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13. ABSTRACT (Maximum 200 Words)

Background: Patients with radiorecurrent prostate cancer have few options. Gene therapy may define a treatment option of both local and systemic value. Pre-clinical studies using adenovirus-mediated (Ad.) transduction of IL-12 (Ad.mIL-12) in a metastatic model of prostate cancer resulted in local growth suppression, survival enhancement and inhibition of pre-established metastases. The basis for these activities include the induction of both innate (neutrophils & NKs) and acquired immunity (T cells).

Objectives/Hypothesis: On the basis of these results, we propose to explore the use of Ad.hIL-12 in patients with clinically localized radiorecurrent prostate cancer in a Phase I trial to explore the safety, induction of immune responses and efficacy following therapy.

Specific Aims/Study Design: In Aim I patients will be placed in escalating dose cohorts with the primary endpoint of the maximum tolerated dose as determined by physical examination, laboratory values of bodily functions and evidence of IL-12 gene transduction by measurement of serum by ELISA. In Aim 2 additional safety data will be recorded through measurement of serum levels of the pro-inflammatory cytokines, TNF-α, IFN-γ and IL-16 by ELISA. In Aim 3 peripheral blood mononuclear cells (PBMCs) will be screened for the induction of T cells, which target the prostate antigens, PSA and PAP via an ELISPOT assay. In Aim 4 evidence of efficacy will be suggested from monitoring of serum PSA.

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As of the Annual report in January of last year we were awaiting final approval of the HSRRB. However, while the HSRBB approved the protocol and consent our IRB has serious issues concerning the new inclusion of the following statement: "If you are hurt or get sick because of this research study, you can receive medical care at an Army hospital or clinic free of charge. You will only be treated for injuries that are directly caused by the research study. The Army will not pay for your transportation to and from the hospital or clinic. If you have questions about this medical care, talk to the principal investigator for this study (name and telephone number of principal investigator). If you pay out-of-pocket for medical care elsewhere for injuries caused by this research study, contact the principal investigator. If the issue cannot be resolved, contact the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of the Staff Judge Advocate (legal office) at (301) 619-7663/2221." The issue of this new policy and the language explaining it in the consent were not resolved by the IRB and Legal until 11/04. At this time, during the annual IRB approval process the new Institutional policy for Conflicts of Interests and the management thereof became an issue for this trial. Dr Shu Hsia Chen, who will be overseeing the T Cell assays on patient specimens is the co-inventor of the vector used in this trial. While she will have no contact with any patient before, during or after the trial, the newly formed COI Committee reviewed the protocol and interviewed the parties involved with the following recommendations, focusing on the DMSB. They have recommended having a member of the DMSB from an outside Instituition and another member from a second DMSB responsible for a similar but different Clinical trial with similar technology and potential conflict. This has been done and we are awaiting final IRB approval. In the interim this project has been given a no cost extension.

In the interim we have prepared for publication a draft of a paper attached below, on the tissue effects of Adenovirus gene therapy on prostate tissue. The toxicity and tissue effects thereof were part of the FDA and RAC protocol and used to guide the injection scheme for this trial.

HISTOLOGICAL EFFECTS OF AN ADENOVIRUS EXPRESSING HERPES SIMPLEX VIRUS THYMIDINE KINASE IN CONJUNCTION WITH SYSTEMIC GANCICLOVIR: RESULTS OF A PHASE I CLINICAL TRIAL AS NEO-ADJUVANT THERAPY BEFORE RADICAL PROSTATECTOMY.

