules that lead to increased recognition and killing by the host.

Rosenberg and associates have developed a human trial based on the use of T-lymphocytes modified to express the MOv-y receptor. This receptor is derived from a monoclonal antibody that recognizes an antigen highly expressed in ovarian cancer. The modified T-lymphocytes are then introduced to the patient (Hwu, 1995). Berchuck and Lyerly (1995) have developed a active immunotherapy trial using IL-2 modified tumor cells. Tumor is collected at surgery, modified to express IL-2, and reintroduced to the patient.

C. Manipulation of drug sensitivity

Drug sensitivity in tumor cells can be manipulated to induce selective toxicity of tumor cells to an agent produced within the cell or to an introduced agent. In addition, the multi-drug resistance gene (MDR1) has been used in gene transfer experiments. This gene, identified in a number of tumors treated with chemotherapy, confers a chemoprotective effect when transferred to hematopoietic cells, allowing higher, presumably more effective doses of chemotherapy to be used (Champlin, 1994).

The trial described in the current report combines the concepts of drug sensitivity manipulation and augmentation of immune function. Gene transfer techniques using the Herpes Simplex Virus-Thymidine Kinase (HSV-TK) gene as a so-called suicide gene, have previously been used to augment immune response in a variety of tumor types (Hasegawa, 1993, Caruso, 1993). Cells carrying this gene are susceptible to the anti-viral drug ganciclovir (GCV), and appear to initiate the bystander effect, in which nearby unmodified tumor cells are killed as well. Ovarian cancer would appear to be an appropriate target for this type of therapy, as the disease is generally confined to the peritoneal cavity, allowing for effective use of an agent with a local/regional therapeutic effect.

This report describes the *in vitro* and murine studies and subsequent Phase I human trial of a gene-modified vaccine using the HSV-TK suicide gene concept for ovarian cancer. In the murine model, soluble factors associated with the immune response are reported, and tumor response to treatment is described. Toxicity and survival data are presented from the human trial and possible mechanisms of action are discussed.

II. Results

A. In vitro studies

The KBALB (murine fibrosarcoma), SKOV-3, and PA-1 (both human ovarian tumor) cell lines were used in this study. The retroviral vector LNL6 (Miller and Rosman, 1990) was transduced into the KBALB line, which was then referred to as KBALB-LNL. The retroviral vector STK (Mo

olten and Wells, 1990) was constructed from the LNL vec tor and contains an SV40 promoted HSV-TK gene. This

