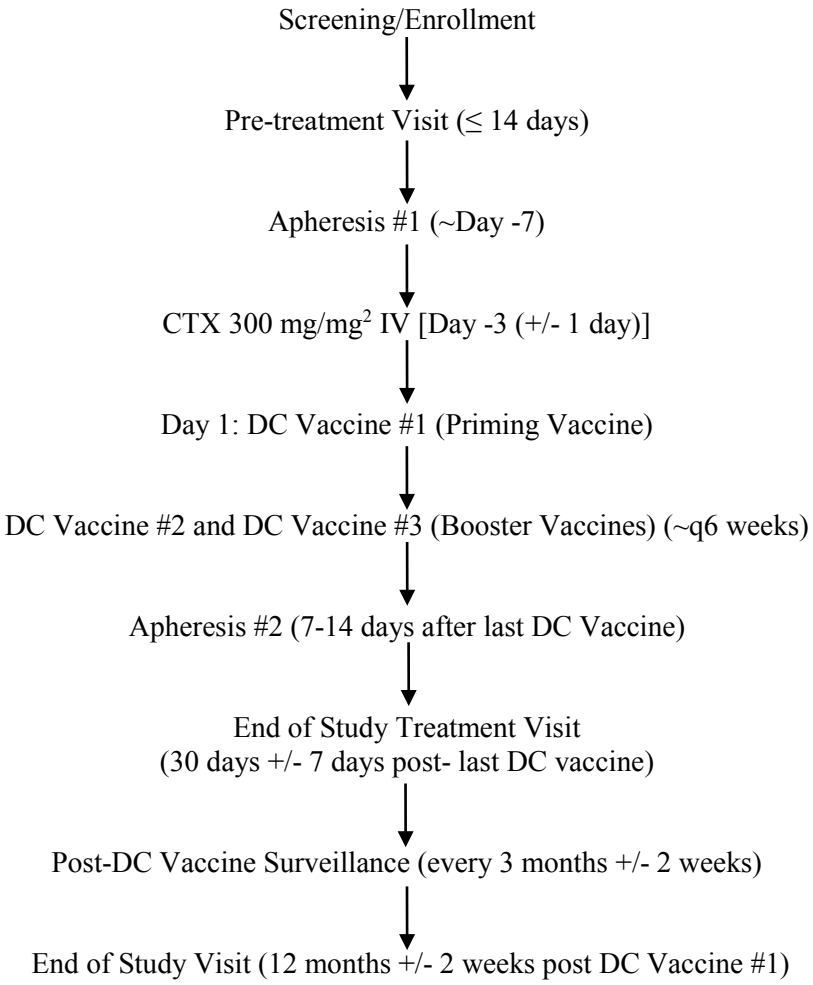
Pilot Study of Mature Dendritic Cell Vaccination for Resected Hypermutated Colorectal Cancer

NCT03730948

Principal Investigator:	Kim Reiss-Binder, MD Division of Hematology Oncology University of Pennsylvania School of Medicine Telephone: [redacted] E-mail: [redacted]
Regulatory Sponsor:	University of Pennsylvania
Sponsor Medical Director:	[redacted]
Sponsor Scientific Advisors:	[redacted]
Funding Organizations:	Parker Institute for Cancer Immunotherapy National Institutes of Health (NIH)
Protocol Number:	UPCC#16218; UPenn IRB# 831453
IND Number:	IND #13590
Main Investigational Product:	Autologous neoantigen peptide-pulsed mature dendritic cell vaccines (mDC3/8 vaccines with and without influenza)
Cell Manufacturing:	Clinical Cell and Vaccine Production Facility University of Pennsylvania
Correlative Laboratory Team:	[redacted]
Biostatistician:	[redacted]

Original Protocol Date: **V1.07-16-2018**
Amended: **V2.09-27-2018**
V3.03-11-2019
V4.10-13-2020
V5.03-11-2021
V6.06-21-2021
V7.11-11-2021

Schema



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1.0 BACKGROUND AND RATIONALE

1.1 Overview

Immunotherapy with checkpoint inhibitors is now widely accepted as an effective therapeutic approach that offers, in some instances, durable complete remission for patients with various types of cancer such as melanoma, lung, and bladder cancer[1]. One unifying theme is the high mutational burden present in tumors analyzed from responding patients providing evidence that the immune system is capable of recognizing genomic alterations (such as missense mutations)[1]. Emerging evidence confirms the activity of checkpoint inhibitors in patients with hypermutated GI malignancies such as MSI-H colorectal cancer (CRC)[2]. This present study aims to elicit T cell immunity to unique tumor encoded neoantigens using a dendritic cell (DC) vaccine platform for the purposes of identifying the genomic alterations that serve as target neoantigens in patients with CRC. The hope is this information will allow investigators to better design the next generation of personalized immunotherapies which are specific for each patient's cancer.

1.2 Colorectal Cancer (CRC) Statistics

CRC continues to be a leading cause of death in North America and worldwide. In 2018, the estimated number of new cases of CRC in the US is 140,250. The estimated number of deaths due to CRC in the US is 50,630 total (27,390 males and 23,240 females)[3]. The lifetime risk of developing invasive colorectal cancer in the US is approximately 4.5% (1/22 individuals). CRC is the fourth leading cause of death due to cancer among all age groups. In the tri-state area (Pennsylvania, New Jersey, Delaware), it is estimated that 3920 deaths due to CRC will occur in 2018[3]. Recent reports suggest that the incidence of CRC is rising in younger adults less than 50 years of age[4, 5].

