PHASE II STUDY OF ADENOVIRUS/PSA VACCINE IN MEN WITH HORMONE - REFRACTORY PROSTATE CANCER

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1. INTRODUCTION

1.1 Background- Immunotherapy in Prostate Cancer

Prostate cancer is the second leading cause of cancer death among males in the United States. There will be an estimated 234,460 new diagnoses of prostate cancer made in the United States in 2006 (1). Treatments for organ-confined prostate cancer include radical prostatectomy and radiation therapy. When the cancer presents de novo, or recurs outside the prostate, first-line systemic treatments typically include hormonal blockade (with LHRH agonists or bilateral orchiectomy), which suppress testosterone levels, limit the growth of androgen-dependent cancer cells, and result in clinical tumor control. After a median time of 2 years, patients progress into a clinical hormone-refractory state, when the prostate specific antigen (PSA) levels rise despite castration, there is proliferation of androgen-independent cancer cells, and there is continued clinical tumor growth that becomes fatal. Therapeutic measures in this situation include further hormonal manipulations or the use of systemic chemotherapy, which has recently shown a small survival benefit in phase III trials. Approximately 30,000 Americans die from prostate cancer each year.

Immunotherapeutic approaches against prostate cancer have been investigated for several years. Most of these studies have concentrated on active non-specific therapy and adoptive or passive therapy, with only recent focus on the induction of antigen-specific immune responses. Viral vectors have been used successfully in both gene transfer and vaccine therapy studies (2). Replication-competent and replication-deficient adenoviruses expressing foreign proteins have been used to elicit immune responses to a variety of tumor antigens (3-7).

We have demonstrated that immunizations with adenovirus, carrying the human PSA gene, can induce vigorous anti-PSA T-cell responses and cause the destruction of PSA-secreting tumors in a pre-clinical mouse model of prostate cancer (8,9). Such active immunization against prostate-cancer associated antigens might be more effective than active non-specific or adoptive/passive immunotherapy. Therefore, we have pursued a vaccination strategy based on an adenovirus that carries the gene for prostate specific antigen (PSA). Results from our Phase I trial of adenovirus/PSA (Ad/PSA) vaccine (section 1.4, below) demonstrated that a single immunization of men with metastatic prostate cancer was able to induce anti-PSA T cell responses. The trial design was a dose escalation study with the vaccine administered subcutaneously (sc) either in an aqueous solution or in a collagen matrix (Gelfoam®). We now propose a Phase II clinical trial using the Ad/PSA vaccine, administered in multiple injections to prostate cancer patients with minimal disease burden.

1.2 Adenovirus vectors

Recombinant adenoviral vectors transduce a wide range of dividing and nondividing cells types, making this gene delivery system valuable as a tool for studying diseases, for vaccine therapy, and for potential clinical use (10). Recombinant adenovirus can be prepared and purified in high titers. In addition, wild-type adenovirus infections are extremely common in the general population, giving adenovirus a well-documented safety record (11). Moreover, adenoviruses are structurally stable and no adverse effects have been reported following the vaccination of US military recruits with wild types, demonstrating their safety for human use (11). Adenoviral vectors for gene therapy and vaccine therapy are adenoviruses which have been genetically modified to allow insertion of foreign genes and to render the virus replication-defective. Current vectors have a deletion in the E1 region or in both the E1 and E2 regions. Adenoviral gene transfer has been

used in a variety of experimental conditions that include transfers to the liver (12), lung (13), central nervous system (14,15), and to cancer cells (16).

There is evidence that the introduction of foreign transgenes by adenovirus induces immune responses to the transgene product, which become ultimately responsible for the elimination of the virus (17,18). While this is disadvantageous for insertion of functional genes into host cells, it is advantageous in the use of viruses carrying foreign genes as immunogens. In the vaccine therapy of cancer, active immunization against a murine colon cancer, breast cancer, and melanoma antigens have been induced by adenoviral vaccines (19-26).

The Ad/PSA vaccine used our laboratory and in our Phase I clinical trial was produced by inserting the gene for the full length pre-pro form of human PSA into a replication deficient adenovirus serotype 5. Replication deficiency was induced by deletion of the E1a and E1b genes of the virus. Details of the vaccine can be found in section 9 of this protocol. Approval for the use of the vaccine in the Phase I trial was obtained from the FDA under IND #9706.

In pre-clinical studies, our group has demonstrated that the Ad/PSA vaccine was able to induce stronger anti-PSA immune responses than other viral PSA vaccines. These include vaccinia viruses, both replication competent and replication deficient, and to a canarypox vaccine (Table 1), The frequency of PSA-specific CD8+ cells T cells generated by the Ad/PSA vaccine was greater than were generated by any of the other vaccines tested.. In addition to the superior immunizing property of the Ad/PSA, the incorporation of Gelfoam, a collagen matrix (section 1.3), has been shown in pre-clinical studies to enhance the ability of the vaccine to induce strong anti-PSA immune responses (8). Lastly, immunization of mice with Ad/PSA in matrix can induce anti-PSA responses even in the presence of high titer anti-adenovirus antibodies (8). This latter finding is important in light of the fact that most humans have pre-existing levels of anti-adenovirus antibodies as a result of prior natural exposure to the virus.

Table 1
Effector Cell Frequency Analysis (ELISPOT)

Vaccine	Virus	Frequency of PSA-Specific CD8+ T Cells
Ad/PSA*	Replication deficient adenovirus	1/455
Prostvac	Replication competent vaccinia	1/2028
NYVAC/PSA	Replication deficient vaccinia	1/3597
ALVAC/PSA	Canarypox	1/35,714

1.3 Gelfoam® Matrix

Gelfoam (Pharmacia & Upjohn Company, Kalamazoo, MI) is a medical device intended for application to bleeding surfaces as a hemostatic agent. It is a water-insoluble, off-white, non-elastic, porous, pliable product prepared from purified pork skin. The Gelfoam gelatin preparation is available either as a cross-linked sponge or as non-cross linked beads. It is able to absorb and hold within its interstices approximately 45 times its weight of blood and other fluids (26). The absorptive capacity of Gelfoam is a function of its physical size, increasing with increasing gelatin volume (27).

The mechanism of action of surface-mediated hemostatic devices is supportive and mechanical (27). Surface-acting devices, when applied directly to bleeding surfaces, arrest bleeding by the formation of an artificial clot and by producing a mechanical matrix that facilitates clotting (28). Jenkins et al have theorized that the clotting effect of Gelfoam may be due to release of thromboplastin from platelets, occurring when platelets entering the Gelfoam become damaged by contact with its myriad of interstices (29). Thromboplastin interacts with prothrombin and calcium to produce thrombin, and this sequence of events initiates the clotting reaction. The authors suggest that the physiologic formation of thrombin in Gelfoam is sufficient to produce formation of a clot, by its action on the fibrinogen in blood (29). The spongy physical properties of Gelfoam hasten clot formation and provide structural support for the forming clot (28,30).

Gelfoam has been used experimentally for the delivery of soluble proteins and drugs, including insulin, antibiotics, and growth factors (31-33). Gelfoam was used for sustained release of insulin in an ocular implant device (31). Delivery of insulin in solution had no effect on blood glucose levels. In contrast, the use of Gelfoam as a sustained release delivery agent provided measurable insulin activity for up to 10 hours after implantation. Glucose levels in the blood stabilized at 60% of the original value, whereas administration of insulin in eye drops had no effect.

MacDonald and Mathews (34) studied Gelfoam implants in canine kidneys and reported that it assisted in healing, with no marked inflammatory or foreign-body reactions. Jenkins and Janda (35) studied the use of Gelfoam in canine liver resections and noted that Gelfoam appeared to offer a protective cover and provide structural support for the reparative process. Correll et al (36) studied the histology of Gelfoam when implanted in rat muscle and reported no significant tissue reaction.

Gelfoam has been used as a hemostatic agent in dog prostate (37). In these studies no gross histological evidence of tissue damage or calcification was induced. In addition, these investigators demonstrated that placement of Gelfoam into the lumen of the bladder resulted in liquefaction of the Gelfoam without any evidence of calculogenesis. Finally, Bischoff and Goerttler (38) used Gelfoam in human prostate therapeutic embolization with success.

Our laboratory, in collaboration with Dr. Timothy Ratliff, has demonstrated that administration of the Ad/PSA vaccine in Gelfoam induces a stronger anti-PSA immune response (Figure 1). In our pre-clinical studies, immunization with the vaccine in an aqueous suspension induces strong immunity with 10^9 pfu with weaker immunity induced with 10^8 and 10^7 pfu. Use of Gelfoam permits the induction of strong responses at the lower dose of 10^8 pfu. In addition, strong anti-PSA T cell responses could be induced by immunization with the Ad/PSA vaccine in Gelfoam even in mice pre-immunized to adenovirus (Figure 2). In the Phase I clinical trial (section 1.4), the addition of Gelfoam to the vaccine immunization did not result in excess serious adverse events.

