

*Abbreviated Title: TARP Peptide Vaccine Prostate Cancer  
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**Abbreviated Title:** TARP Peptide Vaccine Prostate Cancer

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**Title:** A Pilot Study of Vaccination with Epitope-Enhanced TARP Peptide and TARP Peptide Pulsed Dendritic Cells in the Treatment of Stage D0 Prostate Cancer

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**Investigational Agents:**

Drug Name:	TARP 27-35 Peptide (Native Peptide) NSC #740703	Tarp 29-37-9V Peptide (Epitope Enhanced Peptide) NSC # 740704
IND Number:	13925	13925
Sponsor:	CCR	CCR
Manufacturer:	NeoMPS, Inc	NeoMPS, Inc.

**Commercial Agents:**

Interleukin-4, CellGenix, BB-MF 11269

Montanide® ISA 51 VG, Seppic, NSC #737063

Hemocyanin from Keyhole Limpet, Sigma –Aldrich, DMF 21067

Leukine, Sanofi-Aventis

FluMist, MedImmune

Elutra Cell Separation System, Gambro BCT, Inc., BB-MF11922

LPS (Endotoxin, E. Coli), NIH Clinical Center, BB-MF-7294

## **PRÉCIS**

### ***Background***

- T-cell receptor alternate reading frame protein (TARP) is expressed by both normal and malignant prostate cancer tissue and is found in about 95% of prostate cancer specimens. TARP is immunogenic and hence is a target antigen for vaccination.
- The immunogenicity of TARP peptides can be augmented through epitope enhancement that is achieved through amino acid substitutions resulting in increased peptide binding affinity.
- Two HLA-A\*0201 TARP peptide epitopes are associated with generation of catalytic T-cell responses: TARP27-35 and TARP29-37. Substitution of Val for Leu at position 9 in TARP29-37, results in a peptide with increased binding affinity (TARP29-37-9V) that induces antigen specific T cells able to recognize wild type and multiple modified TARP peptides. The affinity of the TARP 27-35 peptide, corresponding to a distinct but overlapping epitope, is high enough that no enhancement was required.
- Stage D0 prostate cancer patients have no evidence of visceral or bony metastatic disease but have persistently elevated or rising PSA levels (biochemical progression) and are at increased risk for disease progression. Since they lack much of the immune dysfunction associated with the high tumor burden characteristic of end-stage metastatic disease, they are an ideal population in which to study therapeutic vaccination to slow or prevent disease recurrence and progression.
- Dendritic cells (DC) are the most potent antigen-presenting cells of the immune system and are being studied extensively for anti-tumor activity in a broad spectrum of cancer patients.
- As the optimal method for therapeutic immunization with peptide vaccines in patients with cancer is unclear, vaccination with TARP peptides in Montanide® ISA 51 VG adjuvant plus Sargramostim will be studied in a randomized fashion with autologous, TARP peptide-pulsed DCs in HLA-A\*0201 Stage D0 prostate cancer patients.

### ***Objectives***

- Determine the safety and toxicity of TARP peptide and TARP peptide-pulsed dendritic cell vaccination in patients with Stage D0 prostate cancer.
- Determine the T-lymphocyte immune responses to TARP peptide vaccination with Montanide® ISA 51 VG plus Sargramostim or autologous dendritic cells as measured by tetramer staining, IFN- $\gamma$  ELISPOT and  $^{51}\text{Cr}$  release CTL assays.

### ***Eligibility***

- Males  $\geq$  18 years of age with histologically confirmed adenocarcinoma of the prostate.
- Must have completed and recovered from all prior definitive therapy (surgery, brachytherapy, cryotherapy or radiotherapy) for the primary tumor, or other definitive-intent local therapy.
- Stage D0 disease with documented biochemical progression documented by rising PSA and no evidence of metastatic disease by physical examination, CT scan or bone scan.
- PSADT  $\geq$  3 months and  $\leq$  15 months:

- Patients must have  $\geq 3$  PSA measurements over  $\geq 3$  months.
- The interval between PSA measurements must be  $\geq 4$  weeks.
- For patients following definitive radiation therapy or cryotherapy: a rise in PSA of  $> 2\text{ng/mL}$  above the nadir (per RTOG-ASSTRO consensus criteria).
- For patients following radical prostatectomy: 2 absolute PSA values  $> 0.3\text{ng/ml}$  (per NCCN guidelines).
- Non-castrate level of testosterone:  $\geq 50$  ng/dL (prior ADT allowed; must be  $\geq 6$  months since last dose of ADT).
- HLA-A\*0201 positive.
- Performance Status: ECOG 0-2 or Karnofsky 70-100% and life expectancy  $\geq 1$  year.
- Hemoglobin  $\geq 10.0$  gm/dL, WBC  $\geq 2,500/\text{mm}^3$ , ALC  $\geq 500/\text{mm}^3$ , ANC  $\geq 1,000/\text{mm}^3$ , platelet count  $\geq 100,000/\text{mm}^3$ , and PT/PTT  $\leq 1.5\text{X ULN}$  unless receiving clinically indicated anticoagulant therapy; SGPT/SGOT  $\leq 2.5\text{X ULN}$ , total bilirubin  $\leq 1.5\text{X ULN}$ ; creatinine  $\leq 1.5\text{X ULN}$  *and* estimated GFR (eGFR)  $\geq 60$  ml/min.
- Hepatitis B and C negative (unless the result is consistent with prior vaccination or prior infection with full recovery); HIV negative.
- No use of investigational agents within 4 weeks of study enrollment or use of immunosuppressive or immunomodulating agents within 8 weeks of study entry.
- No other concurrent anticancer therapy or prior prostate cancer vaccines expressing TARP or HLA A2.
- No alternative medications known to alter PSA (e.g. phytoestrogens and saw palmetto). Note: patients receiving medications for urinary symptoms such as Flomax or 5-alpha reductase inhibitors (finasteride and dutasteride) on a chronic stable dose for at least 3 months are *allowed*.

### **Study Design**

- This is a randomized, prospective, pilot study of vaccination with a mixture of wild type (TARP27-35) and epitope-enhanced (TARP29-37-9V) TARP peptides in HLA-A\*0201 patients with stage D0 prostate cancer.
- Vaccination with TARP peptides admixed with Montanide® ISA 51 VG plus Sargramostim administered by deep subcutaneous injection will be compared with vaccination with TARP peptide-pulsed autologous dendritic cells (DCs) administered intradermally.
- Autologous dendritic cells will be matured from peripheral blood monocytes with Sargramostim, IL-4, IFN- $\gamma$  and LPS and pulsed with wild type and epitope-enhanced TARP peptides.
- Apheresis will be performed on all patients at weeks 0, 24, and 48.
- Randomization and assignment to received TARP peptide vaccine with Montanide® ISA 51 VG plus Sargramostim given by deep subcutaneous injection or TARP peptide-pulsed autologous DCs given ID will be performed at week 0.

- All patients will receive live, attenuated influenza vaccine (FluMist™) when seasonally available at the very end of their week 0 visit as a control vaccine to assess cytotoxic T lymphocyte responses.
- TARP Peptide vaccines will be administered every three weeks at weeks 3, 6, 9, 12, and 15, with a sixth and seventh booster dose of vaccine at Week 48 and 96. Follow-up will be through 144 weeks on study.
- The trial uses an optimal 2-stage design targeting an immunologic response between 10 and 40%. We will initially accrue 9 patients in each arm. If 0-1 patients develop an immunologic response, then no further patients will be enrolled. If 2 or more of these patients develop an immunologic response, we will accrue 11 additional patients for a maximum total of up to 20 patients in each arm. A stopping rule for excessive toxicity will be incorporated.

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## **1 INTRODUCTION**

### **1.1 STUDY OBJECTIVES**

#### 1.1.1 Primary Objectives

- 1.1.1.1 Determine the safety and toxicity of TARP peptide and TARP peptide-pulsed dendritic cell vaccination in patients with Stage D0 prostate cancer naïve to androgen deprivation therapy (ADT).
- 1.1.1.2 Determine the T-lymphocyte immune responses to TARP peptide vaccination with Montanide® ISA 51 VG plus GM-CSF or autologous dendritic cells as measured by tetramer staining, IFN- $\gamma$  ELISPOT and 51Cr release CTL assays.

#### 1.1.2 Secondary Objectives

- 1.1.2.1 Determine the effect of TARP peptide vaccination on serum prostate specific antigen doubling time (PSADT).
- 1.1.2.2 Determine the effect of TARP peptide vaccination on PSA growth rate and regression rate constants.

### **1.2 BACKGROUND AND RATIONALE**

#### 1.2.1 Peptide Vaccines

Elucidation of the crystal structure of the MHC and of the peptides bound<sup>1</sup>, and discovery of anchor-residue sequence motifs accounting for binding specificity of peptides to MHC molecules<sup>2</sup> provided the visual and mechanistic answer to how T cells recognize antigens in the form of short peptides. The observation that short peptide segments (8-10 amino acids) fit into a groove in the MHC molecule, combined with knowledge of the amino acid sequences of tumor epitopes, prompted the use of peptides as therapeutic agents in the treatment of cancer. These observations were followed by cloning of the first human tumor associated antigen and identification of its nonamer peptide sequence<sup>3</sup>. Several strategies have been developed both to improve immunogenicity and to steer the immune system toward desired types of responses. Peptides have been administered loaded on autologous DCs in an attempt to enhance their immunogenicity and improve on peptide vaccination efficacy. However, individual peptides will each be useful only in patients with appropriate HLA molecules presenting that peptide.

In a study of vaccination with p53 peptide-pulsed dendritic cells in HLA-A2+ patients with progressive advanced breast cancer, a significant fraction of breast cancer patients (8/19 evaluable patients) obtained stable disease (SD) or minor regression<sup>4</sup>. The effect of p53-specific immune therapy was supported by: positive correlation between p53 expression of tumor and observed SD; therapy-induced p53 specific T cells in 4/7 patients with SD but only 2/9 patients with PD; and significant response-associated changes in serum YKL-40 and IL-6 levels. In a trial of patients with metastatic melanoma vaccinated with melanoma peptide-pulsed DCs, longer survival was associated with induction of melanoma peptide-specific immunity to at least two of the four melanoma peptides (MART-1/MelanA, tyrosinase, MAGE-3, and gp100) used in the vaccine<sup>5</sup>. Similarly, in a phase I study of HLA-A2-binding MUC1 peptide pulsed DC

vaccination in patients with metastatic renal cell carcinoma, development of MUC1 peptide-specific T-cell responses in peripheral blood mononuclear cells was seen in the six (of twenty) patients with objective responses<sup>6</sup>. Consistent with the observed correlations between induction of peptide-specific immune responses following DC vaccination and clinical outcomes is a trial of CA9-peptide-pulsed mature dendritic cells in patients with renal carcinoma<sup>7</sup>: none of the immunomonitoring assays documented evidence of CA9-peptide-specific immunity and no clinical responses were observed.

### 1.2.2 Epitope Enhancement

Modification of the amino acid sequence of epitopes, commonly referred to as epitope enhancement, can improve the efficacy of vaccines through several means: 1) increasing affinity of peptide for MHC molecules<sup>8-10</sup>, 2) increasing T cell receptor (TCR) triggering<sup>11-13</sup>, or 3) inhibiting proteolysis of the peptide by serum peptidases<sup>8,14,15</sup>. Whenever the peptide sequence is altered, it is important to demonstrate that the T cells induced still recognize the native peptide sequence. There is precedent for epitope-enhanced peptides showing greater efficacy in clinical trials<sup>10</sup>. Epitope-enhanced subdominant peptides can bypass self tolerance because subdominant epitopes do not generally induce tolerance but can be made more immunogenic by epitope enhancement<sup>16</sup>.

### 1.2.3 Clinical Trials of Peptide Vaccines

The best-studied clinical model of peptide vaccination is malignant melanoma. Rosenberg and colleagues evaluated vaccination with native gp100 peptide 209-217 and found that it produced only low levels of T cell reactivity in 2 of 8 melanoma patients analyzed whereas an epitope-enhanced gp100 (209-2M) peptide generated strong T cell reactivity in 10 of 11 patients immunized<sup>10</sup>. Nevertheless, only a single objective clinical response was reported.

Immunization with the epitope-enhanced peptide, 209-2M, combined with high dose IL-2 treatment, produced objective clinical anti-tumor responses in 42% of patients, although T cell reactivity was observed in <10% of patients. In the adjuvant setting, Smith et al.<sup>17</sup> found a vaccination frequency of every two or three weeks resulted in a median frequency of CD8<sup>+</sup> cells binding 209-2M tetramers of 0.34% after 6 months compared with 0.02% before vaccination, whereas less frequent vaccination gave substantially lower responses of 0.03%. The impact of age was striking. In patients under 60 years old the median number of tetramer positive CD8<sup>+</sup> cells induced by vaccination was 0.64%, whereas in those over 60 it was 0.08%. In other studies using this peptide, addition of either IL-12<sup>18</sup> or GM-CSF<sup>19</sup> increased this percentage slightly.

Immunization with tyrosinase peptide has been significantly less effective despite epitope enhancement. Immunization with the tyrosinase 370D peptide in Incomplete Freund's Adjuvant with or without cytokines, including IL-12<sup>18</sup> or GM-CSF<sup>19</sup>, only rarely resulted in low numbers of tetramer positive CD8 cells or cytokine production. The levels of 370D-responsive cells has been low (0.01-0.03% of input cells) by ELISPOT assay<sup>20</sup>. The use of peptide vaccines may be additionally complicated by the choice of adjuvants. Most studies used IFA but Schaed et al.<sup>20</sup> showed no response with 370D peptide in IFA, whereas almost 50% of patients showed low-level ELISPOT responses with QS21 or GM-CSF as adjuvants.

Recent peptide-pulsed DC-based vaccines have yielded more promising and clinically significant results against a variety of tumors when associated with the induction of peptide specific immune responses and include: disease stabilization in pts with p53 expressing advanced breast cancer<sup>4</sup>; prolonged survival in subsets of patients with metastatic malignant melanoma

vaccinated with melanoma peptides<sup>5</sup>; and objective responses and disease stabilization in patients with metastatic renal cell carcinoma<sup>6</sup>. In addition, HER2/*neu* peptide vaccination alone has been associated with reduced recurrence rates in disease-free, conventionally treated, high-risk breast cancer patients<sup>21</sup>. These results must be balanced against clinical trials with negative outcomes: a trial of CA9-peptide-pulsed mature dendritic cells failed to show any clinical benefit in patients with progressive renal cell carcinoma<sup>7</sup>, while a randomized trial of autologous DC vaccines pulsed with MHC class I and II-restricted peptides could not be demonstrated to be more effective than dacarbazine (DTIC) in patients with stage IV metastatic melanoma<sup>22</sup>. These contrasting outcomes reflect the evolving understanding of dendritic cell biology and delivery, a refinement of tumor antigens and peptides selected, and the optimization of patient populations that are candidates for therapeutic dendritic cell vaccination.

The importance of epitope enhancement is supported by the promising results of vaccination with the epitope enhanced carcinoembryonic antigen (CEA) peptide<sup>13</sup>, using peptide-pulsed FLT3 ligand-expanded DCs to immunize patients with advanced colorectal cancer. Five of 12 patients immunized developed > 1% tetramer-positive CD8<sup>+</sup> T cells and two had clinical responses.

