UNIVERSITY OF VIRGINIA
HUMAN IMMUNE THERAPY CENTER

MEL 64

A TRIAL TO EVALUATE THE SAFETY, IMMUNOGENICITY, AND CLINICAL ACTIVITY OF A HELPER PEPTIDE VACCINE PLUS PD-1 BLOCKADE

Abbreviated title: PATHVACS (PD-1 Antibody and T-Helper Vaccine And Correlative Studies)

Product: Pembrolizumab (MK-3475) 2
Protocol: UVA-Mel-64
Version Date: 07/13/18

TITLE: A trial to evaluate the safety, immunogenicity, and clinical activity of a helper peptide vaccine plus PD-1 blockade

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Name	**Signature**	**Date**

INVESTIGATOR'S AGREEMENT

I confirm that I have read this protocol and I agree to conduct the study as outlined herein. I agree to conduct the study in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with Good Clinical Practices, as outlined in ICH E6, and the applicable laws and regulations.

IND NUMBER: 10825

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1.0 TRIAL SUMMARY

Abbreviated Title	6MHP Vaccine Plus Pembrolizumab
Trial Phase	Phase I/II
Clinical Indication	advanced melanoma (stage IIIB-IV at initial presentation or on recurrence),
Trial Type	Open-label
Type of control	N/A
Route of administration	IV (pembrolizumab); SQ and ID (6MHP)
Trial Blinding	N/A
Treatment Groups	Single Group: 6MHP + pembrolizumab
Number of trial subjects	A maximum of 25 to obtain 22 evaluable subjects
Estimated duration of trial	Approximately 47 months.
Duration of Participation	Approximately 71 months
Estimated average length of treatment per patient	Approximately 2 years

2.0 TRIAL DESIGN

2.1 Trial Design

This study is an open-label, phase I/II study of a vaccine comprised of a mixture of 6 synthetic melanoma helper peptides (6MHP) administered in Montanide ISA-51 (incomplete Freund's adjuvant) combined with pembrolizumab (Figure 1). The study will accrue up to 25 subjects to obtain evaluable data on 22 subjects.

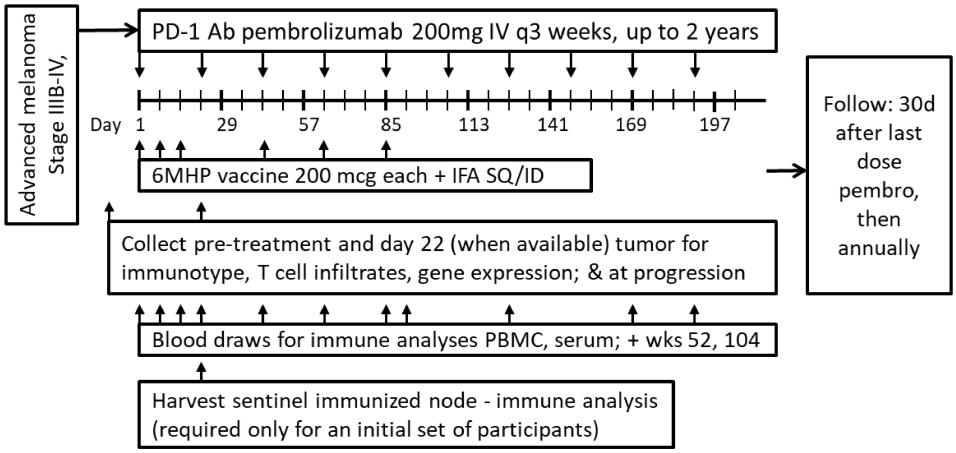
All subjects will receive the same treatment regimen consisting of 6MHP administered intradermally (ID) and subcutaneously (SQ) on days 1, 8, 15, 43, 64, and 85. Pembrolizumab will be administered intravenously (IV) every 3 weeks, beginning on day 1. Duration of pembrolizumab will be up to 2 years.

Biopsy specimens will be collected from the tumor (days 1 and 22) and a sentinel immunized node (SIN; day 22). The SIN biopsy will no longer be required after protocol modification approval to remove the SIN biopsy component. Biopsy and peripheral blood specimens will be used in the immunologic analyses.

Follow-up after last dose of pembrolizumab will occur 30 days post last infusion. Subjects will be followed annually for disease status and survival. Tumor assessments will be completed at screening and again at days 57, 127, and as indicated per standard care. The expectation is that there will be additional follow-up as part of standard of care, either by the study physician or by a local referring physician. Every effort will be made to obtain data on disease status and survival at all such visits, in addition to the mandated study visits.

2.2 Trial Diagram

Figure 1. Clinical Trial Schema



3.0 OBJECTIVES

3.1 Primary Objectives

(1) Safety: To determine whether the administration of pembrolizumab plus a vaccine consisting of 6 melanoma-derived class II MHC-restricted helper peptides (6MHP) is safe.

(2) Immunogenicity: To estimate the immunogenicity of 6MHP in the blood when co-administered with pembrolizumab, with or without removal of a sentinel immunized node.

3.2 Secondary Objectives

To determine whether combined treatment with 6MHP vaccine plus pembrolizumab:

(1) Modifies the tumor microenvironment, including estimation of whether combined treatment:

(a) Increases infiltration of $CD4^+$ and $CD8^+$ T cells into tumor metastases, especially CD4+ T cells reactive to the vaccine peptides.

(b) Increases Th1-dominant immune signatures in the tumor microenvironment.

(2) Induces $CD8^+$ responses to defined melanoma defined antigens (epitope-spreading) and IgG antibody responses to 6MHP in the blood and SIN, when SIN biopsy specimen available.

3.3 Exploratory Objectives

(1) To obtain preliminary data on whether 6MHP vaccine plus pembrolizumab induces CD4+ or CD8+ T cell responses to mutated neoantigens

(2) To obtain preliminary data on whether 6MHP vaccine plus pembrolizumab induces clinical responses (as measured by RECIST 1.1) in patients with or without prior PD-1/PD-L1 blockade therapy.

4.0 BACKGROUND & RATIONALE

4.1 Background

Cancer immunotherapy for solid tumors is coming of age, with FDA-approved immunotherapeutics in prostate cancer, melanoma, and renal cell cancer. Interleukin-2 (IL-2) and the anti-CTLA-4 antibody ipilimumab are approved for melanoma; both induce durable clinical regressions. Recent data also show that blockade of PD-1/PD-L1 induces durable clinical regressions of melanoma, renal cell cancer and non-small cell lung cancer (NSCLC) (1), and the PD-1 antibody pembrolizumab has been approved for treatment of advanced melanoma in patients who have progressed after ipilimumab therapy. Furthermore, antigen-specific adoptive T cell therapy induces clinical regressions that are durable in about 20% of treated patients (2). There is excitement about this growing armamentarium of systemic immunotherapeutics, whose effects are mediated predominantly by T lymphocytes. However, despite the effectiveness of available immunotherapeutic approaches, current therapies still fail in about 70-80% of patients. Thus, there is a need for new combination approaches that build on the demonstrated clinical value of immune therapy.

Cancer vaccines inducing antigen-specific T cell responses are emerging as a component of combination immunotherapy. In the past few years, a cancer vaccine has been approved for prostate cancer, based on improved survival outcomes from two randomized trials (3), and a randomized prospective trial in melanoma patients showed that adding a peptide vaccine to high-dose IL-2 significantly prolonged progression-free survival (PFS) when compared to IL-2 alone (4). Thus, after several decades of development and optimization, there is now evidence that some cancer vaccines may improve clinical outcomes, in particular in combination with other active therapy.

We have developed a vaccine incorporating 6 intermediate-length peptides that induces $CD4^+$ helper T cell (T_H) responses (6 helper peptides, 6MHP), and has clinical activity in patients with advanced melanoma (5). The 6MHP vaccine has also been shown to induce Th1 dominant T cell responses (6) and cross-priming of $CD8^+$ T cells (7). The current protocol is to obtain preliminary data on whether the combination of the 6MHP vaccine with immune checkpoint blockade of PD-1 is safe and improves immunologic and clinical outcomes compared to prior experience with each single agent alone. This trial will incorporate correlative studies of immune responses in blood, lymph nodes, and tumor to obtain a more complete understanding of the host: tumor relationship in the context of these combination therapies. These studies hold promise:

- To modulate T cell infiltration into metastases
- To increase helper T cell responses to vaccine, and
- To increase clinical response rates and to extend the life of patients with melanoma.

4.2 Study Rationale

<u>Evidence for the Role of the Immune System in Protecting Against the Development of Solid Tumors</u>

There has long been evidence of immune responses to cancer, but evidence of impact on tumor progression has not been well demonstrated until recently. The most convincing experimental evidence of the importance of immune surveillance in preventing the development of solid tumors is provided by work in murine models, in which 50-100% of knockout mice lacking STAT1 and/or IFNγ receptor function developed spontaneous solid tumors of various histologies within 12-15 months, whereas normal mice never developed malignancies during the same time period (8). These studies strongly support the role of cellular immune function in the control of cancer progression. These findings are supported in humans by the fact that blockade of immune checkpoints (CTLA-4 and/or PD-1) induces durable clinical responses in patients with melanoma and other cancers (9-11).

The Role of $CD4^+$ Helper T Lymphocytes in Anti-tumor Immune Responses.