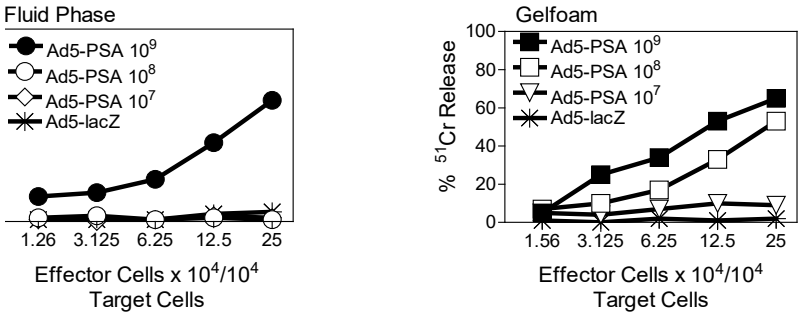

Figure 1 – Anti-PSA cytotoxic activity of spleen cells obtained from mice immunized with Ad/PSA in aqueous (fluid phase) versus Gelfoam.

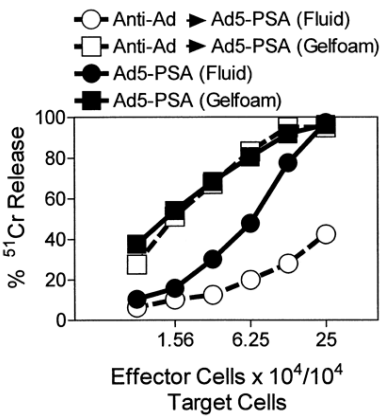

Figure 2. Effect of the presence of anti-adenovirus Ab on Ad5-PSA immunization with and without Gelfoam. Mice were injected i.p. with 10^9 PFU of Ad5-lacZ or PBS 2 wk before immunization with 10^9 PFU Ad5-PSA delivered s.c. in the fluid phase (PBS) or Gelfoam. CTL were tested against RM-11psa and RM-11neo to demonstrate PSA specificity. Cytolytic activity of each group against RM-11neo targets was <10% for all E:T ratios.

1.4 Phase I study

A Phase I clinical trial of the Ad/PSA vaccine has been completed in men with measurable metastatic prostate cancer, with the primary objectives of determining the toxicity profile and maximal tolerated dose (MTD). The ability of the vaccine to induce anti-PSA immune responses and any clinical responses was also evaluated. Funding for the Phase I trial was provided by multiple sources that include the Holden Comprehensive Cancer Center, the Department of Urology, and the Carver College of Medicine at the University of Iowa.

Eligible patients consisted of men with prostate cancer that had measurable metastatic disease, 90% of whom were stage D3. Prior therapies had included androgen depletion, ketaconazole, prednisone, Casodex, Taxotere, and external beam radiation, but the initiation of vaccine therapy was equal to, or greater than, 30 days after the most recent therapy. Patients were treated in successive dose levels and aqueous vs. matrix cohorts, according to the protocol plan. We were able to administer the maximum permitted dose of 10^8 pfu without any serious adverse events by vaccination of the first of 18 patients. These initial 18 patients were followed throughout the one-year period after injection. An additional 14 patients were treated at the MTD dose level, as planned in the protocol and confirmed by a letter to the FDA. The purpose of the additional patients was to have sufficient numbers of patients in the groups to statistically evaluate the anti-PSA immune responses induced by the Ad/PSA vaccine. In summary, 32 patients were treated in the study followed through the one-year period. Two additional patients were enrolled in the study (total number of enrolled patients, 34) but never received the vaccine and were therefore not evaluable. One patient chose to have radiation therapy instead of participating in the trial and the second was diagnosed with a second malignancy (melanoma) shortly after his enrollment in the phase I study.

1.4.1 **Phase I study results**

The median age of the patients was 70.2 years (range, 52 to 89). The vaccine was administered as an aqueous suspension or in a collagen (Gelfoam) matrix to 32 patients. Sixteen (16/32) or 50% of the patients exhibited grade 1 vaccine-related adverse events (AE), 1/32 (3.1%) that exhibited a grade 2 AE, and one patient exhibited a grade 3 AE which was a decrease in neutrophil count. There were no vaccine-related grades 4 or 5 AEs. The following Table 2 is a listing of all AEs, sorted by relationship to the vaccine injection.

Table 2
Ad/PSA Phase I Trial
Adverse Events – by Vaccine Relationship

Patient	System	Day of onset*	Event	Vaccine-related
AP005	Neural	21	Agitation	No
AP006	Musculoskeletal	13	Left hip and thigh pain	No
AP007	Musculoskeletal	22	Left back pain	No
AP007	Neural	14	Situational depression	No
AP009	GI	21	Constipation	No
AP009	Musculoskeletal	14	Joint aches, secondary to fall	No
AP009	Neural	11	Fall, causing head lacerations & loss of consciousness	No
AP010	GU	21	Proteinuria	No
AP010	Skin	21	Edema	No
AP014	Constitutional	22	Fatigue	No
AP014	Musculoskeletal	14	Leg pain (bone – femur)	No
AP015	Respiratory	1	Cough	No
AP016	GU	172	Left nephrolithiasis	No
AP016	GU	183	New primary tumor – papillary bladder	No
AP016	Immune	8	Flu-like symptoms	No
AP016	Musculoskeletal	4	Bone pain – left leg	No
AP016	Musculoskeletal	21	Decreased left leg strength	No
AP018	Musculoskeletal	8	Bilateral rib pain	No
AP018	Neural	8	Insomnia	No
AP020	Neural	168	cord compression	No
AP022	GI	2	constipation	No
AP022	Hematologic	14	decreased lymphocyte count	No
AP022	Hematologic	63	decreased WBC & neutrophils	No
AP022	Musculoskeletal	5	increased bone pain	No
AP022	Neural	16	depression	No
AP023	Musculoskeletal	20	pain in left buttocks	No
AP025	Musculoskeletal	16	bone pain	No
AP026	Cardiovascular	14	Lower extremity edema	No
AP027	GI	1	GI cramping	No
AP027	GI	1	constipation	No
AP034	Respiratory	prior	Edema, wheezing (change in inhaler in same time period)	No
AP035	Constitutional	64	Weight loss	No
AP035	GI	50	Constipation	No
AP035	GU	22	Proteinuria	No
AP035	Hematologic	15	Lymphopenia	No
AP035	Musculoskeletal	22	Increased alkaline phosphatase	No
AP037	GI	73	Nausea	No
AP018	Skin	1	Injection site tenderness	Possible
AP019	Cardiovascular	1	Hypotension	Possible
AP019	Constitutional	1	fever	Possible
AP019	Constitutional	14	fatigue	Possible
AP019	GU	1	Proteinuria	Possible
AP020	GI	21	increased alkaline phosphatase	Possible
AP020	GU	21	ketonuria	Possible

AP020	Metabolic	14	hyponatremia	Possible
AP020	Metabolic	14	hyperglycemia	Possible
AP020	Respiratory	9	viral symptoms	Possible
AP005	Musculoskeletal	1	Left inguinal pain	Possibly
AP008	GU	21	Proteinuria	Possibly
AP008	Respiratory	3	Cold symptoms	Possibly
AP010	Hematologic	1	Decrease in absolute neutrophils count	Possibly
AP027	Liver	21	increased AST	Possibly
AP036	Hematologic	1	Anemia	Possibly
AP036	Hematologic	63	Anemia	Possibly
AP037	Hematologic	1	Lymphopenia	Possibly
AP002	Musculoskeletal	3	Groin pain	Unlikely
AP002	Neural	3	headache	Unlikely
AP002	Skin	1	Itching	Unlikely
AP003	Pulmonary	21	Pleural effusion	Unlikely
AP006	Constitutional	14	Chills	Unlikely
AP006	Neural	14	Migraine headache	Unlikely
AP012	Cardiovascular	21	Hypotension	Unlikely
AP012	GI	0	Heartburn	Unlikely
AP012	Neural	21	Dizziness	Unlikely
AP014	Hematologic	82	Anemia	Unlikely
AP015	Cardiovascular	137	Myocardial infarction	Unlikely
AP018	Respiratory	43	DOE?	Unlikely
AP021	Hematologic	1	decreased lymphocytes	Unlikely
AP021	Hematologic	63	thrombocytopenia	Unlikely
AP023	Immune	16	urinary tract infection	Unlikely
AP023	Liver	21	elevated AST	Unlikely
AP024	Hematologic	14	anemia	Unlikely
AP024	Hematologic	1	anemia	Unlikely
AP025	GI	85	abdominal distention, constipation, apparently related to metastatic disease in the periaortic lymph nodes and periprostatic tumor mass	Unlikely
AP025	Hematologic	14	thrombocytopenia	Unlikely
AP025	Neural	122	Headache	Unlikely
AP027	Immune	21	Infection	Unlikely
AP034	Cardiovascular	14	Hypotension/dizziness	Unlikely
AP034	Musculoskeletal	14	Back pain	Unlikely
AP036	Cardiovascular	63	Edema – bilateral ankles	Unlikely
AP002	Skin	0	hematoma at injection site	Yes
AP003	Skin	0	Bruising at injection site	Yes
AP004	Skin	0	Ecchymosis at injection site	Yes
AP005	Skin	0	Ecchymosis and erythema at injection site	Yes
AP008	Skin	0	Ecchymosis at injection site	Yes
AP009	Skin	0	Ecchymosis at injection site	Yes
AP018	Skin	0	Ecchymosis	Yes
AP025	Skin	0	Ecchymosis at injection site	Yes
AP026	Skin	0	Erythema at injection site	Yes
AP034	Skin	0	Pain at injection site	Yes

* day 0 = day of injection; day 1 = day after injection

We measured the anti-PSA immune responses, both antibody and T cell, in all patients enrolled in the study. Antibody responses to PSA were measured by the binding to PSA-secreting cell lines using the method adapted from Cavacini, et al. (39). Results of those analyses demonstrated that 57% of men immunized with the Ad/PSA vaccine developed measurable anti-PSA antibodies. ELISPOT assays were utilized to measure anti-PSA T cell responses. The results, depicted in Table 3, demonstrate that of the 32 patients, 18 (56.3%) developed anti-PSA T cell responses. The addition of Gelfoam did not appear to affect the development of anti-PSA responses, but in this Phase I study the numbers of patients in each group was too small to make statements of statistical significance of the data. These results demonstrate the ability of men with late stage metastatic prostate cancer, injected one time with Ad/PSA, to respond to the vaccine with the production of anti-PSA T cells.