Despite the small sample sizes and the variable populations treated, some principles emerge. Immunization with native peptide sequences is often insufficient to generate reactive T cells and clinical responses in most patients. Epitope-enhanced peptides can generate T cell responses, but not always clinical tumor responses. Adjuvants, including cytokines and costimulatory molecules, improve the immunogenicity of peptide vaccination. Paradoxically, combining peptide vaccination with IL-2 significantly reduced detection of specific T cells in blood, but nearly half the patients showed objective cancer regressions<sup>10</sup>, possibly due to IL-2-induced innate immunity combined with vaccine immunity. In addition, preclinical studies indicate that high avidity CTL to self-peptides tumor antigens may be deleted by self tolerance<sup>16</sup>. Epitope enhancement of subdominant epitopes allows induction of protective high avidity CTL responses to these tumor antigen epitopes that are not limited by self-tolerance. This provides an important rationale for use of epitope enhanced peptides.

#### 1.2.4 Previous Vaccine Branch Studies of Peptide-Pulsed Dendritic Cells

Although there has been significant progress in the development of peptide vaccines for the treatment of melanoma, for other solid malignancies progress has lagged. We have evaluated mutated *ras* and p53 peptides in patients with lung and colon cancer.

In study 94-C-0031, a phase I/II trial of mutant *ras* and p53 peptides in a variety of cancers, both CTL responses and interferon-gamma responses to the peptide vaccine were statistically significantly correlated with median survival times (393 vs 98 days,  $p = 0.04$ , and 470 vs 88 days,  $p = 0.02$ , for the two types of immune response, respectively)<sup>23</sup>.

In study 99-C-0023, a phase I/II trial of vaccination with mutant *Ras* peptide-pulsed DCs in HLA A2.1 patients with colorectal cancer, two groups of patients were eligible for study participation: patients with evidence of metastatic disease and patients without evidence of disease but at high risk for recurrence. One of 15 patients enrolled in the metastatic arm developed a detectable immunologic response but there were no objective tumor responses, although occasional patients showed regression of individual metastatic lesions. There were no immunologic responses among the four patients accrued to the adjuvant arm without metastatic disease.

In study 99-C-0142, a phase II trial of individualized mutant p53 peptide-pulsed DCs in the treatment of patients with locally advanced non-small cell lung cancer after standard therapy, twelve patients were screened for the study and two patients were eligible for enrollment. Of the two patients enrolled, only one was evaluable for immune responses to p53 vaccination and there was no evidence of vaccine-induced immunologic reactivity. The other patient experienced rapid disease progression following completion of vaccination and could not be evaluated for immune response.

These trials of peptide-pulsed dendritic cells were not successful in demonstrating an acceptable level of immunization with mutated *ras* or p53. This may be due to the relatively low binding affinity of the mutated *ras* peptides for HLA-A\*0201; successful immunization of patients with peptides appears to require epitope-enhanced peptides as discussed earlier. Another challenge that has made use of *ras* and p53 peptides particularly difficult is the need to treat patients whose tumor cells express the mutated sequences for this approach to be beneficial. Only one in three patients screened for eligibility for the *ras* trial was eligible, increasing the amount of screening required to identify potential candidates. In addition, in both these studies, the dendritic cell vaccines were administered intravenously. It has become clear from the emerging literature on therapeutic dendritic cell cancer vaccination that intradermal delivery results in optimal immunogenicity. For this reason we have looked for other peptides that are more universally expressed that would be attractive targets for immunization and are administering the TARP peptide-pulsed dendritic cell vaccines in this study intradermally.

#### 1.2.5 TARP

A novel protein expressed in patients with prostate and breast cancer has recently been described<sup>24</sup>. This 58 amino acid protein, T-cell receptor  $\gamma$  alternate reading frame protein (TARP), was identified with the expressed sequence database. The mRNA is initiated in the J $\gamma$  1 exon of the TCR  $\gamma$  and the protein expressed is initiated in an alternative reading frame than the TCR  $\gamma$  coding sequence. In their initial description of TARP in the human prostate, Pastan et al demonstrated that it originated from epithelial cells and not from infiltrating T lymphocytes, and that it is expressed in normal prostate epithelium, adenocarcinoma of the prostate, and the prostatic adenocarcinoma cell line LNCaP<sup>25</sup>. They subsequently showed that TARP was also expressed in three breast cancer cell lines and breast cancer tissues<sup>26</sup> and determined that TARP is expressed in androgen-sensitive (LNCaP) but not in androgen-independent (PC3) prostate cancer cell lines, implicating the role of TARP in prostate cancer progression<sup>27</sup>. The protein is expressed both by normal and malignant prostate cancer tissue with about 95% of prostate cancer specimens positive for its expression. Oh et al determined two HLA-A2 epitopes that produce cytolytic T cell responses<sup>28</sup>. These sequences map to amino acids 27-35 and 29-37. TARP27-35 was found to bind with an affinity that was 10 times greater than that of TARP29-37. These peptides were demonstrated to be immunogenic by immunizing A2K<sup>b</sup> transgenic mice (expressing human HLA-A\*0201) with dendritic cells pulsed with these peptides or with DNA encoding the peptide. Dendritic cell immunization produced a higher level of immunity than DNA immunization and as expected due to its higher binding affinity, TARP27-35 produced a higher level of CD8+ T cell response than TARP29-37.

Epitope enhancement of the TARP peptides was performed to increase the level of immunity that could be generated with these peptides. Amino acid substitutions in the TARP27-35 peptide did not increase binding affinity but two amino acid substitutions in TARP29-37 did produce

higher binding affinity peptides. For TARP29–37, Arg at position 3 and Leu at position 9 were substituted with Ala (TARP29–37-3A) and Val (TARP29–37-9V), respectively. Substitution at position 3 with Ala in TARP29–37 resulted in the greatest increase in the binding affinity of the peptide. Although TARP29–37-9V showed a lower binding affinity to HLA-A2 than TARP29–37-3A did, substitution of Leu at position 9 with Val did enhance the binding affinity compared with the wild-type peptide, TARP29–37. When the immunogenicity of these peptides was evaluated in A2K<sup>b</sup> transgenic mice both of the epitope-enhanced peptides produced a higher percentage of CD8<sup>+</sup> T cells specific than the wild type sequence. It was also shown that T cells generated with the epitope enhanced TARP29-37 sequences reacted with targets pulsed with the wild type TARP29-37 peptide in the mouse.

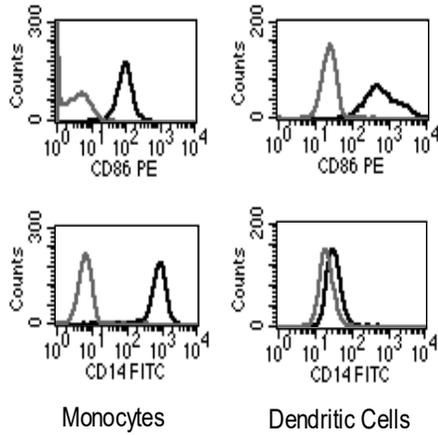
Although immunogenicity of these peptides was demonstrated in the mouse it is important to confirm their immunogenicity and cross reactivity in humans. Studies of these peptides in human cells showed that TARP29-37, TARP29–37-3A, and TARP29–37-9V were immunogenic in human T cells. TARP29–37-9V specific T cells recognize targets pulsed with all three peptides equally well whereas TARP29–37-3A specific T cells recognized only targets pulsed with TARP29–37-3A, and that TARP29–37 specific T cells recognized targets pulsed with the epitope enhanced peptides less well. This would suggest that the TARP29–37-3A peptide would not be appropriate for immunization in humans whereas the TARP29–37-9V would be more likely to generate T cells that recognize the wild type sequence. Human T cells specific for TARP27-35 recognized targets pulsed with that sequence as anticipated. In addition to their ability to kill targets pulsed with TARP peptides, CD8<sup>+</sup> T cells specific for TARP peptides were able to kill human tumor targets that were HLA A2 positive and that expressed TARP sequences. The availability of tetramers that react with CD8<sup>+</sup> T cells specific for TARP provide a simple means of evaluating the ability to stimulate immunity to the TARP peptides. In a limited survey tetramer positive cells ranged from 0.66% to 3.9% of the CD8<sup>+</sup> T cells in prostate and breast cancer patients compared with .01-.6% in normal controls.

#### 1.2.6 Dendritic Cell Vaccination with TARP

This antigen will be presented on the patient's own HLA-A\*0201 positive dendritic cells. Dendritic cells are very potent antigen presenting cells. They have several distinct features making them ideal vehicles for presentation of these tumor specific peptides. Dendritic cells express a high level of both MHC class I and II molecules, as well as, a variety of adhesion and costimulatory molecules that contribute to their ability to effectively stimulate T cells<sup>29</sup>. Dendritic cells have been shown to be more effective in the stimulation of the immune response than B cells or macrophages<sup>30</sup>. In addition, *ex vivo* maturation of dendritic cells may, in part, overcome the inability of cancer patients to direct the immune response toward their own tumor. Dendritic cells within tumor bearing mice have been found to be marginally effective when presenting peptides to the animal's immune system. This is due to the presence of vascular endothelial growth factor, present in many cancers, including colorectal cancers bearing *ras* mutations, that inhibits dendritic cell maturation. However, cells from the same tumor bearing animals, matured *ex vivo*, are extremely effective in presentation to the immune system<sup>31,32</sup>. Dendritic cells can be matured *ex vivo* from human peripheral blood mononuclear cells using interleukin 4 (IL-4) and granulocyte macrophage colony stimulating factor (GM-CSF) followed by CD40 ligand treatment<sup>33,34</sup>. We have conducted pilot experiments along with the Department of Transfusion Medicine using peripheral blood monocytes cells (PBMCs). We have measured

the up regulation of CD83, costimulatory and adhesion molecules as surrogate markers and have measured the function of dendritic cells in a mixed lymphocyte reaction <sup>29,35</sup>.

Figure 1



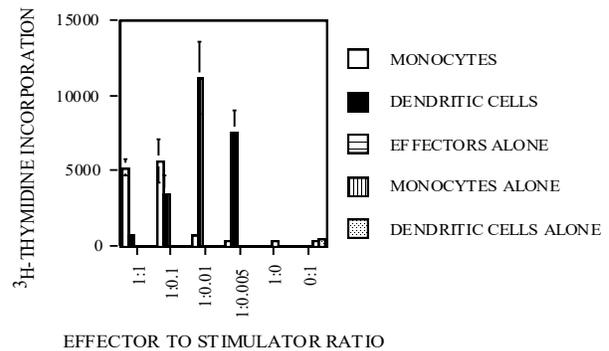
We also plan to further improve our dendritic cell manufacturing process utilizing IFN- $\gamma$  and LPS for dendritic cell maturation. Labeur et al have compared both the cell surface markers and the functional activity of dendritic cells cultured with GM-CSF and IL-4 (immature dendritic cells) and those cultured with GM-CSF and IL-4 plus CD40L or LPS (mature dendritic cells) <sup>26</sup>. The mature dendritic cells when compared to immature dendritic cells demonstrated greater expression of most cell surface markers thought to be important in T cell interaction.

In addition, the mature dendritic cells produced significantly more interleukin 12 than immature dendritic cells. More importantly, in a tumor challenge model, six of six mice that were immunized with antigen pulsed mature dendritic cells did not develop tumors while six of six mice that were immunized with antigen pulsed immature dendritic cells did develop tumors. Further, in an established tumor model, tumor growth was significantly inhibited in mice vaccinated with mature dendritic cells when compared to mice vaccinated with immature dendritic cells <sup>36</sup>. The ability of CD40 ligand to induce the maturation of dendritic cells that are better able to stimulate the T cell response has also been evaluated ex vivo in humans. Morse et al were able to show that dendritic cells treated with CD40 ligand were more effective at stimulating cytotoxic T cells than were immature dendritic cells grown in GM-CSF and IL-4 <sup>37</sup>.

### 1.2.7 Vaccination with Peptides or Peptide-Pulsed Dendritic Cells

There are limited studies that address the most effective means for induction of cytotoxic T cell responses in patients with cancer. Slingluff compared the use of peptide in adjuvant with peptides pulsed on dendritic cells in a small study in patients with melanoma. This study

**COMPARISON OF DENDRITIC CELLS VS. MONOCYTES AS STIMULATORS IN A MIXED LYMPHOCYTE REACTION MLR PULSED W/ TRITIATED THYMIDINE ON DAY 7**



showed a superior response with peptides in adjuvant compared with peptides pulsed on dendritic cells. T cell response was monitored in peripheral blood and draining lymph nodes. In patients vaccinated with peptide in adjuvant T cell responses were seen in 42% of peripheral blood samples and 80% of lymph nodes whereas in patients vaccinated with peptides pulsed on dendritic cells 11% showed response in the blood and 13% in the nodes. Despite the disparity in immunologic response rates objective responses were observed in both arms of the study. This study used immature dendritic cells and previous studies have shown that immature dendritic cells are ineffective in producing a cytolytic T cell response compared with mature dendritic cells. Another possible disadvantage with immature dendritic cells is their ability to induce tolerance. This study will address in a randomized fashion the immunogenicity of TARP peptides in adjuvant compared with TARP peptides pulsed on mature dendritic cells.

## **2 ELIGIBILITY ASSESSMENT AND ENROLLMENT**

### **2.1 ELIGIBILITY CRITERIA**

#### **2.1.1 Inclusion Criteria**

- 2.1.1.1 Males  $\geq$  18 years of age with histologically confirmed adenocarcinoma of the prostate.
- 2.1.1.2 HLA-A\*201 positive
- 2.1.1.3 Patients must have
  - 2.1.1.3.1 Completed and recovered from all prior definitive therapy (surgery, brachytherapy, cryotherapy or radiotherapy) for the primary tumor, or other definitive-intent local therapy.
  - 2.1.1.3.2 Stage D0 disease with documented biochemical progression documented by a rising PSA.
  - 2.1.1.3.3 No evidence of metastatic disease by physical examination, CT scan or bone scan.
  - 2.1.1.3.4 For patients following definitive radiation therapy or cryotherapy: a rise in PSA of  $>2$  ng/mL above the nadir (per RTOG-ASTRO consensus criteria).
  - 2.1.1.3.5 For patients following radical prostatectomy: 2 absolute PSA values  $> 0.3$  ng/mL (per NCCN guidelines)
  - 2.1.1.3.6 Non-castrate level of testosterone:  $\geq 50$  ng/dL (prior ADT allowed; must be  $\geq 6$  months since last dose of ADT).
  - 2.1.1.3.7 A Pre-Enrollment/Baseline PSADT  $> 3$  months and  $\leq 15$  months
    - 2.1.1.3.7.1 Patients must have  $\geq 3$  PSA measurements over  $\geq 3$  months
    - 2.1.1.3.7.2 The interval between PSA measurements must be  $\geq 4$  weeks
    - 2.1.1.3.7.3 For patients receiving 5-alpha reductase inhibitors (5ARI) e.g. finasteride or dutasteride, only PSA values obtained after at least 3 months on therapy may be used to calculate PSADT.
  - 2.1.1.3.8 Performance Status: ECOG 0-2 or Karnofsky 70-100%
  - 2.1.1.3.9 Life expectancy  $\geq 1$  year.
  - 2.1.1.3.10 Hemoglobin  $\geq 10.0$  gm/dL, WBC  $\geq 2,500/\text{mm}^3$ , ALC  $\geq 500/\text{mm}^3$ , ANC  $\geq 1,000/\text{mm}^3$ , platelet count  $\geq 100,000/\text{mm}^3$ .

