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Title: A Pilot Study of Long Term TARP Vaccination Using A Multi-Epitope TARP Peptide Autologous Dendritic Cell Vaccination in Previously Vaccinated Men on NCI 09-C-0139

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Investigational Agent:

Drug Name:	Multi-Epitope (ME) TARP autologous dendritic cell vaccine
IND Number:	16255
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Manufacturer:	Department of Transfusion Medicine, NIH Clinical Center

Commercial Agents: none

Identifying Words: Multi-Epitope (ME) TARP, dendritic cell vaccine, D0 prostate cancer

PRÉCIS

TARP

- T-cell receptor γ alternate reading frame protein (TARP) is an amino acid protein expressed by both normal and malignant prostate cancer tissue; 95% of prostate cancer specimens are positive for TARP expression. TARP is highly expressed in prostate cancers of all Gleason types, in primary as well as metastatic disease, and in hormone sensitive and castrate resistant prostate cancer. Therefore, TARP is an ideal tumor antigen target for a vaccine.
- A prospective, randomized pilot study of 1st generation TARP Peptide vaccination (NCI 09-C-0139) utilizing TARP WT 27-35 and EE29-37-9V peptides was conducted in HLA-A*0201positive men with stage D0 prostate cancer (PSA biochemical recurrence) and a PSA doubling time (PSADT) of ≥ 3 months and ≤ 15 months. TARP vaccination was found to be immunogenic, safe and well tolerated, with adverse events limited to injection site reactions ≤ Grade 2. TARP vaccination was also associated with a decreased slope log PSA compared to pre-vaccination baseline in 72% of subjects reaching 24 weeks and 74% reaching 48 weeks (p=0.0012 and p=0.0004 for overall changes in slope log PSA, respectively); TARP vaccination also resulted in a 50% decrease in calculated tumor growth rate constant: pre-vaccine g = 0.0042/day, post-vaccine g = 0.0021/day (p=0.003); TARP-specific IFN-γ ELISPOT responses were detected in the majority of subjects but did not correlate with decreases in slope log (PSA).

Multi-Epitope (ME) TARP Vaccine

- The vaccine platform includes the original two 9-mer HLA-A*0201 binding TARP peptide epitopes (WT27-35 and EE29-37-9V) utilized in NCI 09-C-0139 as well as an additional five 20-mer TARP peptides overlapping by 10 amino acids for a total of 7 peptides that span the amino acid sequence of the entire TARP protein.
- The advantage of this multi-epitope TARP peptide vaccine platform is that the overlapping epitopes cover the entire TARP protein, resulting in potential for induction of a multi-valent anti-TARP response. In addition, these longer synthetic peptides include TARP-specific MHC class II CD4+ T cell helper epitopes that will allow generation of better CD8+ T cell responses with improved functional avidity and longevity as well as humoral anti-TARP antibody responses.

Study Objective

- To assess the long-term safety of repeated TARP peptide vaccination following the use of a 1st generation bivalent (09-C-0139) and a 2nd generation ME TARP peptide vaccine. Specifically, to document if less than 10% of enrolled patients experience a vaccine-related Grade 3 adverse event (local injection site reactions or systemic reactions).

Eligibility Criteria All Patients

- Males > 18 years of age with histologically confirmed adenocarcinoma of the prostate.
- Prior enrollment in NCI protocol 09-C-0139 with receipt of at least 5 doses of TARP peptide vaccine (i.e. completion of primary vaccination series).
- Performance Status: ECOG 0-1.

- Hemoglobin > 10.0 gm/dL, WBC > 2,500/mm^3, ALC > 500/ mm^3, ANC > 1,000/mm^3, platelet count > 100,000/mm^3, and PT/PTT < 1.5X ULN unless receiving clinically indicated anticoagulant therapy; SGPT/SGOT < 2.5X ULN, total bilirubin < 1.5X ULN; creatinine < 1.5X ULN and estimated GFR (eGFR) > 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.
- Standard of care medical management of current prostate cancer disease status by the patient's local oncologist e.g. androgen deprivation therapy is allowed.
- Must be able/willing to adhere to protocol requirements and vaccination timeline.

Exclusion Criteria All Patients

- Patients with active infection or other significant or uncontrolled medical illness. Patients with a remote history of asthma or active mild asthma may participate.
- Patients on immunosuppressive therapy including systemic corticosteroid therapy for any reason. Patients receiving inhaled or topical corticosteroids may participate.
- Patients who, in the opinion of the Principal Investigator, have significant medical or psychosocial problems that warrant exclusion.

Study Design

- Open label, prospective, non-randomized, long-term follow-up pilot study to assess the long-term safety of repeated TARP vaccination in patients that have already received the first generation TARP vaccine. Sample size: N=40 maximum.
- All patients will undergo an 18L apheresis for mononuclear cell collection at Week 0.
- All patients will receive a total of 6 doses of autologous ME TARP peptide DC vaccine: 20 x10^6 viable cells/dose) delivered intradermally at Weeks 3, 6, 9, 12, 15, and 24.

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective:

To assess the long-term safety of repeated TARP peptide vaccination following the use of a 1st generation bivalent (09-C-0139) and a 2nd generation ME TARP peptide vaccine. Specifically, to document if less than 10% of enrolled patients experience a vaccine-related Grade 3 adverse event (local injection site reactions or systemic reactions).

1.1.2 Secondary Objectives:

1.1.2.1 For subjects who are still Stage D0:

- To assess and compare the change in slope log PSA from pre-study baseline (-12 months to entry on the current study) to the change in slope log PSA at weeks 3-24 and 3-48 post ME TARP vaccination.
- To compare the change in slope log PSA at weeks 3-24 and 3-48 following ME TARP vaccination versus the same change in slope log PSA parameters following 1st generation TARP vaccination on protocol 09-C-0139.

1.1.2.2 To assess reactivity to WT27-35 and EE29-37-9V TARP peptides as measured by IFN-γ ELISPOT at Week 24 and Week 48 following immunization with the 2nd generation ME TARP vaccine platform in individuals that have previously received TARP vaccination.

1.1.2.3 To characterize and compare Week 24 and 48 TARP WT27-35 and EE29-37-9V TARP peptide IFN-γ ELISPOT reactivity following ME TARP vaccination with the Week 24 and 48 responses induced with the 1st generation bivalent TARP vaccine in the same individuals.

1.1.2.4 To assess additional cellular and humoral immune responses associated with ME TARP peptide autologous DC vaccination using anti-TARP antibodies and TARP-specific CSFE (carboxyfluorescein diacetate, succinimidyl ester) proliferation, additional ELISPOT (perforin and Granzyme B), intracellular cytokine staining (ICS) and tetramer assays.

1.2 BACKGROUND AND RATIONALE

1.2.1 Peptide Vaccines

Vaccination with tumor-associated antigens (TAA) is designed to induce T cell responses aimed at eliciting and enhancing specific immune responses that can eradicate tumors in patients with established disease. However, a significant challenge remains in deciding what cancer antigens to target, the native form of the delivered antigens (DNA *vs.* peptide *vs.* protein *vs.* whole tumor lysate), the delivery platform (cellular *vs.* non-cellular approaches), and the co-administration of adjuvants (which ones and how many) as well as overcoming negative immune regulation in the host caused by tumors themselves. Important criteria for an ideal cancer antigen include:

CRITERIA	TOP SUBCRITERIA
Therapeutic function	Superb data controlled vaccine trial suggestive.
Immunogenicity	T-cell an/or antibody responses elicited in clinical trials

Oncogenicity	Associated with oncogenic process (i.e. oncogenic "self" protein)
Specificity	Absolutely specific (e.g., mutated oncogene, idiotype protein, or viral protein)
Expression level and % positive cells	Highly expressed on all cancer cells in patients designated for treatment
Stem cell expression	Evidence for expression on putative cancer stem cells
No. patients antigen-positive cancers	High level of expression in many patients with a particular tumor type
No. epitopes	**Longer antigen with multiple epitopes and the potential to bind to most MHC molecules**
Cellular location of expression	Normally expressed on the cell surface with no or little circulating antigen

Adapted from Cheever MA et al. *Clin Canc Res* 2009;15:5323-37. (**1**)

Historically five categories of tumor-associated antigens (TAA) have been utilized in immunotherapy: mutated antigens (p53 or RAS), over-expressed self-antigens (HER2/*neu* or MUC-1), differentiation antigens (gp100, tyrosinase), cancer testis antigens (MAGE, BAGE or CAGE families, NY-ESO-1) and viral antigens (HPV16 E6 or E7, EBV and others). (**1**). The advantages of therapeutic cancer vaccines utilizing proteins and peptides include the simplicity of production and the relative absence of major safety and regulatory issues.

All cells that express class I MHC can present short peptides (8–11 amino acids) from TAA or viruses whose chronic persistent infection is associated with the development of malignancy (e.g. HPV, hepatitis B and C). However, co-stimulatory signals essential for T cell stimulation and the induction of lasting potent and effective immune responses are often absent due to the lack of induction of specific T-cell help, resulting in suboptimal and short-lived $CD8^+$ T-cell responses caused by a lack of proper T-helper cell-mediated signaling through dendritic cells (DCs) (**2**). In addition, vaccination with restricted MHC class I binding peptides can be associated with induction of peptide specific tolerance rather than tumor-controlling immunity (**3,4**) and the use of a limited number of peptides within any given vaccine platform may allow the development of immune escape. Recent developments in therapeutic cancer vaccine research have included the use of TAA synthetic long peptides (SLP) (**5**) as well as the use of overlapping and/or multi-epitope peptide vaccines (**6**). Examples of multi-epitope peptide cancer vaccine platforms under clinical investigation include folate receptor alpha (NCT01606241), HER2/*neu* (NCT01632332, NCT00266110, NCT00088985) and melanoma (NCTI00580060, NCT 00071981, NCT00471471, NCT00705640, NCTI00085137) peptides.

SLP are synthetic peptides of 20-50 amino acids that because of their length require internalization and processing by DCs. Processing by these professional antigen presenting cells avoids presentation by non-professional antigen-presenting cells that could potentially induce tolerance instead of immunity. Overlapping SLP contain both CD4 and CD8 epitopes that results in parallel stimulation of both CD4+ and CD8+ T cells and a stronger, more effective and optimal immune response (**7-12**). In addition, since overlapping SLP contain all potential epitopes irrespective an individual's MHC type, the use of SLPs is a highly attractive approach to maximize the therapeutic applicability of any given vaccine in a genetically diverse human

population such as that of the United States. Protein vaccination is very suitable for the induction of $CD4^+$ T cell responses and antibodies but it generally induces responses against dominant epitopes (**13, 14**) and often fails to induce proper and effective $CD8^+$ T cell immunity, in contrast to long peptides that induce both (**13**). In addition, processing of SLP is more efficient compared to intact protein, and, specifically relevant to the vaccine platform proposed in this concept, uptake of SLP is more efficient in DCs (**13**). Importantly, the use of SLP or a multi-epitope vaccine is able to induce a broader repertoire of T cell responses, thereby maximizing the diversity of epitopes potentially associated with anti-tumor effector function and minimizing the risk of tumor antigen escape. Recently, the use of the multipeptide vaccine IMA901 (comprised of nine HLA-A*02-restricted tumor associated peptides and one HLA-DR-restricted tumor associated peptide derived from highly over-expressed tumor antigens) delivered following a single dose of cyclophosphamide was reported with improved overall survival in patients with renal cell carcinoma (RCC) (**6**).

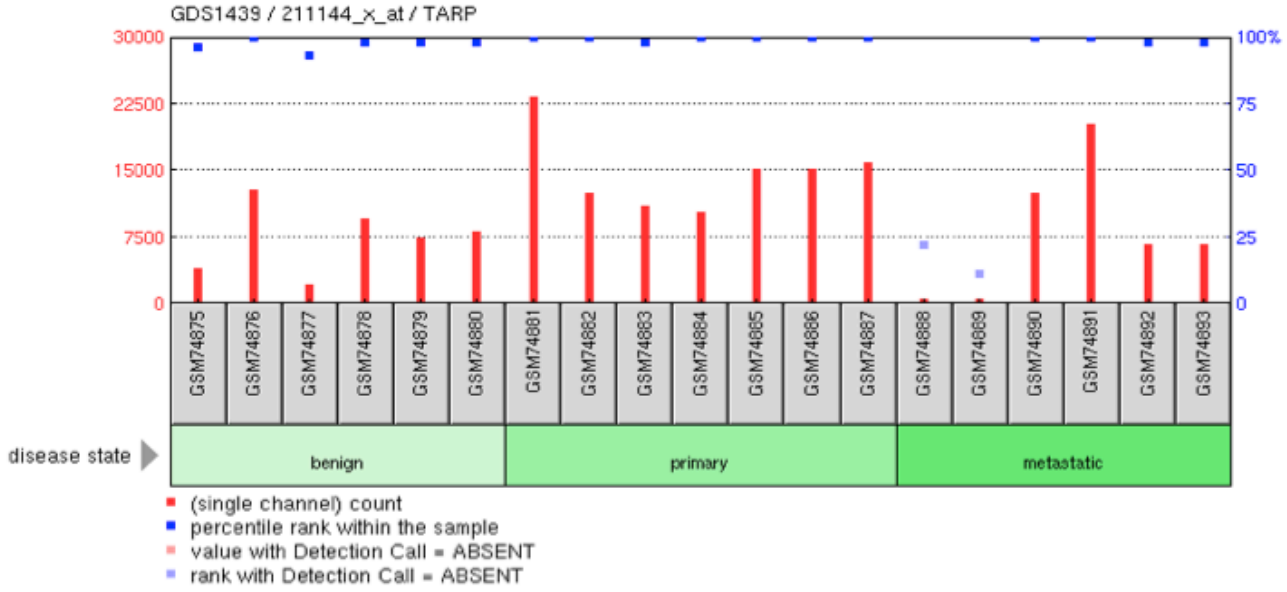
1.2.2 **TARP**

TARP (T-cell receptor γ alternate reading frame protein) is a novel, 58 amino acid protein identified using the expressed sequence database that is over-expressed in patients with prostate and breast cancer (**15**). The mRNA is initiated in the Jγ 1 exon of the TCR γ and the protein expressed is initiated in an alternative reading frame distinct from that of the TCR γ 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, and overexpressed in adenocarcinoma of the prostate, and the prostatic adenocarcinoma cell line LNCaP (**16**). They subsequently showed that TARP was also expressed in three breast cancer cell lines and breast cancer tissues (**17**) and determined that TARP is expressed in some prostate cancer lines (LNCaP) but not in PC3 that also lacks other expected prostate cancer proteins (**18**). Additional work by others has shown that TARP is:

- Highly expressed in primary as well as metastatic prostate cancer (Figure 1)
- Expressed in prostate cancers with a range of Gleason patterns (Figure 2)
- Expressed in both hormone sensitive and castrate resistant prostate cancer (Figure 3)

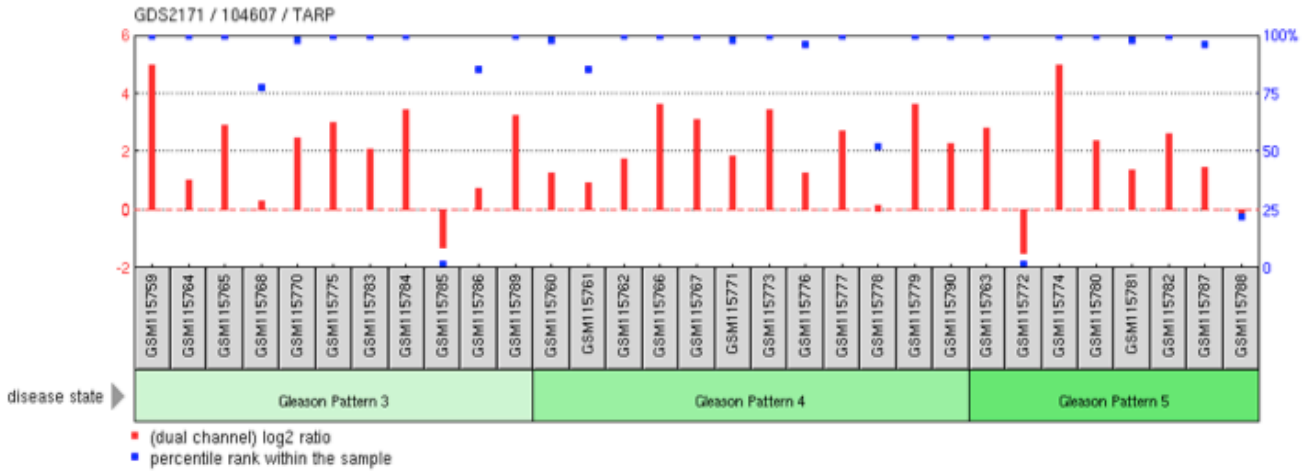
Refer to Figures 1-3 below:

