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PROTOCOL TITLE

A Phase II Trial to Evaluate the Ability of a Dendritic Cell Vaccine to Immunize Melanoma or Epithelial Cancer Patients Against Defined Mutated Neoantigens Expressed by the Autologous Cancer

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Investigational Agents:

Drug Name:	Peptide loaded dendritic cell vaccine
IND Number:	17564
Sponsor:	Center for Cancer Research
Manufacturer:	Surgery Branch Cell Production Facility

PRÉCIS

Background:

- Therapeutic vaccination against cancer has proven very challenging with little clinical benefit.
- Vaccines against non-viral tumors have mainly targeted differentiation antigens, cancer testis antigens, and overexpressed antigens. However negative selection in the thymus against these normal nonmutated antigens severely limits the ability to generate high avidity anti-cancer T cells. Such depletion can impair their antitumor activity and limit tumor elimination.
- The National Cancer Institute Surgery Branch (NCI SB) has developed a pipeline for the identification of immunogenic T cell epitopes derived from neoantigens.
- In recent studies, we identified the neoantigens recognized by TIL that mediated regression in patients with metastatic cancer. Using whole exome sequencing of a resected metastatic nodule followed by high throughput immunologic screening, we were able to demonstrate that tumor regressions were associated with the recognition by the administered TIL of unique somatic mutations that occurred in the cancer.
- We, therefore, aim to use this pipeline to identify immunogenic neoantigens from epithelial cancer patients and to use these defined epitopes for a personalized therapeutic dendritic cell (DC) vaccine.

Objectives:

- Primary objective:
    - To determine the clinical response rate in patients with metastatic melanoma or epithelial cancer who receive this DC vaccine

Eligibility:

- Age $\geq$ 18 years and $\leq$ 70 years
- ECOG 0 – 2
- Evaluable metastatic melanoma or epithelial cancer refractory to standard treatment
- Metastatic melanoma or epithelial cancer lesion(s) that is resectable for TIL or in selected cases, available PBMC

Design:

- Patients with metastatic melanoma or epithelial cancer will undergo surgical resection of tumor followed by exome and RNA sequencing to identify expressed mutations (conducted under the NCI SB companion protocol 03-C-0277).
- Patients will undergo apheresis and DC will be cryopreserved for vaccine preparation.
- Immunogenic neoantigens will be identified from TIL and PBMC by high throughput immunologic screening using long peptides and tandem minigenes covering all mutated epitopes.
- Patient will be vaccinated with autologous mature dendritic cells loaded with long peptides and minimal epitopes from defined neoantigens or highly expressed mutations in tumor suppressor or driver genes.

- DC will be administered intravenously and subcutaneously for four cycles at biweekly intervals.
- Blood samples will be taken every two weeks, and patients will be monitored for the quantity and quality of circulating neoantigen-specific T cells.

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1.1.1 Primary Objective

- To determine the clinical response rate in patients with metastatic melanoma or epithelial cancer who receive this DC vaccine.

1.1.2 Secondary Objectives

- To determine whether administration of a DC vaccine presenting defined mutated neoantigens can increase the quantity and quality of circulating antigen-specific T cells.
- To determine the safety of the vaccine.

1.2 BACKGROUND AND RATIONALE

1.2.1 Cancer Vaccines

Protective vaccination against infectious diseases has proven to be one of the most effective health measures. Therapeutic vaccination against established diseases such as persistent infections and cancer has proven much more challenging. Cancer vaccines are designed to target antigens that can elicit an immune response that selectively attacks cancer cells. Ideally these antigens should be exclusively presented on cancer cells. Until recently, vaccines against non-viral tumors mainly targeted differentiation antigens, cancer testis antigens, and overexpressed antigens. However, central immunological tolerance has limited the generation of high avidity reactivity against these normal antigens[1,2]. Thus, T cells prompted using these vaccines underwent depletion of high avidity clones directed against such antigens. This depletion causes the loss of T cells bearing high-affinity TCRs for their cognate antigens which have the superior cytotoxic capacity, longer persistence in the tumor microenvironment, and decreased susceptibility to immune suppression[3]. Taken together, such depletion can lead to impaired clinical efficiency following vaccine administration. In recent years our group and others have extensively studied the importance of neoantigens as targets for immunotherapy. It is now clear that neoantigen specific T cells are present in most cancers. Neoantigens derived from somatic mutations offer a specific and highly immunogenic target for vaccination. Today, with the rapid development of technologies for DNA sequencing, the identification of those mutations becomes widely feasible. Evidence for the power of neoantigen vaccines in pre-clinical and clinical settings was recently published. The first proof of concept of neoantigen vaccines was shown in the B16 mice melanoma model[4]. By prophylactic vaccination with two mutated epitopes, the researchers achieved complete tumor protection in 40% of the mice. In therapeutic settings, they observed significant tumor growth inhibition induced by mutation-specific peptide vaccination. Recently, the same group published evidence for the potential of neoantigen vaccines as tested in three independent murine models[5]. In this paper, the researchers showed that a considerable fraction of the non-synonymous mutations in the cancer were immunogenic, and the majority of them were recognized by $CD4^+$ T cells. Vaccination with these $CD4^+$ epitopes induced potent tumor control and rejection of established tumors. Similar results were published in another independent mutation based cancer model. In this work the authors developed a pipeline to identify immunogenic mutant peptides using whole-exome and transcriptome sequencing together with mass spectrometry. Again, the researchers were able to show that vaccination with mutation specific peptides induced therapeutically active T cell responses. Results of the current

pre-clinical data support the evaluation of neoantigen vaccines as an effective cancer treatment. Although studied extensively in pre-clinical models, only one limited human trial has been published. In this trial, a group from Washington University in St. Louis reported on a first-in-man clinical trial evaluating neoantigen vaccine in patients with metastatic melanoma[6]. The researchers vaccinated three patients with mature dendritic cells presenting seven predicted mutation-specific HLA-A*02:01 restricted epitopes. They showed that vaccination with high affinity, patient specific mutated epitopes augmented T cell immunity directed against naturally occurring dominant neoantigens and broadened the response by revealing subdominant neoantigens; but no clinical responses were observed. However, this did show that vaccination against mutated epitopes was safe as none of the three patients experienced any autoimmune adverse events.

1.2.2 Identification of Cancer-Specific Immunogenic Epitopes Derived from Somatic Mutations

The NCI SB has developed a process to identify immunogenic T cell epitopes derived from neoantigens (**Figure 1**)**.** The identification of these antigens is done in four main stages: 1) Whole exome sequencing and RNA-seq analysis of the tumor and a matched normal apheresis sample to identify highly expressed somatic mutations; 2) Construction of tandem minigenes (TMGs) and synthesis of long peptides covering these mutations; 3) expression of the constructs in autologous antigen presenting cells; 4) In vitro co-culture assay to identify T cells recognizing mutated epitopes from TIL and PBMC[7]. In earlier studies done by the NCI SB (published and unpublished data), 25 patients with melanoma were screened in the above manner, 64 antigenic somatic mutations were identified with no overlapping between patient tumors[8,9]. Recently, a study to identify antigenic mutations from patients with epithelial cancers including those of the GI tract, genitourinary tract and breast, identified 57 non-overlapping somatic mutations.

In this study, two patients were identified who developed T-cell response against the mutated KRAS oncogene[10]. Work published by the NCI SB in *Nature Medicine* proved for the first time that neoantigen specific T cells can also be isolated from patients' PBMC[7]. By using this process, neoantigen specific lymphocytes were identified in the peripheral blood of three of four patients with melanoma.

Despite their low frequency in the circulation, these CD8+PD-1+ cell populations had lymphocytes that targeted unique patient-specific neoantigens. This method provides a novel noninvasive approach to identify antigenic neoantigens.

Thus far, more than 190 immunogenic epitopes have been identified (**Table 1**) from multiple cancer types including melanoma, ovarian, colorectal, lung and breast cancers[7,8,10-13]. This process, which can be applied to any cancer type and can be completed in several weeks, ensures that the selected antigens are not just expressed in the tumor but can also prompt a significant T cell-mediated immune response.

1.2.3 Preclinical Studies for the Generation of a Dendritic Cell Vaccine

Development of a personalized neoantigen vaccine puts forward several hurdles. The vaccination must address those issues that are unique to the cancer model. The number of epitopes is variable.

- The vaccine should optimally include both CD4 and CD8 epitopes.

- The vaccine may induce a cell-mediated Th1 response.
- The vaccine manufacturing should avoid expensive and time-consuming processes.

To address these issues, ex vivo generated DC will be used. Manufacturing of DC from patient PBMC is a well-established and straightforward method. DC vaccines are proven to induce T cell responses against multiple malignancies and infectious diseases. Numerous clinical trials incorporating and using different administration routes have demonstrated the safety and tolerance of DC vaccines[14]. In addition, the use of DCs both as antigen presenting cells in the initial high throughput screening for recognition of neoantigens and in the delivery of these antigens in the vaccine product, should ensure that all antigens will be properly processed and presented to T cells upon vaccination.

1.2.3.1 DCs Matured with PolyI:C, R848, and IFNγ Upregulate Co-Stimulatory Molecules and Secrete Pro-Inflammatory Cytokines

DCs are one of the most effective antigen-presenting cells to induce T cell immunity. Even though immature DCs (iDCs) can uptake, process, and present antigens, they fail to secrete proinflammatory cytokines, and therefore have been shown to be tolerogenic, or at best weakly immunogenic[15]. Multiple maturation cocktails have been used in the clinic to produce fully mature DCs. Until recently, researchers used mainly immature DCs or cells activated by a cytokine cocktail (Il-1β, IL-6, TNFα, and PGE2). Lately, several reports citing the use of TLR ligands such as PolyI:C and R848 as DCs stimulators have been published[6]. To evaluate which maturation method generated the highest number of DCs secreting IL-12p70/TNFα, day six monocyte-derived DCs were incubated with LPS, CD40L expressing 3T3 cells, TLR mix (PolyI:C, R848, and IFNγ) and cytokine cocktail (Il-1β, IL-6, TNFα, and PGE2). Sixteen hours post incubation the level of costimulatory molecules was measured by flow cytometry and the secretion of multiple cytokines and chemokines was measured by multiplex assay (**Figure 2**). From **Figure 2** it can be clearly seen that the TLR mix induced the secretion of IL-12p70, TNFα, IP-12, MCP-1 and MIP-1β. The TLR cocktail is capable of maturing DCs to secrete TH1 polarizing cytokines and multiple chemokines. Recently, a group from Washington University in St. Louis (WUSTL) used a combination of K562-CD40L cells with PolyI:C, R848, and IFNγ to mature monocyte-derived DCs. To evaluate if the addition of K562-CD40L cells to the TLR mix could induce superior DCs maturation, clinical grade K562-CD40L cells (K602C14) were produced and their contribution to DC stimulationwas evaluated. The original cells used by the WUSTL group (K463H) (**Figure 3**) were used as a positive control. This indicates that TLR mix alone is enough to produce fully mature DCs, and thus no additional stimulation is needed.

1.2.3.2 DCs Loaded with Long Peptides and Minimal Epitopes Stimulate both CD4 and CD8 T Cells

To evaluate the best method of loading DCs with antigenic determinants, mRNA electroporation of TMGs was compared to loading with long peptides and minimal epitopes.

A screening system to evaluate the presentation of three CD8 and three CD4 epitopes in one assay was developed. A series of TMG constructs harboring different signal peptides and targeting signals and encoding both long peptides and minimal epitopes covering all antigenic determinants (**Figure 4**) were produced.

To test antigen presentation, DCs were transfected with the TMG constructs or, loaded with long peptide or minimal epitopes. DCs were co-cultured with PBMC transduced with TCRs recognizing the corresponding antigens and antigen recognition was evaluated by flow cytometry for 41BB expression (**Figure 5**). The results clearly showed that while long peptides and minimal epitopes were recognized by CD4 and CD8 cells, TMGs elicited only CD8 responses likely indicating that CD4 epitopes cannot be processed through cytosolic proteins thus abrogating the use of TMGs as an efficient platform for antigen presentation. To test if mature DCs could efficiently present both CD4 and CD8 epitopes DCs were loaded with long peptides and minimal epitopes and stimulated for 16 hours using the TLR mix (**Figure 6**). As shown the mature DCs efficiently presented both minimal and long epitopes for CD4 and CD8 epitopes. Thus, mature DCs loaded with long peptides or minimal epitopes covering both CD8 and CD4 epitopes will be used in this trial.