Table 3
ELISPOT Analysis of Anti-PSA T Cell Immune Responses

Patient Number	Dose/ Vehicle	Response	Frequency	
			Pre-Immunization	Post- Immunization
AP-002	10^6-aqueous	-	1/985,000	1/258,571
AP-004	10^6-aqueous	-	0	1/311,111
AP-007	10^6-aqueous	+	1/46,901	1/14,804
AP-003	10^6-matrix	+	1/90,000	1/8075
AP-005	10^6-matrix	+	$1/1.2x10^6$	1/152,353
AP-006	10^6-matrix	+	1/93,103	1/15,762
AP-008	10^7-aqueous	-	1/100,000	1/97,419
AP-010	10^7-aqueous	-	0	0
AP-013	10^7-aqueous	+	0	1/60,000
AP-009	10^7-matrix	+	1/52,535	1/30,566
AP-012	10^7-matrix	-	1/13,488	1/254,286
AP-014	10^7-matrix	+	1/562,500	1/120,000
AP-015	10^8-aqueous	+	$1/1.4x10^6$	1/780
AP-016	10^8-aqueous	+	0	1/130,000
AP-017	10^8-aqueous	+	0	1/124,375
AP-025	10^8-aqueous	-	0	0
AP-026	10^8-aqueous	-	0	1/560,000
AP-027	10^8-aqueous	+	0	1/26,364
AP-029	10^8-aqueous	+	0	1/333
AP-032	10^8-aqueous	+	1/250,000	1/3793
AP-018	10^8-matrix	-	0	0
AP-019	10^8-matrix	+	1/14,235	1/336
AP-020	10^8-matrix	+	1/8824	1/1802
AP-021	10^8-matrix	+	1/689	1/431
AP-022	10^8-matrix	+	0	1/180,000
AP-023	10^8-matrix	-	1/320,000	0
AP-030	10^8-matrix	+	1/666.667	1/32,000
AP-034	10^8-matrix	+	1/80,000	1/15,152
AP-035	10^8-matrix	+	1/5295	1/2459
AP-036	10^8-matrix	+	1/62,000	1/9487
AP-037	10^8 matrix	-	$1/2.1x10^6$	1/965,000

The effects of vaccination on serum PSA and on patient survival were also evaluated as a secondary endpoint in the phase I trial. Although there was no sustained decline in individual PSA levels, the PSA doubling times (PSADT, calculated based on 3 pre-enrollment consecutive PSA

measurements) were reduced in 54 percent of the patients compared to pre-vaccine administration, with the best responses occurring in patients immunized with the highest dose of the vaccine (Table 4). In addition, published survival nomograms for patients with hormone refractory prostate cancer were applied to patients in this phase I trial (40,41). Table 5 shows that 57% of all patients at all doses, whether injected with the vaccine as an aqueous suspension or in the collagen matrix, had a survival time longer than that predicted by the nomogram, The range of increased survivals in the different groups was 33% to 100%.

Table 4
PSA Doubling Time (DT) in Phase I Patients

Vaccine Dose & Vehicle	Percent iNcreased PSA DT
10^6 aqueous	33%
10^6 matrix	33%
10^7 aqueous	67%
10^7 matrix	67%
10^8 aqueous	25%
10^8 matrix	62%
Total	**54%**

Table 5
Ad/PSA Phase I Trial
Predicted and Actual Patient Survival

Vaccine Dose	Vehicle	Percent with Longer Than Predicted Survival
10^6	Aqueous	33%
10^6	Matrix	67%
10^7	Aqueous	100%
10^7	Matrix	33%
10^8	Aqueous	56%
10^8	Matrix	50%
Overall		**57%**

1.5 Other immunotherapy clinical trials for prostate cancer

The last several years have seen an increase in the number of clinical trials using vaccine immunotherapy for the treatment of prostate cancer. The trials have used a variety of target antigens that have been shown to be associated with prostate and prostate cancer cells. These include PSA (39,42-49), prostatic acid phosphatase (PAP) (50-53), prostate specific membrane antigen (PSMA) (54-56), telomerase (hTERT) (57,58), Thomsen-Friedenreich antigens (59), mucins (60), carbohydrates (61), and HLA-associated peptides (62). A variety of vectors have been used in the immunization process that include dendritic cells (45,50-58,63), vaccinia virus (39,42,43,47,49), fowlpox virus (39,47), liposomes (44), plasmids, (48), and chemical conjugates (59-61).

Recently, Kaufman et al. recently reported the results of a phase II trial (ECOG 7897) with a prime/boost vaccine using vaccinia virus and fowlpox virus expressing human PSA in patients with hormone-dependent prostate cancer (64). Sixty-four eligible patients with biochemical progression after local therapy were randomly assigned to three treatment arms: (A) fowlpox-PSA (rF-PSA) by intramuscular injection every six weeks for four doses, (B) rF-PSA for three doses

followed by vaccinia-PSA (rV-PSA) given by intradermal injection, or (C) rV-PSA followed by three rF-PSA vaccines. Dreicer et al. reported a randomized phase II study with a recombinant Modified Vaccinia Ankara virus which expresses both MUC1 and IL2 (TG4010) (65). MUC1 is a glycoprotein associated with several malignancies. Eligible patients were required to have no evidence of metastatic disease following curative intent local therapy, evidence of PSA failure (PSA over 2 ng/ml) and PSA doubling time (PSA-DT) less than 10 months. Arm 1 had TG4010 injected sc weekly, at a dose of 10^8 pfu, for six weeks, then every three weeks. Arm 2 had 10^8 pfu TG4010 injected sc every three weeks. Therapy was continued to disease progression or to a maximum of 36 weeks. Lastly, Small et al. recently presented the results of a phase III trial with APC8015, an immunotherapy cellular product consisting of autologous peripheral blood mononuclear cells enriched for a dendritic cell fraction pulsed with PA2024, a Prostatic Acid Phosphatase (PAP)-GM-CSF construct (66). Patients with asymptomatic, metastatic hormone-refractory prostate cancer were randomized (2:1) to receive APC8015 (n=82) or placebo (n=45) every 2 weeks x 3.

ECOG is currently planning a phase III trial using the Vaccinia virus (PROSTVAC-V/TRICOM) followed by Folwlpox virus vaccination (PROSTVAC-F/TRICOM) with GM-CSF, compared with placebo vaccine plus GM-CSF in patients with hormone-refractory prostate cancer with absence of metastatic disease (ECOG 1805, PARADIGM).

In summary, the results from these trials vary in terms of patient populations studied (hormone dependent vs. independent) and in levels of positive results, which include the induction of antigen-specific immune responses, decreases in levels of serum PSA and in rates of change in PSA velocity, and measures of clinical responses. Thus far no single vaccine immunotherapy has proven to be definitely superior to others in terms of clinical benefit, and other phase II and III trials continue to be planned or conducted. The results of some of these vaccine trials raise the question that an increase in PSADT may in the future represent a possible surrogate marker for increased time to progression, or overall survival in immunotherapy studies, and that absolute PSA responses may not constitute an obligatory step for the ultimate demonstration of clinical benefit of immunotherapy approaches in prostate cancer. Furthermore, the T-cell stimulation index may have important correlation with clinical vaccine efficacy, as seen in the phase III trial by Small et al.(66). These developing notions further support the current proposal for clinical development of our Ad/PSA vaccine, also based on the results of our prior phase I trial.

1.6 Proposed phase II clinical trial: rationale

Based on the significant pre-clinical activity of the Ad/PSA vaccine in generating tumor-specific T cells, and the encouraging safety and efficacy results from our phase I study, we propose to continue the clinical development of the Ad/PSA vaccine with the performance of the current phase II trial. It is our contention that the vaccine product and the method of immunization set this therapy apart from other ongoing prostate cancer investigational immunotherapeutic approaches. Specifically, the incorporation of Gelfoam, not present in other vaccine preparations, enhances the induction of strong anti-PSA responses. Immunization of mice with Ad/PSA in Gelfoam matrix was able to induce anti-PSA responses even in the presence of high-titer anti-adenovirus antibodies. Notably, most humans naturally possess high titers of anti-adenovirus antibodies due to natural exposure to adenoviruses.