1.3 Colorectal Cancer (CRC) Genetics

The molecular basis of CRC was initially outlined in 1990 by Fearon and Vogelstein[6]. A multistep model of genetic alterations including the accumulation of key driver mutations (such as KRAS) and loss of various tumor suppressor genes (such as p53) defined a conceptual framework to delineate the origins and evolution of CRC. The original multihit model has been refined and extended to include inherited susceptibility factors, environmental influences and tumor-host interactions, including the role of the immune system[7]. Recent advances now provide compelling evidence linking genetic instability with high mutational burden and recognition by the host immune system which is able to mediate tumor elimination under certain conditions. Despite the fact that most sporadic CRCs have a low mutational burden (1-2 mutations/Mb), it is now appreciated that a distinct subgroup of patients, including individuals with a familial cancer predisposition, have hyper-mutated CRC frequently associated with mismatch repair (MMR) deficiency[8].

The original 1990 model of multistep tumorigenesis defined the clonal nature of adenoma transformation to malignancy followed by metastasis but did not outline the complexities of intratumor heterogeneity as revealed by next generation sequencing methodologies. Data from The Cancer Genome Atlas (TCGA) and related efforts have defined the 4 major consensus molecular subtypes (CMS) of colorectal cancer as shown below[9]:

CMS1 MSI immune	CMS2 Canonical	CMS3 Metabolic	CMS4 Mesenchymal
14%	37%	13%	23%
MSI, CIMP high, hypermutation	SCNA high	Mixed MSI status, SCNA low, CIMP low	SCNA high
BRAF mutations		*KRAS* mutations	
Immune infiltration and activation	WNT and MYC activation	Metabolic deregulation	Stromal infiltration, TGF-β activation, angiogenesis
Worse survival after relapse			Worse relapse-free and overall survival

It is now well established that ~15% of CRCs (CMS1) are hypermutated (>10 mutations/Mb) and associated with inherited cancer syndromes (most often, Lynch syndrome), CpG island methylator phenotype, and microsatellite instability. Interestingly, the CMS1 molecular subgroup of patients is responsive to checkpoint inhibitor therapy. In 2017, both anti-PD-1 agents, pembrolizumab and nivolumab, received regulatory approval as monotherapy for treatment of relapsed/refractory MSI-H CRC that progressed after treatment with 5-FU based chemotherapy[10]. The remaining 3 molecular subtypes [(CMS2, canonical) (CMS3, metabolic) and (CMS4, mesenchymal)] are typically low mutational burden (1-2 mutations/Mb) malignancies and are not responsive to checkpoint inhibition with either an anti-PD-1 agent or anti-CTLA-4 antibody.

CMS1 type CRCs are seen in both sporadic cases of CRC as well as in association with hereditary CRC predisposition syndromes. The most common inherited CRC syndrome is Lynch syndrome which accounts for 3% of all CRC and is characterized by a germline mutation in one of the four DNA mismatch repair genes including *MSH2*, *MLH1*, *MSH6*, and *PMS2*, or in *EPCAM*, which can lead to epigenetic silencing of *MSH2*[8]. Current guidelines recommend universal testing of primary CRC specimens for MMR deficiency in all patients [11, 12]. Congenital mismatch repair deficiency (CMMRD) is a rare syndrome with inherited mutation in both alleles of a given MMR gene resulting in cancer predisposition at a younger age and increased risk for CMS1 type CRCs. Finally, a distinct familial syndrome is polymerase proofreading associated polyposis (PPAP) which is a rare autosomal dominant syndrome involving a mutation in *POLE* or *POLD1* and is characterized by ultra-hypermutated cancers (>100 mutations/Mb)[13].

Despite many CMS1 type CRCs being related to hereditary syndromes, the majority of CMS1 type CRCs are sporadic, with microsatellite instability (MSI) resulting from CpG island methylation of a DNA mismatch repair gene – typically, MLH1 – with loss of expression resulting in DNA mismatch repair deficiency. Approximately 12% of CRC patients exhibit a sporadic MSI-H phenotype resulting in the accumulation of mutations most notably at repetitive DNA sequences known as satellites[8]. By employing routine (standard of care) IHC methods to assess for MMR loss (MLH1/MSH2/MSH6/PMS2) in conjunction with molecular microsatellite testing, we are now able to universally screen all CRC patients in order to identify hypermutated CRC genomes.

1.4 Cancer Neoantigens

In 2005, Galon and colleagues published a landmark report describing the immune landscape within primary CRCs and concluded that the presence of infiltrating memory CD8+ T cells correlated with improved overall survival [14]. A follow up report with different patient cohorts confirmed this finding one year later and advanced the concept that the type, density and location of CD8+ T cells (Immunoscore) in the primary tumor was a superior predictor of outcome compared to the widely accepted TNM classification [15]. Moreover, it further supported the notion that a type-1 biased (Th1) immune response correlated with improved outcomes. Despite this important finding, it has been difficult to translate this finding into new personalized therapeutic approaches for CRC, in part, because few target antigens are well defined. However, new universal testing methods to detect mismatch repair deficiency (by IHC) and the related microsatellite instability (by PCR) can easily identify both somatic MMR deficient/MSI-H CRC as well as familial cancer syndromes (such as Lynch Syndrome). Characterization of mutational burden using NGS platforms have collectively simplified our ability to find target neoantigens in CRCs and thus, have opened the door to new therapeutic strategies[12].