Figure 1: TARP is Highly Expressed Primary Prostate Tissue: Benign *vs.* Localized PC *vs.* Metastatic PC



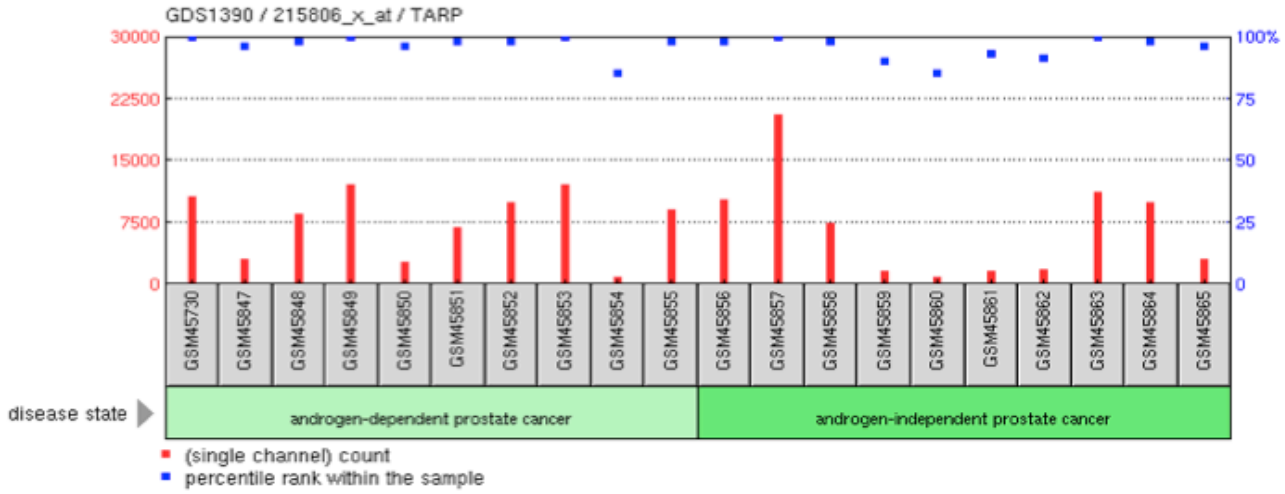
NCBI Dataset Record GDS1439
Citation: Varambally S, Yu J, Laxman B, Rhodes DR et al. Integrative genomic and proteomic analysis of prostate cancer reveals signatures of metastatic progression. Cancer Cell 2005 Nov;8(5):393-406. PMID: 16286247

Figure 2: TARP is Expressed in Prostate Tissues With A Range of Gleason Scores:



NCBI Dataset Record GDS2171
Citation: True L, Coleman I, Hawley S, Huang CY et al. A molecular correlate to the Gleason grading system for prostate adenocarcinoma. Proc Natl Acad Sci U S A 2006 Jul 18;103(29):10991-6. PMID: 16829574

Figure 3: TARP is Expressed in Both Hormone Sensitive *and* Castrate Resistant Prostate Tissue:



NCBI Dataset Record GDS1390
Citation: Best CJ, Gillespie JW, Yi Y, Chandramouli GV et al. Molecular alterations in primary prostate cancer after androgen ablation therapy. Clin Cancer Res 2005 Oct 1;11(19 Pt 1):6823-34. PMID: 16203770

As shown in Figure 1, TARP is expressed both by normal and malignant prostate cancer tissue, with about 95% of prostate cancer specimens reported to be positive for its expression, both primary and metastatic. A recently published study of TARP protein expression in primary prostate cancer specimens from 621 patients who underwent radical prostatectomy (median age 62, median PSA 7.2), documented that TARP was over-expressed in the vast majority (~85%) in comparison to non-neoplastic prostate tissue and its expression was associated with conventional markers of unfavorable and more aggressive tumor behavior (**19**). As shown in Figs 2 and 3, it is also expressed in prostate cancers of all Gleason types and both androgen-dependent and independent. ***Therefore, TARP is an ideal tumor antigen target for a vaccine that could be applied to any stage of prostate cancer.***

Oh et al (our lab) determined two HLA-A2 epitopes that produce cytolytic T cell responses (**20**). 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 $A2K^b$ 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.

1.2.3 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 (**21-23**), 2) increasing T cell receptor (TCR) triggering (**24-26**), or 3) inhibiting proteolysis of the peptide by serum peptidases (**21, 27, 28**). Whenever the peptide

sequence is altered, it is important to demonstrate that the T cells induced still recognize the native peptide sequence. 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 (**29**).

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 $A2K^b$ transgenic mice, both of the epitope-enhanced peptides produced a higher percentage of specific $CD8^+$ T cells 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 was necessary 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 TARP29–37 specific T cells recognized targets pulsed with the epitope-enhanced peptides less well. This to us suggested 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, $CD8^+$ T cells specific for TARP peptides were able to kill human tumor targets that were HLA-A2 positive and that expressed TARP sequences, confirming that TARP was endogenously processed and presented in human tumor cells. The availability of tetramers that react with $CD8^+$ 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 $CD8^+$ T cells in prostate and breast cancer patients compared with .01-.6% in normal controls.

1.2.4 **Clinical Translation: Therapeutic Vaccination Utilizing Wild Type (WT) and Epitope-Enhanced (EE) TARP Peptides, NCI 09-C-0139**

NCI 09-C-0139 was a prospective, randomized pilot study examining TARP vaccination in HLA-A*0201 positive men with Stage D0 prostate cancer (PSA biochemical recurrence without evidence of visceral or bony metastatic disease). Since the optimal method for therapeutic immunization with peptide vaccines in patients with cancer is unclear, patients were randomized to receive vaccination with TARP peptides in Montanide® ISA 51 VG adjuvant plus GM-CSF (Arm A) or as an autologous,TARP peptide-pulsed dendritic cell (DC) vaccine. The primary

objective was to determine the safety and immunogenicity (as measured by IFN-γ ELISPOT, ICS and tetramer assays) of TARP vaccination. The secondary objectives were to determine the effect of TARP peptide vaccination on PSA doubling time (PSADT) (**30**) and PSA growth rate and regression rate constants. All study participants had to have a baseline PSADT (calculated using PSA values within 12 months of study entry) ≥ 3 months and ≤ 15 months.

Study accrual (N = 41) was completed on 12/19/11, within 28 months of enrollment of the first patient. ***A total of 40 patients completed the series of five primary TARP vaccinations and would be eligible for this study***. Base line demographics for all patients are as follows:

	Age	GS	PSA	Vit D	ALC	CD4%	CD4#
Arm A (N = 21)							
Median	64	7	3.44	26	1360	40.4	584
Range	45 - 74	6 - 9	0.64 - 16.70	6 - 79	610 - 2160	28.5 - 58.4	206 - 915
Arm B (N = 20)							
Median	60	7	2.74	28.5	1230	44.7	536
Range	50 – 82	4 – 9	0.48 – 30.70	5- 70	690 – 3270	26.5 – 62.9	288 - 1283
All Patients (N = 41)							
Median	62	7	3.44	28	1270	42.5	547
Range	45 - 82	4 – 9	0.48 – 30.70	5 – 79	610 – 3270	26.5 – 62.9	206 - 1283

TARP vaccine was administered by deep subcutaneous injection (Arm A) or intradermally (Arm B, $20x10^6$ *viable* cells/vaccine) at Weeks 3, 6, 9, 12, and 15, with an optional sixth dose of vaccine at Week 36 based on changes in PSADT (≥ 50% increase over pre-vaccine PSADT) or immune parameters (3-fold increase in TARP-specific reactivity as measured by IFN-γ ELISPOT reactivity at at least two time points) at Week 24.

1.2.5 TARP Vaccine Safety and Clinical Outcomes

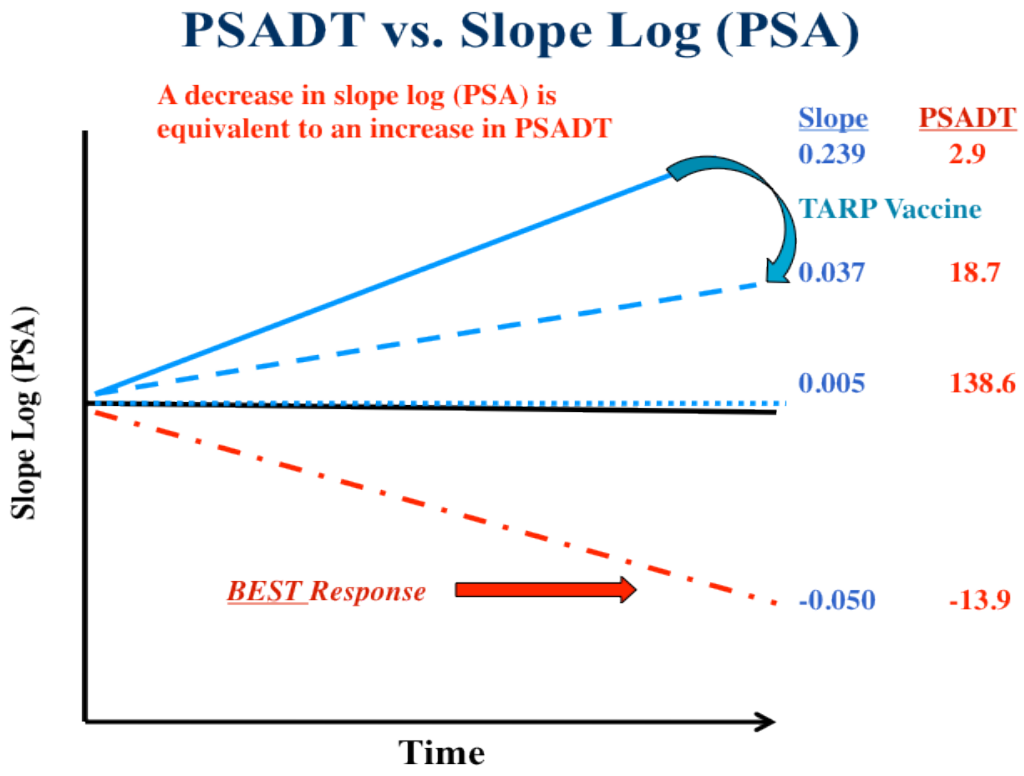
TARP vaccination was found to be safe and well tolerated over the time span evaluated, with adverse events limited to injection site reactions ≤ Grade 2. There were no systemic or immediate hypersensitivity reactions or laboratory abnormalities associated with vaccination.

As of 10/01/13, 8 patients currently remain on Study and 33 patients have gone off study (Arm A N = 17, Arm B N= 16) for the following reasons:

Off Study Reason	**Median Off Study Week 102 (range 8-144 weeks)**
Completed Study	N=12
DC Vaccine Viability Issues	N=1
Lost to Follow-Up	N=1
Patient Request	N=4 (3 wanted to start hormone therapy, 1 leaving country)
Progression	N=12 (6 local recurrence, 6 bony metastatic disease)
PSADT Criteria	N=1
Secondary Malignancy	N=2 (superficial MM, invasive SCC of tongue (smoker)

1.2.6 Impact of TARP Vaccination on PSADT and Slope Log PSA

Because of responses observed in increases/lengthening of PSADT, the original 48 week study was extended twice to a total of 144 weeks, with additional booster doses of TARP vaccine administered at Weeks 48 and 96. PSADTs were calculated using the Memorial Sloan Kettering Cancer Center nomogram (http://nomograms.mskcc.org/Prostate/PsaDoublingTime.aspx). 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 over time. Since the PSADT goes to infinity as the slope approaches 0 and becomes meaningless when the slope is negative, and several patients had dramatic responses to TARP vaccination resulting in *negative* PSADTs, the slope log (PSA) parameter was utilized for the formal statistical analysis since the slope log (PSA) is a continuous variable whether positive, zero, or negative. A representative diagram documenting the relationship of PSADT (in months) to slope log (PSA) is included below.



Week 24 and Week 48 slope log (PSA) responses to TARP vaccination through 06/18/12 are summarized below:

Patients Reaching Week 24*		
TOTAL N =	**39**	
Arm A	21	
Arm B	18	
Decreased Slope	**28**	**71.8% of All Patients**
Arm A	13	61.9% of Arm A Pts
Arm B	15	83.3% of Arm B Pts
Increased Slope	**11**	
Arm A	8	
Arm B	3	
Slope Difference Slope 3-24 *minus* Pre-NIH Slope		
Min	-0.175	
Max	0.144	

Patients Reaching Week 48^		
TOTAL N =	**29**	
Arm A	16	
Arm B	13	
Decreased Slope	**21**	**72.4% of All Patients**
Arm A	10	62.5% of Arm A Pts
Arm B	11	84.6% of Arm B Pts
Increased Slope	**8**	
Arm A	6	
Arm B	2	
Slope Difference Slope 3-48 *minus* Pre-NIH Slope		
Min	-0.178	
Max	0.066	

***At Week 24:Patient 213 excluded due to mixed vaccines**
Patient 219 off study @ Wk 9 due to ↓PSADT

^At Week 48: 3 patients Off Study- 203, 213 and 219

In September 2012, a formal interim analysis was performed on 39 patients with data through 07/31/12 to assess decrease in slope log (PSA) (equivalent to an increase / lengthening in PSADT). For this analysis:

- Baseline = slope log (PSA) Pre-NIH (outside PSAs from -12 months to entry)
- Wk3-24 = slope log (PSA) @NIH (PSAs from Weeks 3 to 24)
- Wk3-48 = slope log (PSA) @ NIH (PSAs from Weeks 3 to 48)

The primary statistical analysis using a Hochberg adjustment for the pooled cohort of patients from both arms revealed a statistically significant decline in the slope log (PSA):

N = 39 patients at Week 24.

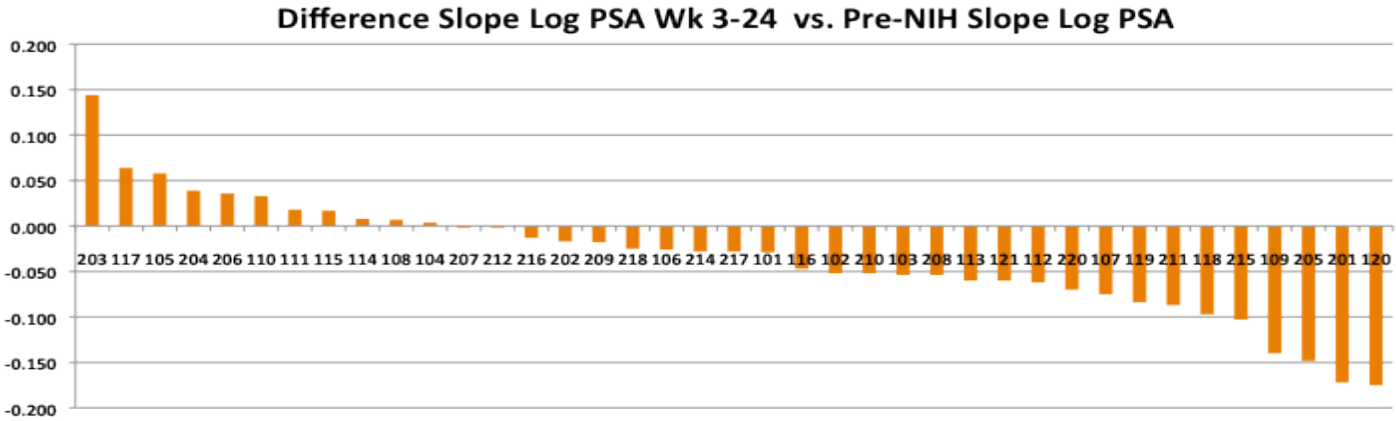
- 28 of 39 patients (71.8%%) exhibited a decrease in slope log (PSA) at Week 24
- Among pooled patients in both arms, slope log (PSA) from 3-24 weeks *declined significantly compared to baseline, p = 0.0012*. Median slope decline (range): -0.028 (-0.175 to 0.144)
- *Within each arm* the decrease in the slope log PSA was *statistically significant:* p = 0.023 for Arm A and p = 0.026 for Arm B.