We plan to enroll prostate cancer patients with hormone-refractory metastatic disease into this Phase II clinical trial. This is similar to the population that constituted the majority of patients in our Phase I toxicity trial of the Ad/PSA vaccine. Patients in this trial will have low burden of disease, despite the fact that they are hormone refractory, i.e., have negative bone scans and/or

low serum PSA. We will be comparing the clinical and immunologic response of these patients with two other patient populations in the trial. The latter populations, described in a separate protocol, will be patients with newly recurrent disease, either hormone naïve or during androgen depletion therapy. Although the patients with recent recurrences will have a smaller tumor burden than will the patients in this protocol that have metastatic disease, the observation period to detect a therapeutic effect of the vaccine will be shorter in the latter patient population than the former population. It is for this important reason that we will enroll patients with metastatic disease on this protocol. Patients will be eligible if they have recent evidence of hormone refractory disease (D3) and either (a) have a positive bone scan or CT scan with a PSA doubling time of $\geq$6 months, and total PSA of <10 ng/ml, and asymptomatic; or (b) have a negative bone scan and CT scan with any PSA doubling time, asymptomatic, and not a candidate for chemotherapy.

We will enroll and treat patients at two affiliated and adjacent medical centers, the University of Iowa Hospitals and Clinics (UIHC) and the Iowa City Veterans Affairs Health Care System (ICVAHCS). There is a single Institutional Review Board (IRB) that approves protocols for both institutions. After the University of Iowa's IRB has approved the protocols the Research and Development Committee of the ICVAHCS meets to provide their approval for the study.

Patient Recruitment: The UIHC and ICVAHCS draw from a large catchment area that will ensure the enrollment of sufficient numbers of men into the protocol. We will use recruitment methods similar to the methods used to obtain subjects for our Phase I clinical trial. That includes contacting patients that are being or have been treated in the Departments of Urology, Internal Medicine, and Radiation Oncology at the UIHC and ICVAHCS and letters written to urologists, oncologists, and medical oncologists in Iowa and the neighboring states of Wisconsin, Illinois, Missouri, Nebraska, Minnesota, and South Dakota. Drs. J. Brown and M. O'Donnell and ARNP E. Brown will assist in the recruitment of patients from the Department of Urology, Dr. Vaena from Internal Medicine, and Dr. Smith from Radiation Oncology. Additional recruitment of patients will occur through a collaboration with Mercy Medical Center, Des Moines, IA (Hereafter referred to as DMMCC). Once accrual has been completed, no further recruitment methods will be utilized.

A physician from outside the UIHC or ICVAHCS may inform patients of the study. If a patient expresses interest in learning more about the trial, the physician will provide him with contact information for the clinical trial team. When a patient contacts the clinical trial team, a member of the trial team will briefly describe the study and request the patient's mailing address in order to mail him a copy of the study informed consent form.

After the patient has had time to become familiar with the study and discuss it with his family, a member of the research team will follow up with a telephone call to discuss the study and answer any questions the patient may have. At this time, if the patient is still interested in participating, he will be asked to provide verbal consent to allow the research team to review information from his medical record to evaluate his eligibility for the trial. The researcher will document the verbal consent process in the research file, and will contact the patient's physician to request the medical record for review. The patient will be required to sign a HIPAA privacy form and a Release of Medical Information form at his physician's office prior to the physician releasing his records to the research team.

After reviewing the relevant portions of the medical record, a member of the research team will again contact the patient. Eligible individuals will be scheduled for a visit to the UIHC or ICVAHCS to undergo additional eligibility testing. At that visit, informed consent for participation in the clinical trial will be obtained. The subject will then be further evaluated for study eligibility

through a medical history and physical exam, blood tests, and scans. Final determination of eligibility will occur at a weekly meeting of the clinical trial team.

2. OBJECTIVES

2.1 Primary Objective

To evaluate the response rates (PSA responses and changes in PSADT) following immunization with the Ad/PSA vaccine using a prime-boost immunization strategy, in patients with hormone refractory metastatic disease.

2.2 Secondary objectives

2.2.1 To evaluate the development of anti-PSA immune responses in study patients.

2.2.2 To evaluate biochemical (PSA recurrence) and radiographic (bone scans) time to progression and overall survival in evaluable patients receiving the Ad/PSA vaccine.

3. SELECTION OF PATIENTS

As described in Section 1.6 prostate cancer patients with hormone refractory metastatic disease will be enrolled in the study.

3.1 Inclusion criteria:

3.1.1 Men with prostate cancer who present with evidence of hormone refractory disease (D3).

3.1.2 Men with a positive bone scan or a positive CT scan (with obvious soft tissue metastasis or lymph nodes >1 cm), a PSA doubling time of $\geq$6 months, and a total PSA of <10 ng/ml, and asymptomatic OR men with a negative bone scan and a negative CT scan with any PSA doubling time and asymptomatic.

3.1.3 Scans must be obtained within 6 weeks of initiation of treatment.

3.1.4 Written informed consent.

3.1.5 Age $\geq$ 18 years.

3.1.6 Required laboratory values (obtained within 2 weeks of initiation of treatment)

3.1.6.1 Serum creatinine $\leq$ 2.0 mg/dL

3.1.6.3 Adequate hematologic function: granulocytes $\geq$ 1800 per mm^3, platelets $\geq$ 100,000 per mm^3, WBC $\geq$3700, and lymphocytes >590.

3.1.6.4 Adequate hepatocellular function: AST <3x ULN and total bilirubin <1.5 mg/dl (unless bilirubin elevation is consistent with Gilbert's syndrome).

3.1.6.5 Castrate levels of testosterone of $\leq$50 ng/ml.

3.1.7 PSA used as an eligibility criterion must be drawn within 28 days prior to injection number 1 and will be redrawn on Day 1 for use as a baseline value.

3.2 Exclusion criteria:

3.2.1 Active or unresolved clinically significant infection.

3.2.2 Parenteral antibiotics <7 days prior to initiation of treatment.

3.2.3 Evidence of prior or current CNS metastases. Specific imaging is not necessary in the absence of signs or symptoms.

3.2.4 Co-morbid medical conditions which would result in a life expectancy (participation) of less than 1 year.

3.2.5 Patients with compromised immune systems; congenital, acquired, or drug-induced (immunosuppressive agents) will be excluded from the study. Use of prednisone at doses higher than 10 mg daily (or equipotent steroid doses) for more than 7 days within 3 months of initiation of treatment is not allowed.

3.2.6 Pre-existing malignancies that required treatment within the past 5 years except for basal or squamous cell cancers of the skin.

3.2.8 Prior participation in any vaccine studies for non-infectious diseases.

3.2.9 Prior chemotherapy, defined as prior cytotoxic chemotherapy for prostate cancer (or any cancer unless more than 5 years have elapsed). Examples of cytotoxic chemotherapy are mitoxantrone/prednisone and taxanes. Drugs such as Casodex or ketoconazole treatment must have been completed at least 6 weeks prior to initiation of treatment.

3.2.10 The inability to understand the English language and the clinical protocol.

3.2.11 Allergy or religious objection to pork products; Gelfoam is produced from pork.

Patients that have been on bisphosphonate treatment for greater than 1 month prior to entry into the trial will be able to continue the medication. Patients that have not been on bisphosphonate therapy will not start on the medication for at least 30 days after the first vaccination.

4. Registration Procedures

4.1 All patients will be registered through the Department of Urology at the University of Iowa Hospitals and Clinics (UIHC) or the Urology Service at the Iowa City Veterans Affairs Health Care System (ICVAHCS).

4.2 Patients who are candidates for enrollment into the study will be evaluated for eligibility by the clinical investigators to ensure that the criteria outlined in Section 3 have been satisfied and that the patient is eligible for participation in this clinical investigation. The University of Iowa will provide a patient eligibility case report form for this evaluation.

4.3 Informed Consent - Signed informed consent for enrollment in this protocol will be obtained from eligible patients by the attending physician, study coordinator, or clinical trial coordinator before the start of research intervention. At the preadmission consultation, patients will be fully informed of the purpose and potential risks and benefits of participating in the study. Patients have the opportunity to have questions answered to their satisfaction before signing the consent.

4.4 Eligible patients must be registered Monday through Friday between 8:00 a.m. and 4:30 p.m. (Central Time). Patients at the University of Iowa Clinical Cancer Center will be registered by calling Pamela Zehr, RN, MA at 319-353-8914. Patients at the ICVAHCS will be registered by calling Karen Clark Griffith, RN, PhD at 319-338-0581, ext. 7519. Information from the eligibility form will be provided by the investigator or the investigator's research staff to the University of Iowa Cancer Center at this time, and the patient will be registered and assigned a unique patient number.

4.5 No patient may be enrolled or begin research intervention prior to registration and assignment of a patient number. As a follow-up, University of Iowa Cancer Center will provide the investigator with written confirmation of each patient's registration.