Somatic gene mutations within cancer cells may be translated into peptides that are processed and presented on the surface of tumor cells. These mutated peptides can serve as foreign epitopes, or neoantigens, that may be recognized by the host immune system [16]. Neoantigen-specific T cell responses have been well documented in patients for whom immune checkpoint blockade therapy has been successful. Recent studies - including our own published work in melanoma - have demonstrated neoantigen-specific T cell responses can be elicited in cancer patients and interestingly, administration of anti-PD-1 antibody after neoantigen vaccination can result in complete melanoma regression with durable remissions [17-19]. Adoptive transfer of neoantigen-specific T cell products can also result in tumor regression in patients with melanoma as well as various GI cancers as published in case reports [16]. Thus, targeting cancer neoantigens has proven to be feasible and in certain instances, clinically effective based on durable tumor regression. Based on new scientific advances related to immuno-oncology and unmet medical need for improved adjuvant therapy, there appears to be strong rationale to investigate hypermutated CRC as a new disease indication for neoantigen directed immunotherapy[10].

1.5 Adjuvant Treatment for CRC: Current Standard

A role for adjuvant treatment of high risk, surgically resected stage III CRC was established in 1990 with the publication of a randomized controlled clinical trial with 5-fluorouracil (5-FU) and levamisole [20]. The NIH consensus panel supported the routine use of adjuvant chemotherapy for patients with stage III resected CRC but not stage II resected disease[21]. It was later shown that 5-fluorouracil/leucovorin was superior to 5-fluorouracil/levamisole and this was adopted as the standard of care by the mid-1990's [21] .

The Multicenter International Study of Oxaliplatin/5-FU/Leucovorin in the Adjuvant Treatment of Colon Cancer (MOSAIC) [22] was a landmark trial which provided clear evidence in support of adding oxaliplatin to 5-FU/Leucovorin for stage III resected CRC with a hazard ratio for recurrence, 0.77; P=0.002. A second trial (NSABP C-07)[23] provided confirmation for the superiority of oxaliplatin/5-FU/Leucovorin (FOLFOX) over 5-FU/Leucovorin; however, high rates of neurotoxicity and diarrhea/dehydration related to oxaliplatin remain a concern. Recent non-inferiority analysis of multiple trials presented at ASCO 2017 meeting Plenary session (LBA1) reviewed data from >13,000 patients and concluded that 3 months of adjuvant chemotherapy is sufficient for the specific subset of patients with low risk stage III disease (T3N1) and decreases the risk of neurotoxicity without necessarily compromising efficacy. It is important to note that

only 20% of stage III CRC patients receive benefit from adjuvant chemotherapy and thus, new treatment options are needed.

Data from multiple randomized clinical trials evaluating the use of adjuvant chemotherapy, including FOLFOX, fail to convincingly show benefit for patients with stage II resected CRC. However, for high risk stage II patients such as those with T4 lesions, perforation or obstruction at presentation, poorly differentiated histology, lymphovascular invasion, perineural invasion or fewer than 12 lymph nodes examined during surgery, chemotherapy may be considered. An important exception to this is the MSI-H or MMR deficient stage II CRC patients. In this particular group, regardless of other high risk features, there is no established benefit to adjuvant chemotherapy and in fact, literature suggests that 5-FU adjuvant therapy may indeed be detrimental in this subgroup[24, 25].

1.6 Dendritic Cells

Antigen presentation to T cells in the context of class I and II major histocompatibility molecules is critical for priming neoantigen-specific CD8+ and CD4+ T cell responses, respectively. Antigen presentation is mediated by antigen presenting cells which include dendritic cells, B cells and macrophages. Dendritic cells (DC) are the most potent antigen presenting cells given their ability to express high levels of costimulatory molecules and secrete Th1 polarizing cytokines (e.g. IL-12) that are essential for the generation of cytotoxic T cell responses[24]. A variety of technical advances involving the isolation of human DC as well as an improved understanding of DC biology have led investigators to study autologous DC as adjuvant for peptide vaccination in cancer. Various clinical studies have demonstrated the safety and tolerability of DC immunization and serious adverse events related to DC vaccination are rare [26].

1.7 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 (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. Several randomized trials have confirmed that vaccines administered along with cyclophosphamide have enhanced immunogenicity. Low dose cyclophosphamide, which may be administered orally or intravenously, is an effective strategy to eliminate Treg in patients prior to vaccination [27].

1.8 Organoid Cultures

As part of participation on a separate tissue acquisition trial, subjects will have fresh tissue from both benign colonic mucosa as well as from the resected colon cancer, collected at the time of routine care surgery and transported to the labs of Dr. Anil Rustgi and Dr. Chris Lengner for organoid culture establishment. Collection of tumor tissue samples will occur prior to participation on this interventional trial.

For establishment of organoids from benign colonic tissue, healthy colonic epithelium will be mechanically fragmented and separated from underlying submucosal tissues, and subsequently intestinal crypts will be isolated as previously described [29]. Once isolated these crypts will be plated and grown in a mixture of Matrigel and Wnt-containing cell culture media. For establishment of organoids from neoplastic tissue, the tumor will first be mechanically fragmented and then chemically dissociated from surrounding tumor stroma. Further mechanical dissociation will be performed through a combination of pipetting and vortexing, and cells will be plated and

grown in a mixture of Matrigel and Wnt-free cell culture media as previously described [30] After growth of organoid cultures from both benign and neoplastic tissue is established, these organoids will be passaged as needed, and will be cryopreserved to allow for future experimental use including morphologic and biochemical viability assays in the presence of subject's post-DC vaccination T-cells.