N = 31 patients at Week 48.

- 23 of 31 of patients (74.2%) exhibited a decrease in slope log (PSA) at Week 48.
- Among pooled patients in both arms, slope log (PSA) from 3-48 weeks *declined significantly compared to baseline, p = 0.0004*. Median slope decline (range): -0.035 (-0.178 to 0.066)

- In Arm A the decrease in the slope log PSA showed a statistically significant trend, p = 0.056; *in Arm B the decrease in the slope log PSA was highly statistically significant, p = 0.0069.*

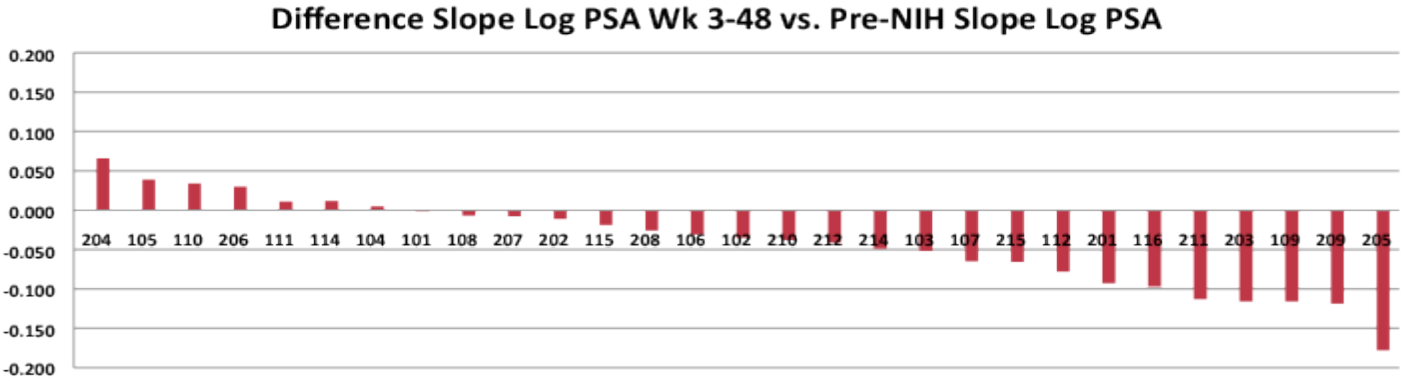
These changes in slope log (PSA) at Week 24 and at Week 48 are graphically highlighted in waterfall plot data below for individual study patients (data through 07/31/12):



Waterfall Plot Week 24 Data (through 06/18/12): **TOTAL N = 39***

Patients with *Decreased* Slope Log PSA: **YES = 28 (71.8%)**

No = 11 (28.9%)



Waterfall Plot Week 48 Data (through 06/18/12): **TOTAL N = 29^**

Patients with *Decreased* Slope Log PSA: **YES = 21 (72.4%)**

No = 8 (27.6%)

^Three patients off study by Week 48: 203 and 213 (patient request); 219 (PSADT)

In summary there were no statistically significant differences between Arm A and Arm B when examining differences in slope log (PSA) between Weeks 3-24 or 3-48 minus the pre-NIH slope, allowing us to pool them for the final analysis. *In the pooled analysis*, a statistically significant decrease was observed in the slope log PSA compared to the Pre-NIH baseline at both 3-24 and 3-48 weeks: at 24 weeks, the decrease in the slope log (PSA) was statistically significant within each arm (p = 0.023 for Arm A and p = 0.026 for Arm B); at 48 weeks, the decrease in the slope

log (PSA) showed a statistical trend in Arm A (p = 0.056) and was highly statistically significant (p = 0.0069) in Arm B. at either 24 or 48 weeks. In addition, the effect of decreased slope log (PSA) at Weeks 3-24 doesn't wane significantly over time and isn't impacted by an additional vaccine dose at Week 36. Finally, *there were no correlations or associations with change in slope that were strong or statistically significant using any baseline variables* including $CD4^+$ T cell percent/absolute count, $CD8^+$ T cell percent/absolute count, 25-OH vitamin D levels, Gleason score, PSA or a baseline PSADT < 6 *vs.* ≥6 months.

Although TARP vaccination was associated with a decline in the slope log (PSA) in the majority of patients at Week 24 and Week 48, *it was associated with an absolute decrease in PSA in only a minority of patients* (6/39, 15.4% at Week 24; 4/31, 12.9% at Week 48) as highlighted in the table below:

***Week 24 vs. Week 48 Absolute PSA Values* thru 07/31/12**

Patients Reaching Week 24*			Patients Reaching Week 48^		
TOTAL N =	**39**		**TOTAL N =**	**31**	
Arm A	21		Arm A	16	
Arm B	18		Arm B	15	
Decreased PSA	**6**	**15.4% of All Patients**	**Decreased PSA**	**4**	**12.9% of All Patients**
Arm A	3	14.3% of Arm A Pts	Arm A	1	6.3% of Arm A Pts
Arm B	3	16.7% of Arm B Pts	Arm B	3	20.0% of Arm B Pts
Increased or No Change in PSA	**33**		**Increased or No Change in PSA**	**27**	
Arm A	18		Arm A	15	
Arm B	15		Arm B	12	
Greatest Decrease in PSA			**Greatest Decrease in PSA**		
Arm A	- 0.48		Arm A	- 0.36	
Arm B	- 1.60		Arm B	- 0.61	

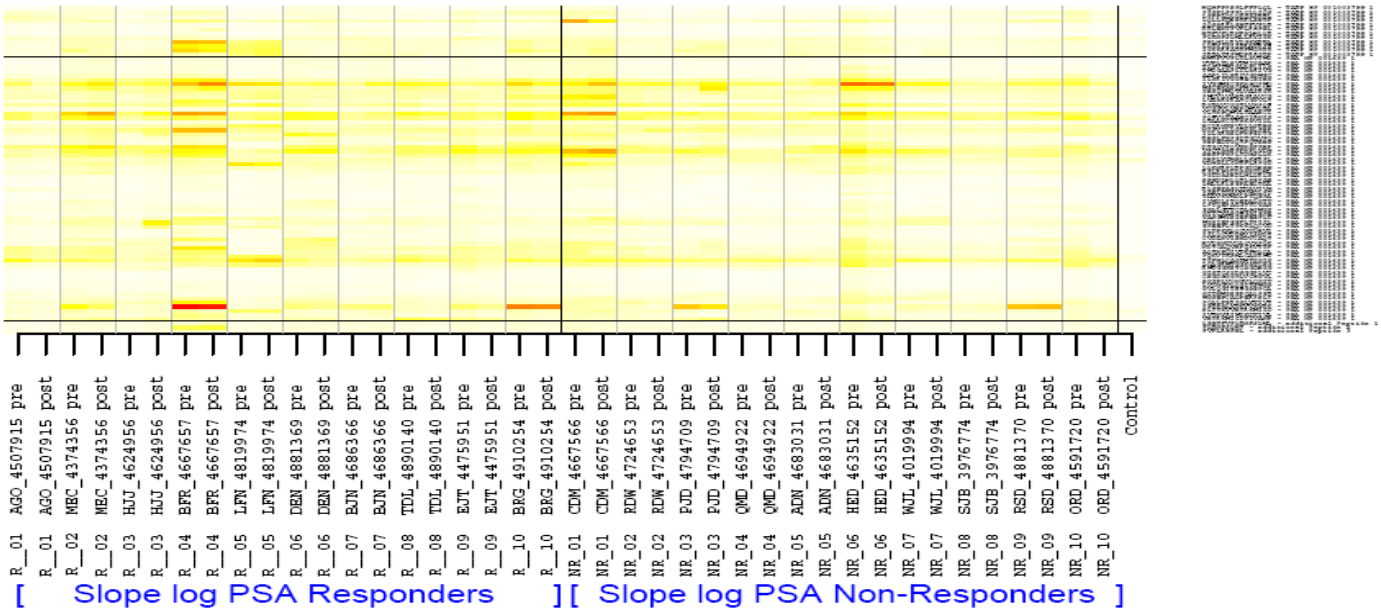
***At Week 24:Patient 213 excluded due to mixed vaccines** **^At Week 48: Two patients Off Study- 203 and 213**
Note: ***Patient 109 on Arm A, and Patients 201 and 209 had sustained decreases in Week 24 and 48 PSAs when compared to Week 0.***

1.2.7 Immune Responses Following TARP Vaccination

Immunologic responses to TARP vaccination were examined in *ex vivo* and 7 day *in vitro* stimulation (IVS) IFN-γ ELISPOT assays. While increased TARP-specific reactivity was demonstrated using *ex vivo* PBMC in a few patients, utilization of the 7 day IVS ELISPOT assay proved to be more sensitive in detecting reactivity to TARP WT27-35 and EE29-37-9V in addition to WT29-37. Induction of TARP-specific IFN-γ ELISPOT responses (characterized by

a 3-fold increase in TARP specific reactivity over at least 2 time points) was observed in a majority (77.5%) of patients. These increased responses over baseline were highly statistically significant at 12, 18, and 24 weeks ($p < 0.0001$) at all time points for all TARP peptides tested. However, there was no statistically significant difference between arms or between response groups i.e. between PSADT responders (decreasing slope log PSA) vs. PSADT non-responders (increasing slope log PSA). Interestingly, reactivity was documented to non-vaccine TARP protein WT 29-37 following vaccination, replicating what was observed in pre-clinical mouse models. Additional studies to date examining functional avidity of TARP ELISPOT responses as well as TARP tetramer responses have been unrevealing. Studies of polyfunctional intracellular cytokine staining (ICS) (including Granzyme A and perforin) for assessment of TARP-specific cellular reactivity are in progress.

An investigation of anti-TARP antibody responses using microarray platform technology spanning the entire TARP protein revealed that patient specific binding patterns were detected. However, for some patients, only slight differences were observed for the two time points and no cross-reactivity of the secondary (anti-PSA) antibody was detected. In addition, a heat map analysis (shown below) for anti-TARP and anti-PSA antibodies documented no difference between the top 10 PSADT responders and bottom 10 PSADT non-responders.



1.2.8 **Rationale for Proposed Follow-up Study Design**

The focus of this study is to assess the long-term safety of repeated TARP vaccination in patients that have already received the first generation vaccine and to allow HLA-A*0201 patients who received the 1st generation TARP vaccine on protocol 09-C-0139 to receive the 2nd generation Multi-Epitope (ME) TARP vaccine regardless of current treatment or disease status. The safety of repeated peptide immunizations over multiple years has not been well studied, but such long-term vaccination may be necessary to maintain anti-tumor activity and control. In addition, many patients that received the first generation TARP vaccine are anxious to receive the ME

TARP vaccine with the hope of broadening and enhancing their anti-TARP and potentially anti-tumor immune response as a result of vaccination.

The rationale for this study design is to assess the long-term safety of repeated TARP peptide vaccination by taking advantage of individuals who have already received 2 years of TARP peptide immunizations, to shorten by two years the time required to obtain such long-term safety data for vaccinations continued for 3 or 4 years, as well as to study immunologic responses to the 2nd generation ME TARP vaccine in the same population of patients immunized with the 1st generation TARP vaccine and compare them to vaccine-induced responses observed on protocol 09-C-0139 as each patient serves as his own control. Because the ME TARP vaccine platform has been expanded to seven peptides and also includes the *original* two HLA-A*0201 restricted, 9-mer TARP WT27-35 and EE29-37-9V peptides, plus an additional five *new* 20-mer peptides that cover the whole TARP protein, administering the ME TARP vaccine in this previously vaccinated population provides a unique opportunity to assess the impact of re-vaccination on boosting and sustaining the TARP-specific responses to the original peptides observed with the original vaccine. It will be very valuable to know whether continued immunizations with the TARP peptides will increase or sustain the immune response over a number of years, and this cohort of patients all immunized first at least 2 years earlier will provide a valuable opportunity to examine the effect of such repeated immunizations with these peptides over several years. These patients from the earlier protocol will give us about a 2-year head start in gathering such data (already boosted and bled at weeks 48 and 96), whereas it would take 2 years longer to learn that with any new patients.

If the TARP vaccine is successful, we will need to make some decisions about how long to keep immunizing patients; *the longer-term data provided by this study will be very helpful to make those decisions*. Identical to the 09-C-0139 study, patients will undergo a series of five primary immunizations three weeks apart with the ME TARP vaccine. In contrast to the 09-C-0139 study where an optional booster dose was delivered at Week 36 based on Week 24 response parameters, in this study all patients will receive a booster dose of vaccine at Week 24. This will then allow direct comparison of TARP specific IFN-γ ELISPOT responses to WT27-35 and EE-29-37 at Weeks 24 and 48, generated in the *absence* of TARP-specific CD4 help with the first generation bivalent vaccine, with these same responses in the *presence* of TARP-specific help available through the second generation ME vaccine. The presence of TARP-specific CD4 help at the time of immunization may boost the magnitude of TARP-specific immune responses as well as their duration, allowing us to gain useful information in this population that will all be at least two years out from their primary immunization series with the first generation TARP vaccine.

In addition, we will seek to determine if ME TARP vaccination broadens the number of TARP peptide cellular immune responses (assessed by overlapping peptide microarrays) and to continue to try and identify immune correlates of slowing in PSA velocity in those patients who remain Stage D0. In these D0 patients, for a given individual we will also assess the change in

slope log PSA following ME TARP vaccination and compare it with the change in slope log PSA following bivalent vaccination.

A separate prospective, randomized 2:1 study will assess and confirm the changes in slope log PSA that were observed in response to TARP vaccination documented in our initial pilot 09-C-TARP study.

1.2.9 Multi-Epitope (ME) TARP Vaccine Description

The 2nd generation ME TARP vaccine is based on the amino acid sequence of the entire TARP protein annotated below. The vaccine platform *includes the original two 9-mer HLA-A*0201 binding TARP peptide epitopes* (WT27-35 and EE29-37-9V) utilized in NCI 09-C-0139 as well as *an additional proposed five 20-mer TARP peptides* overlapping by 10 residues for a total of 7 peptides that span the entire TARP sequence:

Amino Acid Sequence of TARP (58 residues)

MQMFPPSPLFFFLQLLKQSSRRLEHTFVFLRNFSLMLLRGIGKKRRATRFWDPRRGTP
1 11 20 21 30 31 40 41 50 58

Original Epitopes in the 1st Generation TARP Vaccine Platform

FVFLRNFSL = WT HLA-A*0201-binding peptide TARP 27-35
FLRNFSLMV= EE HLA-A*0201-binding peptide TARP 29-37-9V

Additional Epitopes 2nd Generation TARP Vaccine Platform

- **This multi-epitope TARP peptide vaccine consists of 20-mer peptides overlapping by 10-mer spanning the entire 58 amino acid TARP protein as outlined below.**

Amino Acid Sequence of TARP Overlapping Peptides

- **TARP 1-20: MQMFPPSPLFFFLQLLKQSS**
- **TARP 11-30: FFLQLLKQSSRRLEHTFVFL**
- **TARP 21-40: RRLEHTFVFLRNFSLMLLRG**
- **TARP 31-50: RNFSLMLLRGIGKKRRATRF**
- **TARP 41-58*: IGKKRRATRFWDPRRGTP**

***Note: this last peptide is only 18 mer**

The advantage of this multi-epitope TARP peptide vaccine platform is that the overlapping epitopes cover the entire TARP protein, resulting in potential for induction of a multi-valent anti-TARP response. In addition, these longer synthetic peptides include MHC class II CD4+ T cell helper epitopes that will allow generation of better CD8+ T cell responses with improved functional avidity and longevity (42) as well as humoral anti-TARP antibody responses.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Males ≥ 18 years of age with histologically confirmed adenocarcinoma of the prostate. Histology confirmation must be documented with a formal pathology report. Notes from an outside physician documenting histology may be used in cases where another tissue biopsy would be necessary for formalize a pathology diagnosis.

2.1.1.2 Prior enrollment in NCI protocol 09-C-0139 with receipt of at least 5 doses of TARP peptide vaccine (i.e. completion of primary vaccination series).