4.6 All investigators will be notified by the Chair of the Protocol Review and Monitoring Committee or by the trial's Data and Safety Monitoring Board if the study is placed on administrative hold, and when the study is completed or closed to further patient enrollment.

4.7 Patients must begin the vaccine protocol within 30 days of registration (date the study committee approves patient eligibility).

5. RESEARCH INTERVENTION PLAN

5.1 Administration Schedule

Ad/PSA

All patients will receive three injections of 0.16 ml. (0.125 ml. vaccine + 0.035 ml. Gelfoam) of the Ad/PSA subcutaneously in the right thigh unless patient condition precludes the use of that leg, in which case the left thigh will be used for all three injections. The dose of the vaccine, based upon our results from the Phase I trial, will be 1×10^8 pfu (4.4×10^9 particles) in the Gelfoam matrix. The Gelfoam comes in sterile patient-ready packages. The virus will be suspended in sterile saline and the Gelfoam powder added in a ratio of 30 mg of powder per ml. of virus suspension. Injections will be spaced apart by 30 days, such that each patient will receive the vaccine on days 1, 30, and 60. The use of the matrix has been shown in collaborative pre-clinical experiments to enhance infection of host cells by the virus. Results from the Phase I trial indicated that the injection of the vaccine in Gelfoam did not produce any adverse events greater than those produced by the vaccine in an aqueous suspension. The vaccine induced anti-PSA immune response in patients injected as an aqueous or Gelfoam vaccination. Injections will be carried out in the University of Iowa Clinical Research Unit (CRU). Each subject will be required to stay in the CRU for two hours and observed for early signs of toxicities.

5.2 5.2 Design and Stages

The ideal design would be a two-stage design of Simon (1989) requiring 32 patients. But due to the complexity of the trial, a more conservative approach consisting of using 32 patients in a one-stage design at efficacy level and a two looks at toxicity level as means of stopping rules will be carried out. By using a one-stage design at efficacy endpoint, we are making the probability of early termination for efficacy purposes to be zero. The reason for this design is due to the nature of the research intervention as outlined in section 10. Stopping design based on toxicity is as follow: one look after 17 subjects are treated and another look after 25 subjects are treated. If 5 out of 17 show vaccine-related grade 3 toxicity or higher, the trial will stop. Otherwise, an additional 8 subjects will enter the trial. If 7 out of 25 show grade 3 toxicity or higher, the trial will stop otherwise we will proceed to full registration of 32 patients to assess efficacy.

6. Adverse Events

Toxicity will be graded according to the NCI common toxicity criteria "Common Terminology Criteria for Adverse Events" (NCI-CTCAE v3.0 can be accessed at website: http://ctep.cancer.gov/forms/CTCAE_Index.pdf). Non-hematologic and Hematologic dose limiting toxicity (DLT) will be defined as described in Section 8.5.1.

6.1 Definitions

Adverse Event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this research intervention. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

This will also include intercurrent diseases and accidents observed during the research intervention period as well as corresponding events during drug-free, pre- and post-intervention periods, under placebo or in a reference group receiving drug or non-drug therapy.

Serious adverse event (SAE) is any untoward medical occurrence that:

a. results in death
b. is life-threatening[B]
c. requires inpatient hospitalization or prolongation of existing hospitalization
d. results in persistent or significant disability or incapacity
e. is a congenital anomaly / birth defect or
f. is another medically important condition.[C]

[B] The term "life-threatening" in the definition of "serious" refers to an event in which the patient is at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death if it were more severe.

[C] Medically important conditions that may not result in death, be life-threatening or require hospitalization may be considered as SAE when, based upon appropriate medical judgment, they may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above. Examples of such events are intensive treatment in an emergency room or at home for allergic

6.2 Procedures of documentation of AEs

All AEs occurring during the study must be documented, regardless of the assumption of a causal relationship, on the respective AE CRF. All events which occurred after signed informed consent should be documented. The investigator should ensure that all events are recorded that occurred within at least 4 weeks after the last exposure to the study drug (through Day 90).

Documentation of AEs includes: date of onset and offset, intensity, frequency, seriousness, related interventions and outcome. The investigator will also evaluate the probability of a causal relationship of the adverse event to the study medication as being: "definite, probable, possible, unlikely, or unrelated."

All unanticipated problems involving risk to subjects or others, serious adverse events related to participation in the study and subject deaths related to participation in the study will be promptly reported by phone (301-619-2165), by email (hsrrb@det.amedd.army.mil), or by facsimile (301-619-7803) to the USAMRMC, Office of Research Protections, Human Research Protection Office. A complete written report will follow the initial notification. In addition to the methods above, the complete report will be sent to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-ZB-PH, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

The medical monitor is required to review all unanticipated problems involving risk to volunteers or others, serious adverse events, and all volunteer deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the medical monitor should comment on the outcomes of the event or problem, and in the case of a serious adverse event or death, comment on the relationship to participation in the study. The medical monitor should also indicate whether he/she concurs with the details of the report provided by the study investigator. Reports for events determined by either the investigator or medical monitor to be possibly or definitely related to participation and reports of events resulting in death should be promptly forwarded to the HSRRB.

Expedited reporting

The investigator must immediately report serious adverse events (SAE) occurring or observed during the course of the study and within 4 weeks of last administration of the study drug to the FDA, IRB, OBA, and CRU.

The order of reporting of SAEs is the following: reported to the Principal Investigator within 24 hours of the event; the PI will then report the event to the FDA, OBA, Institutional Biosafety Committee (IBC), and DOD within 7 days of the event and to the IRB within 10 days of the event. After notifying the proper agencies by telephone of an SAE the "Serious Adverse Event Report "must also be sent by fax to the agencies whether or not complete information is available at the time. If complete information is unavailable the investigator must provide follow-up information to the agencies as soon as it is known.

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bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse. N.B.: The term "severe" is often used to describe the intensity (severity) of an event (such as: mild, moderate, or severe e.g., pain). The event itself may be of relatively minor medical significance (such as severe headache). This is not the same as "serious", which is based on patient/event outcome or action criteria usually associated with events that pose a threat to patient's life or vital functions. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

In particular, the investigator must inform the agencies and committees by phone and fax using the timeline described above of occurrence of immediately life-threatening SAEs or SAEs with fatal outcome. SAEs must be reported to the site's IRB according to the IRB's requirements.

Important: The investigator must report any SAE to the FDA, IRB, OBA, and CRU, regardless of causality.

Reports will be evaluated by the Medical Monitor/Sponsor. FDA/HPB and investigators will be informed as required by the regulations. The same information will also be made available to all participating investigators as well as to other investigators participating in different clinical trials utilizing the same study medication.

It should be noted that, although the Ad/PSA vaccine contains the gene for PSA, there is no need for patients to utilize contraceptive practices. The adenovirus will not be transmitted from patient to his partner.

7 MEASUREMENT OF CLINICAL AND IMMUNOLOGICAL EFFICACY

7.1 Methods of Malignant Disease Evaluation - Each patient will have a baseline evaluation prior to the injection of the Ad/PSA vaccine. The measurements will include physical exam, temperature, weight, serum PSA, blood chemistries, urinalysis, a quantitative bone scan for bone metastases, chest x-ray, and CT for soft tissue metastases, and performance status for quality of life.

Patients will be seen in the Urology and Oncology Clinics (see Table 6 for schedule). The injection site will be examined for evidence of erythema, induration and necrosis and patients will have their temperature and weight recorded and the patients will be interviewed to determine whether they experienced any adverse reactions. Blood samples will also be taken for standard laboratory testing and measurement of PSA and anti-PSA immune responses (see Table 6). Patients recruited from the DMMCC will return to that facility for their follow-up examinations and laboratory testing on days 44, 74, and at 6, 9, 12 months and all subsequent visits. At the 6 month, 9 month, 12 month, and subsequent semi-annual visits each patient will be evaluated comparing the baseline visit measurements to those obtained at the specific visit.

Because changes to the clinical status of the patients will be prolonged, continued follow up will be important. Therefore, patients will return to the clinic for two additional visits, at 18 and 24 months, unless travel distance is a prohibitive factor. Follow-up of patients for survival will occur every 6 months by telephone.

7.2 Scans – Bone scans will be performed at 90 days, 6 months, and 12 months during the first year as well as the 24 month visit and annually until progression. If there is measurable disease by CT scan at registration then CT scans will also be performed using the same schedule as the bone scans.

7.3 Use of Serum PSA for Disease Evaluation – Based upon our pre-clinical experiments and the results from the Phase I clinical trial we expect the immunized men to produce anti-PSA antibodies. The levels of antibody will be measured by a flow cytometry assay as described by Cavacini, et al. used in our Phase I clinical trial (40). We will also explore the use of a second serum marker for prostate cancer, hK2 in collaboration with Donald Tindall, Charles Young, and George Clee at the Mayo Clinic. Investigators at Mayo, along

with Hybritech, Inc. have been exploring hK2 and published a number of papers in recent years on the subject (67-70). Patient sera from each clinic visit will be sent to Mayo where they will measure the levels of hK2. We will use the data to evaluate the effect of anti-PSA antibodies on both PSA and hK2 in the sera of vaccinated patients.