- 2.1.1.3.11 PT/PTT  $\leq$  1.5X ULN unless receiving clinically indicated anticoagulant therapy.
- 2.1.1.3.12 SGOT/SGPT  $\leq$  2.5X ULN, total bilirubin  $\leq$  1.5X ULN, Cr  $\leq$  1.5X ULN, estimated GFR (eGFR)  $>$  60 ml/min.
- 2.1.1.4 Hepatitis B and C negative, unless the result is consistent with prior vaccination or prior infection with full recovery.
- 2.1.1.5 HIV negative
- 2.1.1.6 No use of investigational agents within 4 weeks of study enrollment.
- 2.1.1.7 No use of immunosuppressive (cytotoxic chemotherapy, systemic steroids) or immunomodulating agents (including IVIG) within 8 weeks of study entry. Note: topical and intranasal steroid therapy is permitted.
- 2.1.1.8 No other concurrent anticancer therapy.
- 2.1.1.9 No alternative medications known to alter PSA (e.g. phytoestrogens and saw palmetto). Note: patients receiving medications for urinary symptoms such as Flomax or 5-alpha reductase inhibitors (finasteride and dutasteride) on a chronic stable dose for at least 3 months are *allowed*.
- 2.1.1.10 No prior prostate cancer vaccines expressing TARP or HLA A2.
- 2.1.1.11 Able to understand and provide Informed Consent.
- 2.1.2 Exclusion Criteria
  - 2.1.2.1 HLA-A\*201 negative
  - 2.1.2.2 Patients with an active second malignancy other than adequately treated squamous or basal cell carcinoma of the skin, or superficial bladder carcinoma.
  - 2.1.2.3 Patients with active infection.
  - 2.1.2.4 Patients with brain, visceral or bony metastatic disease.
  - 2.1.2.5 Patients in who live attenuated intranasal influenza vaccine (FluMist™) is contraindicated<sup>38</sup> including individuals with asthma or reactive airways disease, cardiovascular or pulmonary disease, chronic metabolic diseases (including diabetes mellitus), renal dysfunction or hemoglobinopathies.

## **2.2 RESEARCH ELIGIBILITY EVALUATION**

- 2.2.1 Protocol screening evaluation, other than the determination of HLA typing, will be performed within 2 weeks of study entry.
- 2.2.2 All patients will undergo a baseline history and physical examination including review of systems, performance status and life expectancy assessment. Radiologic evaluation (performed within 8 weeks of study) will include a, CT scan of the, chest, abdomen and pelvis, and a bone scan and may be performed at outside institutions. Laboratory studies will include a CBC with differential, platelet count, PT/PTT, chemistries (Acute Care Panel, Mineral Panel, Hepatic Panel, total protein, CK, Uric acid, LDH), PSA, testosterone, 25 hydroxy vitamin D, HIV-1 antibody, Hepatitis B Surface antigen (HBSAg) and antibody to Hepatitis B Surface antigen (anti-HBSag), Hepatitis C antibody (anti-HCV) and HTLV-1 antibody (anti-HTLV-1).
- 2.2.3 Refer to [Appendix 1](#) for the Schedule of Study Clinical, Laboratory and Radiographic Evaluations.

## **2.3 REGISTRATION PROCEDURES**

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (<http://home.ccr.cancer.gov/intra/eligibility/welcome.htm>) must be completed and faxed to 301-480-0757. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

### **Randomization Procedures**

Once registration is confirmed, enrolled patients will be randomized 1:1 to receive TARP peptides with Montanide® ISA 51 VG adjuvant plus GM-CSF administered by deep subcutaneous (SC) injection or autologous, TARP peptide-pulsed dendritic cells administered intradermally (ID):

Arm A (TARP peptides with Montanide® ISA 51 VG plus GM-CSF): study numbers 101-120

Arm B (TARP peptide pulsed dendritic cells): study numbers 201-220

All patients will undergo apheresis for immunologic monitoring. The apheresis will also be used for preparation of dendritic cells by elutriation of monocytes for dendritic cell culture, maturation and vaccine generation for those randomized to receive peptide-pulsed dendritic cells.

## **3 STUDY IMPLEMENTATION**

### **3.1 STUDY DESIGN**

This is a randomized, prospective pilot study of TARP peptide vaccination in HLA-A\*201 patients with Stage D0 prostate cancer. HLA-A\*201 positive eligible patients with *no evidence*

of brain, visceral or bony metastatic disease, a non-castrate testosterone level  $\geq 50$  ng/dL (prior ADT allowed; must be  $\geq 6$  months since last dose of ADT), and a PSADT  $> 3$  months and  $\leq 15$  months will be randomized to receive TARP peptides with Montanide® ISA 51 VG adjuvant plus GM-CSF administered by deep subcutaneous (SC) injection or autologous, TARP peptide-pulsed dendritic cells administered intradermally (ID).

TARP peptides or TARP-pulsed dendritic cells will be administered every three weeks at weeks 3, 6, 9, 12, and 15 for a total of five vaccinations with a booster dose of TARP vaccine given at Weeks 48 and 96. PSADT will be calculated in months at the following study time points: Pre-Enrollment/Baseline, weeks 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, and 144. PSADT will be calculated using the Memorial Sloan-Kettering Cancer Center cancer information prostate nomogram for PSA doubling time found at:

<http://www.mskcc.org/mskcc/shared/forms/Nomograms/flash/load.cfm?type=Prostate&width=586&height=505&title=Prostate%20Nomogram> as recommended in the Prostate Specific Antigen Working Group Guidelines.<sup>39</sup> Cumulative PSA values within 12 months inclusive of the study time point will be used for calculation of the baseline/Week 0 PSADT.

Exploratory comparisons of percent change in PSADT will be done among the following time points:

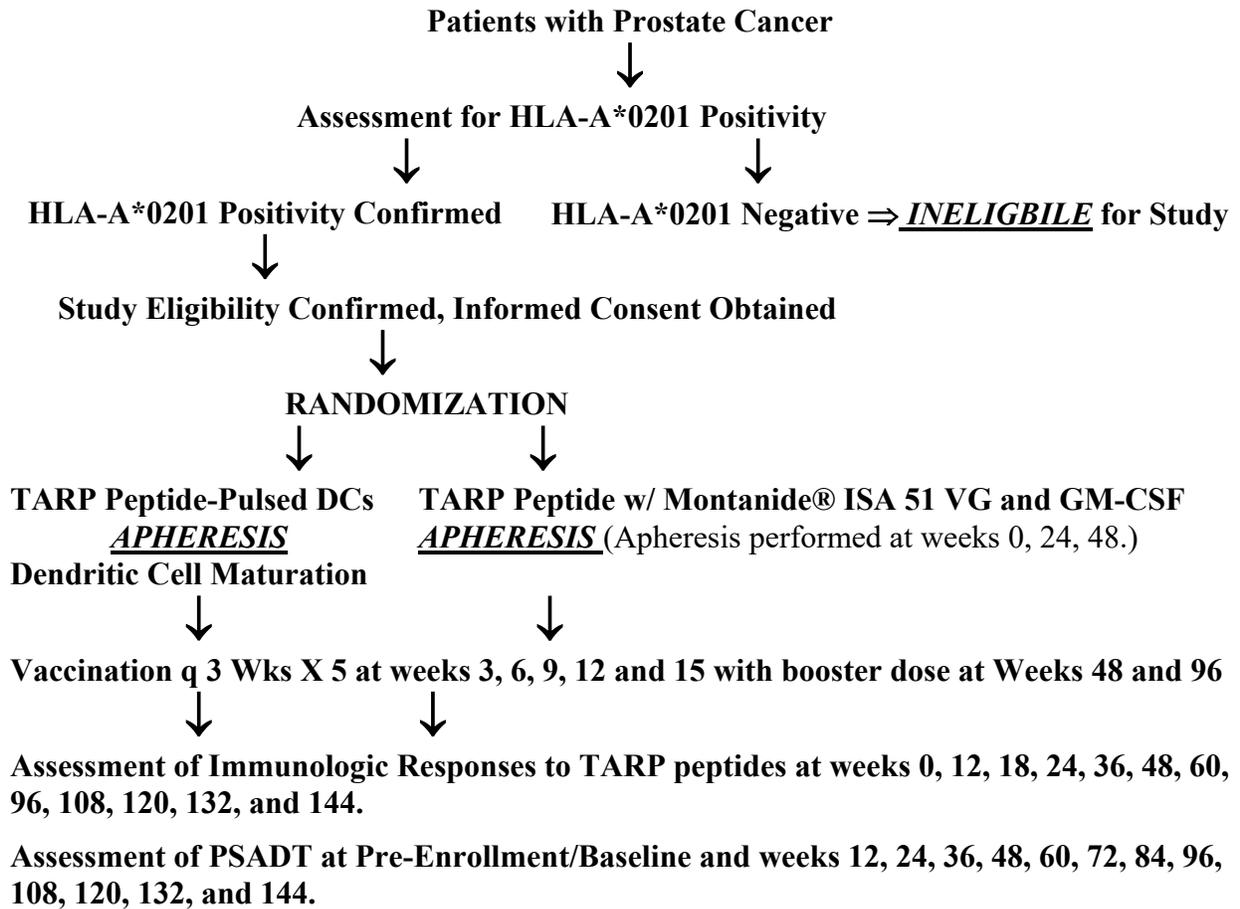
- Pre-Enrollment/Baseline PSADT vs. Week 12 PSADT
- Pre-Enrollment/Baseline PSADT vs. Week 24 PSADT
- Pre-Enrollment/Baseline PSADT vs. Week 36 PSADT
- Pre-Enrollment/Baseline PSADT vs. Week 48 PSADT
- Week 24 PSADT vs. Week 36 PSADT
- Week 36 PSADT vs. Week 48 PSADT
- Additional exploratory analyses of PSADT will be performed of Pre-Enrollment/Baseline PSADT vs. Weeks 60, 72 and 96 and Week 48 PSADT vs. Weeks 96, 108 and 144 PSADT.

Week 24 PSADT responders will be allowed to receive an additional dose of TARP peptide vaccine at Week 36. All patients will receive a booster of vaccine at Weeks 48 and 96.

Assessment of humoral and cellular immune responses to TARP peptide vaccination will be performed at weeks 0, 12, 18, 24, 36, 48, 60, 96, 108, 120, 132, and 144. Antigen-specific T-lymphocyte responses will be assessed by tetramer staining, IFN- $\gamma$  ELISPOT and  $^{51}\text{Cr}$  release CTL assays following exposure to native wild type and epitope-enhanced TARP peptides.

The schema for treatment is detailed below.

### 3.1.1 Study Schema



### 3.2 VACCINE ADMINISTRATION

Following apheresis to remove peripheral blood monocytes for dendritic cell preparation as well as peripheral blood mononuclear cells for flow cytometry and immunologic studies at their week 0 visit, all patients will undergo intranasal vaccination with live attenuated influenza vaccine (FluMist™) when seasonally available. Attenuated influenza vaccine is being administered as a control vaccine to characterize the patient's innate ability to generate immune responses to vaccination. Conserved HLA-A\*0201 restricted cellular and humoral responses to flu matrix peptides have been extremely well characterized regarding antibody titers and the range of ELISPOT responses that are typically observed in normal healthy individuals. Following receipt of the intranasal flu vaccine, patients will be immediately discharged to home and precautions will be taken to minimize the risk of potential transmission to other Clinical Center patients as outlined in Section 9.5

Because live attenuated influenza viruses replicate in the nasopharynx of recipients and can be shed in respiratory secretions, the possibility exists that the vaccine viruses can be transmitted to susceptible individuals who have not received the vaccine. Studies in children 8-36 months of age attending daycare who were randomized to receive a single dose of influenza virus vaccine live intranasal or placebo indicated that 80% of vaccine recipients shed at least 1 vaccine strain; the mean duration of shedding was 7.6 days (range: 1-21 days).<sup>38</sup> The frequency and duration of shedding of vaccine virus strains by individuals 5-49 years of age who receive influenza virus

vaccine live intranasal have not been established. Consequently, study patients will return to the CC at study week 3 which should provide an adequate window to ensure that recipients are unlikely to be shedding attenuated viral vaccine strains.

Patients in Arm A will receive an admixture of the wild-type and epitope-enhanced TARP peptides emulsified with Montanide® ISA 51 VG and GM-CSF administered by deep subcutaneous (SC) injection. Patients in Arm B will receive peptide-pulsed dendritic cells administered intradermally in two vaccination sites. Each peptide will be pulsed on dendritic cells separately in order to assure adequate binding of the peptide and cells will not be washed to remove free peptide after pulsing. Following verification of mature dendritic cell validation markers and release standards, the separately peptide-pulsed dendritic cells will be recombined for administration. Refer to [Appendix 2](#) for details concerning the production of peptide –pulsed dendritic cells by the NIH Clinical Center Department of Transfusion Medicine under the supervision of Drs. Marianna Sabatino, Hanh Khuu, Susan Leitman, & David Stroncek.

For both groups vaccinations will be administered at weeks 3, 6, 9, 12 and 15 for a total of five vaccinations with a booster dose of vaccine at Weeks 48 and 96. Patients will be monitored for immediate adverse event vaccine reactions (VS, clinical assessment) for 1 hours following their first TARP peptide vaccine dose. If no adverse reactions are observed with the first vaccination, patients will be monitored for 15 minutes for all subsequent vaccinations. If adverse reactions are observed following the first vaccine, the duration of post-vaccination monitoring with subsequent vaccinations will be determined by the Principal Investigator and Lead Associate Investigator as clinically indicated depending on the severity of the initial vaccine reaction. Since this protocol involves TARP vaccination in humans for the first time, enrollment will be staggered to allow for safety monitoring. For the first 3 patients on each study arm, enrollment of the next patient can only proceed until the preceding study subject is 2 weeks status-post their first vaccination. If no adverse events are observed in the two week window following the first vaccination in these 6 patients, enrollment of additional patients may proceed as quickly as is logistically feasible. Cells used for subsequent dendritic cell maturation will be derived from monocytes frozen during the initial apheresis.