2.1.1.3 Performance Status: ECOG 0-1.

2.1.1.4 Hemoglobin ≥ 10.0 gm/dL, WBC ≥ 2,500/mm^3, ALC ≥ 500/mm^3, ANC ≥ 1,000/mm^3, platelet count ≥ 100,000/mm^3.

2.1.1.5 PT/PTT ≤ 1.5X ULN unless receiving clinically indicated anticoagulant therapy.

2.1.1.6 SGOT/SGPT ≤2.5X ULN, total bilirubin ≤ 1.5X ULN, Cr ≤ 1.5X ULN, estimated GFR (eGFR) ≥ 60 ml/min.

2.1.1.7 Hepatitis B and C negative, unless the result is consistent with prior vaccination or prior infection with full recovery.

2.1.1.8 HIV negative

2.1.1.9 No use of investigational agents within 4 weeks of study enrollment or use of immunosuppressive or immunomodulating agents (including IVIG) within 8 weeks of study entry. Note: Use of topical, inhaled and intranasal steroid therapy is permitted.

2.1.1.10 Greater than or equal to 6 weeks since the receipt of chemotherapy or radiation therapy.

2.1.1.11 Standard of care medical management of current prostate cancer disease status by the patient's local oncologist, e.g. *androgen deprivation therapy is allowed*.

2.1.1.12 Able to understand and provide Informed Consent.

2.1.1.13 Must be able and willing to adhere to protocol requirements, visits and vaccination timeline.

2.1.2 Exclusion Criteria

2.1.2.1 Patients with a second malignancy requiring active treatment other than adequately treated squamous or basal cell carcinoma of the skin.

2.1.2.2 Patients with an active infection.

2.1.2.3 Patients on immunosuppressive therapy including:

- Systemic corticosteroid therapy for any reason. Patients receiving inhaled, intranasal or topical corticosteroids may participate.

2.1.2.4 Other significant or uncontrolled medical illness. Patients with a remote history of asthma or active mild asthma may participate.

2.1.2.5 Patients who, in the opinion of the Principal Investigator, have significant medical or psychosocial problems that warrant exclusion.

2.1.3 **Recruitment Strategies**

This protocol will recruit patients previously enrolled on the prior 09-C-0139 pilot TARP vaccine study that have received at least 5 doses of TARP peptide vaccine (i.e. completion of primary vaccination series). The maximum number of patients eligible to enroll in this study is 40. Patients that remain Stage D0 disease will have recruitment priority, followed by other patients who completed the entire 144 week study and those that came off study prior to Week 144.

2.2 SCREENING EVALUATION

Note: Screening evaluation testing/procedures are conducted under the separate screening protocol, 01-C-0129 (Eligibility Screening and Tissue Procurement for the NIH Intramural Research Program Clinical Protocols).

At screening, all patients will undergo a history and physical including height, weight, vital signs, review of systems, performance status, review of concomitant medications and life expectancy assessment.

To determine eligibility, the screening evaluations below will be completed within 60 days of enrollment and must be completed at the NIH except imaging studies as described below.

- Laboratory evaluations: CBC with differential counts, PT/PTT, acute care panel, hepatic panel, mineral panel, PSA and testoterone
- TTV Serology (Anti-HIV-1/2 Ab, anti-HCV Ab, HBsAg, HBs Ab, anti-HTLV-1/2 Ab, West Nile, T. Cruzi and RPR) and ABO typing if not done at NIH before.

  NOTE: TTV serology must be drawn within 30 days of apheresis and may need to be repeated due to the apheresis schedule if patient consents. Only HIV and viral hepatitis (HBV and HCV) serology results are required for consenting and pending results status is acceptable for the rest of the serologic tests. Historical ABO typing results are acceptable.
- EKG
- Imaging studies: CT scan of the chest, abdomen and pelvis, Technetium^{99} bone scan

  NOTE: NIH imaging studies are preferred, however, outside scans may be substituted if deemed to be an acceptable quality. If the quality of outside imaging is not felt to be acceptable according to the standards of NIH Radiology and Imaging Services and the NCI Vaccine Branch Clinical Trials Team, new imaging studies should be obtained at the NIH.

2.3 BASELINE EVALUATION

Baseline evaluations should occur within 30 days of the first vaccination. All patients will undergo a baseline history and physical which will include height, weight, vital signs, review of systems, performance status, review of concomitant medications and life expectancy assessment.

- Laboratory evaluations: CBC with differential, PT/PTT, acute care panel, hepatic panel, mineral panel, amylase, lipase, lipid panel, TSH, 25-OH Vitamin D, lymphocyte phenotyping, urinalysis

NOTE: If amylase, lipase, lipid panel, TSH, 25-OH Vitamin D, lymphocyte phenotyping, urinalysis have already been completed within 30 days of the first dose of administration of investigational product, they do not need to be repeated at the baseline timepoint.

Refer to **Appendix 1** for the Study Calendar Schedule of Study Clinical, Laboratory and Radiographic Evaluations.

2.4 REGISTRATION PROCEDURES

Authorized staff must register an eligible candidate with the NCI Central Registration Office (CRO) within 24 hours of signing the consent document. A Confirmation Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) will also be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

2.4.1 Treatment Assignment and Procedures

Cohorts

Number	Name	Description
1	Cohort 1	Males with histologically confirmed adenocarcinoma of the prostate who were previously enrolled on 09C0139 and receive at least 5 doses of TARP peptide vaccine.

Arms

Number	Name	Description
1	Lead-in TARP DC vaccine treatment	Six doses of autologous ME TARP DC vaccine (20 x 10^6 viable cells/dose) every 3 weeks at weeks 3, 6, 9, 12, 15 and 24.

Arm Assignment
Patients in cohort 1 will be directly assigned to arm 1.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is an open label, prospective, non-randomized, long-term follow-up pilot study to assess the long-term safety of repeated TARP vaccination in patients that have already received the 1st generation TARP vaccine on protocol 09-C-0139 to receive the 2nd generation Multi-Epitope (ME) TARP vaccine regardless of current treatment or disease status. It is hoped that ME TARP vaccination will enhance and broaden patients' existing anti-TARP immunity.

Enrollment will be staggered every three weeks for the first three patients that allows a 3 week interval for safety assessment before the next enrolled patient is scheduled to receive their first dose of ME TARP DC vaccine.

- The first three patients will be assessed for any acute unanticipated safety issues possibly, probably or definitely related to vaccination occurring within 3 weeks of receipt of the first ME TARP DC vaccine dose. It is anticipated that *all patients* will experience self-limited vaccine-related local injection site reactions.
- Assessment of safety will be performed *in real time* by the Protocol Principal Investigator. The FDA and the NCI IRB will be notified in real time if there is any evidence of an unanticipated safety signal that arises.
- If there is no adverse safety signal identified in these first 3 patients, enrollment of the remaining study subjects will proceed as quickly as feasibly possible on or after 9 weeks after the *first* study subject has received their first vaccine dose and 3 weeks after the *third* study subject has received their first vaccine study e.g. 03/16/15 in the example above.

All patients will receive a total of 6 doses of autologous ME TARP DC vaccine (20 x 10^6 *viable* cells/dose) administered intradermally every three weeks at weeks 3, 6, 9, 12, 15 and 24 and undergo restaging at Weeks 48 and 96.

All patients will have a history and physical exam, routine monitoring labs, PSA and testosterone levels performed at study week visits 0, 3, 6, 9, 12, 15, 18, 24, 36, 48, 60, 72, 84 and 96. (Note: testosterone levels will *not* be obtained in patients on hormone therapy).

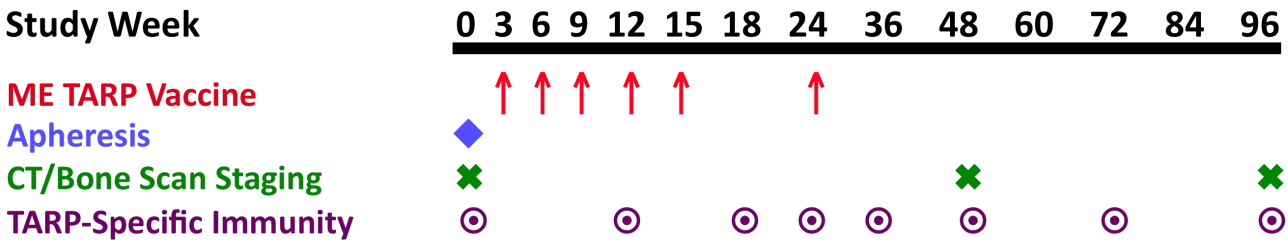
For men who are still Stage D0 disease, PSA Doubling Time (PSADT) and slope log (PSA) will be calculated at Pre-enrollment/baseline (-12 months to entry on the current study) and at every study visit using the PSADT Memorial Sloane Kettering nomogram found at: http://nomograms.mskcc.org/Prostate/PsaDoublingTime.aspx.

For men who still have Stage D0 disease but are on Androgen Deprivation Therapy, PSADT and slope log will not be calculated.

Immunologic responses (CFSE, IFN-γ ELISPOT, ICS and tetramer assays, anti-TARP antibodies) to ME TARP peptide vaccination will be examined at the following time points: Weeks 0, 12, 18, 24, 48, 72 and 96.

The schema for treatment is detailed below.

3.1.1 **Study Schema**



3.1.2 **Immunization-Related Dose Limiting Toxicity (DLT)**

The following assessment guidelines for the management of dose limiting toxicity (DLT) are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE and DLT reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

Immunization-related DLT is defined by the parameters outlined below will be classified by the Principal Investigator as follows for determination of relatedness to ME TARP DC vaccination:

- Unrelated
- Unlikely
- Possibly related
- Probably related
- Definitely related

For the purposes of this study, the definitions of dose-limiting toxicities attributed as possibly, probably or definitely related to ME TARP DC vaccination include:

3.1.2.1 Any Grade 2 or Grade 3 or greater allergic/hypersensitivity reaction

3.1.2.2 Any Grade 2 or greater rash consistent with erythema multiforme

3.1.2.3 Grade 3 or greater hematologic or non-hematologic toxicity, excluding lymphopenia. Abnormal laboratory studies will be repeated to verify toxicity

3.1.2.4 Grade 3 or greater acute vascular leak syndrome: respiratory compromise or fluids indicated.

3.1.2.5 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 ≥ 50% BSA.
- Urticaria: intervention indicated for ≥ 24 hrs.

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

- Pruritis/itching: intense or widespread and interfering with ADL lasting ≤ 72 hrs
- Fatigue: severe fatigue interfering with ADL lasting ≤ 72 hrs
- Fever: > 40.0° C for ≤ 24hrs
- Local lymphadenopathy lasting ≤ 1 week

3.1.3 **Dose Escalation**

There is no ME TARP vaccine dose escalation on this study.

3.2 VACCINE ADMINISTRATION

The vaccine manufacturing schema is described in **Appendix 2** and manufacturing procedures will follow the NIH Center for Cellular Engineering (CCE) Protocol Specific Instructions (PSI) and Standard Operation Procedure (SOP). All patients will undergo 15-18L 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. Cells used for subsequent dendritic cell maturation will be derived from monocytes frozen during the initial apheresis. Apheresis may be repeated any time if additional plasma or cell aliquots are needed to manufacture the vaccine. Please note, this will be repeated only 1 more time with a minimum of 48-hour interval with platelet recovery. Eligible subjects will receive autologous ME TARP dendritic cell vaccine beginning at Week 3. Each dose can be delivered starting from -1 week of the target date until the maximum delay described in Section **3.2.1**. Patients will be assessed prior to each dose, no earlier than 10 days prior to the administration of the investigational product. This assessment will include a CBC, acute care panel and liver panel. 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 cGMP production of peptide-pulsed dendritic cells by the NIH Clinical Center Department of Transfusion Medicine.

- Autologous ME TARP DC vaccine preparations will be assessed for release standards (nucleated cell content and concentration, appearance, flow cytometric verification of DC validation markers, viability $\geq$ 60%, and product sterility and safety testing) prior to release for vaccine administration to the patient.

- The investigational product will be administered intradermally in two sites on the forearm with a maximum volume of 0.5ml per injection. Administration will be alternated between the left and right forearms with each vaccination, with the exception of patients with a contraindication to using a particular arm. In these instances, consecutive doses in the same arm will be allowed. All patients will receive a total of 6 doses of vaccine (20 x10^6 *viable* cells/dose, +/- 20%) delivered at Weeks 3, 6, 9, 12, 15, and 24 and will undergo scans at Weeks 48 and 96 for restaging. Patients who have more advanced disease than D0 prostate cancer will undergo scans guided by their clinical condition at the NIH or through their primary oncologist's office.

- Patients will be monitored for immediate adverse event vaccine reactions (VS, clinical assessment) for 1 hour 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 an adverse reaction is observed following the first vaccine, the reaction will be characterized and a determination made as to whether it is considered a dose limiting toxicity (DLT) as outlined in section **3.1.2**) If the adverse reaction is determined *not* to be a DLT, the duration of post-vaccination monitoring for 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.

- All patients will be given a ME TARP DC Vaccine Report Card (refer to **Appendix 3**) and instructed on how to complete it, following each ME TARP DC vaccine dose.

3.2.1 Delay in Vaccination

Patients who are unable to receive their vaccine injection as scheduled due to adverse events, toxicity, failed vaccine release criteria 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 not more than 6 weeks after the date of the scheduled dose that was missed. 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. If the delay in the vaccination administration is from cell processing scheduling issues or any other regulatory issues not related to patient's medical condition, delay up to 6 weeks is allowed from the scheduled target date of vaccine administration.

3.2.2 Dose Modifications

No dose modifications will be made in patients receiving autologous ME TARP DC vaccination. Subjects will cease to receive immunization if they experience dose-limiting toxicity (DLT) as outlined in Section **3.1.2**. Toxicity will be assessed according to NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.0 that is available at:
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

3.2.3 Patients That Experience Disease Progression While On Study

Stage D0 At Enrollment
Patients who are Stage D0 at enrollment and are subsequently documented to meet criteria for removal from protocol vaccine therapy as outlined in criteria 3.4.1.3, 3.4.1.4, and 3.4.1.5 will be allowed to receive androgen deprivation therapy through their local oncologist and remain on study for monitoring of long-term safety and immune responses *unless they require active chemotherapy or radiation therapy as outlined in Off Study Criteria 3.4.2.2.*

Patients with Documented Locally Recurrent or Metastatic Disease at Enrollment
Patients with previously documented locally recurrent or metastatic disease at enrollment will be allowed to receive androgen deprivation therapy through their local oncologist as clinically indicated. If they are documented to have further disease progression on protocol re-staging studies that *requires active chemotherapy or radiation therapy as outlined in Off Study Criteria **3.3.3.2**, they will be removed from the study.*

3.2.4 Study Stopping Rules

The study will be halted pending discussing with the FDA and the NCI IRB if real time safety assessments in the first six lead-in study subjects include the following:

- Study enrollment will be halted if 1 or more of the first 3 subjects (101-103) experiences a Grade II or greater adverse event designated as possibly, probably or definitely related to vaccination with the exception of local injection site reactions.

- Study enrollment will be halted if 2 or more of the first 6 subjects (101-106) experiences a Grade II or greater adverse event designated as possibly, probably or definitely related to vaccination with the exception of local injection site reactions.

3.3 STUDY CALENDAR AND ON STUDY EVALUATION

Refer to **Appendix 1** for Study Calendar Schedule of Clinical, Laboratory and Radiographic Evaluations.

3.3.1 Criteria For Removal from Protocol Therapy and Off Study Criteria

A safety visit is required approximately 30-60 days following the last dose of study therapy. A telephone follow-up will take place should a patient be unable to travel to NIH for a clinic visit.

3.3.2 Criteria for Removal From Protocol Vaccine Therapy

3.3.2.1 Persistent failure of autologous ME TARP DC or placebo vaccine to meet DTM release criteria as determined by DTM and the Principal Investigator.

3.3.2.2 Delay in vaccination beyond timeframes established in section **3.2.1**.

3.3.2.3 For Stage D0 Patients: Patient develops evidence of visceral or bony metastatic disease. The patient may receive androgen-deprivation therapy as managed by their local oncologist and may continue on study for monitoring of long-term safety and immune responses.

3.3.2.4 For Stage D0 Patients: Calculated PSADT of <3 months after a minimum of 15 weeks on study or 4 doses of vaccine. Prior to removal from study, PSA check and PSADT calculation should be repeated at the next visit in 3 to 6 weeks from the date PSADT < 3 months is identified for an off-study decision. The patient may receive androgen-deprivation therapy as managed by their local oncologist and may continue on study for monitoring of long-term safety and immune responses.