1.9 Study Rationale

Recent advances provide compelling evidence that the immune system plays a significant role in controlling cancer. Breakthrough treatments such as immune checkpoint inhibitors and CAR T cells demonstrate new therapeutic strategies which can translate into durable disease control rates, responses and even complete remissions in patients with advanced malignancies. For patients with stage II, MMR deficient CRC, no accepted adjuvant regimen exists. Although recurrence rates are low in this population, the development of therapies to further improve upon outcomes hold appeal. Patients with hypermutated CRC defined by the CMS1 subgroup represent a unique opportunity to evaluate personalized cancer vaccines which have been shown in melanoma to elicit tumor-specific T cells and mediate complete regression of metastatic disease after administration of anti-PD-1 antibody in select cases [17, 18]. Moreover, personalized cancer vaccines are safe and well tolerated[17, 18, 19].

1.10 Study Design

This is a pilot study to assess the safety and tolerability, as well as the immune response rate, of mDC3/8 vaccine in patients with colorectal cancer. The duration of a subject's participation in this study will be approximately 12 months from receipt of the first mDC3/8 vaccine infusion. Approximately 12 subjects will be enrolled in this study.

Additional secondary/exploratory objectives are described below.

2.0 OBJECTIVES

2.1 Primary Objective

1. To assess the immune response rate to DC vaccination in subjects with hypermutated CRC.
2. To determine the safety of DC vaccination in subjects with surgically resected hypermutated CRC.

2.2 Secondary Objective

1. To determine the percentage of CD8+ cells in the primary tumor tissue.

2.3 Exploratory Objectives

1. To evaluate tumor and immune biomarkers and their association with treatment outcomes.
2. To isolate and characterize TIL from metastatic tumors that relapse after study vaccine treatment.

3.0 ELIGIBILITY CRITERIA

Patients must fulfill the following eligibility requirements:

3.1 Inclusion Criteria

1. Pathologically-confirmed stage I and II hypermutated colorectal cancer (CRC). Hypermutated CRC is defined by one of the following categories:
   a. Microsatellite instability – high (MSI-H) by PCR. MSI-H is defined as two or more of the five markers for microsatellite instability being positive. These markers are: BAT25, BAT26, D2S123, D5S346 and D17S250.
   b. Mismatch repair deficient (MMRd) as defined by loss of expression of MMR protein(s) (MLH1, MSH2, MSH6, and/or PMS2) on MMR immunohistochemistry (IHC), which includes hereditary syndromes such as Lynch syndrome and congenital mismatch repair deficiency (CMMRD) as well as CRCs with somatic MMRd.
   c. Those arising in patients with polymerase proofreading associated polyposis (PPAP) with inherited mutations in either *POLE* or *POLD1*. [Hypermutated malignancies including CRC typically harbor >10 mutations/Mb].
2. Surgically resected disease
3. Male or female patients age ≥ 18 years
4. ECOG performance status 0-1
5. Required initial laboratory values (performed within 14 days prior to eligibility confirmation by physician-investigator):
   a. WBC >3 THO/µL
   b. Hg ≥ 9.0 gm/dl
   c. Platelets >75 THO/µL
   d. Serum Total Bilirubin ≤ 2.0 mg/dl; unless the subject has known or suspected Gilbert's syndrome for which ≤ 3 mg/dl is permitted.
   e. Serum Creatinine < 2.0 mg/dl
6. 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.
7. Provide written informed consent.

3.2 Exclusion Criteria

1. Prior malignancy within 3 years that, in opinion of the physician-investigator, would put subject at additional risk
2. Pregnant or nursing (lactating) women
3. Concurrent treatment with systemic immunosuppressants including corticosteroids (e.g prednisone), calcineurin inhibitors (e.g tacrolimus, cyclosporine), antiproliferative agents (e.g mycophenolate mofetil, azathioprine) within 2 weeks of eligibility confirmation by physician-investigator. Local (inhaled or topical) steroids or replacement dose prednisone (≤10 mg daily) are permitted.
4. Known allergy to eggs
5. Any uncontrolled intercurrent illness or active ongoing infection that, in the opinion of the physician-investigator, would put the subject at additional risk

4.0 REGISTRATION

Assignment of subject numbers will occur at consent and will be in ascending order (16218-01, 16218-02, etc). No numbers will be omitted. This subject identification number will be used as the primary identifier for that subject throughout his/her participation in the trial. 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.

At the time a subject consents to participate in this study, a Consent Notification Form should be completed. When eligibility of the subject is confirmed by a physician-investigator, an Enrollment Notification should be completed. Both completed forms should be emailed in real-time to:

Protocol Monitor and Sponsor Project Manager
Center for Cellular Immunotherapies (CCI)

Once subject eligibility has been confirmed by a physician-investigator, apheresis collection and the manufacturing of the study vaccine may commence.