### **3.3 TREATMENT MODIFICATIONS AND IMMUNIZATION STOPPING RULES**

No dose modifications will be made in patients receiving vaccination. Subjects will cease to receive immunization if they experience dose-limiting toxicity (DLT). Toxicity will be graded according to NCI Common Terminology Criteria for Adverse Events (CTCAE) v3.0 ([http://ctep.info.nih.gov/reporting/ctc\\_v30.html](http://ctep.info.nih.gov/reporting/ctc_v30.html))

#### **3.3.1 Immunization-Related Dose Limiting Toxicity (DLT)**

Immunization-related DLT is defined by the parameters outlined below attributed by the Principal Investigator or Protocol Chairperson as possibly, probably or definitely related to vaccine administration:

- 3.3.1.1 Any Grade 2 or Grade 3 or greater allergic/hypersensitivity reaction
- 3.3.1.2 Any Grade 2 or greater rash consistent with erythema multiforme.
- 3.3.1.3 Grade 3 or greater hematologic or non-hematologic toxicity, excluding lymphopenia. Abnormal laboratory studies will be repeated to verify toxicity
- 3.3.1.4 Grade 3 or greater acute vascular leak syndrome: respiratory compromise or fluids indicated.

3.3.1.5 The following Grade 3 reactions commonly associated with immunization **will** be dose-limiting:

- Injection site reactions: ulceration or necrosis that is severe; operative intervention indicated.
- Skin rash/desquamation: severe, generalized erythroderma or macular, papular or vesicular eruption; desquamation covering  $\geq 50\%$  BSA.
- Urticaria: intervention indicated for  $\geq 24$  hrs.

3.3.1.6 The following Grade 3 reactions commonly associated with immunization **will not** be dose-limiting:

- Pruritis/itching: intense or widespread and interfering with ADL lasting  $\leq 72$  hrs
- Fatigue: severe fatigue interfering with ADL lasting  $\leq 72$  hrs
- Fever:  $> 40.0^{\circ}$  C for  $\leq 24$ hrs
- Local lymphadenopathy lasting  $\leq 1$  week

### 3.3.2 Stopping for Excessive toxicity

If more than one of the first nine subjects in either arm of the trial experiences dose limiting toxicity, enrollment to that arm will cease pending discussion and review with the FDA and the IRB.

### 3.3.3 Delay in Vaccination

Patients who are unable to receive their vaccine injection as scheduled due to adverse events, toxicity or unforeseen personal or medical circumstances and who are otherwise eligible to continue on protocol, may be continued on vaccine therapy provided that their next vaccine is within three weeks of the previous vaccine. Patients will be advised of their options concerning alternative treatments before proceeding with the completion of their study vaccinations. The reason for the change in vaccination schedule will be documented in the patient's chart.

## 3.4 ON STUDY EVALUATION

Refer to [Appendix 1](#) for the Schedule of Clinical, Laboratory and Radiographic Evaluations.

### 3.4.1 Clinical Evaluation

(weeks 0, 3, 6, 9, 12, 15, 18, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132 and 144.)

3.4.1.1 Review of systems and documentation of symptoms

3.4.1.2 Assessment of ECOG Performance Status

3.4.1.3 Vital signs and physical examination

3.4.1.4 Assessment of injection site reactions

3.4.1.5 Provision of Vaccination Report Card to study subject (when TARP vaccine administered)

### 3.4.2 Laboratory Evaluation

(weeks 0, 3, 6, 9, 12, 15, 18, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, and 144.)

3.4.2.1 CBC with differential and platelets

3.4.2.2 Chemistries (Acute Care Panel, Mineral Panel, Hepatic Panel, total protein, CK, Uric acid, LDH),

3.4.2.3 PSA, testosterone

3.4.3 Radiographic Evaluation

(Weeks 0, 48, 96 and 144. No further restaging unless clinically indicated. )

3.4.3.1 CT Scan of the chest/abdomen/pelvis

3.4.3.2 Bone Scan

3.4.4 Immunologic Responses

(Weeks 0, 12, 18, 24, 36, 48, 60, 96, 108, 120, 132, and 144.)

3.4.4.1 Lymphocyte Subsets: CD4 and CD8 percent and absolute counts (done at Clinical Center)

3.4.4.2 Anti-TARP antibody responses

3.4.4.3 NKT Cell analysis (weeks 0, 12, 18 and 24 only)

3.4.4.4 IFN- $\gamma$  ELISPOT of PBMCs

3.4.4.5 Tetramer staining and  $^{51}\text{Cr}$  release CTL assay of PBMCs

3.4.5 Apheresis

Weeks 0, 24, 48.

3.4.6 Research Specimens for Assessment of Immunologic Responses

3.4.6.1 Cryopreserved PBMCs (6 tubes sodium heparin 10ml GTT) for tetramer, IFN-g ELISPOT and CTL assays. Weeks 0, 3 (FluMist responses), 12, 18, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132 and 144. Note: PBMCs are for storage only at Weeks 72 and 84.

3.4.6.2 Serum Storage (10ml RTT) for anti-TARP antibody responses. Weeks 0, 12, 18, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132 and 144.

3.4.6.3 NKT Cell Analysis (2 tubes 8ml blue tiger top sodium citrate tubes). Weeks 0, 12, 18, 24,

**3.5 CONCURRENT THERAPIES**

3.5.1 Contraindicated Therapies

3.5.1.1 Patients may not receive chemotherapy, hormonal therapy or radiation therapy while on study.

3.5.1.2 Systemic administration of steroids is not allowed with the exception of oral corticosteroids for management of acute allergic reactions or contact hypersensitivity of  $\leq 14$  days duration in patients who require them. Topical steroids as well as intranasal steroids are permitted. Patients must not have received systemic steroids within 14 days to TARP peptide vaccination.

3.5.1.3 Pharmacologic doses of immune modulating agents including IVIG, recombinant cytokines or growth factors.

3.5.1.4 Immunosuppressive agents such as cytotoxic chemotherapy.

3.5.1.5 Other investigational agents.

### 3.5.2 Allowed Therapies

Study subjects will be allowed to take multivitamins, analgesics/antipyretics/antihistamines for local and systemic injection site reactions, and other medications as clinically indicated. Use of herbal or nutritional supplements, known to alter PSA e.g. phytoestrogens or saw palmetto is not allowed.

## 3.6 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

### 3.6.1 Criteria for removal from protocol therapy

- 3.6.1.1 Patient experiences a Grade 3 or greater toxicity attributed as possibly, probably or definitely related to vaccine as described in Section 3.3 Treatment Modifications and Immunization Stopping Rules.
- 3.6.1.2 Patient is taken off treatment by the Principal Investigator or Lead Associate Investigator for reasons other than toxicity e.g. insufficient recovery of dendritic cells to generate required vaccine dose, failure to adhere to scheduled study visits, etc.

### 3.6.2 Post Treatment Evaluation

This study is 144 Weeks in duration.

- 3.6.2.1 Clinical Evaluation: physical examination and assessment of performance status
- 3.6.2.2 Laboratory Evaluation: CBC with differential, platelets, PT/PTT, chemistries (Acute Care Panel, Mineral Panel, Hepatic Panel, total protein, CK, Uric acid, LDH), PSA.
- 3.6.2.3 Radiographic Evaluation: CXR, CT scan of the brain/chest/abdomen/pelvis, bone scan.
- 3.6.2.4 Immunologic Evaluation: CD4 and CD8 percent and absolute counts.

### 3.6.3 Off Study Criteria

- 3.6.3.1 Patient elects to withdraw from participation in the study at any time.
- 3.6.3.2 Patient is removed from study after resolution of Grade 3 or greater toxicities attributed as possibly, probably or definitely related to vaccine as described in Section 3.3 Treatment Modifications and Immunization Stopping Rules.
- 3.6.3.3 Patient develops evidence of brain, visceral or bony metastatic disease.
- 3.6.3.4 Patients with a calculated PSADT that has *decreased* by  $\geq 50\%$  compared to their Pre-Enrollment/Baseline PSADT. PSADT is calculated as specified in Section 3.1.
- 3.6.3.5 Patients with a calculated PSADT of  $\leq 3$  months at any of the outlined PSADT assessment time points i.e. weeks 12, 24, 36 or 48. PSADT is calculated as specified in Section 3.1.
- 3.6.3.6 For those patients receiving them, a discontinuation or dose reduction of their Flomax or 5-alpha reductase inhibitors.
- 3.6.3.7 Patient completed study

### 3.6.4 Off-Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off-study. An off-study form from the web site (<http://home.ccr.cancer.gov/intra/eligibility/welcome.htm>) main page must be completed and faxed to 301-480-0757.

## **4 SUPPORTIVE CARE**

We do not anticipate significant systemic or organ toxicities associated with TARP peptide vaccination using either Montanide® ISA 51 VG plus GM-CSF or peptide- pulsed dendritic cells. We have not observed any clinically significant toxicities in our previous trials investigating other class I peptides in patients with lung or colon cancer. Non-steroidal anti-inflammatory medication or other analgesics and possibly antihistamines may be needed for local injection site reactions to vaccination.

## **5 DATA COLLECTION AND EVALUATION**

### **5.1 DATA COLLECTION**

The Vaccine Branch clinical research team will perform real time data collection. The Principal Investigator will be responsible for oversight of the protocol.

All patients enrolled on study will be centrally registered at the NCI Central Registration Office. All on study evaluations must be performed within one week of their scheduled time point as outlined in the study schema ([Appendix 1](#)). Delay in vaccine administration is allowed for up to 3 weeks as outlined in Section [3.3.3](#). Modifications in this policy can be discussed on an individual basis with the Lead Associate Investigator. Research data will be collected in the NCI C3D database.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until satisfactory resolution. AEs should be reported up to 30 days following the last dose of study drug.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact

**End of study procedures:** Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

**Loss or destruction of data:** Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

### **5.2 RESPONSE CRITERIA**

#### **5.2.1 Immunologic Response Criteria**

Immunologic responses to TARP peptide vaccination will be assessed at weeks 0, 12 (after 3 vaccines), 18 (after 5 vaccines), 24, 36, 48, 60 and 96; Note: apheresis will be performed at weeks 0, 24, 48 and 96. Refer to [Appendix 3](#) for details of specimen tube, collection and

processing and to [Appendix 4](#) for descriptions of the immunology assays. Additional exploratory analyses of TARP immunologic responses will be performed at Weeks 60, 72, 96, 108, 120, 132 and 144.

A positive immunological response to class I TARP peptides is defined as:

- A three-fold increase over baseline in the number of positive cells by tetramer staining  
**OR**
- A three-fold increase over baseline in the number of positive cells by IFN- $\gamma$  ELISPOT assay.
- Positive assays must be confirmed at two study time points to be considered a definitive, vaccine-induced positive result.
- Week 24 immunologic *responders* will be allowed to receive an additional dose of TARP peptide vaccine at Week 36.
- The study time points at which a positive immunologic response is documented will be noted for every patient enrolled on the study.

Hence a patient will be considered a responder if they demonstrate a positive response as described above in either the tetramer or the IFN- $\gamma$  ELISPOT assays.  $^{51}\text{Cr}$  release cytotoxic T lymphocyte (CTL) assays using T2 cells as targets will only be performed in patients documented to have a positive tetramer or ELISPOT response and will not be utilized in the assessment of immunologic response.

A positive  $^{51}\text{Cr}$  release CTL assay will be defined as:

- The percent lysis observed against TARP peptides is at least 2 standard deviations greater than the percent lysis observed against control peptides AND
- Greater than or equal to a 10% difference in the TARPT peptide percent lysis vs. control peptide percent lysis at more than one effector to target cell ratio.

### 5.2.2 PSADT Response Criteria

PSADT was chosen as a surrogate marker for biochemical response to vaccination based on the Prostate-Specific Antigen Working Group's consensus Guidelines on PSADT.<sup>39</sup> Utilization of PSADT as a predictive factor of disease progression and prostate cancer death is based on level II evidence derived from cohort- or case-controlled analytic studies or controlled, non-randomized trials that have been validated in multiple data sets,<sup>40-42</sup> PSADT will be calculated using the Memorial Sloan-Kettering Cancer Center cancer information prostate nomogram for PSA doubling time found at:

<http://www.mskcc.org/mskcc/shared/forms/Nomograms/flash/load.cfm?type=Prostate&width=586&height=505&title=Prostate%20Nomogram> recommended in the Prostate Specific Antigen Working Group Guidelines. The PSADT is calculated assuming an exponential increase in serum PSA and first order kinetics. The formula takes into account the natural logarithm of 2 divided by the slope obtained from fitting a linear regression of the natural log of PSA on time. All PSA values used in the calculation should be 0.20 ng/ml or greater and follow an increasing trend. PSA values need not be consecutively increasing and all values obtained during a maximum period of 12 months prior to a given protocol time point will be included in the calculation. The maximum period of the last 12 months is recommended to reflect current disease activity because in some men PSADT may change over time. Multiple studies concur that men with a PSADT < 3 months are at extremely high risk for metastatic disease progression

and death with prostate cancer mortality approaching 100%; for this reason they have been excluded from participation in this study. In contrast, men with a slow PSADT i.e. > 15 months have an extremely low risk of death from prostate cancer.

The following general guidelines<sup>39</sup> regarding PSA determinations and calculation of PSADT used in this protocol will be adhered to (refer to [Appendix 5](#), PSADT Calculation Guidelines)

#### **Pre-Enrollment/Baseline and On Study PSADT:**

- PSADT will be calculated using the Memorial Sloan-Kettering Cancer Center cancer information prostate nomogram for PSA doubling time found at:  
<http://www.mskcc.org/mskcc/shared/forms/Nomograms/flash/load.cfm?type=Prostate&width=586&height=505&title=Prostate%20Nomogram>
- Minimum requirements for PSADT include  $\geq 3$  PSA measurements over  $\geq 3$  months.
- The interval between PSA measurements must be  $\geq 4$  weeks.
- For patients receiving 5-alpha reductase inhibitors (5ARI) e.g. finasteride or dutasteride, only PSA values obtained after at least 3 months on therapy may be used to calculate PSADT.
- Calculated PSADT values will be reported in months
- The same laboratory must have performed PSA values used in the calculation of PSADT.
- All PSA values used in the calculation should be  $\geq 0.20$  ng/mL and follow a rising trend although all values need not be consecutively rising.
- All values obtained over a maximum period of 12 months prior to any given protocol time point assessment should be included in the PSADT calculation to reflect the patient's current disease activity.
- PSA values obtained within  $\leq 2$  weeks of instrumentation, documented infection (prostatitis), or radiation proctopathy should be *omitted* from inclusion in PSADT calculations.

On Study PSADT will be calculated at baseline and weeks 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, and 144.

Exploratory comparisons of percent change in PSADT will be done among the following time points:

- Pre-Enrollment/Baseline PSADT vs. Week 3-12 PSADT/
- Pre-Enrollment/Baseline PSADT vs. Week 24 PSADT
- Pre-Enrollment/Baseline PSADT vs. Week 36 PSADT
- Pre-Enrollment/Baseline PSADT vs. Week 48 PSADT
- Week 24 PSADT vs. Week 36 PSADT
- Week 36 PSADT vs. Week 48 PSADT
- Additional exploratory analyses of PSADT will be performed of Pre-Enrollment/Baseline PSADT vs. Weeks 60, 72 and 96 and Week 48 PSADT vs. Weeks 96, 108 and 144 PSADT.

#### **5.2.2.1 PSADT Response & Failure**

**(assessed in all patients at weeks 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, and 144.)**

- PSADT *response* is defined as:
  - a  $\geq 50\%$  increase in calculated PSADT OR

- a PSADT > 15 months
- Patient's whose PSADT is *decreased* by  $\geq 50\%$  will be considered PSADT *failures*.
- Week 24 PSADT *responders* will be allowed to receive an additional dose of TARP peptide vaccine at Week 36.

