Abbreviated Title: TARP Phase II
CC Protocol #: 15-C-0075 H
Version Date: February 11, 2020
NCT Number: NCT02362451

Title: A Randomized, Placebo-Controlled Phase II Study of Multi-Epitope TARP Peptide Autologous Dendritic Cell Vaccination in Men with Stage D0 Prostate Cancer

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Investigational Agent:

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

Commercial Agents: none

Identifying Words: Multi-Epitope TARP, autologous dendritic cell vaccine, D0 prostate cancer, elutriated monocyte placebo vaccine, prostate specific antigen doubling time (PSADT)

PRÉCIS

TARP

- T-cell receptor γ alternate reading frame protein (TARP) is a 58 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 difference in the slope log (PSA) for Weeks 3-24 minus that formed for the 12 months prior to enrollment on study (referred to as slope 324 – 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 – pre-slope) in patients naïve to TARP vaccination receiving active, multi-epitope TARP vaccination *vs.* placebo.

Eligibility

- Males ≥ 18 years of age with histologically confirmed adenocarcinoma of the prostate.
- Stage D0 disease with documented biochemical progression documented by rising PSA and no evidence of metastatic disease by physical examination, CT scan or bone scan.
- PSADT ≥ 3 months and ≤ 15 months:
    - Patients must have ≥ 3 PSA measurements over ≥ 3 months.
    - The interval between PSA measurements must be ≥ 4 weeks.
- Performance Status: ECOG 0-1.
- No other concurrent anticancer therapy or prior prostate cancer vaccines expressing TARP.

Study Design:

- Phase II, prospective, single-blinded, randomized, placebo controlled study in men with Stage D0 prostate cancer. Men with a PSADT ≥ 3 months and ≤ 15 months will be randomized 2:1 to receive ME TARP autologous DC vaccination or a control elutriated monocyte vaccine placebo.
- An initial lead-in of 6 patients will be enrolled to allow preliminary assessment of the safety of the ME TARP vaccine platform through 12 weeks before enrollment of prospectively randomized subjects blinded to treatment assignment begins.
- All patients will receive a total of 6 doses of vaccine (20 x10^6 *viable* cells/dose) delivered intradermally at Weeks 3, 6, 9, 12, 15, and 24. All patients will undergo a 15-18L apheresis at Week 0 and restaging at Weeks 48 and 96 to confirm maintenance of Stage D0 disease.
- Sample size: N = 72 (6 lead-in patients for safety assessment, 2:1 randomization: TARP N = 44; placebo N =22).

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective:

- To assess the difference in the slope log (PSA) for Weeks 3-24 minus that formed for the 12 months prior to enrollment on study (referred to as slope 324 – 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 – pre-slope) in patients naïve to TARP vaccination receiving active, multi-epitope TARP vaccination *vs.* placebo.

1.1.2 Secondary Objectives:

- To assess the safety of multi-epitope TARP peptide autologous DC vaccination.
- To characterize cellular and humoral immune responses associated with multi-epitope TARP peptide autologous DC vaccination as measured by anti-TARP antibodies and TARP-specific CSFE (carboxyfluorescein diacetate succinimidyl ester) proliferation, ELISPOT (IFN-γ, perforin and Granzyme B), intracellular cytokine staining (ICS) and tetramer assays.
- To conduct an exploratory comparative analysis of slop 324 – pre-slope and slope 348 – pre-slope differences in HLA-A*0201 patients versus <u>non</u>-HLA*A0201 patients.
- To conduct an exploratory assessment of progression free survival (PFS) at 96 weeks in patients receiving multi-epitope TARP peptide autologous DC vaccination

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 and/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

CRITERIA	TOP SUBCRITERIA
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 antigens 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 (9 – 11 mers) 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-

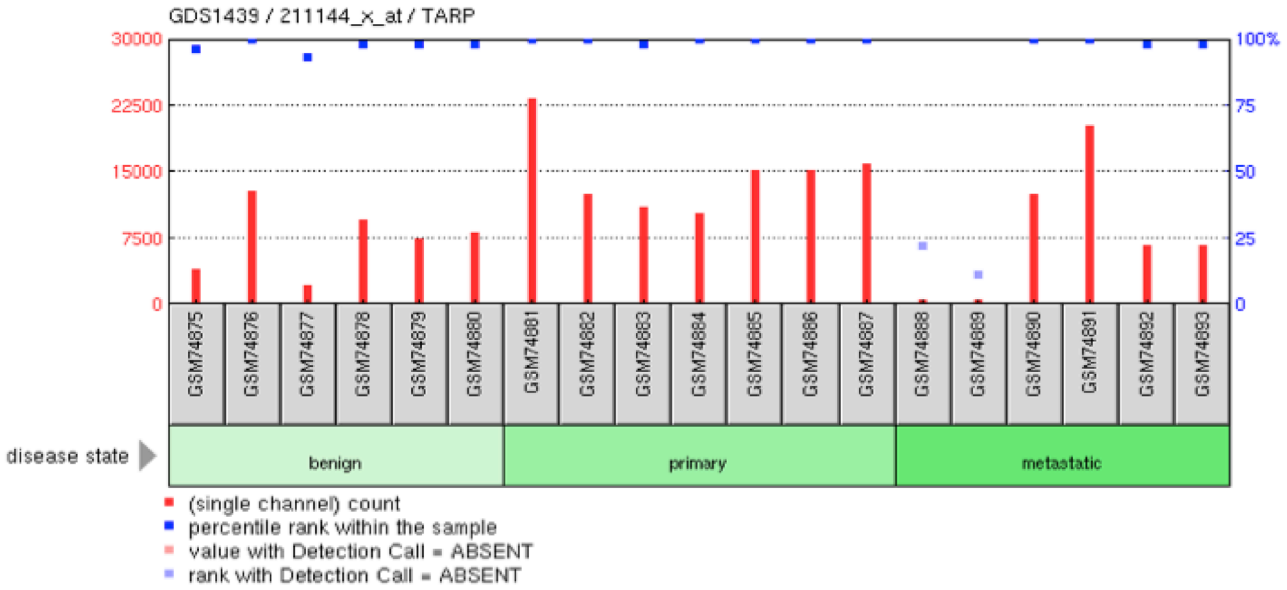
restricted tumor associated peptide derived from highly over-expressed tumor antigens) delivered following a single dose of cyclophosphamide was reported with improved overall survival in patients with renal cell carcinoma (RCC). (**6**)

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:

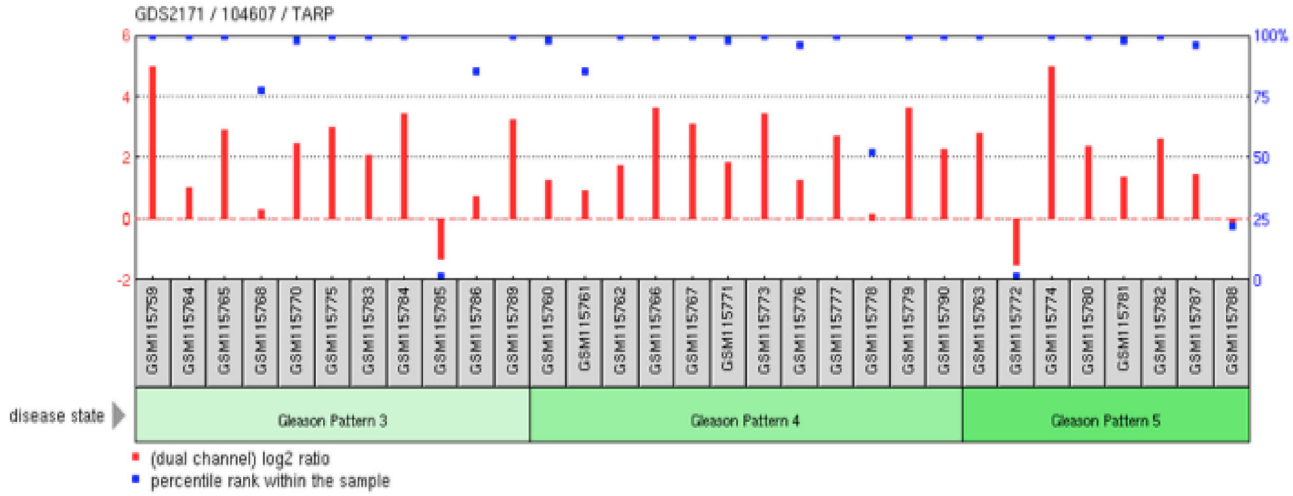
- Highly expressed in primary as well as metastatic prostate cancer (**Figure 1**)
- Expressed in prostate cancers with a range of Gleason patterns (**Figure 2**)
- Expressed in both hormone sensitive and castrate resistant prostate cancer (**Figure 3**)