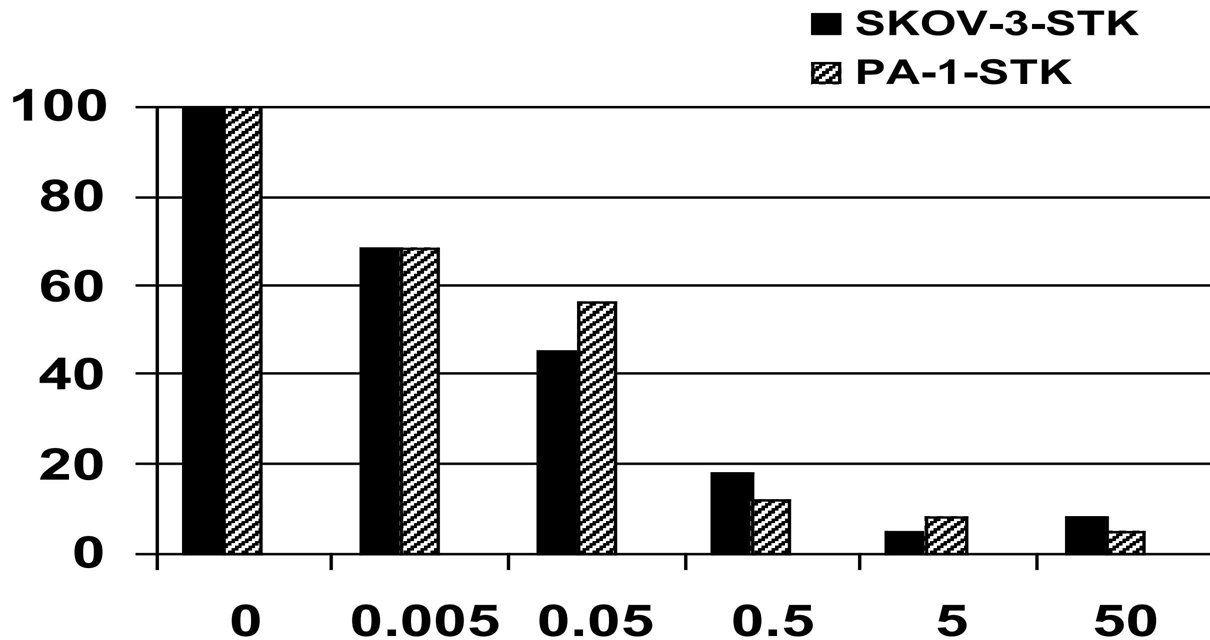

Figure 1. Colony Formation in cell lines SKOV-3-STK and PA-1-STK exposed to various concentrations of ganciclovir.

vector was transduced into all cell lines, which were desi gnated as KBALB-STK, SKOV-3-STK, and PA-1-STK. Colon y counts were performed 10-14 days following exposure of plated SKOV-3, SKOV-3-STK, and PA-1-STK cells to var ying concentrations of GCV as described below. Addition al cells from these lines were plated concurrently but not exposed to GCV in order to serve as controls. The numbe r of live colonies was expressed as a percentage of maxim al colony formation. The maximal toxic effect on the SKO V-3-STK and PA-1-STK cells was similar, and occurred at a GCV concentration of 5 mM. (**Figure 1**).

B. Murine studies

Tumors were established subcutaneously (s.c.) in mic e by injecting 1x10^6 KBALB-LNL or KBALB-STK tumor cel ls alone or in combination. After three days, the mice wer e randomly assigned to study (treatment) or control grou ps. Mice receiving treatment were injected with GCV. Mur ine tumors derived from KBALB-STK cells were harveste d at varying time intervals (1, 2, and 4 days) after treatm ent with GCV for analysis by RT-PCR. Multiple cytokines, i ncluding tumor necrosis factor-alpha (TNFa), interleukin (IL)-1a, IL-6, granulocyte macrophage-colony stimulatin g factor (GM-CSF), and interferon-gamma(IFN-g) were de tected. (**Table 2**). These factors were expressed sequenti ally in the following pattern: IL-1, TNF, and IL-6- day 1 th rough day 4; GM-CSF- days 2 and 4; IFN-day 4 only.

The ability of the animals to manifest the bystander ef fect was then tested in relation to immune competency. I mmune competent (Balb/C) and immune deficient (nude) mice were injected with subcutaneous tumors consistin g of various ratios of HSV-TK gene-modified tumor cells a nd nonmodified tumor cells (0, 50, or 100%). After three days the tumors measured approximately 10 mm^2. The a nimals were then treated with GCV and tumor size was m easured. Regression occurred in immune competent mic e when the tumor was composed of only 50% HSV-TK ge ne-modified cells. However, regression occurred in only 25% of immune deficient mice with tumors consisting of 100% HSV-TK gene-modified cells, and no regression wa s seen with tumors consisting of 50% HSV-TK gene-modi fied tumor cells (**Table 1**)

C. Phase I human trial

Eighteen patients were enrolled to the trial. The mean age of the patients was 57.3 years. 15 patients were Cauc asian and three were African-American. 17 had Stage III disease at diagnosis, and one had stage II disease. All pati ents had received either cis- or carboplatin and paclitaxel . Many received a variety of other cytotoxic agents as wel l. Entry demographic and clinical characteristics of the pa tients are summarized in **Table 1**.

% KBALB-STK	Immune Competent	Immune Deficient
100% (a)	100%	25%
50% (b)	100%	0%
0%	0%	0%

(a) Mice Injected with 100% KBALB-STK tumor cells
(b) Mice Injected with 50% KBALB-STK tumor cells and 50% KBALB tumor cells
(c) Mice Injected with 100% KBALB tumor cells

Table 1. Regression of KBALB-STK tumor in Immune-Competent vs. Immune Deficient Mice Treated with Ganciclovir.