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Introduction:

Prostate cancer is now the most commonly diagnosed organ cancer in American males leading to an estimated 30,000 deaths[1]. Public awareness of prostate cancer and use of prostate specific antigen (PSA) testing has resulted in diagnosis of men with lower stage of disease which are more amenable to curative therapy. However, over 30% of patients undergoing radical surgery experience recurrence as measured by PSA. These failures are due to either local extension determined pathologically until after surgery or occult metastases which emerge later. On average clinical manifestation of metastasis occurs within 8 years of a detectable PSA and death follows 5 years later. Nomograms have been developed and validated which can predict those at high, intermediate or low risk recurrence based on preoperative parameters of clinical stage, PSA level and Gleason sum score. It is quite sobering to note that 34% of intermediate-low risk group, 58% of intermediate-high risk group and 83% of high risk group undergoing surgery will suffer PSA relapse following surgery. In some instances these failures can be linked to local extension. Analysis of radical prostatectomy specimens has shown that from 27 to 68% of patients with stage T2b and T2c cancer have tumor extending through the prostatic capsule, extending to the surgical margin in 17 to 43% of such patients [2,3]. Extracapsular extension alone correlates with biochemical failure rates in 26 to 34% of patients within the first five years post surgery[4,5]. Therefore, identifying new therapeutic strategies for high risk patients to be used in conjunction with standard therapies may benefit many prostate cancer patients. Ideally such an approach would have both locals and systemic effect, not compromise standard therapy and be minimally toxic.

The technologies of gene therapy are being exploited to treated localized and metastatic cancer through a variety of means. One such example, transduction of genes which activate prodrugs to produce cytotoxicity, termed pro-drug activation gene therapy, has been tested extensively in preclinical studies. Specifically, cellular expression of the Herpes Simplex virus thymidine kinase (HSV-tk) gene phosphorylates exogeneous ganciclovir (GCV), initiating a pathweay converting this fairly invert compound into an inhibitor of DNA synthesis, thereby killing the cell. This approach involves a defined "bystander effect," whereby the number of cells killed significantly exceeds the number of cells transduced with the foreign gene[7], thereby bypassing a significant limitation of in situ gene therapy. The combination of an adenovirus-mediated (Ad) delivery HSV-tk into cells in combination with GCV showed marked cytotoxicity in both mouse and human prostate cancer cells *in vitro*. *In vivo* studies in subcutaneous and orthotopic mouse prostate cancers demonstrated treatment-induced cytotoxicity, associated with areas of necrosis with inflammatory infiltration, and increased apoptotic activity following direct injection[8]. Furthermore, treatment of a primary tumor with Ad.HSV-tk+GCV resulted in inhibition of spontaneous metastasis and induced metastases, both if present before or after therapy [11]. Mechanistic studies identified the induction of anti-tumor Natural Killer cells (NK) following Ad.HSV-tk+GCV treatment within the primary tumor, which wewre found to mediate to some degree cytotoxicity within the injected tumor and completely of the anti-metastatic effect. [9] The finding of both localized and systemic activity following local expression of HSV-tk indicated the potential benefit of this regimen in the neo-adjuvant setting for patients with higher risk prostate cancer.

In this study, we examined Adv.HSV-tk + GCV as neoadjuvant therapy in higher risk patients with clinically localized prostate cancer about to undergo radical prostatectomy. The primary aim of this Phase I trial were to assess the toxicity of escalating doses of Ad.HSV-tk with fixed doses of GCV. Secondarily, because our study population underwent surgery in the weeks following gene therapy, we were able to assess the quality of immuno-infiltrate to an extent that prostate biopsies do not permit. We aim to further characterize the nature of the cytotoxicity of this local therapy through histopathological and immunohistochemical studies of the prostatectomy specimens.

Materials and Methods:

Vector

A replication-defective recombinant Adenovirus (Ad.) containing the HSV-tk gene under the transcriptional control of the Rous Sarcoma virus (RSV) long terminal repeat promoter was constructed based on an adenoviral genus C serotype 5 backbone as previously described (Max paper +Chen et al., 1994). Clinical grade vector lots were purchased from the Baylor College of Medicine Gene Vector Laboratory. Cohort 1 received virus from lot 00495A at a dose of 1×10^{10} IU (VP:IU=24 – 2.4×10^{11} vp) and cohort 2 received virus from lot 02797ATKA at a dose of 1×10^{11} IU (VP:IU=9 – 9×10^{11} vp). Ganciclovir was donated by Roche Laboratories , Inc exclusively for use in this trial.