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



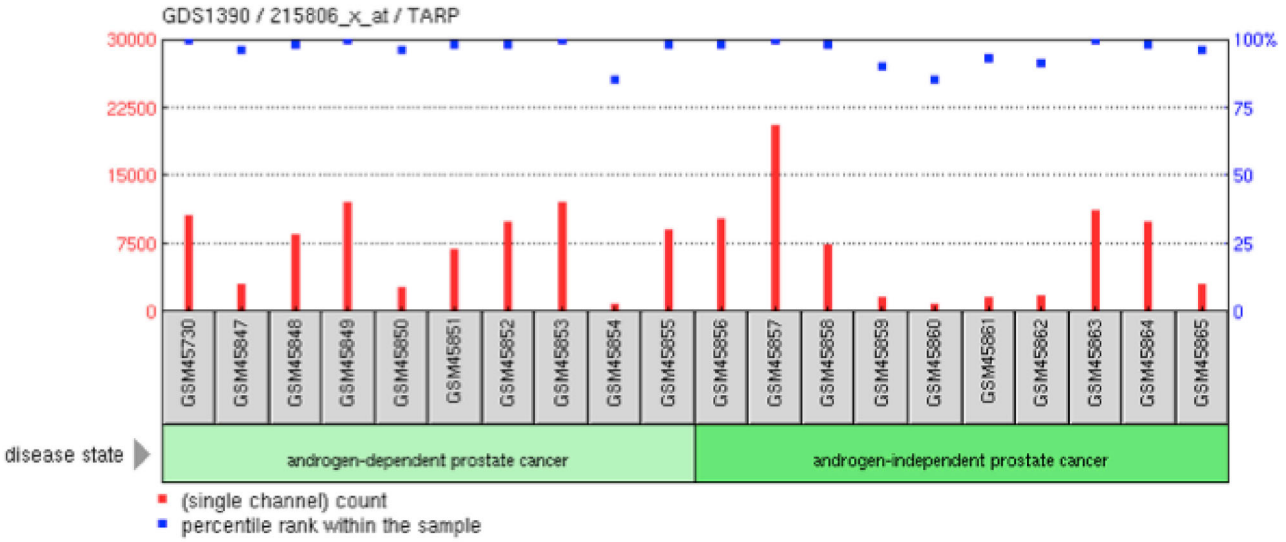
NCBI Dataset Record GDS1439
Citation: Varambally S, Yu J, Laxman B, Rhodes DR et al. Integrative genomic and proteomic analysis of prostate cancer reveals signatures of metastatic progression. Cancer Cell 2005 Nov;8(5):393-406. PMID: 16286247

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 **Figure 2** and **Figure 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 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 $CD8^+$ T cells specific than the wild type sequence. It was also shown that T cells generated with the epitope-enhanced TARP29-37 sequences reacted with targets pulsed with the wild type TARP29-37 peptide in the mouse.

Although immunogenicity of these peptides was demonstrated in the mouse it 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 is 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. Base line demographics for all patients are highlighted in **Table 1**:

	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 at least two time points) at Week 24. TARP vaccination was found to be safe and well tolerated, with adverse events limited to injection site reactions ≤ Grade 2. There were no

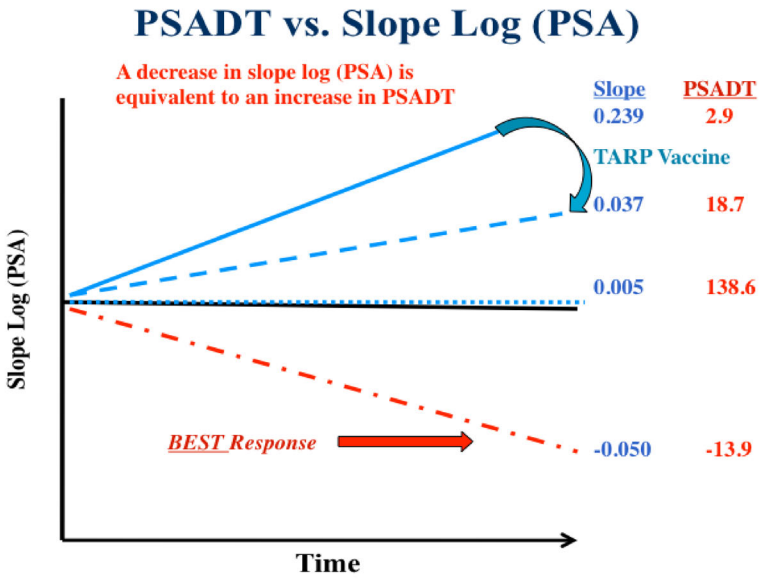
systemic or immediate hypersensitivity reactions or laboratory abnormalities associated with vaccination.

As of 09/02/14, 2 patients currently remain on study for follow up. Patients have gone off study for the following reasons:

Off Study Reason	**Median Off Study Week 75 (range 9-144 weeks)**
Completed Study	N=18
DC Vaccine Viability Issues	N=1
Lost to Follow-Up	N=1
Patient Request	N=4
Progression	N=12 (bone metastasis, LAN w/ obstructive uropathy, nodal involvement)
PSADT Criteria	N=2
Secondary Malignancy	N=3 (superficial MM, invasive SCC of tongue (smoker) colon adenocarcinoma)

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 in **Figure 4** below.

Figure 4



Week 24 and Week 48 slope log (PSA) responses to TARP vaccination through 07/31/12 are summarized in **Table 2** 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**	**28.2% of All Patients**
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 =	**31**	
Arm A	16	
Arm B	15	
Decreased Slope	**23**	**74.2% of All Patients**
Arm A	10	62.5% of Arm A Pts
Arm B	11	86.7% of Arm B Pts
Increased Slope	**8**	**25.8 % of All Patients**
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 final formal 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)
- Wk 3-24 = slope log (PSA) @NIH (PSAs from Weeks 3 to 24)
- Wk 3-48 = 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, although there was *no* statistically significant difference between the two arms.

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,* although there was *no* statistically significant difference between the two arms.