1.2.3.3 Mature DCs can Stimulate Both Memory, Effector, and Naïve Neoantigen Specific T Cells

An efficient vaccine should be able to augment neoantigen specific T cells and to expand the breadth of the anti-tumor immune response. To test if DCs matured with TLR mix are capable of stimulating naïve, antigen-experienced and terminally differentiated effector cells, an in vitro stimulation experiment was conducted. Apheresis samples from a patient with colorectal cancer were thawed and then incubated in an appropriately sized tissue culture flask. Adherent monocytes were differentiated into DCs, loaded with neoantigen peptide pools and matured using a TLR mix. Non-adherent cells were collected, and memory, naïve and terminally differentiated effector memory cells (T_{EMRA}) were sorted and in vitro stimulated with mature peptide-pulsed autologous DCs (**Figure 7**). Ten days after the first stimulation T cells were restimulated with DCs loaded with all peptide pools, and sorted based on 41BB and OX40 expression to enrich for neoantigen-specific cells. Cells were then tested for neoantigen specific recognition using single peptides derived from the most reactive peptide pools. By applying this method, neoantigen specific T cells from naïve, antigen-experienced and T_{EMRA} cells (**Figure 8**) could be identified. These results show that peptide-loaded, TLR mix stimulated DCs are capable of in vitro activation of T cells derived from memory or naïve precursors.

1.2.4 Rationale for Selecting Defined, Tumor Suppressor and Driver Neoantigens to use in this Trial

Historically, preclinical and clinical neoantigen vaccines used epitopes predicted in silico. Although in silico prediction can select for proper antigens, in many cases there is no evidence that those antigens are processed and presented by the tumor or antigen presenting cells in the microenvironment. As described above, patient TIL and PBMC will be screened to identify defined mutated antigens recognized by T cells guaranteeing that those antigens are processed and presented for T cells in the tumor or its draining lymph nodes.

There will be one exception for using defined neoantigens in this trial. There is growing evidence to suggest that targeting tumor driver and suppressor genes can elicit superior clinical response. Mutations in these genes are highly common and have been found in many tumors. When mutations are identified in KRAS, NRAS, p53, EGFR, IDH1, PIK3CA or any other potential driver mutations, peptides covering those epitopes will be synthesized and used in the vaccine composition. The method/process for epitope selection and synthesis is shown in **Figure 9**.

Augmenting T cell responses against defined neoantigens, should increase the likelihood of a significant clinical response.

1.2.5 Relevant Preclinical and Clinical Experience to Support the Proposed Route and Dose of the DC Vaccine

As shown by the NCI SB, neoantigen specific cells can be found both in the tumor and in the blood of cancer patients[7,10]. To boost responses against defined neoantigens, we are using a systemic vaccine dose and schedule covering both the blood and peripheral tissues. For that purpose, we decided to use two routes of administration: Intravenous (IV) to stimulate T cells that reside in the lungs, spleen and the bone marrow of the patients and subcutaneously (SQ) to stimulate T cells residing in peripheral lymph nodes. This concept was also shown before in a mouse model[16]. Recently, a group from Washington University in St. Louis (WUSTL) reported on a first-in-man clinical trial evaluating neoantigen vaccine in patients with metastatic melanoma[6]. The researchers vaccinated three patients IV with mature dendritic cells presenting seven predicted mutation-specific HLA-A*02:01 restricted epitopes. They showed that vaccination with patient specific mutated epitopes augmented T cell immunity directed against naturally occurring dominant neoantigens and broadened the response by revealing subdominant neoantigens. The initial priming dose they used was $1.5x10^7$ DC per peptide (9 peptides in total, $1.35X10^8$ DC total), and all the subsequent doses were $5.0x10^6$ DC per peptide ($4.5X10^7$ DC total). In our trial, we plan to load DC with a peptide pool containing all defined epitopes recognized by TIL. Therefore, we chose a dose level similar to the doses used during the WUSTL trial for the IV administration. In a different phase I/II clinical trial of sequential SQ and IV delivery of a dendritic cell vaccine for refractory multiple myeloma[17], patients were vaccinated with increasing doses of 5 × -, 10× -, $50×10^6$ cells and 10× -, $50×10^6$ cells respectively. The treatment was well tolerated, and no severe adverse events occurred. Therefore, we decided to vaccinate with DC doses which are not higher than $50×10^6$ for the SQ and the IV route of administration. We are planning to give the maximal dose possible according to our manufacturing yields to allow efficient activation of circulating and tissue resident neoantigen specific cells.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

a. Metastatic melanoma or epithelial cancer with at least one lesion that is resectable **or** in selected cases, available PBMCs.

b. Measurable and evaluable metastatic disease per RECIST 1.1 criteria.

c. Confirmation of the diagnosis of metastatic cancer by the Laboratory of Pathology of NCI.

d. All patients must be refractory to approved standard systemic therapy.

e. Patients with 3 or fewer brain metastases that are less than 1 cm in diameter and asymptomatic are eligible. Lesions that have been treated with stereotactic radiosurgery must be clinically stable for one month after treatment for the patient to be eligible. Patients with surgically resected brain metastases are eligible.

f. Age ≥ 18 years and ≤ 70 years.

g. Clinical performance status of ECOG 0, 1, 2.

h. Patients of both genders must be willing to practice birth control from the time of enrollment on this study and for four months after treatment.

i. Serology:

- Seronegative for HIV antibody. (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who are HIV seropositive can have decreased immune-competence and thus are less responsive to the experimental treatment and more susceptible to its toxicities.)
- Seronegative for hepatitis B antigen, and seronegative for hepatitis C antibody. If hepatitis C antibody test is positive, then the patient must be tested for the presence of antigen by RT-PCR and be HCV RNA negative.

j. Hematology

- Absolute neutrophil count > 1000/mm^3 without the support of filgrastim
- WBC ≥ 3000/mm^3
- Platelet count ≥ 100,000/mm^3
- Hemoglobin > 8.0 g/dL. Subjects may be transfused to reach this cut-off.
- CD4 count > 200/uL

k. Chemistry:

- Serum ALT/AST < 5.0 x ULN
- Serum creatinine ≤ 1.6 mg/dL
- Total bilirubin ≤ 2.0 mg/dL, except in patients with Gilbert's Syndrome, who must have a total bilirubin ≤ 3.0 mg/dL.

l. More than four weeks must have elapsed since any prior systemic therapy at the time the patient receives the vaccine, and patients' toxicities must have recovered to a grade 1 or less (except for toxicities such as alopecia or vitiligo).

Note: Patients may have undergone minor surgical procedures within the past 3 weeks, as long as all toxicities have recovered to grade 1 or less.

m. Ability of subject to understand and the willingness to sign a written informed consent document.

n. Subjects must be co-enrolled on protocol 03-C-0277.

2.1.2 Exclusion Criteria

a. Women of child-bearing potential who are pregnant or breastfeeding because of the potentially dangerous effects of the treatment on the fetus or infant.

b. Any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease).

c. Concurrent opportunistic infections. (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who have decreased immune

competence may be less responsive to the experimental treatment and more susceptible to its toxicities).

d. Active systemic infections requiring anti-infective treatment, coagulation disorders or any other active or uncompensated major medical illnesses.

e. Patients who are receiving any other investigational agents.

2.2 SCREENING EVALUATION

Note: Testing for screening evaluation is conducted under the NCI SB companion protocol, 99-C-0128 (Evaluation for NCI Surgery Branch Clinical Research Protocols).

Note: The date of enrollment is the date the subject signs the written informed consent document. The patient will be consented and enrolled on this protocol once the patient has adequate leukapheresis material banked by the Surgery Branch Cell Production Facility (SB CPF) and prior to administration of the first vaccine.

2.2.1 Within 3 Months Prior to Enrollment

- HIV antibody titer, HBsAg determination, and anti HCV
- Confirmation of diagnosis of metastatic melanoma or epithelial cancer by the Laboratory of Pathology of NCI. (Note: Testing is permitted to be conducted at any time prior to enrollment.)
- Anti CMV antibody titer, HSV serology, and EBV panel. (Note: Patients who are known to be positive do not need to be retested.)

2.2.2 Within 4 Weeks Prior to Enrollment

- Complete history and physical examination. (Note: Patient history may be obtained within 8 weeks prior to enrollment.)
- Baseline imaging to determine the status of disease. This may include CT, MRI, PET, and/or photography.

2.2.3 Within 7 Days Prior to Enrollment

- Physical examination, including ECOG of 0, 1, or 2
- Vital signs
- CBC w/differential
- Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST/GOT, ALT/GPT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric Acid, Creatinine Kinase, Lactate Dehydrogenase, Total Protein
- PT/PTT
- β-HCG pregnancy test (serum or urine) on all females of child-bearing potential
- Urinalysis, with culture if indicated
- Chest x-ray
- EKG

2.3 REGISTRATION PROCEDURES

2.3.1 Prior to Registration for this Protocol

Patients will initially sign the consent for and enroll on the NCI SB companion protocol, 03-C-0277 (Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols), prior to tumor harvest and leukapheresis for generation of the cell product. Patients will sign the consent for and enroll on this treatment protocol once the patient has adequate leukapheresis material banked by the SB CPF and prior to administration of the first vaccine.

Immature and mature dendritic cells must pass the FACS and sterility or gram stain assay as noted in the Certificates of Analysis (CoAs) (**Appendix 2** and **Appendix 3**). If cells do not meet CoA requirements then the patient will be taken off-study (Section **3.6.2**). Once it is confirmed that immature and mature dendritic cells have met the CoA requirements (**Appendix 2** and **Appendix 3**), the vaccines will be administered to the patient.

2.3.2 Registration Procedure

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

2.4 TREATMENT ASSIGNMENT PROCEDURES

2.4.1 Cohorts

Subjects will be enrolled in one of two cohorts:

Number	Name	Description
1	*Melanoma*	Patients with metastatic melanoma
2	*Epithelial*	Patients with epithelial cancer

2.4.2 Arms

Number	Name	Description
1	*Experimental Therapy*	Peptide loaded dendritic cell vaccine on days 0, 14, 28, and 42

2.4.3 Randomization and Arm Assignment

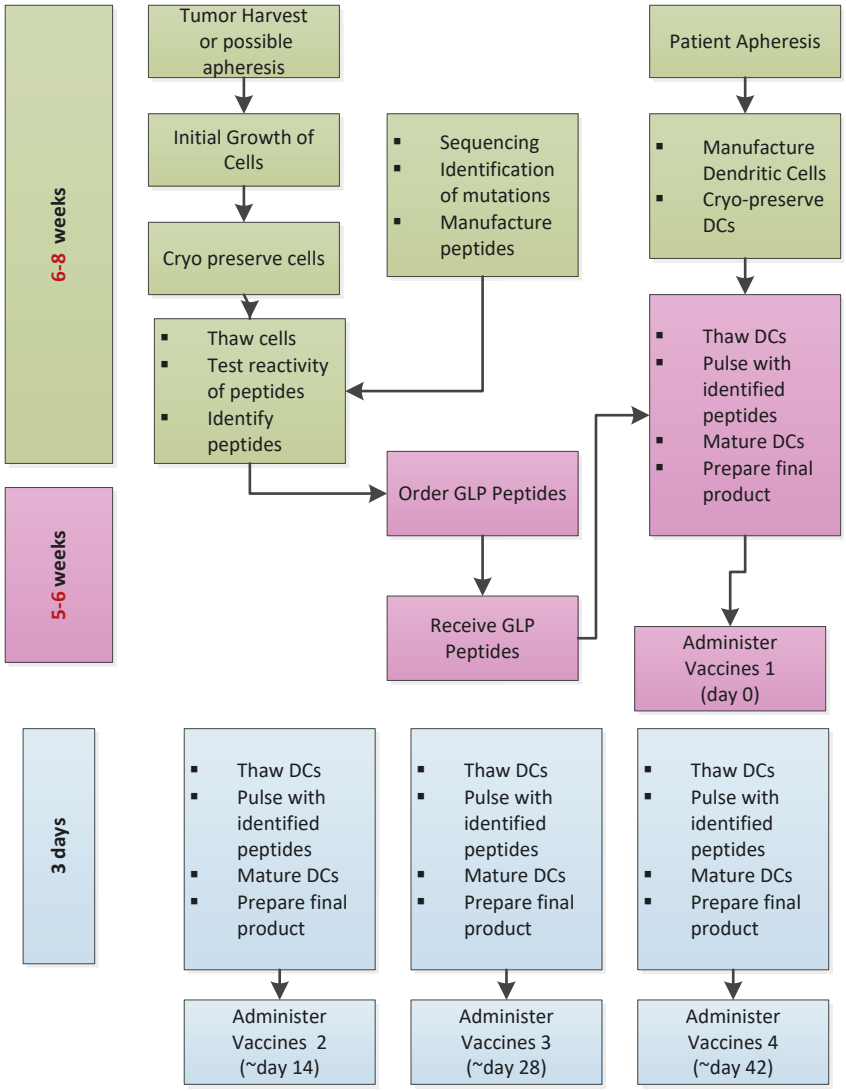
This is a non-randomized study. All subjects will be directly assigned based on cohort as follows:

- Subjects in Cohorts 1-2 will be directly assigned to Arm 1.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

3.1.1 Pre-Treatment Phase: DC Preparation



Under 03-C-0277, patients with evaluable metastatic melanoma or epithelial cancers will undergo resection of the tumor and apheresis. Whole exome and RNA sequencing will be performed to identify all cancer mutations using our standard procedures. TIL will be grown and expanded for this trial according to standard operating procedures submitted in the IND. After a sufficient yield of TIL ($5x10^7$ cells) is expanded and PBMCs are available, immunogenic neoantigens will be identified by high throughput immunologic screening using long peptides and tandem minigenes covering all mutated epitopes to identify the exact mutations recognized by autologous T cells. For vaccine preparation, patients will undergo apheresis and DCs will be produced and cryopreserved for vaccine preparation. Peptides (15-25mers with the mutation at positions 13) or minimal epitopes (9-10mers) will be synthesized and pulsed on autologous antigen presenting cells (DC). The antigen pulsed DC will then be matured using a TLR mix.