Initially, the majority of cancer vaccines were designed to activate the $CD8^+$ cytotoxic T cell arm of the host immune system. However, more recent approaches target the activation of $CD4^+$ T_h cells. This is based in part on results from earlier studies which demonstrated that depletion of $CD4^+$ T-cells abrogates all or part of protective immune response to vaccines (12). Furthermore, adoptive therapy with $CD4^+$ T-cells has been shown to induce tumor protection in some model systems (13,14).

There are several mechanisms by which T_h cells mediate anti-tumor responses. T_h cells can activate dendritic cells (DC) for heightened antigen presentation, causing the DC to secrete IL-2 and other cytokines and co-stimulatory molecules that support the induction of $CD8^+$ T cell immune responses (15-18). In addition, T_h responses support the establishment of CD8+ T cell memory responses (19-22). Furthermore, strong T_h1 help produces a cytokine milieu which is critical to the induction of immune-mediated tumor destruction (23,24).

4.2.1 Pharmaceutical and Therapeutic Background

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades (25). Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies (26-30). In particular, the presence of $CD8^+$ T-cells and the ratio of $CD8^+$ effector T-cells / $FoxP3^+$ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2) (31-34). The structure of murine PD-1 has been resolved (35). PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade (31,36). The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4, as both molecules regulate an overlapping set of signaling proteins (37,38). PD-1 was shown to be expressed on activated lymphocytes including

peripheral $CD4^+$ and $CD8^+$ T-cells, B-cells, T regs and Natural Killer cells (39,40). Expression has also been shown during thymic development on $CD4^-CD8^-$ (double negative) T-cells as well as subsets of macrophages and dendritic cells (41). The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors (37,42-44). Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues (37). Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL) (45). This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Pembrolizumab (previously known as SCH 900475 and MK-3475) is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2.

4.2.2 Preclinical and Clinical Trial Data

Refer to the Investigator's Brochure for Preclinical and Clinical data.

4.3 Rationale

4.3.1 Rationale for the Trial and Selected Subject Population

Melanoma is the fifth most common cancer in the United States, and over 70,000 new cases will be diagnosed this year (46). Worldwide, approximately 50,000 people die each year from melanoma. When diagnosed and treated for localized disease, a patient's prognosis is often favorable; however, approximately 12% of patients with localized disease eventually recur with metastatic disease. Factors associated with an increased risk of recurrence include the presence of ulceration, microsatellitosis, intransit disease and lymph node involvement. Prior to the current era of checkpoint blockade therapy, the median survival time for patients with stage IV metastatic melanoma was only about 6-10 months (47).

$CD4^+$ T-helper cells have been shown to play an important role in anti-tumor immunity. Anti-tumor responses can occur via direct interactions between $CD4^+$ T cells and tumor cells or alternatively, through indirect mechanisms including the licensing and activation of antigen presenting cells (15,16,48). The 6MHP vaccine incorporates known MHC class II-restricted peptides derived from melanoma-associated tumor antigens and administration of the vaccine in an emulsion with Montanide ISA-51 induces T_h1-dominant $CD4^+$ T cell responses to the peptides (5,7). T cell responses against the vaccine have been induced in 40-80% of patients, and RECIST-defined clinical response rates and disease control rates have been observed in 8% and 30%, respectively. In prior melanoma studies, Mel41 and E1602, durations of SD and clinical responses have been durable, ranging from 1 to 7 years (5,49) (Table 1). Also, there has been a strong and significant association between immune response to the 6MHP and survival, in patients with advanced melanoma in the ECOG trial E1602 (univariate, p = 0.005, multivariate p =0.038, HR 0.50) (49)(Reed 2015 [3])(Figure 2).

Table 1. Clinical Response Rates to 6MHP Vaccines in Subjects with Advanced Melanoma in Mel41 and E1602 Trials (RECIST)

Study	*N*	*CR+PR*	*CR+PR+SD*	*RR*	*DCR*
Mel41	17	2	4	12%	24%
E1602 Arm D (6MHP)	42	3	15	7.1%	36%
E1602 Arm C	32	2	8	6.3%	25%
All studies	**91**	**7**	**27**	**7.7%**	**30%**

The programmed cell death receptor-1 (PD-1) is found on activated T cells, and binding of this receptor with its ligands results in the down-regulation of T cell responses (50). Pembrolizumab blocks the interaction between PD-1 and its ligands, preventing down-regulation of the T cell response. Clinical responses with pembrolizumab in the melanoma setting have been observed in approximately 26% of patients who have progressed after ipilimumab therapy (51). Combining pembrolizumab with the 6MHP vaccine may lead to enhanced targeted T cell responses against defined tumor antigens and result in the induction of improved clinical response rates in patients with metastatic disease. The goals of this phase I study are to evaluate the safety of the combination of 6MHP and pembrolizumab and also to evaluate the T cell response against the 6MHP vaccine in the context of pembrolizumab administration. Clinical responses will also be measured.

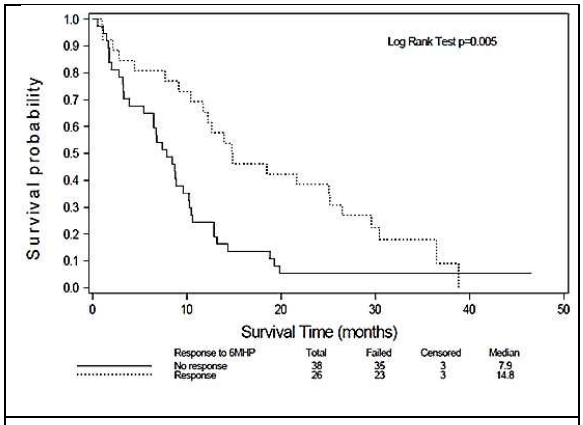


Figure 2: Overall Survival on E1602
Overall survival by helper T cell response to 6MHP in analyzable patients on arms C and D of E1602 trial (n = 64).

To improve the clinical benefit of this and other vaccines, it is critical to support T cell infiltration and function in the tumor microenvironment. We have reported that melanoma metastases can be categorized into 3 Immunotypes based on immune cell infiltrates, where Immunotype A melanomas (29%) lack infiltrates, Immunotype B (63%) have immune cell infiltrates cuffing intratumoral vessels but not infiltrating tumor nests, and only Immunotype C melanomas (8%) have diffuse immune infiltrates (52). Blockade of PD-1/PD-L1 can increase T cell infiltration and combination with a vaccine should improve tumor targeting. Also, recent data have revealed

increased PD1 expression on exhausted $CD4^+$ T cells (53); thus, blocking PD1/PDL1 is expected to support $CD4^+$ T cell function.

The current proposal is for a clinical trial combining this active 6MHP vaccine with pembrolizumab. Primary endpoints include safety and immune response. Secondary endpoints will include assessment of changes in the tumor microenvironment. Exploratory objectives will include assessment of objective response rate, overall clinical outcome and association between immune response and survival. There is great interest in obtaining preliminary data on whether the combination of 6MHP vaccine and pembrolizumab may increase objective clinical response rates, PFS, or overall survival. However, patterns of care for patients are changing rapidly as new immunotherapy agents are becoming available. On one hand, the overall response rate for patients pretreated with ipilimumab is about 23%, but for patients treated with pembrolizumab as first-line therapy is 33%. Thus, powering the study for response rate would depend on limiting enrollment to only one of these approaches. In addition, combination immunotherapy with PD-1 blockade plus CTLA-4 blockade is being employed, with response rates of about 50%, but with much higher toxicity than with pembrolizumab alone. Patients may be selected for one of these 3 regimens (pembrolizumab first, CTLA-4 blockade first, or combination therapy) for reasons that may affect the patient populations, including patient frailty, age, or biomarker assessment of metastases. Thus, the present study is designed to obtain pilot data on clinical outcome but to power the study for safety, detection of effects on the immune responses (in all patients), and for detection of changes in the tumor microenvironment (in a subset of patients).

4.3.2 Rationale for Dose Selection/Regimen/Modification

Pembrolizumab:

The rationale for further exploration of 2 mg/kg and comparable doses of pembrolizumab in solid tumors is based on: 1) similar efficacy and safety of pembrolizumab when dosed at either 2 mg/kg or 10 mg/kg Q3W in melanoma patients, 2) the flat exposure-response relationships of pembrolizumab for both efficacy and safety in the dose ranges of 2 mg/kg Q3W to 10 mg/kg Q3W, 3) the lack of effect of tumor burden or indication on distribution behavior of pembrolizumab (as assessed by the population PK model) and 4) the assumption that the dynamics of pembrolizumab target engagement will not vary meaningfully with tumor type.

The choice of the 200 mg Q3W as an appropriate dose for the switch to fixed dosing is based on simulations performed using the population PK model of pembrolizumab showing that the fixed dose of 200 mg every 3 weeks will provide exposures that 1) are optimally consistent with those obtained with the 2 mg/kg dose every 3 weeks, 2) will maintain individual patient exposures in the exposure range established in melanoma as associated with maximal efficacy response and 3) will maintain individual patients exposure in the exposure range established in melanoma that are well tolerated and safe.

A fixed dose regimen will simplify the dosing regimen to be more convenient for physicians and to reduce potential for dosing errors. A fixed dosing scheme will also reduce complexity in the logistical chain at treatment facilities and reduce wastage.