PSA measurements, CT and bone scans are routinely used to follow disease recurrence and/or progression in individual prostate cancer patients and as such would be considered standard of care. Laboratory measurements such as hematology, liver function and kidney function chemistries, while are routinely used to follow the health of a prostate cancer patient, would not normally be performed at the frequency proposed in this trial to assess possible vaccination toxicities. Therefore, they would be considered part of the research protocol.

7.4 Experimental Evaluation of the Ad/PSA Vaccination

7.4.1 Blood will be collected prior to each injection of the Ad/PSA vaccine and at return dates indicated in Table 6, page 26. Two separate samples will be collected; one in red top tubes to allow collection of serum from coagulated blood and a second in heparinized tubes to permit collection of lymphocytes.

7.4.2 Levels of PSA, hK2, anti-PSA antibodies, and anti-adenovirus antibodies will be measured in the serum.

7.4.3 Anti-PSA T cell immune responses will be measured by ELISPOT analysis using the methods developed for, and used in, our Phase I clinical trial. In addition to measuring the anti-PSA T cell activity, we will also measure anti-adenovirus T cell activity as well as reactivity to stimulation with cytomegalovirus (CMV). A non-specific stimulus will be provided by PMA and ionomycin for each patient's lymphocytes.

7.5 Definitions of Response –

7.5.1 Primary Endpoint - PSA doubling-time response

7.5.1.1 Definition: a 50% increase in the PSADT compared to pre-enrollment PSADT.

7.5.1.2 PSADT will be calculated based on the MSKCC calculator, available at http://www.mskcc.org/mskcc/html/10088.cfm.

7.5.1.3 PSADT response will be measured at 9 and 18 months after initiation of the research intervention.

7.5.1.4 Three measurements of PSA, spaced at least 2 weeks apart, will be required prior to study enrollment. Post-intervention PSADT will be based on PSA levels at 3, 6 and 9 months (9 month PSADT calculation) and 3, 6, 9,12,15,18 month levels (18 month PSADT calculation).

7.5.2 Secondary Endpoint – PSA response

7.5.2.1 Definition: a 50% reduction in the pre-research intervention PSA value, verified with a second measurement >28 days later.

7.5.3 Progression:

Progression of disease is defined for 3 different categories of disease: measurable lesions; non-measurable/non-target lesions; and PSA-only disease. The first category of disease to progress will meet the definition of progression in this trial, as follows:

7.5.3.1 Progression for measurable disease:

Definition: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) and is equal or greater than 20 mm with conventional techniques (CT, MRI, x-ray) or equal or greater than 10 mm with spiral CT scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters)

All measurable lesions (up to a maximum of 10) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repetitive measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

All other lesions, including non-measurable small lesions as defined above and bone metastasis, will be identified as non-target lesions (see definition of progression for non-target lesions below).

Progression is defined if any of the criteria below are met:

- at least a 20% increase in the sum of the LDs of target lesions (taking as a reference the smallest sum LD recorded since the treatment started) or the appearance of two or more new lesions
- development of an indication for radiation therapy while on treatment

7.5.3.2 Progression for non-measurable disease/non-target lesions:

Non-measurable disease/non-target lesions: progression of disease is defined as any of the following:

7.5.3.2.1 - worsening bone scan as evidenced by the appearance of more than two new lesions, not felt to be consistent with a tumor flare; worsening of pre-existing lesions (increase in intensity or size of a lesion) may be difficult to interpret and in such a case will not be considered the evidence of progressive disease; or

7.5.3.2.2 - appearance of new metastatic lesions outside the bone; or unequivocal progression of existing non-target lesions. Because a clear progression

of non-target lesions only is exceptional, such circumstances should be discussed with the study co-investigator's; or

7.5.3.2.3 - development of an indication for radiation therapy while on treatment

7.5.3.3 Progression for patients with PSA-only disease

PSA progression will be evaluated as follows:

7.5.3.3.1 In patients that had any PSA decline from baseline:

7.5.3.3.1.1 - increasing PSA by 25% above the nadir plus

7.5.3.3.1.2 - an increase in PSA by a minimum of 2 ng/ml from the nadir plus

7.5.3.3.1.3 - confirmation by second consecutive rising PSA >28 days later

7.5.3.3.2 - in patients whose PSA has not declined, progression is defined as

7.5.3.3.2.1 - an increase in PSA by 25% above the pretreatment level plus

7.5.3.3.2.2 - an increasing PSA by a minimum of 2 ng/ml relative to the study entry PSA value, plus

7.5.3.3.2.3 - confirmation by second consecutive rising PSA >28 days later.

7.5.4 Onset of Response – In patients that present with radiologic evidence of metastases onset of response is the onset of PR or CR. For patients with biochemical disease only the onset of response is defined by the first drop in serum PSA.

7.5.5 Duration of Response – In patients that present with radiologic evidence of metastases, duration of response is time from onset of PR or CR, whichever occurs first, (even if the patient later has a CR) until objective evidence of progression. In patients with PSA-only disease, duration of response is time from first drop in serum to objective evidence of progression. Progression is defined in Section 7.5.3, beginning on page 20.

7.6 Timing of Toxicity Assessments - Toxicity assessment will occur as stated in the calendar.

8 STUDY PARAMETERS

8.1 Scans or x-rays used to document measurable or evaluable disease must be done with 6 weeks prior to the initiation of treatment.

8.2 CBC with differential, LFT's must be done ≤2 weeks before study entry. Castrate levels of testosterone must be drawn at initiation of treatment.

8.3 All chemistries must be done ≤2 weeks before the study entry, unless specifically required on day 1 as per protocol. If abnormal, they can be repeated within 48 hours prior to the initiation of treatment.

8.4 Hgb, Hct, WBC, Plt must be done ≤2 weeks before study entry but, if abnormal, they can be repeated <48 hours prior to initiation of treatment.

8.5 REMOVAL OF PATIENTS FROM STUDY (Criteria for discontinuation of a patient's study participation)

8.5.1 Adverse events: Study subjects will be monitored for adverse events for 30 days beyond the final study injection, or longer if existing unresolved AEs are being monitored. In the event of a vaccine-associated unmanageable or irreversible toxicity, that would include hematological and non-hematological toxicities, the investigator will withdraw a patient from further research intervention and notify the Study Chair immediately. *In addition, the FDA and the IRB and other agencies listed on page 17 will be notified of the adverse events.* If unmanageable or irreversible toxicities occur the patients will receive the best possible medical care according to the recommendations of the treating physicians. The treatment plan will depend upon what unmanageable or irreversible toxicities may occur. In the event of an emergency the patient will be instructed to seek immediate care by calling 911, his local physician, the University of Iowa or Iowa City VA Health Care System urology staff on call.

8.5.1.1 Management of Toxicities

8.5.1.1.1 Hematological Toxicity Management

8.5.1.1.1.1 Patients with grade 2 or 3 hematological toxicity will not receive the subsequent dose of vaccine until there is normalization of cbc parameters until grade 1 or less toxicity is reached. An exception to withholding a dose of vaccine would be if the cbc parameters were stable (at or near the pre-vaccination values).

8.5.1.1.1.2 In case of grade 2 or 3 hematological toxicity detected any time during protocol therapy, CBC with differential will be checked on a weekly basis until grade 1 or less toxicity is reached.

8.5.1.1.1.3 If the subsequent vaccine dose needs to be postponed for more than 2 weeks, those patients will be removed from protocol therapy.

8.5.1.1.1.4 Patients with grade 4 hematological toxicity at any time will be permanently removed from protocol therapy.

8.5.1.1.2 Non-Hematological Toxicity Management

8.5.1.1.2.1 Patients with grade 2 or 3 non-hematological toxicity will not have the subsequent dose of vaccine administered until the toxicity ameliorates down to a grade 1 level.

8.5.1.1.2.2 Patients will be evaluated once a week with study team assessments, if a grade 3 non-hematological toxicity occurs.

8.5.1.1.2.3 If the subsequent dose of vaccine needs to be postponed for more than 2 weeks, those patients will be permanently removed from protocol therapy.

8.5.1.1.2.4 Patients with grade 4 non-hematological toxicity at any time will be permanently removed from protocol therapy.

Patients may also be removed from protocol therapy at any time based on assessment of any other risks, at the discretion of the study investigators.

8.5.2 Disease Progression: Patients will be taken off-study if they have progressive disease (PD) or clinically significant deterioration at any time during the study if the investigator feels that (a) alternative prostate cancer therapy might benefit the patient, or (b) to continue on study might be unsafe for the patient. Patients receiving alternative prostate cancer therapies will be followed for toxicity for 30 days beyond the third AdPSA injection only unless existing unresolved AEs are being monitored. Patients will be asked in the Informed Consent document whether they would return every three months up to 12 months from study entry for immunologic evaluations. Patients who are being treated with prednisone at doses higher than 10 mg daily (or equipotent steroid doses) will be excluded from these every three month returns.

8.5.3 Allergic Reactions: Patients must be removed from the study should they develop grade II allergic reactions.

8.5.4 Personal Reasons: As stated in the informed consent, patients may withdraw from the study at any time.

8.5.5 Clinical Judgment: A patient may be withdrawn from the study, if, in the opinion of the investigators, it is not in the patient's best interest to continue (e.g. an adverse experience, intercurrent illness, non-compliance, etc.)