No patients were removed from therapy for treatment-related toxicity. All patients had temperature elevations during treatment, including 10 grade II and 7 grade I fevers. These episodes occurred within 36 hours of receiving the intraperitoneal vaccine and were not accompanied by other symptoms. All temperature elevations resolved spontaneously with the use of oral acetaminophen. No delayed fevers or other signs of infection or sepsis were noted. Seven patients had grade I abdominal discomfort and/or nausea. This occurred within 30 minutes to one hour of administration of the vaccine, and was usually described as a feeling of bloating. These symptoms resolved spontaneously within 2-3 hours. All patients tolerated a minimum of 1.5 total liters of fluid given through the port to optimize distribution.

One patient developed grade I shortness of breath, and one patient developed grade I anemia. Both of these findings resolved spontaneously. One patient developed a grade III renal toxicity based on elevations of her kidney function tests. This patient had a long history of hypertensive disease and mild kidney dysfunction prior to therapy. She was apprehensive about abdominal discomfort during the treatment and had minimal oral intake during the first 1-2 days of treatment as a result. Her serum creatinine rose to 3.5 mg/dl during this time. Following slow intravenous fluid administration, her serum creatinine fell to 1.1 mg/dl over the next 2-3 days. Her urine output remained stable throughout this episode (**Table 2**).

The mean survival for all patients was 11.9 (range 2-26) months. Kaplan-Meier analysis of survival is plotted in **Figure 2**. Eight patients (BP, ES1, TA, ES2, JS, MH, RC, DM) had evidence of disease progression during or within one month of treatment. Three patients developed pleural effusions, three had rising serum CA-125 levels, and two developed abdominal tumor masses. The remaining ten patients had no evidence of disease progression during treatment. Three (SV, PN, PA) of these patients had mildly elevated CA-125 levels that remained stable during treatment, but rose approximately 3 months later. Three other patients had CA-125 levels <35 at the beginning of treatment. One (EB) died of breast cancer 24 months following treatment. Another (GR) had laparoscopy performed four months after treatment for symptoms of abdominal bloating and was found to have ascites with small volume disease, and the third (MG) has had slowly rising CA-125 levels but remains asymptomatic after 7 months.

Four patients had resolution of physical findings or decreases in CA-125 levels while receiving treatment. One patient (MM), whose CA-125 levels were consistently <10, had resolution of abdominal bloating and ascites during therapy. She remained asymptomatic for one year before relapsing and dying of disease at 23 months. Two patients had falls in CA-125 levels and died of other causes (myocardial infarction (LI) and pulmonary embolus (CM)) approximately 4-6 months following therapy. The final patient (SK) experienced a fall in CA-125 levels initially followed by a rebound three months after therapy. The CA-125 levels are summarized in **Table 3**.

III. Discussion

Ovarian cancer, like many other malignancies, appears to result from a complex interaction of acquired (and some inherited) genetic rearrangements. Traditional therapies, including surgery and cytotoxic chemotherapy, are directed at cellular reproduction in a very broad manner. This results in significant toxicity to normal tissues and a variable degree of therapeutic benefit, depending on the type and stage of tumor. Gene transfer technology using viral vectors has been greatly refined in the last decade, a

llowing for much more precisely directed antitumor effec ts. In view of the limited

Initials	Race	Age	Stage at Diagnosis	Histology	Toxicity	Max. Dose Admin. (# of cells)
CM	W	50	IIIb	endometriod	fever, gr. II	$3x10^8$
PA	W	43	IIIc	*pap. serous	fever, gr. II	$3x10^8$
BP	W	65	IIIc	*pap. serous	fever, gr. I nausea, gr. I	10^8
EB	W	74	IIIb	*pap. serous	fever, gr. I	10^8
ES1	W	66	IIIc	*pap. serous	fever, gr. II abd. dis., gr. I**	$3x10^8$
SV	W	50	IIIc	*pap. serous	fever, gr. .II	10^9
LI	W	73	IIb	*pap. serous	fever, gr. I S.O.B.***	10^9
TA	W	50	IIIc	*mucinous	fever, gr. I abd. dis., gr. I**	$3x10^8$
ES2	B	49	IIIc	*pap. serous	fever, gr. II	10^9
PN	W	67	IIIc	*pap. serous	renal tox., gr. III fever, gr. I	$3x10^9$
MM	B	51	IIIc	*pap. serous	fever, gr. II	$3x10^9$
JS	W	55	IIIc	*pap. serous	fever, gr. I abd. dis., gr. I**	10^9
MH	W	71	IIIc	*pap. serous	fever, gr. I nausea, gr. I	$3x10^9$
GR	W	42	IIIc	*pap. Serous	fever, gr.II anemia, gr. I	$3x10^9$
SK	W	63	IIIc	*pap. Serous	fever, gr. I nausea, gr. I	10^{10}
MG	W	60	IIIb	*pap. serous	fever, gr. II abd. dis., gr. I**	10^{10}
RC	W	49	IIIc	*pap. serous	fever, gr. I	10^9
DM	B	54	IIIc	*pap. serous	fever, gr. II	$3x10^9$