Standard of care in the metastatic/unresectable setting is administration of pembrolizumab for up to two years. Regulatory approval does not limit the time on therapy. In this study, we will continue pembrolizumab for 2 years of therapy in the setting of stable disease or response without limiting toxicity.

6MHP vaccine:

The 6MHP vaccine was tested at 3 dose levels (200, 400, 800 mcg per vaccine) and no differences were observed in immunogenicity, Th1 profiles or in toxicity (6). Thus the lowest dose of 200 mcg will be used in this protocol.

4.3.3 Rationale for Endpoints

The 6MHP vaccine has been administered to over 200 subjects and overall has been well-tolerated. A summary of toxicities related to 6MHP administration may be found in Table 2.

Table 2. Reported Toxicities for 6MHP Vaccines in Humans (n=207)

Toxicity (based on max grade)	Grade 1	Grade 2	Grade 3	Grade 4
LOCAL, INJECTION SITE				
Injection site reaction	18%	56%	2.4%	
Ulceration		5%	1.4%	
CONSTITUTIONAL				
Fatigue	43%	8%	3.4%	
Headache	27%	1.4%	0.5%	
Rigors, Chills	24%	1.4%	--	
Nausea	23%	2%	0.5%	
Sweating	19%	1%		
Myalgias	18%	0.5%		
Arthralgias	17%	0.5%		
Fever	16%	2%		
Dizziness	13%			
Anorexia	13%	3%		
Diarrhea	12%	2%		
Cough	13%			
Allergic rhinitis	11%			
Nasal/paranasal reactions	11%			
Pain larynx/throat	10%			
Flushing	10%			
Pruritis	9%	0.5%		
Rash	6%	3.4%		
Dyspnea	5%	1%	0.5%	
Vomiting	5%	1%	0.5%	
Flu-like syndrome	6%			
Mucositis	6%			
Constipation	5%			
Autoimmune reaction	4%	0.5%		
Wound, non-infectious	4%			
Pain, other	2%	1%	0.5%	
Abdominal pain	1.4%		0.5%	
Tinnitus		0.5%	0.5%	
Tumor pain	0.5%		0.5%	
Hearing (without monitoring program)			0.5%	
CLINICAL LABORATORY				
Hyperglycemia (not fasting)	22%	1%		
Hemoglobin, low	17%	1%	0.5%	
Hyperkalemia	13%			
Lymphopenia	9%	2.9%	1%	

Toxicity (based on max grade)	Grade 1	Grade 2	Grade 3	Grade 4
Leukocytes	8%	1%		
Hyponatremia	7%			
Increased creatinine	6%	0.5%		
Hypoglycemia	6%			
AST, SGOT	5%			0.5%
ALT, SGPT	4%		1%	
Neutrophils	3%	2%		
Metabolic, Other	3%	0.5%	0.5%	
Alk phos	4%	0.5%		
Bilirubin	3%		0.5%	

Pembrolizumab has been evaluated in multiple studies with doses ranging from 1 mg/kg to 10 mg/kg Q2W to Q3W. Safety data from the prior PN001 phase I clinical trial study (479 subjects) suggest that pembrolizumab is well-tolerated, with 4.2% of study subjects discontinuing study treatment due to a treatment-related AE (Investigator's Brochure).

The described trial is the first evaluation of pembrolizumab combined with a peptide-based vaccine (6MHP) and the first evaluation of any PD-1/PD-L1 antibody in combination with a helper-peptide vaccine. Thus, safety is included as a primary endpoint for this trial. Additional endpoints include measures of immunogenicity and clinical efficacy.

4.3.3.1 Efficacy Endpoints

From prior clinical trials with the 6MHP vaccine, we determined that we can induce T cell responses to the peptides following vaccination (5,49,54). In addition, vaccination with 6MHP led to clinical responses in a modest number of subjects (5,49). Administration of pembrolizumab with 6MHP may enhance $CD4^+$ T cell responses directed against tumor-derived peptides within the vaccine and lead to improved disease control by preventing down-regulation of the T cell response following vaccination. Accordingly, we have included endpoints to obtain preliminary data on whether combination of 6MHP with immune checkpoint blockade improves immunologic and clinical outcomes compared with historical data for each single agent alone.

Biomarker Research

In order to optimize combinatorial immunotherapy approaches for the treatment of cancer, we must obtain a more complete understanding of the host: tumor relationship in the context of these combination therapies. The following analyses are included as part of this study to evaluate the biology of host immune responses in the peripheral blood, within a lymph node immediately draining a vaccine site (sentinel immunized node (SIN)), and within sites of tumor. Blood and tumor tissue samples will be taken pre- and post- study drug administration to evaluate changes over time. A SIN biopsy will be taken one week following the third vaccine for a subset of patients.

Measuring T cell responses to Melanoma Antigens in the Vaccine: To estimate whether the immunogenicity of 6MHP, and induction of epitope-spreading, will be increased by co-administration with pembrolizumab.

We anticipate that co-administration of pembrolizumab with a helper peptide vaccine may increase the $CD4^+$ T cell response to the vaccines. We have also observed induction of $CD8^+$ T cells following vaccination with 6MHP, presumably due to epitope spreading (7). Thus, we hypothesize that CD4 and CD8 T cell responses will increase in frequency, magnitude, and duration with pembrolizumab.

Immune responses in PBMC and SIN will be measured by ELIspot assay, proliferation assay, and/or flow cytometry. Increases in immune response rate, magnitude, and duration will all be of interest. Of greatest interest will be the induction of durable helper T cell responses, defined as responses in PBMC at 2 or more of the blood draws after day 85. Also, we will test for T cell responses to multiple antigens, using overlapping 15-mers from NY-ESO-1, MelanA, MAGE-A1, MAGE-A3, tyrosinase, and gp100, as well as for $CD8^+$ T cell responses to a panel of at least 14 defined epitopes from these proteins. This will also include analysis of immune responses in the tumor microenvironment, as detailed below, with the expectation of tumor biopsies in at least 14 of the patients enrolled on this trial.

Since we have observed induction of epitope spreading to peptide antigens not in the vaccine, we hypothesize that vaccination with helper peptides will also induce epitope spreading to mutated neoantigens, and that the activity of T cells reactive to them in the tumor may be supported by co-administration with pembrolizumab. Thus, we propose exploratory studies to identify putative neoantigens in these patients and to test whether patients with immune responses to the vaccine also have epitope spreading to recognition of those mutated neoantigens, and whether T cells reactive to those antigens may be identified among tumor infiltrating lymphocytes.

Impact of sentinel immunized node biopsy, and presence of tumor, on circulating immune response. The induction of immune responses to vaccines are presumed to be mediated by interactions of T cells and antigen presenting cells in the vaccine-draining node (sentinel immunized node, SIN). Measurement of circulating T cell responses thus may be affected by removal of the SIN at week 3 in the first set of patients enrolled on this trial. They may also be affected by homing to metastatic tumor. For those tumors that permit infiltration by vaccine-reactive T cells, it would be ideal if T cells induced by vaccination primarily home to tumor and are relatively depleted from circulation. Thus, we are interested in obtaining preliminary data on whether circulating immune responses differ, after the SIN is harvested, for the first patients enrolled, who undergo SIN biopsy, compared to those who do not undergo SIN biopsy. To address this, the protocol is revised for the latter group of patients not to have SIN biopsy.

Tumor microenvironment. To determine whether Th1 $CD4^+$ T cells induced by vaccination infiltrate the tumor microenvironment and whether they are associated with a favorable immune signature.

Induction of T cells reactive to melanoma antigens in the circulation is necessary but not sufficient for tumor control. We will test whether $CD4^+$ T cells induced by the 6MHP vaccine infiltrate melanoma metastases, for the subset of patients (at least 12 patients) who have tumor accessible for biopsy (typically skin metastases, superficial nodes, and soft tissue masses). Tumor tissue can be limiting; so isolation of viable cells in adequate number from metastatic tumor biopsies can be challenging. An alternate approach is to identify T cell receptors of the T cells induced by the vaccines. We hypothesize that there will be increased infiltration of T cells induced by 6MHP vaccine when co-administered with pembrolizumab, compared to pre-treatment biopsies. We also hypothesize that expansion of Th1 $CD4^+$ cells will support expansion of tumor-reactive $CD8^+$ T cells in the tumor microenvironment, which will be evaluated simultaneously.

We will determine if there is evidence of a T cell response in PBMC. Immune responses are anticipated in more than 50% of patients. The PBMC samples with responses and corresponding pretreatment PBMC samples will be prepared as isolated $CD4^+$ T cell populations by negative selection using Miltenyi beads. Those cells will be submitted for T-cell receptor (TCR) sequence profiling by ImmunoSeq (Adaptive Biotechnologies, Seattle, WA), which will identify TCRs enriched in the PBMC and SIN as T cells responding to the vaccines.

We will also send frozen (or FFPE) tumor tissue for the same analysis, as a measure of whether T cells induced by vaccination are then found infiltrating the tumors. In cases where tumor-infiltrating lymphocytes can be isolated in adequate quantity to perform functional analyses, this will be done as well, as validation of the TCR sequencing studies, but we expect that to be an uncommon event. The TCR molecular analysis will be feasible in a larger proportion of patients. Associations with clinical outcome will be noted as pilot data.