These changes in slope log (PSA) at Week 24 and at Week 48 are graphically highlighted in waterfall plot data in **Figure 5** and **Figure 6** below for individual study patients (data through 07/31/12):

Figure 5

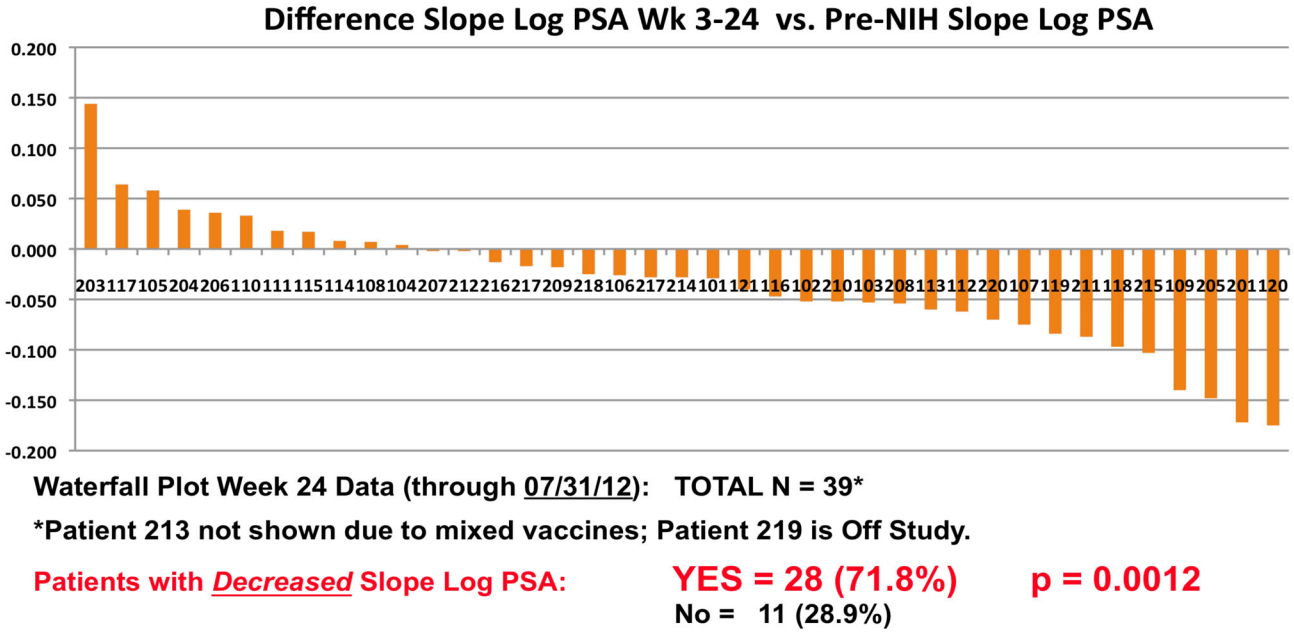
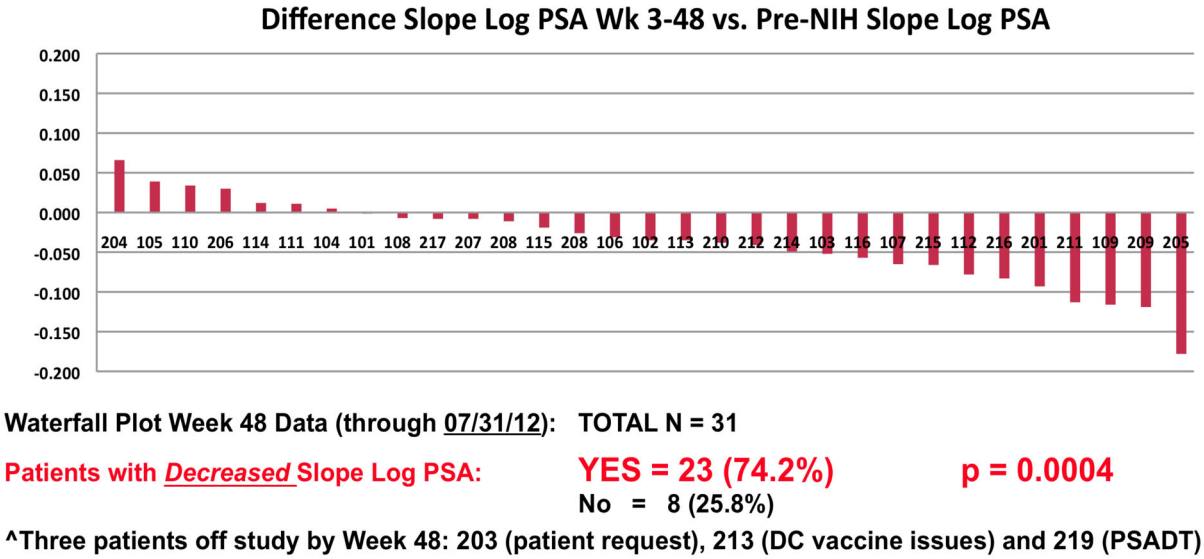


Figure 6



Within each arm the decrease in the slope log (PSA) was *not* statistically significant, except in Arm B at 48 weeks, which was highly statistically significant (p = 0.0069). However, there was no formal statistically significant difference observed between Arm A and Arm B. at either 24 or 48 weeks. Hence, although the arms may not be equal, our analysis does not demonstrate a significant difference between the two. Importantly, the effect of decreased slope log (PSA) at Weeks 3-24 is similar to that at later time periods i.e. it doesn't wane significantly over time and isn't impacted by an additional vaccine dose at Week 36. In addition, *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 24, *it was associated with an absolute decrease in PSA in only a minority of patients* as highlighted in **Table 3** 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	

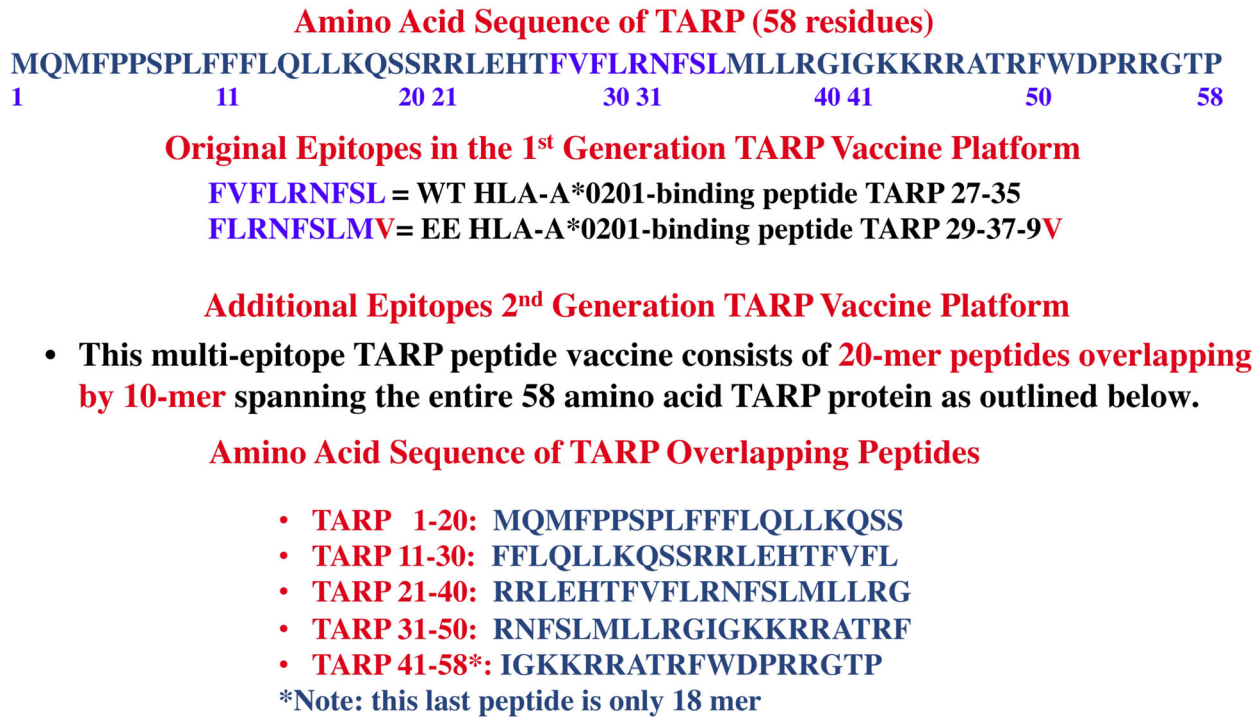
***At Week 24:Patient 213 excluded due to mixed vaccines** **^At Week 48: Two patients Off Study- 203 and 213**
Note*: Patient 109 on Arm A, and Patients 201 and 209 had sustained decreases in Week 24 and 48 PSAs when compared to Week 0.*

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. However, increases in TARP-specific reactivity were seen in PSADT responders (declining slope log (PSA)) as well as PSADT non-responders (increasing slope log (PSA)). Additional studies to date examining functional avidity of ELISPOT responses, demonstrated no differences between slope log PSA responders and non-responders. Studies of tetramer responses and polyfunctional intracellular cytokine staining (ICS) (including Granzyme A and perforin) for assessment of TARP-specific cellular reactivity are in progress. Investigation of anti-TARP antibody responses using microarray platform technology spanning the entire TARP protein is also underway.