Eligibility criteria

The protocol for the trial was approved by the Institutional Review board of Mount Sinai Medical Center the FDA (IND# 7140) and the Recombinant DNA Advisory Committee (RAC) . Patients reviewed the informed consent document and were individually counseled before agreeing to participate in this study. Patients were eligible for this Phase I trial if they had intermediate or high risk clinically localized, T1c or T2b/c, prostate cancer and had previously agreed to undergo radical prostatectomy. Inclusion and exclusion criteria are summarized in Table 1. Higher risk parameters included PSA levels of greater than 10, clinical stage T2b or c, or Gleason sum score of 7 or higher on biopsy.

Vector Injection

Each dose of vector was diluted with 0.9% saline to a volume of 1ml. Following a prostate nerve block of 10cc of 1% lidocaine vector is injected via three separate transrectal ultrasound (TRUS) guided needle tracts (21 gauge Chiba needle), directed by the presence of a palpable lesion, prior biopsies, and/or TRUS evaluation,. Only one lobe, the right or left, is injected with vector. As viewed in the transverse plain the needle was place towards the bladder neck beginning just lateral to the midline and 333ul was injected as the needle was removed. The next 2 injections were carried out ~1cm laterally from each other.

Patient Monitoring

Following injection patients were admitted to the GCRC and began ganciclovir 24 hours later via intravenous infusion, 5 mg/kg BID for seven days. Patients were monitored daily for constitutional symptoms and physical problems. Additional potential toxicities were screened via CBC, serum electrolytes, and liver function tests drawn on days 2, 4, and 7 of GCV therapy. Adverse events as reported by patients, or obtained from physical exam or laboratory report, are graded based upon the Common Terminology Criteria for Adverse Events v.3.0 (CTCAE) from the Cancer Therapy Evaluation Program (CTEP)[14]. Following a seven day course of drug therapy patients were discharged home to return 7-10 days later for planned radical prostatectomy. Patients then returned as per routine post-surgery for catheter removal and every 3 months thereafter.

Treatment Response

The primary goal of this Phase I clinical trial was the validation of the safety of this treatment before radical prostatectomy. Within the study potential influence of Ad.HSV-tk+GCV on therapy can be gleaned through the surrogate endpoints of PSA responses and tissue effects. For study purposes serum levels of PSA were sampled the day of vector injection, days 2, 4 and 7 post-vector injection and the day of radical prostatectomy.

As per routine, the removed prostate was divided into right and left, anterior and posterior and sectioned every 2 cm. Representative sections were stained with hematoxylin and eosin for pathological analysis. Further serial sections were stained for CD 8 T cells (anti-XXXXX) or NK cells (anti-CD57, XXXX). Apoptotic activity was assessed by TUNEL treatment of embedded sections as per manufactures instructions (XXXXX). [15, 8]. This analysis focused on areas of inflammation, subdivided to that involving benign prostatic hyperplasia and that with cancer, and adjacent non-infiltrated tissues. Treated sections were viewed at high power, 400x, and the number of apoptotic bodies per high power field counted and averaged across sections.

Results:

Patient Characteristics

Six patients were treated with intraprostatic injections of Ad-hsv-tk prior to scheduled radical retropubic prostatectomy (RRP). Patient characteristics are summarized in Table 2. Cohort 1 (pt 1-3) received 1 x 10^{10} IU of adenovirus, and cohort 2 (pt 4-6) received 1 x 10^{11} IU of virus. The mean age of the treated patients was 64 with a range of 54-74. All patients had clinically localized disease, mean Gleason Scores of 7, and mean PSA of 10.6. All of the patients were clinically staged as either T1C or T2C.

Adenovirus & Ganciclovir related-Events

No dose limiting toxicity was encountered in this trial with only one serious unexpected adverse event experienced (see below). Four patients experienced fevers (patient 3, and all patients in cohort 2), all of which resolved by post-vector day 3. Patient 5 had a fever classified as a Grade 2. These fevers coincided with complaints of experienced malaise, chills, and chills and nausea, in patients 3, 4 and 5 respectively.

