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Mature Dendritic Cell Vaccination Against Mutated Antigens in Patients with Advanced Melanoma

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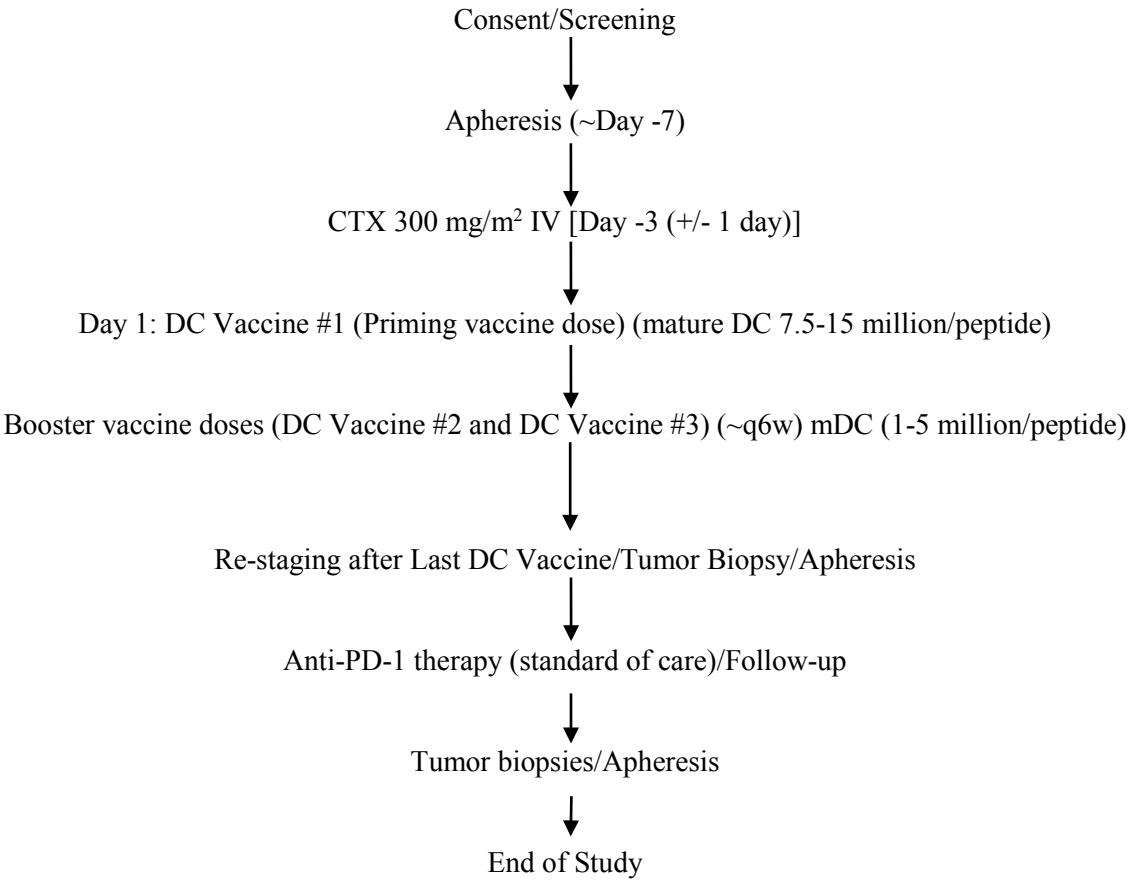
Schema



***Immune monitoring:** Beginning on Day 1/Week 1 and collected weekly thereafter through Week 18 (~Day 120), then every third week for 12 additional weeks, for a total of 30 weeks. Please refer to **Section 8.5** for additional details.

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1.0 BACKGROUND AND RATIONALE

1.1 Overview

In 2016, it is estimated that 76,380 new cases of melanoma will be diagnosed in the US (1). Although disease will be detected early and cured in many individuals with surgery, approximately 10,130 patients will die from metastatic melanoma. Patients with metastatic (stage IV) melanoma suffer a poor prognosis. Since 2011, two new treatments have become available for patients with metastatic melanoma. Checkpoint inhibitors such as Ipilimumab, Nivolumab, and Pembrolizumab serve to block negative inhibitory signals during T cell activation and allow tumor-specific T cells to recognize and eliminate cancer. The 3 approved checkpoint inhibitors have all been shown in randomized controlled trials to improve overall survival when compared to chemotherapy(2-4). The second new treatment targets the BRAF V600E mutation which is present in approximately 50% of patients with cutaneous melanoma. Vemurafenib is a selective oral inhibitor of the mutated (V600E) BRAF kinase. A randomized phase 3 controlled clinical trial demonstrated improved OS in patients receiving vemurafenib compared to dacarbazine (5). Vemurafenib was approved in August 2011. In addition, the combination of a BRAF inhibitor plus a MEK inhibitor (dabrafenib/trametinib, GSK) was granted regulatory approval in 2014 based upon improved ORR and overall survival(6,7). Despite approval of these new agents, metastatic melanoma remains an incurable malignancy with a median OS estimated at 24 months.

1.2 Chemotherapy for Melanoma

Chemotherapy has little impact on the natural history of metastatic melanoma. Dacarbazine received FDA approval in 1975 and remains a treatment option for patients with stage IV melanoma. The response rate to dacarbazine in most studies is 10-15%, primarily partial responses that tend to be of short (2-3 months) duration. Few durable complete responses are seen after treatment with dacarbazine. A meta-analysis of 74 studies published between 1974-1995 reviewed the ineffectiveness of dacarbazine-based chemotherapy as the median survival of enrolled patients was 8.8 months (8). Addition of other cytotoxic agents to dacarbazine adds little clinical benefit and increases toxicity (9).

1.3 Cytokines for Melanoma

Interleukin-2 received FDA approval in 1998 as treatment for metastatic melanoma despite concerns about toxicities and poor tolerability. In a series of single arm clinical trials (n=270 patients), the response rate to high dose Interleukin-2 is 16% (6% complete response rate). As reported, ten of the 16 patients who achieved a complete response remain disease free at a median follow up of 5 years (10). Six deaths occurred due to toxicity. This published experience using high dose IL-2 suggests that immunomodulation has a role in the treatment of this disease and in fact, may be curative in a small group of patients.

1.4 Vaccines for Melanoma

Since 1970, investigators have pursued the hypothesis that melanoma is inherently immunogenic and elicits an immune response that can be, in some instances, protective (11). As a result, therapeutic immunization strategies have evolved over the past decade to develop more contemporary approaches based on molecular techniques of antigen identification and immune monitoring. As one example, the molecular cloning of several melanocyte-lineage antigens (MLA)

has prompted investigators to identify and isolate immunogenic peptides that can be used in vaccination protocols (13). These candidate melanoma tumor antigens include: gp100/pMe117, MART-I, Melan-A, tyrosinase as well as antigens from the cancer-testis family (MAGE family) which are known to be expressed only in testis and placenta in normal tissues (14, 15). In normal adult tissues, each MLA is expressed at low levels with expression restricted to melanocytes and retina pigmented epithelium (16).

1.4.1 Gp100 Melanoma Antigen Vaccines

The gp100 antigen has been studied in detail. Three candidate peptides (G154, G209-2M and G280-9V) that bind HLA-A2 (the most common class I allele expressed by ~50% of the population) have been identified and each is known to be immunogenic in patients with melanoma (14)). One peptide named (G209-2M (also called gp100:209-217) has been studied extensively and shown to be immunogenic when given subcutaneously with adjuvant (17). In our work, the (G280-9V (also called gp100:280-288) peptide was given with autologous dendritic cells (without IL-2 or any other cytokine) and shown to be immunogenic in patients with advanced melanoma (18). The third peptide epitope named G154 has also been studied in some detail (19). Other gp100 related peptides that are restricted to HLA-A3 are known but are less well studied (20).