5.0 SUBJECT RECRUITMENT AND SCREENING

Approximately 12 evaluable subjects will be enrolled in this study. All subjects who receive at least one Dendritic Cell Vaccine at the minimum acceptable dose for infusion will be considered evaluable for primary endpoint analysis. Subjects who fail apheresis or who do not receive a vaccine infusion for any other reasons will not be considered evaluable and will be replaced.

Subjects 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 subjects 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 enrollment and within 30 days of the subject's 1st DC vaccine (Day 1).

Subjects 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 subject 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

Subjects 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 subject 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, supplements/vitamins, devices, and herbal and nutritional supplements taken by the subject during the 30 days prior to eligibility confirmation by a physician investigator will be recorded. At every visit following enrollment, concomitant medications will be recorded in the medical record. Any additions, deletions, or changes of these medications will be documented.

For all subjects, a 28-day washout period from any prior systemic chemotherapy is required prior to the first vaccine dose.

Concomitant medications and therapies deemed necessary for the supportive care and safety of the subject are allowed. However, the following concomitant therapy guidelines must be adhered to during the study:

- Treatment with systemic immunosuppressants including corticosteroids (e.g. prednisone), calcineurin inhibitors (e.g. tacrolimus, cyclosporine), antiproliferative agents (e.g. mycophenolate mofetil, azathioprine) is prohibited. Local (inhaled or topical) steroids or replacement dose prednisone (≤ 10 mg daily or equivalent) are permitted.

7.0 INVESTIGATIONAL PRODUCTS

7.1 Dendritic Cell Vaccines (mDC3/8 vaccine 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 Vaccine #1 – hereafter identified as DC Vaccine #2
- Booster Vaccine #2 – hereafter identified as DC Vaccine #3

[redacted]

Please refer to the Investigator Brochure for additional information.

7.1.2 Mechanism of Action

7.1.3 Pharmacodynamics/Kinetics

Immunological monitoring is planned to assess the response to DC immunization.

7.1.4 Formulation

Synthetic peptides (>95% purity) will be obtained commercially.

7.1.5 Availability

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

7.1.6 Preparation

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

Vaccines will be made starting from fresh peripheral blood mononuclear cells (PBMC) in accordance with the Investigator's Brochure.

7.1.7 Packaging and Labeling

The investigational products 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 Vaccines) will be administered intravenously at the following doses:

- DC Vaccine #1 (Priming Vaccine): Protocol-specified dose of $7.50 \times 10^6 – 1.50 \times 10^7$ DC per peptide; Minimum acceptable dose for infusion is 1.00×10^6 DC per peptide.
- DC Vaccine #2 & #3 (Booster Vaccines): $1.00 \times 10^6 – 5.00 \times 10^6$ DC per peptide;

Minimum acceptable dose for infusion is 1.00 x 10^6 DC per peptide. Two doses will be administered approximately 6 weeks apart.

Please see **Section 8.3** for additional details.

7.1.9 Potential Toxicities

Please refer to the Investigator 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 subject 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 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 the FDA approved package insert. Full details on its mechanisms of action and toxicity profile can be found in the 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 subjects 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 3 days (+/- 1 day) prior to the first DC Vaccine.

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.

8.0 STUDY PROCEDURES

8.1 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 1-2x10^{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. Apheresis may occur any time after physician-investigator confirmation of eligibility but must occur at least 7 days prior to the anticipated vaccination. 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 (performed after last DC Vaccine) 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.

8.2 Treg Depletion

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

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

A total of 3 DC vaccines will be administered over a ~12-week period with priming DC Vaccine #1 administered on Day 1, followed by booster DC Vaccine #2 and DC Vaccine #3 administered ~6 weeks apart. Vaccines cannot be administered until all toxicities ≥ 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 post-infusion, with vital signs assessed every 30 minutes (+/- 5 minutes) from the end

of the infusion. After each subsequent infusion, subjects will be observed for 30 minutes after the end of the infusion, with vital signs assessed at 30 minutes (+/- 5 minutes) post-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 subject 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.

8.4 Immune Monitoring

Research blood samples for immune monitoring will be collected in accordance with the Study Calendar in **Section 10.0**. This testing requires five 10mL green top tubes (BD vacutainer sodium heparin tube) for a total of ~50 ml of blood. These blood samples will be used for immunologic monitoring studies (e.g. IFN-y ELIspot, peptide-HLA (p-HLA) multimer staining and cytotoxicity assays). The blood samples will be transported to Dr. Carreno's laboratory (SPE 8-309, bays 305B-307B).

Subjects will undergo an additional large-volume apheresis procedure (approximately 15 liter volume) ~7-14 days after their last DC Vaccine to collect lymphocytes for functional laboratory assays, to determine immunity against neoantigen peptides and TCR sequencing. Note: As per **Section 8.1** above, this apheresis collection may be moved up to an earlier study time point if deemed necessary for manufacturing purposes.

This additional apheresis procedure will be performed at the HUP Apheresis Unit according to standard procedures. These cells will then be transported to Dr. Carreno's laboratory for processing, storage, and exploratory analysis.

8.4.1 Flow Cytometry / p-HLA multimer Staining

Assessment of cellular immune activity may occur via the application of flow cytometry. Flow cytometric assays will include an examination of the influence of immunotherapy on the ability of subject T cells to exhibit phenotypic markers associated with cytolytic potential (e.g. IFN-y, IL-2, TNF-alpha, Granzyme B) after short-term stimulation by mutated peptide and p-HLA multimer staining. PBMC responses against a pool of known antigenic Cytomegalovirus, Epstein Barr Virus and Influenza epitopes will be evaluated in order to track general cellular immune competence during the study.