1.2.5 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 10mer for a total of 7 peptides that span the entire TARP sequence as outlined in **Figure 7**:

Figure 7



The advantage of this multi-epitope TARP peptide vaccine platform is that the overlapping epitopes cover the entire TARP protein, eliminating the need for HLA restriction in study participants that will greatly facilitate the rate of study accrual. 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.

1.2.6 Rationale for Proposed Phase II Study Design

The rationale for the current phase II study design is to definitively establish that the observed declines in the difference in the slope log (PSA) documented in our initial pilot 09-C-0139 TARP study are indeed real and not due to spontaneous or random slowing in the rate of PSA rise. The observed decreased PSA slope in over 70% of all the patients with a vaccine that has no clinically significant adverse effects other than mild local injection site reactions (< Grade II) is an extremely promising result for any cancer therapy that needs to be confirmed in a prospective, randomized study. Therefore, it is essential to carry out a randomized, placebo-controlled phase II study. However, to accrue a large enough population for such a randomized phase II study, enrollment cannot be restricted to HLA-A2 positive patients and the excessive screening that entails. We must be able to include patients of all HLA types. We therefore propose to expand the vaccine platform coverage to include the whole TARP protein, as has been done for other tumor antigens like PSA, CEA, and melanoma antigens in the CCR and elsewhere once the value of the antigen target was demonstrated first in HLA-A2 positive patients. (**31-38**) The whole protein will have all the possible epitopes that can be presented by any HLA molecule and therefore would be suitable for vaccinating the entire population of prostate cancer patients,

making adequate accrual feasible. For the reasons stated earlier, overlapping long peptides such as the 20-mers overlapping by 10 residues have been widely used to represent a whole protein because they contain all the potential epitopes of the whole protein but are more amenable to processing for both class I and class II HLA presentation to $CD4^+$ and $CD8^+$ T cells. (**13, 39-41)** We have followed this widespread practice in proposing to include an additional five 20-mer peptides overlapping by 10 residues covering the whole protein.

Although there was not a formal statistical difference found between the two arms in the pilot study, we have selected the autologous DC vaccine platform in this study because of the statistical trend at both 24 and 48 weeks that suggested it might be better and the logistics of manufacturing a multi-epitope vaccine. All of the peptide vaccine emulsions (TARP WT27-35 and EE19-37-9V) delivered on Arm A in the 09-C-0139 study were generated manually using a syringe method of emulsification by pharmacists in the Investigational Drug Service. This approach is not logistically feasible for 7 different peptides and extensive additional studies would be required to determine if peptides could be combined to generate emulsions.

The proposed phase II clinical trial is a prospective, single-blinded, randomized, placebo controlled study of 96 weeks duration in men with Stage D0 prostate cancer. Men with a PSADT $\geq$ 3 months and $\leq$ 15 months will be randomized 2:1 to receive multi-epitope TARP autologous DC vaccination or a control elutriated monocyte vaccine placebo. An initial lead-in of 6 patients will be enrolled to allow preliminary assessment of the safety of the multi-epitope TARP vaccine platform through 12 weeks before enrollment of prospectively randomized subjects blinded to treatment assignment begins.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Males $\geq$ 18 years of age with histologically confirmed adenocarcinoma of the prostate. Histology confirmation must be documented with a formal pathology report. Notes from an outside physician describing the pathologic findings (based on a prior review of the full pathology report) may be used if unable to obtain the original pathology report. This will eliminate the need for an additional invasive tissue biopsy.

2.1.1.2 Must have completed and recovered from all prior definitive therapy (surgery, brachytherapy, cryotherapy or radiotherapy) for the primary tumor, or other definitive-intent local therapy.

2.1.1.3 Stage D0 disease with documented biochemical progression documented by rising PSA and no evidence of metastatic disease by physical examination, CT scan or bone scan.

2.1.1.4 PSADT $\geq$ 3 months and $\leq$ 15 months:

- Patients must have $\geq$ 3 PSA measurements over $\geq$ 3 months.
- The interval between PSA measurements must be $\geq$ 4 weeks.

2.1.1.5 For patients following definitive radiation therapy or cryotherapy: a rise in PSA of > 2ng/mL above the nadir (per RTOG-ASSTRO consensus criteria).

2.1.1.6 For patients following radical prostatectomy: 2 absolute PSA values > 0.2 ng/mL.

2.1.1.7 Non-castrate level of testosterone: ≥ 50 ng/dL (prior ADT allowed; must be ≥ 6 months since last dose of ADT).

2.1.1.8 Performance Status: ECOG 0-1 (Refer to **Appendix B**).

2.1.1.9 Hemoglobin ≥ 9.0 gm/dL, WBC ≥ 2,500/mm^3, ALC ≥ 500/ mm^3, ANC ≥ 1,000/mm^3, platelet count ≥ 75,000/mm^3, and PT/PTT ≤ 1.5X ULN unless receiving clinically indicated anticoagulant therapy; SGPT/SGOT ≤ 3X ULN, total bilirubin ≤ 1.5X ULN; creatinine ≤ 1.5X ULN *and* estimated GFR (eGFR) ≥ 60 mL/min.

2.1.1.10 Hepatitis B and C negative (unless the result is consistent with prior vaccination or prior infection with full recovery); HIV negative.

2.1.1.11 No use of investigational agents within 4 weeks of study enrollment or use of immunosuppressive or immunomodulating agents within 8 weeks of study entry.

2.1.1.12 No other concurrent anticancer therapy or prior prostate cancer vaccines expressing TARP.

2.1.1.13 No alternative medications or nutriceuticals known to alter PSA (e.g. phytoestrogens and saw palmetto). Note: patients receiving medications for urinary symptoms such as Flomax or 5-alpha reductase inhibitors (finasteride and dutasteride) on a chronic stable dose for at least 3 months prior to study enrollment are *allowed*.

2.1.2 Exclusion Criteria

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

2.1.2.2 Patients with active infection.

2.1.2.3 Patients on immunosuppressive therapy including:

- Systemic corticosteroid therapy for any reason. Patients receiving inhaled 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

We anticipate accrual to this phase II to be brisk given the level of interest in our initial pilot TARP vaccination study NCI 09-C-0139. Over 220 patients were screened for HLA-A*0201 and PSADT criteria to achieve the final accrual of 41 patients that was accomplished within 28 months. The multi-epitope (ME) TARP peptide vaccine platform covering the entire TARP protein to be used in this study eliminates the need for HLA restriction in study participants and will greatly facilitate the rate of study accrual. In addition, multiple community-based oncology providers with a substantial number of patients enrolled in the initial 09-C-0139 clinical trial have already begun referring patients in anticipation of this phase II ME TARP DC vaccine study.

In addition to the standard postings on the CCR website, as the previous Principal Investigator of the ME TARP DC vaccine study, Dr. Wood developed a brief 2-minute video that would provide an informational overview to both patients and health care providers describing the scientific rationale for and design of the clinical study.

This video would be posted to the YouTube websites of the NCI Office of Communications. Given that so much of consumer media information is now provided in video format that can easily be accessed via the web using computers and smart phone platforms, we believe that this could serve as a novel recruitment tool for patients interested in participating in clinical research studies.

Additional recruitment strategies will involve the use of flyers, posters and letters generated with the guidance of the NIH Clinical Center – Office of Patient Recruitment. Study information will be posted on NIH websites and social media forums.