Patients will receive peptide-loaded mature dendritic cells intravenously and subcutaneously at biweekly intervals for 4 cycles. Immunologic tests will be performed as described in Section **5.5**.

3.1.2 Treatment Phase

The protocol will consist of two cohorts: cohort 1, patients with melanoma and cohort 2, patients with epithelial cancer.

The study will utilize an optimal design where initially 21 evaluable patients will be enrolled to each cohort. If 0 or 1 of the 21 patients in a given cohort experiences a clinical response, then no further patients will be enrolled; if 2 or more of the first 21 evaluable patients enrolled have a clinical response, then accrual will continue until a total of 41 evaluable patients have been enrolled to that cohort.

Patients will receive no other experimental agents while on this protocol. All patients will receive one course of treatment (4 vaccination cycles). The start date of the course will be the start date of the first vaccination; the end date will be the day of the first post-treatment evaluation (week 8 if the patient receives all 4 cycles).

Enrollment will be staggered within each cohort with three weeks between start dates for the first three patients to allow for a 3-week interval for safety assessment before the next enrolled patient is scheduled to receive their first dose of the DC vaccine.

3.1.3 Protocol Stopping Rules

New subject enrollment to the protocol will be temporarily halted if any of the following conditions are met, and discussions will be had with the FDA or NIH IRB regarding protocol revisions if applicable:

- If one or more treatment-related death occurs due to vaccination, we will promptly discuss this with the NCI IRB and the FDA.
- Two or more patients develop a grade 3 or greater toxicity at any point in the study not attributable to disease progression (or circumstances unrelated to the study).
    - The following grade 3 reactions commonly associated with immunization **will not** be included in this protocol stopping rule:
        - Pruritus/itching: intense or widespread and interfering with ADL lasting <72 hours
        - Fatigue: severe fatigue interfering with ADL lasting < 72 hours
        - Fever: > 40.0°C for < 24 hours
        - Local lymphadenopathy lasting < 1 week
- If one of the first three patients (or 2 of the first 6 patients, or 3 of the first 9 patients, or 4 of the first 12 patients) develop grade 3 autoimmunity, that cannot be resolved to less than or equal to a grade 2 autoimmune toxicity within 10 days, or any grade 4 or greater autoimmune toxicity.

3.2 DRUG ADMINISTRATION

Patients will receive peptide pulsed dendritic cell vaccines on days 0, 14 (± 5 days), 28 (± 5 days), and 42 (± 5 days). The vaccine will be administered as both an intravenous (IV) infusion

and a subcutaneous (SQ) injection. The total dose will be divided equally between IV and SQ containing $1.0x10^7$ to $8.0x10^7$ cells per cycle depending upon the manufacturing yield.

The patient's dendritic cell products will be delivered to the Patient Care Unit by a staff member from the NCI SB. Prior to infusion, the cell product identity labels on the syringe and cell infusion bag will be double-checked by two authorized staff (MD or RN), an identification of the product and documentation of administration are entered in the patient's chart, as is done for blood banking protocols.

- Cells for SQ injection will be administered first, followed by cells for IV infusion.
- Cells for SQ injection will be administered in a total of 1.0 mL ($0.5x10^7$ to $4.0x10^7$ cells) into the deep subcutaneous tissue of a proximal extremity. Injections will preferentially be administered in the anterior thigh. Injections will not be given into extremities that have had radical lymph node dissections. The same extremity will be used for each cycle of the peptide loaded dendritic cell vaccine administration.
- Cells for IV administration ($0.5x10^7$ to $4.0x10^7$ cells) will be infused over 20 to 30 minutes, or as clinically determined by an investigator for patient safety, via non-filtered tubing, gently agitating the bag during infusion to prevent cell clumping. See **Appendix 1** for cell infusion instructions.
- Vital signs will be monitored hourly (±15 minutes) for two hours. The post-administration monitoring period may be adjusted by the PI.
- All patients will receive their peptide pulsed dendritic cell vaccines in the NIH Clinical Center. The first three patients will receive their Day 0 SQ injection and IV infusion on the In-Patient Care Unit, and then will receive their subsequent SQ injections and IV infusions (days 14, 28, and 42) as outpatient procedures in the 3SE-S Day Hospital. All patients enrolled after the first three patients will receive their full course of SQ injections and IV infusions (days 0, 14, 28, and 42) as outpatient procedures in the 3SE-S Day Hospital. This schedule may be adjusted by the PI as clinically appropriate.

3.3 On-Study Evaluation

3.3.1 Within 7 Days Prior to the 1st Vaccination (Day 0)

- Repeat all screening procedures from Section **2.2.3** if not completed within 7 days prior to the 1st vaccination (Day 0).

3.3.2 Within 2 Days Prior to the 1st Vaccination (Day 0)

- Lymphocyte phenotyping (TBNK)
- Review of baseline symptoms
- Research samples as described in Section **5**

3.3.3 Within 2 Days Prior to Each Subsequent Vaccination (Days 14, 28, and 42 (± 5 days))

- Physical examination, including ECOG
- Vital signs
- CBC w/differential

- Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric Acid, Creatinine Kinase, Lactate Dehydrogenase, Total Protein
- β-HCG pregnancy test (serum or urine) on all females of child-bearing potential
- Toxicity assessment, including a review of systems
- Research samples as described in Section **5**

3.3.4 After Each Vaccination

- Vital signs will be monitored hourly (± 15 minutes) for two hours. The post-administration monitoring period may be adjusted by the PI.

3.4 POST-TREATMENT EVALUATION (FOLLOW-UP)

3.4.1 1st Follow-up Evaluation (Day 56 ± 5 days)

All patients will return to the NIH Clinical Center for their first follow-up evaluation on day 56 (±5 days), which is approximately 2 weeks from the 4th vaccination.

At the first follow-up evaluation, patients will undergo:

- Physical examination, including ECOG
- Vital signs
- CBC w/differential
- Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric Acid, Creatinine Kinase, Lactate Dehydrogenase, Total Protein
- Lymphocyte phenotyping (TBNK)
- β-HCG pregnancy test (serum or urine) on all females of child-bearing potential
- Toxicity assessment, including a review of systems
- Research samples as described in Section **5**
- Imaging studies as performed at baseline to determine tumor response. If clinically indicated, other scans or x-rays may be performed, e.g., brain MRI, bone scan.
- A 5-liter leukapheresis may be performed. If the patient is unable to undergo leukapheresis, approximately 96 mL of blood may be obtained. This will be conducted under 03-C-0277.

3.4.2 Subsequent Follow-up Evaluations

The second follow-up evaluation will take place 1 month (± 5 days) after the first follow-up evaluation (Day 56). Subsequent follow-up evaluations will take place every 1-2 months for the first year, and then annually or as clinically appropriate as determined by the PI. As noted in Section **3.4.1**, additional leukapheresis may be performed only at the first follow-up evaluation. It will not be performed at any other post-treatment evaluations.

Note: Patients may be seen more frequently as clinically indicated.

At each scheduled follow-up evaluation, patients will undergo:

- Physical examination, including ECOG
- Vital signs
- CBC w/differential
- Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric Acid, Creatinine Kinase, Lactate Dehydrogenase, Total Protein
- Lymphocyte phenotyping (TBNK)
- β-HCG pregnancy test (serum or urine) on all females of child-bearing potential (complete at all follow-up evaluations within 4 months after the last vaccine)
- Toxicity assessment, including a review of systems
- Research samples as described in Section **5**
- Imaging studies as performed at baseline to determine tumor response. If clinically indicated, other scans or x-rays may be performed, e.g., brain MRI, bone scan.

Note: Patients who are unable or unwilling to return for follow-up evaluations may be followed via phone or e-mail contact. Patients may be asked to send laboratory, imaging and physician exam reports performed by their treating physician.

3.5 STUDY CALENDAR

Assessments	Screening/Baseline (prior to enrollment on treatment protocol)			On-Study/Course of Treatment (1 vaccine = 1 cycle)				Post-Treatment Follow-up	
	Within 3 Months	Within 4 Weeks	Within 7 Days	Day 0[1]	Day 14 (±5 d)	Day 28 (±5 d)	Day 42 (±5 d)	Day 56 (±5 d)	Subsequent Follow-up Visits[2]
Informed consent[3]									
Confirmation of diagnosis by NCI Lab of Pathology[4]	X								
Medical history[5]		X							
Physical exam		X	X	X	X	X	X	X	X
Performance score (ECOG)[6]			X	X	X	X	X	X	X
Vital signs[7]			X	X	X	X	X	X	X
β-HCG pregnancy test[8]			X	X	X	X	X	X	X
Urinalysis[9]			X	X					
EKG			X	X					
Toxicity assessment					X	X	X	X	X
Laboratory Procedures									
Viral testing[10]	X								

1 Assessments **(except TBNK and research samples)** may be done within 7 days prior to the first vaccination on Day 0. **Lymphocyte phenotyping (TBNK) and research samples must be drawn within 2 days prior to the first vaccination on Day 0.**
2 The second follow-up evaluation will take place 1 month (± 5 days) after the first follow-up evaluation (Day 56). Subsequent follow-up evaluations will take place every 1-2 months for the first year, and then annually or as clinically appropriate as determined by the PI. Patients may be seen more frequently as clinically indicated. Patients who are unable or unwilling to return for follow-up evaluations may be followed via phone or email contact. Patients may be asked to send laboratory, imaging and physician exam reports performed by their treating physician.
3 The date of enrollment is the date the subject signs the written informed consent document. The patient will be consented and enrolled on this protocol once the patient has adequate leukapheresis material banked by the SB CPF and prior to administration of the first vaccine.
4 Confirmation of diagnosis of metastatic melanoma or epithelial cancer. Testing is permitted to be conducted at any time prior to enrollment.
5 Patient history may be obtained within 8 weeks prior to enrollment.
6 ECOG of 0, 1, or 2.
7 After each vaccination, vital signs will be monitored hourly (± 15 minutes) for two hours. The post-administration monitoring period may be adjusted by the PI.
8 Serum or urine; on all females of child-bearing potential. Complete at all follow-up evaluations within 4 months after the last vaccine.
9 With culture if indicated.
10 HIV antibody titer, HBsAg determination, anti HCV, anti CMV antibody titer, HSV serology, and EBV panel. Patients who are known to be positive for CMV, HSV and/or EBV do not need to be retested.

