Study Title: A Pilot Study to Assess the Immunogenicity of Candidate PSA Peptides for a Prostate Cancer Vaccine
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Background and Rationale

Prostate cancer is the most commonly diagnosed cancer in men in the United States. It accounted for over 218,890 new cases and 27,050 deaths in 2007. Approximately 70% of patients will have metastases at some time during the course of their disease. Treatment varies based on stage and grade of the disease from active surveillance, surgery, radiation for early stage disease to hormonal therapy and chemotherapy for more advanced stage.

A prostate cancer vaccine (Sipuleucel-T) was approved based on its survival benefit on castration resistant patients. In this group of patients there was a 4.1-month improvement in median survival (25.8 months in the sipuleucel-T group vs. 21.7 months in the placebo group). There was no effect on the time to disease progression (1). Sipuleucel-T is an active cellular immunotherapy, a type of therapeutic cancer vaccine consisting of autologous peripheral-blood mononuclear cells (PBMCs), including antigen-presenting cells (APCs), that have been activated ex vivo with a recombinant fusion protein (PA2024). PA2024 consists of a prostate antigen, prostatic acid phosphatase, that is fused to granulocyte–macrophage colony-stimulating factor, an immune-cell activator. This treatment although is FDA approved has raised significant criticism partly due to its price and modest benefit. Better treatments are urgently needed for patients with prostate cancer.

The goal of this project is to collect information in regards to the immunogenicity of PSA peptides in order to develop a novel therapeutic vaccine. This vaccine will consist of prostate specific antigen (PSA) peptide and Candida skin test reagent. Candida has recently been shown to be a promising new vaccine adjuvant for promoting T-cell responses (2). It can induce interleukin-12 (promotes T-cell response) secretion by Langerhans cells, the main antigen presenting cells in skin (3). In a Phase I clinical trial treating women with biopsy-proven high-grade squamous intraepithelial lesions (HSILs), precursors of cervical cancer, a combination of human papillomavirus peptides with Candida was demonstrated to be safe, to induce immune responses to human papillomavirus, and to promote T-helper type 1 (Th-1) response (promotes cell-mediated immunity) in vaccine recipients (4).

For treating prostate cancer, PSA is an ideal antigen as it is expressed in prostate cancer but not in any other organs. The characteristics of peptides that can effectively be used in therapeutic vaccines are their **solubility** in a single solution, **immunogenicity** in terms of containing large number of T-cell epitopes (so the vaccine can be used for all patients and not just a few that express certain HLA tissue types), and **ability to mature Langerhans cells** which in turn promotes T-cell activity.

In this protocol we focus on the immunogenicity of candidate peptides.

Specific Aims

To measure antigen-specific interferon- secretion by enzyme-linked immunospot (ELISPOT) assay, which measures antigen-specific interferon- secretion

Study Design and Procedures

Sixty ml of whole blood will be drawn in tubes containing sodium heparin from patients (n=10) diagnosed with prostate cancer. Blood will be drawn at the same time patients are having routine standard of care labs done. Up to 20 subjects could be screened in order to reach the enrollment goal of 10 subjects.

Five PSA peptides have been selected for (1) having neutral or positive charges and (2) containing <30% hydrophobic residues: PSA 1-40 (Ac-MWVPVVFLTLSVTWIGAAPLILSRIVGGWECEKHSQPW QV-NH2), PSA 41-80 (Ac-LVASRGRAVCGGVLVHPQWVLTAAHCIRNKSVILLGRHSL-NH2), PSA 161-200 (Ac-EPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTKFMLCAG-NH2), PSA 81-120 (Ac-FHPEDTGQVFQVSHSPHP LYDMSLLKNRFLRPGDDSSHD-NH2), PSA 201-240 (Ac-RWTGGKSTCSGDSGGPLVCNGVLQGITSWGSEPCA LPER P-NH2), PSA 241-261 (Ac-SLYTKVVHYRKWIKDTIVANP-NH2).

Peripheral blood mononuclear cells (PBMCs) will be purified using the ficoll gradient centrifugation method. 96-well plates (MultiScreen-HA; Millipore, Bedford, MA) will be coated with 5µg/ml of primary anti-interferon-gamma monoclonal antibody (Mabtech, Stockholm, Sweden) for capture in 50µl/well of phosphate-buffered saline (PBS) and stored at 4°C overnight. The plates will be washed four times with PBS and blocked using RPMI 1640 with 5% pooled human serum for 1h at 37°C. Three hundred thousand PBMC per well in triplicate will be presented with 10microM each of the PSA peptides described above.