### **5.3 TOXICITY CRITERIA**

This study will utilize NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0, published June 10, 2003, for toxicity and adverse event monitoring and reporting. (Refer to [http://ctep.info.nih.gov/reporting/ctc\\_v30.html](http://ctep.info.nih.gov/reporting/ctc_v30.html)). All clinical treatment areas will have access to a copy of CTCAE version 3.0.

## **6 STATISTICAL CONSIDERATIONS**

### **6.1 OVERALL STATEMENT OF OBJECTIVES**

The primary objective of this study, on which the sample size will be based is to determine the proportion of patients who will generate a specific immune response following vaccination as tested in tetramer, ELISPOT and cytotoxicity assay, and to evaluate if this is adequate for future consideration. Determination of safety of the vaccination is also an important endpoint. It is also of interest to assess risk of progression among patients with metastatic Stage D0 prostate cancer naïve to androgen deprivation therapy vaccinated with these peptides using PSA doubling time as a surrogate marker of this risk.

### **6.2 PRIMARY OBJECTIVE AND STATISTICAL JUSTIFICATION FOR SAMPLE SIZE**

This study will be conducted as a prospective, randomized two arm, pilot trial to explore the utility of TARP peptide vaccination alone or vaccination with TARP peptide-pulsed dendritic cells in patients with Stage D0 prostate cancer.

The primary objective is to obtain a reliable estimate of the immunologic response rate to vaccination with either peptide vaccine or dendritic cells in patients with biochemical progression and to determine if the rate of immunologic response in either arm is adequate to warrant further investigation in subsequent studies. Evaluation of safety and toxicity will also be a primary goal. The study is not designed to have adequate power to identify if one arm is associated with a significantly improved immunologic response rate as compared to the other, as such detection may require very large numbers of patients given the potential similarity of the arms. Furthermore, there is no requirement that only one arm be selected for further development. Should both arms demonstrate adequate immunologic responses, then future studies will consider either or both arms depending on immune responses, feasibility, toxicity and other outcomes such as PSADT.

Patients will initially be screened for HLA-A\*0201 positivity. HLA-A\*0201 patients will then undergo comprehensive protocol eligibility screening and if found to be eligible, will be enrolled in the study. Upon enrollment patients will be randomized in a 1:1 manner to receive TARP peptide vaccination with Montanide® ISA51 VG plus GM-CSF (Arm A) or TARP peptide-pulsed dendritic cells (Arm B).

For each arm, using an acceptable immunologic response rate of 40% ( $p_1=0.40$ ) and an unacceptable rate of 10% ( $P_0=0.10$ ) and using a two-stage optimal design (Simon R, *Controlled Clinical Trials* 10:1-10, 1989) with  $\alpha = 0.05$  and  $\beta = 0.10$ , we will initially accrue 9 patients in each arm. An unacceptable response rate of 10% (or less) and an acceptable response rate of 40% were determined based on historical clinical trial response rates to vaccines and immune-based therapies for cancer.<sup>4-7</sup> If 0-1 patients develop an immunologic response as defined in Section 5.2.1, then no further patients will be enrolled. If 2 or more of these 9 patients develop an immunologic response, we will accrue 11 additional patients for a maximum total of 20 patients per arm. If 2-4 of 20 has an immunologic response, then the results from this arm will be considered inadequate for further investigation. If 5 or more of these 20 has an immune response, we will be able to conclude that the vaccine on the given arm is worthy of further development in this population on the basis of immune response. Under the null hypothesis ( $p_0=0.20$ ), the probability of early termination is 77%.

There is no requirement that only one arm be selected for further development; should both arms demonstrate adequate immunologic responses, then future studies will consider either or both arms depending on immune responses, feasibility, toxicity and other outcomes such as PSADT.

As stated in Section 3.3.2, an early stopping rule will be used to ensure that the study subjects do not have exposure to excessive toxicity: If more than one of the first nine subjects on a given arm experiences dose limiting toxicity (defined in Section 3.3.1), enrollment will cease for that arm and revisions to the treatment will be discussed with the IRB and FDA. This is because 1/9 has an upper one-sided 90% confidence interval limit of 37%, while 2/9 has an upper one-sided 90% confidence interval limit of 49%; the former would be marginally acceptable as a rate of toxicity while the latter is not. In addition, any unusually severe toxicity noted may result in temporary suspension of accrual pending re-evaluation of the agent. All toxicities noted will be tabulated by type and grade, for each arm. Any such toxicities that appear in at least 5 patients on either arm will also have the distribution of grades of that toxicity compared between the arms using an exact Cochran-Armitage test.

The percent of patients who meet PSADT response criteria as outlined in section 5.2.2 will also be evaluated as a secondary endpoint. In addition, exploratory comparisons of PSADT among study time points will be performed as outlined in Sections 3.1 and 5.2.2. Given that there will be 6 separate comparisons of PSADT, we will only report differences with  $p < 0.01$  as being statistically significant while other differences with  $0.01 < p < 0.05$  will be considered to be trends. Appropriate 95% confidence intervals will be constructed and reported which takes into consideration the two-arm nature of the design. No formal comparison will be undertaken since the study does not have adequate power to address this comparison properly.

It is expected that 1-2 patients per month may be enrolled onto this study. Based on accrual of 18-20 patients per year, accrual should be completed in approximately 2 to 2.5 years if all 40 patients were to be required. To allow for the possibility of inevaluable patients, an accrual ceiling of 43 will be used.

## **7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN**

At each study visit, patients will be evaluated for new or evolving AEs. Symptoms will be elicited through the use of open-ended questions, followed by appropriate questions that clarify the patient's verbatim description of AEs or changes in concomitant medications. A review of systems will be performed.

The Principal Investigator or Lead Associate Investigator will assess whether the AE or SAE is associated with the administration of TARP peptide vaccine:

- Unrelated, Unlikely, Possible, Probably, Definite

### **7.1 DEFINITIONS**

#### **7.1.1 Adverse Event**

Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research

- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

#### **7.1.2 Suspected adverse reaction**

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

#### **7.1.3 Unexpected adverse reaction**

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

"Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

#### **7.1.4 Serious**

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

#### 7.1.5 Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

#### 7.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

#### 7.1.7 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

#### 7.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB-approved research protocol.

#### 7.1.9 Non-Compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

#### 7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to
  - (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and
  - (b) the characteristics of the subject population being studied; **AND**
- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a *greater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

## **7.2 NIH INTRAMURAL IRB AND CLINICAL DIRECTOR REPORTING**

### **7.2.1 NIH Intramural IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths**

The Protocol PI will report in the NIH Problem Form to the NIH Intramural IRB and NCI Clinical Director:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

### **7.2.2 NIH Intramural IRB Requirements for PI Reporting at Continuing Review**

The protocol PI will report to the NIH Intramural IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:
  - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
  - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
  - All Grade 5 events regardless of attribution;
  - All Serious Events regardless of attribution.

**NOTE:** Grade 1 events are not required to be reported.

### **7.2.3 NIH intramural IRB Reporting of IND Safety Reports**

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NIH Intramural IRB.

## **7.3 IND SPONSOR REPORTING CRITERIA**

An investigator must **immediately** report to the sponsor, using the mandatory MedWatch form 3500a, any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event.

- All Grade 5 (fatal) events (except death due to progressive disease) must be reported via email within 24 hours. A complete report must be submitted within one business day.
- All other serious adverse events including deaths due to progressive disease must be reported within one business day

Study endpoints that are serious adverse events (e.g. all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the drug and the event (e.g. death from anaphylaxis). In that case, the investigator must immediately report the death to the sponsor.

Events will be submitted to Center for Cancer Research (CCR) at: [CCRsafety@mail.nih.gov](mailto:CCRsafety@mail.nih.gov) and to the CCR PI and study coordinator.

## **7.4 DATA AND SAFETY MONITORING PLAN**

### **7.4.1 Principal Investigator/Research Team**

The Principal Investigator and Lead Associate Investigator will monitor this pilot, prospective randomized study, as required by the FDA as part of the Sponsor Investigator IND held by the Principal Investigator. Based on the minimal risk and the limited number of subjects to be studied, monitoring by a Data Safety Monitoring Board is not warranted for this trial. There will be continuous close monitoring of protocol-related toxicity by the Lead Associate Investigator and Principal Investigator. The study research nurse coordinator and/or data manager will generate a bi-weekly (or weekly) reports of all on-study laboratory data with correspondent toxicity grading according to CTCAE v3.0 as well as a summary of all clinical and Vaccine Report Card (VRC) surveillance adverse event data for review by the Principal Investigator and The Lead Associate Investigator in conjunction with Associate Investigators. In addition, study summaries will be generated every 6 months and forwarded to the FDA. Adverse events will be reported to the IRB as described in section 7.2. Decisions regarding revision of the protocol or consent will be promptly communicated to the IRB and FDA.

### **7.4.2 Sponsor Monitoring Plan**

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary study endpoints; adherence to the protocol, regulations, and SOPs; and human subjects protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring
- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by an NCI contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

## **8 HUMAN SUBJECTS PROTECTION**

### **8.1 RATIONALE FOR SUBJECT SELECTION**

All subjects enrolled in this study will be male as the disease under study does not affect women or children. We will make every effort to accrue patients from all racial and ethnic groups. Since our referral population is the nation, we should obtain referrals representative of the national composition of races. In addition, HLA-A\*0201 positivity is not restricted to a particular racial or ethnic group: it is present in 49.54% of Caucasians, 33.74% of African-Americans, and 55.87% of Orientals. Hence the requirement for HLA-A\*0201 positivity should not preclude our ability to accrue a racially and ethnically diverse population representative of individuals affected by prostate cancer.<sup>15</sup>

Participants will be accrued through web-based advertisements, community-based contacts and referrals of patients from other studies at the Clinical Center. This protocol will also be advertised on the NCI PDQ Clinical Trials database as well as through ClinicalTrials.gov.

### **8.2 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS**

The benefit of vaccination with peptides is unknown. Although several patients in our previous Phase I peptide vaccine studies mounted immune responses to various peptides, the benefit or clinical significance of these immune responses remains unknown. However, in animal models, immune responses have been correlated with both a decrease in measurable disease and a subsequent resistance to tumor challenge (Ref). In this study, we will determine the impact of vaccination on PSADT, a well established surrogate marker that is a predictive factor of longer time to metastases, prostate-cancer death and all-cause adverse clinical outcomes.<sup>30-33</sup> The risks of vaccination are also unknown. However, no severe or systemic toxicity in response to vaccination with peptides alone has been observed and the most frequent adverse event is local injection site reactions, an observation common to all vaccines.

### **8.3 CONSENT AND ASSENT PROCESSES AND DOCUMENTATION**

Consent forms are attached. Two consent forms will be used for this study. Participants will initially agree to allow their blood to be tested for HLA-A\*0201 positivity. The process for obtaining patients' consent for HLA typing may involve telephone contact only. The study will be explained to the prospective patient and any questions answered. The consent form will be signed and witnessed and returned with the blood sample for HLA typing to the NIH by FEDEX. If the patient is HLA-A\*0201 positive they will be invited for a screening visit at the NCI to be evaluated for protocol eligibility. Following eligibility screening, each patient will be discussed with the Principal Investigator or the Lead Associate Investigator. Eligible patients will be presented with a detailed description of the study protocol plan and treatment. The specific requirements, objectives, risks, alternatives, time commitments and potential benefits will be reviewed with the patient. A copy of the Informed Consent will be provided to the patient for their review. The patient will be reassured that participation in this study is entirely voluntary and that they may withdraw or decide against receipt of vaccination at any time without adverse consequences. The Principle Investigator, Lead Associate Investigator or their designee will summarize the screening results and answer any questions prior to obtaining Informed Consent.

## 9 PHARMACEUTICAL INFORMATION

### 9.1 SARGRAMOSTIM

#### 9.1.1 Product Description

The GM-CSF to be used in this study is glycosylated, recombinant human Granulocyte-Macrophage Colony Stimulating Factor (rhu GM-CSF) (brand name Leukine®). This rhu GM-CSF is an altered form of the native molecule: the position 23 arginine has been replaced with a leucine to facilitate expression of the protein in yeast (*Saccharomyces cerevisiae*) and the carbohydrate moiety may be different from the native protein. The Clinical Center Pharmacy will purchase commercially available sargramostim. Rhu GM-CSF will be used both as an ancillary product for the maturation of dendritic cells and for administration to patients with peptide vaccination.

#### 9.1.2 Formulation and Preparations

- A.)** Lyophilized LEUKINE is a sterile, white, preservative-free powder (250 mcg) that requires reconstitution with 1 mL Sterile Water for Injection, USP or 1 mL Bacteriostatic Water for Injection, USP. Reconstituted lyophilized LEUKINE is a clear, colorless liquid suitable for subcutaneous injection or intravenous infusion. The vial of lyophilized LEUKINE contains 250 mcg ( $1.4 \times 10^6$  IU/vial) sargramostim, 40 mg/mL mannitol, USP; 10 mg/mL sucrose, NF; and 1.2 mg/mL tromethamine, USP, as excipients. Biological potency is expressed in International Units (IU) as tested against the WHO First International Reference Standard. The specific activity of LEUKINE is approximately  $5.6 \times 10^6$  IU/mg.
- B.)** Sargramostim solution is a sterile, preserved (1.1% benzyl alcohol) injectable 500 mcg/ml solution.

#### 9.1.3 Stability and Storage

- A.)** **Lyophilized** LEUKINE should be refrigerated at 2 to 8° C (36 to 46° F) and is stable for at least eighteen months. It should not be frozen, shaken or used beyond the expiration date printed on the vial. LEUKINE reconstituted with 1.0 ml of Sterile Water for Injection, USP (without preservative) should be administered as soon as possible and within 6 hours following reconstitution. Reconstituted solutions prepared with Bacteriostatic Water for Injection USP (0.9% benzyl alcohol) may be stored for up to 20 days at 2 to 8° C prior to use. The contents of vials reconstituted with different diluents should not be mixed together.
- B.)** **Sargramostim solution: store intact vials in the refrigerator (2°C-8°C). Do not freeze or shake the vial.**

#### 9.1.4 Administration Procedures

- A.) Lyophilized LEUKINE will be used *in vitro* by the Department of Transfusion Medicine for the generation of dendritic cells by the Department of Transfusion Medicine.
- B.) Sargramostim solution (110 mcg or 0.22ml) will be given with Montanide® ISA 51 with TARP peptides as described in Sections 3.2 Vaccine Administration and Sections 9.3.4 and 9.4.4.

#### 9.1.5 Incompatibilities:

Refer to the package insert/PDR/Formulary.

### **9.2 INTERLEUKIN-4 CELLGENIX**

#### 9.2.1 Product Description

Interleukin-4 (IL-4) used in this study is investigational. It is manufactured and supplied by CellGenix (Master File cross reference BB-MF 11269). It will be used as an ancillary product to mature dendritic cells *in vitro* and will not be administered directly to patients. IL-4 exerts important effects on B cells, T cells, macrophages, eosinophils, hematopoietic progenitor cells, endothelial cells and promotes the maturation of dendritic cells. The complementary DNA clone (cDNA), when expressed in E.coli yields a 129 amino acid protein with a molecular weight of 14,957 daltons. IL-4 is a highly purified ( $\geq 95\%$  chromatographically pure), sterile, water-soluble protein.