Assessments	Screening/Baseline (prior to enrollment on treatment protocol)			On-Study/Course of Treatment (1 vaccine = 1 cycle)				Post-Treatment Follow-up	
	Within 3 Months	Within 4 Weeks	Within 7 Days	Day 0[1]	Day 14 (±5 d)	Day 28 (±5 d)	Day 42 (±5 d)	Day 56 (±5 d)	Subsequent Follow-up Visits[2]
CBC w/differential			X	X	X	X	X	X	X
Chemistry panel[11]			X	X	X	X	X	X	X
PT/PTT			X	X					
Lymphocyte phenotyping (TBNK)				X				X	X
Additional leukapheresis[12]								X	
Correlatives[13]									
CPT tubes (8) (SB CPF)				X	X	X	X	X	X
SST tube (1) (Figg Lab)				X	X	X	X	X	X
Imaging									
CT, MRI, PET, and/or photography[14]		X						X	X
Chest x-ray			X	X					
Treatment/Intervention									
Peptide loaded dendritic cell vaccine[15]				X	X	X	X		

11 Chemistry panel: Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid
12 A 5 liter leukapheresis may be performed. If the patient is unable to undergo leukapheresis, approximately 96 ml of blood may be obtained. This will be conducted under 03-C-0277, and will only be performed at the first follow-up evaluation.
13 Research samples, as described in Section **5**, prior to each vaccination and at each scheduled follow-up evaluation. Includes evaluation of specific lysis and cytokine release, metabolomic and bioenergetic studies (using Seahorse), intracellular FACS of cytokine production, ELISA-spot assays, and lymphocyte subset analysis may be used to evaluate the immunological correlates of treatment.
14 As performed at baseline to determine tumor response. If clinically indicated, other scans or x-rays may be performed, e.g., brain MRI, bone scan.
15 Administered subcutaneously and intravenously.

3.6 Criteria for Removal from Protocol Therapy and Off-Study Criteria

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the first follow-up evaluation on Day 56.

3.6.1 Criteria for Removal from Protocol Therapy

Patients will be taken off treatment for the following:

- Completion of treatment
- Grade 3 or 4 toxicity due to the vaccine, excluding those known events such as local injection site reactions, skin rash, pruritus, fatigue, fever and local adenopathy.
- Progression of disease
- Participant requests to be withdrawn from active therapy
- Investigator discretion
- Positive pregnancy test

3.6.2 Off-Study Criteria

Patients will be taken off study for the following:

- Cells do not meet CoA requirements
- Completed study follow-up period
- Patient requests to be withdrawn from the study
- There is significant noncompliance
- Lost to follow-up
- Investigator discretion
- Progression of disease
- Death

All patients will be co-enrolled on the NCI SB companion protocol, 09-C-0161 (Follow up Protocol for Subjects Previously Enrolled in NCI Surgery Branch Studies). Patients who are taken off-study for progressive disease or study closure on the treatment protocol may be followed on protocol 09-C-0161.

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

3.6.3 Off Protocol Therapy and Off-Study Procedure

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

4 CONCOMITANT MEDICATIONS/MEASURES

Over the counter medications, such as ibuprofen, may be used to provide relief of local injection site reactions.

5 BIOSPECIMEN COLLECTION

Blood and tissue are tracked at the patient level and can be linked to all protocols on which the patient has been enrolled. Samples will be used to support the specific objectives listed in the treatment protocol(s), e.g., immunologic monitoring, cytokine levels, persistence. Patients who agree to have specimens and data stored to support long-term research efforts within the NCI SB will be consented and enrolled on 03-C-0277.

The amount of blood that may be drawn from adult patients for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period.

Samples will be ordered in CRIS and tracked through Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without IRB notification and an executed MTA.

5.1 SAMPLES SENT TO FIGG LAB

- Venous blood samples will be collected in either a 4 mL or an 8 mL SST tube to be processed for serum and stored for future research. Record the date and exact time of draw on the tube. Blood tubes may be kept in the refrigerator until pick up.
- For sample pick up, page 102-11964.
- For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).
- For questions regarding sample processing, contact Julie Barnes by e-mail or at 240-760-6044.
- The samples will be processed, barcoded, and stored in Dr. Figg's lab until requested by the investigator.

5.2 SAMPLES SENT TO SURGERY BRANCH CELL PRODUCTION FACILITY

- Venous blood samples will be collected in 8 mL CPT tubes to be processed and stored for future research. Record the date and exact time of draw on the tube. Blood tubes are kept at room temperature until pick up.
- Samples will be picked up by the research nurse or designee and transported to the SB CPF within 24 hours of blood draw.
- The samples will be processed, barcoded, and stored in the SB CPF.

5.3 PRIOR TO EACH VACCINATION

- 8 CPT tubes (8 mL each): SB CPF
- 1 SST tube (8 mL): Figg Lab

5.4 AT EACH SCHEDULED FOLLOW-UP EVALUATION

- 8 CPT tubes (8 mL each): SB CPF
- 1 SST tube (8 mL): Figg Lab

5.5 IMMUNOLOGICAL TESTING

- A variety of tests including evaluation of specific lysis and cytokine release, metabolomic and bioenergetic studies (using Seahorse), intracellular FACS of cytokine production,

ELISA-spot assays, and lymphocyte subset analysis may be used to evaluate the immunological correlates of treatment. In general, differences of 2-3 fold in these assays over the baseline measurement are indicative of true biologic differences.

Note: The collection and analysis of research labs will be monitored by the TIL lab and not by the Center for Cancer Research's data contractor.

5.6 Sample Storage, Tracking and Disposition For Surgery Branch Cell Production Facility

Blood and tissue collected during the course of this study will follow the Cell Tracking and Labeling System established by the SB CPF. The Cell Tracking and Labeling System is designed to unambiguously ensure that patient/data verification is consistent. The patients' cell samples (blood or tissue) are tracked by distinct identification labels that include a unique patient identifier and date of specimen collection. Cryopreserved blood and tissue samples also bear the date the sample was frozen. All cryopreserved samples are tracked for freezer location and storage criteria. All samples are stored in monitored freezers/refrigerators in 3NW NCI SB laboratories at specified temperatures with alarm systems in place. Serum samples will be sent to the Blood Processing Core (BPC) for storage. Samples will be barcoded and stored on site or offsite at NCI Frederick Central Repository Services in Frederick, MD. All collected samples (blood or tissue) are entered into a central computer database with identification and storage location, and this database is backed up every night.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed and reported as such to the IRB. Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e. broken freezer allows for extensive sample thawing, etc.) will be reported as such to the IRB.

Blood and tissue collected during the course of this study will be stored, tracked and disposed of as specified in protocol 03-C-0277.

5.7 Sample Storage, Tracking and Disposition for Dr. Figg's Lab

5.7.1 Sample Data Collection

All samples sent to the BPC will be barcoded, with data entered and stored in the LABrador (aka LabSamples) utilized by the BPC, and data will be updated to the NCI SB central computer database weekly. This is a secure program, with access to LABrador limited to defined Figg lab personnel, who are issued individual user accounts. Installation of LABrador is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen. All Figg lab personnel with access to patient information annually complete the NIH online Protection of Human Subjects course.

LABrador creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without LABrador access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.7.2 Sample Storage and Destruction

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in LABrador. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed and reported as such to the IRB. Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e. broken freezer allows for extensive sample thawing, etc.) will be reported as such to the IRB.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the LABrador. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

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

All adverse events (AEs), including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Patients will be followed for adverse events from the time the patient receives the investigational agent/intervention to the time of the second follow-up evaluation, or until off-study, whichever comes first.

An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study

- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

All AEs must be recorded on the AE case report form.

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

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

6.1.1 Exclusions to Routine Adverse Event Recording

There are no exclusions to routine adverse event reporting/recording for this protocol.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

De-identified human data generated for use in future and ongoing research will be shared through a NIH-funded or approved repository (ClinicalTrials.gov) and BTRIS. At the completion of data analysis, data will be submitted to ClinicalTrials.gov either before publication or at the time of publication or shortly thereafter. Data may also be used to support long-term research efforts within the NCI SB and de-identified data may also be shared with collaborators as specified in protocol 03-C-0277.

6.2.2 Genomic Data Sharing Plan

The NIH Genomic Data Sharing Policy does not apply to this study.

6.3 RESPONSE CRITERIA

For the purposes of this study, patients should be re-evaluated for response approximately two weeks following the last vaccination, then one month later x1, and then every 1-2 months for the first year, and then annually or as clinically indicated. In addition to a baseline scan, confirmatory scans should also be obtained at least 4 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1)[18]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

6.3.1 Definitions

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment with peptide loaded dendritic cell vaccine.

Evaluable for objective response: Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response: Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

6.3.2 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as:

- By chest x-ray: ≥ 20 mm;
- By CT scan:
  - Scan slice thickness 5 mm or under: ≥ 10 mm
  - Scan slice thickness > 5 mm: double the slice thickness
- With calipers on clinical exam: ≥ 10 mm.

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. If the investigator thinks it appropriate to include them, the conditions under which such lesions should be considered must be defined in the protocol.

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be >15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease: All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Target lesions: All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters

will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions: All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

6.3.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment 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. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans). Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

FDG-PET: While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

- Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
- No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
- FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication.

However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

6.3.4 Response Criteria

6.3.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum of diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum of diameters while on study.

6.3.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or PI).

6.3.4.3 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). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST v1.1 manuscript for further details on what is evidence of a new lesion.

** Only for non-randomized trials with response as primary endpoint.

*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: 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.

6.3.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event

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

7.1.2 Suspected Adverse Reaction

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

7.1.3 Unexpected Adverse Reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected" also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

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

7.1.5 Serious Adverse Event

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

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

7.1.6 Disability

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

7.1.7 Life-Threatening Adverse Drug Experience

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

7.1.8 Protocol Deviation (NIH Definition)

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

7.1.9 Non-Compliance (NIH Definition)

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

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

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

7.2 NCI-IRB Reporting and Clinical Director (CD) Reporting

7.2.1 NCI-IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths

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

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

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

7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:

- All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
- All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
- All Grade 5 events regardless of attribution;
- All serious events regardless of attribution.

Note: Grade 1 events are not required to be reported.

7.2.3 NCI-IRB Reporting of IND Safety Reports

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

7.3 IND Sponsor Reporting Criteria

From the time the subject receives the investigational agent/intervention to the time of the second follow-up evaluation, the investigator must immediately report to the sponsor, using the mandatory MedWatch form 3500a or equivalent, any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event. For serious adverse events that occur after the second follow-up evaluation, only those events that have an attribution of at least possibly related to the agent/intervention will be reported.

Required timing for reporting per the above guidelines:

- Deaths (except death due to progressive disease) must be reported via email within 24 hours. A complete report must be submitted within one business day.
- Other serious adverse events as well as deaths due to progressive disease must be reported within one business day

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

7.3.1 Reporting Pregnancy

7.3.1.1 Maternal Exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately and the pregnancy reported to the Sponsor. The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agents(s) should be documented in box B5 of the MedWatch form "Describe Event or Problem".

Pregnancy itself is not regarded as an SAE. However, as patients who become pregnant on study risk intrauterine exposure of the fetus to agents which may be teratogenic, the CCR is requesting that pregnancy should be reported in an expedited manner as **Grade 3** "***Pregnancy, puerperium and perinatal conditions - Other (pregnancy)***" under the ***Pregnancy, puerperium and perinatal conditions*** SOC.

Congenital abnormalities or birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

If any pregnancy occurs in the course of the study, then the investigator should inform the Sponsor within one day, i.e., immediately, but **no later than 24 hours** of when he or she becomes aware of it.

The designated Sponsor representative will work with the investigator to ensure that all relevant information is provided to the Sponsor within 1 to 5 calendar days for SAEs and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

7.3.1.2 Paternal Exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 120 days after the last vaccination.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until 120 days after the last dose should, if possible, be followed up and documented.

7.4 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

7.4.1 Serious Adverse Event Reports to IBC

Serious adverse events are not required to be reported to the NIH IBC, as the research is not subject to the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)*. Therefore, the reporting requirements as described in Appendix M do not apply for this study.

7.4.2 Annual Reports to IBC

Within 60 days after the one-year anniversary of the date on which the IBC approved the initial protocol, and after each subsequent anniversary until the trial is completed, the Principal Investigator (or delegate) shall submit the information described below. Alternatively, the IRB continuing review report can be sent to the IBC in lieu of a separate report. Please include the IBC protocol number on the report.

7.4.2.1 Clinical Trial Information

A brief summary of the status of the trial in progress or completed during the previous year. The summary is required to include the following information:

- the title and purpose of the trial
- clinical site
- the Principal Investigator
- clinical protocol identifiers;
- participant population (such as disease indication and general age group, e.g., adult or pediatric);
- the total number of participants planned for inclusion in the trial; the number entered into the trial to date whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons

- the status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed,
- if the trial has been completed, a brief description of any study results.