* papillary serous
** abdominal discomfort
*** shortness of breath

Table 2. Patient characteristics and toxicities.

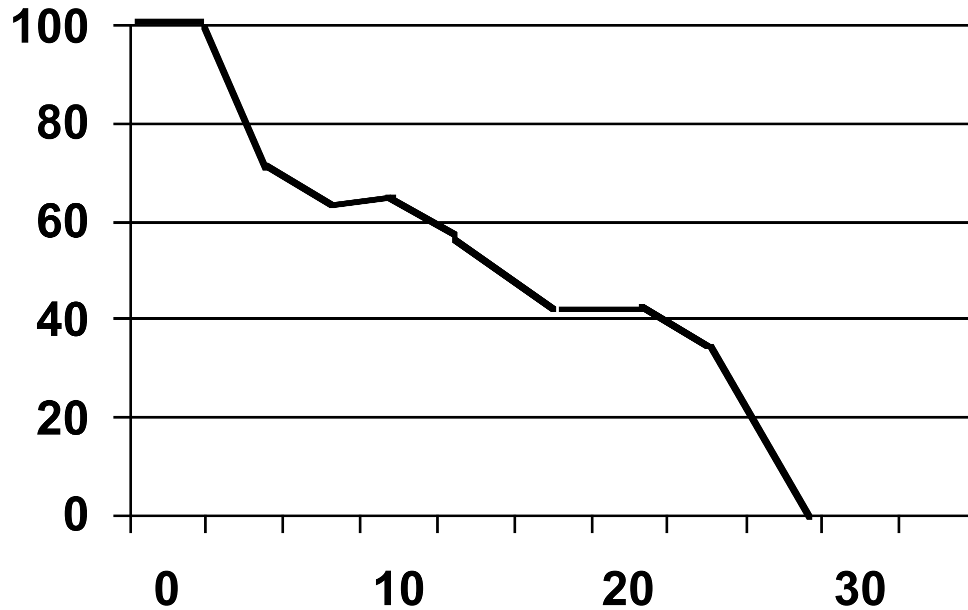

Figure 2. Kaplan-Meier analysis of survival of patients treated with PA-1-STK gene-modified vaccine.

therapeutic benefit associated with current treatments, ovarian cancer would appear to be an appropriate target for clinical trials of the introduction of therapeutic genetic material.

Suicide gene therapy, as used in this trial, refers to a process in which chemotherapy-resistant tumor cells are modified to express a gene that renders a new drug sensitivity phenotype to the tumor and under appropriate circumstances will be lethal to tumor cells.

It has been previously demonstrated that tumor cells transfected with the HSV-TK gene and exposed to GCV can be killed in vitro (Moolten, 1986). Studies of tumor-bearing animals inoculated with HSV-TK-positive cells and treated with GCV showed tumor regression as well (Moolten, 1990). The mechanism by which HSV-TK cells cause cell death is phosphorylation of GCV into a toxic nucleotide analogue which functions as a DNA chain terminator by interfering with DNA polymerase activity.

In the preclinical data from the current report, we confirm these findings by demonstrating that tumor regression occurred when tumors were genetically modified to express the Herpes Simplex virus thymidine kinase gene (HSV-TK) and treated with the anti-viral pro-drug ganciclovir. This anti-tumor effect occurred when only a fraction of the tumor expressed the HSV-TK gene. This is the basis of the bystander effect, a complex biological process consisting of three interrelated phases: (i) chemosensitization of some tumor cells, (ii) hemorrhagic tumor necrosis caused by release of soluble factors from the dying HSV-TK gene-modified tumor cells, and (iii) generation of an anti-tumor immune response.