8.4.2 Cytotoxicity Assays

The cytolytic activity of neoantigen-specific T cells will be assessed by chromium release cytotoxicity assays. Target cells are co-cultured in the presence of CD8+ T cells isolated from vaccinated subjects. Cytotoxicity will be measured of Cr release from lysed target cells. When available, HLA class I matched tumor cell lines will be used as the target cell population.

8.4.3 TCR Sequencing

TCR sequencing will be performed as previously described. Briefly, single-cell sorting will be performed on tetramer-positive cells identified from apheresis collection 7-14 days following last DC Vaccine. RT-PCR will be performed to provide template DNA for PCR-based TCR gene amplification. PCR products with then be gel purified and sequenced. The functionality of TCR sequences will then be confirmed by cloning into an expression vector for transduction of human T cells for *in vitro* assays.

8.5 Post-DC Vaccine Surveillance

Following the End of Study Treatment visit, subjects will continue to be followed for safety and the collection of immune monitoring samples for up to 1 year post-DC Vaccine #1. The first Post-DC Vaccine Surveillance Visit will occur approximately 6 months after DC Vaccine #1.

During Post-DC Vaccine Surveillance, only protocol-defined adverse events (PDAEs) will be collected and reported. This includes any adverse events that are ongoing at the End of Treatment Visit as well as any adverse events determined to be at least possibly related to the investigational product (See **Section 12.1**).

Blood samples will continue to be collected for immune monitoring analysis every 3 months as per the Study Calendar in **Section 10.0**. Tissue samples obtained as part of standard of care procedures may also be used for research analysis.

Subjects will not be formally followed for response, however if the subject experiences documented disease recurrence during Post-DC Vaccine Surveillance, this data will be collected for research purposes.

In the event that a subject cannot return to the University of Pennsylvania for follow-up visits, the subject's local provider may also be asked to provide information from the subject's medical record and assist in the collection of protocol-required immune monitoring blood samples, which will be sent to the University of Pennsylvania Carreno Lab.

9.0 POTENTIAL TOXICITY AND DOSE MODIFICATIONS

All adverse events should be recorded and reported as per protocol **Section 12**. 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 ≥ grade 2 have resolved to baseline.

9.1 Dose Limiting Toxicity (DLT)

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 or 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 12.1**. Dose-limiting 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.

Please refer to **Section 12.7** for study stopping rules.

10.0 STUDY CALENDAR

Tests and Observations	Screening/ Enrollment[l]	Pre-Treatment (≤ 14 days of DC Vaccine #1)[l]	Apheresis #1 (~Day -7)	Cyclo-phosphamide Day -3 (+/- 1 day)	DC Vaccine #1: Priming Dose (Day 1)	DC Vaccine #2: Booster Dose (Day 43 +/- 14 days)	DC Vaccine #3: Booster Dose (Day 85 +/- 14 days)	Apheresis #2 (~7-14 days after the last DC Vaccine)	End of Study Treatment Visit (30 +/- 7 days post-last DC Vaccine)	Post-DC Vaccine Surveillance (Every 3 months +/- 2 weeks; beginning 6 months post-DC Vaccine #1)	End of Study Visit[a] (12 months post-DC Vaccine #1 +/- 2 weeks)
Informed Consent	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[b]	X	X			X[b]	X[b]	X[b]		X		
Apheresis			X[c]					X[d]			
Cyclophosphamide				X[e]							
Dendritic Cell Vaccine Administration					X[f]	X[f]	X[f]				
CBC[g], CMP[h]	X	X			X	X	X		X		
CEA	X										
PT/PTT	X										
LDH (Serum)	X										
Immune Monitoring[j]	X[j]				X[j]	X[j]	X[j]	X[j]	X[j]	X[j]	X[j]
Pregnancy Test[k]	X	X									

Tests and Observations	Screening/ Enrollment[l]	Pre-Treatment (≤ 14 days of DC Vaccine #1)[l]	Apheresis #1 (~Day -7)	Cyclo-phosphamide Day -3 (+/- 1 day)	DC Vaccine #1: Priming Dose (Day 1)	DC Vaccine #2: Booster Dose (Day 43 +/- 14 days)	DC Vaccine #3: Booster Dose (Day 85 +/- 14 days)	Apheresis #2 (~7-14 days after the last DC Vaccine)	End of Study Treatment Visit (30 +/- 7 days post-last DC Vaccine)	Post-DC Vaccine Surveillance (Every 3 months +/- 2 weeks; beginning 6 months post-DC Vaccine #1)	End of Study Visit[a] (12 months post-DC Vaccine #1 +/- 2 weeks)
AE Assessment[i]			X----------X							X----------X[i]	
Concomitant Medications	X----------X										
Survival										X----------X	

a. End of Study visit will take place approximately 1 year after the first DC vaccine infusion on Day 1.

b. All subjects will have vital signs (including temperature, respiration rate, pulse, blood pressure, oxygen saturation) assessed prior to each infusion. After DC Vaccine #1, subjects will be observed for 2 hours post-infusion, with vital signs assessed every 30 minutes (+/- 5 minutes) from the end of the infusion. After each subsequent infusion, subjects will be observed for 30 minutes post-infusion, with vital signs assessed 30 minutes (+/- 5 minutes) post-infusion.