Laboratory adverse events are summarized in Table 3. By post-vector injection day 2 Patients 1, 2, and 5 experienced Grade 1 leukopenia, with patient 2 experiencing a Grade 2 neutropenia, and patient 5, a Grade 1 neutropenia. Patient 5 also experienced a Grade 2 thrombocytopenia. Ganciclovir was re-dosed at 2.5mg/kg for the remainder of the drug course with resolution of both abnormalities. In patient 2 the abnormalities resolved without re-dosing. Patient 3 by post-vector injection day 2 experienced a grade 2 rise in creatinine which resolved following redosing of ganciclovir to 2.5mg/kg. The remaining minor laboratory changes resolved without changing drug dose or altering treatment.

Surgical Procedure

Five of six patients presented for surgery as planed without incident. When patient 3 presented for surgery he complained of a swollen leg for several days. A Doppler ultrasound diagnosed a DVT. A Greenfield filter was placed and his surgery delayed until 38 days after vector injection, when his leg was no longer symptomatic. No difficulty was encountered during sugery in any patient which could be attributed to the injections if adenovirus. Surgical pathology of each patient is summarized in Table 4.

PSA Response

PSA responses following vector injection are summarized in Table 5. In first week following Ad-HSV-tk injection 2 of 3 patients in the high dose group experienced an acute rise in PSA, one 1.8-fold, the other 2-fold. This rise returned to baseline by the time of surgery in both patients. One patient (patient 3) had a significant reduction in PSA post-therapy from 11.5 to 5.4; his surgery had been delayed due to his developing a DVT.

Histomorphologic and immunohistochemical studies

Histopathological examination of surgical specimens noted an inflammatory response confined to the lobe originally injected in 5/6 patients, consisting almost exclusively with lymphocytes. The extent of inflammation was variable, sometimes

extending across several centimeters of tissue, while in other instances, remaining rather limited. In 2 patients this infiltration was within areas containing malignant glands, most extensively in patient 3. In most instances the inflammatory infiltrate distorted the appearance of both benign and malignant glands. In areas with less intense infiltration lymphocytes were noted in and around glands and not within the surrounding stroma. Immunohistochemical staining demonstrated that the CD8+ T lymphocytes represented most of the inflammation in all sites of involvement, both benign and malignant, while few NK cells were detected. Apoptotic activity was highest within tissues containing both malignant cells and inflammatory infiltrate, 1.83+/-0.29 apoptotic bodies per HPF. This nearly four fold higher than in BPH tissue, 0.5+/-0.1 ($p=0.018$, t-test) and three fold higher than BPH with inflammation, 0.633+/-0.14 ($p=0.039$, t-test) or cancer without inflammation, 0.62+/-0.03 ($p=0.03$, t-test).

Discussion:

A number of Phase I/II clinical trials over the past several years have looked at safety and efficacy of intraprostatic injections in prostate cancer patients. To date, including our trial, 150 patients have been given intraprostatic injections of HSV-tk followed by GCV therapy (Table 6: Summary of clinical trials to date). With isolated exceptions, patients have experienced only low-grade adverse events, rendering reassuring safety data. In our study, toxicity findings were similarly reassuring. All AE were of the Grade 1 or Grade 2 subtype. Four of our six patients had some combination of the following hematological adverse events: leucopenia, neutropenia, anemia, and thrombocytopenia. These are known adverse effects of ganciclovir, and in most cases the episodes were resolved or resolving by the end of post-vector week one.

As in prior clinical trials, a number of patients experienced fever and constitutional symptoms following vector injection and GCV therapy initiation. All of the patients in cohort 2 experienced a low-grade fever, compared with 1/3 in cohort 1, and in all cases the fever was resolved by post-vector Day 3. Although this sample size is not statistically powered to make this assertion, there is the suggestion that the fever could be a dose-related effect of ADV injection. The constitutional symptoms reported were mild, all resolved very early in the post-vector course, and can be attributed to GCV adverse effect profile. The DVT experienced by one patient is not likely attributable to the therapy we are studying.