3.3.2.5 For Stage D0 Patients: Calculated PSADT that has decreased by >50% of their calculated Pre-NIH PSADT at any outlined assessment time points. Prior to removal from study, PSA check and PSADT calculation should be repeated at the next visit in 3 to 6 weeks from the date PSADT decreased by > 50% is identified for an off-study decision.

3.3.2.6 The patient may receive androgen-deprivation therapy as managed by their local oncologist and may continue on study for monitoring of long-term safety and immune responses.

3.3.2.7 Patients experiencing a Grade 3 or greater toxicity outlined in sections **3.1.2.1** to **3.1.2.6** attributed as possibly, probably or definitely related to ME TARP DC vaccination.

3.3.3 Off-Study Criteria

3.3.3.1 Completion of study

3.3.3.2 Patient experiences disease progression requiring active chemotherapy or radiation therapy for treatment of locally recurrent or metastatic prostate cancer.

3.3.3.3 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 **3.1.2** Dose Limiting Toxicity.

3.3.3.4 Development of a second malignancy other than basal cell or squamous cell carcinoma of the skin that is amenable to local treatment.

3.3.3.5 Patient elects to withdraw from study participation at any time.

3.3.3.6 Patient is removed from study by the Principal Investigator or Lead Associate Investigator for reasons other than toxicity e.g. failure to adhere to study visits or due to the presence of an intercurrent medical condition, etc.

3.3.3.7 Investigator decision to end the study

3.3.3.8 Death

Once a subject is taken off study, no further data can be collected.

3.3.4 Off Protocol Therapy and Off Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off protocol therapy and when a subject is taken off-study. A Participant Status Update Form from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov.

4 CONCOMITANT MEDICATIONS /MEASURES

- Study subjects will be allowed to take multivitamins, analgesics (NSAIDS or acetaminophen), antipyretics, and antihistamines for symptomatic relief of local or systemic injection site reactions.
- Patients may receive standard of care medical management e.g. androgen deprivation/hormonal therapy, including changes in therapy (as long as they do not include chemotherapy or radiation therapy) through their local oncologist as clinically indicated.
- Patients are allowed to continue on medications as clinically indicated for treatment of chronic medical conditions e.g. hypertension, diabetes, hypercholesterolemia, etc.
  - *Excluded* Therapy:
    - Chemotherapy: Concomitant use of chemotherapy is not allowed during this trial.
    - Anti-Cancer Radionuclides: Concomitant use of anti-cancer radionuclides or radiation therapy is not allowed during this trial.
    - Corticosteroids: Concomitant, *chronic systemic corticosteroids are not allowed* during this trial (excepting emergent use for clinical indications such as allergic reactions or contact hypersensitivity). *If systemic steroids are given for clinical indications as noted, patients must not have received systemic steroids within 14 days of planned ME TARP peptide DC vaccination.* However, the use of inhaled corticosteroids, intranasal sprays and topical creams on limited body areas *is allowed*.

Vitamin D3 (Cholecalciferol) Supplementation

Vitamin D when ingested is metabolized in the liver to 25-OH vitamin D. Inside cells, it is metabolized further by 1-hydroxylase where it is transformed into a seco-steroid hormone that is important to a host of critical cellular and immune functions within the body. Within cells is a second enzyme 24-hydroxylase whose function is to decrease vitamin D, thereby maintaining intracellular vitamin D homeostasis. The classic function of vitamin D is to regulate calcium homeostasis and in turn, bone formation and resorption. However, additional functions of vitamin D have been demonstrated and include effects on immune response by promoting cellular

apoptosis and differentiation. The exact role of vitamin D deficiency in prostate, breast, colon and other cancers has been controversial, with some laboratory studies suggesting there is a role and other epidemiological studies suggesting that there is no role or even possibly that supplementation should be avoided. In a recent study by Marshall and colleagues (**43**) vitamin D supplementation of 4.000 IU per day for one year was examined in men with low risk prostate cancer (Gleason score of 6, 1-6 cores positive out of 12 possible and a PSA <10) under active surveillance. After one year upon re-biopsy, 60% showed a decrease in the number of positive cores, Gleason score or both and in 6% these factors remained unchanged. In addition, PSA levels did not rise. In another study reported by Wagner and colleagues at AACR in April 2012 (**44**), 66 men scheduled to undergo radical prostatectomy were randomly assigned to receive a daily vitamin D dose of 400, 10,000 or 40,000 IU daily for 3 to 8 weeks prior to surgery. Calcitriol levels in the prostate increased progressively with increasing vitamin D dosing and corresponded with lower levels of Ki67 as well as higher levels of specific growth-inhibitor microRNAs.

Several studies have shown that women with low vitamin D levels have an increased risk of breast cancer incidence and mortality, but research is lacking investigating vitamin D levels and prognostic variables e.g. hormone receptor status, Oncotype DX etc. in this patient population. In a case control study of 194 women s/p breast cancer surgery and 194 cancer-free controls conducted by Peppone and colleagues at the University of Rochester **(45**), women with breast cancer were found to have significantly lower 25-OH vitamin levels than controls (32.7ng/mL vs 37.4 ng/mL respectively, P=0.02). Importantly, women with suboptimal 25-OH vitamin D levels (<32 ng/mL) had significantly increased odds of having ER-negative (OR = 2.59, 95% confidence interval [95% CI] = 1.08-6.23) and triple-negative cancer (OR = 3.15, 95% CI = 1.05-9.49) than those with optimal 25-OH D concentrations. In addition, women with basal-like phenotype had lower 25-OH vitamin D levels than women luminal A phenotype (24.2 ng/mL vs. 32.8 ng/mL, respectively P = 0.04). In summary, women with a more aggressive breast cancer molecular phenotype (basal-like) and worse prognostic indicators (ER- and triple-negative) had lower mean 25-OH vitamin D levels.

Given its critical role in immune function and possible role in cancer pathophysiology, all patients will have serum 25-OH vitamin D levels obtained at baseline. Although there is debate about the target level of 25-OH vitamin D for optimum health, many vitamin D experts agree that it should be greater than the 20 ng/mL recommended by the Institute of Medicine (IOM) and U.S. Food and Nutrition Board (**46**) and that levels of at least 30 ng/mL are necessary for optimum skeletal health as well as the other potential non-skeletal benefits of vitamin D (**47**).

- All patients with 25-OH vitamin D levels < 40 ng/mL will be initiated on oral supplements of Vitamin D3 (cholecalciferol) per standard clinical care guideline.

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH/PHARMACOKINETIC STUDIES

This study does not involve any investigation of pharmacokinetic or pharmacodynamic parameters.

Exploratory analyses will be performed to identify vaccine characteristics using microarrays and TARP-specific cellular or humoral responses. We will also examine circulating tumor cells (CTC) and immune subsets in peripheral blood mononuclear cells including iNKT and myeloid

derived suppressor cells (MDSCs) with Jane Trepel. CTCs will also be analyzed in parallel using technology from RareCyte, Inc. to compare the quantitative parameters with those obtained using the standard techniques in the Trepel lab as part as the CRADA # 03048 (see section **11**). In addition, the under the CRADA, molecular characterization of individual CTCs pre and post vaccination will also be performed.

We will also conduct exploratory analyses of anti-TARP cellular and humoral antibody responses using JPT RepliTope™ TARP peptide microarray platforms to identify immunodominant TARP epitopes associated with ME TARP vaccination in this HLA *0201 patient population. The microarrays comprise purified synthetic peptides derived from the TARP antigen that are chemoselectively and covalently immobilized to a glass surface. An optimized hydrophilic linker moiety is inserted between the glass surface and the antigen derived peptide sequence to avoid false negatives caused by sterical hindrance. After incubation of the peptide microarray with human patient serum, anti-TARP antibodies can be detected using fluorescently labeled secondary antibodies. These resulting antibody signatures represent unique insights into the individual patient's humoral immune responses as well as their antibody specificities. In addition to assessing TARP-specific INF-**γ ELISPOT** responses utilizing a 7 day in vitro stimulation assay (described in detail in Appendix 4), we will also consider further characterizing as indicated cellular responses using TARP-specific CSFE (carboxyfluorescein diacetate, succinimidyl ester) dilution as a measure of proliferation, ELISPOT (perforin and Granzyme B), intracellular cytokine staining (ICS) and tetramer assays.

5.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

5.2.1 Collection and Storage of Research Samples

For research samples obtained for investigation, the Clinical Support Laboratory, Leidos Biomedical Research 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 I.D. 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 I.D. 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. These facilities are operated, under a subcontract to Leidos Biomedical Research. Access to stored clinical samples is restricted. Investigators establish sample collections under "Source Codes" and the investigator responsible for the collections, the protocol Principal Investigator and/or Lead Associate Investigator, specifies who has access to the collection.

When requests are submitted by the NCI investigator for shipment of samples outside of the NIH it is the policy of the laboratory to request documentation that a Material Transfer Agreement (MTA) is in place that covers the specimen transfer. The laboratory does not provide patient identifier information as part of the transfer process but may, at the discretion of the NCI investigator, group samples from individual patients when that is critical to the testing process. The NCI investigator responsible for the sample collection is responsible for ensuring appropriate

IRB approvals are in place and that a MTA has been executed prior to requesting the laboratory to ship samples outside of the NIH.

5.2.2 Future Use / Protocol Completion / Sample Destruction

Blood, urine 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. If new risks are associated with the research (e.g., analysis of germ line genetic mutations.) the Principal Investigator must amend the protocol and obtain informed consent from all research subjects.

Following completion of this study, samples will remain in storage as detailed above unless a patient has opted out of the future use of specimens and data. Currently, there is no plan to use these samples outside of the use described in the protocol.

If the patient withdraws consent the participant's data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2.1**.

6 DATA COLLECTION AND EVALUATION

6.1 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 and its implementation. All on study evaluations must be performed within ± one week for scheduled time points through Week 24 and within ± two weeks of scheduled time points thereafter as outlined in the study calendar (**Appendix 1**). Delay in vaccine administration is allowed as outlined in Section **3.2.1**. Modifications in this policy can be discussed on an individual basis with the Principal Investigator.

1. Each patient must meet all eligibility requirements and a completed registration must be sent to the NCI Central Registration Office (CRO).
2. The Consent Document must be signed prior to registration with the CRO.
3. Treatment will be given according to protocol (on-study and treatment notes, reports of adverse events and documentation of any deviation from the study protocol).
4. Data will be entered into a secure software system (C3D Database) produced by Oracle™ Corporation (Redwood Shores, CA). Data will be collected based on protocol-specific requirements, verified for accuracy and completeness. Any hard copy data will be kept in locked secure area in the Vaccine Branch Clinical Trials Offices.
5. Toxicity will be assessed according to the protocol using the CTCAE v4.0 that is available at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

6. **For Stage D0 patients not receiving Androgen Deprivation Therapy**, response to autologous ME TARP DC or PMBC vaccination will be assessed by calculation of slope log (PSA)/PSADT at every protocol study time point and specifically used to examine:
    - The slope log (PSA for Weeks3-24 minus that formed for the 12 months prior to enrollment on study (referred to as slope324 – pre-slope) as well as the slope log (PSA) for weeks 3-48 versus the same pre-treatment slope log (PSA) (referred to as slope 348 – preslope) in patients receiving active, multi-epitope TARP vaccination.
7. **For Stage D0 patients,** results of re-staging scans at Weeks 48 and 96 will be documented and verified that the patient remains Stage D0 without evidence of progressive disease unless the scans or clinical findings demonstrate otherwise.
8. Drug accountability records will be maintained for each patient.
9. Vaccine report cards (VRC) associated with each autologous ME TARP DC vaccination will be obtained for each patient (see **Appendix 3**). The information recorded in the VRC will also be documented in CRIS.
10. Personal identifiers will not be used when collecting and storing data.
11. An enrollment log will be maintained in the regulatory binder/file that is the only location of personal identifiers with unique subject identification number.

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system (C3D) and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

AEs will be documented starting with the first study intervention through 60 days following the last administration of vaccine. Adverse events that are serious need to be recorded after 60 days following the last intervention, only if they are serious and related to the study intervention.

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
- 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.

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, this will be reported expeditiously per requirements in section **7.2.1**.

6.2 RESPONSE CRITERIA

6.2.1 Immunologic Response Criteria

Immunologic responses to ME TARP peptide vaccination will be assessed at Weeks 0, 12 (after 3 vaccines), 18 (after 5 vaccines), 24, 36 (after 6 vaccines), 48, 72 and 96 in all patients. Descriptions of the primary immune response assays (inclusive but not comprehensive of all immune assays that may be utilized) are found in **Appendix 4.**

A positive immunological response to TARP peptides is defined as:

- A three-fold increase over baseline in the number of positive cells by IFN-γ ELISPOT assay **OR**
- A three-fold increase over baseline in anti-TARP antibody concentrations (measured as mcg/ml) or a 4-fold increase in anti-TARP antibody dilution titers over baseline at the time points measured.
- Positive assays must be confirmed at two study time points to be considered a definitive, ME vaccine-induced positive result.
- 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 IFN-γ ELISPOT or anti-TARP antibody assays. Additional cellular immune response assays such as CSFE, additional ELISPOT (perforin and Granzyme B), intracellular cytokine staining (ICS) and tetramer assays will be explored to further characterize responses to ME TARP vaccination.

6.2.2 Slope Log PSA Response Criteria (Analysis in Stage D0 Patients not receiving Androgen Deprivation Therapy)

For those patients who are still stage D0, the change in slope log PSA from pre-study baseline (-12 months to entry onto the current study) will be compared to the change in slope log PSA at weeks 3-24 and 3-48. **A *negative difference* *in the change in slope log PSA* will be considered a *positive* response.** Slope Log PSA/PSADT will be calculated using the Memorial Sloan-Kettering Cancer Center cancer information prostate nomogram for PSA doubling time found at: http://nomograms.mskcc.org/Prostate/PsaDoublingTime.aspx 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 (*using the same assay*) during a maximum period of 12 months prior to enrollment in the current study will be used as the Pre-Study Baseline. The maximum period of the last 12 months is recommended to reflect current disease activity because in some men PSADT may change over time.

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

6.3 SAFETY AND TOXICITY CRITERIA

The primary objective of this study is to document the frequency of vaccine-related Grade 3 adverse events (local injection site reactions or systemic reactions) and determine if it is less than 10% in this study population previously immunized with TARP vaccine. The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. All enrolled patients will be evaluated for safety and toxicity. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

7 NIH REPORTING REQUIREMENTS / DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found here.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found here. Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found here.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reported to the OHSRP/IRB in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention **s**hould be reported via email to the Clinical Director unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to Dr. Dahut at NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 NIH REQUIRED DATA AND SAFETY MONITORING PLAN

7.4.1 Principal Investigator/Research Team

The clinical research team will meet on a weekly basis when patients are being actively treated on the trial to discuss each patient. Decisions about enrollment will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the Principal Investigator or a Lead Associate Investigator. Events meeting requirements for expedited reporting as described in section **7.2.1** will be submitted within the appropriate timelines.

The Principal Investigator will monitor in real time the safety assessment of the first 6 subjects enrolled, 101-106, for acute or subacute adverse events (other than local injection site reactions) determined to be possibly, probably or definitely related to vaccination occurring within 3 weeks of receipt of the first ME TARP DC vaccine dose. Initial staggered enrollment of the first 3 study subjects and subsequent enrollment will proceed as previously described.

The Principal Investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The Principal Investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.4.2 Safety Monitoring Committee (SMC)

This protocol will require oversight from the Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual NCI-IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period. Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

8 SPONSOR SAFETY REPORTING

8.1 DEFINITIONS

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2))

8.1.2 Serious Adverse Event (SAE)

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 event (see section **8.1.3**)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient convenience) is not considered a serious adverse event.
    - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.
- 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.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32)

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 4.0.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 Assessment of Safety Events

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.

- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to section **6.1**.

8.3 Reporting of Serious Adverse Events

Any AE that meets protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form.

All SAE reporting must include the elements described in section **8.2**.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at:
https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=157942842
Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 REPORTING PREGNANCY

8.4.1 Maternal exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy becomes known,-Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (section **8.1.2**) should be reported as SAEs.
The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

8.4.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 30 after the last vaccine dose.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until 30 days after the last dose should, if possible, be followed up and documented.