In phase 1, the transfer of the HSV-TK gene to tumor cells chemosensitizes the tumor cell to GCV. This is accomplished in the human trial by the intraperitoneal administration of non-native tumor cells that have been transduced with HSV-TK. We have previously demonstrated the ability of tumor cells, introduced to the peritoneal cavity, to home to native tumor deposits (Freeman, 1994). The introduced cells in effect become part of the native tumor by their proximity, and thereby sensitize the tumor to GCV. This initiates phase 2, the generation of a generalized hemorrhagic tumor necrosis (HTN) produced by the release of soluble factors from the dying HSV-TK gene-modified tumor cells. The HTN leads to disruption of the tumor blood supply, and thus loss of nutrients which leads to killing of the majority of tumor cells. The final phase of this process, cytokine production by the dying HSV-TK cells, appears to result in the death of any remaining tumor cells by initiating a host immune response. We have previously demonstrated that HTN stimulates a cellular immune response, leading to a lymphocytic infiltration of the tumor (Freeman, 1994), and results in upregulation of a variety of co-stimulatory molecules including ICAM-1, B7-1, and B7-2 (Ramesh, in press).

To summarize, we hypothesize that cytokine production by the HSV-TK cells results in the transformation of the tumor microenvironment from immunoresistant to immuno-

Patient	Initial	During Therapy	Completion of Therapy
CM	163	35	31
PA	120	112	114
BP	385	446	1,588
EB	10	7	12
ES1	1,357	2,710	-
SV	308	349	473
LI	119	73	35
TA	79	90	398
ES2	81	50	60
PH	67	72	57
MM	17	20	15
JS	144	237	-
MH	600	609	749
GR	11	11	7
SK	311	158	103
MG	25	17	24
RC	23	34	26
DM	497	1,282	-

Table 3. CA-125 levels during therapy with a gene-modified vaccine.

stimulatory, allowing tumor infiltrating lymphocytes, generated by HTN, to have a lethal effect on any remaining viable tumor cells. Thus, a chemotherapy-resistant tumor can undergo complete regression if only a fraction expresses the HSV-TK gene because the augmented host immune response acquires the ability to eradicate any residual cells.

Supporting the role of the host immune system in this mechanism, the current data demonstrates that immune deficient mice had some anti-tumor effect following treatment with the HSV-TK system, but far less than immune competent mice. We have also demonstrated in the current data that the killing effect of GCV can be achieved *in vitro* at a concentration of 5.0 mM. Human pharmacokinetic data show that intravenous administration of GCV at the recommended therapeutic dose of 5mg/kg easily exceeds this level (Faulds and Heel, 1990).

The results of the human trial reported here demonstrate that intraperitoneal vaccine therapy is technically feasible in this setting. Administration of the vaccine was tolerated by all patients, and no technical problems with the use of the intraperitoneal ports were encountered. The only consistently seen toxicity was fever, which occurred in all patients, but was mild and well tolerated. Nausea and abdominal discomfort associated with infusion of the vaccine diluent were seen in approximately one third of the patients. We found that mild sedation with a benzodiazepene such as Ativan or Valium immediately prior to infusion eliminated these symptoms.

As would be expected, survival in these heavily pretreated, presumed chemotherapy-resistant patients was poor. Objective response data was difficult to interpret, as we did not require post treatment histologic verification of the presence of tumor. Most of these patients had undergone multiple surgical procedures and were understandably reluctant to agree to additional operative intervention. Estimation of tumor response was therefore based on physical findings and CA-125 results. 4/18 patients had some evidence of response, based on these criteria, with a mean survival of just under one year. We feel this is consistent with results from studies of cytotoxic chemotherapy in the salvage setting (Vergote, 1992, Eisenhauer, 1994, Creemers, 1996).

In summary, suicide gene therapy as described here results in significant tumor regression *in vitro* and in murine studies. The mechanism of action appears applicable to human ovarian cancer based on these studies as well. A Phase I human trial demonstrates that this method of therapy is feasible and well tolerated. Patient survival is similar to that seen using standard cytotoxic chemotherapy. A Phase II trial is indicated to more accurately estimate disease response.