1.4.2 Melanoma Neoantigens

Among cancer genomes, cutaneous melanoma harbors the largest number of non-synonymous mutations. Ultraviolet light exposure is a major risk factor for cutaneous melanoma and whole genome sequencing reveals the high rate of UV light induced mutational alterations (21). We have hypothesized that UV light induced non-synonymous mutations generate patient-specific neoantigens which could be targeted by vaccination to elicit melanoma-specific T cells. Based on our published report to identify tumor-specific neoantigens from melanoma patients samples, we have been able to identify >20 candidate peptide neoantigens restricted to the HLA-A*0201 allele with ~50% promoting an immune response upon incorporation into a vaccine (22). Thus, inclusion of candidate patient-specific neoantigen peptides alone or in combination with the gp100 melanoma shared peptides into a vaccine is predicted to improve the breadth of the immune response directed against the tumor.

1.5 Dendritic Cells

Dendritic cells (DC) serve a critical role in capturing and presenting antigen in the initiation of immunity against infectious pathogens and cancer. A variety of technical advances involving the isolation of human DC as well as an improved understanding of DC biology have led investigators to propose using autologous DC as adjuvant for peptide vaccination in cancer, including melanoma (23). The initial clinical reports conclude that DC immunization is safe and well tolerated; serious adverse events related to DC vaccination are rare (24). Interestingly, clinical responses have been documented by numerous investigators in a variety of malignancies including melanoma (18,25).

1.6 Regulatory T Cells

Recent advances in immunology confirm the presence of a small population of circulating CD4+CD25+ T cells (known as regulatory T cells or Treg) that function to suppress T cell immunity toward pathogens and cancer. In healthy adults, approximately 5% of peripheral blood CD4+ T cells are Treg based on co-expression of CD25 and the transcription factor FoxP3 (26). Depletion of Treg by administration of low dose cyclophosphamide is sufficient to allow tumor rejection after peptide vaccination in model systems. This strategy was attempted clinically in the 1980's using cyclophosphamide together with a first generation (whole cell, irradiated) vaccine and shown to be safe and well tolerated. Several randomized trials later confirmed that vaccine given with cyclophosphamide is more immunogenic (27). Currently, the use of low dose cyclophosphamide which can be given either by infusion or orally is an effective strategy to eliminate Treg in patients prior to vaccination and is currently being evaluated at multiple centers.

1.7 Study Rationale

Cancer vaccines represent a promising area of clinical investigation in solid tumors based on evidence of clinical activity and minimal toxicity (28). Recent progress in immuno-oncology supports continued clinical evaluation of cancer vaccines in melanoma patients especially in combination with checkpoint inhibitors (29). The central hypothesis is that checkpoint inhibition will permit vaccine-elicited T cells to expand and traffic to the tumor microenvironment for more efficient elimination of the cancer cells.

The primary endpoint is immunological response based on tetramer assays performed on longitudinal blood samples obtained at multiple time points. Immunological response is based on measuring increased numbers of antigen peptide-specific CD8+ T cells as measured by the tetramer assay. In the proposed study, the tetramer assay will be performed at the designated time points. The additional laboratory correlative assays such as the ^{51}Cr release assay and functional assays will be performed after the third dose. The primary endpoint will also include evaluating the safety and tolerability of the mature dendritic cell vaccine. Secondary study endpoints are described in **Section 2.2**.

We propose to obtain tumor tissue to evaluate various biomarkers including the expression of PD-L1 antigens and characterize the tumor infiltrating lymphocytes, and perform exploratory studies to discover markers of resistance using tumor biopsy specimens collected at the indicated time points. These correlative laboratory studies are viewed as a high priority that will allow scientists to better understand the mechanisms of response/resistance to therapy.

1.8 Investigational Product Update: September 2016

Since the IND was open, we have published two reports documenting the safety, tolerability, and immunogenicity of the vaccine. Our initial publication reported the initial cohort of patients that received the mature DC (mDC) vaccine with the three gp100 peptides restricted to HLA-A*02:01. Maturation of DC was performed by activation of cells with CD40-ligand (CD40L) and Interferon-γ (IFN-γ). Of the seven patients treated, six developed sustained T cell immunity to all 3 melanoma peptides (30). Importantly, 3 patients developed a confirmed clinical response by RECIST criteria. DC vaccine derived IL-12p70 levels positively correlated with time to progression (TTP) as did type-1 immunity as assessed by IFN-γ/IL-13 ratios. A pathway specific defect in IL-12p35 transcription was identified in clinical non-responder patients and gp100 specific T cells were type-2 polarized. Incorporation of TLR3 (poly I:C) and TLR8 (R848) agonists into the CD40L/IFN- γ

activation protocol for DC maturation corrected the IL-12p70 production defect.

The second published report involved the incorporation of neoantigen peptides together with 2 gp100 peptides using autologous DC matured with CD40L/IFN-γ/TLR3/TLR8 agonists (refer to as mDC3/8) (22). Our group employed next generation sequencing methods with a novel bioinformatics pipeline to identify missense mutations in surgically resected melanoma metastases from patients with advanced disease that previously received ipilimumab. The DC vaccine led to an increase in the frequency of naturally occurring neoantigen-specific immunity and revealed previously undetected HLA class I restricted neoantigens in patients. Vaccination promoted a diverse neoantigen-specific T cell receptor repertoire and most importantly, was safe and well tolerated. Please refer to the Investigator Brochure for current toxicity information.

We have treated two additional patients since this publication using the same vaccine protocol. One patient (MEL66) had progression after ipilimumab followed by dabrafenib/trametinib combination and the residual disease was surgically resected. She received the vaccine as adjuvant therapy, tolerated the treatment well with no adverse events and remains alive with no measurable disease 18 months later. The other patient (MEL69) had widely metastatic relapsed/refractory disease at the start of the vaccine protocol. He received two vaccine doses without side effect or toxicity but had documented disease progression and was removed from the study. He died a few weeks later due to disease progression.

We have received funding from NCI and the Parker Institute for Cancer Immunotherapy to accrue additional patients for enrollment into the vaccine protocol. The hypothesis to be tested is that anti-PD-1 therapy inhibits adaptive immune resistance by enhancing neoantigen-specific T cell immunity within the tumor microenvironment leading to more effective tumor regression.

1.9 Major Study Update: November 2017

The results of the BMS 238 clinical trial evaluating nivolumab versus ipilimumab as adjuvant therapy for surgically resected stage 3/4 melanoma have been published (31). FDA has accepted for priority review its supplemental Biologics License Application for nivolumab to treat patients with melanoma in the adjuvant setting and FDA approval is anticipated. Based on this publication, the study has been further amended to allow for subjects without measurable disease (i.e. disease that has been completely resected) to enroll on this study.

2.0 OBJECTIVES

2.1 Primary Objective

1. To determine the immunological response based on measuring increased numbers of peptide specific T cells as calculated by the tetramer assay.

2. To assess the safety and tolerability of the mature dendritic cell vaccine (mDC3/8 vaccines).

2.2 Secondary Objectives

1. To determine the clinical response rate using RECIST 1.1 criteria

2. To determine the time to progression

3. To assess regulatory T cell depletion after cyclophosphamide administration.

4. To perform exploratory biomarker analysis of accessible tumors

5. To determine the safety and side effect profile of the dendritic cell vaccine (mDC3/8 vaccines) administered to patients given after a single dose of cyclophosphamide.

3.0 ELIGIBILITY CRITERIA

Patients must fulfill the following eligibility requirements:

3.1 Inclusion Criteria

1. Histologically confirmed stage III and stage IV M1a/M1b/M1c melanoma. Measurable disease is not required for eligibility and patients with completely resected disease are permitted.

2. Male or female patients age ≥ 18 years

3. ECOG performance status 0-2

4. Required initial laboratory values (performed within 14 days prior to eligibility confirmation by physician-investigator):
   a. WBC >3 THO/µL
   b. Hg ≥ 9.0 g/dL
   c. Platelets >75 THO/µL
   d. Total Bilirubin < 2.0 mg/dl; unless the subject has known or suspected Gilbert syndrome for which ≤ 3 mg/dl is permitted
   e. Serum Creatinine < 2.0 mg/dl

5. Subjects of reproductive potential must agree to use a medically accepted birth control method during the trial and for at least two months following the trial. Please see **Section 5.0** for additional details.