Phytohemagglutinin will be used as a positive control while media containing no peptide will serve as a negative control. Human recombinant interleukin-2 (R&D Systems, Inc., Minneapolis, MN) (20U/ml) will be added to all wells. After 40 +/- 2h incubation at 37°C, the plate will be washed four times with PBS containing 0.05% Tween-20. A total of 50microl of secondary antibody (biotin-conjugated interferon-γ monoclonal antibody) (Mabtech) in PBS at a final concentration of 1microg/ml will be added, and the plate will be incubated for 2h at 37°C. The plate will then be washed four times with PBS containing 0.1% Tween-20. Avidin-bound biotinylated horseradish peroxidase H (Vectastain Elite ABC kit; Vector Laboratories, Inc., Burlingame, CA) will be added for 1h at 37°C. After four washings with PBS containing 0.1% Tween-20, stable diaminobenzene (Open Biosystems, Huntsville, AL) will be added to develop the reaction. The plates will be washed with distilled water three times and air-dried overnight.

The spots formed by interferon-gamma-secreting T-cells will be counted with an automated ELISPOT analyzer (AID ELISPOT Classic Reader; Autoimmun Diagnostika GmbH, Strassberg, Germany). The average spot-forming units (SFU) per antigen will be calculated. A response will be considered positive when the average SFU in wells with a given peptide was at least twice that of the average SFU in the no-peptide control wells.

Data analysis: Number of patients testing positive for each PSA peptide will be described. The statistical significance of the response will be assessed using paired t-test comparing SFU in peptide containing wells and those in no-peptide wells.

Study Population

Inclusion Criteria:

- Histological documented diagnosis of prostate cancer
- 18 years of age or older
- Signed informed consent form approved by the University of Arkansas for Medical Sciences (UAMS) IRB

Exclusion Criteria:

- Subjects must have no other current malignancies. Subjects with prior history at any time of any basal or squamous skin cancer are eligible, provided they are disease-free at the time of registration. Subjects with other malignancies are eligible if they have been continuously disease free for ≥ 5 years prior to the time of registration

Consent Process

Potential subjects with prostate cancer will be identified in the UAMS Medical Oncology or Urology clinic during routine clinic appointments. Upon identification, patients will be informed regarding the study by their physician along with the research staff. Patients will be allowed to review the IRB-approved informed consent form, and if they would like to participate in the study, they will sign the informed consent form.

During the consent process, the individual consenting patients will clearly be informed that their participation in this study is voluntary and that the decision whether or not to participate in the study will have no effect on future clinical care. The research subject will be encouraged to have family or friends participate in any or all of the process. The research subject will be provided sufficient time to ask questions, and will be questioned to ensure they understand the information. If they agree to proceed, the subject will sign the consent form. The consent process will be documented in the medical record. A copy of the informed consent document will be provided to the research subject, and additional copies will be sent to the medical records department. The original informed consent will be filed with the subject file in Cancer Clinical Trials Office (CCTO). The consent process will occur in a private exam room or in the private office of the research nurse.

Risks and Benefits

This is a minimal risk study (risk of phlebotomy; bruising, pain etc.) Potential benefits of this study may include the ability to contribute further research in this area. We anticipate that the data will help us better understand how to develop a prostate cancer vaccine.

Data Handling and Recordkeeping

The Principal Investigator will carefully monitor study procedures to protect the safety of research subjects, the quality of the data and the integrity of the study. All study subject material will be assigned a unique identifying code or number. Only the research staff will have access to the code and information that identifies the subject in this study. All study data will be kept on password-protected computers maintained by UAMS IT and accessible only to the study team.

Ethical Considerations

This study will be conducted in accordance with all applicable government regulations and University of Arkansas for Medical Sciences research policies and procedures. This protocol and any amendments will be submitted and approved by the UAMS Institutional Review Board (IRB) prior to conducting the study.

Dissemination of Data

Results of this study may be used for presentations, posters, or publications. The publications will not contain any identifiable information that could be linked to a participant.

References

1. Kantoff P et al. Sipuleucel-T Immunotherapy for Castration-Resistant Prostate Cancer N Engl J Med 2010; 363:411-422July 29, 2010DOI: 10.1056/NEJMoa1001294
2. Wang X et al Candida skin test reagent as a novel adjuvant for a human papillomavirus peptide-based therapeutic vaccine Vaccine. 2013 Dec 2;31(49):5806-13. doi: 10.1016/j.vaccine.2013.10.014. Epub 2013 Oct 14.
3. Nakagawa M et al IL-12 secretion by Langerhans cells stimulated with Candida skin test reagent is mediated by dectin-1 in some healthy individuals. Cytokine. 2014 Feb;65(2):202-9. doi: 10.1016/j.cyto.2013.11.002. Epub 2013 Dec 2.
4. Nakagawa M A phase I dose-escalation clinical trial of a peptide-based human papillomavirus therapeutic vaccine with candida skin test reagent as a novel vaccine adjuvant for treating women with biopsy-proven cervical intraepithelial neoplasia 2/3. ASCO 2015 Chicago, IL (poster presentation)