c. To obtain mononuclear cells for DC generation. Apheresis #1 will be performed ~7 days prior to the first DC vaccine with an approximate collection volume of 15-20 liters. 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 (performed after last DC Vaccine) may be moved up to an earlier study timepoint to allow for required manufacturing. Please refer to **Section 8.1** for additional details.

d. To obtain mononuclear cells for immune monitoring. Apheresis #2 will be performed ~7-14 days after last DC Vaccine with an approximate collection volume of 15 liters. Please refer to **Section 8.4** for additional details.

e. A single dose of 300 mg/m^2 cyclophosphamide will be administered via intravenous infusion Please see **Section 8.2** for additional details

f. Please see **Section 8.3** for additional details

g. Complete Blood Count (CBC) includes White Blood Cell count with differential, Hemoglobin / Hematocrit and Platelet count.

h. Comprehensive Metabolic Panel (CMP) includes Glucose, BUN, Creatinine, Sodium, Potassium, Calcium, Total Protein, Albumin, Total Bilirubin, Alkaline phosphatase, ALT (SGPT), AST (SGOT)

i. 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 Treatment Visit. After the End of Study Treatment Visit, subjects will continue to be followed for protocol-defined adverse events only. Please refer to **Section 12.0** for additional information.

j. Research blood for immune monitoring studies will be collected in 10mL green top tubes (BD vacutainer tube). Five 10 mL green top tubes (Up to 50 mL total) will be drawn at required study visits. Please see **Section 8.4** for additional details.

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

l. 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 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 EARLY WITHDRAWAL OF SUBJECTS

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

- The judgment of the principal investigator that the subject is too ill to continue if this occurs prior to the vaccine dose.
- 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 enrollment but before administration of the vaccine dose. This will be done at the judgment of the PI, and could include for example, disease recurrence requiring alternative treatment, or a serious adverse event.
- Subject withdraws consent
- Termination of the study

Subjects who receive DC Vaccine #1 may be discontinued from receiving additional study treatment/primary follow-up for any of the following reasons:

- Subject withdraws consent.
- The PI decides to discontinue the study treatment (i.e. for non-compliance with the protocol or disease recurrence requiring urgent therapeutic intervention).
- Subjects who develop DLT (as defined in **Section 9.1**).
- Subjects who develop an allergic reaction to the dendritic cell vaccine (mDC3/8 vaccines).
- Pregnancy
- Termination of the study

All subjects who complete/prematurely discontinue from the study after receipt of at least one DC vaccine infusion will be asked to complete an End of Study Treatment visit no sooner than 30 days after their last mDC3/8 Vaccine. Following the End of Study Treatment visit, subjects will continue to be followed for safety and the collection of immune monitoring samples for up to 1 year post-DC Vaccine #1. The first Post-DC Vaccine Surveillance Visit will occur approximately 6 months after DC Vaccine #1. Immune monitoring samples will continue to be collected every 3 months during Post-DC Vaccine Surveillance Visit until the End of Study Visit.

Subjects may be discontinued from Post-DC Vaccine Surveillance Follow-up for the following reasons:

- Subject withdraws consent
- Death
- Investigator Discretion- Subjects will be asked to complete an End of Study Visit.
- Termination of the study
- Completion of Post-DC Vaccine Surveillance (12 months post-DC Vaccine #1)- Subjects will be asked to complete an End of Study Visit.

12.0 SAFETY AND ADVERSE EVENTS

12.1 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 or PDAE 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 subject'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 subject, 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 (UADE)
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.

Protocol defined adverse events (PDAEs)
During Post-DC Vaccine Surveillance, only protocol-defined adverse events (PDAEs) will be collected and reported. Protocol-defined adverse events that are determined to be serious as defined above will be considered protocol-defined serious adverse events (PDSAEs) and also require expedited reporting to the Sponsor per **Section 12.3**.

The PDAEs are as follows:

- Any adverse events ongoing at the End of Study Treatment. These events must be followed until resolution or End of Study.
- Any adverse events determined to be possibly related to the investigational product.

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.

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).

12.2 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 5.0 at each study visit.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 through interval medical history evaluations, physical examinations, and clinical laboratory assessments as per the Study Calendar (**Section 10**). Adverse events will be collected continuously throughout the subject's participation; using testing/examinations, non-directive questioning (e.g. review of systems), subject self-reporting, etc. Information on all adverse events should be recorded in the source documentation. Information on all adverse events should be recorded in the source documentation. All clearly related signs, symptoms, and abnormal diagnostic procedure 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 12.1** above) must be recorded. If there are no adverse events identified during a study visit occurring after the AE reporting period commences, physician-investigator confirmation of the absence of adverse should be documented.

Adverse events that 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 the following information:

1. The severity grade (CTCAE Grade 1-5)
2. Duration
3. Its relationship to the study treatment (as defined in **Section 12.1**)
4. Expectedness to study treatment(as defined in **Section 12.1**)
5. Action taken with respect to study or investigational treatment
6. Whether medication or therapy was administered
7. Whether it is serious (as defined in **Section 12.3**)

Physician-investigator assessment of whether an adverse event is serious (as defined by **Section 12.1**) must occur within 24 hours from the date of knowledge of the adverse event, in order to meet SAE reporting requirements as described in **Section 12.3**. Additional assessment of non-serious adverse events, including grade and relationship to study treatment, should occur within 7 days from the date of knowledge of the adverse event or from the date of the study visit where the absence of adverse events was confirmed. Accelerated timelines for adverse event assessments and reporting may be requested in the event of emergent safety concerns and/or to address time-sensitive requests from the FDA.