In a study of men with radio-recurrent prostate cancer who received single lobe injections of ADV/H SV-tk followed by GCV therapy, three of eighteen patients whose PSAs had been rising for >100 days prior to treatment, experienced a >50% reduction in PSA that in one case lasted almost one year[16]. When this study was extended to more accurately assess PSA response and systemic T-cell activation, a significant prolongation of the PSA doubling time (PSADT) was seen relative to controls[12]. In our study, we saw an early rise in PSA in 2/3 patients in the higher infectious dose cohort. These results suggest a dose-dependant effect of adenovirus on prostatic tissue. Only one patient had a drop in PSA, and that was the same patient who had a delay in surgery due to the development of a DVT. However it should be noted that the time between vector injection/GCV therapy and prostatectomy was relatively short. Indeed, an important method of action of phosphorylated GCV involves the inhibition of DNA synthesis, thus targeting proliferating cells. Given the low proliferative index of prostate cancer, a longer period of time is needed to exploit this action than is accounted for in this study.

Previous investigators were also able to give additional credence to the association between immune response and tumor cell cytotoxicity. They found a positive correlation between the number of apoptotic bodies and the density of CD8+ T-cells in biopsies that contained cancerous cells. Supporting this observation was the additional finding that activated T-cells (CD8+ DR+) in the periphery increased following vector injection[12]. A later trial combining gene therapy with the established modality of radiation therapy (RT) in one of its three arms reiterated these results and showed the greatest increase in peripheral CD8+ DR+ T-cells to occur in those patients receiving both modalities[17].

In the past, pathologic examination of prostates from patients who underwent gene therapy prior to surgery yielded the following findings: 1) circumscribed necrotic nodules within the tumor, or groups of decaying cells surrounded by fibrous stroma. 2) mononuclear infiltrate in portion of prostate not affected by tumor with cancerous areas focally infiltrated. 3) nuclear changes: lack of chromatin detail, absent nucleoli. It was described that these changes were found in 11-57% of total tumor volume and normal prostatic tissue was rarely affected[18]. In the past, interpretation of tissue effects was limited to TRUS biopsies, but we have demonstrated from analysis of prostate specimens a sometimes impressive lymphocytic infiltrate surrounding benign and malignant tissue with necrosis specific to the malignant cells. Importantly, these findings were limited to the injected lobe. However, this was only on needle biopsy material Although the number of patients in our study is small, there also appears to be evidence of efficacy related to therapy based on histopathological evidence of inflammation, necrosis and apoptosis within the injected area of the prostate. It is also clear that the effects of viral treatment are fairly limited to the needle tract as discussed in the next section.

Efficacy: Vector distribution

In preclinical animal models, efficacy of gene therapy was related to the ability to transduce sufficient cells within the prostate documenting the need to increase the number of injection sites. (WHAT PRECLINICAL STUDIES DESCRIBED THIS). Similar statements can be made about clinical efficacy. Our pathological observations showed that none of the gene therapy mediated changes occurred in the non-injected prostate lobe. Supporting this finding, other investigators have reported an inverse relationship between the size of the area affected by gene therapy and the size of the injected tumor and gland[18].

Our protocol called for a single injection of vector into one lobe of the prostate. Since our trial began, other investigators have asserted that diffuse distribution of vector throughout the gland is important for clinical efficacy. With this in mind, trials subsequent to when ours began have injected viral doses similar to ours in four divided injections throughout all lobes.

The ideal method of distribution vector has yet to be established. It has been suggested that injecting increasing amounts of vector into hypoechoic regions of the prostate might be appropriate, as these regions are increasingly correlated with cancer[19].

Acknowledgements\

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References

1. Parker SL., Tong T, Bolden S, and Wingo PA. Cancer statistics, 1996. CA Cancer J Clin, 46: 5-27, 1996.

2. Ohori M, Wheeler TM, Kattan MW, Goto Y, and Scardino PT. Prognostic significance of positive margins in radical prostatectomy specimens. J Urol, 154: 1818-1824, 1995.

3. Epstein JI, Walsh PC, and Bendler CB. Radical Prostatectomy for impalpable prostate cancer: The Johns Hopkins experience with tumors found on transurethral resection (stage T1a and T1b) and on needle biopsy (stage T1c). J Urol, 152:1721-1729, 1994.