7.4.2.2 Progress Report and Data Analysis

Information obtained during the previous year's clinical and non-clinical investigations, including:

- a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system
- a summary of all serious adverse events submitted during the past year
- a summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications
- if any deaths have occurred, the number of participants who died during participation in the investigation and causes of death
- a brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

7.5 DATA AND SAFETY MONITORING PLAN

7.5.1 Principal Investigator/Research Team

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

All data will be collected in a timely manner and reviewed by the Principal Investigator. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS.

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

7.5.2 Sponsor Monitoring Plan

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

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

- Response assessment

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

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

8 STATISTICAL CONSIDERATIONS

8.1 STATISTICAL HYPOTHESIS

8.1.1 Primary Efficacy Endpoint

The primary objectives of this trial is to determine the clinical response rate in patients with metastatic melanoma or epithelial cancers who receive this dendritic cell vaccine.

The objective response rate (responses/evaluable patients) is the measurable endpoint, and it will be of interest to determine if it exceeds 10% and is consistent with 25%.

8.1.2 Secondary Efficacy Endpoint

The secondary objectives are to determine the safety of the vaccine and to determine whether treatment administration can increase the quantity and quality of circulating antigen-specific T cells.

8.2 SAMPLE SIZE DETERMINATION

Assays to assess quality and quantity of the vaccine induced cells will include Eliza and Elispot assays assessing reactivity to the mutated peptide compared to the non-mutated peptide. Patients will be enrolled into 2 individual cohorts; melanoma and common epithelial cancers. For each of the 2 cohorts, the study will be conducted using a phase II optimal design[19] (Simon R, Controlled Clinical Trials 10:1-10, 1989). The objective will be to determine if the vaccine is able to be associated with a clinical response rate that can rule out 5% (p0=0.05) in favor of a modest 20% PR + CR rate (p1=0.20).

In patients in each of the two cohorts, the following design will be used. For each cohort, with alpha=0.05 (5% probability of accepting a poor therapy) and beta=0.10 (10% probability of rejecting a good therapy,), initially 21 evaluable patients will be enrolled. If 0 or 1 of the 21 patients experiences a clinical response, then no further patients will be enrolled. If 2 or more of the first 21 evaluable patients enrolled have a clinical response, then accrual will continue until a total of 41 evaluable patients have been enrolled. As it may take several weeks to determine if a patient has experienced a clinical response, a temporary pause of up to 6 months in the accrual to the trial may be necessary to ensure that enrollment to the second stage is warranted. If 2 to 4 of the 41 have a clinical response, then this will be considered inadequate for further investigation. If 5 or more of 41 patients have a clinical response, then this will indicate that this strategy provides a new approach that may be worthy of further consideration. Under the null hypothesis (5% response rate), the probability of early termination is 72%.

A total of up to 86 patients may be required (allowing up to 2 inevaluable patients per cohort). Provided that about 1-2 patients per month will be able to be enrolled onto this trial, approximately 6 years may be needed to accrue the maximum number of required patients.

However, as adequate responses to proceed to the second stage of accrual may not occur, the trial may end up accruing as few as 21 patients in each cohort.

8.3 POPULATION FOR ANALYSES

Modified intention to treat analysis dataset. Patients who receive adequate treatment (defined as the administration of peptide pulsed antigen presenting cells) will be considered evaluable and included in the statistical analyses

8.4 STATISTICAL ANALYSES

8.4.1 General Approach

Descriptive statistical analyses will be performed without any formal hypothesis testing.

8.4.2 Analysis of the Primary Endpoints

For each cohort, the clinical response rate (PR+CR/number of evaluable subjects) will be determined and reported along with the corresponding 95% two-sided confidence interval.

8.4.3 Analysis of the Secondary Endpoint(s)

To determine whether treatment administration can increase the quantity and quality of circulating antigen-specific T cells, by measuring the reactivity of circulating cells against the immunizing peptides in Elispot and 41BB upregulation assays.

8.4.4 Safety Analyses

Safety will be monitored by identifying the type and severity of any adverse events. The fraction of patients who experience a DLT will be identified, with information reported about the number and grade of each type of DLT identified.

8.4.5 Baseline Descriptive Statistics

Not applicable.

8.4.6 Planned Interim Analyses

As noted in Section **8.2**, results after the first stage of a Simon optimal two-stage design in each cohort will be examined to determine if the minimum number of responses has been obtained.

8.4.7 Sub-Group Analyses

There are no planned analyses based on demographic characteristics since the power to do subgroup analyses would be limited and make interpretation difficult.

8.4.8 Tabulation of individual Participant Data

None planned.

8.4.9 Exploratory Analyses

None planned.

9 COLLABORATIVE AGREEMENTS

None.

10 HUMAN SUBJECTS PROTECTIONS

10.1 Rationale for Patient Selection

Subjects from both genders and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one group compared to another. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on the one hand and the need to explore gender and ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to gender or to ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.

We will limit enrollment to patients 70 years of age or less because based on our admittedly limited experience with prior ACT clinical trials, younger patients tolerate and recover from these toxicities better than elderly patients.

10.2 Participation of Children

Since the efficacy of this experimental procedure is unknown, it does not seem reasonable to expose children to this risk without further evidence of benefit. Should results of this study indicate efficacy in treating metastatic cancer, which is not responsive to other standard forms of therapy, future research can be conducted in the pediatric population to evaluate potential benefit in that patient population.

10.3 Participation of Subjects Unable to Give Consent

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (Section **10.5**), all subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team for evaluation. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MAS Policy 87-4 for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

10.4 Evaluation of Benefits and Risks/Discomforts

The experimental treatment has a chance to provide clinical benefit though this is unknown. The NCI SB has extensive experience with ACT following treatment with high-dose aldesleukin, however this experimental treatment does not include the standard NCI SB preparative regimen or high-dose aldesleukin. Although we have seen responses to prior NCI SB ACT treatments, we do not know if this change in our process will improve patient outcome. The risks associated with ACT are substantial, including, a delay in treatment due to the need to harvest and grow the cells, a surgical procedure (possible major) to obtain tumor for the cell product, the possibility

that a cell product cannot be generated, infection due to the surgical procedure, and death. The risks in this treatment are detailed in Section **11**. The risks associated with taking blood draw samples are pain, bruising, or infection.

10.5 Risk/Benefit Analysis

Because all patients in this protocol have metastatic or recurrent/refractory locally advanced cancer and limited life expectancies the potential benefit is thought to outweigh the potential risks.

10.6 Consent Process and Documentation

If the patient meets the thorough screening for eligibility, the patient, with family members or friends at the request of the patient, will be presented with a detailed description of the protocol treatment. The specific requirements, objectives, and potential advantages and disadvantages will be presented. The informed consent document is given to the patient, who is requested to review it and to ask questions prior to agreeing to participate in the treatment portion of this protocol. The patient is reassured that participation on trial is entirely voluntary and that he/she can withdraw or decide against treatment at any time without adverse consequences. The Principal Investigator, associate investigator, or clinical fellow is responsible for obtaining written consent from the patient.

10.6.1 Informed Consent of Non-English Speaking Subjects

If there is an unexpected enrollment of a research participant for whom there is no translated extant IRB approved consent document, the Principal Investigator and/or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, OHSRP SOP 12, 45 CFR 46.117 (b) (2), and 21 CFR 50.27 (b) (2). The summary that will be used is the English version of the extant IRB approved consent document. Signed copies of both the English version of the consent and the translated short form will be given to the subject or their legally authorized representative and the signed original will be filed in the medical record.

Unless the PI is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation (using either the long translated form or the short form). Preferably someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of the relevant consent documents well before the consent conversation with the subject (24 to 48 hours if possible).

We request prospective IRB approval of the use of the short form process for non-English speaking subjects and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form.

11 PHARMACEUTICAL INFORMATION

11.1 Cell Preparation

The procedure for growing and expanding the autologous DC and the Certificate of Analysis are similar to those approved by the Food and Drug Administration and used in other NCI SB clinical studies. This product will be provided for investigational use only under a Sponsor IND. The Certificate of Analysis is in **Appendix 2** and **Appendix 3**, and the Standard Operating

Procedures for the growth of dendritic cells is in the IND and in the NCI SB BB Master File 13782. DC will be administered at a dose of between $1x10^7$ to $8x10^7$ dendritic cells.

11.2 Subcutaneous Injection and Intravenous Infusion Preparation

Classification: Peptide Loaded Dendritic Cell Vaccine

Mode of Action: Autologous monocyte derived DC pulsed with peptide neoantigens are recognized in vivo by tumor-specific cytotoxic T-lymphocytes, leading to a major histocompatibility complex-restricted cytotoxic response against tumor cells bearing these neoantigens.

Product Description: The vaccine is derived from autologous DCs produced ex vivo from monocytes isolated from an apheresis product. DCs are produced using clinical grade cytokines (IL-4 and GM-CSF). Neoantigens will be identified from a sequence comparison of a patient's tumor and normal DNA sample. An algorithm will predict mutated sequences with a high probability of binding to a patient's specific HLA repertoire. Neoantigens will be prepared as peptides, either as 25mers, with the mutated amino acid residue situated at position 13, or as minimal epitopes. The patient's tumor infiltrating lymphocytes, cultured from a biopsy sample or PBMC, will be screened for their ability to recognize these predicted neoantigens pulsed onto DCs. Neoantigens recognized by the patient's TIL or PBMC will be used to formulate the vaccine. The vaccine will be formulated for each administration as follows. The identified peptides will be pulsed onto thawed, previously cryopreserved DCs overnight. The following day, the DCs pulsed with peptides will be matured with Poly I:C, R848 and IFN-γ (TLR mix). The DCs will be washed and $1\text{-}8x10^7$ will be loaded into syringes for IV and SQ administration.

How Supplied: Neoantigen Peptides: The peptides will be manufactured by Peptides and Elephants (PE) GmBH, Potsdame, Germany. The peptides are supplied as a sterile, GLP grade, lyophilized powder (TFA salt) in vials. Interferon-γ is purchased from Miltenyi as a lyophilized powder. Poly I:C will be purchased from a qualified supplier as a lyophilized powder. R848 will be purchased from a qualified supplier as a lyophilized powder.

Preparation: Peptides for pulsing DCs will be reconstituted in sterile water for DC pulsing to a final concentration of 1 mg/mL. Poly I:C is reconstituted in sterile water at a concentration of 1mg/ml. R848 is reconstituted in sterile water for injection at a concentration of 1mg/mL. Interferon-γ is reconstituted in sterile water for injection at a concentration of $4x10^6$ units per mL.

Storage: Peptides for Pulsing: Lyophilized vials will be stored at -20°C. Reconstituted peptide solutions will be stored at -20°C. DCs will be cryopreserved in Cryostor10 and maintained in vapor phase liquid nitrogen (-140 to -180°C) until used. Poly I:C and R848 are stored at -20°C as lyophilized powders and as reconstituted solutions. Interferon-γ is stored as lyophilized powder at -20°C and as a reconstituted solution at 4°C.

Stability: The peptides, Poly I:C, R848 and DC are stable at the recommended storage temperatures for at least one year. Interferon-γ will be made up fresh for each maturation process. The long-term stability of these vaccine components is under evaluation through an on-going stability testing program.

Route of Administration:

SQ Injection: A total of 1 mL (0.5 to $4x10^7$) DCs will be administered as an injection into the deep SQ tissue of a proximal extremity. Injections will preferentially be administered in the

anterior thigh. Injections will not be given into extremities that have had radical lymph node dissections. The same extremity will be used for each cycle of the peptide loaded dendritic cell vaccine administration.

IV Infusion: A total of (0.5 to $4x10^7$) cells for IV administration will be infused over 20-30 minutes via non-filtered tubing, gently agitating the bag during infusion to prevent cell clumping.

Potential Adverse Events and Risks: The peptide antigens pulsed on to the DCs will be unique to each patient and may mimic portions of natural proteins found naturally throughout the body, there is a chance for the development of autoimmune reactions to cells containing these proteins.

Special Handling: The peptides are NOT a cytotoxic or infectious agent and require no special handling. The DCs are a blood derived product. All personnel should treat blood derived products as potentially infectious material. Universal precautions and infection control should be practiced at all times.