To understand whether the combination therapy induces Th1-biased immune signatures in the tumor microenvironment, we will perform gene expression analysis on pretreatment tumors day 1 and treated tumors day 22. Tumor specimens placed in RNA-Later at the patient bedside, typically within 5 min of excision and stored at -80^{o}C, provide source material. RNA will be isolated by our laboratory staff from those specimens. RNA amplification, array application using HUMAN GENE 2.0 ST chips from Affymetrix Inc, RNA-seq, or comparable analysis, and data analyses will be performed using Partek Genomic Suite & Ingenuity Pathway Analysis (IPA) software to provide principal component analysis, identification of genes differentially expressed on day 22 (vs. day 1) to mark effects of the combination therapy. Changes will be evaluated in canonical pathways, gene network, and transcription factor expression. Gene expression of pretreatment samples will be correlated with clinical responses.

The histology evaluations will be compared to the TCR expression data, to assess whether infiltration with vaccine-reactive CD4^{+} T cells is associated with increased infiltration of CD4+ cells or lymphocytes overall, or with Immunotype C infiltration patterns (55) in particular. Immunohistochemical stains to clarify these observations may be performed on tumors.

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Diagnosis/Condition for Entry into the Trial

Subjects with unresectable stage IIIB or IIIC melanoma, or stage IV metastatic melanoma that have clinical or radiological evidence of disease. Stage definition may occur at original diagnosis or after recurrence. These subjects may have had cutaneous, uveal, mucosal primary melanoma, or an unknown primary melanoma. Staging must be confirmed by cytological or histological examination. Staging of cutaneous melanoma will be based on the AJCC v7 staging system (Appendix 12.4) (56). Subjects must be eligible to be treated with pembrolizumab based on clinician judgement.

5.1.2 Subject Inclusion Criteria

In order to be eligible for participation in this trial:

1. A subject must be willing and able to provide written informed consent/assent for the trial.
2. A subject must be ≥ 18 years of age on day of signing informed consent.
3. A subject may be naïve for immunotherapy agents or have received interferon-alpha, ipilimumab or other CTLA-4 antibody, PD-1 antibody (or anti-PD-L1 antibody), interleukin-2, or prior cancer vaccines other than the 6MHP vaccine. Patients who have received a PD-1/PD-L1 antibody may be enrolled in either of the following settings:
    a) A patient who has experienced progression of melanoma during that therapy or after having completed that therapy,

b) A patient who fails to experience an objective clinical response (partial response or complete response by RECIST 1.1 criteria) after either 12 weeks of continuous anti-PD1 antibody or anti-CTLA4/anti-PD1 combination therapy, and is a candidate to receive pembrolizumab therapy

4. A subject must have measurable disease based on RECIST 1.1.

   Subjects will be required to have radiological studies to define radiologically evident disease. Required studies include:

   - Chest CT scan,
   - Abdominal and pelvic CT scan, and
   - Head CT scan or MRI

   PET/CT fusion scan may replace scans of the chest, abdomen, and pelvis.

5. Subjects who have metastatic melanoma available for biopsy pretreatment and on day 22 must consent to having those biopsies. These melanoma lesions may be in nodes, skin, soft tissue, liver, or other sites that can be accessed safely by needle biopsy, incisional or excisional biopsy, with or without image guidance. The lesions to be biopsied must be specified at study enrollment and not included as target lesions for RECIST calculations. In instances where disease that is accessible to biopsy is limited, archival tissue specimens collected after a subject's last systemic therapy may be used for baseline measures.

   Subjects must have measurable disease in addition to the lesion(s) to be biopsied.

6. Subjects who have had brain metastases will be eligible if all of the following are true:

   - Each brain metastasis must have been completely removed by surgery or each unresected brain metastasis must have been treated with stereotactic radiosurgery.
   - There has been no evident growth of any brain metastasis since the most recent treatment
   - No brain metastasis is > 2 cm in diameter at the time of registration.
   - Neurologic symptoms have returned to baseline,
   - There is no evidence of new or enlarging brain metastases,
   - Subjects are not using steroids for at least 7 days prior to registration.

7. The most recent surgical resections or gamma-knife therapy for malignant melanoma must have been completed ≥ 1 week prior to registration.

8. A subject must have a performance status of 0 or 1 on the ECOG Performance Scale.

9. A subject must demonstrate adequate organ function as defined in Table 3, all screening labs should be performed within 28 days of treatment initiation.

Table 3. Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X

System	Laboratory Value
(GFR can also be used in place of creatinine or creatinine clearance)	institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Creatinine clearance should be calculated per institutional standard.	

10. Female subjects of childbearing potential should have a negative urine or serum pregnancy test within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
11. Female subjects of childbearing potential (Section 5.7.2) must be willing to use an adequate method of contraception as outlined in Section 5.7.2 – Contraception, for the course of the study through 120 days after the last dose of study medication.

    Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

12. Male subjects of childbearing potential (Section 5.7.2) must agree to use an adequate method of contraception as outlined in Section 5.7.2 – Contraception, starting with the first dose of study therapy through 120 days after the last dose of study therapy.

    Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

13. Blood is to be collected for HLA typing (Class I and Class II), which will be analyzed as part of the immunologic endpoints, but HLA type will not be an inclusion/exclusion criterion.
14. A subject must have at least two intact (undissected) axillary and/or inguinal lymph node basins.

5.1.3 Subject Exclusion Criteria

The subject must be excluded from participating in the trial if the subject:

1. Is currently participating in or has participated in a study of an investigational agent or using an investigational device within 4 weeks of the first dose of treatment.
2. Is currently receiving Interferon (e.g. Intron-A®), growth factors (e.g. Procrit®, Aranesp®, Neulasta®), or interleukins (e.g. Proleukin®), or has received these agents within 4 weeks of the first dose of treatment.
3. Is currently receiving nitrosureas or has received this therapy within the preceding 6 weeks of first dose of treatment.

4. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to registration with the following exceptions (which are permitted):
    - replacement steroid doses in patients with adrenal or pituitary insufficiency
    - Intra-articular steroid injections
    - Inhaled steroids (e.g.: Advair®, Flovent®, Azmacort®) at low doses (less than 500 mcg fluticasone per day, or equivalent)
    - Topical and nasal corticosteroids
    - Non-steroidal anti-inflammatory drugs
5. Has had a prior monoclonal antibody within 3 weeks prior to study Day 1 or who has not recovered (i.e. ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.
6. Has had prior chemotherapy, targeted small molecule therapy, or external beam radiation therapy within 2 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.
    - Note: Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study.
    - Note: If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.
7. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include carcinoma in situ of the breast (DCIS or LCIS), basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or in situ cervical cancer that has undergone potentially curative therapy.
8. Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis.
9. Has an active autoimmune disease requiring systemic treatment within the past 3 months or a documented history of clinically severe autoimmune disease, or a syndrome that requires systemic steroids or immunosuppressive agents. Exceptions to this criterion include:
    - Subjects with vitiligo or other depigmenting illness.
    - Resolved childhood asthma/atopy
    - Intermittent use of bronchodilators or local steroid injections
    - Hypothyroidism stable on hormone replacement or Sjogren's syndrome
    - The presence of laboratory evidence of autoimmune disease (e.g. positive ANA titer) without symptoms
    - Mild arthritis requiring NSAID medications
10. Has a history of (non-infectious) pneumonitis that required steroids or current evidence of interstitial lung disease or pneumonitis.
11. Has an active infection requiring systemic therapy.
12. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full

duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

13. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
14. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment.
15. Is HIV positive or has evidence of active Hepatitis C virus (testing to be done within 6 months of study entry) or active Hepatitis B virus.
16. Has received a live vaccine or allergy desensitization injections within 30 days prior to the first dose of trial treatment.
17. Has known or suspected allergies to any component of the vaccine.
18. Has been vaccinated previously with any of the synthetic peptides included in this protocol.
19. Is classified according to the New York Heart Association classification as having Class III or IV heart disease (Appendix 12.5).
20. Has uncontrolled diabetes, defined as having a HGBA1C > 8.5%.
21. Has a body weight < 110 pounds (without clothes) at enrollment, due to the amount and frequency with which blood will be drawn.

5.2 Trial Treatments

The treatment to be used in this trial is outlined below in Table 4.

Table 4. Trial Treatment

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
Pembrolizumab[1]	200 mg	Q3W starting at Day 1	IV infusion	Up to 2 years	Experimental
6MHP	200 mcg each peptide	Days 1, 8, 15, 43, 64, 85	SQ and ID Injection	Through Day 85	Experimental
[1]The pembrolizumab dosing interval may be increased as described in Section 5.2.1.3.					

Trial treatment should begin within 14 days of registration.

5.2.1 Dose Selection/Modification

5.2.1.1 Dose Selection

The rationale for selection of doses to be used in this trial is provided in Section 4.0 – Background and Rationale.

5.2.1.2 Study Drug Preparation and Administration

Pembrolizumab
Details on the preparation and administration of pembrolizumab are provided in the Pharmacy Manual for MK-3475 (pembrolizumab).

6MHP

Details on the preparation of 6MHP will be provided on vaccine mixing forms. The prepared peptide vaccines will be stored in a plastic syringe and delivered to the clinicians in a plastic bag. This bag with the syringe will be stored at room temperature until the vaccine is administered. Ideally, the vaccine should be administered within 1-2 hours after mixing. If the vaccine is not administered within 4 hours after mixing, it should be discarded.