8.5.6 The date of discontinuation and the reason(s) for patient discontinuation from the study will be recorded in the CRF. All efforts will be made to conduct evaluations that are required at the follow-up for each patient who discontinues research intervention, regardless of the reason.

Regulatory and Reporting Requirements

The Data and Safety Monitoring Committee (DSMC) of the Holden Comprehensive Cancer Center will provide data and safety monitoring for this study. “The Data and Safety Monitoring Plan of the Holden Comprehensive Cancer Center” provides standard operating procedures to monitor all clinical cancer trials at the UIHC. All investigator-initiated trials are automatically monitored by the DSMC. A detailed data and safety monitoring plan for this study is on file with the DSMC and the Clinical Research Safety Officer (CRSO).

Data Management, Quality Control and Data Security

In order to protect confidentiality the subject will be assigned an identification number. This number will be used on all specimens from the subject and will be used for documentation purposes.

Data management for the optimal entry, processing, storage, and retrieval for this protocol's data will be accomplished by the principal investigator. The database will be located on a computer or in a locked cabinet in a locked office. This computer will be secured, accessible only by the research team. There will be more than one copy of the database. The second, secured, copy of the protocol data will be stored in a locked room accessible only by the research team. For quality control, auditing, and checking data for integrity, there will be a regular accounting of data periodically performed. The medical record and research record will be linked by the study identification number. The data managers in this trial will be responsible for verification of the accuracy of all data transferred from the medical record to the research record. These records will be audited quarterly by the Data Safety Monitoring Committee. All Data Safety Monitoring Committee reports will be provided to the USAMRMC Office of Research Protections, Human Research Protection Office as they become available.

Data will be kept on file on each patient for at least two years past the termination of that patient’s participation in the trial. All records will be stored long-term in a secure location. UIHC records will be kept for at least 15 years and then confidentially destroyed. VAHCS records will be retained until disposition instructions are approved by National Archives and Records Administration.

Information from the medical records of patients referred by physicians outside the University of Iowa and the VA Health Care System required to determine eligibility for this protocol will be placed in a research folder, identified by the patient code and will be available only to members of the clinical trial team (identified on pages 30 and 31). The signed HIPAA forms will be kept in the patient’s folder in the office of the referring physician. The records of such patients that are deemed ineligible following a screening procedure will have their signed Release of Information forms, informed consent document, and eligibility form kept in a research folder under similar security conditions. The records for ineligible subjects will be stored with the other research records as indicated above.

Table 6
Study Design and Testing

	Std. of Care or Res.	Prior to Reg.[1]	Day 1 First Inj.	30 d. 2nd Inj.	44 d.	60 d. 3rd Inj.	74 d.	90 d.	6 mo	9 mo	12 mo	18 mo.	24 mo.	Annual to prog.	Follow-up[7]
Immunization[2]	R		X	X		X									
Physical Examination	S		X	X		X		X							
Adverse Event Assessments	R		X	X		X		X							
Performance Status	S		X	X		X		X	X	X	X				
Vital Signs	S		X	X		X		X	X	X	X				
Weight	S		X	X		X		X	X	X	X				
Blood for PSA & PAP[3]	S/R		X	X (R)		X (R)		X	X	X	X	X	X		
anti-PSA Ab, and lymphocytes for cellular immunity	R	X	X	X	X	X	X	X	X[6]	X[6]	X[6]	X	X		
CBC, differential	R	X		X		X		X	X	X	X	X	X		
AST, ALT, LDH, alkaline phosphatase, bilirubin	R	X		X		X		X							
Creatinine	R	X		X		X		X							
Urinalysis	R	X		X		X		X							
Serum testosterone	R	X													
Chest x-ray	R	X		X[4]		X[4]		X[4]							
Bone scan	S	X						X	X		X		X	X	
Abdominal/pelvic CT	S	X							X[5]		X[5]				
Survival assessment	R														X[7]

S = Procedures considered “Standard of Care” for prostate cancer patients.
R = Procedures considered “Research” as part of this Clinical Trial.

[1] Reg. = Registration, the date patient eligibility approved by the study team.
[2] Androgen deprivation therapy must continue for the duration of study participation.
[3] If the PSA meets the definition of progression, partial or complete response, confirm the PSA results >/= 28 days.
[4] will be repeated only if abnormal at screening or if patient develops a fever.
[5] will be repeated only if abnormal at screening.
[6] to be performed even if there is disease progression (up to 12 months from study entry) if the patient has consented to this in the informed consent (question in section “How long will I be on the study?).”
[7] Follow-up for survival every 6 months by telephone.

In the event of holidays or other extenuating circumstances the times on the table may vary by $\pm$ 4 days for the first 90 days. Beyond that every effort will be made to see the patient within 2 weeks of the expected date of return, but the investigator is willing to allow for other arrangements for the convenience of the patient.

9 DRUG FORMULATION AND PROCUREMENT

9.1 Drug Name

Adenovirus/PSA (Ad/PSA)

9.2 Classification

Vaccine

9.3 Mode of Action

The adenovirus is a replication-deficient virus unable to produce virus progeny in the infected cells. The virus will infect cells in the location of the injection site, the PSA gene will produce the protein product which will be recognized as an antigen by the immune system and produce anti-PSA immune responses. Based upon our pre-clinical studies in an animal model of human prostate cancer, these responses, mainly the CD8+ CTL response, will cause the destruction of PSA-secreting prostate tumors.

9.4 Dose Specifics and Route of Administration

The route of injection, vehicles for the vaccine, and dose schedules have been outlined in Section 5.1 of this protocol.

9.5 Availability

Produced and provided by Molecular Medicine, LLC, San Diego, CA

9.6 Storage

The vaccine is stored in the UIHC's Pharmacy Department in a -70^{o}C temperature-monitored and controlled access freezer. Only the Investigational Pharmacist will remove the vaccine from the freezer and enter the amount removed in a trial-specific log.

9.7 Injection Procedures & Observation

At the weekly meetings of the clinical trial team at which the eligibility of the patients is decided, orders for the initial vaccine injection will be written by clinician members of the team. Orders for the second and third vaccine injections will be written at the meetings as close to the date of the scheduled injections as possible. On the designated days of injections and immediately prior to vaccination, the Investigational Pharmacist will mix the Ad/PSA (10^8 pfu or 4.4×10^9 particles) with the Gelfoam powder (30 mg/ml). Each vial of the vaccine contains twice the volume needed for each injection. One half of the vial will contain the proper pfu and particles required for the vaccination. The injection material will be taken from the pharmacy in a 1 ml. syringe with 25 gauge needle and presented to staff in the Clinical Research Unit. Each patient will be injected with approximately 0.16 ml. of the vaccine/Gelfoam material subcutaneously in the thigh. The exact volume of the vaccination mixture (Ad/PSA in Gelfoam) is not as important as the exact number of pfu or particles and that is controlled by using the required volume from the dose vial. The empty syringe will be disposed of in a biohazard container.

Patients will remain in the CRU for two hours after each injection to be monitored for signs of adverse events.

9.8 Manufacturing

9.8.1 The PSA cDNA provided by Donald Tindall, Mayo Clinic, Rochester, MN, was placed 3' to the CMV promoter in a shuttle vector containing Ad5 DNA. The sequence inserted was the pre-pro form of PSA described by Lundwall (71) that encodes 262 amino acids with a predicted molecular weight of 28.8 kDa. Using methods previously described (72), the shuttle vector and E1a-E1b deletion mutant Ad5 DNA were transfected into HEK 293 cells, and recombination between the DNA species was allowed to occur. The amplification and purification of Ad/PSA was performed by the University of Iowa Gene Transfer Vector Core as previously described (73). Ad/lacZ used as a control was also obtained from the Gene Transfer Vector Core and is previously described (74).

9.8.2 The Principal Investigator provided the Ad/PSA vaccine used for the pre-clinical studies to Molecular Medicine, LLC of San Diego, CA for the production of the clinical grade product. Information on the manufacturing of the GMP Vaccine by Molecular Medicine, LLC is found in the accompanying documents supplied by the company.

10 STATISTICAL CONSIDERATIONS

The ideal endpoint would be a clinical outcome that is of particular relevance to the patient such as increased time to tumor progression, increased time to death or reducing the proportion of death. This trial is using a surrogate endpoint as a substitute to the clinically meaningful outcome since the tumors cannot be accessed directly. The association between the surrogate and survival rate had not been clearly established by any phase I & II trial. The trial consists of using Ad/PSA vaccine administered in multiple injections to prostate cancer patients with hormone—refractory metastatic prostate cancer—with the goal to induce anti-PSA T cells responses. Three injections of equal dose are proposed. The previous phase I trial consisting of a single injection in men with hormone-refractory metastatic prostate cancer was able to induce anti-PSA T cells responses. The Phase I consisting of a single injection using dose escalation protocol of the vaccine in an aqueous or matrix delivery vehicle did not show any significant AE. Additional pre-clinical pharmacology /toxicology studies required by the FDA did not show any significant side effects using the three-injection schedule. The primary endpoint is the serum PSADT.