8.5 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected in an expedited manner to the FDA in accordance to 21 CFR 31.2.32. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

9 CLINICAL MONITORING

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 and secondary study endpoints; adherence to the protocol, regulations, ICH E6, 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 a CCR 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.

10 STATISTICAL CONSIDERATIONS

10.1 OVERALL STATEMENT OF OBJECTIVES

This protocol will consist of patients enrolled on the prior TARP 09-C-0139 pilot trial that completed the primary series of 5 TARP vaccinations and allow them to receive the updated, 2nd generation ME TARP vaccine under the present study, including patients that may have come off study for disease progression and gone on to receive hormonal treatment or other therapies. The primary objective of this study is the long-term safety of repeated TARP vaccination, in a population that will have received both the bivalent 1st generation and the multi-epitope 2nd generation TARP vaccine.

10.2 PRIMARY OBJECTIVE AND STATISTICAL JUSTIFICATION FOR SAMPLE SIZE

This study will be conducted as a prospective, open label, non-randomized pilot trial to explore the long-term safety of repeated TARP peptide vaccination. As all patients on the prior study may be eligible for inclusion on this study, this cohort may theoretically include up to 40 patients from the prior study, but many may not be available to enroll. (Note: one patient of the 41 patients enrolled on 09-C-0139 came off study prior to completing all 5 doses of TARP vaccine and hence only 40 patients are eligible for the current proposed study). Consequently, all of the analyses conducted as part of this study will be exploratory and descriptive in nature.

Assessment of safety, specifically vaccine-related adverse events (AEs) will be captured for all study patients. Vaccine-related Grade II and III local injection site, systemic, clinical and laboratory AEs will be reported with confidence intervals. We will specifically note the percent of patients with $\geq$ Grade 3 AEs. An acceptable long-term safety profile will be if $\leq$ 10% of patients (e.g. 4 of 40, 3 of 30 or 2 of 20, depending on the total number of subjects that enroll) develop Grade 3 vaccine-related AEs. An ***unacceptable*** response is defined as > than 10% of patients with a $\geq$ Grade 3 vaccine-related AE and will be considered an unacceptable level of toxicity for repeated TARP vaccine exposure and long-term safety. Accrual to the study will stop if 3 or more of the first 20 patients (or 2 or more of the first 10 patients) develop this level of toxicity.

For all patients, several immune parameters obtained at Week 24 post vaccination with the original pilot TARP vaccine will be compared to Week 24 immune parameters post vaccination obtained with the current second generation ME TARP vaccine. It is unknown how many of the 40 patients will be available, but 34 patients with paired data would yield 80% power to detect a difference of 0.5 SD (effect size 0.5) using a paired t-test with a 0.05 two-sided significance level for any given test, and 20 patients would yield 88% power for a 0.75 SD difference.

For previously enrolled patients who continue to have Stage D0 disease, the changes in log slope (PSA) from 3-24 weeks from the first study vs. the second study will be compared within all available patients using a paired analysis, such as a Wilcoxon signed rank test. The actual number of these patients to enroll in this study is unknown and estimated to between 18-22. If there are 16 or more of these patients who enroll on the present study who were on the prior study, there is at least 80% power to find a difference equal to 0.75 SD (0.75 effect size) between the two paired log slope (PSA) differences using a paired t-test with a 0.05 two-sided significance level. Since all of these analyses are considered exploratory, there will be no adjustments for multiple comparisons, but results will be reported and discussed in the context of the number of tests performed and the set of findings obtained.

Thus, up to 40 patients may accrue to this study. While the active accrual window for the study is expected to be 12 months, it is anticipated that accrual will be completed in 36 months due to constraints associated with limited DTM vaccine manufacturing capacity, the NIH PDS quarantine that began in June 2015 and its subsequent impact on enrollment and investigational ME TARP peptide inventories.

11 COLLABORATIVE AGREEMENTS

11.1 COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA)

11.1.1 **CRADA # 03039** was previously in place between the Vaccine Branch, CCR, NCI and PDS Biotechnology, Newark New Jersey in order to carry out ongoing studies of chemistry, manufacturing, control and optimization of the ME TARP peptide dendritic cell cancer vaccine platform utilizing PDS' proprietary Versamune® immunotherapeutic technology to optimize peptide antigen uptake and processing. This will be accomplished by studies in the Cell Processing Section in the department of Transfusion Medicine: parallel DC vaccine manufacturing runs and final product parameters will be compared to document whether there is better antigen expression without compromise of the FDA mandated release criteria. The vaccines were manufactured in parallel with Versamune® will *NOT* be administered to study subjects.

NOTE: As of Amendment E, this CRADA has been discontinued with PDS Biotechnology.

11.1.2 **CRADA # 03048** is in place between the Vaccine Branch, CCR, NCI and RareCyte, Inc to investigate the use of RareCyte's technology as described in section **5.1.**

12 HUMAN SUBJECTS PROTECTION

12.1 RATIONALE FOR SUBJECT SELECTION

All subjects enrolled in this study will be male as the disease under study does not affect women or children. This study is restricted to participants in NCI 09-C-0139 that received at least 5 doses of the 1st generation bivalent TARP peptide vaccine and all subjects are HLA-A*0201 positive.

Participants will be contacted by the Vaccine Branch Clinical Trials Team and assessed for their interest in participation in this long-term pilot follow-up study. Enrollment priority will be given to those subjects that remain Stage D0, followed by those who completed 144 weeks on study and finally those who came off study prior to Week 144.

12.2 PARTICIPATION OF CHILDREN

Children < than 18 years of age are not eligible for this study as the incidence of disease is not applicable to this population.

12.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section **12.4**), all subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team for evaluation. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in NIH HRPP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

12.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

The potential benefit of vaccination with multi-epitope TARP peptides in patients who have previously undergone TARP vaccination is unknown. However, based on immunologic principles of immune boosting with repeated vaccination and the five additional 20 mer peptides included in the 2nd generation ME TARP vaccine platform, we anticipate that ME TARP vaccination will result in enhancement in the magnitude and breadth of TARP-specific responses. This will in turn diminish the likelihood of successful immune escape and evasion by tumor. Specifically, we will be able to determine if ME TARP vaccination will boost and sustain the responses to the WT27-35 and EE29-37-9V TARP peptides included in the original vaccine. It will be very valuable to know whether continued immunizations with the TARP peptides will increase or sustain the immune response over a number of years, and this cohort of patients, *all immunized over at least 2 years ago*, will provide a valuable opportunity to examine the effect of such repeated immunizations with these peptides over several years. In addition, inclusion of longer peptides will allow the generation of humoral anti-TARP antibody responses as well as potentially improve the functional avidity and longevity (**42**) of TARP cellular responses by providing TARP-specific CD4 help at the time of vaccination.

Although a majority of patients (~72%) in the 09-C-0139 TARP peptide vaccine study exhibited a decline in the slope log PSA compared to their Pre-NIH slope that was sustained out to 48 weeks, these responses were associated with absolute declines in PSA levels in only a minority of patients (~15% of responders). Although a smaller cohort of subjects still with Stage D0 will enroll in this trial compared to the original study, immunizing patients that are still Stage D0

with the ME TARP vaccine platform will allow an immediate comparison as to whether the broader antigen repertoire that includes the entire TARP amino acid sequence, results in a more dramatic slowing of PSA velocity or even lowering of absolute PSA values, since each patient services as their own control for comparison of the changes in slope following the 1st and 2nd generation TARP vaccine.

It is unknown and unproven whether slowing PSA velocity through vaccination or any other treatment intervention, will in turn impact clinical outcomes. Continuing long-term observation out to 144 weeks on the final stages of the 09-C-0139 study document that responses to TARP vaccination appear to wane in some individuals (but not others). Boosting these pre-existing responses may lead to more optimal control of tumor growth. Importantly, there were study subjects who had objective evidence of clinical progression following an initial, favorable slowing in PSA velocity. As previously noted, this second generation ME TARP vaccine comprised of longer synthetic peptides will include MHC class II CD4+ T cell helper epitopes that will allow generation of better CD8+ T cell responses with improved functional avidity and longevity (**42**) as well as humoral anti-TARP antibody responses.

TARP peptide vaccination was shown to be safe and very well tolerated in the 09-C-0139 study and we think it is highly likely that the multi-epitope TARP vaccine will have a similar, favorable long-term safety and tolerability profile. Although bivalent TARP vaccination was also associated with induction of TARP-specific cellular immune responses in a majority of patients (~75%), their clinical significance remains unknown as they did *not* correlate with changes in Slope Log PSA. In this study, we will continue to explore immunologic correlates of changes in Slope Log PSA including CSFE, ICS, and anti-TARP antibody responses in addition to IFN-γ ELISPOT reactivity.

12.5 RISKS / BENEFITS ANALYSIS

The potential risk to the study population of patients previously immunized with bivalent TARP vaccine is reasonable in relation to the anticipated potential benefits of immunization with the second generation ME TARP vaccine because

- There was no significant adverse event or safety signal other than local injection site reactions seen in the NCI 09-C-0139 of the first generation TARP vaccine.
- TARP vaccination was documented to be immunogenic and associated with a highly statistically significant decrease in slope log (PSA) at both 24 and 48 weeks post vaccination compared to the Pre-NIH slope log (PSA) baseline.
- There is a reasonable expectation that important knowledge will be gained regarding the safety of repeated and long-term TARP immunization as well as whether boosting and expansion in the breadth of TARP-specific immune responses occurs.
- In the sub-population of study participants that remains D0, there will be an immediate opportunity to assess whether ME TARP vaccination results in a more rapid or greater magnitude of decrease in the slope log (PSA) at 24 and 48 weeks, compared to that seen with the first generation TARP vaccine.

12.6 CONSENT PROCESSES AND DOCUMENTATION

Eligible patients will be presented with a detailed description of the study protocol plan and treatment and provided with a copy of the IRB-approved Informed Consent to review in advance

of any discussions with the clinical trials team. The specific requirements, research objectives, risks, alternatives, time commitments and potential benefits will be reviewed with the patient. 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. All questions about the study will be answered and alternatives to participation will also be discussed. The Principal Investigator, Lead Associate Investigator or their designee is responsible for obtaining a signed protocol Informed Consent using the current version approved by the NCI IRB and posted on the web. The original signed informed consent will be placed in the patient's medical record and a copy will be provided to the patient.

12.6.1 **Telephone re-consent procedure**

Re-consent on this study may be obtained via telephone according to the following procedure: the informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent.

The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone.

A fully executed copy will be returned via mail for the subject's records.

The informed consent process will be documented on in the medical record.

13 PHARMACEUTICAL INFORMATION

13.1 AUTOLOGOUS MULTI-EPITOPE (ME) TARP DENDRITIC CELL VACCINE DESCRIPTION:

The 2nd generation ME TARP vaccine is based on the amino acid sequence of the entire TARP protein annotated below. The vaccine platform *includes the original two 9-mer HLA-A*0201 binding TARP peptide epitopes* (WT27-35 and EE29-37-9V) utilized in NCI 09-C-0139 as well as *an additional proposed five 20-mer TARP peptides* overlapping by 10mer for a total of 7 peptides that span the entire TARP sequence:

- WT TARP 27-35 (9 mer, HLA-A*0201 restricted)
- EE TARP 29-37-9V (also called TARP 29-37 (37V)) (9 mer HLA-A*0201 restricted)
- TARP 1-20: MQMFPPSPLFFFLQLLKQSS (20 mer, HLA *non*-restricted)
- TARP 11-30: FFLQLLKQSSRRLEHTFVFL (20 mer, HLA *non*-restricted)
- TARP 21-40: RRLEHTFVFLRNFSLMLLRG (20 mer, HLA *non*-restricted)
- TARP 31-50: RNFSLMLLRGIGKKRRATRF (20 mer, HLA *non*-restricted)
- TARP 41-58: IGKKRRATRFWDPRRGTP (18 mer, HLA *non*-restricted)

Autologous ME TARP DC vaccine and autologous PBMC placebo vaccine will be generated utilizing cGMP manufacturing conditions by the Department of Transfusion Medicine as outlined in **Appendix 2**.

13.2 INTERLEUKIN-4 CELLGENIX

13.2.1 **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 (≥ 95% chromatographically pure), sterile, water-soluble protein.

13.2.2 **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 at -20°C to -80°C. per manufacturer's storage condition recommendations (http://cellgenix.com/products/recombinant-human-il-4/). .

13.2.3 **Stability and Storage:**

The reconstituted product should be refrigerated as follows:

Store a 250 µg/ml reconstituted cytokine solution:
• 4 weeks at 2°C to 8°C under sterile conditions after reconstitution. Store in the original container.
• 4 months at -20°C to -80°C under sterile conditions after reconstitution. Store in 60 µl aliquots in polypropylene cryogenic vials.
Avoid repeated freeze/thaw cycles

13.2.4 **Administration Procedures:**

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

13.2.5 **Incompatibilities:**

None known in culture.

13.3 KLH (KEYHOLE LIMPET HEMOCYANIN)

13.3.1 **Product Description:**

KLH protein (Product Code KLH-02NV) was purchased from Stellar Biotechnologies, Inc. It will be dispensed to Center for Cellular Engineering in Department of Transfusion Medicine, NIH Clinical Center for use in dendritic cell culture. The KLH formulation to be utilized by NIH has been purified as native molecules and designated as High Molecular Weight KLH (HMW-KLH).

The only source for KLH is the hemolymph of Giant Keyhole Limpets, *Megathura crenulata*, an ocean mollusk. Stellar's clinical grade HWM-KLH is purified under cGMP conditions, and sourced from giant keyhole limpets cultured by Stellar under controlled aquaculture systems.

KLH protein is expressed in two subunit isoforms (KLH1 and KLH2) of approximately 360,000 and 400,000 monomeric molecular weight, respectively. The KLH monomers are each composed of 7 or 8 functional unit domains; each functional unit contains an

oxygen binding site containing two copper atoms. Both KLH isoforms assemble into native homo-decamers and didecamers of 4,000,000 to 8,000,000 molecular weight in hemolymph.

13.3.2 **Formulation and Preparation:**

Stellar high molecular weight KLH is provided in soluble form in a buffer solution that is composed of 10mM sodium phosphate, 135mM NaCl, 1mM $CaCl_2$ and 0.5mM $MgCl_2$ as a bulk drug substance at 5mg/mL and then diluted and vialed into single use vials at 2mg/mL, 250 µL/vial.

13.3.3 **Drug Procurement:**

Stellar Biotechnology's KLH will be purchased from Stellar Biotechnology. It will be dispensed by PDS to the NIH Department of Transfusion Medicine (DTM) Center for Cellular Engineering (CCE) for use in dendritic cell culture.

13.3.4 **Stability and Storage:**

HMW-KLH is stable for at least 12 months when stored at 2 to 8°C. Further extension after 12 months of manufacturing will occur upon review of appropriate stability test results by NIH DTM CCE, according to an established stability testing program.

13.3.5 **Administration Procedures:**

HMW-KLH will be used in vitro by CCE at a concentration of 10mcg/mL for the generation of dendritic cells.

13.3.6 **Incompatibilities:**

No Information Available.

13.4 TARP 27-35 (WILD TYPE) PEPTIDE NSC#740703

13.4.1 **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

13.4.2 **Formulation and Preparation:**

The peptide is vialed in 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. Vaccine preparation will occur according to Protocol Specific Instructions (PSI) and established Standards of Practice (SOP) of The Center for Cellular Engineering, NIH/CC.

13.4.3 **Stability and Storage:**

Store the finished injectable dosage forms in the freezer (-70˚C or below) for long-term storage. Intact vials are stable for at least 6 months when stored at controlled room temperature (15ºC – 30ºC) or in the refrigerator (2ºC – 8ºC), and for at least 36 months when stored in the freezer (-10ºC to -25ºC and -70ºC). The peptide vial contains no

preservatives; once the peptide vial is entered, discard unused peptide solution after 3 hours. Stability will be monitored according to an established NIH DTM CCE stability program.

13.4.4 **Administration Procedures:**

Autologous peptide-pulsed dendritic cell vaccines will be prepared under GMP conditions from cryopreserved patient monocytes. 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-γ. A fraction of autologous dendritic cells will be pulsed separately with TARP WT27-35 peptide. After removing peptide-pulsing media, dendritic cells, individual fractions will be combined and will be concentrated down at 40 x 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.