6. Provide written informed consent.

3.2 Exclusion Criteria

1. Prior treatment with more than one line of cytotoxic chemotherapy; prior treatment with one line of cytotoxic chemotherapy is permitted. Prior treatment with targeted therapy (such as ipilimumab, anti-PD-1, or BRAF + MEK inhibitor combination) is permitted.

2. Active untreated CNS metastasis

3. Active infection

4. Prior malignancy (except non-melanoma skin cancer) within 3 years

5. Pregnant or nursing (lactating) women

6. Concurrent treatment with high-dose systemic corticosteroids; local (inhaled or topical) steroids are permitted

7. Known allergy to eggs

8. Prior history of uveitis or autoimmune inflammatory eye disease

9. Known positivity for hepatitis BsAg, hepatitis C antibody, or HIV antibody

4.0 REGISTRATION

At the time a subject consents to participate in this study, the subject should be registered in the PennCTMS (i.e. Velos). Once subject eligibility has been confirmed by a physician-investigator, apheresis collection and the manufacturing of the study vaccine may commence. Patients who fail apheresis or who do not receive vaccine due to other reasons will be replaced and will not be considered evaluable.

Each Subject is identified in the study by a Subject Number that is assigned when the subject is first consented and is retained as the primary identifier for the subject throughout his/her entire participation in the trial. The Subject Number consists of the Protocol Number with a sequential subject number suffixed to it, so that each subject is numbered uniquely across the entire database. Once assigned, the Subject Number must not be reused for any other subject and the Subject Number for that individual must not be changed, even if the subject is re-screened. If the subject fails screening for any reason, the reason will be entered into the End of Study eCRF.

5.0 PATIENT RECRUITMENT AND SCREENING

Approximately 12 evaluable subjects will be enrolled in this study. Patients who fail apheresis or who do not receive vaccine due to other reasons will not be considered evaluable and will be replaced.

Patients will be identified through the clinical practices of the investigator or sub-investigators and through referrals from outside hospitals and physicians. The study will be posted on clinicaltrials.gov, and publicized via University of Pennsylvania or Abramson Cancer Center press releases. No direct-to-patient advertising will be performed.

Female patients of reproductive potential (women who have reached menarche or women who have not been post-menopausal for at least 24 consecutive months, i.e., who have had menses within the preceding 24 months, or have not undergone a sterilization procedure [hysterectomy or bilateral oophorectomy]) must have negative pregnancy test performed at the time of screening and within 30 days of the subject's 1st DC vaccine (Day 1).

Patients must agree not to participate in a conception process while participating in this study (e.g., active attempt to become pregnant or to impregnate, sperm donation, in vitro fertilization). Additionally, if participating in sexual activity that could lead to pregnancy, the study patient must agree to use at least one reliable method of contraception during their participation in the study.

Acceptable birth control includes one of the following methods:

- Total abstinence (no sexual relations)
- Female sterilization- surgical removal of both ovaries (woman's reproductive system that stores and releases eggs for fertilization and produces female sex hormones), or tubal ligation (having your "tubes tied") at least six weeks prior to signing this consent.
- Male sterilization (i.e. vasectomy)
- Condoms (male or female) with or without a spermicidal agent
- Diaphragm or cervical cap with spermicide
- Intrauterine device (IUD)
- Hormonal-based contraception

Patients who are not of reproductive potential (women who have been post-menopausal for at least 24 consecutive months or have undergone hysterectomy, salpingectomy, and/or bilateral oophorectomy or men who have documented azoospermia) do not require the use of contraception. Acceptable documentation of sterilization, azoospermia, and menopause is specified below:

Written documentation by clinician or clinician's staff through one of the following:

1. Physician report/letter
2. Operative report or other source documentation in the patient record (a laboratory report of azoospermia is required to document successful vasectomy)
3. Discharge summary of sterilization procedure or hysterectomy, and/or salpingectomy, oophorectomy
4. Laboratory report of azoospermia
5. Follicle stimulating hormone measurement elevated into the menopausal range

6.0 CONCOMITANT THERAPY

All prescription and nonprescription medication, vitamins, herbal and nutritional supplements, taken by the patient within 14 days prior to eligibility confirmation by a physician-investigator will be recorded. Concomitant medications will continue to be collected at every visit thereafter, until the subject has discontinued participation in the study. Any additions, deletions, or changes of these medications will be documented.

For all subjects, a 28-day washout period for any prior therapy (including adjuvant interferon) is required prior to the first vaccine dose. This does not include adjunctive therapies (i.e. steroids, growth factors, etc); just prior therapies for their melanoma.

High-dose systemic corticosteroids (i.e., prednisone >10 mg daily or equivalent) are prohibited.
Low-dose systemic corticosteroids (i.e. prednisone ≤10 mg daily or equivalent) are permitted, as well as local steroids (inhaled or topical).

7.0 INVESTIGATIONAL PRODUCTS

7.1 Dendritic Cell Vaccines (mDC3/8 vaccines with and without influenza)

7.1.1 Agent Description

For this study, Dendritic Cell Vaccines (mDC3/8 vaccines) are named as follows:

- Priming Vaccine – hereafter identified as DC Vaccine #1
- Booster Vaccines– hereafter identified as DC Vaccine #2 and DC Vaccine #3

Autologous dendritic cells pulsed with 12 (or fewer) melanoma tumor-specific peptides. For DC Vaccine #1 only, inactivated influenza vaccine is added to the DC preparation to provide a source of class II antigen to induce CD4+ helper T cells. For patients who are not HLA-A2 positive, the gp100 peptides are omitted since they will not bind. Total number of peptides used will remain at a maximum of 12.

Please refer to the Investigator's Brochure for additional information.

7.1.2 Mechanism of Action

Induction of antigen-specific CD8+ T cells that function to promote tumor regression through multiple mechanisms including 1) direct tumor cytolysis requiring cell to cell contact; 2) secretion of cytokines such as Interferon-γ and tumor necrosis factor. Administration of cyclophosphamide 3-4 days prior to the first immunization will deplete regulatory T cells that serve to inhibit the generation of immunity. Multiple immunizations are necessary to elicit and sustain CD8+ T cell immunity.

7.1.3 Pharmacodynamics/Kinetics

Immunological monitoring is planned to assess the response to DC immunization. Regulatory T cell (Treg) counts will be assessed pre-treatment.

7.1.4 Formulation

Synthetic peptides (>98% purity) will be obtained commercially.

7.1.5 Availability

Cells used to manufacture the DC vaccines will be obtained by apheresis prior to administration of cyclophosphamide.

7.1.6 Preparation

The manufacture and release testing of the vaccine product will be performed by the Clinical Cell and Vaccine Production (CVPF) at the University of Pennsylvania. The vaccine product will be manufactured from the patients' apheresis product. The vaccine products are not released from the CVPF until release criteria are met.

Vaccine will be made starting from fresh peripheral blood mononuclear cells (PBMC) in

accordance with the Investigator's Brochure. DC preparations that fail to pass each release criteria will be held and not released for patient administration.

7.1.7 Packaging and Labeling

The investigational product will be released at room temperature in an infusion bag and affixed with a label containing information regarding the dose, the number of cells and volume and the following statements "FOR AUTOLOGOUS USE ONLY" and "Caution: New Drug-Limited by Federal Law to Investigational Use". In addition, the label will have at least two unique identifiers.

7.1.8 Administration

Dendritic cells (mDC3/8 Vaccine) will be administered intravenously at the following doses:

- DC Vaccine #1 (Priming Vaccine Dose): maximum of 7.5-15 million DC per peptide
- DC Vaccine #2 and #3 (Booster Vaccine Doses): maximum of 1-5 million DC per peptide x 2 doses administered ~ q6 weeks apart.

Please see **Section 8.4** for additional details.

7.1.9 Potential Toxicities

Please refer to the Investigator's Brochure for toxicity information.

7.1.10 Return or Destruction of Study Drug

The investigational product may need to be returned to the manufacturing facility for a variety of reasons, including but not limited to: 1) Mislabeled product; 2) Condition of patient prohibits infusion/injection, and 3) Subject refuses infusion. Any unused product will be returned to CVPF for freezing by CVPF personnel. Final disposition of the investigational product must be documented appropriately.