For efficacy purposes, we expect at least 50% of the patients to show 50% increase in PSADT. This proportion is judged clinically important and anything less than 30% can stop the trial for futility. The ideal design would be a two-stage design of Simon (1989) requiring 32 patients. After testing the research intervention on 12 patients, if 3 did not have a 50% increase in PSADT, the trial will be terminated. Otherwise, an additional 20 patients will be recruited for the trial. The expected sample size will be 19.73 and the probability of early termination, 0.72. If the research intervention is not effective, there is 0.1 probability of concluding that it is. If the research intervention is effective, there will be 0.2 probability of concluding that it is not. However, due to the complexity of the trial, we will not use Simon (1989) but a more conservative approach consisting of using 31 patients in a one-stage design at efficacy level and a two-stage design at toxicity level for stopping rules. By using a one-stage design at efficacy endpoint, we are making

the probability of early termination for efficacy purposes to be zero. Early termination will be based on toxicity only. The reason for this design is due to the nature of the research intervention and is explained below. Enrollment will not stop unless stopping rules based on toxicity are satisfied:
"After the third vaccine dose is administered, toxicity will be evaluated 90 days into the trial"

Due to the nature of the research intervention, anti-tumor activity will be potentially delayed and the primary efficacy endpoint will be determined 18 to 20 months after initial patient accrual. Since we will be able to assess PSADT after nearly 20 months into the study, it will be unreasonable to stop the study due to unsatisfactory results prior to that point. This is the main reason why we will carry a one-stage design for the primary endpoint and a two-stage design for toxicity. Criteria for proceeding with enrollment into a subsequent stage prior to the two-year efficacy evaluation will be based on assessment of toxicity in the patient cohorts since this can be done as early as 90 days into the trial.

For toxicity purposes, we will test the null hypothesis that the toxicity level is less than 15% versus the alternative that it is greater than 35%. The trial will be stopped whenever the null hypothesis is rejected. We will conduct these tests in 2 different looks—one after an enrolment of 17 subjects and the second after 25 subjects are enrolled.

If 5 out of 17 show grade 3 vaccine-related toxicity or higher, the trial will stop (this will correspond to testing the toxicity hypotheses at level alpha=0.05 with a power 77%); otherwise, an additional 8 subjects will enter the trial. If 7 out of 25 show a grade 3 toxicity or if 2 out of 17 show a grade 4 toxicity or higher, the trial will stop (this will be equivalent to testing the toxicity hypotheses at level alpha =0.05 with a power 82%); otherwise we will proceed to full registration. The overall type I error for this test is 0.09. In addition, the trial will stop if any vaccine-related deaths occur. The determination of a death related to the vaccine will be made by the clinical trial team and an audit by the Data and Safety Monitoring Board.

The reasons for using different looks at toxicity level is because we are using surrogate endpoints that had not been proven formerly to have an ease of predictability of an outcome of direct relevance to patients; also, it is not quite clear how theses surrogates relate to the pathway of the natural disease and to overall survival rate.

Simon, Richard. "Optimal Two-Stage Designs for Phase II Clinical Trials," Controlled Clinical Trials, 1989, Volume 10, pages 1-10.
De Gruttola Victor et Al., "Considerations in the Evaluation of Surrogate Endpoints in Clinical Trials: Summary of NIH workshop" Controlled Clinical Trials, 2001, Volume 22, pages 485-502

Addendum
PHASE II STUDY OF ADENOVIRUS/PSA VACCINE IN MEN WITH HORMONE - REFRACTORY PROSTATE CANCER
Food and Drug Administration (FDA) Investigational New Drug (IND) #9706
Department of Defense, Prostate Cancer Research Program A-14059.2

The following are reporting requirements and responsibilities of the Principal Investigator to the United States Army Medical Research and Materiel Command's (USAMRMC) Office of Research Protections (ORP), Human Research Protection Office (HRPO).

1. The protocol will be conducted in accordance with the protocol submitted to and approved by the USAMRMC ORP HRPO and will not be initiated until written notification of approval of the research project is issued by the USAMRMC ORP HRPO.

2. Accurate and complete study records will be maintained and made available to representatives of the U.S. Army Medical Research and Materiel Command as a part of their responsibility to protect human subjects in research. Research records will be stored in a confidential manner so as to protect the confidentiality of subject information.

3. All unanticipated problems involving risk to subjects or others, serious adverse events related to participation in the study and subject deaths related to participation in the study should be promptly reported by phone (301-619-2165), by email (hsrrb@det.amedd.army.mil), or by facsimile (301-619-7803) to the USAMRMC, Office of Research Protections, Human Research Protection Office. A complete written report will follow the initial notification. In addition to the methods above, the complete report will be sent to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-ZB-PH, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

4. Any deviation to the protocol that may have an effect on the safety or rights of the subject or the integrity of the study must be reported to the USAMRMC ORP HRPO as soon as the deviation is identified.

5. Major modifications to the research protocol and any modifications that could potentially increase risk to subjects must be submitted to the USAMRMC ORP HRPO for approval prior to implementation. All other amendments will be submitted with the continuing review report to the USAMRMC ORP HRPO for acceptance.

6. A copy of the approved continuing review report and the local IRB approval notification will be submitted to the USAMRMC ORP HRPO as soon as these documents become available. A copy of the approved final study report and local IRB approval notification will be submitted to the USAMRMC ORP HRPO as soon as these documents become available.

7. The knowledge of any pending compliance inspection/visit by the FDA, OHRP, or other government agency concerning clinical investigation or research, the issuance of Inspection Reports, FDA Form 483, warning letters or actions taken by any Regulatory Agencies including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to USAMRMC ORP HRPO. "

Roles and Responsibilities of Study Personnel:

David M. Lubaroff, PhD, Principal Investigator – Dr. Lubaroff, along with Dr. Williams, will manage all aspects of the trial, from assisting in patient recruitment, co-chairing the clinical trial meetings, to supervising the immunologic testing of the patients' sera and lymphocytes for anti-PSA immune responses. He will work together with Dr. Williams the Co-Principal Investigator on all important clinical issues for the trial.

Daniel Vaena, MD; Co-Principal Investigator – Dr. Vaena is a Medical Oncologist who cares for prostate cancer patients following recurrences of their disease. He will manage all patient care activities associated with this proposal and will work together with Dr. Lubaroff on managing the trial. Dr. Vaena will function as co-chair of the weekly trial team meetings. He will be a major participant in the clinical management of prostate cancer patients that includes recruitment, patient management, assessing clinical response and any adverse events and long-term follow-up during the trial.

James Brown, MD, Co-Investigator – Dr. Brown, a clinical urologist on the clinical trial team, will also participate in the recruitment and clinical management of the patients. As Chief of Urology at the Iowa City Veterans Affairs Health Care System (ICVAHCS) Dr. Brown will be an active participant in the treatment and follow-up of patients at that hospital. He will work closely with Dr. Vaena in that capacity.

Mark C. Smith, MD, Co-Investigator – Dr. Smith is a Radiation Oncologist who is responsible for radiation therapy of men with prostate cancer. He will assist in the recruitment and care of patients in the trial.

Michael O'Donnell, MD, Co-Investigator – Dr. O'Donnell is an urologist at the UIHC who will contribute to the recruitment of new patients and care of enrolled patients in the study.

Sundeep Deorah, MD, Co-Investigator – Dr. Deorah is an urologist at the UIHC who will contribute to the recruitment of new patients and care of enrolled patients in the study.

Amit Gupta, MD, Co-Investigator – Dr. Gupta is an urologist at the UIHC who will contribute to the recruitment of new patients and care of enrolled patients in the study.

Kenneth Nepple, MD, Co-Investigator – Dr. Nepple is an urologist at the UIHC who will contribute to the recruitment of new patients and care of enrolled patients in the study.

Pamela Zehr, RN, MA - Clinical Trial Coordinator – Ms. Zehr participates in clinical trials in the Holden Comprehensive Cancer Center at the University of Iowa. She has and will continue to guide the clinical protocol through the institutional and federal other regulatory approval processes. She will coordinate follow-up visits of the patients.

Mary Schall, RN – Clinical Trial Coordinator - Ms. Schall also participates in clinical trials in the Holden Comprehensive Cancer Center at the University of Iowa. She will act as a backup for Ms. Zehr.

Karen Clark Griffith, RN, PhD - Clinical Trial Coordinator VAMC – Ms. Griffith will be responsible for trial for Veteran patients enrolled in the clinical trial at the VA Health Care System. She will work closely with all members of the trial team and attend the weekly meetings to discuss eligibility and follow-up of patients.

Steven Varga, PhD, Collaborator – Dr. Varga is an immunologist and will assist in the immunologic evaluation of patient's immune responses to the vaccine.

Lyse Norian, PhD, Collaborator – Dr. Norian is an immunologist and will assist in the immunologic evaluation of patient's immune responses to the vaccine.

Gideon Zamba, PhD – Biostatistician – Dr. Zamba participated in the discussions pertinent to the development of the clinical protocols, performed the power analysis, and constructed the section on statistical considerations for the trial. He will assist in the data analysis and statistical interpretation of the patient data.

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