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 recorded and reported.

12.3 Reporting of Serious Adverse Events

Every SAE, UADE, and PDSAE (during post-DC vaccine surveillance), **regardless of suspected causality**, occurring during the adverse event reporting period defined in **Section 12.1** 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/UADEs/PDSAEs 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 subject continued or withdrew from study participation.

Report serious adverse events 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)

12.3.1 Investigator Reporting: Local Regulatory Review Committees

Report events to local regulatory review committees per institutional policy.

12.4 Pregnancies

To ensure subject safety, each pregnancy occurring while the subject 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.

12.5 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.

12.6 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.

12.7 Study Stopping Rules

This trial will be paused if the following events occur, pending further discussion with the Sponsor Medical Director, IRB and FDA:

- Any death that may be related to the investigational products.
- 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 above statistical stopping rule was developed such as the rule will be triggered if the lower limit of the two-sided 90% confidence interval of the event rate exceeding 20%. Under this rule, we would stop the study early with a probability of 1.6%, 8.8%, and 23.8% if the true event rate is 10%, 20%, and 30%, respectively. These probabilities were calculated from a simulation study.

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.

13.0 STATISTICAL CONSIDERATIONS

Approximately 12 evaluable subjects will be enrolled in this study. All subjects who receive at least one Dendritic Cell Vaccine at the minimum acceptable dose for infusion will be considered evaluable for primary endpoint analysis. Subjects who fail apheresis or who do not receive vaccine due to other reasons will not be considered evaluable and will be replaced. The sample size is determined based on availability of the funding. Early stopping rules will be implemented as described in **Section 12.7**.

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 flow cytometric-based intracellular cytokine or tetramer staining[15]. For each peptide, the intracellular cytokine and tetramer assays are performed on blood samples obtained at the indicated time points. Data are presented as the absolute number or percentage of $CD8^+$ T cells positive for IFN-y, IL-2, TNF-alpha and granzyme B secretion when stimulated with peptide or tetramer binding based on gating variables set using the iMASC reagent kit (Beckman Coulter). The lower limit of detection (LLD) is 0.03% per 100,000 cells for flow cytometric assays. For the analysis, a negative control peptide or tetramer (e.g. HIV gag peptide) will be used to normalize each assay. Each sample is incubated or stained with control peptide or tetramer and the number of positive $CD8^+$ T cells are subtracted from each experimental point. The mean and SD at baseline are obtained for each subject. Based on our prior experience, the expected analytical CV (standard deviation/mean) is 10%. A positive intracellular cytokine or tetramer response is defined as an increase greater than 3SD above the baseline value. For each peptide, the proportions of $CD8^+$ T cell responses (positive or negative) and the associated 90% exact confidence intervals (CI) will be reported for each time point. With 12 subjects, the width of the 90% exact CI will be no more than 25% away from the observed proportions.

The primary endpoint will also include evaluating the safety and tolerability of the mature dendritic cell vaccine using proportions and exact 90% confidence intervals. Safety data from all subjects will be analyzed together regardless of the actual infusion dose received.
Adverse events will be tabulated by grades and body system. The frequency of grade 3 and 4 AEs will be summarized separately. With 12 evaluable subjects, the half-width of the exact 90% confidence interval (CI) will be no more than 26%. The probability of observing at least one DLT will be 72%, 93%, and 99% if the true DLT rate is 10%, 20%, and 30%, respectively.

For the secondary objective, the percentage of $CD8^+$ T-cell responses 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 positivity versus negativity by time, dose and time by dose interaction. These models are intended to be descriptive, and no preliminary data exist which estimate longitudinal trends, so no power calculation is attempted.

For the exploratory aim, the association between treatment outcomes and tumor/immune biomarkers will be examined by t-test. The treatment outcomes are binary variables including immune response rate as measured by dextrameter/tetramer assay as described previously and any grade 3 or higher AEs. Tumor and immune biomarkers are primarily continuous variables. Due to exploratory nature of the analyses, no adjustment of multiple testing will be performed.

Additional correlative assays will be performed (Cr^{51} release); however, these data will be not be used to determine the primary end point but may be used as surrogate assays to evaluate the functionality of neoantigen peptide-specific $CD8^+$ T cells. These assays will be performed using $CD8^+$ T cells from baseline and post-vaccination blood samples. A positive Cr^{51} assay is defined as an increase in specific lysis > 15% above background at effector:target ratio of 5:1.

14.0 DATA HANDLING AND RECORDKEEPING

14.1 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 and Sponsor, by regulation, retain the ability to use all information collected prior to the revocation of subject 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.

14.2 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, subjects' 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, subject 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 subject's file. The investigator must also keep the original signed informed consent form, and a signed copy must be given to the subject.

14.3 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.

14.4 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.

15.0 STUDY MONITORING, AUDITING, AND INSPECTING

15.1 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.

15.2 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.

16.0 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.

17.0 STUDY FINANCES

17.1 Funding Source

This study will be funded by the Parker Institute for Cancer Immunotherapy and the National Institutes of Health (NIH).

17.2 Conflict of Interest

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

17.3 Subject Stipends or Payments

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

18.0 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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