7.2 Cyclophosphamide

7.2.1 Agent Description

Cyclophosphamide is an FDA-approved chemotherapeutic agent. Cyclophosphamide is considered an investigational agent on this protocol since it is not the standard of care in this patient population and is not being used in accordance with its approved labeling. However, while administered for research purposes as part of this study, it will be prepared and infused in accordance with its approved package insert. Full details on its mechanisms of action and toxicity profiles can be found in the approved package insert.

7.2.2 Receipt and Storage

Commercial cyclophosphamide will be obtained through the site-designated research pharmacy for research purposes. It will be stored according to the manufacturing instructions in the approved package insert.

7.2.3 Premedication

All patients receiving cyclophosphamide may be premedicated with an anti-emetic. The choice of premedication will be left to discretion of the treating physician. Cyclophosphamide is associated with moderate to high emetic potential, and antiemetics are recommended to prevent nausea and vomiting. Combining NK1R antagonist (e.g. aprepitant or fosaprepitant) with 5-HT3 receptor antagonist (e.g. ondansetron) is recommended, but may be modified per investigator discretion and institutional standards. Glucocorticoids (e.g. dexamethasone) should be avoided as these agents may affect vaccine efficacy.

7.2.4 Administration

A single dose of 300 mg/m^2 cyclophosphamide will be administered via intravenous infusion on Day -3 (+/- 1 day) prior to the first dendritic cell vaccine (mDC3/8).

7.2.5 Potential Toxicities

Please refer to the cyclophosphamide package insert for toxicity information. The package insert describes the risks of cyclophosphamide when given as part of routine care. We expect similar side effects when administered as part of this study.

7.3 Anti-PD-1 (Pembrolizumab)

Pembrolizumab is FDA approved for the treatment of metastatic melanoma. Beginning as early as 14 days after the subjects' last DC Vaccine, subjects will commence treatment with pembrolizumab (anti-PD-1 therapy) as per standard of care. The timing of pembrolizumab administration will be at the discretion of the physician investigator. Treatment will be administered per the FDA approved package insert. As administration and duration of treatment will be driven by routine care and not per protocol requirements, these drugs will not be supplied by the study and will be obtained commercially through the subjects' insurance provider. Please refer to the pembrolizumab package insert for toxicity information.

8.0 STUDY PROCEDURES

8.1 On-study Biopsies

Onstudy biopsies will be performed as follows:

- Post DC Vaccine Biopsy #1: Will occur after the subject's last DC vaccine, and prior to initiation of treatment with Anti-PD-1 therapy.
- Post DC Vaccine Biopsy #2: 4 weeks (+/- 5 days) after the subject's first dose of Anti-PD-1 therapy.

- Post DC Vaccine Biopsy #3: 8 weeks (+/- 1 week) after the subject's first dose of Anti-PD-1 therapy.

Onstudy biopsies will only be performed in patients with accessible lesions. This will be determined by the treating investigator.

8.2 Dendritic Cell (mDC3/8) Preparation

After eligibility has been confirmed by a physician-investigator, subjects will undergo a large volume apheresis procedure (approximately 15-20 L volume) at the HUP Apheresis Unit according to standard procedures for autologous transplantation, to allow for a target of 1.5-2.0 x 10^{10} mononuclear cells to be collected. The actual volume apheresis procedure to be performed will be at the discretion of the physician-investigator in consultation with transfusion medicine. The apheresis product will be transported to the CVPF manufacturing facility and manufactured in accordance with the current mDC3/8 Investigator's Brochure.

If the initial apheresis collection does not yield an adequate number of cells required for manufacturing all required DC Vaccines, the second study apheresis procedure (typically performed after the last DC Vaccine and prior to administration of Anti-PD-1 therapy) may be moved up to an earlier study timepoint to allow for required manufacturing. An approximate 15 liter volume procedure will be performed, and the product processed as indicated in the Investigator's Brochure.

Cells remaining after manufacturing is complete may be banked for research purposes.

8.3 Treg Depletion

Patients will return to the Hospital of the University of Pennsylvania to receive a single dose of IV Cyclophosphamide (300 mg/m^2) on Day -3 (+/- 1 day) prior to DC#1 dose, in order to deplete circulating Treg prior to Dose #1. Patients may receive premedication at the discretion of the treating physician. Only a single dose of cyclophosphamide is given to each patient during the initial treatment phase prior to DC vaccine #l.

8.4 Administration of Dendritic Cell Vaccines (mDC3/8 vaccines with and without influenza)

A total of 3 DC vaccines will be administered, approximately 6 weeks apart (+/- 2 weeks) beginning on Day 1. The timing of the DC Vaccine Infusions will be calculated from the 1st DC Vaccine Infusion (i.e. Day 1, also known as Week 1). Thus, the 2nd DC Vaccine Infusion is planned for 6 full weeks after Day 1/Week 1 at ~Day 43 (i.e. the end of Week 6 and the beginning of Week 7). Similarly, the 3rd DC Vaccine Infusion is planned for 12 full weeks after Day 1/Week 1 at ~Day 85 (i.e. the end of Week 12 and the beginning of Week 13). Vaccines cannot be administered until all toxicities $\geq$ grade 2 have resolved to baseline.

All infusions will take place at the Hospital of the University of Pennsylvania by a licensed Registered Nurse. Each DC vaccine is administered by intravenous infusion through either a peripheral venous line or a central venous line. A macrodrip intravenous tubing will be used to infuse DC vaccine by gravity (i.e. no infusion pump will be used), therefore each infusion will take less than 15 minutes. All subjects will have vital signs (including temperature, respiration rate, pulse, blood pressure, oxygen saturation) assessed prior to each infusion. After the first infusion,

subjects will be observed for 2 hours after the end of the infusion, with vital signs assessed every 30 minutes (+/- 5 minutes) from the start of the infusion. After each subsequent infusion, subjects will be observed for 30 minutes after the end of the infusion, with vital signs assessed every 30 minutes (+/- 5 minutes) from the start of the infusion. If subjects develop temperature greater than 38ºC, acetaminophen 650mg PO should be given and the PI notified the same day. After the required observation period is complete, subjects may be discharged to home from the treatment area.

Administration of the DC Vaccine may be delayed for a number of reasons, including toxicity, investigator discretion, etc. Treatment delays of up to 21 days will be allowed. If administration of the next planned dose is delayed greater than 21 days, the patient will discontinue the study vaccine and will not be eligible to receive additional treatment on this protocol. Treatment delays should be to be kept to a minimum and every effort is to be made to maintain a planned schedule.

8.5 Immune Monitoring

Starting on Day 1/Week 1 (DC vaccine #1) and weekly thereafter through Week 18 (~Day 120), up to four 10mL green top tubes (BD vacutainer sodium heparin tube, ~ 40 mL) will be obtained by venipuncture for immune monitoring analysis. After the Week 18 collection, the frequency of peripheral blood draws will shift to q3 weeks for up to12 additional weeks (i.e. at Week 21, Week 24, Week 27, and Week 30). Sample collection will continue on this schedule, regardless of the timing of the 2nd and 3rd DC Vaccinations and any subsequent Anti-PD-L1 therapy. Any missed sample collections must be documented appropriately. These blood samples will be used for tetramer assay and determination of Treg cell counts. The blood samples will be transported to the Dr. Carreno's laboratory (SPE 8-309, bays 305B-307B).

Patients will also undergo two additional apheresis procedures to collect lymphocytes for functional laboratory assays, to determine immunity against the melanoma peptides, at the following timepoints:

- Apheresis #2: Collected after the subject's last DC Vaccine and prior to anti-PD-1 therapy being administered. Note: As per **Section 8.2** above, this Apheresis Collection may be moved up to an earlier study timepoint if deemed necessary for manufacturing purposes.
- Apheresis #3 (Optional): Approximately 4-8 weeks after initiation of anti-PD-1 therapy (Optional).

The additional apheresis procedures #2 and #3 noted above will be performed at the HUP Apheresis Unit according to standard procedures. At each timepoint, an approximate 15 liter volume apheresis procedure will be performed. These cells will then be transported to Dr. Carreno's laboratory for processing.