IV. Material and methods

A. Cell lines and retroviral vectors

The KBALB, (murine fibrosarcoma) SKOV-3, and PA-1 (both human ovarian tumor) cell lines were used in this study. They were obtained from American Type Culture Collection (ATCC, Rockville, MD) and maintained as described elsewhere (Freeman, 1993). The retroviral vector LNL6 (Miller and Rosman, 1990) was transduced into the KBALB line, which was then referred to as KBALB-LNL. The retroviral vector STK (Moolten and Wells, 1990) was constructed from the LNL vector and contained an SV40-promoted HSV-TK gene. This vector was transduced into all cell lines, which were designated as KBALB-STK, SKOV-3-STK, and PA-1-STK.

To determine the *in vitro* sensitivity of these cell lines to GCV, 10^3 cells from lines SKOV-3, SKOV-3-STK, and PA-1-STK were plated separately and exposed to concentrations of GCV of either 0, 0.005, 0.05, 0.5, 5.0, or 50 mM. 10-14 days later the plates were stained with methylene blue and colonies were counted.

B. Murine studies

Female BALB/c mice (Charles River Laboratories, Wilmington, MA) obtained at 5-6 weeks of age were maintained pathogen-free according to established guidelines. BALB/C athymic nude mice (nu/nu) were also obtained from the same source. Tumors were established subcutaneously (s.c.) in all mice by injecting $1x10^6$ KBALB-LNL or KBALB-STK tumor cells alone or in combination using a 26-gauge needle. After three days, the mice were randomly assigned to study (treatment) or control groups. Mice receiving treatment were injected with GCV twice a day, for 5-10 doses (150 mg/Kg). Animals not used for survival studies were sacrificed on days 1, 2 and 4 after initiation of GCV treatment. Visible tumors were isolated aseptically under sterile conditions, snap frozen, and stored at -70^0C. Reverse transcriptase polymerase chain reaction (RT-PCR) was then performed using RNA extracted from the tumors as described elsewhere (Freeman, 1995a) using RNAzol B (Biotecx Laboratories, Houston, TX). The final concentration of the extracted RNA was adjusted to 1mg/ml. First strand complimentary DNA (cDNA) was synthesized from total RNA by reverse transcription using 50 picomoles of 3' downstream primer (antisense) for each of the cytokines (TNF, IL-1, IL-2, IL-4, IL-6, IL-10, IFN-g and GM-CSF) tested and PCR performed (Freeman, 1995a). The amplified PCR product was detected by agarose gel electrophoresis and confirmed by Southern hybridization.

C. The phase I human trial

All patients had a histologically proven diagnosis of ovarian cancer with clinical evidence of recurrent, progressive or residual disease confined to the peritoneal cavity following treatment with combination chemotherapy to include cis-platin or carboplatin and paclitaxel. Southwest Oncology Group (SWOG) performance status for all patients was 0-1. At least six weeks had to have passed since the most recent exposure to chemotherapy, and patients could not have tumor masses larger than 2 cm prior to treatment. Tumor size and location were determined by surgery and/or imaging study. The Tulane Instituitional Review Board and the Food and Drug Administration reviewed and approved this trial. All federal, state and institutional regulations regarding consent were fulfilled.

All patients underwent placement of an intraperitoneal port-a-cath type device. The reservoir of the port was placed in the subcutaneous fat on the chest wall below the breast and secured with permanent sutures. The silicon tubing was tunneled through the subcutaneous tissue of the abdominal wall and inserted through the fascia and peritoneum approximately 3 cm lateral to the umbilicus. The tubing was secured to the abdominal wall fascia with absorbable suture at this point. The intraperitoneal portion of the tubing, including the fenestrated section, was directed into the pelvis. Complete access to the peritoneal cavity was verified at this time. Any adhesions which obstructed access were dissected.

The gene-modified human ovarian cancer cell line PA-1-STK was selected for use as the vaccine and tested for contaminants including bacteria, fungi, and viruses. PA-1-STK cells were lethally irradiated prior to administration to eliminate any intrinsic oncogenic potential of the vaccine. The survival of the irradiated vaccine cells was 3-4 days in vitro. The PA-1-STK cells were also tested for and found to be free of replication-competent virus prior to administration (Freeman, 1995b).