Designation of Vaccine Sites

Evidence suggests nodes proximal to a tumor site may be relatively immunosuppressed; therefore, the vaccination sites will be selected to be distant from the sites of tumor whenever possible. In general, subjects will be vaccinated in upper arm or thigh sites with intact draining nodes. Vaccines will be administered at the designated site(s). Chronic inflammatory reactions are expected to occur in patients at their vaccine sites. Induration may persist for months, but is not expected to require additional therapy. Sterile abscesses may occur in some patients. These are not a basis for discontinuation of the vaccines unless they lead to ulceration of greater than 2 cm diameter, require antibiotics, or require surgical debridement.

Administration of 6MHP

The vaccine regimen will be the same for all subjects. Days 1, 8, 15, 43, 64, 85: 200 mcg each of the 6 peptides (Table 5), emulsified in Montanide ISA-51 adjuvant (2 ml total will be administered). Each of vaccines 1-6 will be administered subcutaneously (50%) and intradermally (50%). Vaccines 1-3 will be administered at one skin site. Vaccines 4-6 will also be administered at one skin site, but in a separate location than vaccines 1-3. If either vaccine site has severe inflammation or ulceration after 1-2 vaccines, the next vaccine(s) may be placed near the original site.

Table 5. Class II-Restricted Melanoma Peptides

Allele	Sequence	Epitope
HLA-DR4	AQNILLSNAPLGPQFP	Tyrosinase $_{56\text{-}70}$#
HLA-DR15	FLLHHAFVDSIFEQWLQRHRP	Tyrosinase $_{386\text{-}406}$
HLA-DR4	RNGYRALMDKSLHVGTQCALTRR	Melan-A/MART-1$_{51\text{-}73}$
HLA-DR11	TSYVKVLHHMVKISG	MAGE-3 $_{281\text{-}295}$
HLA-DR13	LLKYRAREPVTKAE	MAGE-1,2,3,6 $_{121\text{-}134}$
HLA-DR4 & -DR1	WNRQLYPEWTEAQRLD	gp100 $_{44\text{-}59}$

An alanine residue was added to the N-terminus to prevent cyclization.

Pre-medications

None required.

Post-Vaccination Observation

All subjects will be closely observed for adverse events for at least 20 minutes following each vaccination. Any time thereafter, subjects should report any adverse events to the research coordinator or research clinician.

5.2.1.3 Dose Modification

6MHP vaccines: There will be no dose modifications of the vaccine components.

In circumstances where assessment of an AE is limited, such as by intercurrent illness, or when laboratory studies are required to assess for other causes of toxicity, the vaccine schedule may be interrupted for up to 21 days. Delay of one vaccine administration by up to 21 days will not be considered a protocol violation if due to an AE, regardless of attribution. If more than one vaccine is delayed by 21 days due to an AE, regardless of attribution, vaccine treatment should be discontinued, though the other systemic therapy may be continued.

Pembrolizumab: Pembrolizumab will be withheld for drug-related AEs as detailed below and in Table 6. In the event that the dose of pembrolizumab is held, subjects may continue receiving the 6MHP vaccine if the investigators do not believe the toxicities are attributable to the vaccines.

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table 6, below. See Section 5.6 and Table 8 for supportive care guidelines, including use of corticosteroids.

Table 6. Dose Modification and Toxicity Management Guidelines for Immune-related AEs Associated with Pembrolizumab

General instructions:

1. Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.
2. For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to Grade 0-1 and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤10 mg prednisone or equivalent per day within 12 weeks.
3. For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.

Immune-related AEs	Toxicity grade or conditions (CTCAEv4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment • Add prophylactic antibiotics for opportunistic infections
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus). • Participants with ≥ Grade 2

	Grade 4	Permanently discontinue		diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.
AST / ALT elevation or Increased bilirubin	Grade 2	Withhold	• Administer corticosteroids (initial dose of 0.5- 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable
	Grade 3 or 4	Permanently discontinue	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold	• Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated.	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hyper-thyroidism	Grade 2	Continue	• Treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders.
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyroinine) per standard of care	• Monitor for signs and symptoms of thyroid disorders.
Nephritis and Renal dysfunction	Grade 2	Withhold	• Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1 or 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
All other immune-related	Intolerable/ persistent Grade 2	Withhold	• Based on type and severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude

AEs	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Gullain-Barre Syndrome, encephalitis		other causes
	Grade 4 or recurrent Grade 3	Permanently discontinue		

1. Withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician.

NOTE:
For participants with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required, pembrolizumab may be resumed when AE resolves to ≤ Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM).

Dosing interruptions are permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor-Investigator. The reason for interruption should be documented in the patient's study record.

5.2.1.4 Delayed Visit for Reasons Other Than Toxicity

A schedule for return visits should be established at the first visit. If a subject misses a treatment, the missed treatment will be administered as soon as possible, and treatment will be continued for an additional time period so that the subsequent vaccinations are given in the appropriate intervals. Subjects who are vaccinated outside of the established schedule should return to the original schedule as soon as possible.

The table below (Table 7) defines what constitutes a delayed visit, whether the subject should continue to be treated, and whether a protocol violation should be reported and recorded. The range of days is counted from the original scheduled date.

Table 7. Delayed Visit for Reasons other than Toxicity

Treatment Period	Range of Days	Subject Treatment	Protocol Deviation
*Biopsy/Vaccine 1**/Pembrolizumab			
Day 1	± 2 days	Vaccine, Labs, Pembrolizumab	No
	± 3 to 7 days	Vaccine, Labs, Pembrolizumab	Yes
	± 8 or more days	Labs	Yes
*Vaccines 2-3**			
Days 8, 15	± 2 days	Vaccine/Labs	No
	± 3 to 7 days	Vaccine/Labs	Yes
	± 8 or more days	Labs	Yes
Biopsy/Pembrolizumab			
Day 22	± 2 days	Labs, Biopsies, Pembrolizumab	No

	± 3 to 7 days	Labs, Biopsies, Pembrolizumab	Yes
	± 8 or more days	Labs	Yes
*Vaccine 4**/Pembrolizumab			
Days 43	± 2 days	Vaccine, Labs, Pembrolizumab	No
	± 3 to 7 days	Vaccine, Labs, Pembrolizumab	Yes
	± 8 or more days	Labs	Yes
Assessment			
Day 57	± 7 days	Scans**	No
	± 8 to 14 days	Scans	No
	± 15 or more days	Scans	Yes
*Vaccine 5**/Pembrolizumab			
Day 64	± 7 days	Vaccine, Labs, Pembrolizumab	No
	± 8 to 14 days	Vaccine, Labs, Pembrolizumab	Yes
	± 15 or more days	Labs	Yes
*Vaccine 6**/Pembrolizumab			
Day 85	± 7 days	Vaccine, Labs, Pembrolizumab	No
	± 8 to 14 days		Yes
	± 15 or more days	Labs	Yes
Assessment			
Day 92	± 7 days	Labs	No
	± 8 to 14 days	Labs	Yes
	± 15 or more days	Labs	Yes
Pembrolizumab			
Day 106+	± 7 days	Labs/Pembrolizumab	No
	± 8 to 14 days	Labs/Pembrolizumab	Yes
	± 15 or more days	Labs, Pembrolizumab/Scans**	Yes
Safety Follow-up			
30-days after last dose of pembrolizumab	± 14 days	Labs	No
	± 15 or more days	Labs	Yes

* A subject will be taken off protocol treatment if more than one vaccination is delayed [± 3 to 7 days] during the treatment period.

** CT scans on days 57 and 127 (weeks 8 and 18) should also be completed within these time limits. Subsequent scans should be completed per clinical care. The scan at week 8 is to measure response, with confirmatory scans if needed. Scans beyond week 18 may be performed earlier or more frequently than specified in the protocol if indicated as part of clinical care.

5.2.2 Timing of Dose Administration

Trial treatment should be administered after all procedures/assessments have been completed as detailed on the Trial Flow Chart (Section 6.0). All trial treatments will be administered on an outpatient basis.

Pembrolizumab will be administered as a 30 minute IV infusion (treatment cycle intervals may be increased due to toxicity as described in Section 5.2.1.3. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

The Pharmacy Manual for MK-3475 contains specific instructions for pembrolizumab dose calculation, reconstitution, preparation of the infusion fluid, and administration.

6MHP will be administered as a subcutaneous and intradermal injection.

On days when both pembrolizumab and vaccine are administered, the vaccine may be administered before or after pembrolizumab, but preferably before.

5.2.3 Trial Blinding/Masking

This is an open-label trial; therefore, the Sponsor-Investigator and subject will know the treatment administered.

5.3 Randomization or Treatment Allocation

All subjects will receive 6MHP plus pembrolizumab following the same treatment regimen.

5.4 Stratification

Patients will be monitored for post entry stratification by whether or not biopsies are obtained at baseline and day 22.

5.5 Concomitant Medications/Vaccinations (allowed & prohibited)

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the Sponsor-Investigator. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician. However, the decision to continue the subject on trial therapy or vaccination schedule requires the mutual agreement of the Investigator, the Sponsor-Investigator, and the subject.