Please refer to the Study Calendar in **Section 10.0** for additional information related to study timepoints.

8.6 Assessment of Clinical Response

Patients will undergo routine clinical monitoring, blood work and physical examinations. RECIST 1.1 criteria [56] will be used for tumor response assessment after the subject's last vaccine dose. CT of the chest, abdomen, and pelvis is the preferred imaging modality for assessment. In certain instances, MRI scans or other imaging modalities may be performed at physician-investigator discretion. For cutaneous lesions, direct measurement is also acceptable and visible lesions should

be photographed. After initiation of Anti-PD-1 therapy, subjects will be assessed for clinical response according to routine care guidelines. Data from any response evaluations performed while the subject remains onstudy will be collected for research purposes.

Please refer to the Study Calendar in **Section 10.0** for complete details.

8.7 Anti-PD-1 Administration

Beginning as early as 14 days after the subjects' last DC Vaccine, subjects will commence treatment with pembrolizumab (anti-PD-1 therapy) as per standard of care. The timing of pembrolizumab administration will be at the discretion of the physician investigator. Treatment with pembrolizumab will be administered per the FDA approved package insertand will not be driven by protocol requirements.

During anti-PD-1 administration subjects will continue to have blood samples collected for immune monitoring purposes in accordance with **Section 8.5**, and will undergo apheresis and biopsy procedures for research purposes as outlined above.

If a subject dose not receive pembrolizumab per routine care post-DC Vaccine, either at the physician-investigator's discretion or per subject decision, the subject will complete their End of Study Treatment Visit as scheduled and continue to have their immune monitoring samples collected per protocol. However, the subject would not undergo the Post-Anti-PD-1 Therapy Visit #1 or Visit #2. The subject would then complete the End of Study visit once immune monitoring sample collection is completed per protocol requirements.

9.0 POTENTIAL TOXICITY AND DOSE MODIFICATIONS

Any toxicities of grade 2 or higher, regardless of attribution/expectedness, should be reported immediately to the Principal Investigator. Additional vaccines cannot be administered until all toxicities $\geq$ grade 2 have resolved to baseline.

9.1 Dose Limiting Toxicity

Is defined as any of the below events determined to be at least possibly related to the dendritic cell vaccines (mDC3/8 vaccine):

- Any Grade 3 or greater hematological and non hematological toxicities
- Any Grade 3 or greater allergic reaction
- Any Grade 3 or greater autoimmunity that involves vital organ (heart, kidneys, brain, eye, liver, colon and adrenal gland).

If a subject experiences a DLT, they will be discontinued from additional study treatment. Subjects discontinued from study treatment due to a DLT will continue to be followed per the Study Calendar in **Section 10.0** until the End of Study visit, and for adverse events per **Section 13.1**. Dose-limited toxicities will be confirmed by the Sponsor Medical Director. The Medical Director will also assess the impact of dose-limiting toxicities on subsequent enrollment/ treatment activity. In the event of a DLT and treatment discontinuation, immune monitoring blood samples will continue to be collected until the EOS visit. Please refer to **Section 13.7** for study stopping rules.

10.0 STUDY CALENDAR

Tests and Observations	Screening/ Enrollment[w]	Pre-Treatment (≤ 14 days prior to 1st DC Vaccine)[w]	Cyclo-phosphamide Day -3 (+/- 1 day)	DC Vaccination Visits (Every 6 weeks +/- 2 weeks)[v]			End of Study Treatment Visit (~10-28 Days.After Last DC Vaccine and Prior to Initiation of Anti-PD-1 Therapy)[s]	Post-DC Vaccine Follow-up		
				DC Vaccine #1 (Priming Vaccine)	DC Vaccine #2 (Booster Vaccine)	DC Vaccine #3 (Booster Vaccine)		Post Anti-PD-1 Therapy-Visit #1 (4 weeks after initiation of Anti-PD-1 Therapy +/- 5 days)[t]	Post Anti-PD-1 Therapy-Visit #2 (8 weeks after initiation of Anti-PD-1 Therapy +/- 1 week)[t]	End of Study Visit[p] (~Week 30 Post-DC Vaccine #1)
				Day 1 (Week 1)	~Day 43 (+/- 14d)	~Day 85 (+/- 14d)				
Signed informed consent	X									
Tumor Biopsy[a]							X[q]	X	X	
Medical History	X----------X									
Physical Examination	X	X		X	X	X	X			
ECOG Performance Status	X	X		X	X	X	X			
Vital Sign Assessment[k]	X	X		X[k]	X[k]	X[k]	X			
Apheresis		X[b]					X[b, c]	X[f]		
Cyclophosphamide			X[i]							
Dendritic Cell Vaccination				X[n]	X[n]	X[n]				
Routine Anti-PD-1 Therapy							X[o]----------X[o]			
Tumor measurements[r]	X[g]	X[h]					X	X[m]----------X		
CBC, CMP[l], LDH	X[u]	X		X	X	X				
PT/PTT	X	X								
Immune monitoring[v]	X[d]			X[d]----------X[d]				Q3 weeks[d]		X[p]
Pregnancy test	X[e]	X[e]								
AE assessment[j]		X ---------- X[j]								
Concomitant Medications	X	X ---------- X								

Tests and Observations	Screening/ Enrollment[w]	Pre-Treatment (≤ 14 days prior to 1st DC Vaccine)[w]	Cyclo-phosphamide Day -3 (+/- 1 day)	DC Vaccination Visits (Every 6 weeks +/- 2 weeks)[v]			End of Study Treatment Visit (~10-28 Days After Last DC Vaccine and Prior to Initiation of Anti-PD-1 Therapy)[s]	Post-DC Vaccine Follow-up		
				DC Vaccine #1 (Priming Vaccine)	DC Vaccine #2 (Booster Vaccine)	DC Vaccine #3 (Booster Vaccine)		Post Anti-PD-1 Therapy-Visit #1 (4 weeks after initiation of Anti-PD-1 Therapy +/- 5 days)[t]	Post Anti-PD-1 Therapy-Visit #2 (8 weeks after initiation of Anti-PD-1 Therapy +/- 1 week)[t]	End of Study Visit[p] (~Week 30 Post-DC Vaccine #1)
				Day 1 (Week 1)	~Day 43 (+/- 14d)	~Day 85 (+/- 14d)				
Survival										X

a. Please refer to **Section 8.1** for additional details. Performed in patients with accessible lesions as determined by the treating investigator.

b. To obtain mononuclear cells for DC generation. Apheresis #1 will be performed with an approximate volume collection of 15-20 liters ~7 days prior to the first DC vaccine. If the initial apheresis collection does not yield an adequate number of cells required for manufacturing all required DC Vaccines, the second study apheresis procedure (typically performed after the last DC Vaccine and prior to administration of Anti-PD-1 therapy) may be moved up to an earlier study timepoint to allow for required manufacturing. An approximate 15 liter volume procedure will be performed, and the product processed as indicated in the Investigator's Brochure.

c. To obtain mononuclear cells for immune monitoring. Apheresis #2 will be performed after DC vaccine #3 and prior to administration of anti-PD-1 therapy at the End of Study Treatment Visit. Please refer to **Section 8.5** for additional details.

d. Immune monitoring studies will be drawn in 10mL green top tubes (BD vacutainer tube). At screening, up to four 10mL green top tubes will be collected. Starting on Week 1/Day 1 (DC vaccine #1) and weekly thereafter through Week 18 (~Day 120), up to four 10mL green top tubes (~40 mL total) will be drawn for immune monitoring analysis. After the Week 18 collection, the frequency of peripheral blood draws will shift to q3 weeks for up to 12 additional weeks (i.e. at Week 21, Week 24, Week 27, and Week 30). Sample collection will continue on this schedule, regardless of the timing of the 2nd and 3rd DC Vaccinations and any subsequent Anti-PD-1 Therapy. Any missed sample collections must be documented appropriately. Please see **Section 8.5** for additional details.

e. Females of childbearing potential. A serum or urine pregnancy test will be accepted. A pregnancy test must be performed within 30 days prior to DC Vaccine #1 (Day 1).