2.2 PRE-SCREENING AND ELIGIBILITY EVALUATION

2.2.1 Pre-Screening

All potential patients must provide written documentation of outside PSA values obtained within 12 months of study entry for calculation of PSADT as outlined in **2.1.1** Inclusion Criteria. Pre-NIH calculated PSADT using the Memorial Sloane Kettering nomogram (http://nomograms.mskcc.org/Prostate/PsaDoublingTime.aspx) must be ≥ 3 months and ≤ 15 months for patients to be brought to the NIH Clinical Center for further eligibility screening and baseline evaluation.

2.2.2 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, ECOG performance status and review of concomitant medications.

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 testosterone.
- TTV Serology (Anti-HIV-1/2 Ab, anti-HCV Ab, HBsAg, HBs Ab, anti-HTLV-1/2 Ab, West Nile, T. Cruzi and RPR)

  Note: TTV serology must be drawn within 30 days of apheresis and may need to be repeated if there is a lapse in apheresis schedule. Only HIV and viral hepatitis (HBV and HCV) serology results are required for consenting. A pending result status is acceptable for the rest of the serologic tests.
- ABO typing (CRIS order "Type and Screen"), if not previously done at NIH
- EKG
- Imaging studies: CT scan with IV contrast of the chest, abdomen and pelvis, Technetium^{99} whole body 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 CC Radiology and Imaging Services and

the NCI Vaccine Branch Clinical Trials Team, new imaging studies should be obtained at the NIH. If outside imaging is utilized, the imaging should be uploaded and reported at NIH CC Radiology and Imaging Services.

2.3 BASELINE EVALUATION

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

- Laboratory evaluations: CBC with differential, acute care panel, hepatic panel, mineral panel, amylase, lipase, lipid panel, TSH, 25-OH Vitamin D, lymphocyte phenotyping, urinalysis and HLA-ABC, DR (if not previously completed at NIH)

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 A** for the Schedule of Study Clinical, Laboratory and Radiology Evaluations and visits.

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.

2.5 TREATMENT ASSIGNMENT AND RANDOMIZATION PROCEDURES:

Randomization and Arm Assignment

After confirmation of eligibility, the initial lead-in cohort of 6 subjects will receive enrollment study numbers 101-106. These lead-in subjects will be enrolled to allow preliminary assessment of the safety of the autologous multi-epitope TARP DC vaccine platform. After safety and immunogenicity have been established through 12 weeks in this initial lead-in cohort of 6 patients; Central Registration Office (CRO) will prospectively randomize enrolled subjects blinded to study treatment on a 2:1 basis to receive either autologous multi-epitope TARP DC vaccine or an autologous elutriated monocyte vaccine placebo.

Cohorts

Number	Name	Description
1	Lead in cohort 1	Lead in cohort
2	Cohort 2	Patients who will be randomized

Arms

Number	Name	Description
1	Lead-in TARP DC vaccine treatment	All patients to receive autologous multi-epitope TARP DC vaccine before randomization
2	Active TARP DC vaccine treatment	Autologous multi-epitope TARP DC vaccine after randomization
3	Placebo	Autologous elutriated monocyte vaccine placebo after randomization

Randomization and Arm Assignment

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

Patients in cohort 2 will be randomized between arms 2 and 3 on a 2:1 basis.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

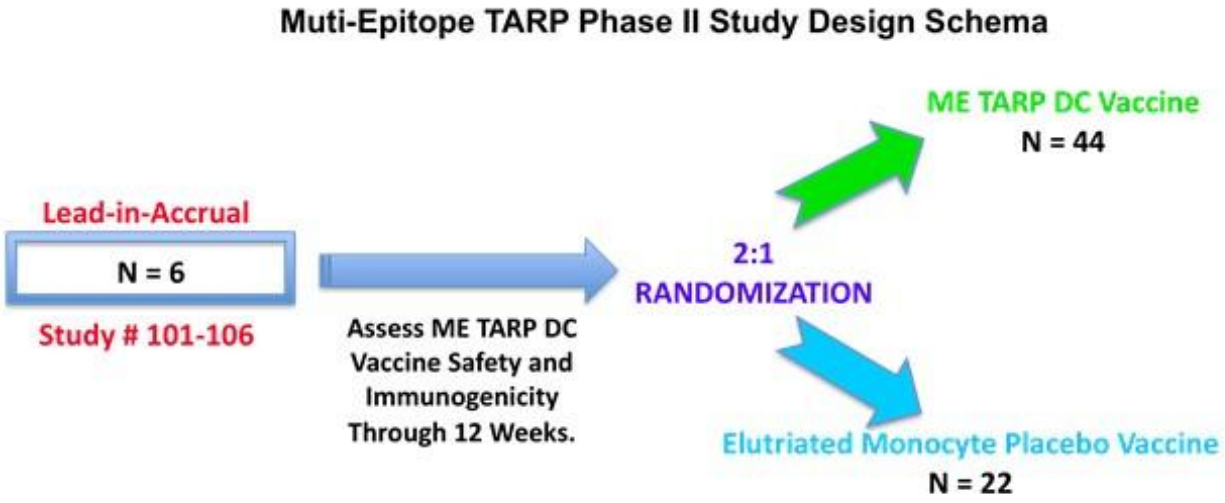
Enrollment will be staggered every three weeks for the first three patients that allows a 3 week interval for safety assessment before the next enrolled patient is scheduled to receive their first dose of ME TARP DC vaccine.

- 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 NIH Intramural 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 3 lead in subjects 104-106 and subsequent randomization of 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 dose e.g. 03/16/15 in the example above.
- All study subjects will continue to be monitored in real time for adverse safety events by the protocol Principal Investigator.

After the lead in period portion of the study is complete eligible patients (blinded to treatment assignment) will be prospectively randomized 2:1 to receive either autologous TARP multi-epitope DC vaccine or an autologous elutriated monocyte vaccine placebo after safety and immunogenicity have been established through 12 weeks in an initial lead-in cohort of 6 patients as outlined below in **Figure 8**.

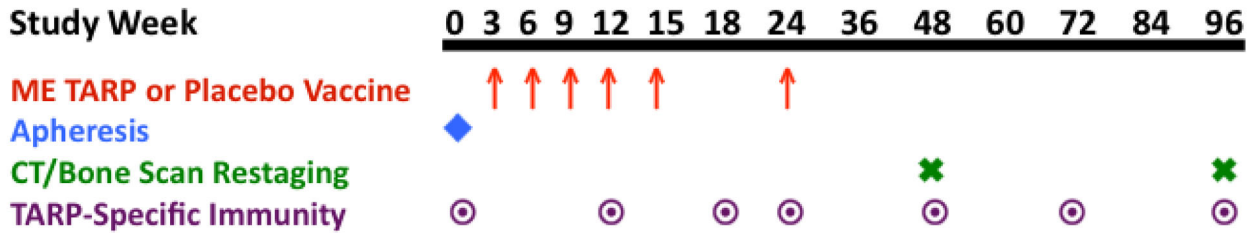
3.1.1 Schema

Figure 8



All patients will receive a total of 6 doses of vaccine (20 x10^6 *viable* cells/dose) delivered intradermally at Weeks 3, 6, 9, 12, 15, and 24. All patients will undergo. The on study monitoring schedule of clinical assessments, laboratory and imaging studies is outlined below in **Figure 9** and in **Appendix A**.

Figure 9



3.2 VACCINE ADMINISTRATION

The vaccine manufacturing schema is described in **Appendix C** 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. Eligible subjects will receive autologous ME TARP dendritic cell or elutriated monocyte vaccine placebo beginning at Week 3. Each dose can be delivered starting from -1 week of the target date until the maximum delay described in Section **3.3.2**. 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. Refer to **Appendix C** for details concerning the cGMP production of peptide-pulsed dendritic cells and elutriated monocyte placebo by the NIH Clinical Center Department of Transfusion.