4. Catalona WJ and Smith DS. 5 year recurrence rates after anatomical radical retropubic prostatectomy for prostate cancer. J Urol, 152: 1837-1842, 1994.

5. Zincke H, Oesterling JE, Blute ML, Bergstralh EJ, Myers RP, and Barrett DM. Longterm (15 years) results after radical prostatectomy for clinically localized (stage T2c or lower) prostate cancer. J Urol, 152: 1850-1857, 1994

6. Paulson DF. Impact of radical prostatectomy in the management of clinically localized disease. J Urol, 152: 1826-1830, 1994

7. Freeman SM, Abboud CN, Whartenby KA, et al. The "bystander effect:" Tumor regression when a fraction of the tumor mass is genetically modified. Cancer Res 1993;53:5274-5283.

8. Eastham JA, Chen SH, Sehgal I, et al. Prostate cancer gene therapy: Herpes simplex virus thymidine kinase gene transduction followed by ganciclovir in mouse and human prostate cancer models. Hum Gene Ther 1996;7:515-523.

9. Hall SJ, Sanford MA, Atkinson G, and Shen SH. Induction of potent antitumor natural killer cell activity by herpes simplex virus-thymidine kinase and ganciclovir therapy in an orthotopic mouse model of prostate cancer. Cancer Res, 58, 3221-3225, 1998.

10. Hall, S.J., Mutchnik, S.E., Chen, S-H., Woo, S.L.C., and Thompson, T.C. Adenoviral-mediated herpes simplex virus thymidine kinase gene and ganciclovir therapy leads to systemic activity against spontaneous and induced metastasis in an orthotopic model of prostate cancer. Int., J. Cancer, 70: 183-187, 1997.

11. Hall SJ, Mutchnik SE, Yang G, et al. Cooperative therapeutic effects of androgen ablation and adenovirus-mediated herpes simplex virus thymidine kinase gene and ganciclovir therapy in experimental prostate cancer. Cancer Gene Ther 1999;6:54-63.

12. Miles BJ, Shalev M, Aguiler-Cordova E, et al. Prostate specific antigen response and systemic T cell activation after in situ gene therapy in prostate cancer patients failing radiotherapy. Hum Gene Ther 2001;12:1955-1967.

13. Carter, H.B., Morrell, C.H., Pearson, J.D., Brant, L.J., Plato, C.C., Metter, E.J., Chan, D.W., Fozard, J.L., and Walsh, P.C. (1992). Estimation of prostatic growth using serial prostate-specific antigen measurements in men with and without prostate disease. Cancer Res. 52, 3323-3328.

14. Cancer Therapy Evaluation Program, Common Terminology Criteria for Adverse Events, Version 3.0, DCTD, NCI, NIH, DHHS. March 31, 2003 (http://ctep.cancer.gov), Publish Date: December 12, 2003.

15. Gavrielli, Y., Y., S., and Ben-Sasson, S.A. (1992). Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J. Cell Biol. 119, 493-501.

16. Herman JR, Adler HL, Aguilar-Cordova E, Rojas-Martinez A, Woo S, Timme TL, Wheeler TM, Thompson TC, and Scardino PT. (1999) In situ gene therapy for adenocarcinoma of the prostate: a phase I clinical trial. Hum Gene Ther, 10:1239-1249.

17. Satoh T, Teh BS, et al. (2004) Enhanced systemic T cell activation after in situ gene therapy with radiotherapy in prostate cancer patients. Int J Rad Onc Biol Phys, 2:562-571.

18. Ayala G, Wheeler TM, Shalev M, et al. Cytopathic effect of in situ gene therapy in prostate cancer. Hum Pathol 2000;31:866-870.

19. Teh BS, Ayala G, Aguilar L, et al. Phase I/II trial evaluating combined intensity-modulated radiotherapy and in situ gene therapy with or without hormonal therapy in treatment of prostate cancer—interim report on PSA response and biopsy data. Int J Rad Onc Biol Phys 2004, 58:1520-1529.