5.5.1 Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care including the following:

- Nonsteroidal anti-inflammatory agents
- Anti-histamines (e.g. Claritin®, Allegra®)
- Topical and nasal corticosteroids or steroids for the reasons cited in sections 5.5.2.
- Short-term therapy for acute conditions not specifically related to melanoma
- Chronic medications except those listed in section 5.5.2
- Influenza vaccines (excluding the live version of the vaccine) are permitted, but should be administered at least 2 weeks prior to or at least 2 weeks after administration of study drug.

All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If

changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs as defined in Section 7.2.

5.5.2 Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase of this trial:

- Anti-cancer systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents; Note: pembrolizumab and 6MHP are permitted during the treatment phase, per the protocol.
- Radiation therapy; Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed after consultation with Sponsor-Investigator.
- Allergy desensitization injections
- Systemic steroid therapy or any other form of immunosuppressive therapy with the following exceptions (which are permitted):
    - replacement steroid doses in patients with adrenal or pituitary insufficiency
    - Inhaled steroids (e.g.: Advair®, Flovent®, Azmacort®) at low doses (less than 500 mcg fluticasone per day, or equivalent)
- Street drugs
- Live vaccines within 30 days prior to the first dose of trial treatment, while participating in the trial and for at least 2 weeks after the last dose of study drug. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chicken pox, yellow fever, rabies, BCG, Flu-Mist ®, and typhoid (oral) vaccine.

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial. Subjects may receive other medications that the investigator deems to be medically necessary.

The Exclusion Criteria describes other medications which are prohibited in this trial.

There are no prohibited therapies during the Post-Treatment Follow-up Phase.

5.6 Rescue Medications & Supportive Care

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines

are intended to be applied when the investigator determines the events to be related to pembrolizumab.

Note: if after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance (as outlined below). Refer to Section 5.2.1 for dose modification.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event.

- **Pneumonitis**:
    - For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
    - For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
    - Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.
- **Diarrhea/Colitis**:
  Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).
    - All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
    - For **Grade 2 diarrhea/colitis** that persists greater than 3 days, administer oral corticosteroids.
    - For **Grade 3 or 4 diarrhea/colitis** (or Grade 2 diarrhea that persists > 1 week), treat with intravenous steroids followed by high dose oral steroids.
    - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
- **Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or ≥ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)**
    - For **T1DM** or **Grade 3-4** Hyperglycemia
        - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.
        - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.
- **Hypophysitis**:
    - For **Grade 2** events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
    - For **Grade 3-4** events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be

started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hyperthyroidism or Hypothyroidism**:
  Thyroid disorders can occur at any time during treatment. Monitor patients for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.
  - **Grade 2** hyperthyroidism events (and **Grade 3-4** hypothyroidism):
    - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
    - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyronine, is indicated per standard of care.
  - **Grade 3-4** hyperthyroidism
    - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hepatic**:
  - For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).
    - Treat with IV or oral corticosteroids
  - For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours.
  - When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

- **Renal Failure or Nephritis**:
  - For **Grade 2** events, treat with corticosteroids.
  - For **Grade 3-4** events, treat with systemic corticosteroids.
  - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Management of Infusion Reactions**: Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

  Table 8 shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab (MK-3475).

Table 8. Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to:	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (MK-

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	3475) with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

5.7 Diet/Activity/Other Considerations

5.7.1 Diet

Subjects should maintain a normal diet unless modifications are required to manage an AE such as diarrhea, nausea or vomiting.

5.7.2 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm.

For this trial, male subjects will be considered to be of non-reproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

Female subjects will be considered of non-reproductive potential if they are either:

(1) postmenopausal (defined as at least 12 months with no menses without an alternative medical cause; in women < 45 years of age a high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.);

OR

(2) have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion, at least 6 weeks prior to screening;

OR

(3) has a congenital or acquired condition that prevents childbearing.

Female and male subjects of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving study drug and for 120 days after the last dose of study drug by complying with one of the following:

(1) practice abstinence† from heterosexual activity;

OR

(2) use (or have their partner use) acceptable contraception during heterosexual activity.

Acceptable methods of contraception are‡:

Single method (one of the following is acceptable):

- intrauterine device (IUD)
- vasectomy of a female subject's male partner
- contraceptive rod implanted into the skin

Combination method (requires use of two of the following):

- diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- cervical cap with spermicide (nulliparous women only)
- contraceptive sponge (nulliparous women only)
- male condom or female condom (cannot be used together)
- hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

†Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle and if

considered acceptable by local regulatory agencies and ERCs/IRBs. Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

‡If a contraceptive method listed above is restricted by local regulations/guidelines, then it does not qualify as an acceptable method of contraception for subjects participating at sites in this country/region.

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study, subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 120 days after the last dose of trial therapy. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

5.7.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor-Investigator and to Merck without delay and within 24 hours if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn). The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor-Investigator. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the Sponsor-Investigator and to Merck and followed as described in Section 7.2.8.

5.7.4 Use in Nursing Women

It is unknown whether pembrolizumab is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, subjects who are breast-feeding are not eligible for enrollment.

5.8 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator or the Sponsor-Investigator if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding discontinuation or withdrawal are provided in Section 7.1.4 – Other Procedures.

Protocol treatment includes both the 6MHP vaccine and pembrolizumab. Either or both may be discontinued for any of the following reasons.

- The subject or legal representative (such as a parent or legal guardian) withdraws consent.
- Confirmed radiographic disease progression

Note: A subject may be granted an exception to continue on treatment with confirmed radiographic progression if clinically stable or clinically improved. Patients with progressive disease should undergo a second scan at least 4 weeks later to confirm progression and exclude the possibility of a tumor flare reaction, according to immune-related response criteria (irRC) guidelines.

- Any dose-limiting toxicity (DLT), as defined in Section 7.2.7, warrants discontinuation of the agent to which the DLT is attributed. If a DLT attributed to one intervention is not believed to be related to the other treatment, only the one to which the DLT is attributed must be discontinued, and other protocol interventions may be continued as close to the original schedule as possible. However, management of toxicities attributed to PD-1 antibody should follow current standard practice guidelines, including use of steroids when indicated. Also, if a patient is discontinued from PD-1 antibody but not from vaccine, the visits every 3 weeks for PD-1 antibody, and the associated safety studies (e.g.: CBC, chemistries) do not need to be performed.
- Disease progression requiring other therapy (e.g. surgery under general anesthesia, radiation, chemotherapy, or steroid therapy). The appearance of small metastases or recurrent tumor deposits will not be a basis for discontinuing the vaccinations. Biopsy to determine the nature of new lesions, or minor surgical procedures to excise a new lesion, will not be a basis for discontinuing vaccinations. Also, surgery to perform a biopsy of tumor at day 22, in accord with the protocol, will not be a basis for discontinuing therapy, whether done under local anesthesia or general anesthesia.
- Intercurrent illness that prevents further administration of treatment
- Initiation of cytotoxic chemotherapy, radiation therapy, or targeted therapy for melanoma or other cancer.
- Initiation of systemic steroid therapy, or other systemic immunosuppressive therapy, except that steroid or immunosuppressive therapy for management of toxicities of pembrolizumab is allowed without discontinuation of vaccines.
- Any other potential adverse reaction deemed sufficiently serious to warrant discontinuation of therapy by the Principal Investigator or one of the Associate Investigators.
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Completed 24 months of uninterrupted treatment with pembrolizumab or 35 administrations of study medication, whichever is later.

  Note: 24 months of study medication is calculated from the date of first dose. Subjects who stop pembrolizumab after 24 months may be eligible for additional pembrolizumab treatment as part of their clinical care if they progress after stopping study treatment.

- Administrative reasons

The End of Treatment and Follow-up visit procedures are listed in Section 6 (Protocol Flow Chart) and Section 7.1.5 (Visit Requirements). After the end of treatment, each subject will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment as described in section 7.1.5.3.1 and 7.2.7.8). Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing

consent or becoming lost to follow-up (every 8 weeks post discontinuation up to 2 years or until the toxicity resolves). After documented disease progression each subject will be followed by telephone annually for overall survival until death, withdrawal of consent, or until study closure, whichever occurs first.

5.9 Subject Replacement Strategy

A subject who is enrolled but does not receive any pembrolizumab or 6MHP, or any of the study-related procedures may be replaced. Every attempt will be made to evaluate data for endpoint assessment from subjects who withdrawal from the study.

5.10 Clinical Criteria for Early Trial Termination

Early trial termination will be the result of the criteria specified below:

1. Quality or quantity of data recording is inaccurate or incomplete as determined by the Sponsor-Investigator.

2. Poor adherence to protocol and regulatory requirements

3. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects

4. Plans to modify or discontinue the development of the study drug

In the event of Merck decision to no longer supply study drug, ample notification will be provided so that appropriate adjustments to subject treatment can be made.