1. Study design

This Phase I study was designed as an escalating dose trial to determine the maximally tolerated dose (MTD) of use of the PA-1-STK cell line as a vaccine for ovarian cancer. The objectives were: (i) to evaluate the safety and side effects of the treatment, (ii) to determine the technical feasibility of intraperitoneal vaccine administration activated by intravenous ganciclovir, and (iii) to observe for clinical effects on the cancer. All toxicities were graded according to the National Cancer Institute common toxicity criteria. Disease status was documented using physical examination and serum CA-125 levels. Patients were followed until death and survival was plotted using a Kaplan-Meier survival curve.

2. Treatment plan

The maximum cell dose each patient received is listed in **Table 1**. Treatments were planned on 21 day cycles for three total treatments. The dose escalated with each treatment unless any grade 3 or higher toxicity occurred. For grade 3 or 4 toxicity, the dose was not elevated If these grade 3 or 4 toxicities did not resolve within one week, the patient was taken off study. If more than one grade 3 or 4 toxicity occurred in any group, the next lower dose level would be considered the MTD.

The PA-1-STK cells were suspended in 500 cc of normal saline and administered through the intraperitoneal port. Additional normal saline (maximum-1500 cc) was then administered to patient tolerance through the port to assure optimal distribution. Ganciclovir was administered intravenously beginning no more than one hour following administration of the vaccine at a dose of 5mg/kg in patients with a creatinine clearance (CrCl) >80. Patients w

hose CrCl was 50-79 received 2.5mg/kg, and those with CrCl <50 were excluded. Ganciclovir was given twice dail y for seven days following each treatment with the vaccin e.

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References

Becker JC, Brabltz T, Czerny C, Termeer C, Brocker EB (**1993**) T umor escape mechanisms from immunosurveillance: induct ion of unresponsiveness in a specific MHC-restricted $CD4^+$ h uman T cell clone by the autologous MHC class II+ melanom a. **Int Immunol** 5, 1501-1508.

Berchuck A and Lyerly H (**1995**) A phase I study of autologous human interleukin 2 (IL-2) gene modified tumor cells in pat ients with refractory metastatic ovarian cancer. **Human Ge ne Transfer Protocols**, Office of recombinant DNA activitie s. Bethesda, Maryland, National Institutes of Health.

Berek JS, Hacker NF, Lichenstein AK, Jung T, Spina C, Knox RM, Brady J, Greene T, Ettinger LM, Lagasse LD (**1985**) Intraperi toneal recombinant a-interferon for salvage immunotherap y in stage III epithelial ovarian cancer: a Gynecologic Oncolo gy Group study. **Cancer Res** 45, 4447-4451.

Caruso M, Panis Y, Gagandeep S, Houssin D, Salzmann JL, Klatz mann D (**1993**) Regression of macroscopic liver metastases after in situ transduction of a suicide gene. **Proc Natl Acad Sci USA** 90, 7024-7028.

Champlin F, Kavanaugh J, Deisseroth A (**1994**) Use of safety-mo dified retrovirus to induce chemotherapy resistance sequen ces into normal hematopoietic cells for chemoprotection du ring the therapy of ovarian cancer:A pilot trial. **Hum Gene Ther** 5, 1507-1522.

Creemers GJ, Bolis G, Gore M, Scarfone G, Lacave AJ, Guastalla JP , Despax R, Kreinberg R, Van Belle S, Hudson I, Verweij J, Te n Bokkel H (**1996**) Topotecan, an active drug in the second-line treatment of epithelial ovarian cancer:results of a large European Phase II study. **J Clin Oncol** 14, 3056-3061.

Deshane J, Loechel F, Conry R, Siegal G, Alvarez R, King C, Curiel DT (**1996**) Intracellular antibody directed against erbB-2 m ediates targeted tumor cell eradication by inducing apoptos is. **Cancer Gene Ther** 3, 89-98.

Eisenhauer EA, Wim W, Ten Bokkel H, Swenerton KD, Gianni L, Myles J, Van Der Berg MEL, Kerr I, Vermoken JB, Buser K, Co lombo N, Bacon M, SantabarbaraP, Onetto N, Winograd B, C anetta R (**1994**) European-Canadian randomized trial of pal itaxel in relapsed ovarian cancer:high dose versus low dose and long versus short infusion. **J Clin Oncol** 12, 2654-2666.