f. Apheresis #3 (optional) will be performed ~ 4-8 weeks after initiation of anti-PD-1 therapy. Please refer to **Section 8.5** for additional details.

g. Must be completed within 28 days prior to physician-investigator confirmation of eligibility.

h. CT scans do not have to be repeated at pre-treatment as long as screening CT scans were within 28 days of the 1st DC vaccine.

i. Please see **Sections 7.2** and **8.3** for additional details.

j. Collection of AEs will begin at the time of the first apheresis procedure (Apheresis #1) and continue until subject discontinuation or the End of Study Visit. Please refer to **Section 13.1** for additional information.

k. Vital sign assessments include temperature, respiration rate, pulse, blood pressure, and oxygen saturation. Vital signs will be assessed prior to each infusion. 1st DC Vaccine Infusion: Subjects will be observed for 2 hours after the end of the infusion, with vital signs assessed every 30 minutes (+/- 5 minutes) from the start of the infusion. Subsequent DC Vaccine Infusions: Subjects will be observed for 30 minutes after the end of the infusion, with vital signs assessed every 30 minutes (+/- 5 minutes) from the start of the infusion.

l. Comprehensive metabolic panel including Glucose, BUN, Creatinine, Sodium, Potassium, Calcium, Total Protein, Albumin, Total Bilirubin, Alkaline phosphatase, ALT (SGPT), AST (SGOT)

m. Response Follow-up: After initiation of Anti-PD-1 therapy, subjects will be assessed for clinical response according to routine care guidelines. Patients who are removed from therapy for reasons other than progressive disease will be followed according to standard of care procedures until disease progression. Patients who discontinue study therapy in the absence of progressive disease to pursue alternative therapies will also be followed according to standard of care procedures until disease progression. Data from any response evaluations performed while the subject remains onstudy will be collected for research purposes.

n. Please see **Section 8.4** for additional details.

o. Beginning as early as 14 days after the subject's last DC vaccine, subjects will commence treatment with Pembrolizumab (Anti-PD-1) as per standard of care. The timing of pembrolizumab administration will be at the discretion of the physician investigator. Treatment will be administered per the FDA approved package insert and will not be driven by protocol requirements.

p. The end of study visit will occur when the last immune monitoring blood sample is collected ~ 30 weeks after the 1st DC Vaccine (Day 1). If the subject is prematurely discontinued for any reason (e.g. Investigator discretion, Subject withdrawal of consent, etc), this must be documented appropriately and an End of Study Visit should be performed at the time of discontinuation.

q. Biopsy to be collected after the last DC vaccine dose and prior to beginning Anti-PD-1 therapy.

r. Please refer to **Sections 8.6** and **11** for additional information on response assessments.

s. The End of Treatment Visit will occur ~10-28 days after the last DC Vaccine. However this visit and associated study procedures must also occur **prior** to administration of Anti-PD-1 therapy. Anti-PD-1 therapy may commence as early as 14 days after the subject's last DC Vaccine.

t. If a subject dose not receive pembrolizumab per routine care post-DC Vaccine, either at the physician-investigator's discretion or per subject decision, the subject will complete their End of Study Treatment Visit as scheduled and continue to have their immune monitoring samples collected per protocol. However the subject would not undergo the Post-Anti-PD-1 Therapy Visit #1 or Visit #2. The subject would then complete the End of Study visit once immune monitoring sample collection is completed per protocol requirements.

u. Must be performed within 14 days prior to physician-investigator confirmation of eligibility.

v. Study timepoints will be calculated from the 1st DC Vaccine Infusion (i.e. Day 1, also known as Week 1). Thus, the 2nd DC Vaccine Infusion is planned for 6 full weeks after Day 1/Week 1 at ~Day 43 (i.e. the end of Week 6 and the beginning of Week 7). Similarly, the 3rd DC Vaccine Infusion is planned for 12 full weeks after Day 1/Week 1 at ~Day 85 (i.e. the end of Week 12 and the beginning of Week 13). This same principle applies to the timing/identification of immune monitoring sample collection.

w. Tests/procedures used to evaluate the subject's eligibility to participate must be performed prior to physician-investigator confirmation of eligibility and within 28 days prior to physician-investigator confirmation of eligibility unless otherwise specified. Tests/procedures performed for screening/enrollment which also fall within the 14 day window required for Pre-Treatment evaluations may be used to fulfill this additional study requirement and do not need to be repeated unless clinically indicated.

11.0 CRITERIA FOR RESPONSE (RECIST Criteria v1.1)

11.1 Tumor Measurement

Measurable disease: the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

Measurable lesions: lesions that can be accurately measured in at least one dimension with the longest diameter ≥2.0 cm. With a spiral CT scan, the lesion must be ≥1.0 cm in at least one dimension.

Non-measurable lesions: all other lesions, including small lesions (longest diameter < 2.0 cm with conventional techniques or <1.0 cm with spiral CT scans) and other non-measurable lesions. These include: bone lesions; leptomeningeal disease; ascites; pleural/pericardial effusion; inflammatory breast disease; lymphangitis cutis/pulmonis; abdominal masses that are not confirmed and followed by imaging techniques; and cystic lesions.

All measurements should be recorded in metric notation, using a ruler or calipers. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules, palpable lymph nodes). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesions is recommended.

11.2 Baseline Documentation of Target and Non-target Lesions

All measurable lesions up to a maximum of 10 lesions representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline.

Target lesions should be selected on the basis of their size (lesions with the longer diameter) and their suitability for accurate repetitive measurements (either by imaging techniques or clinically).

A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as "present" or "absent."

11.3 Response Criteria

11.3.1 Evaluation of Target Lesions

Complete response (CR)–disappearance of all target lesions.

Partial response (PR)–at least a 30% decrease in the sum of the LD of the target lesions taking as reference the baseline sum LD.

Progression (PD)–at least a 20% increase in the sum of the LD of the target lesions taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.

Stable disease (SD)–neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD since the treatment started.

11.3.2 Evaluation of Non-target Lesions

Complete response (CR)–disappearance of all non-target lesions and normalization of tumor marker level.

Non-complete response (non-CR)/non-progression (non-PD)–persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits.

Progressive disease (PD)–appearance of one or more new lesions. Unequivocal progression of existing non-target lesions. Although a clear progression of non-target lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed later on by a review panel (or principal investigator).

11.4 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). In general, the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "symptomatic

deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment.

In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends upon this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

11.5 Confirmation

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat studies that should be performed no less than 4 weeks after the criteria for response are first met.

In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of 8-12 weeks.

12.0 EARLY WITHDRAWAL OF SUBJECTS

Subjects who enroll but do not receive DC Vaccine #1 will be prematurely discontinued from the study, will not be followed (with the exception of monitoring of adverse events felt to be related to research procedures), and will be replaced in the study. Reasons for premature discontinuation prior to receipt of DC Vaccine #1 may include, but are not limited to the following:

- Screen failures
- The judgment of the principal investigator that the patient is too ill to continue if this occurs prior to the vaccine dose.
- Pregnancy
- Technical difficulties are encountered in the manufacturing process that preclude generation of a vaccine dose that meets all Quality Control criteria.
- If a subject develops a condition that precludes treatment after eligibility confirmation by physician-investigator but before administrative of the vaccine dose. This will be done at the judgment of the PI, and could include for example, the occurrence of an intercurrent illness, significant and rapid progression of malignancy requiring alternative treatment, or a serious adverse event.
- Patient withdraws consent
- Termination of the study

Patients may also be discontinued from Protocol Therapy for any of the following reasons:

- Patient withdraws consent
- The PI decides to remove the patient from the study (i.e. for non-compliance with the protocol or rapid clinical progression requiring urgent therapeutic intervention).
- Termination of the study
- Patients who develop DLT (as defined in **Section 9.1**)
- Patients who develop an allergic reaction to the dendritic cell vaccine (mDC3/8 vaccines).
- Pregnancy

If a subject is discontinued from Protocol Therapy for any reason other than withdrawal of consent, they will remain in protocol follow-up for 30 days after the last mDC3/8 Vaccine to monitor for any potential

adverse events. During this 30 day follow-up period, immune monitoring samples will continue to be collected and subjects may be treated per routine care at the discretion of their treating physician.