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6.0 TRIAL FLOW CHART

6.1 Study Flow Chart

Trial Period:	**Active Treatment**																
Day	Main Study Screening	1	8	15	22	43	57	64	85	92	106	127	148	169	190	365	730
Week		0	1	2	3	6	8	9	12	13	15	18	21	24	27	52	104
Scheduling Window (Days):			± 2	± 2	± 2	± 2	± 7	± 7	± 7	± 7	± 7	± 7	± 7	± 7	± 7	± 7	± 7
Administrative Procedures																	
Informed Consent	X[a]																
Pathology Review at UVA	X[a]																
Inclusion/Exclusion Criteria	X[b]																
Laboratory Procedures																	
CBC with Differential [m]	X[b]	X[f]			X	X		X	X		X	X	X	X	X	X	X
Comprehensive Serum Chemistry Panel [m,v]	X[b,e]	X[f]			X	X		X	X		X	X	X	X	X	X	X
HGBA1C	X[b]																
Urinalysis	X[b]																
Pregnancy Test: Serum or urine β-HCG in women	X[c]	X[u]															
T3, FT4 and TSH		X				X			X			X		X		X	X
PT/INR and aPTT	X																
HIV/HCV/HBV	X[d]																
ANA/RF		X				X			X							X	X
Clinical Assessments																	
Demographics and Medical History	X[b]																
Physical Exam	X[b]	X[f]	X	X	X	X		X	X	X		X		X	X	X	X

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Trial Period:	**Active Treatment**																
Day	Main Study Screening	1	8	15	22	43	57	64	85	92	106	127	148	169	190	365	730
Week		0	1	2	3	6	8	9	12	13	15	18	21	24	27	52	104
Scheduling Window (Days):			± 2	± 2	± 2	± 2	± 14	± 7	± 7	± 7	± 7	± 7	± 7	± 7	± 7	± 7	± 7
Prior and Concomitant Medication Review	X[b]	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X
Record Baseline Symptoms		X															
Review Adverse Events			X	X	X	X		X	X	X	X	X	X	X	X	X	X
Designation of potential vaccination sites	X[b]																
Assessment of skin and nodal basins for evidence of disease	X[b]																
Assessment of skin for vitiligo		X			X				X					X	X	X	X
Assessment of hair and eye color		X			X				X					X	X	X	X
Visual acuity exam/color vision		X															
Patient diary reviewed and/or distributed		X	X	X	X	X		X	X	X	X	X					
Efficacy Measures/Tumor Imaging																	
CT chest/abdomen/pelvis or PET-CT[r]	X						X[h]					X[h]			X[j]	X[j]	X[j]
Head MRI/CT[r]	X						X[i]					X[i]			X[i]	X[i]	X[i]
Digital images of cutaneous lesions[r]	X						X[h]					X[h]			X[j]	X[j]	X[j]
Research Specimen Collection and Procedures																	
120 cc blood- green top tube		X[k]															
80 cc blood- green top tube			X	X	X	X		X	X	X		X		X	X	X	X
20 cc blood- red top tube		X	X	X	X	X		X	X	X		X		X	X	X	X
Tumor Biopsy		X			X												
SIN biopsy (thru protocol v04-04-17 only)					X												
Study Drug Administration																	
6MHP		X	X	X		X		X	X								
Pembrolizumab every 3 weeks		X			X	X		X	X		X	X	X	X	X	X	X[t]

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	End of Treatment	Post-Treatment		
	Discontinuation of pembrolizumab or of both drugs[l]	Safety Follow-up Discontinuation of pembrolizumab or of both drugs[l]	Follow Up Visits [o] After discontinuation for reasons other than disease progression	Survival Follow-Up[q] After discontinuation of therapy for disease progression and/or initiation of new antineoplastic therapy
Scheduling Window (days)		± 14 Days	± 15 Days	± 28 Days
	At time of discontinuation of pembrolizumab	30 days post discontinuation of pembrolizumab	Every 8 weeks post discontinuation up to 2 years or until resolution of toxicity[s]	Annually
Laboratory Procedures				
CBC with Differential	X	X		
Comprehensive Serum Chemistry Panel[v]	X	X		
Urinalysis	X			
T3, FT4 and TSH	X	X		
Clinical Assessments				
Physical Exam	X	X	X	
Prior and Concomitant Medication Review	X	X		
Review Adverse Events	X	X	X	
Efficacy Measures/Tumor Imaging				
CT chest/abdomen/pelvis or PET-CT	X[j]		X[j]	
Head MRI/CT	X[j]			
Digital images of cutaneous lesions	X[j]		X[j]	
Research Specimen Collection and Procedures				
80 cc blood- green top tube	X	X	X[s]	
20 cc blood- red top tube	X	X	X[s]	

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	End of Treatment	Post-Treatment		
	Discontinuation of pembrolizumab or of both drugs[l]	Safety Follow-up Discontinuation of pembrolizumab or of both drugs[l]	Follow Up Visits [o] After discontinuation for reasons other than disease progression	Survival Follow-Up[q] After discontinuation of therapy for disease progression and/or initiation of new antineoplastic therapy
Scheduling Window (days)		± 14 Days	± 15 Days	± 28 Days
	At time of discontinuation of pembrolizumab	30 days post discontinuation of pembrolizumab	Every 8 weeks post discontinuation up to 2 years or until resolution of toxicity[s]	Annually
Tumor Biopsy	X[n]			X[n]
Survival Status				
Survival Status	X	X	X	X
Start of Antineoplastic therapy				X

[a] Any point prior to registration
[b] Pre-study within 6 weeks of registration
[c] Within 2 weeks of registration (for childbearing women)
[d] Within 6 months of registration
[e] To include fasting glucose
[f] History & physical, comprehensive chemistry, and CBC with differential, for Day 1 are not required if the pre-study assessment was completed within 10 calendar days of day 1.
[g] For patients who have tumor accessible to biopsy, biopsies will be completed 1) at screening or up until day 1, and 2) at day 22. Additional biopsies may be performed during the study or during follow-up if subjects are removed from the study or experience progression.
[h] Tumor imaging (window of ± 14 Days) should be performed when clinically needed to verify objective clinical responses (per RECIST v1.1) and every 8-12 weeks as indicated per standard care.
[i] Head imaging should be completed when clinically indicated.
[j] Tumor imaging should be completed as indicated per standard care.
[k] Blood for HLA typing is included in the research bloods
[l] If a subject is discontinued and the assessments have been completed as part of a regularly scheduled visit, the assessments do not need to be repeated. If a subject is discontinued from 6MHP but continues with pembrolizumab, the safety follow-up visit for 6 MHP will occur at the time of their next infusion of pembrolizumab.
[m] Repeat CBC and chemistries every 3 weeks, just prior to the next dose of pembrolizumab. If there is a delay in pembrolizumab treatment for toxicity or other reasons, the CBC and chemistries should be delayed comparably, to enable those blood studies on the same day (before) each dose of pembrolizumab.

[n] If subjects develop superficial metastatic deposits accessible to biopsy/excision with minimal morbidity, an optional biopsy may be collected for research purposes. If surgical resection or biopsies are completed for clinical care, the tumor tissue may be collected for study related analyses.
[o] Follow-up visits every 8 weeks will not be required beyond 2 years from study entry. If a subject is managed locally and is not able to return to UVA every 8 weeks after discontinuation for reasons other than disease progression, because of toxicity or intercurrent health problems, every effort will be made to obtain records that supply data comparable to what is specified in the table.
[p] Head MRI/CT may be performed every 6 months or as clinically indicated.
[q] At the time of disease progression or if a subject begins receiving a new antineoplastic therapy, the subject will move into survival follow-up. Subjects may be contacted by telephone for survival follow-up status. The expectation is that there will be additional follow-up as part of standard of care, either by the study physician or by a local referring physician. Every effort will be made to obtain data on disease status and survival at all such visits, in addition to the mandated study visits.
[r] Patients with progressive disease should undergo a second scan at least 4 weeks later to confirm progression and exclude the possibility of a tumor flare reaction, according to irRC guidelines.
[S] If a subject discontinues pembrolizumab for toxicity, that patient should be followed every 8 weeks until resolution of the toxicity or until 2 years from study entry, whichever is longer. If the toxicity resolves before 2 years, then the patient should continue with visits requiring research blood draws and tumor biopsies.
[t] Week 52 is last planned clinical trial intervention other than continued dosing of pembrolizumab every 3 weeks; pembrolizumab is to be continued every 3 weeks between week 26 and week 52, and beyond that to 2 years, along with safety labs as needed in routine clinical care.
[u] Must be completed within 72 hours prior to receiving the first dose of study drug. If the screening test is completed within 72 hours prior to Day 1, this test does not need to be repeated.
[v] Refer to Table 9 for a complete list of analyses to be completed as part of the comprehensive chemistry panel.

7.0 TRIAL PROCEDURES

7.1 Trial Procedures

The Trial Flow Chart - Section 6.0 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

Furthermore, additional evaluations/testing may be deemed necessary by the Sponsor-Investigator and/or Merck for reasons related to subject safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g., HIV, Hepatitis C, etc.), and thus local regulations may require that additional informed consent be obtained from the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations.

7.1.1 Administrative Procedures

7.1.1.1 Informed Consent

The Investigator must obtain documented consent from each potential subject prior to participating in a clinical trial.

7.1.1.1.1 General Informed Consent

Consent must be documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion.

A copy of the signed and dated consent form should be given to the subject before participation in the trial.

The initial informed consent form, any subsequent revised written informed consent form and any written information provided to the subject must receive the IRB/ERC's approval/favorable opinion in advance of use. The subject or his/her legally acceptable representative will be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the subject's dated signature or by the subject's legally acceptable representative's dated signature.

Specifics about a trial and the trial population will be added to the consent form template at the protocol level.

The informed consent will adhere to IRB/ERC requirements, applicable laws and regulations and Sponsor-Investigator requirements.

7.1.1.2 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial.

7.1.1.3 Registration

All subjects must sign the consent form prior to determination of eligibility for this study. Registration will occur following verification of eligibility by the treating physician. Subjects will be registered in the OnCore database and should receive their first study treatment within 2 weeks of registration.