#### 9.2.2 Formulation and Preparation

RhIL-4 Sterile Powder for Injection is supplied in 100 mcg and 200 mcg vials (containing a total of 120mcg and 240mcg of IL-4, respectively) as a sterile lyophilized powder formulated with glycine, human serum albumin, citric acid, and sodium citrate. Un-reconstituted IL-4 should be kept refrigerated at 2-8°C. 1.2 mL of Sterile Water for Injection USP should be added to each vial of rhIL-4 Sterile Powder for Injection. The vial should be gently agitated to completely dissolve the powder and should be inspected visually for discoloration and particulates prior to use.

#### 9.2.3 Stability and Storage

The reconstituted product should be refrigerated at 2-8°C and should be used within 24 hours.

#### 9.2.4 Administration Procedures

To be used in dendritic cell culture, not administered directly to patients.

#### 9.2.5 Incompatibilities:

None known in culture.

### **9.3 TARP 29-37-9V PEPTIDE (EPIPOPE-ENHANCED) NSC #740704**

#### 9.3.1 Product Description

TARP 29-37-9V is investigational. TARP 29-37-9V is a synthetic HLA-A2-restricted 9-amino acid epitope of the tumor associated protein TARP, with a single amino acid substitution (valine at position 37, instead of leucine) to increase its binding affinity and immunogenicity. Amino acid sequence: Phenylalanine-Leucine-Arginine-Asparagine-Phenylalanine-Serine-Leucine-Methionine-Valine (FLRNFSLMV)

Molecular Weight: 1126.4

#### 9.3.2 Formulation and Preparation

The peptide is manufactured by NeoMPS, Inc., 9395 Cabot Drive, San Diego, CA 92126. The peptide is vialled by the Clinical Center Pharmacy as a 5 mL siliconized sterile amber type 1 glass vial with a Teflon-lined stopper containing 0.5 mL of a sterile clear solution. Each mL

contains 2.2 mg of TARP:29-37(37V) Peptide and 0.5 mL of trifluoroacetate 0.05% v/v.

### 9.3.3 Stability and Storage

Store the finished injectable dosage forms in the freezer (-70°C) for long-term storage. Intact vials are stable for at least 6 months when stored at controlled room temperature (15°C – 30°C), at least 9 months when stored in the refrigerator (2°C – 8°C), and for at least 36 months when stored in the freezer (-10°C to -25°C and -70°C). The peptide vial contains no preservatives; once the peptide vial is entered, discard unused peptide solution after 3 hours. Vaccine emulsions consisting of peptide admixed with Montanide® ISA 51 VG are stable for up to 3 hours, but should be administered as soon as possible following preparation.

### 9.3.4 Administration Procedures

Preparation and administration of Epitope-Enhanced TARP with Montanide® ISA 51 VG and sargramostim: Each vial of TARP 29-37-9V peptide is in aqueous solution containing 0.5ml of peptide at a concentration of 2.2 mg/ml, for a total of 1.1mg peptide per vial. Sargramostim solution, 110 micrograms, (0.22 mL) and 0.1 mL of 10x saline will be added to the vial of peptide for a total aqueous peptide volume of 0.82 ml. Prepare the peptide emulsion by injecting the 0.82ml aqueous TARP 29-37-9V peptide solution into 0.8 mL of Montanide® ISA 51 VG as outlined in Section 9.6.6 Syringe Method of Emulsification. The total volume of peptide emulsion will be 1.62 ml. Withdraw a total of 1.5 mL (~1.0 mg of peptide) for administration by deep subcutaneous injection in one or two equal volumes into the anterior thigh.

Administration: Injections will preferentially be administered in the anterior thigh. The upper arm may be used if necessary. The same extremity will be used for each dose of peptide administered on any given day but the extremities may be rotated.

### 9.3.5 Incompatibilities

None known.

### 9.3.6 Reported Adverse Events and Potential Risks

Common adverse events reported with peptides emulsified with Montanide® ISA 51 VG have consisted of fatigue, fever, flushing, injection site reactions including pain, erythema and induration; localized swelling, rare granuloma formation. Other reported possible adverse events include chills/rigors, transient increases in liver transaminases, headache, rash, and pruritis. TARP is a 58 amino acid protein found on cells of prostate origin and is over expressed in over 90% of patients with prostate cancer and about half of patients with breast cancer. Because the peptide mimics portions of a prostate protein found naturally in the body, there is a chance for development of an autoimmune reaction to it and may result in the possible development of inflammation in the prostate gland.

### 9.3.7 Special Handling

The peptide is NOT a cytotoxic or infectious agent and requires no special handling.

## 9.4 TARP 27-35 (WILD TYPE) NSC#740703

### 9.4.1 Product Description

TARP 27-35 is a synthetic HLA-A2-restricted 9-amino acid epitope of the tumor-associated protein TARP.

Amino acid sequence: Phenylalanine-Valine-Phenylalanine-Leucine-Arginine-Asparagine-Phenylalanine-Serine-Leucine (FVFLRNFSL)

Molecular Weight: 1142.4

#### 9.4.2 Formulation and Preparation

The peptide is manufactured by NeoMPS, Inc., 9395 Cabot Drive, San Diego, CA 92126. The peptide is vialled by the Clinical Center Pharmacy as a 5 mL siliconized sterile amber molded glass vial containing a sterile white lyophilized powder. Each vial contains 1.1 mg of TARP:27-35 peptide and Mannitol.

#### 9.4.3 Stability and Storage

Store the finished injectable dosage forms in the freezer (-70°C) for long-term storage. Intact vials are stable for at least 6 months when stored at controlled room temperature (15°C – 30°C) or in the refrigerator (2°C – 8°C), and for at least 36 months when stored in the freezer (-10°C to -25°C and -70°C). The peptide vial contains no preservatives; once the peptide vial is entered, discard unused peptide solution after 3 hours. Vaccine emulsions consisting of peptide admixed with Montanide® ISA 51 are stable for up to 3 hours, but should be administered as soon as possible following preparation.

#### 9.4.4 Administration Procedures

Preparation and administration of WT TARP with Montanide® ISA 51 VG and sargramostim: Each vial of WT TARP 27-35 peptide contains 1.1 mg as a lyophilized powder. It is reconstituted in 0.5 mL of Sterile Water for Injection, USP to yield a final concentration of 2.2 mg/mL. Sargramostim solution, 110 micrograms, (0.22 mL) and 0.1 mL of 10x saline will be added to the vial of peptide for a total aqueous peptide volume of 0.82 mL. Prepare the peptide emulsion by injecting the 0.82 mL aqueous WT TARP 27-35 peptide solution into 0.8 mL of Montanide® ISA 51 as outlined in Section 9.6.6 Syringe Method of Emulsification. The total volume of peptide emulsion will be 1.62 mL. Withdraw a total of 1.5 mL (~1.0 mg of peptide) for administration by deep subcutaneous injection in one or two equal volumes into the anterior thigh.

Administration: Injections will preferentially be administered in the anterior thigh. The upper arm may be used if necessary. The same extremity will be used for each dose of peptide administered on any given day but the extremities may be rotated.

#### 9.4.5 Incompatibilities

None known.

#### 9.4.6 Reported Adverse Events and Potential Risks

Common adverse events reported with peptides emulsified with Montanide® ISA 51 VG have consisted of fatigue, fever, flushing, injection site reactions including pain, erythema and induration; localized swelling, rare granuloma formation. Other reported possible adverse events include chills/rigors, transient increases in liver transaminases, headache, rash, and pruritis. TARP is a 58 amino acid protein found on cells of prostate origin and is over expressed in over 90% of patients with prostate cancer and about half of patients with breast cancer. Because the peptide mimics portions of a prostate protein found naturally in the body, there is a chance for development of an autoimmune reaction to it and may result in the possible development of inflammation in the prostate gland.

#### 9.4.7 Special Handling

The peptide is NOT a cytotoxic or infectious agent and requires no special handling.

### 9.5 INFLUENZA VIRUS VACCINE LIVE INTRANASAL

#### 9.5.1 Product Description

The influenza virus vaccine live intranasal (FluMist™) is a live trivalent nasally administered vaccine intended for active immunization for the prevention of influenza. The product is manufactured by MedImmune and will be purchased by the Clinical Center Pharmacy. The previous season's vaccine might be used until the supply for the current influenza season becomes available.

#### 9.5.2 How Supplied

This vaccine contains live attenuated, cold adapted virus from both influenza A and B. Each 0.2 mL dose is formulated to contain  $10^{7.0 \pm 0.5}$  TCID<sub>50</sub> (median tissue culture infectious dose) of live attenuated influenza virus reassortants of the strains recommended by the U.S. Public Health Service (USPHS).

#### 9.5.3 Stability and Storage

Influenza virus vaccine live intranasal can be stored for extended periods at 2-8 °C. Influenza virus vaccine live intranasal is a clear to slightly cloudy, colorless to pale yellow liquid; some proteinaceous particulates may be present. The thawed vaccine should not be refrozen.

#### 9.5.4 Administration Procedures

Patients should receive 0.2 ml intranasally of influenza virus vaccine (0.1ml in each nostril) during vaccination. Under no circumstances should the vaccine be administered parenterally. If the vaccinee sneezes after administration of the intranasal vaccine, the dose should *not* be repeated. Influenza virus vaccine live intranasal is contraindicated in patients with known hypersensitivity to any component of the vaccine, including egg or egg products, individuals with a history of Guillan-Barre Syndrome, a known or suspected immunodeficiency disease/condition and individuals with asthma or reactive airway disease or other chronic pulmonary, cardiovascular or metabolic conditions. Vaccine administration should be postponed for a least 72 hours after the acute phase of a febrile and /or respiratory illness.

#### 9.5.5 Special Precautions

Because live attenuated influenza viruses replicate in the nasopharynx of recipients of influenza virus vaccine live intranasal and can be shed in respiratory secretions, the possibility exists that the vaccine viruses can be transmitted to susceptible individuals who have not received the vaccine. The manufacturer states that individuals receiving influenza virus vaccine live intranasal should avoid close contact with immunocompromised individuals for at least 21 days after vaccination. To minimize risk of transmission of attenuated vaccine virus to other Clinical Center patients the following procedures will be adhered to:

- influenza virus vaccine live intranasal will be administered immediately prior to the patient's departure from the Clinical Center.
- The patient will be asked to wear a mask in the process of exiting the building and will be immediately discharged to home.

- Transportation by private taxi to the airport will be authorized to eliminate exposure to other CC patients traveling on the NIH shuttle.
- Patients will be provided with a hand hygiene product as well as tissues.
- Patients will not be authorized to return to the CC until at least 21 days following receipt of their vaccine.
- While away from the CC they will be advised to avoid contact with severely immunosuppressed patients for 7 days after vaccination.

## **9.6 MONTANIDE® ISA 51 VG (NSC 737063) CLASSIFICATION: ADJUVANT**

### 9.6.1 Product Description

Montanide® ISA 51 VG (vegetable-grade) is an oil-based adjuvant composed of a highly refined mannide monooleate (Montanide 80), a surfactant, in a mineral oil solution (Drakeol 6VR). The mannide monooleate is synthesized from raw materials of vegetable origin. Montanide® ISA 51 VG, when mixed with an equal part of an aqueous solution, is designed to form an injectable water-in-oil emulsion. Comparative analysis of the chemical compositions of the previous animal-grade formulation (Montanide® ISA 51) and current vegetable-grade formulation (Montanide® ISA 51 VG) oleic acid sources shows a final product with similar chemical and applicative properties (e.g., emulsion stability and viscosity).

### 9.6.2 How Supplied

Montanide® ISA 51 VG is supplied in 5 mL amber glass vials containing 3 mL of a sterile, clear yellow, oil solution. The agent is manufactured by Seppic, Inc. and will be supplied through the Pharmaceutical Development Service, Clinical Center Pharmacy Department.

### 9.6.3 Storage

Store intact vials at controlled room temperature.

### 9.6.4 Stability

Montanide® ISA 51 VG is stable at room temperature until the expiration date printed on the agent label. The solution contains no preservatives; once the vial is entered, discard any remaining solution after 3 hours. Withdraw and administer peptide vaccine emulsions immediately after preparation.

### 9.6.5 Toxicity

Toxicities related to Montanide® ISA 51 VG have included erythema at the injection site, rare granuloma formation and low-grade fever.

### 9.6.6 Peptide Vaccine Preparation

To prepare a water-in-oil emulsion vaccine, consisting of the peptide (aqueous phase) and the adjuvant Montanide® ISA 51 VG (oil phase), combine equal parts of the peptide and the adjuvant and subject the mixture to high shear forces using the following method:

#### ***Syringe Method of Emulsification (Investigator's Preference): Arm A Patients 101-110***

For preparation of emulsions of water-soluble peptide antigens in an adjuvant oil phase for immunization a modification of the "double hubbed needle method" will be employed. In this method two sterile 2.5 ml glass Hamilton syringes (TLL (PTFE Luer Lock) w/o slots #81420, Hamilton Co., Reno, Nev) with Teflon plungers and male Luer Lock tip connectors are used.

The materials are kept sterile at all times. The adjuvant, Montanide® ISA 51 VG (0.8 ml) is drawn up completely in one syringe using a sterile 23 gauge needle. The needle is removed and any air bubbles displaced. A sterile double ended female Teflon connector (3/4 inch, 24 gauge, #86510, Hamilton Co., Reno, Nev) is attached to the adjuvant syringe and the plunger is pushed carefully to allow the adjuvant to fill the connector space. The peptide aqueous solution (0.82 ml total) is then drawn up into the other glass syringe with a sterile 23 gauge needle. The needle is discarded and any air bubbles displaced. The syringe containing the aqueous peptide solution is securely attached to the free female end of the Teflon connector.

**It is very important that the first pass be aqueous peptide antigen into oil adjuvant to produce the correct water-in oil-emulsion.** A water-in-oil emulsion is obtained by briskly forcing the aqueous peptide solution through the connector into the adjuvant and passing the material back and forth between the two syringes 40-50 times. This is best achieved by holding the connector between thumb and forefinger of one hand with the syringe held securely in the palm of the hand and the other syringe held securely in the palm of the other hand and firmly pressing the plunger of the syringe on a non-slippery surface or blue diaper. The material is passed back and forth between the two syringes by a rocking motion in which the plungers are alternately pushed in and out by forced pressure of the plunger on the bench top. As the emulsion forms, increased resistance should be noted, the emulsion should appear homogeneous (opaque white) with no trapped air bubbles. Following emulsification, almost the entire emulsion (1.5 ml) should be drawn up in one syringe, detached from the Teflon connector assembly and a sterile 23 gauge needle attached to the syringe. Gently push the emulsion into the needle until a small drop is visible at the tip. The peptide-adjuvant emulsion is now ready for immunization.