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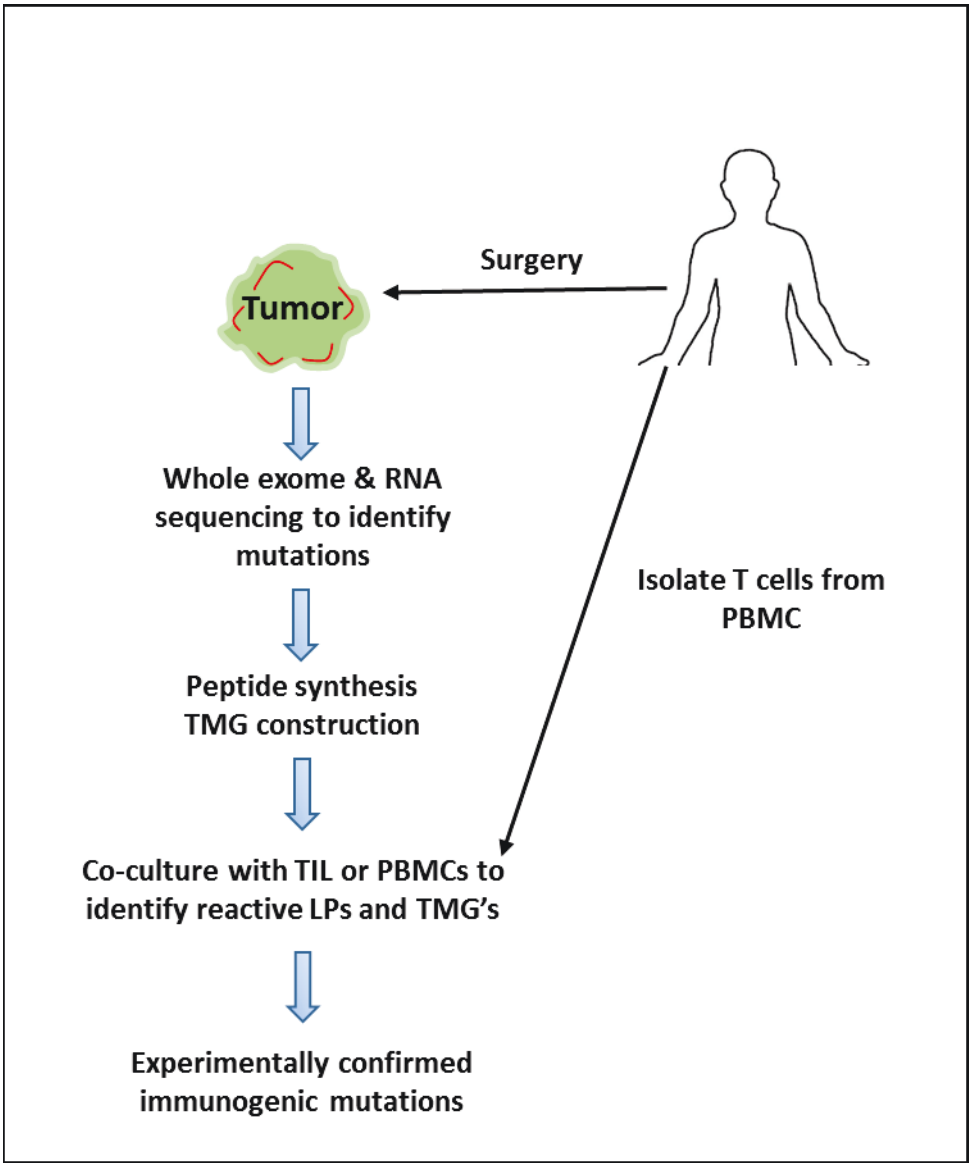
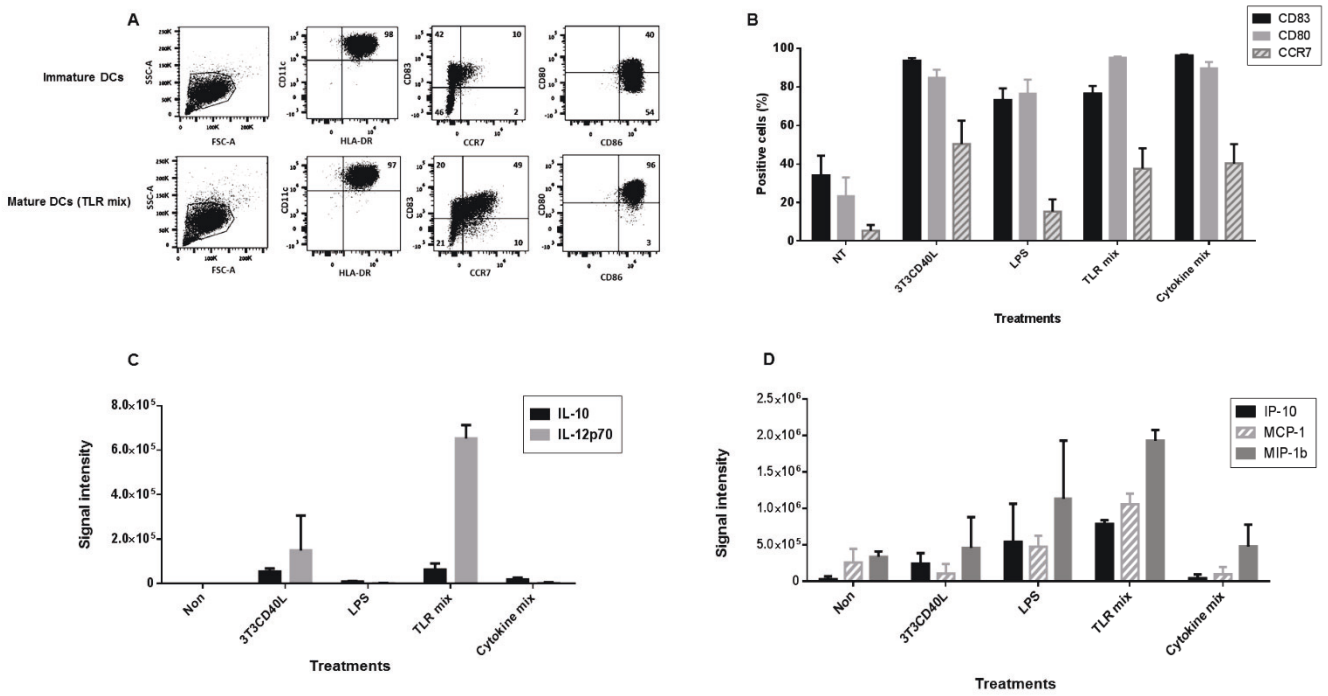
13 FIGURES AND TABLES

13.1 FIGURE 1: A PROCESS FOR THE IDENTIFICATION OF IMMUNOGENIC SOMATIC MUTATIONS

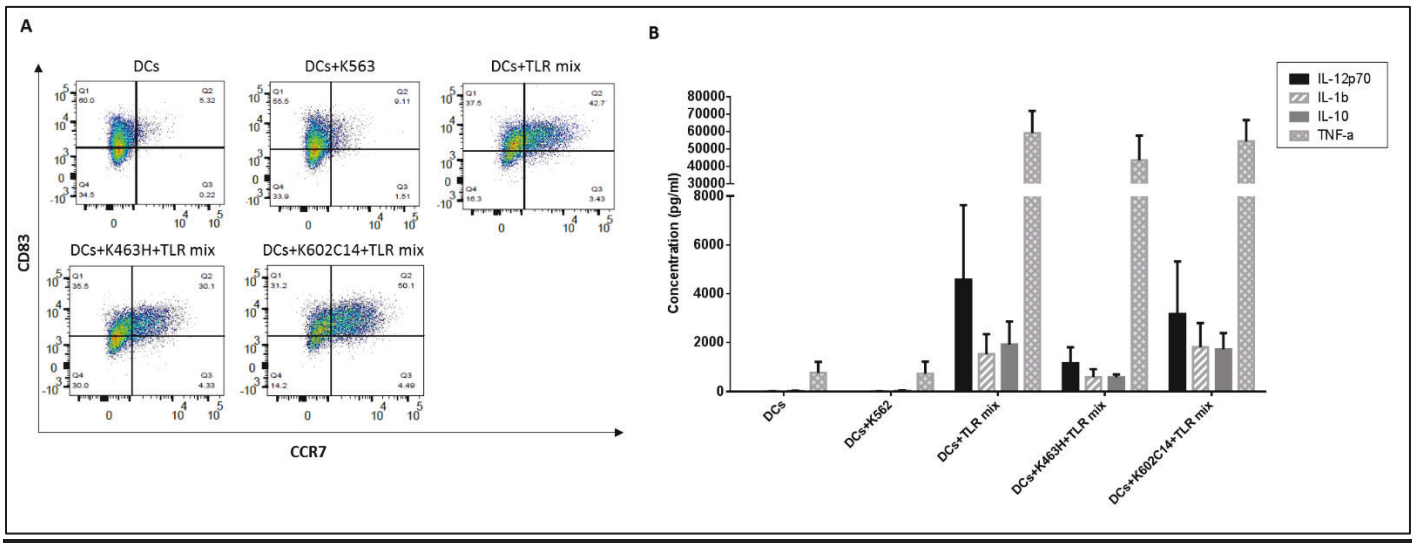


13.2 FIGURE 2: TLR MIX INDUCES THE SECRETION OF IL-12P70, TNFα, AND MULTIPLE CHEMOKINES



Monocyte derived dendritic cells were stimulated with TLR mix (PolyI:C, R848, and IFNγ), 3T3CD40L cells, LPS or a cytokine mix (Il-1β, IL-6, TNFα, and PGE2), Non-treated cells (NT) were used as negative control. Sixteen hours post incubation the level of costimulatory molecules was evaluated by flow cytometry (A, B) and the secretion of multiple cytokines and chemokines was measured by multiplex assay (C, D).

13.3 FIGURE 3: TLR MIX ALONE IS SUFFICIENT TO INDUCE POTENT DC MATURATION



Monocyte derived dendritic cells were stimulated with TLR mix (PolyI:C, R848, and IFNg), K463H+TLR mix and K602C14 cells + TLRmix. As a negative control, we used DCs alone or DCs stimulated with K562 cells. Sixteen hours post incubation the level of costimulatory molecules was evaluated by flow cytometry (A) and the secretion of multiple cytokines was measured by multiplex assay (B).

13.4 FIGURE 4: LONG PEPTIDES, MINIMAL EPITOPES AND TMG MRNA USED TO EVALUATE ANTIGEN PRESENTATION FOR CD4 AND CD8 CELLS

A. **List of minimal epitopes and corresponding long peptides**

Antigen	*HLA restriction*	*Minimal epitope*	*Minigene (25mer)*
MAGE-A3 $_{243-258}$	DP04	QHFVQENYLEY	ILGDPKKLLT**QHFVQENYLEY**RQVP
gp100 $_{44-59}$	DRB1*0401	WNRQLYPEWTEAQRLD	LRTKA**WNRQLYPEWTEAQRLD**CWRG
Tyrosinase $_{450-462}$	DRB1*0401	SYLQDSDPDSFQD	DLGYDY**SYLQDSDPDSFQD**YIKSYL
ppp1R3B $_{172m}$	HLA-A1	YTDFHCQYVK	MTFDTWKS**YTDFHCQYVK**DTYAGSD
HPV16E6 $_{29-38}$	HLA-A2	TIHDIILECV	QLCTELQT**TIHDIILECV**YCKQQLL
HPV16E7 $_{11-19}$	HLA-A2	YMLDLQPET	GDTPTLHE**YMLDLQPET**TDLYCYEQ