- Autologous ME TARP DC and elutriated monocyte placebo vaccine preparations will be assessed for release standards (nucleated cell content and concentration, appearance, flow cytometric verification of DC validation markers, viability ≥ 60%, and product sterility and safety testing) prior to release for vaccine administration to the patient.
- For both groups, the investigational product will be administered intradermally in two sites on the forearm with a maximum volume of 0.5 mL 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 (20×10^6 *viable* cells/dose, +/- 10%) delivered at Weeks 3, 6, 9, 12, 15, and 24 and will undergo scans at Weeks 48 and 96 for restaging.
- 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 **3.3.1**. 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 D**) and instructed on how to complete it, following each ME TARP DC or placebo vaccine dose.

Since this protocol involves multi-epitope TARP vaccination in humans for the first time, enrollment of blinded, randomized subjects will not begin until safety has been established in an initial lead in of 6 patients. If no adverse events are observed through Week 12 following the first vaccination in these 6 patients, enrollment of additional patients may proceed as quickly as is logistically feasible.

3.3 Dose Modification and Immunization Stopping Rules

No dose modifications will be made in patients receiving autologous multi-epitope TARP DC or elutriated monocyte placebo vaccination. Subjects will cease to receive immunization if they experience dose-limiting toxicity (DLT) as outlined in Section **3.3.1**. 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

3.3.1 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 include:

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

3.3.1.2 Any Grade 2 or greater rash consistent with erythema multiforme

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

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

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

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

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

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

3.3.2 Delay in Vaccination

3.3.3 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 within 3 weeks of the scheduled target date of vaccine. Patients will be advised of their options concerning alternative treatments before proceeding with the completion of their study vaccinations. The reason for the change in vaccination schedule will be documented in the patient's chart. 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. Study weeks will be numbered to match vaccine dose weeks.Study Stopping Rules

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

- One or more of the first three 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.
- Two or more of the first six subjects (101-106) experience a Grade II or greater adverse event designated as possibly, probably or definitely related to vaccination with the exception of local injection site reactions.

3.4 STUDY CALENDAR

The protocol study calendar of clinical and laboratory monitoring events is as outlined in **Appendix A.**

Research assays for anti-TARP antibody and TARP-specific cellular responses will be conducted as outlined in **Appendix E**.

3.5 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

A safety visit is required approximately 3-6 weeks 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.

3.5.1 Criteria for removal from protocol vaccine therapy

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

Delay in vaccination beyond timeframes established in section **3.3.2**

3.5.1.2 Patients experiencing a Grade 3 or greater toxicity outlined in sections **3.3.1.3** to **3.3.1.5** attributed as possibly, probably or definitely related to ME TARP DC vaccination.

3.5.1.3 Completion of protocol therapy (6 doses), including safety visit.

3.5.2 Off-Study Criteria

3.5.2.1 Completion of study.

3.5.2.2 Removal from protocol vaccine therapy as outlined in Section **3.5.1**.

3.5.2.3 Patient develops evidence of visceral or bony metastatic disease.

3.5.2.4 Patients with a 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.

3.5.2.5 Patients with a calculated PSADT that has decreased by > 50% from their calculated Pre-NIH PSADT on or after 24 weeks on study. 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.

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

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

3.5.2.8 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.

3.5.2.9 Investigator decision to end the study

3.5.2.10 Death

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

3.5.3 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 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 is not allowed during this trial. Patients will be allowed to co-enroll in studies of novel radionuclide imaging agents e.g. F-18 NaF PET/CT for quantification of metastatic burden in prostate cancer.
    - Hormonal Therapies: Concomitant use of anti-androgen hormonal agents is not allowed.
    - Alternative Medications or Nutriceuticals Known to Alter PSA: Concomitant use of agents known to alter PSA e.g. phytoestrogens and saw palmetto is not allowed. Note: Patients receiving medications for urinary symptoms such as Flomax or 5-alpha reductase inhibitors (finasteride and dutasteride) on a chronic stable dose for at least 3 months prior to study enrollment are *allowed.*
    - Corticosteroids: Concomitant, *chronic systemic corticosteroids are not allowed* during this trial (excepting emergent use for clinical indications). 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 40 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. Specific gene expression profiling of autologous ME DC vaccine products will examine genes involved in antigen uptake, apoptosis, cytokine and chemokine expression. We will also examine circulating tumor cells (CTCs) and immune subsets in peripheral blood mononuclear cells including iNKT and myeloid derived suppressor cells (MDSCs) with Jane Trepel. This will help to determine if the autologous ME TARP DC vaccination is associated with a reduction in CTCs as compared to placebo and to assess the impact of vaccination on quantitative and qualitative parameters of iNKT cells. 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 **11.1.2)**. In addition, the under the CRADA, molecular characterization of individual CTCs pre and post vaccination will also be performed.

Preliminary assessment of immune status of subjects enrolled in the first generation 09-C-0139 TARP peptide vaccine study demonstrated normal absolute lymphocyte counts, CD4 and CD8 T-cell subset absolute counts, percentages and ratios as well as excellent immunity to CEF peptide pooled antigens (CMV, EBV and Flu matrix peptide) utilized as positive controls in IFN-γ ELISPOT assays investigating TARP-specific reactivity. We will plan to prospectively expand our characterization of immune status and senescence in subjects by exploring investigation of T-cell receptor v-beta repertoire analysis, CD4+CD25+FoxP3+ T-regulatory cell subsets and potentially T-cell receptor excision circles (TREC) as potential correlates of changes in slope log PSA/PSADT and clinical outcomes.

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, typically 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. Samples will not be sent outside NIH without IRB notification and an executed MTA.

Once primary research objectives for the protocol are achieved, intramural researchers can request access to remaining samples provided they have an IRB-approved protocol and patient consent. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

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**.

5.3 SAMPLES FOR GENETIC/GENOMIC ANALYSIS

No genetic/genomic analyses will be performed as part of this clinical investigation.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

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. Response to autologous ME TARP DC or elutriated monocyte placebo 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 Weeks 3-24 minus that formed for the 12 months prior to enrollment on study (referred to as slope 324 – 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 naïve to TARP vaccination receiving active, multi-epitope TARP vaccination *vs.* placebo.

7. 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 (VRCs) associated with each autologous ME TARP DC or elutriated monocyte placebo vaccination will be obtained for each patient (see **Appendix D)**. If the VRC is not available, investigators may describe the grade and duration of the injection site reaction based on the patient's verbal report. The information recorded on the VRC will also be documented in the medical record.
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. Serious adverse events that occur more than 6 weeks after the last administration of investigational agent/intervention and have an attribution of at least possibly related to the agent/intervention should be recorded and reported as per sections Error! Reference source not found. and **7.4**.

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

Since Stage D0 prostate cancer patients have biochemical PSA recurrence, with no evidence of bony or visceral metastatic disease traditional RECIST or Immune-Related Response Criteria (irRC) will not be utilized in this study.

6.2.1 Progression-Free Survival

An exploratory assessment of progression free survival (PFS) will be conducted in patients receiving autologous ME TARP DC vs. autologous elutriated monocyte placebo vaccine. PFS is defined as the duration of time from start of vaccine treatment to time of progression or death.

6.3 TOXICITY CRITERIA

The following adverse event management guidelines 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 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 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 lead in accrual subjects 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 and Lead Associate Investigator will be responsible for overseeing the Data and Safety Monitoring Plan with the assistance of personnel employed by a CCR contractor. As information is gathered from this trial, clinical results will be shared with the patients. Laboratory and clinical data will be gathered on a bi-weekly basis and any new significant observation(s) found during the course of the research that may affect patient safety or a patient's willingness to participate further will be explained at the time patients are consented for the study.

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.

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**. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor.

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 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 SECTION

Sample Size/Statistical Considerations: by Seth Steinberg, PhD, OCD, CCR, NCI

The primary objective is to determine if the use of autologous multi-epitope TARP peptide vaccine administered using autologous dendritic cells is able to be associated with a significant difference in the rate of PSA change in patients with stage D0 prostate cancer compared to patients who receive an autologous elutriated monocyte placebo control vaccine.