***Modified Syringe Method of Emulsification: Arm A Patients 111-120***

For preparation of emulsions of water-soluble peptide antigens in an adjuvant oil phase for immunization, a modification of the "double hubbed needle method" will be employed. In this method two sterile 3 ml Norm-Ject plastic Luer Lock tip syringes (manufactured by Henke Sass Wolf in Tuttlingen, Germany) are used. The materials are kept sterile at all times. The adjuvant, Montanide® ISA 51 VG (0.8 ml) is drawn up completely in one syringe using a sterile 23 gauge needle. The needle is removed and discarded and any air bubbles displaced. A sterile Discifix, 3-way stopcock (manufactured by B. Bruan Medical, Inc., Bethlehem, PA) is attached to the adjuvant syringe and the plunger is pushed carefully to allow the adjuvant to fill the connector space. The peptide aqueous solution (0.82 ml total) is then drawn up into the other plastic syringe with a sterile 23 gauge needle. The needle is discarded and any air bubbles displaced. The syringe containing the aqueous peptide solution is securely attached to the opposite end of the 3-way stopcock.

**It is very important that the first pass be aqueous peptide antigen into oil adjuvant to produce the correct water-in oil-emulsion. Peptide will then be emulsified as follows:**

- 20 "pre-emulsion cycles" will be performed (a cycle is defined as one pass back and forth between the two syringes). The pre-emulsion cycles are to be performed *slowly*, such that 20 cycles are performed over approximately 1 minute. *The pre-emulsion cycles are a necessary and important step in the quality of the final emulsion.*
- Following the pre-emulsion cycles, 100 additional cycles will be performed such that the additional 100 cycles are performed over approximately 1 minute.

- Withdraw a total of 1.5 ml (~1.0mg of peptide) for administration into one of the syringes, disconnect from the 3-way stopcock and cap the syringe.
- Label the syringe with the CRIS label (a “butterfly” label may be used).

#### 9.6.7 Administration Procedures

Divide peptide vaccine emulsion dose volumes of greater than 1.5 mL into two or more injections. Administer peptide vaccine emulsions prepared with Montanide® ISA 51 VG by deep subcutaneous injection using a 23 gauge needle. Injections will be preferentially given into the anterior thigh but the upper arm may be used if necessary. The same extremity will be used for each dose of peptide administered on any given day but the extremities may be rotated.

### 9.7 KLH (KEYHOLE LIMPET HEMOCYANIN)

#### 9.7.1 Product Description

Stellar Biotechnology’s KLH is a potent form of clinical grade KLH that is manufactured by Sigma-Aldrich. It is purified from the hemocyanin of the giant keyhole limpet, *Megathura crenulata*. The denatured subunit of KLH is a glycoprotein with a molecular weight of 400-450,000 daltons. The native form of KLH is a dodecamer, which consists of twenty (20) subunits of KLH with a molecular weight of 6-9000.000 daltons. In the hemocyanin, at least 50% of the KLH exists as a dodecamer and the remainder can be found as dodecamer aggregates. Stellar Biotechnology’s KLH is purified as native molecules with high molecular weight and designated as KLH-HMW.

#### 9.7.2 Formulation and Preparation

Stellar Biotechnology’s KLH is provided in soluble form in a buffer solution that is composed of 10mM sodium phosphate, 135mM NaCl, 1mM CaCl<sub>2</sub> and 0.5mM MgCl<sub>2</sub>. It is provided by the manufacturer in 600mg containers at 5mg/mL. It has been received by the Clinical Center Pharmacy Department and re-vialed into single use vials at 2mg/mL, 250 µL/vial.

#### 9.7.3 Drug Procurement

Stellar Biotechnology’s KLH will be purchased from Stellar Biotechnology and then vialled by Clinical Center Pharmacy Development Service (PDS). It will be dispensed by PDS to DTM for use in dendritic cell culture.

#### 9.7.4 Stability and Storage

KLH-HMW is stable for at least 12 months when stored at 2 to 8°C.

#### 9.7.5 Administration Procedures

KLH-HMW will be used in vitro by the Department of Transfusion Medicine at a concentration of 10mcg/mL for the generation of dendritic cells. Cells will be extensively washed before administration.

#### 9.7.6 Incompatibilities

Refer to the package insert.

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**Appendix 1 Schedule of Study Clinical, Laboratory and Radiographic Evaluations**

Schedule study Events Weeks 0-48												
	Screen D-14 to D-1	Wk 0 D1	Wk 0 D3 or D5	Wk 3	Wk 6	Wk 9	Wk 12	Wk 15	Wk 18	Wk 24	Wk 36	Wk48
HLA-A*0201 Testing Prior to Screening												
Informed Consent	X											
Medical History & ROS	X			X	X	X	X	X	X	X	X	X
Physical Examination	X			X	X	X	X	X	X	X	X	X
Performance Status	X			X	X	X	X	X	X	X	X	X
Height	X			X	X	X	X	X	X	X	X	X
Weight	X			X	X	X	X	X	X	X	X	X
Vital Signs	X			X	X	X	X	X	X	X	X	X
Apheresis			X							X		X
FluMist™ Flu Vaccine			X									
TARP Peptide Vaccine				X	X	X	X	X			X*	X
Vaccine Report Card				X	X	X	X	X			X*	X
CBC/differential, plts	X			X	X	X	X	X	X	X	X	X
PT / PTT	X											
Acute Panel	X			X	X	X	X	X	X	X	X	X
Hepatic Panel	X			X	X	X	X	X	X	X	X	X
Mineral Panel	X			X	X	X	X	X	X	X	X	X
Lipid Panel	X											X
Amylase / Lipase	X											X
Vitamin D, 25 hydroxy	X											
PSA	X			X	X	X	X	X	X	X	X	X
PSADT Calculation	X						X			X	X	X
Serum Testosterone	X			X	X	X	X	X	X	X	X	X
Urinalysis	X						X			X		X
HIV Serology	X											
Hepatitis B Serology	X											
Hepatitis C Serology	X											
HTLV-1 Serology	X											
VDRL	X											
EKG	X											
CT Scan Chest/Abd/Pelvis	X											X
Bone Scan	X											X
Lymphocyte Subsets	X						X			X		X
Anti-TARP Antibody		X					X		X	X	X	X
NKT Cell Assessment		X					X		X	X		
Tetramer Staining		X					X		X	X	X	X
IFN-γ ELISPOT		X					X		X	X	X	X
<sup>51</sup> Cr CTL Assay#		X					X		X	X	X	X
PBMCs for Storage		X		X			X		X	X	X	X
Serum for Storage		X					X		X	X	X	X

Schedule study Events Weeks 60-144									
	Wk 60	Wk 72	Wk 84	Wk 96	Wk 108	Wk 120	Wk 132	Wk 144	
HLA-A*0201 Testing Prior to Screening									
Informed Consent									
Medical History & ROS	X	X	X	X	X	X	X	X	X

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Physical Examination	X	X	X	X	X	X	X	X
Performance Status	X	X	X	X	X	X	X	X
Height	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X
Apheresis								
FluMist™ Flu Vaccine								
TARP Peptide Vaccine				X				
Vaccine Report Card				X				
CBC/differential, plts	X	X	X	X	X	X	X	X
PT / PTT								
Acute Panel	X	X	X	X	X	X	X	X
Hepatic Panel	X	X	X	X	X	X	X	X
Mineral Panel	X	X	X	X	X	X	X	X
Lipid Panel				X				X
Amylase / Lipase				X				X
Vitamin D, 25 hydroxy								
PSA	X	X	X	X	X	X	X	X
PSADT Calculation	X	X	X	X	X	X	X	X
Serum Testosterone	X	X	X	X	X	X	X	X
Urinalysis				X				X
HIV Serology								
Hepatitis B Serology								
Hepatitis C Serology								
HTLV-1 Serology								
VDRL								
EKG								
CT Scan /Chest/Abd/Pelvis				X				X
Bone Scan				X				X
Lymphocyte Subsets	X			X				X
Anti-TARP Antibody	X			X				X
NKT Cell Assessment								
Tetramer Staining	X	X	X	X	X	X		X
IFN-γ ELISPOT	X	X	X	X	X	X		X
<sup>51</sup> Cr CTL Assay#	X	X	X	X	X	X		X
PBMCs for Storage	X	X	X	X	X	X		X
Serum for Storage	X	X	X	X	X	X		X

Notes:

- Screening CT scans of the Chest/Abd/Pelvis and Bone Scans may be performed up to 8 weeks prior to enrollment and at outside institutions.
- All on study assessments can be obtained within +/- one week of scheduled measurement to allow for holidays and unscheduled delays.
- Screening for HLA-A\*0201 positivity will be completed before protocol eligibility screening is initiated. Only HLA-A\*0201 positive patients are brought to NIH for further screening and eligibility assessment.
- FluMist™ vaccination will be given with the seasonal influenza preparation when it is available at the time of enrollment.
- X\*: a 6<sup>th</sup> dose of TARP peptide vaccine will be administered to those patients with a ≥ 50% increase in PSADT at week 24 or who have confirmed immunologic responses to vaccination by tetramer or IFN-g ELISPOT assays as outlined in Section 5.2.1 Immunologic Response Criteria. A booster dose of TARP peptide vaccine will be administered to all patients at Week 48 and 96 who elect to continue on the extension phase of the study through Week 144..
- #: <sup>51</sup>Cr CTL Assay will be performed at the time points indicated only in those patients with evidence of immunologic reactivity by tetramer staining and/or IFN-γ ELISPOT assays.

## **Appendix 2 Standard Operating Procedure for Dendritic Cell Vaccine Preparation**

### *Autologous Cell Harvest*

Blood collection shall be by standard lymphapheresis. 8 to 15 liters of whole blood will be processed in order to collect peripheral blood mononuclear cells (MNC) with a target number of at least  $2.2 \times 10^9$  monocytes. Lymphocytes will also be cryopreserved. Apheresis will be performed in the Clinical Center (CC) Department of Transfusion Medicine (DTM) using approved standard operating procedures. Bilateral peripheral venous access will be used for apheresis whenever possible. Alternatively, a temporary femoral central venous catheter (CVL) will be placed for collection. If this is necessary, the patient will be admitted to the hospital for CVL placement prior to starting apheresis. The CVL will be inserted by appropriately trained personnel (e.g., Critical Care, Interventional Radiology, General Surgery) with the assistance of anesthesiology as indicated. The patient will remain hospitalized until the CVL is removed. Prophylactic intravenous CaCl<sub>2</sub> and MgSO<sub>4</sub> infusions may be administered during apheresis to treat or prevent citrate toxicity at the discretion of the DTM physician per routine.

### **Patient Cell Processing**

All cell processing will be conducted in accordance with approved DTM policies and procedures.

### **TARP Peptide-Pulsed Dendritic Cells**

**Background:** Autologous dendritic cells prepared from peripheral blood monocytes will be loaded with 2 different TARP-derived peptides. These peptides are made of amino acids 27-35 and 29-37, namely TARP 27-35 and TARP 29-37-9V, comprised of HLA-A2-restricted epitopes that are involved in the cytolytic T cell response. TARP 29-37-9V contains a substitution that has been shown to enhance its binding affinity. Different fractions of autologous dendritic cells will be pulsed individually with only one of these peptides and the two fractions will be combined before administration to the patient.

**Formulation and Preparation:** Autologous peptide-pulsed dendritic cell vaccines will be prepared under GMP conditions from cryopreserved patient monocytes. Autologous monocytes for dendritic cell culture will be enriched from peripheral blood MNC apheresis collections by counter-flow elutriation, aliquoted into at least 6 vials with  $\sim 333 \times 10^6$  cells/vial and cryopreserved for future preparation of the dendritic cell products. After thaw, the monocytes will be placed into a 5 day culture with rhIL-4 and rhGM-CSF to generate immature dendritic cells, followed by pulse with KLH and maturation with LPS and IFN- $\gamma$ , and pulsed with TARP peptide. After removing peptide-pulsing media, dendritic cells will be concentrated down at  $40 \times 10^6$  cells/ml in infusion media (Plasma-Lyte A containing 10% autologous heat inactivated plasma). The final peptide-loaded, volume-reduced mature dendritic cell product will be prepared in sterile syringes for fresh administration intradermally. A validated manufacturing process described in the Department of Transfusion Medicine, Clinical Center, NIH standard operating procedures will prepare the dendritic cell vaccine product. Detailed standard operating procedures for processing, labeling, storage, and quality assays are available on site in the Cell Processing Section of the Department of Transfusion Medicine.

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***Stability and Storage:*** Autologous peptide-pulsed dendritic cell vaccines will be harvested from the 5-day culture product and packaged for fresh administration on the same day according to Standard Operating Procedures of the Department of Transfusion Medicine. A fixed autologous peptide-pulsed dendritic cell vaccine dose of  $20 \times 10^6$  cells/ in 0.25ml or 0.5ml will be administered immediately upon receipt in the clinical setting. Post packaging tests indicated that the product was stable for at least 2hours.

### **Appendix 3 Handling and Processing of Research Specimens**

The majority of the testing on this study will be done in the NIH Clinical Center clinical laboratory following their guidelines for blood collection and tube type. The appropriate tube for uncommon laboratory tests and immunologic research specimens and where they should be sent are as follows:

**HLA Typing:** 1 Yellow Top to HLA Laboratory, Department of Transfusion Medicine

**FACS Analysis for Lymphocyte Subsets:** 1 Purple Top to the Clinical Laboratory for lymphocyte subsets

**NOTE:** FACS of dendritic cells utilized for TARP peptide-pulsed dendritic cell vaccine preparations will be done in the cell processing section of the blood bank.

#### **Tetramer & IFN- $\gamma$ ELISPOT Assays:**

3 10ml Green Top Heparinized Tubes (30 ml total)  
Send via Frederick Courier to NCI Frederick Clinical Support Laboratory (Dr. William Kopp) for specimen processing and freezing.

Tetramer Assay: Will be performed by the flow cytometry unit in the laboratory of Dr. William Kopp.

IFN- $\gamma$  ELISPOT Assay: Will be performed in the Laboratory of Cell Mediated Immunity- Dr. Anatoly Malaguine

**<sup>51</sup>Cr Release CTL Assay:** 3 10ml Green Top Heparinized Tubes (30 ml total)  
Send via Frederick Courier to NCI Frederick Clinical Support Laboratory (Dr. William Kopp) for specimen processing and freezing.

Assay will be performed in the Laboratory of Cell Mediated Immunity- Dr. Anatoly Malaguine

**NKT Cell Analysis:** 2 8ml Blue Tiger Top Sodium Citrate Tubes (16 ml total)  
Deliver to laboratory of Dr. Jane Trepel Bldg.10, Rm. 12N230

301-496-1547

**NOTE:** DTM will send aliquots of PBMCs following apheresis at weeks 0, 24, 48 and 96, 108, 120, 132 and 144 and shipped to NCI Frederick as described above. Multiple (N=6) heparinized green top tubes for immune assays will be drawn at *non-apheresis* time points (weeks 12, 18, 36, 60, 72, 84, 108, 120, 132 and 144) and processed in an identical manner.