13.4.5 **Incompatibilities:**

None Known

13.4.6 **Reported Adverse Events and Potential Risks:**

As the TARP peptide is being delivered as a vaccine, likely adverse events include local injection site reactions commonly associated with vaccination. TARP is found in normal prostate tissue as well and over expressed prostate 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 (if not removed with radical prostatectomy). However, this was not observed in the initial pilot 09-C-0139 study investigating TARP peptide vaccination.

13.4.7 **Special Handling:**

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

13.5 TARP 29-37-9V PEPTIDE (EPITOPE-ENHANCED) NSC #740704

13.5.1 **Product Description:**

TARP 29-37-9V is investigational. TARP 29-37-9V, also called TARP 29-37 (37V) 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 9 in this peptide or position 37 in full TARP protein, instead of leucine) to increase its binding affinity and immunogenicity. Amino acid sequence: Phenylalanine-Leucine-Arginine-Asparagine-Phenylalanine-Serine-Leucine-Methionine-Valine (FLRNFSLMV)

13.5.2 **Formulation and Preparation:**

The peptide is vialed in 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 mcL of trifluoroacetate 0.05% v/v. Vaccine preparation will occur according to Protocol Specific Instructions (PSI) and established SOPs of The Center for Cellular Engineering, NIH/CC.

13.5.3 **Stability and Storage:**

Store the finished injectable dosage forms in the freezer (-70˚C or below) for long-term storage. Intact vials are stable for at least 6 months when stored at controlled room temperature (15ºC – 30ºC), at least 9 months when stored in the refrigerator (2ºC – 8ºC), and for at least 36 months when stored in the freezer (-10ºC to -25ºC and -70ºC). The peptide vial contains no preservatives; once the peptide vial is entered, discard unused peptide solution after 3 hours. Stability will be monitored according to an established NIH DTM CCE stability program.

13.5.4 **Administration Procedures:**

Autologous peptide-pulsed dendritic cell vaccines will be prepared under GMP conditions from cryopreserved patient monocytes. 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-γ. A fraction of autologous dendritic cells will be pulsed separately with TARP WT27-35 peptide. After removing peptide-pulsing media, dendritic cells, individual fractions will be combined and will be concentrated down at 40 x 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.

13.5.5 **Incompatibilities:**

None known.

13.5.6 **Reported Adverse Events and Potential Risks:**

As the TARP peptide is being delivered as a vaccine, likely adverse events include local injection site reactions commonly associated with vaccination. TARP is found in normal prostate tissue as well and over expressed prostate 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 (if not removed with radical prostatectomy). However, this was not observed in the initial pilot 09-C-0139 study investigating TARP peptide vaccination.

13.5.7 **Special Handling:**

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

13.6 TARP 1-20 PEPTIDE

13.6.1 **Product Description:**

TARP 1-20 is investigational. Amino Acid Sequence: H-Met-Gln-Met-Phe-Pro-Pro-Ser-Pro-Leu-Phe-Phe-Phe-Leu-Gln-Leu-Leu-Lys-Glyn-Ser-Ser-OH Acetate.

13.6.2 **Formulation and preparation:**

The peptide is vialed in a 2 mL clear type-1, borosilicate glass vial with a 13 mm gray, chlorobutyl, polytetrafluoroethylene (PTFE) “Teflon” lined stopper, and a 13 mm aluminum flip-off seal. Vial contains 1.2 mL of a 1 mg/mL sterile solution of TARP 1-20 Peptide (MPS-479) in dimethylsulfoxide (DMSO) with 0.1% trifluoroacetic acid

(TFA). Vaccine preparation will occur according to Protocol Specific Instructions (PSI) and established SOPs of The Center for Cellular Engineering, NIH/CC.

13.6.3 **Stability and Storage:**

Peptide is stored at -70°C or below. Stability will be monitored according to an established NIH DTM CCE stability program.

13.6.4 **Administration procedures:**

Autologous peptide-pulsed dendritic cell vaccines will be prepared under GMP conditions from cryopreserved patient monocytes. 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-γ. A fraction of autologous dendritic cells will be pulsed separately with TARP 1-20 peptide. After removing peptide-pulsing media, dendritic cells, individual fractions will be combined and will be concentrated down at 40 x 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.

13.6.5 **Incompatibilities:**

None known.

13.7 TARP 11-30 PEPTIDE

13.7.1 **Source:**

TARP 11-30 is investigational. Amino Acid Sequence: H-Phe-Phe-Leu-Gln-Leu-Leu-Lys-Gln-Ser-Ser-Arg-Arg-Leu-Glu-His-Thr-Phe-Val-Phe-Leu-OH Acetate

13.7.2 **Formulation and preparation:**

The peptide is vialed in a 2 mL clear type-1, borosilicate glass vial with a 13 mm gray, chlorobutyl, polytetrafluoroethylene (PTFE) "Teflon" lined stopper, and a 13 mm aluminum flip-off seal. Vial contains 1.2 mL of a 1 mg/mL sterile solution of TARP 11-30 Peptide (MPS-480) in dimethylsulfoxide (DMSO) with 0.1% trifluoroacetic acid (TFA). Vaccine preparation will occur according to Protocol Specific Instructions (PSI) and established SOPs of The Center for Cellular Engineering, NIH/CC.

13.7.3 **Stability and Storage:**

Peptide is stored at -70°C or below. Stability will be monitored according to an established NIH DTM CCE stability program.

13.7.4 **Administration procedures:**

Autologous peptide-pulsed dendritic cell vaccines will be prepared under GMP conditions from cryopreserved patient monocytes. 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-γ. A fraction of autologous dendritic cells will be pulsed separately with TARP 11-30 peptide. After removing peptide-pulsing media, dendritic cells, individual fractions will be combined and will be concentrated down at 40 x 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.

13.7.5 **Incompatibilities:**

None known.

13.8 TARP 21-40 PEPTIDE

13.8.1 **Source:**

TARP 21-40 is investigational. Amino Acid Sequence:: H-Arg-Arg-Leu-Glu-His-Thr-Phe-Val-Phe-Leu-Arg-Asn-Phe-Ser-Leu-Met-Leu-Leu-Arg-Gly-OH Acetate

13.8.2 **Formulation and preparation:**

The peptide is vialed in a 2 mL clear type-1, borosilicate glass vial with a 13 mm gray, chlorobutyl, polytetrafluoroethylene (PTFE) "Teflon" lined stopper, and a 13 mm aluminum flip-off seal. Vial contains 1.2 mL of a 1 mg/mL sterile solution of TARP 21-40 Peptide (MPS-481) in sterile water for injection. Vaccine preparation will occur according to Protocol Specific Instructions (PSI) and established SOPs of The Center for Cellular Engineering, NIH/CC.

13.8.3 **Stability and Storage:**

Peptide is stored at -70°C or below. Stability will be monitored according to an established NIH DTM CCE stability program.

13.8.4 **Administration procedures:**

Autologous peptide-pulsed dendritic cell vaccines will be prepared under GMP conditions from cryopreserved patient monocytes. 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-γ. A fraction of autologous dendritic cells will be pulsed separately with TARP 21-40 peptide. After removing peptide-pulsing media, dendritic cells, individual fractions will be combined and will be concentrated down at 40 x 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.

13.8.5 **Incompatibilities:**

None known.

13.9 TARP 31-50 PEPTIDE

13.9.1 **Source:**

TARP 31-50 is investigational. Amino Acid Sequence: Sequence: H-Arg-Asn-Phe-Ser-Leu-Met-Leu-Leu-Arg-Gly-Ile-Gly-Lys-Lys-Arg-Arg-Ala-Thr-Arg-Phe-OH Acetate

13.9.2 **Formulation and preparation:**

The peptide is vialed in a 2 mL clear type-1, borosilicate glass vial with a 13 mm gray, chlorobutyl, polytetrafluoroethylene (PTFE) "Teflon" lined stopper, and a 13 mm

aluminum flip-off seal. Vial contains 1.2 mL of a 1 mg/mL sterile solution of TARP 31-50 Peptide (MPS-482) in sterile water for injection. Vaccine preparation will occur according to Protocol Specific Instructions (PSI) and established SOPs of The Center for Cellular Engineering, NIH/CC.

13.9.3 **Stability and Storage:**

Peptide is stored at -70°C or below. Stability will be monitored according to an established NIH DTM CCE stability program.

13.9.4 **Administration procedures:**

Autologous peptide-pulsed dendritic cell vaccines will be prepared under GMP conditions from cryopreserved patient monocytes. 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-γ. A fraction of autologous dendritic cells will be pulsed separately with TARP 31-50 peptide. After removing peptide-pulsing media, dendritic cells, individual fractions will be combined and will be concentrated down at 40 x 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.

13.9.5 **Incompatibilities:**

None known.

13.10 TARP 41-58 PEPTIDE

13.10.1**Source:**

TARP 41-58 is investigational. Amino Acid Sequence: H-Ile-Gly-Lys-Lys-Arg-Arg-Ala-Thr-Arg-Phe-Trp-Asp-Pro-Arg-Arg-Gly-Thr-Pro-OH Acetate

13.10.2 **Formulation and preparation:**

The peptide is manufactured by NeoMPS, Inc., 9395 Cabot Drive, San Diego, CA 92126. The peptide is vialed in a 2 mL clear type-1, borosilicate glass vial with a 13 mm gray, chlorobutyl, polytetrafluoroethylene (PTFE) "Teflon" lined stopper, and a 13 mm aluminum flip-off seal. Vial contains 1.2 mL of a 1 mg/mL sterile solution of TARP 41-58 Peptide (MPS-483) in sterile water for injection. Vaccine preparation will occur according to Protocol Specific Instructions (PSI) and established SOPs of The Center for Cellular Engineering, NIH/CC.

13.10.3 **Stability and Storage:**

Peptide is stored at -70°C or below. Stability will be monitored according to an established NIH DTM CCE stability program. Administration procedures: Autologous peptide-pulsed dendritic cell vaccines will be prepared under GMP conditions from cryopreserved patient monocytes. 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-γ. A fraction of autologous dendritic cells will be pulsed separately with TARP 41-58 peptide. After

removing peptide-pulsing media, dendritic cells, individual fractions will be combined and will be concentrated down at 40 x 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.

13.10.4 **Incompatibilities:**

None known.

13.11 VITAMIN D3 SUPPLEMENTS

13.11.1 **Product Description:**

Vitamin D3 (also known as cholecalciferol) is available as an over-the-counter nutritional/vitamin supplement in capsule, tablet and liquid drop form. For study subjects identified to have 25-OH vitamin D levels < than 40 ng/mL on screening, vitamin D3 supplementation will be recommended rather than vitamin D2 since cholecalciferol is the form of vitamin D naturally produced by the skin in response to sun exposure.

13.11.2 **Formulation and Preparation:**

Vitamin D3 supplements are manufactured by multiple suppliers and come in standard dose formulations ranging from 100 to 5000 IU per dose in capsule, tablet or liquid drop form.

13.11.3 **Stability and Storage:**

Oral vitamin D3 supplements should be stored according to the manufactures specifications.

13.11.4 **Administration Procedures:**

Patients will receive Vitamin D3 according to standard of care guidelines. The dose of 2000 IU daily is consistent with the recommended daily dose of 1500 – 2000 IU by the Endocrine Society (**48**) and is also less than the upper limit of 4000 IU/day set by the U.S. Food and Nutrition Board (**46**).

13.11.5 **Incompatibilities:**

None known.

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15 APPENDICES

15.1 APPENDIX 1 SCHEDULE OF STUDY CLINICAL, LABORATORY AND RADIOGRAPHIC EVALUATIONS

Study Procedures	Screening[6] /Apheresis	Wk 3[7] (Baseline[8])	Wk 6[7]	Wk 9[7]	Wk 12[7]	Wk 15[7]	Wk 18[7]	Wk 24[7]	Wk 36[7]	Wk 48[7]	Wk 60[7]	Wk 72[7]	Wk 84[7]	Wk 96[7]	Post Therapy Follow up[9]
NIH Advance Directives Form[1]	X														
History & Physical	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
ECOG Status	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Height	X	X													
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
CBC w/ diff	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
PT/PTT	X	X													
Acute/Hepatic /Mineral Panel	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
TTV Screen[2]	X														
PSA / Testosterone	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
PSADT Calculation[3]	X					X	X	X	X	X	X	X	X	X	
Bone Scan	X									X				X	
CT Scan (C/A/P)	X									X				X	
EKG	X														
Informed Consent	X														
Apheresis[4]	X														
Urinalysis		X								X				X	
TSH		X			X			X		X		X		X	
25-OH Vit D level		X			X			X		X		X		X	
Amylase/Lipase/ Lipid panel		X								X				X	
ABO typing[5]		X													
Lymphocyte phenotyping		X			X		X	X		X	X	X	X	X	
DC vaccine		X	X	X	X	X		X							
Vaccine Report Card		X	X	X	X	X		X							
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	

Study Procedures	**Screening[6] /Apheresis**	**Wk 3[7] (Baseline[8])**	**Wk 6[7]**	**Wk 9[7]**	**Wk 12[7]**	**Wk 15[7]**	**Wk 18[7]**	**Wk 24[7]**	**Wk 36[7]**	**Wk 48[7]**	**Wk 60[7]**	**Wk 72[7]**	**Wk 84[7]**	**Wk 96[7]**	**Post Therapy Follow up[9]**
Phone/mail follow up or communication with local oncologist															X
Research Correlatives															
Anti-TARP Ab		X			X		X	X		X	X	X	X	X	
CTCs, immune subsets		X			X		X	X		X	X	X	X	X	
PBMCs Cellular Responses		X			X		X	X		X	X	X	X	X	

1. As indicated in section **12.3**, all subjects ≥ age 18 will be offered the opportunity to complete an NIH Advance Directives form. This should be done preferably at baseline but can be done at any time during the study as long as the capacity to do so is retained. The completion of the form is strongly recommended but is not required.
2. TTV Screening (Anti-HIV-1/2 Ab, anti-HCV Ab, HBsAg, HBs Ab, anti-HTLV-1/2 Ab, West Nile, T. Cruzi and RPR) must be drawn at the time of screening but for the consent, only HIV, HBV and HCV results are required to enroll. If the day of apheresis is >30 days from the initial TTV screening, TTV screening needs to be repeated to be compliant with DTM requirements.
3. PSADT will be calculated only when the patient is in D0 prostate cancer.
4. Apheresis should occur within 6 weeks after consent. TTV screening will need to be repeated if >30 days from screening TTV lab draws, per DTM requirements. Apheresis may be repeated any time if additional plasma or cell aliquots are required to manufacture the vaccine.
5. Historic record is acceptable if previous NIH results are available.
6. Screening studies including scans should occur within 60 days of enrollment at the NIH unless otherwise specified (see section **2.2**).
7. Evaluation to proceed with vaccine administration should occur within 10 days prior to administration. Study weeks will be numbered so that they match vaccine dose weeks, in case off-target dates in investigational product administration occur. The dosing interval should be at least 14 days.
8. Evaluations at Week 3 serves as baseline. If amylase, lipase, lipid panel, TSH, 25-OH Vitamin D, lymphocyte phenotyping, urinalysis have already been completed within 30 days prior to the first dose of administration of investigational product, they do not need to be repeated at the baseline (Week 3) timepoint.
9. Post-therapy follow up will occur annually until disease progression or death.

15.2 APPENDIX 2 MANUFACTURING SUMMARY FOR DENDRITIC CELL VACCINE AND ELUTRIATED MONOCYTE PLACEBO

Autologous Cell Harvest

Blood collection shall be a standard leukapheresis. Total volume of 15 to 18 liters of whole blood will be processed in order to collect peripheral blood mononuclear cells (MNC) with a target number of at least 2.2×10^9 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 venous catheter will be placed as an outpatient, if indicated, for collection on the day of apheresis. The venous catheter will be inserted by appropriately trained personnel in special procedures with removal of the catheter by 3SE day hospital or Vaccine Branch clinical staff. Prophylactic intravenous CaCl2 and MgSO4 infusions may be administered during apheresis to treat or prevent citrate toxicity at the discretion of the DTM physician per routine. If the collected plasma volume or cells is not enough to make necessary aliquots for vaccine doses, one additional apheresis to meet this need can be performed during the study period.

Patient Cell Processing
All cell processing will be conducted in accordance with approved Protocol Specific Instructions (PSI) and established Standards of Procedures (SOP) of The Center for Cellular Engineering, NIH/CC/DTM.