Faulds D and Heel RC (**1990**) Ganciclovir: a review of its antivir al activity, pharmacokinetic properties, and theraputic effic acy in cytomegalovirus infections. **Drugs** 39, 597-638.

Freeman SM, Abboud AN, Whartenby KA, Packman CH, Koeplin DS, Moolten FL, Abraham GN (**1993**) The bystander efect : tumor regression when a fraction of the tumor mass is ge netically modified. **Cancer Res** 53, 5274-5283.

Freeman SM, Mcune C, Robinson WR, Abboud CN, Abraham GN, Angel C, Marrogi A (**1995**) The treatment of ovarian cancer with a gene modified cancer vaccine: A phase I study. Huma n Gene Ther. 6, 927-939.

Freeman SM, Ramesh R, Marrogi AJ, Jensen A, Abboud CN (**199 4**) In vivo studies on the mechanism of the bystander effect. **Cancer Gene Ther** 1, 326-332.

Freeman SM, Ramesh R, Shastri M, Munshi A, Jensen AK, Marro gi AJ (**1995**) The role of cytokines in mediating the bystand er effect using HSV-TK xenogeneis cells. **Cancer Letters**. 92, 167-174.

Gall S, Bundy B, Beecham J, Whitney C, Homesley H, Lifshitz S, A dcock LL (**1986**) Therapy of stage III (optimal) epithelial ca rcinoma of the ovary with melphalan or melphalan plus cor ynebacterium parvum (a Gynecologic Oncology Group stud y) **Gynecol Oncol** 25, 26-30.

Hasegawa Y, Emi N, Shimokata K, Abe A, Kawabe T, Hasegawa T , Kirioka T, Saito H (**1993**) Gene transfer of herpes simplex virus type I thymidine kinase gene as a drug sensitivity gen e into human lung cancer cell lines using retroviral vectors. **Am J Respir Cell Mol Biol** 8, 655-661.

Hwu P, Yang J, Cowherd R, Treisman J, Shafer J, Eshhar Z, Rosen berg SA (**1995**) In vivo antitumor activity of T-cells redirect ed with chimeric antibody/T-cell receptor genes. **Cancer R es** 55, 3369-3373.

Miller AD, Rosman GJ (**1990**) Improved retroviral vectors for g ene transfer and expression. **Biotechniques** 7, 980-985.

Moolten FL (**1986**) Tumor chemosensitivity conferred by inser ted herpes simplex thymidine kinase genes. **Cancer Res** 46, 5276-5281.

Moolten FL (**1990**) Mosaicism induced by gene insertion as a m eans of improving chemotheraputic selectivity **Crit Rev Im munol** 10, 203-233.

Moolten FL and Wells JM (**1990**) Curability of tumors bearing h erpes thymidine kinase genes transferred by retroviral vect ors. **J Natl Cancer Inst** 82, 297-300.

Morrow CP, Curtain JP, Townsend DE (**1993**) **Synopsis of Gyne cologic Oncology**, 4th ed. New York: Churchill Livingstone, 7.

Parker SL, Tong T, Bolden S, Wingo PA (**1997**) Cancer Statistics, 1997. CA-A **Cancer Journal for Clinicians**. 47, 5-27.

Ramesh, R., A. Munshi, C.N. Abboud, A.J. Marrogi and S.M. Freem an. Expression of co-stimulatory molecules: B7 and ICAM u pregulation following treatment with a suicide gene. **Cance r Gene Therapy**, in press.

Venesmaa P (**1994**) Epithelial ovarian cancer: Impact of surger y and chemotherapy on survival during 1977-1990. **Obstet Gynecol** 84, 8-11.

Vergote I, Himmelmann A, Frankendal B, Scheistroen M, Vlacho s K, Trope C. (**1992**) Hexamethylmelamine as second line th erapy in platin-resistant ovarian cancer. **Gynecol Oncol** 47, 282-286.

Whartenby, K.A., C.N. Abboud, A.J. Marrogi, R. Ramesh and S.M. Freeman (**1995**) **Biology of Disease**: The Biology of Cancer Gene Therapy, Lab. Invest., 72: p. 131-145.