13.0 SAFETY AND ADVERSE EVENTS

13.1 Definitions

Adverse Event
An ***adverse event*** (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Intercurrent illnesses or injuries should be regarded as adverse events.

Serious Adverse Event
Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- leads to a persistent or significant disability or incapacity or substantial disruption of the ability to conduct normal life functions
- a congenital anomaly or birth defect
- an important medical event

Note that hospitalizations that meet the following criteria should not be reported as serious adverse events:

- Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, such as preplanned study visits and preplanned hospitalizations for study procedures or treatment administration
- Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent
- Social reasons and respite care in the absence of any deterioration in the patient's general condition

Note: Treatment on an emergency outpatient basis that does not result in hospital admission and involves an event not fulfilling any of the definitions of a SAE given above is not a serious adverse event.

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the patient, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result-in patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Unanticipated Adverse Device Effect
Unanticipated adverse device effect means any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

Unexpected adverse events
An adverse event is considered unexpected if the event, and the severity (grade) and/or frequency of the event, is not described in the investigator brochure or protocol. Please refer to the investigator brochure for additional detail related to severity and/or frequency of a particular event.

Related adverse events
An adverse event is considered related to participation in the research if there is a reasonable possibility that an event was caused by an investigational product, intervention, or research-required procedures. For the purposes of this study, "reasonable possibility" means there is evidence to suggest a causal relationship. The relationship of the event to the study will be classified as possibly related, probably related, and definitely related.

- Possibly Related: There is some evidence to suggest a causal relationship; however, other factors may have contributed to the event.
- Probably Related: There is evidence to suggest a causal relationship, and the influence of other factors is unlikely.
- Definitely Related: There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out.

Adverse Event Reporting Period
For this study, collection of AEs will begin at the time of the first apheresis procedure (Apheresis #1) and continue until subject discontinuation or the End of Study Visit.

If a subject is taken off study within 30 days of study treatment, any event that is determined to be probably or definitely related regardless to time at which it occurs after infusion(s), will be reported to the Sponsor Medical Director for assessment.

Preexisting Condition/General Physical Examination Findings
A preexisting condition is one that is present at the start of the Adverse Event Reporting Period. All clinically significant abnormalities should be recorded as a preexisting condition on the medical history eCRF. During the course of the study, a preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens. Preexisting conditions that improve should also be recorded appropriately.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if <u>any one of the following</u> conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose,

discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Laboratory abnormalities that meet the criteria for Adverse Events should be followed until they have returned to normal or an adequate explanation of the abnormality is found. When an abnormal laboratory or test result corresponds to a sign/symptom of an already reported adverse event, it is not necessary to separately record the lab/test result as an additional event. Laboratory abnormalities that do not meet the definition of an adverse event, should not be reported as adverse events. Whenever possible, a diagnosis, rather than a symptom should be provided (i.e. anemia instead of low hemoglobin).

13.2 Recording of Adverse Events

Safety will be assessed by monitoring and recording potential adverse effects of the treatment using the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 at each study visit. Patients will be monitored by medical histories, physical examinations, and blood studies to detect potential toxicities from the treatment. If CTCAE grading does not exist for an adverse event, the severity of mild, moderate, severe, life-threatening, and death, corresponding to Grades 1-5, will be used whenever possible. Subjects will be monitored by medical histories, physical examinations, and blood studies to detect potential toxicities from the treatment.

At each contact with the subject, the investigator must seek information on adverse events by non-directive questioning and, as appropriate, by examination. Adverse events also may be detected when they are volunteered by the subject during the screening process or between visits, or through physical examination, laboratory test, or other assessments. Information on all adverse events should be recorded in the source documentation. All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document, though should be grouped under one diagnosis. To the extent possible, adverse events should be recorded as a diagnosis and symptoms used to make the diagnosis recorded within the diagnosis event. Do not list symptoms separately if a diagnosis can be assigned. The safety team may require events be reported separately if they occur as SAEs (or in the context of a SAE) even if they can also be considered a constituent of another AE.

All adverse events occurring during the adverse event reporting period (defined in **Section 13.1** above) must be recorded. Adverse events that begin in Primary Follow-up and are ongoing at the time the subject enters the Post-DC Vaccine Surveilliance phase of the study will continue to be followed until: a) the adverse event resolves; b) the subject discontinues participation (i.e. End of Study); or c) there is a change in the adverse event that would normally require the event be captured as a new event (i.e. change in attribution). Please refer to the CRF Completion Guidelines (CCG) for specific instructions on data entry.

As much as possible, each adverse event should be evaluated to determine:

1. The severity grade (CTCAE Grade 1-5)
2. Its duration (start and end dates)
3. Its relationship to the study treatment- [Reasonable possibility that AE is related: No (unrelated/ not suspected) or Yes (a suspected adverse reaction)]. If yes (suspected)- is the event possibly, probably or definitely related to the investigational treatment?
4. Expectedness to study treatment- [Unexpected- if the severity and/or frequency is not described in the investigator brochure or protocol].

5. Action taken with respect to study or investigational treatment (none, dose adjusted, temporarily interrupted, permanently discontinued, unknown, not applicable)
6. Whether medication or therapy taken (no concomitant medication/non-drug therapy, concomitant medication/non-drug therapy)
7. Whether it is serious, where a serious adverse event (SAE) is defined as in **Section 13.1**.

All adverse events should be treated appropriately. If a concomitant medication or non-drug therapy is given, this action should be recorded. Once an adverse event is detected, it should be followed until its resolution or until it is judged to be permanent, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study treatment, the interventions required to treat it, and the outcome.

Adverse events that occur concurrently with the progression of malignancy but that are not related to disease progression (i.e. deep vein thrombosis or hemoptysis) will be reported as an adverse event as described above. Progression of malignancy resulting in death should be reported as a serious adverse event.

Serious adverse events that are still ongoing at the end of the adverse event reporting period must be followed to determine the final outcome. Any serious adverse event that occurs after the adverse event reporting period and is considered to be possibly related to the study treatment or study participation, should be promptly recorded and reported.

13.3 Reporting of Serious Adverse Events

Every SAE and UADE, **regardless of suspected causality**, occurring during the adverse event reporting period defined in **Section 13.1** must be reported to the Sponsor Team within 24 hours of learning of its occurrence. The original SAE notification may take place by email to meet the 24 hour reporting window.

Within 3 business days of initial knowledge of the event, the investigator must submit a complete SAE form to the Sponsor along with any other diagnostic information that will assist the understanding of the event. The Investigator will keep a copy of this SAE Form on file at the study site.

New or follow-up information on SAEs should be promptly reported as updates become available.

At a minimum follow-up SAE Forms should be submitted:

- Within 1 week of ICU admission or any life-threatening event
- Within 2 weeks of hospital discharge

Follow-up information should be submitted as an amendment to the initial SAE form, and should include both the follow-up number and report date. The follow-up information should describe whether the event has resolved or continues, if there are any changes in assessment, if and how it was treated, and whether the patient continued or withdrew from study participation.

Report serious adverse events by email to:

Attention: Clinical Safety Manager or designee
Center for Cellular Immunotherapies
University of Pennsylvania

At the time of the initial notification, the following information should be provided:

- Study identifier
- Subject number
- A description of the event
- Date of onset
- Current subject status
- Whether study treatment was discontinued
- The reason the event is classified as serious
- Investigator assessment of the association between the event and study treatment
- Expectedness relative to investigational product(s)

13.3.1 Investigator Reporting: Local Regulatory Review Committees

Report events to local regulatory review committees per institutional requirements.

13.4 Pregnancies

To ensure patient safety, each pregnancy occurring while the patient is on study treatment must be reported to protocol sponsor within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. If a pregnancy occurs on study, this will be reported as an SAE using an SAE Report Form.

Pregnancy outcomes must be collected for the female partners of any males who took study treatment in this study. Consent to report information regarding these pregnancy outcomes should be obtained from the mother.