B. **Basic TMG backbone**

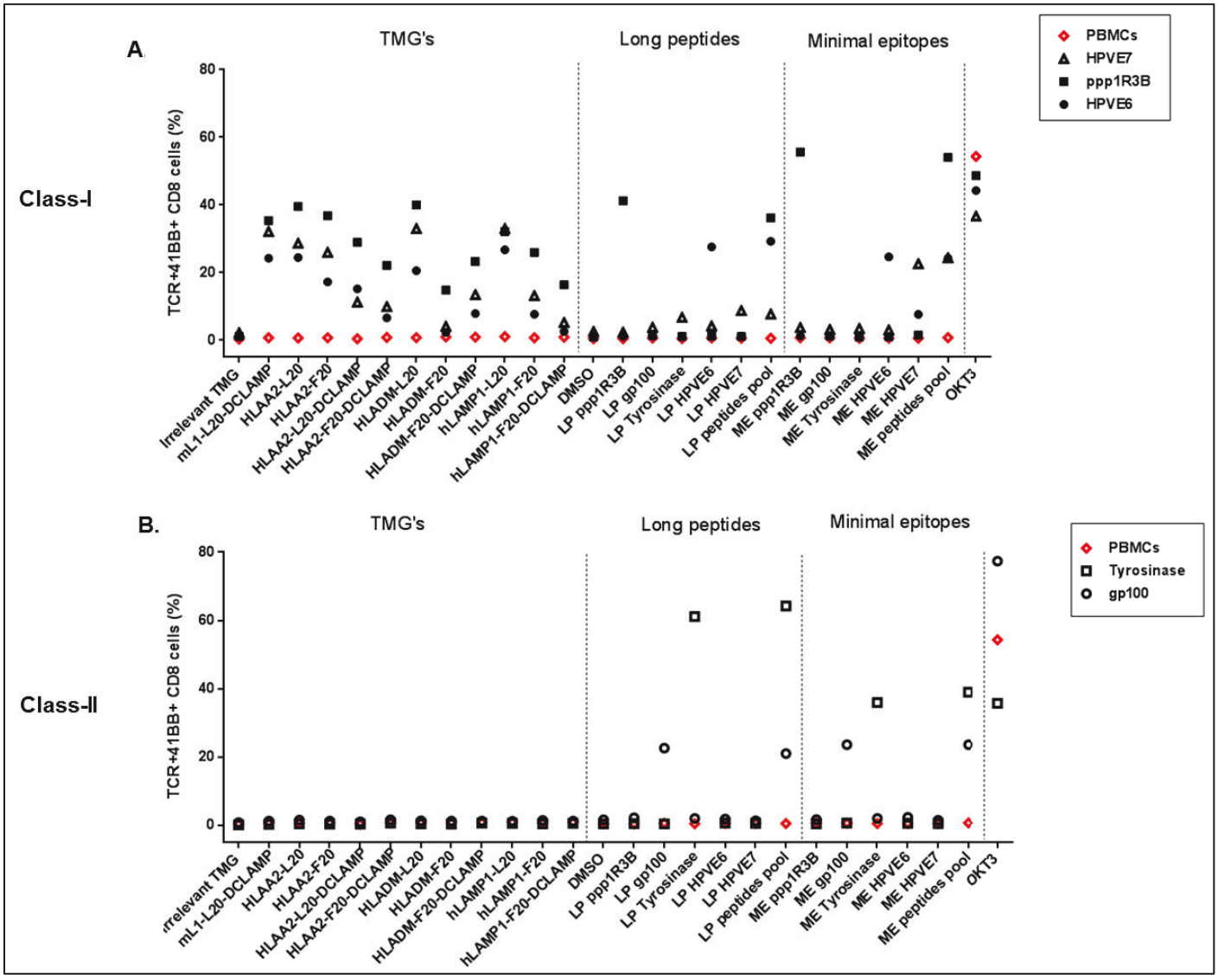
C. **Table summarizing all constructs**

Number	Name	No. of minigenes	Signal peptide	Linker	Targeting sequence	Backbone vector
1	**mL1-L20-DCLAMP**	**20**	**Mouse LAMP-1**	**Non**	**DC-LAMP**	**PST1**
2	HLAA2-L20	20	HLAA2	Non	Non	PST1
3	HLAA2-F20	20	HLAA2	**Furin cleavage site****	Non	PST1
4	HLAA2-L20-DCLAMP	20	HLAA2	Non	DC-LAMP	PST1
5	HLAA2-F20-DCLAMP	20	HLAA2	**Furin cleavage site**	DC-LAMP	PST1
6	HLADM-L20	20	HLA-DM	Non	Non	PST1
7	HLADM-F20	20	HLA-DM	**Furin cleavage site**	Non	PST1
8	HLADM-F20-DCLAMP	20	HLA-DM	Non	DC-LAMP	PST1
9	hLAMP1-L20	20	Human LAMP1	**Furin cleavage site**	Non	PST1
10	hLAMP1-F20	20	Human LAMP1	Non	Non	PST1
11	hLAMP1-F20-DCLAMP	20	Human LAMP1	**Furin cleavage site**	DCLAMP	PST1

*** Proprotein convertase cleavage sites selected based on [*J Biol Chem. 2008 Jul 25; 283(30): 20897–20906*]**

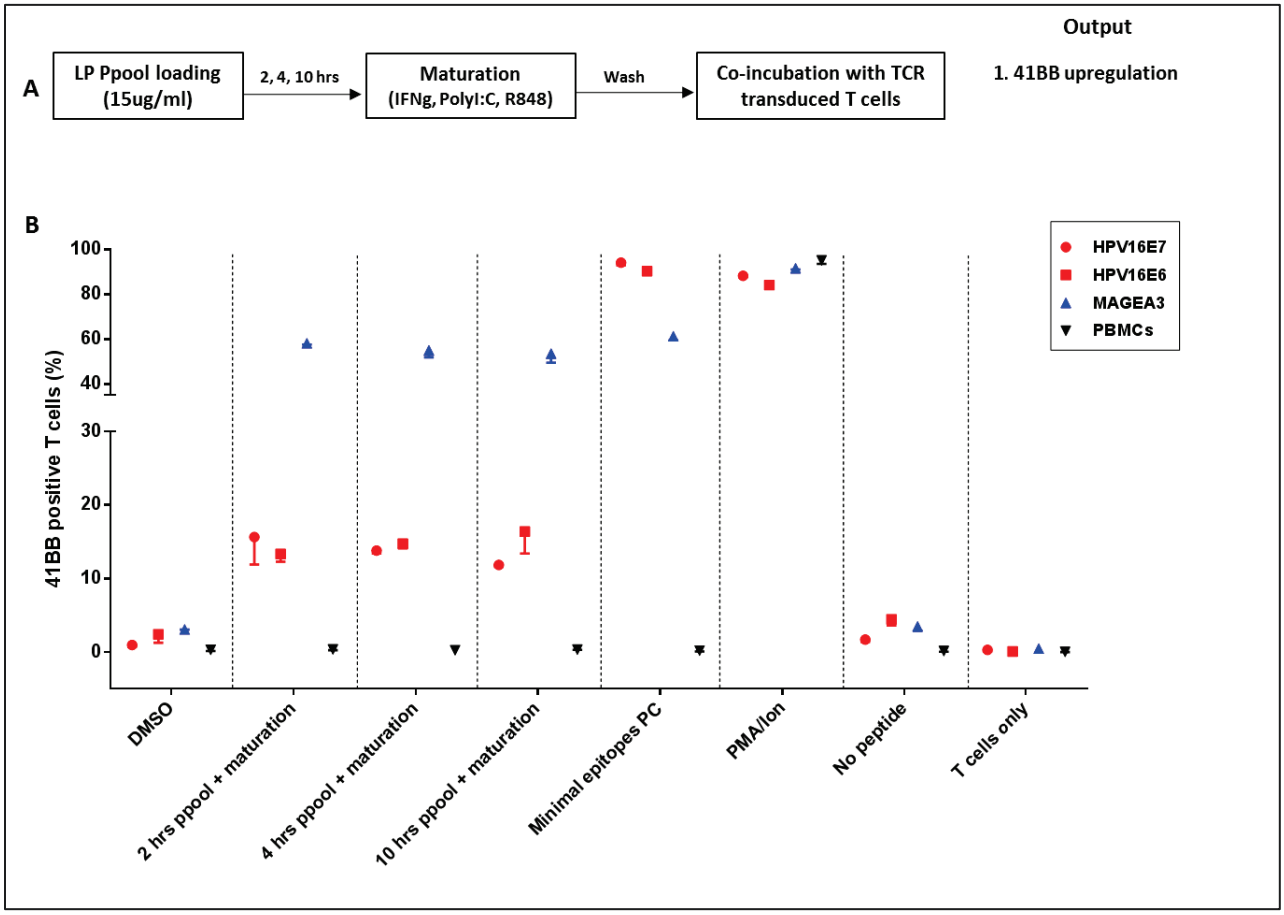
****See appendix I for backbone sequences.]**

13.5 FIGURE 5: LONG PEPTIDES AND MINIMAL EPITOPES ARE SUPERIOR TO TMG MRNA IN PRESENTING ANTIGENS TO CD4 AND CD8 CELLS



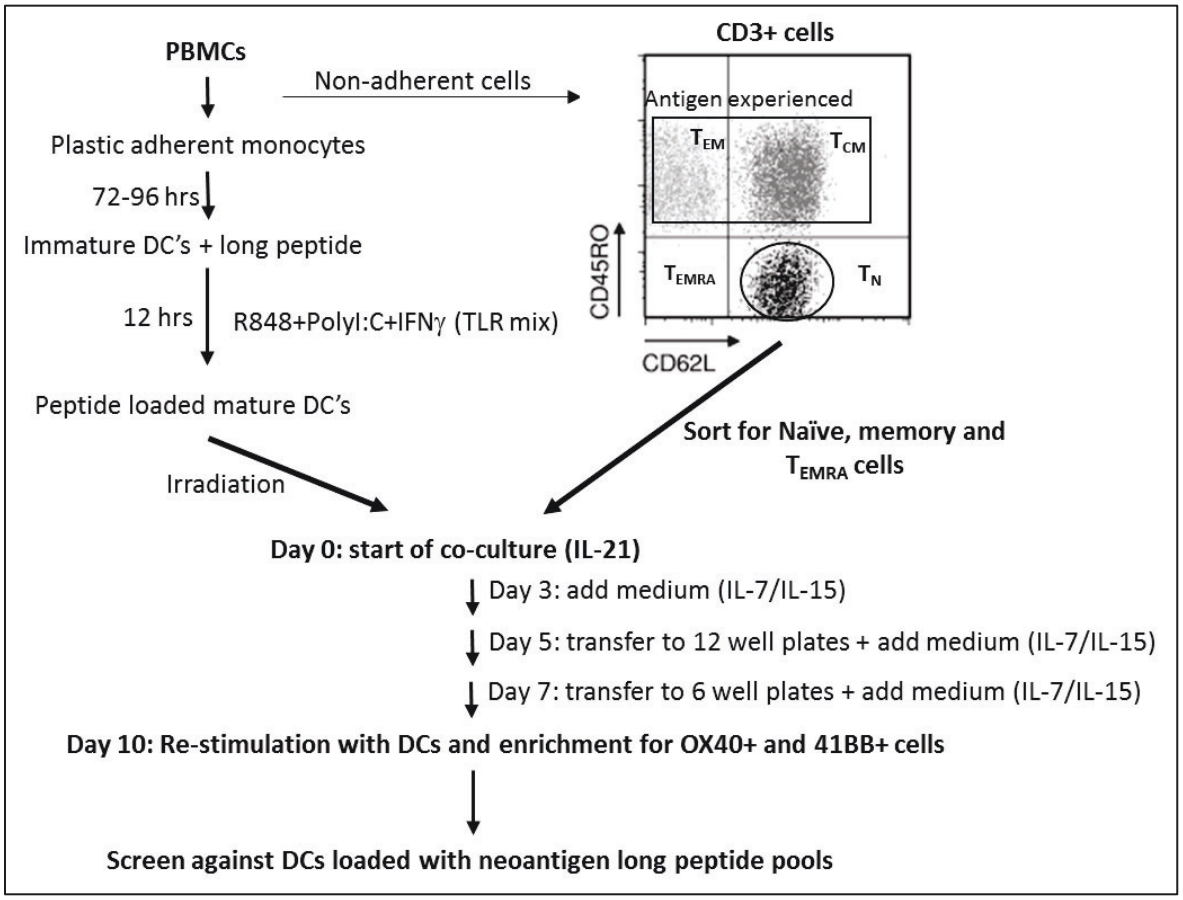
DCs were transfected with the TMG constructs or loaded with long peptide and minimal epitopes for 12 hours. DCs were co-cultured with PBMC transduced with TCR's recognizing the corresponding antigens for 18 hours, and antigen recognition was evaluated for Class-I epitopes (A) and Class-II epitopes (B) by flow cytometry for 41BB expression. Irrelevant TMG and DMSO were used as negative control for TMG transfection and peptide loading, respectively.