After an initial run–in accrual of 6 patients to allow for preliminary assessment of ME TARP DC vaccine safety and immunogenicity, patients blinded to treatment assignment will be randomized in a 2:1 fashion between receiving ME TARP peptide autologous DC vaccine vs. autologous elutriated monocyte placebo vaccine. The primary endpoints will be the difference in the slope log (PSA) for weeks 3-24 minus that formed for the 12 months prior to enrollment on study (referred to as slope 324 – pre-slope), as well as the slope log (PSA) for weeks 3-48 vs. the same pre-treatment slope log (PSA) (referred to as slope 348 – pre-slope). As this is intended to demonstrate that at least a trend toward benefit is identified, the study will be designed using a phase 2.5 approach, in which each endpoint would be considered to have found a trend if $p<0.10$ from a one-tailed test. With two endpoints, the goal will be to design the study to be large enough to potentially find a difference with a 0.05 one-tailed test for each of the two endpoints in order to hold the overall error rate to 0.10.

From the prior trial of TARP peptide vaccine, the dendritic cell arm was associated with a mean +/- SD slope 324 – pre-slope of -0.033 +/- 0.072 based on 18 patients. The slope 348 –pre-slope was -0.049 +/- 0.062 based on 15 patients. It is anticipated that in the absence of the vaccine

being given, that the slope 324 – pre-slope may be at least 0.010 or greater, such as 0.015, as it would be expected that the log slope (PSA) would likely very slowly increase, as a minimum, in the absence of treatment. Choosing the smaller difference would suggest that the difference in means for the two groups could be approximately -0.033 vs. +0.015, or a difference of -0.048.

Assuming a common standard deviation of 0.072 (as was approximately found on the TARP arm B in the prior trial), this would mean that detecting an effect size of 0.67 SD (0.048//0.072=0.67) could be a reasonable goal. With a 2:1 randomization (2 getting vaccine to 1 getting placebo), with 44 on the active arm and 22 on the placebo arm, there is 81% power to detect a difference between the two arms on the order of 0.048 slope log PSA units, assuming the parameters from the prior study may be approximately true and a 0.05 one-sided significance level is sought. The analysis is assumed to be done with a two- group t-test, while in practice a Wilcoxon rank sum test will be used if the values in the two arms are not normally distributed ($p<0.05$ by a Shapiro-Wilks test). In addition, although using a 0.05 significance level for each test assumes that a Bonferroni correction will be used to adjust the two tests for multiple comparisons, in practice, a less overly conservative Hochberg test may be used to interpret the results. It should also be noted that since the two primary endpoints involve overlapping intervals of time, the results may not be independent of one another and thus this should factor into the interpretation at the conclusion of the study.

In addition to the two primary endpoints, the change in log slope (PSA) at later time points, such as weeks 48-96, may also be evaluated as a secondary endpoint. As this is a prospective, single-blinded, randomized placebo controlled trial, the NCI/CCR DSMB will be used to monitor the trial. This monitoring will be done for both futility and for efficacy, as well as for safety.

The futility monitoring will be performed as follows: at the first DSMB meeting after which half of the projected patients have been evaluated (33 total patients have been evaluated for both the changes to 24 weeks and the changes to 48 weeks), the slope differences will be calculated on both arms. If at this point, the slope difference for the placebo arm indicates the same or greater benefit compared to that for the active arm, then the trial will not accrue any further patients since the results to date would indicate lack of benefit from using the active vaccine.

The evaluation for better than expected efficacy will be performed as follows: at the first DSMB meeting after which half of the projected patients have been evaluated (33 total patients have been evaluated for both the changes to 24 weeks and the changes to 48 weeks), the slope differences will be calculated on both arms. If at this point, the one tailed p-value is<0.0054, then the trial will stop accrual for better than expected efficacy. By using this interim evaluation for monitoring, the final significance level would need to be <0.0492 to declare the result to be significant at the 0.05 level.

Safety and evaluation of other immune parameters will also be included in the analyses, as secondary objectives. These will be explored primarily with descriptive statistics and any comparisons between the two arms will be reported without any formal adjustment for multiple comparisons.

With 66 patients as an objective for the randomized portion of the study (N = 44 ME TARP vaccine and N = 22 elutriated monocyte placebo vaccine) and the initial lead in of 6 patients, a total of 72 patients may be required. In order to allow for a small number of inevaluable subjects, the accrual ceiling will be set at 75. For the data collection and analysis, we will define the

evaluable patients as receiving at least 3 doses of vaccines. It is anticipated that up to 2-3 years may be required to enroll 66 patients to the randomized portion of the study.

11 COLLABORATIVE AGREEMENTS

11.1 COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA)

11.1.1 CRADA # 03039 was previously 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 H, 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 PROTECTIONS

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. We will make every effort to accrue patients from all racial and ethnic groups. Since our referral population is the nation, we should obtain referrals representative of the national composition of races. Since this trial is investigating a multi-epitope TARP peptide vaccine platform, there is no need for HLA restriction as was required in the 09-C-0139 pilot study of HLA-A*0201 restricted WT27-35 and EE29-37-9V TARP peptides

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

12.2 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 (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the

capacity to appoint a surrogate. 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.

12.3 PARTICIPATION OF CHILDREN

Since the safety of Multi-Epitope TARP has not been adequately assessed in children, patients under the age of 18 years will not be eligible to participate.

12.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

The potential benefit of vaccination with multi-epitope TARP peptides is unknown. Although a majority of patients (~72%) in our first 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). In addition, it is unknown and unproven whether slowing PSA velocity through vaccination or any other treatment intervention, will in turn impact clinical outcomes. Ongoing long-term observation out to 144 weeks on the 09-C-0139 study suggests that responses to TARP vaccination may wane. In addition, there were study subjects who had objective evidence of clinical progression following an initial, favorable slowing in PSA velocity. We believe that this second generation multi-epitope 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 safety and tolerability profile. Vaccination was also associated with induction of TARP-specific cellular immune responses in a majority of patients (~75%) as well. However the clinical significance of these immune responses remains unknown. In this study, we will attempt to definitely establish whether the observed declines in the difference in the slope log (PSA) documented in the 09-C-0139 study are real and not due to spontaneous or random slowing in the rate of PSA rise. We will also conduct an exploratory assessment of progression free survival (PFS) at 96 weeks to see if there is any indication of efficacy regarding clinical outcomes in patients receiving autologous ME TARP DC vaccination. The most significant risk for study subjects randomized to receive the autologous elutriated monocyte vaccine placebo is the apheresis required to collect cells, as autologous, minimally manipulated thawed elutriated monocytes are unlikely to be associated with any adverse reactions other than local injection site discomfort.

12.5 RISKS/BENEFITS ANALYSIS

This study offers patients the prospect of direct benefit and the treatment has an acceptable risk profile. Therefore, the benefits outweigh the risks. The risk/benefit analysis is the same for patients unable to consent as it is for less vulnerable patients.

12.6 CONSENT PROCESS 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 NIH Intramural 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 consent procedure

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 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 elutriated monocyte placebo vaccine will be generated utilizing cGMP manufacturing conditions by the Department of Transfusion Medicine as outlined in **Appendix C**.

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:

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

KLH was purchased from Stellar Biotechnology and is dispensed by the Center for Cellular Engineering (CCE), Department of Transfusion Medicine (DTM), NIH for use in dendritic cell vaccine manufacturing.

13.3.2 Formulation and Preparation:

Stellar high molecular 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 microliter/vial.

13.3.3 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.

13.3.4 Administration Procedures:

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

13.3.5 Incompatibilities:

Refer to the package insert.

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 CCE, NIH Clinical Center.

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 a stability program approved by the NIH CC Pharmacy.

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)

Molecular Weight: 1126.4

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 Clinical Center.

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 a stability program approved by the NIH CC Pharmacy.

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 by 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 CC, NIH Clinical Center.

13.6.3 Stability and Storage:

Peptide is stored at -70°C or below. Stability will be monitored according to a stability program approved by the NIH CC Pharmacy.

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 Product Description:

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 Clinical Center.