ME TARP Peptide-Pulsed Dendritic Cells

Background: Autologous dendritic cells prepared from peripheral blood monocytes will be loaded with the following 7 different TARP-derived peptides:

- WT TARP 27-35 (9 mer, HLA-A*0201 restricted)
- EE TARP 29-37-9V (also called TARP 29-37 (37V)) (9 mer HLA-A*0201 restricted)
- TARP 1-20: MQMFPPSPLFFFLQLLKQSS (20 mer, HLA *non*-restricted)
- TARP 11-30: FFLQLLKQSSRRLEHTFVFL (20 mer, HLA *non*-restricted)
- TARP 21-40: RRLEHTFVFLRNFSLMLLRG (20 mer, HLA *non*-restricted)
- TARP 31-50: RNFSLMLLRGIGKKRRATRF (20 mer, HLA *non*-restricted)
- TARP 41-58: IGKKRRATRFWDPRRGTP (18 mer, HLA *non*-restricted)

Different fractions of autologous dendritic cells will be pulsed individually with only one of these peptides and the seven fractions will be combined before administration to the patient.

Formulation and Preparation ME TARP DC Vaccine*:* Autologous peptide-pulsed dendritic cell vaccines will be prepared under cGMP conditions from cryopreserved patient monocytes obtained at during the original Week 0 apheresis. Autologous monocytes for dendritic cell culture will be enriched from peripheral blood MNC apheresis collections by counter-flow elutriation, aliquoted into at least 8 vials with ~ 333 x 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-γ, and pulsed with TARP peptide. After removing

peptide-pulsing media, dendritic cells will be concentrated down at 40 x 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.

Stability and Storage: Autologous ME TARP peptide-pulsed dendritic cell vaccines will be harvested from the 5-day culture product and will be packaged for fresh administration on the same day according to Standard Operating Procedures of the Department of Transfusion Medicine. A fixed autologous ME TARP peptide-pulsed dendritic cell vaccine dose of 20 X 10^6 *viable* cells/ in 1.0 ml delivered as two 0.5ml intradermal injections will be administered immediately upon receipt in the clinical setting. Post packaging tests indicated that the product is stable for at least 2 hours.

15.3 APPENDIX 3 VACCINE BRANCH MULTI-EPITOPE TARP VACCINE REPORT CARD

Date of Administration (Day 1):_____Administered By: _____,RN

Vaccine Dose #: ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 [$20x10^6$ viable dendritic cells]

Total Injection Volume ☐ 0.5 x 2 = 1.0 (ml)

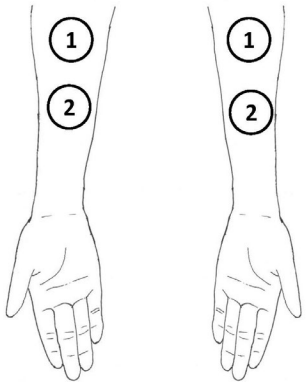
NOTES:

1. For dose #1, vital signs should be documented pre-dose and 15, 30, 45 and 60 min after vaccine administration. For all subsequent doses, vital signs should be documented pre-dose and 15 minutes after vaccine administration.
2. Investigational product labels must be placed on a progress note and sent to the Health Information Management Department to be scanned into CRIS.

	Injection Site: Forearm	**Injection Volume**	**# Cells / Injection**	**ID Wheal Present?**	**Today Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Date resolved**
Site #1	☐Left ☐Right	☐ 0.5 mL	$10x10^6$/0.5 ml	☐Present ☐Absent	☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ 0 ☐ 1 ☐ 2 ☐ 3	
Site #2	☐Left ☐Right	☐ 0.5 mL	$10x10^6$/0.5 ml	☐Present ☐Absent	☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ 0 ☐ 1 ☐ 2 ☐ 3	

- Use back of this page if reaction lasts more than 5 days or need more space to describe the symptoms.

☐ Right ☐ Left



Injection Site Reaction Grading (NCI CTCAE v4.03)

☐ **0** No Reaction
☐ **1** Tenderness *with or without* associated warmth, redness, itching
☐ **2** Pain, swelling (edema), lipodystrophy (shrunken area of the skin due to loss of fat), or phlebitis (inflammation of veins in the skin)
☐ **3** Ulceration or necrosis (skin break down); severe tissue damage. Operative intervention indicated.
☐ **4** Life-threatening consequences. Urgent intervention indicated.

Reported by (print name):_____

Instructions to team: 1) Request that patient submits VRC via mail or at next visit 2) Keep a hard copy in the study chart 3) Document the findings in CRIS using free text VRC template.

15.4 APPENDIX 4 DESCRIPTION OF ANTI-TARP ANTIBODY AND CELLULAR IMMUNE RESPONSE ASSAYS

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:

Quantitative Anti-TARP Antibody Testing: Weeks 3, 12, 18, 24, 48, 60, 72, 84 and 96

Purpose: To determine the immunogenicity of autologous multi-epitope TARP dendritic cell vaccination as measured by a 3-fold increase in anti-TARP antibody concentration (measured as mcg/ml) or a 4-fold increase in antibody dilution titers over baseline.

Specimen Processing: 1 10ml Red Top Clot activator

Specimens will be processed by Dr. Jon Inglefield at the Clinical Support Laboratory Leidos Biomedical Research. Serum will be aliquoted into vials and cryopreserved until ready for interrogation in batched specimen assays.

TARP-Specific Cellular Responses*:* Weeks 3, 12, 18, 24, 48, 60, 72, 84 and 96

CFSE Proliferation, ICS, ELISPOT (IFN-γ, Granzyme B, Peforin) & Tetramer Assays:

6 10ml Green Top Heparinized Tubes (60 ml total)
Send via Frederick Courier to NCI Frederick Clinical Support Laboratory (Dr. Jon Inglefield) for specimen processing and freezing.
Note: PBMCs collected via apheresis that have completed processing by DTM and been subsequently cryopreserved by NCI Frederick, will be used for Week 0 cellular response assays.

Assays will be performed by the flow cytometry unit in the laboratory of Dr. Jon Inglefield.

The Clinical Support Laboratory, Leidos Biomedical Research, 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. These facilities are operated by ATCC under subcontract to Leidos Biomedical Research. Access to stored clinical samples is restricted. Investigators establish sample collections under "Source Codes" and the investigator is responsible for the collections, 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. The PI will record any loss or unanticipated destruction of samples as a deviation. Reports will be made per the requirements of section **7.2**.

Description of 7 Day IVS TARP IFN-γ ELISPOT Assay

Peptides

- TARP WT 27-35
- TARP WT 29-37
- TARP EE 29-37
- HIV-gag
- Human T-lymphotropic virus, type I (HTLV-I) Tax 11-19 (New England Peptide, Gardner, MA)
- Influeza Matrix Peptode FluM1 58-66 (FMP, American Peptide Company, Sunnyvale, CA)
- CEF peptide pool: 23 peptides from MHC class I-restricted T-cell epitopes from human CMV, EBV and influenza virus, designed to stimulate T cells from donors with a variety of HLA types (Mabtech, Mariemont, OH)

Cell Culture Conditions

Frozen patient PBMC are thawed, resuspended and plated into wells of 24-well tissue culture plates (Corning, NY) at 2-3e6 viable cells/well in CTL media containing a 1:1 ratio of RPMI (Gibco, Chicago, IL) and Click's Medium (Sigma, St. Louis, MO), 10% human AB serum (Gemini `Bio-Products, West Sacramento, CA), 1% Pen/Strep-L-glutamine (Gibco, Chicago, IL), 1% sodium pyruvate (Gibco, Chicago, IL) 35mM HEPES (Gibco, Chicago, IL) and 50uM 2-mercaptoethanol (Sigma, St. Louis, MOO. The PBMC are stimulated simultaneously with TARP WT 27-35, TARP WT 29-37 and TARP EE 29-37 peptides at 10ug/mL in a 37°C humidified 10% CO_2 atmosphere in the presence of 1000u/mL recombinant human IL-7 (Peprotech, Rocky Hill, NJ) on day 0. Recombinant human IL-2 (Tecin; Roche, Nutley, NJ) was added on day 3 at 20u/mL. On day 5-6, half of the culture supernatant is replaced with fresh CTL media without anyadditional cyotokines and then the cells are harvested on day 7-8. Additional patient PBMC and normal controls are stimulated in a similar way with 7.5ug/mL FMP.

Enzyme-Linked Immunosorbent Spot (ELISPOT) Assay.

All ELISPOT assays are performed at the Laboratory of Cell-Mediated Immunity at Leidos Biomedical, Inc. (formerly SAIC-Frederick, Inc.) which is certified by Clinical Laboratory Improvements Amendments (CLIA). Two frozen normal donor controls with known response values are run with each assay to assure quality control of the assay results. For all assays, at least one of the two controls is within 2SD of the lab-generated means for FMP and CEF. All assays are performed on 7-8 day in vitro stimulated PBMC (100K or 25K/well) as the effectors and peptide-pulsed autologous day 0 PBMC (100K or 25K/well) as the antigen presenting cells (APC) at a 1:1 ratio in ELISPOT media containing RPMI (Gibco, Chicago, IL), 5% human AB serum (Gemini Bio-Products, West Sacramento, CA), 1% Pen/Strep-L-glutamine (Gibco, Chicago, IL) and 2.5% HEPES (Gibco, Chicago, IL). Briefly, the day before assay setup, 96-well polyvinylidene fluoride (PVDF) membrane, HTS opaque plates (Millipore, Billerica, Massachusetts) are coated overnight with a 1:100 dilution of anti-human IFN-γ capture antibody (1mg/mL, Mabtech Inc., Mariemont, OH) in DPBS at room temperature. Antibody-coated plates are washed four times in DPBS the next day and blocked with 5% human AB ELISPOT medium

at 37°C for approximately 2 hours. The effectors and APC are plated and incubated for 18-20 hours at 37°C and 5% CO_2. The next day, the plates are manually washed six times with 0.05% Tween 20 in DPBS, followed by a 2-hour incubation at room temperature with a 1:2000 dilution of the biotinylated secondary antibody, anti-human IFN-γ (1 mg/mL Mabtech Inc., Mariemont, OH) in DPBS/1% bovine serum albumin/0.05% Tween. After incubation and four washes in DPBS to remove excess antibody, a 1:3000 dilution of streptavidin alkaline phosphatase (Mabtech, Mariemont, OH) in DPBS/1% bovine serum albumin, is added to each well for 1 hour at room temperature followed by 4 manual washes in DPBS. Finally, the BCIP/NPT substrate, 100 ul/well, (KPL, Gaithersburg, Maryland) is added for 7-10 minutes, resulting in the development of spots. The reaction is stopped by washing three times in distilled water. Plates are dried overnight and the spots are visualized and counted using the ImmunoSpot Imaging Analyzer system (Cellular Technology Ltd., Cleveland, OH). ELISPOT results are expressed as the "number of spots per 10^6 responder cells" after subtracting background spots obtained in wells of effectors with non-pulsed PBMC. In addition to the analysis of *in-vitro* stimulated cells, all patient PBMC from weeks 0 and 24 and normal control PBMC are analyzed for responses to CEF and PHA and appropriate donors are also analyzed for their responses to FMP and KLH. For each patient, PBMC from multiple timepoints are analyzed in the same assay to avoid inter-assay variability.

15.5 APPENDIX 5 GUIDELINES AND WORKSHEET FOR CALCULATIONS OF SLOPE LOG PSA / PSADT-STAGE D0

Pre-Enrollment/Baseline and On Study Slope Log / PSADT Calculation:

- ☐ PSADT will be calculated using the Memorial Sloan-Kettering Cancer Center cancer information prostate nomogram for PSA doubling time found at: http://nomograms.mskcc.org/Prostate/PsaDoublingTime.aspx
- ☐ Minimum requirements for PSADT include ≥ 3 PSA measurements over ≥ 3 months.
- ☐ The interval between PSA measurements must be ≥ 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.
- ☐ **PSA values used in the calculation of PSADT must have been *performed by the same laboratory or methodology.***
- ☐ All PSA values used in the calculation should be ≥ 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 a given protocol time point will be included in the Slope Log PSA / PSADT calculation to reflect the patient's current disease activity.

Date Range of PSA Values (should not exceed 24months): _____

Cumulative Total Months of PSA Values (should not exceed 24 months):_____

Receiving Flomax: ☐ YES ☐ NO **Start Date:**_____**On at least 3 Months:** ☐ YES ☐ NO

Receiving 5ARI: ☐ YES ☐ NO **Start Date:**_____ **On at least 3 Months:** ☐ YES ☐ NO

DATE	PSA ng/mL	TESTOSTERONE ng/dL	PSADT

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

Calculated Pre-Enrollment/Baseline Slope Log PSA / PSADT (in months):_____

Calculated Pre-Enrollment/Baseline Slope Log PSA / PSADT (in months):_____

A Slope Log PSA Response is Defined As:

- **A *negative difference* *in the change in slope log PSA* (Pre-NIH Slope Log PSA minus Wk3-24 or Wk3-48 Slope Log PSA) will be considered a *positive* response.**

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

PSADT/Slope Log PSA will be calculated Weeks15, 18, 24, 36, 48, 60, 72, 84, 96.

DATE	STUDY WEEK	PSA ng/mL	TESTOSTERONE ng/dL	Calculated PSADT / Slope Log PSA
	0			**This value *NOT* used in calculations**
	3			**PSADT is not calculated at this point**
	6			**PSADT is not calculated at this point**
	9			**PSADT is not calculated at this point**
	12			**PSADT is not calculated at this point**
	15			
	18			
	24			
	36			
	48			
	60			
	72			
	84			
	96			

On Study *Sequential* PSADT Calculated *for Statistical Analysis*			
Analysis Window	**Study Wk PSA Values Included**	**Calculated PSADT / Slope Log PSA**	**Pre-NIH Slope Log PSA Minus Wk3-24 /Wk3-48**
Pre-NIH	***Outside* PSAs -24 Months to Entry**		
Week 3 to Week 15	Wks 3, 6, 9, 15		
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		

15.6 APPENDIX 6 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:

IFN-γ ELISPOT, CFSE, ICS, Tetramer Assays:

6 10ml Green Top Heparinized Tubes (60 ml total)
Send via Frederick Courier to NCI Frederick Clinical Support Laboratory of Dr. Jon Inglefield for specimen processing and freezing.

Tetramer Assay: Will be performed by the flow cytometry unit in the NCI Frederick Clinical Support laboratory of Dr. Jon Inglefield.

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

Multiparameter Flow Cytometric Analysis of Circulating Tumor Cells (CTC) and Other Immune Cell Subsets: Jane Trepel, DTB

Analysis will be performed by the laboratory of Jane Trepel. CTC cells and other immune cell subsets will be identified by mulitparameter flow cytometry. The order of priority for immune subset analysis is T, B, NK, NKT, Tregs, MDSC and dendritic cells.

CTC Analysis: One 10ml lavender top tube
(Weeks 3, 12 (s/p 3 doses of vaccine), 18 (s/p 5 doses of vaccine), 24, 48, 60, 72, 84, and 96)

Highly multiparametric flow cytometry with on-line physical isolation employing a Miltenyi Biotec platform will be utilized for interrogation of CTC with customized parameters including tumor markers and hematopoietic markers.

Immune Cell Subset Analysis: One 10ml lavender top tube
(Weeks 3, 12, 18, 24, 48, 60, 72, 84 and 96)

Multiparameteric flow cytometry using a Miltenyi Quant flow cytometer. The order of priority for immune subset analysis is T, B, NI, NKT, Tregs, MDSC and dendritic cells.

iNKT Cell Analysis: One 10ml lavender top tube
(Weeks 3, 12, 18, 24, 48, 60, 72, 84 and 96)

Sample Logistics for both Function-Associated mRNAs and CTC and Other Immune Subsets:

- **Notify the Trepel lab via email** when the clinical sample is scheduled to be drawn:
  - Sunmin Lee (lees@pop.nci.nih.gov)
  - Min-Jung Lee (leemj@mail.nih.gov)
  - Jane Trepel (trepel@helix.nih.gov)

- Label the clinical specimen tubes and *include the study week number*.
- **Note: *specimen should be drawn before 1pm*** to allow adequate time for processing.
- **Phone the Trepel lab at 240-760-6330** when the specimen is drawn for pick up by the Trepel lab.
- The laboratory of Jane Trepel where specimens will be processed and cryopreserved is in Bldg.10, Rm. 12C208.