13.6 FIGURE 6: PEPTIDE LOADED, TLR STIMULATED DC'S EFFICIENTLY PRESENT BOTH CD4 AND CD8 EPITOPES

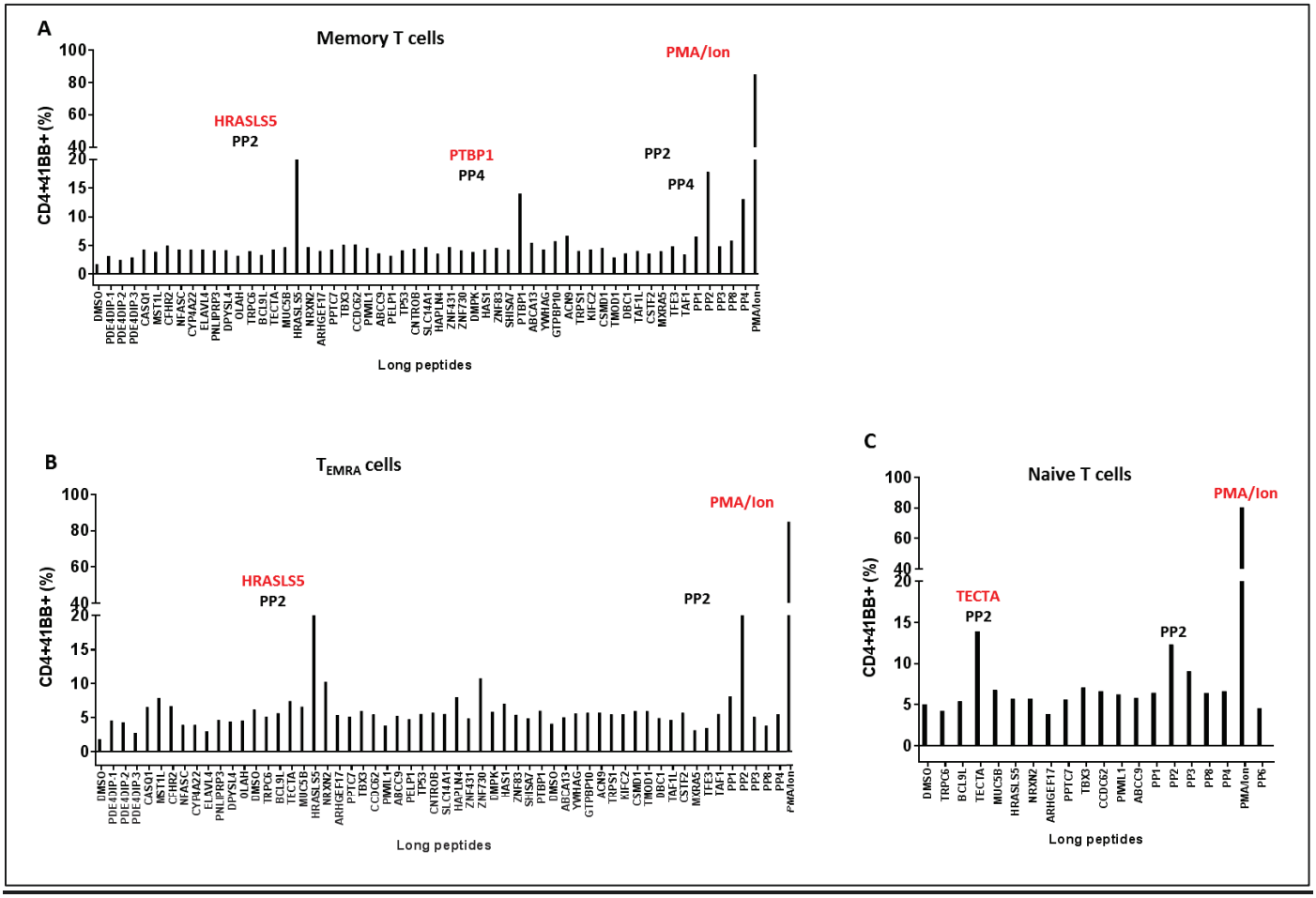


DCs were loaded with long peptides for 2, 4, and 10 hours and matured using a TLR mix. DCs were washed and co-cultured with PBMC transduced with TCRs recognizing the corresponding antigens for 18 hours (A); antigen recognition was evaluated by flow cytometry for 41BB expression (B).

13.7 FIGURE 7: OVERVIEW OF THE METHOD USED TO EVALUATE THE ACTIVATION OF NEOANTIGEN SPECIFIC T CELLS

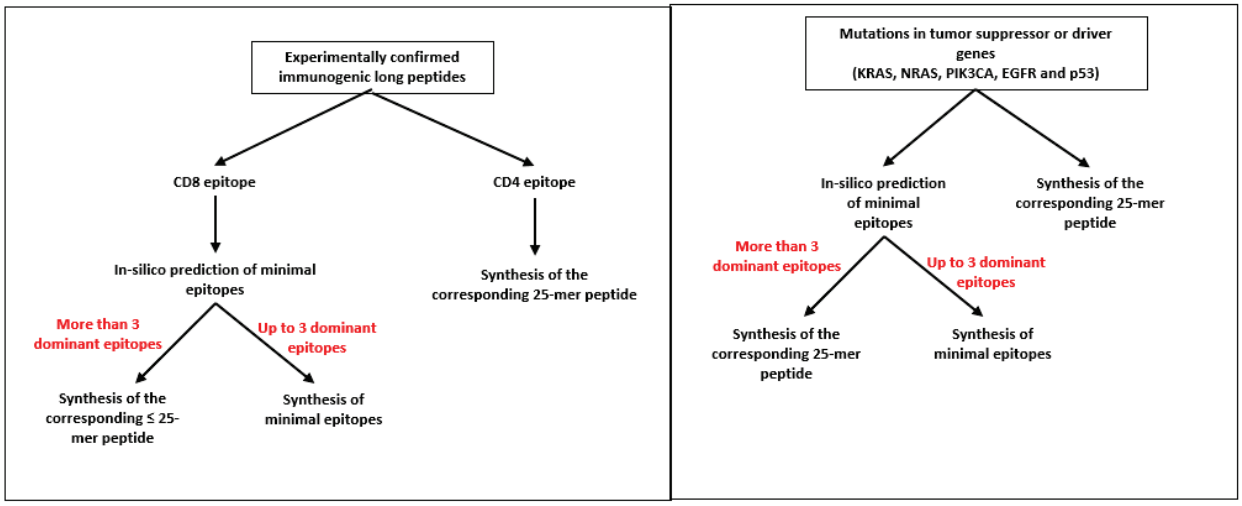


13.8 FIGURE 8: DCS MATURED WITH TLR MIX ARE CAPABLE OF STIMULATING NAÏVE, ANTIGEN-EXPERIENCED AND TERMINALLY DIFFERENTIATED EFFECTOR CELLS



Apheresis samples from a colorectal cancer patient were thawed and then incubated in an appropriately sized tissue culture flask. Adherent monocytes were differentiated into DCs, loaded with neoantigen peptide pools and matured using a TLR mix. Non-adherent cells were collected, and memory, naïve and terminally differentiated effector memory cells (TEMRA) were sorted, and in vitro stimulated with mature peptide-pulsed autologous DCs. Ten days after the first stimulation T cells were re-stimulated with DCs loaded with all peptide pools, and sorted based on 41BB and OX40 expression to enrich for neoantigen-specific cells. Sorted cells were stimulated by DCs loaded with all peptide pools and tested for upregulation of 41BB (data not shown). Memory (A), TEMRA (B), and naïve(C) T cells were then tested for neoantigen specific recognition by 41BB upregulation following co-culture with DCs loaded with single peptides derived from the most reactive peptide pools.

13.9 FIGURE 9: PROCESS FOR EPITOPE SELECTION AND SYNTHESIS



13.10 Table 1: Defined Mutated Antigens Recognized by TIL Identified at the Surgery Branch

Cancer type	No. of mutations recognized by TIL
Colorectal	38
Pancreas	4
Bile Duct	11
Ovary	7
Endometrium	2
Lung	26
Bladder	3
Esophagus	12
Breast	14
Melanoma	76

14 APPENDICES

14.1 APPENDIX 1: CELL INFUSION INSTRUCTIONS

Necessary equipment:

- Primary IV tubing (2) **(DO NOT USE AN INLINE FILTER FOR CELLS)**
- Secondary IV tubing (1)
- NS (sodium chloride 0.9%)
- IV infusion pump
- Gloves

Steps:	**Key Points:**
1. Patient RN will be informed of the approximate time of cell arrival at the bedside.	
2. Verify the physician orders: ▪ to administer the cells ▪ for the date of administration	
3. Verify that the consent form for the protocol is signed.	
4. Ensure that emergency and monitoring equipment are available in the patient's room: ▪ oxygen ▪ suction ▪ vital sign monitor with pulse oximeter and thermometer	
5. Provide patient education covering infusion procedure, potential complications and associated symptoms to report.	
6. Measure and record baseline vital signs, respiratory and circulatory assessments.	
7. Verify the patency of the patient's IV access.	Optimally, a central line or peripheral IV of 20G or larger should be used.
8. Hang a primary line of NS at a kvo rate - **NEW** bag and **NEW** tubing. This **MUST** be ready and infusing **prior** to the cells being delivered to the unit. Have a second bag of NS and tubing connected to the patient as an emergency line.	The primary line will be the dedicated NS line for infusing the cells. Under no circumstances are any other substances to be infused into the line. **Do not use an inline filter for cells.** Cell death occurs quickly – the infusion must be initiated promptly.

	Do not infuse medication during the cell infusion. If emergency meds must be administered, use the emergency NS IV line. The second bag of NS will be the emergency IV solution and can be used for medication administration.
9. The patient RN will be notified approximately 10 minutes before the cells arrive on the unit. The cells will be hand delivered to the bedside.	It is important to be at the bedside awaiting the arrival of the cells for infusion; have baseline VS, assessment, and IV lines hooked up when the cells arrive. Cell death occurs quickly after the cells are removed from the laboratory. Initiate the infusion as quickly as possible.
10. Prior to spiking the cell bag, two RNs will perform the identification procedure. **Both** RNs must sign the tag on the cell bag.	
11. Infuse the cells by infusion pump over 20-30 minutes or as clinically indicated. a. Piggyback the cells into the dedicated NS line; use the backflush technique to prime the line. b. While the cells are infusing, **gently** agitate the bag of cells **every few minutes**. When the cell bag is empty, backflush NS to rinse the bag and infuse this at the same rate as the cells; rinse bag until NS runs clear.	Gently agitating the bag prevents the cells from clumping in the bag.
12. Measure and record VS before and after the cell infusion, then hourly (± 15 minutes) from the end of the infusion for two hours.	
13. Documentation: a. After the cells have infused, remove the adhesive backed "cell therapy product" tag from the cell bag and place it on a progress note. Sign and date the progress note then send it to medical records. b. Document the cell infusion in CRIS using the Surgery Branch Cell Product Administration Flowsheet.	

c. Two separate Surgery Branch Cell Product Administration Flowsheets should be completed: one for the IV and a second one for the SQ cell administration.	

14.2 APPENDIX 2: CERTIFICATE OF ANALYSIS FOR IMMATURE DENDRITIC CELLS

Personalized Dendritic Vaccine Patient:
Date of preparation of final product:

Tests performed on final product:

Test	*Method*	*Limits*	*Result*	*Tests Performed by*	*Initials/Date*
Cell viability[1]	trypan blue exclusion	>50%			
Total viable cell number[1]	visual microscopic count	$>10^7$			
Identity2	FACS	> 60 % CD11C+CD86+			
Microbiological studies	aerobic culture[1]	no growth			
	anaerobic culture[1]	no growth			
	Fungal Culture[1]	no growth			
	gram stain[1]	no micro-organisms seen			
	mycoplasma test[1]	negative			

[1] Performed on the final product immediately prior to cryopreservation.

Prepared by: _____ Date: _____

QC sign-off: _____ Date: _____
Qualified laboratory or Clinical Supervisor

QA sign-off: _____ Date: _____

14.3 APPENDIX 3: CERTIFICATE OF ANALYSIS FOR MATURE PEPTIDE PULSED DENDRITIC CELLS

Personalized Dendritic Vaccine Patient:
Date of preparation of final product:

Tests performed on final product:

Test	*Method*	*Limits*	*Result*	*Tests Performed by*	*Initials/Date*
Cell viability[1]	trypan blue exclusion	>50%			
Total viable cell number[1]	visual microscopic count	between 10^7 and 8 X 10^7			
Identity[1]	FACS	> 60 % CD11C+CD86+			
Microbiological studies	aerobic culture[2]	no growth			
	anaerobic culture[2]	no growth			
	gram stain[1]	no micro-organisms seen			
	fungal culture	no growth			
	mycoplasma test[2]	negative			
Endotoxin[1]	limulus assay	≤5 E.U./kg			

[1] Performed on the final product prior to infusion. Results are available at the time of infusion.
[2] Sample for test collected on the final product prior to infusion. Results will not be available before cells are infused into the patient.

Prepared by: _____ Date: _____

QC sign-off: _____ Date: _____
Qualified laboratory or Clinical Supervisor

QA sign-off: _____ Date: _____