13.7.3 Stability and Storage:

Peptide is stored at -70°C or below. Stability will be monitored according to a stability program approved by the NIH CC Pharmacy. .

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 Product Description:

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 Clinical Center.

13.8.3 Stability and Storage:

Peptide is stored at -70°C or below. Stability will be monitored according to a program approved by the NIH CC Pharmacy. 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.

13.8.4 Incompatibilities:

None known.

13.9 TARP 31-50 PEPTIDE

13.9.1 Product Description:

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 Clinical Center.

13.9.3 Stability and Storage:

Peptide is stored at -70°C or below. Stability will be monitored according to a program approved by the NIH CC Pharmacy.

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 Product Description:

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 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 Clinical Center.

13.10.3 Stability and Storage:

Peptide is stored at -70°C or below. Stability will be monitored according to a program approved by the NIH CC Pharmacy. .

13.10.4 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 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.

13.10.5 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 manufacturer's 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 A: STUDY CALENDAR OF CLINICAL AND LABORATORY EVENTS

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														
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														
Acute/Hepatic /Mineral Panel	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
TTV Screen[2], ABO[3]	X														
PSA / Testosterone	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
PSADT Calculation[4]	X					X	X	X	X	X	X	X	X	X	
Bone Scan	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]**
CT Scan (C/A/P)	X									X				X	
EKG	X														
Informed Consent	X														
Apheresis[5]	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	
HLA typing		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	
Phone/mail follow up															X
Research Correlatives															

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]**
Anti-TARP Ab		X		X[10]	X		X	X	X	X	X	X	X	X	
CTCs, immune subsets		X			X		X	X		X		X		X	
PBMCs Cellular Responses		X		X[10]	X		X	X	C	X	X	X	X	X	

1. As indicated in section **12.2**, 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. Historic records are acceptable if previous NIH results are available.
4. PSADT will be calculated only when the patient is in D0 prostate cancer.
5. Apheresis should occur within 3 weeks after consenting. The first dose of investigational drug should be scheduled within 6 weeks after the consent is signed. Apheresis may be repeated if additional plasma or cell aliquots are required to manufacture the vaccine.
6. Screening studies including scans should occur within 60 days of enrollment at the NIH unless otherwise specified (see section **2.2.2**).
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. Historic HLA-ABC, DR results from NIH may be used for baseline.
9. Post-therapy follow up, with the patient or patient's local oncologist, will occur annually until disease progression or death.
10. Research correlatives on week 12 (post 3 doses of investigational treatment) may be collected 7 days (+/- 3 days) post 3rd dose of investigational treatment, if patient is available locally.

15.2 APPENDIX B: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale	
Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

15.3 APPENDIX C: 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 $\times 10^9$ monocytes. Lymphocytes will also be cryopreserved. Apheresis will be performed in the Clinical Center (CC) Department of Transfusion Medicine (DTM) using approved standard operating procedures. Bilateral peripheral venous access will be used for apheresis whenever possible. Alternatively, a 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 (CCE), 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 (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.

Standard Operating Procedure for Elutriated Monocyte Placebo Vaccine Preparation

Autologous Cell Harvest

Blood collection shall be by standard lymphapheresis.10 to 15 liters of whole blood will be processed in order to collect peripheral blood mononuclear cells (PBMC). Lymphocytes will also be cryopreserved. Apheresis will be performed in the Clinical Center (CC) Department of Transfusion Medicine (DTM) using approved standard operating procedures. Bilateral peripheral venous access will be used for apheresis whenever possible. Alternatively, a temporary femoral central venous catheter (CVL) will be placed as an outpatient, if indicated, for collection on the day of apheresis. The CVL will be inserted by appropriately trained personnel in special procedures with removal of the CVL by 3SE day hospital or DTM 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.

Patient Cell Processing

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

Formulation and Preparation Elutriated Monocyte Placebo Vaccine*:* Autologous elutriated monocyte placebo cell vaccines will be prepared under cGMP conditions from cryopreserved patient monocytes obtained during the original Week 0 apheresis. Autologous monocytes for these placebo vaccines 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 elutriated monocyte placebo cell vaccine products. Elutriated monocytes will be thawed the morning of scheduled vaccine delivery. After thaw, elutriated monocytes 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, volume-reduced elutriated monocyte 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 elutriated monocyte placebo 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 autologous elutriated monocyte placebo vaccine from the single day thaw product. Both 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 or elutriated monocyte placebo vaccine dose of 20 X 10^6 <u>*viable*</u> cells/ in 0.25mL or 0.5mL will be administered immediately upon

receipt in the clinical setting. Post packaging tests indicated that the product is stable for at least 2 hours.

15.4 APPENDIX D: VACCINE BRANCH VACCINE REPORT CARD (VRC)

Date of Administration (Day 1) _____

Investigational Drug Dose # ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 [a total of 20 x 10^6 viable cells]

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

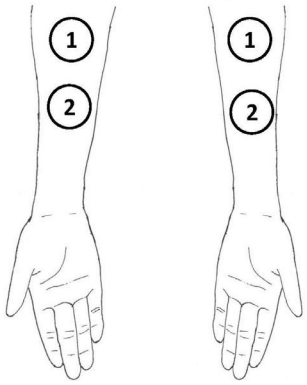
NOTES:

1. For dose #1, vital signs should be documented pre-dose and 15, 30, 45 and 60 min after investigational drug 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 (HIMD) to be scanned into CRIS.

Site	Injection Site	Injection Volume	ID Wheal	Today Day 1	Day 2	Day 3	Day 4	Day 5	Date Resolved
#1	Forearm ☐Left ☐Right	☐ 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	
#2		☐ 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



Administered by (RN printed name & signature): _____

ISR reported by (patient or on behalf of the patient): _____

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

15.5 APPENDIX E: ANTI-TARP ANTIBODY AND CELLULAR RESPONSES

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, 36, 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 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 Response*:* 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 for specimen processing and freezing.

Note: PBMCs collected via apheresis that have completed monocyte elutriation 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 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, typically the protocol Principal Investigator who has access to the collection. Blood and tissue specimens collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study. 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**.

15.6 APPENDIX F: EXPLORATORY CORRELATIVE STUDIES

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, MDSC and dendritic cells. A total of 30 mL of blood in lavender top tubes will be collected.

CTC Analysis:
Weeks 3 (baseline), 12 (s/p 3 doses of vaccine), 18 (s/p 5 doses of vaccine), 24, 48, 72 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:
Weeks 3, 12, 18, 24, 48, 72 and 96
Multiparameteric flow cytometry using a Miltenyi Quant flow cytometer. The order of priority for immune subset analysis is T, B, NI, NKT, MDSC and dendritic cells.

iNKT Cell Analysis:
Weeks 3, 12, 18, 24, 48, 72 and 96

Sample Logistics for CTC and 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 for batch testing is in Bldg.10, Rm. 12C208.

15.7 APPENDIX G: GUIDELINES AND WORKSHEET FOR CALCULATIONS OF PSADT

Study Number: _____

Pre-NIH and On Study 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, when possible.
- ☐ All PSA values used in the calculation should be ≥ 0.2 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 enrollment will be utilized to calculate the patient's Pre-NIH PSADT.

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

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.

NIH PSA values in blue.

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

Value is > 3.0 Months and ≤15 Months: ☐ YES- Eligible

☐ NO- Ineligible for Study

Study Number: _____

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

PSADT/Slope Log PSA will be calculated at Weeks 15, 18, 24, 36, 48, 60, 72, 84 & 96. Calculate using PSAs from both the entire study period and the prior 6 months.

Date	Study Week #	PSA ng/mL	Testosterone (ng/dL)	PSADT
	0			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			

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

PSADT Calculated for Statistical Analysis			
Analysis Window	**Study Wk PSA Values Included**	**PSADT/Slope Log**	**Percent Change PSADT**
Pre-NIH	Time of Entry		
Week 3 to Week 15	Wks 3, 6, 9, 12, 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		