The Clinical Support Laboratory, processes and cryopreserves samples in support of IRB-approved, NCI clinical trials. The laboratory is located in a controlled access building and laboratory doors are kept locked at all times. Upon specimen receipt, each sample is assigned a unique, sequential laboratory accession ID number. All products generated by the laboratory that will be stored either in the laboratory freezers or at a central repository facility are identified by this accession ID. An electronic database is used to store information related to patient samples processed by the laboratory. Vial labels do not contain any personal identifier information. Samples are stored inventoried in locked laboratory freezers and are routinely transferred to the NCI-Frederick repository facilities for long-term storage. Access to stored clinical samples is

restricted. Investigators establish sample collections under “Source Codes” and the investigator is responsible for the collections, typically the protocol Principal Investigator who has access to the collection. Blood and tissue specimens collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study. However, this research may only be done if the risks of the new questions were covered in the consent document, the patient has provided consent for the research use of their clinical specimens, and the proposed research has undergone prospective IRB review and approval. The NIH Intramural IRB will be notified when samples are destroyed.

## **Appendix 4 Description of Immune Response Assays**

Monitoring of humoral and cellular immunologic responses will be performed at study weeks 0, 12, 18, 24, 36, 48, 60, 96, 108, 120, 132, and 144. Apheresis to ensure adequate numbers of cells for dendritic cell vaccine preparation (subjects randomized to Arm B) and for assessment of immunologic responses (all subjects) will be performed at weeks 0, 24, 48 and 96. The procedures involved in each assay are outlined below.

### **Separation of PBMCs**

At week 0 and subsequent *non-apheresis* time points (weeks 12, 18, 36, 60, 72, 84, 108, 120, 132, and 144) blood will be drawn for immunological assays in 6 10ml green top tubes containing heparin. This will be divided into aliquots, diluted with PBS 1:2 and 12 mls of Ficoll-Hypaque will be layered under 30 ml of the diluted patient sample. The cells will be spun at 2000 rpm for 30 minutes. The cells at the interface of the ficoll and the serum will then be collected and washed X 2.

### **Tetramer Assay (All Patients)**

Utilizing HLA A2.1 tetramers containing TARP peptide sequences, cells obtained prior to vaccination and those obtained post vaccination will be stained with these tetramers and the precursor frequency (if any) will be determined at baseline and following TARP vaccination.

### **IFN- $\gamma$ ELISPOT Assay (All Patients)**

Lymphocytes will be separated from heparinized blood using density gradient centrifugation and then be placed in human AB serum with 10% DMSO for storage in liquid nitrogen. At week 24, samples from weeks 0, 12, 18 and 24 will be assessed concurrently utilizing the ELISPOT assay. The ELISPOT measures the number of IFN- $\gamma$  cells and will be used for determination of CTL precursor frequency to TARP peptides in both pre- and post-vaccination peripheral blood mononuclear cells (PBMC). Additional ELISPOT assays will be preformed using samples from weeks 0, 24 and 48 as well as extension year (48-96 and 96-144) timepoints.

Briefly, 96-well MultiScreen<sup>TM</sup> -HA (nitrocellulose) plates are coated with capture mAb against human IFN- $\gamma$  overnight at RT. Effectors will be thawed and cultured overnight in RPMI 1640 plus 10% human AB serum. Plates are washed and blocked for 2 h with RPMI 1640 plus 10% human AB serum. C1R.A2, antigen presenting cells (APC), are irradiated with 20,000 rad and pulsed for 2 h at 37°C with either TARP peptide, Flu matrix peptide, a negative control peptide (TAX peptide) or a positive control peptide (CEF peptide pool- see notes below). Non-pulsed C1R.A2 APC will be incubated in tandem and will serve as a negative control.  $1 \times 10^5$  PBMC are added to each well followed by pulsed- or non-pulsed C1R.A2 cells at an effector:APC ratio of 1:1. Each sample is run in six replicates to control for variability. HLA-A2 binding Flu matrix peptide 59-66 serves as a positive control for inherent cellular reactivity. In addition, PBMC from two "normal" HLA-A2-positive individuals with previously determined levels of FMP- or CMV-peptide T-cell precursors are run as assay controls. Cells are incubated for 24h and lysed with phosphate buffered saline (PBS)-Tween (.05%). Biotinylated antihuman IFN- $\gamma$

antibody in PBS-Tween containing 1% bovine serum albumin (BSA) is added and the plates are incubated overnight at 4°C. Plates are then washed and incubated with streptavidin-alkaline phosphatase for 2 h at RT. Spots are visualized with BCIP-NBT substrate and subjected to automated evaluation using the ImmunoSpot Imaging system (Cellular Technology LTd, Cleveland, OH). ELISPOT assays will be performed by the Laboratory of Cell Mediated Immunity (Dr. Anatoly Malyguine) at NCI Frederick.

NOTE: The CEF peptide pool from Mabtech may be used as HLA.A2 positive control peptides as alternatives to flu matrix peptide (FMP) as an HLA.A2 positive control. The CEF peptide pool is a pool of 23 HLA class I restricted T cell epitopes from human cytomegalovirus, Epstein-Barr virus and influenza virus. The peptide has been shown to stimulate the release of IFN- $\gamma$  from CD8<sup>+</sup> T-cells in individuals with certain highly frequent HLA types (HLA: A1, A2, A3, A11, A24, A68, B7, B8, B27, B35, B44). Chosen epitopes are presented by the most common Caucasian HLA types, whose cumulative frequencies represent >100% of Caucasian individuals. The 23 peptides cover 11 HLA types. The CEF peptide pool has proven useful as a peptide specific positive control in ELISPOT, CTL and intracellular cytokine assays using human PBMC. Original pool described by Currier et. al

### **<sup>51</sup>Cr Release CTL Assay (Patients with Evidence of Positive Tetramer and IFN- $\gamma$ ELISPOT Responses)**

3 X 10<sup>6</sup> cells/well in a 24 well plate will be in vitro pulsed for 7 days in the presence of the following conditions:

- 1) 10  $\mu$ M of the TARP 27-35 peptide (Native/WT peptide)
- 2) 10  $\mu$ M of the TARP 29-37-9V peptide (Epitope Enhanced Peptide)
- 3)  $\square\square\square\square$  flu matrix peptide (lower concentration due to potent stimulation peptide).

On day one 1000 U/ml IL-7 will be added and on day three cultures will be supplemented with 20 IU IL-2/ml. On day 7, cells will be harvested and plated to result in fixed effector to target cell ratios. Cells initially cultured with native or epitope enhanced TARP peptides will be tested against targets pulsed with 50  $\mu$ M of their respective TARP peptide, 50  $\mu$ M of TAX negative control peptide, and 50  $\mu$ M of CEF positive control peptide. Cells initially cultured with flu matrix peptide will be tested against targets pulsed with 1  $\mu$ M flu matrix peptide and 50  $\mu$ M of TAX negative control peptide, and 50  $\mu$ M of CEF positive control peptide. Targets will be labeled with 150  $\mu$ Ci [<sup>51</sup>Cr] sodium dichromate. All target cells will then be washed three times and 3,000 labeled targets aliquoted into each well in a 96-well tissue culture plate that has already been plated with effector cells. At this point, both the effector and target cells will be suspended in complete T cell medium with 10% fetal calf serum and with a 10 fold excess of K562s (over targets). The plate will be incubated at 37° C for four hours. Supernatants will be harvested using a Skatron supernatant harvesting device and the samples counted on a gamma counter. Spontaneous release values will be obtained by incubating targets alone and maximum release values obtained by lysing the targets with Triton X-100.

The percent specific lysis is determined, on triplicate samples, by the following formula: percent specific lysis = 100 X (experimental release - spontaneous release)/(maximum release -

spontaneous release). The percent specific lysis of targets with TARP and control peptide will be compared to the percent specific lysis of targets without peptide. If these are significantly different using student's t test and if there is a greater than 10% difference at more than one effector:target ratio, we will consider the CTL assay to be positive.

NOTE Regarding Immunologic Response Testing: Assays will be run in batch for specimens from weeks 0, 12, 18 and 24. Samples with reactivity at week 24 will be used as a comparator control for batch assays for specimens run from weeks 36, 48, 60 and 96.

### **Restimulation Assay Following Apheresis at Weeks 0, 24, 48 and 96 (All Patients)**

The restimulation assay will be performed by the lab of Cell Mediated Immunity at NCI Frederick. Once apheresis is collected, *prior to monocyte elutriation* (week 0 only), 10 ml of apheresed PBMCs should be sent to the NCI Frederick Clinical Support Laboratory for processing. These cells will be placed in a three week cytotoxicity assay in which they are stimulated repeatedly with the mixture of TARP peptides with which they were vaccinated and flu matrix peptides as a control. A fraction will also be cryopreserved for future immunologic assays. Following these repeated stimulations, cells will then be tested on targets pulsed with each of these three peptides and on HLA A2.1 positive tumor cells expressing TARP.

### **Lymphocyte Subsets for FACS Analysis**

Peripheral blood mononuclear cells will be tested for the following markers via flow cytometry; CD3, CD4, CD8, CD3-/CD16/56+, CD3+/CD16/CD56+. Additional testing will be done on all patients in the research laboratory. We plan to use these markers to determine whether vaccination has caused a change in the number of these cells circulating in the peripheral blood and in the expression of cell surface markers. This assay will require three purple top tubes.

### **Multiparameter Flow Cytometric Analysis of NKT Cells**

Analysis will be performed by the laboratory of Dr. Jane Trepel. NKT cells will be identified by multiparameter flow cytometry. Markers may include, but are not limited to, Va24, Vb11, CD3, CD1d tetramer, CD4, CD8, and Hoechst 33258 for identification of viable cells. If sample permits, PBMC may be placed in culture for NKT cell activation, i.e. with  $\alpha$ -galactosylceramide, incubated at 37°C, and intracellular cytokines such as IFN- $\gamma$ , IL-4, IL-13 may be evaluated using multiparameter flow cytometry. If sample permits, immune cell subsets that contribute to regulation of the NKT axis, i.e. Tregs, may be analyzed.

#### ***When the patient is scheduled please notify the Trepel Lab by email:***

Yeong Sang Kim; kimye@mail.nih.gov and cc: to  
Jane Trepel: trepel@helix.nih.gov

#### ***When the blood is drawn:***

Please label the tubes including week number  
Contact the Trepel lab at 301-496-1547

***Abbreviated Title: TARP Peptide Vaccine Prostate Cancer  
CC Protocol 09-C-0139 L, Version Date: 01/10/2019***

Leave the blood for pickup at room temperature in the soiled utility room, in a container labeled "Trepel Lab".



*Appendix 5B Guidelines and Worksheets for Calculation of PSADT*

**Calculated Pre-Enrollment/Baseline PSADT (in months):** \_\_\_\_\_

**PSADT Response is Defined As:**

- A  $\geq 50\%$  increase over baseline PSADT OR
- A PSADT > 15 months

PSADT Response will be assessed at weeks 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144.

<b>On Study PSADT Calculated at Weeks 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144</b>					
<b>DATE</b>	<b>STUDY WEEK</b>	<b>PSA ng/mL</b>	<b>TESTOSTERONE ng/dL</b>	<b>Calculated PSADT</b>	<b>PSADT Responder?</b>
	0				
	3				
	6				
	9				
	12				
	15				
	18				
	24				
	36				
	48				
	60				
	72				
	84				
	96				
	108				
	120				
	132				
	144				

**Record PSA values with a maximum of 2 digits after the decimal point.**

<b>On Study <i>Sequential</i> PSADT Calculated <i>for Statistical Analysis</i></b>			
<b>Analysis Window</b>	<b>Study Wk PSA Values Included</b>	<b>Calculated PSADT/Slope Log</b>	<b>Percent Change PSADT</b>
Pre-NIH	<i>Outside</i> PSAs -12 Months to Entry		
-12Months to Week 3	-12 Months plus Wks 0, 3		
Week 0 to Week 3	NIH PSAs <i>only</i> Wks 0, 3		
Week 3 to Week 12	Wks 3, 6, 9, 12		
Week 3 to Week 24	Wks 3, 6, 9, 12, 15, 18, 24		
Week 3 to Week 48	Wks 3, 6, 9, 12, 15, 18, 24, 36, 48		
Week 24 to Week 48	Wks 24, 36, 48		
Week 48 to Week 72	Wks 48, 60, 72		
Week 48 to Week 96	Wks 48, 60, 84, 72, 96		
Week 96 to Week 120	Wks 96, 108, 120		
Week 96 to Week 144	Wks 96, 108, 120, 144		

**Appendix 6: TARP Peptide Vaccination Report Card**

<b>MEDICAL RECORD</b>	<b>Outpatient Progress Notes</b>
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**TARP PEPTIDE DENDRITIC CELL VACCINE VACCINATION REPORT CARD**

Date/Time Vaccine Administered: \_\_\_\_\_ Administered By: \_\_\_\_\_

Protocol Week: \_\_\_\_\_ Vaccine Dose #: \_\_\_\_\_ Vaccine Cohort: \_\_\_\_\_

	Injection Volume (in ml)	# Cells / Injection	Arm B patients: ID Wheal Present?	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	Date resolved
Site #1				<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3	
Site #2				<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3	
Site #3				<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3	
Site #4				<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3	<input type="checkbox"/> 0 <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3	

Total Injection Volume: \_\_\_\_\_ Total Peptide-Pulsed Cells Delivered: \_\_\_\_\_

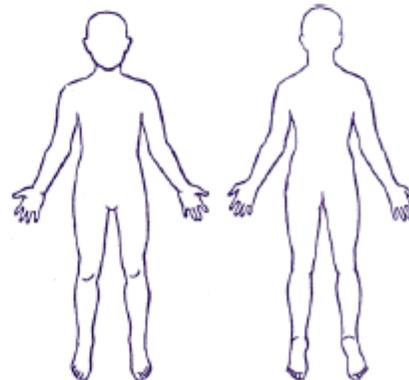
**Injection Site Reaction Grading (NCI CTCAE v3.0)**

0 No Reaction

1 Pain, itching or redness

2 Pain or swelling with significant inflammation (redness, tenderness or phlebitis)

3 Ulceration or necrosis (skin break down) that is severe; operative intervention indicated.



Outpatient Identification	Outpatient Progress Note NIH-532- 10 (8-00) P.A. 09-25-0099 File in Section 2: Progress Notes
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## **Appendix 7: Exploratory Correlative Studies**

### **Measurement of Function-Associated mRNAs in Whole Blood: Dr. Masato Mitsuhashi Hitachi Chemical.**

To be performed on the last 10 subjects enrolled in the 09-C-0139 TARP vaccine study.

**Function-Associated mRNAs:** One 10ml green top sodium heparin tube  
**(Weeks 0, 12, 18 and 24)**

- Blood will be drawn into one 10ml green top tube.
- Add 60 µL each of blood into three 8-well strips (provided by Dr. Mitsuhashi). Strips contain PHA, HAG, PBS, rIL2, rIFN $\alpha$ , CEF, LPS/ZA, TARP27-35 and TARP EE-29-37).
- Incubate at 37°C for 4 hours.
- Store in -80°C freezer until ready for shipment to Dr. Mitsuhashi
- **Note: specimen should be drawn before 1pm** to allow adequate time for specimen processing and four hour incubation by Trepel lab staff.