13.5 Protocol Exceptions/Deviations

Exception:
A one time, **intentional** action or process that departs from the IRB-approved study protocol, intended for **one** occurrence. If the action disrupts the study progress, such that the study design or outcome (endpoints) may be compromised, or the action compromises the safety and welfare of study subjects, **advance** documented approval from the Regulatory Sponsor and local regulatory review committees per institutional guidelines is required. Approval from the Regulatory Sponsor must be received prior to submission to local regulatory review committees for approval.

Deviation:
A one time, **unintentional** action or process that departs from the approved study protocol, involving one incident and **identified retrospectively**, after the event occurred. If the impact on the protocol disrupts the study design, may affect the outcome (endpoints) or compromises the safety and welfare of the subjects, the deviation must be reported to the Regulatory Sponsor within 10 business days of PI knowledge, and to local regulatory review committees per institutional guidelines. Acknowledgement from the Regulatory Sponsor must be received prior to submission to local regulatory review committees.

Other deviations should be appropriately documented per site policies/procedures (such as a subject missing a visit is not an issue unless a critical/important treatment or procedure was missed and must have been done at that specific time).

Include the following information on the Sponsor supplied exception/deviation form: protocol number, subject study number, comprehensive description of the exception/deviation from the protocol, rationale, and corrective and preventative action plan (deviations only). Ensure all completed exception/deviation forms are signed by the Principal Investigator (or physician sub-investigator) and submitted to the Sponsor Project Manager for review.

Attention: Sponsor Project Manager
Center for Cellular Immunotherapies (CCI)
University of Pennsylvania

Once approval of the exception request or acknowledgement of the deviation has been granted by the Regulatory Sponsor, the exception or deviation will be submitted to all applicable committees for review and approval/acknowledgement per institutional guidelines.

13.6 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at the clinical site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

13.7 Study Stopping Rules

This trial will be paused if:

- Any death that may be related to the investigational product.
- DLT is observed in 2 out of the first 2 subjects, 3 out of the first 4 subjects, 4 out of the first 7, 5 out of the first 10, and 6 at any time.

The study may be discontinued at any time by the IRB, the Sponsor, the FDA, or other government agencies as part of their duties to ensure that research subjects are protected.

14.0 STATISTICAL CONSIDERATIONS

Approximately 12 evaluable patients will be enrolled in this study. Patients who fail apheresis or who do not receive vaccine due to other reasons will not be considered evaluable and will be replaced.

The primary end point of the study is to determine the post-vaccine immune response based on measuring increased numbers of peptide-specific CD8 T cells as calculated by the tetramer assay. For each peptide, the tetramer assay is performed on blood samples obtained at the indicated time points. Data are presented as the percentage of CD8+ T cells positive for tetramer binding based on gating variables set using the iMASC reagent kit (Beckman Coulter). The lower limit of detection (LLD) is 0.03% based on the acquisition of 100,000 gated events in the lin-CD8+ gate. The HIV gag HLA-A*0201 restricted peptide (SLYNTVATL) is used as the non-binding control peptide. Each sample is stained with the HIV gag tetramer and the number of HIV gag positive CD8+ T cells is subtracted from each experimental point (ref 24). The mean and SD at baseline is obtained for each patient. Based on our prior experience, the expected

analytical CV (standard deviation/mean) is 10%. A positive tetramer response is defined as an increase greater than 3SD above the baseline value. For each gp100 peptide, the proportions of tetramer response (positive or negative) and the associated 90% exact confidence intervals (CI) will be reported for each time point. With 12 patients, the width of the 90% exact CI will be no more than 25% away from the observed proportions. Tetramer counts (percentage of CD8+ T cells positive for tetramer binding) are expected to be approximately Gaussian on the original or a transformed scale (e.g., log transformation). Linear mixed models will be used to describe the change in counts by time, dose and the time by dose interaction and pattern over time will be examined graphically. Dose is treated as a fixed effect as the doses to be administered cover the range about which conclusions will be drawn. We will explore whether Time should be treated as a random effect, as the time points represent a sample from an ongoing process. In addition, clustered logistic regression will be used to model the probability of tetramer positive versus negative by time, dose and time by dose interaction. These models are intended to be descriptive, and no preliminary data exist with which estimate longitudinal trends, so no power calculation is attempted.

The primary endpoint will also include evaluating the safety and tolerability of the mature dendritic cell vaccine using proportions and confidence intervals. Adverse events will be tabulated by grades and body system.

Additional correlative assays will be performed (Elispot and Cr51 release); however, these data will be not be used to determine the primary end point but be used as surrogate assays for the tetramer assay to evaluate the functionality of gp100 peptide induced CD8+ T cells. These assays will be performed using CD8+ T cells from baseline and post-vaccination blood samples. A positive elispot assay is defined as an increase greater than 3 SD above the baseline value. A positive Cr51 assay is defined an increase in specific lysis > 15% above background at effector: target ratio of 5:1.

For the secondary objective, clinical response rate will be computed with 90% CI. Time to progression (TTP) will be described by Kaplan-meier method and median survival time and the associated 90% CI will be reported. TTP is defined as time from the first DC dose administration to date of first documented disease progression. Patients without disease progression will be censored at time of last disease evaluation. Patients who die without evidence of progression will be censored at date of death. Regulatory T cells (Treg) are defined as CD4+CD25+foxP3+ (triple positive) cells. At the indicated time points, the percentage of Treg cells is determined by 3 color flow cytometry. The depletion of Treg is defined as follows [(Treg **baseline** – Treg **nadir)**/ Treg **baseline** x 100= % depletion] and will be analyzed as a continuous variable.

15.0 DATA HANDLING AND RECORDKEEPING

15.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of patient authorization. For subjects that have revoked authorization to collect or use PHI, attempts

should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

15.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, patients' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, patient files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

The investigator must maintain source documents for each subject in the study, consisting of case and visit notes (hospital or clinical medical records) containing demographic and medical information, laboratory data, and the results of any other tests or assessments. All information recorded on the eCRFs must be traceable to source documents in the patient's file. The investigator must also keep the original signed informed consent form, and a signed copy must be given to the patient.

15.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All entries will be entered into an electronic data capture system (EDC). The Principal Investigator is responsible for assuring that the data entered into eCRF is complete, accurate, and that entry and updates are performed in a timely manner.

15.4 Records Retention

It is the Investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents may be retained for a longer period if required.

16.0 STUDY MONITORING, AUDITING, AND INSPECTING

16.1 Study Monitoring Plan

This study will be monitored according to the Sponsor Data and Safety Monitoring Plan.

Interim Monitoring Visits will be conducted during the course of the study. The Monitors will assure that submitted data are accurate and in agreement with source documentation; verify that investigational products are properly stored and accounted for; verify that subject consent for study participation has been properly obtained and documented; confirm that research subjects entered into the study meet inclusion and exclusion criteria; and assure that all essential documentation

required by Good Clinical Practices (GCP) guidelines are appropriately filed. At the end of the study, Monitors will conduct a close-out visit and will advise on storage of study records and disposition of unused investigational products.

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

16.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the Sponsor, government regulatory bodies, and University compliance groups. The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance offices.

The Principal Investigator must notify the Sponsor in real-time if an audit/inspection notification is received.

17.0 ETHICAL CONSIDERATIONS

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject is submitted to any study procedure.

The protocol is listed on clinicaltrials.gov.

18.0 STUDY FINANCES

18.1 Funding Source

NCI R21 [redacted]

NCI R01 [redacted]

Parker Institute for Cancer Immunotherapy

18.2 Conflict of Interest

All University of Pennsylvania Investigators will follow the University of Pennsylvania Policy on Conflicts of Interest Related to Research.

18.3 Patient Stipends or Payments

There is no patient stipend/payment for participation in this protocol.

19.0 PUBLICATION PLAN

Publication of the results of this trial will be governed by University of Pennsylvania policies. Neither the complete nor any part of the results of the study carried out under this protocol will be published or passed on to any third party without the consent of the Sponsor. Any investigator involved with this study is obligated to provide the Sponsor with complete test results and all data derived from the study.

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