7.1.1.4 Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the Investigator. Details regarding the disease for which the subject has enrolled in this study will be recorded separately and not listed as medical history.

7.1.1.5 Prior and Concomitant Medications Review

7.1.1.5.1 Prior Medications

The investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the subject within 42 days before starting the trial. Treatment for the disease for which the subject has enrolled in this study will be recorded separately and not listed as a prior medication.

7.1.1.5.2 Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the subject during the trial. All medications related to reportable SAEs and ECIs should be recorded as defined in Section 7.2.

7.1.1.6 Disease Details and Treatments

7.1.1.6.1 Disease Details

The investigator or qualified designee will obtain prior and current details regarding disease status.

7.1.1.6.2 Prior Treatment Details

The investigator or qualified designee will review all prior cancer treatments including systemic treatments, radiation and surgeries.

7.1.1.6.3 Subsequent Anti-Cancer Therapy Status

The investigator or qualified designee will review all new anti-neoplastic therapy initiated after the last dose of trial treatment. If a subject initiates a new anti-cancer therapy within 30 days after the last dose of trial treatment, the 30 day Safety Follow-up visit must occur before the first dose of the new therapy. Once new anti-cancer therapy has been initiated the subject will move into survival follow-up.

7.1.1.7 Trial Compliance (Medication/Diet/Activity/Other)

Treatment compliance may be evaluated through drug accountability assessments and through the evaluation of subject medical records and CRF documents.

7.1.2 Clinical Procedures/Assessments

The following evaluations will be performed on an outpatient basis.

7.1.2.1 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. Toxicity diaries will also be distributed to subjects and reviewed by study personnel. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 4.0 (see Section 12.2). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

All AEs of unknown etiology associated with pembrolizumab exposure should be evaluated to determine if it is possibly an event of clinical interest (ECI) of a potentially immunologic etiology (irAE). See Section 5.6.1 regarding the identification, evaluation and management of AEs of a potential immunological etiology.

Please refer to section 7.2 for detailed information regarding the assessment and recording of AEs.

7.1.2.2 Physical Exams

The investigator or qualified designee will perform a complete physical exam during the screening period. Clinically significant abnormal findings should be recorded as medical history.

Physical exams will include the following:

- vital signs (temperature, pulse, respiratory rate, blood pressure)
- weight,
- ECOG performance status,
- medication review,
- neurologic function-general

Height will be measured at screening only

7.1.2.3 Vital Signs

The investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment and at treatment discontinuation as specified in the Trial Flow Chart (Section 6.0). Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only.

7.1.2.4 Eastern Cooperative Oncology Group (ECOG) Performance Scale

The investigator or qualified designee will assess ECOG status (see Section 12.1) as specified in the Trial Flow Chart.

7.1.2.5 Designation of Vaccine Sites

Evidence suggests nodes proximal to a tumor site may be relatively immunosuppressed; therefore, the vaccination sites will be selected to be distant from the sites of tumor whenever possible. In general, subjects will be vaccinated in upper arm or thigh sites with intact draining nodes. Vaccines will be administered at the designated site(s).

7.1.2.6 Visual Assessments

Visual acuity will be assessed using the Snellen chart.

Color vision will be assessed using an Ishihara eye chart.

7.1.2.7 Tumor Imaging and Assessment of Disease

Tumor Imaging

Tumor imaging may include CT/PET-CT scans and/or MRI. These will complement physical exam and other imaging as required, but the primary measures of clinical response will be based on CT/PET-CT and/or MRI and will use RECIST v1.1 criteria. For each subject, the same method of assessment will be used to evaluate tumor burden at baseline and throughout the course of the study.

Tumor Measurements

RECIST 1.1 Criteria will be used to evaluate tumor burden (Section 12.3).

7.1.2.8 Tumor Tissue Collection and Correlative Studies Blood Sampling

7.1.2.8.1 Tumor Biopsies

Tumor biopsies will be completed in subjects who have adequate and accessible metastatic tumor in addition to at least one site of measurable disease. Biopsy sites may be in nodes, skin, soft tissue, liver, or other sites that can be accessed by needle biopsy, incisional or excisional biopsy. Biopsies may be completed with or without image guidance.

Size Requirements

A critical component of this protocol is the histologic and cytologic evaluation of changes in immune effectors and the tumor microenvironment after vaccination and systemic therapy. A minimum of 0.16 cm^3 but ideally 0.3 cm^3 or more of tumor tissue will be needed for each biopsy time point as described in the inclusion criteria. Biopsies may be taken from a single lesion or multiple lesions at each of the time points depending on the size of each lesion. If taken from multiple lesions, those lesions should be similar. For example, three non-ulcerated skin metastases would be considered similar; one bleeding small bowel metastasis would not be considered similar to a subcutaneous nodule).

Sampling

The biopsies will vary based on the clinical scenario and may include six core biopsies, an incisional biopsy or an excisional biopsy as outlined in the inclusion criteria.

Procedure

When appropriate (and we anticipate the majority of cases) the biopsies will be performed under local anesthesia (typically lidocaine HCl 1% and epinephrine 1:100,000 injection + or - 8.4% sodium bicarbonate), in the outpatient clinic or comparable procedure room, using sterile technique. In cases when clinical standard of care requires a larger procedure the biopsies may be performed in the operating room under standard technique.

To minimize errors in analysis due to sampling error and specimen heterogeneity, each study biopsy specimen will be divided into several components and randomly allocated into various preservation conditions. Ideally, tissue will be divided into the following preservation conditions, using core needle biopsies (19 mm long and 2 mm diameter; about 80 mm^3), or incisional or excisional biopsies with at least the same minimum tissue volume:

It is most critical to obtain the following:

- Formalin: 1 core biopsy or similar tissue volume (about 80 mm^3 or greater) will be fixed in formalin, then paraffin-embedded (for histology/immunohistology)

- Quick-frozen: 2 core biopsies or similar tissue volume (each about 80 mm^3 or greater) quick-frozen processed for protein studies, histology, or nucleic acid studies. If only one core can be obtained, this portion should be provided as two specimens (eg cut the core biopsy specimen in half).

When sufficient tissue is available, the following should also be obtained:
- RNA-later: 1 core biopsy or similar tissue volume (about 80 mm^3 or greater) will be placed in RNA-later (for RNA/RT PCR)
- Viable cell suspension: 2 core biopsies or similar tissue volume (total about 160 mm^3 or greater) will be processed for single-cell suspension by mechanical disaggregation, then enzymatic digestion (collagenase, hyaluronidase, DNAase). The resulting suspensions will be cryopreserved in FBS serum and DMSO (for cellular immune function and flow cytometry).
- If there is additional tissue, it may be processed for additional immunologic studies.

The incisions will be sutured closed. Toxicities related to the biopsies will be recorded.

Based on our experience in prior clinical trials (Mel48 (NCT00705640), Mel51 (NCT00977145), and Mel53 (NCT01264731)), it is adequate, for each specimen, to have at least the equivalent of a core biopsy specimen that is 19 mm long and 2 mm in diameter (about 80 mm^3), or a cubic specimen 5 mm in width. Thus, for 1 FFPE and 1-2 QF specimens, we need about 160-240 mm^3 (2-3 cores, or 1 lesion about 7-10 mm in diameter). For those with larger specimens (>1 cm diameter), some will also be saved as viably cryopreserved single cell suspensions.

A 5 micron section of each tumor specimen will be stained by H&E and reviewed to assess the extent and quality of viable tumor. For FFPE specimens, only those with at least 4 mm^2 viable tumor (on cross-section) will be considered evaluable for histologic and immunohistologic studies. FFPE tissue will be evaluated for immunotype and for immune cell infiltrates. For QF specimens, those with at least 70% tumor will be considered evaluable.

Evaluations

Tissue samples may be screened for antigen expression or protein profiles using tests such as Western blots, immunohistochemistry, PCR, flow cytometry or gene chip analysis.

Tumor escape mechanisms may also be evaluated.

Specimens will be used in immunological assays to assess T cell function or antibody response. Assays generally used for this type of testing include, but are not limited to, ELIspot assays, ELISAs, chromium-release assays, proliferation assays, intracellular cytokine staining, and T cell receptor sequencing.

Specimens may be used to study the immunologic aspects of the tumor microenvironment or as targets or controls in laboratory assays.

Specimens may be used to establish cell lines for long-term studies.

This tissue may also be compared to lesions resected prior to enrollment, which will be requested from the pathology department of each institution as paraffin-embedded tissue samples, and these tissues may be banked for use in future studies.

HLA typing (Class I and Class II) will be analyzed as part of the immunologic endpoints. (8 ml)

7.1.2.8.2 Optional Tumor Biopsies (at the time of progression, after study completion and withdrawal)

If during the study, participants develop superficial metastatic deposits accessible to biopsy/excision with minimal morbidity, an optional biopsy may be collected for research purposes.

If tumor samples are collected when a Mel 64 participant is no longer participating in the Mel 64 study and the participant consents to allow their tissue to be collected under the IRB #10598 tissue banking study to be analyzed for Mel 64, the tissue may be analyzed as part of the Mel 64 analysis.

Optional biopsy samples may be evaluated as described in section 7.1.2.8.1.

7.1.2.8.3 Tumor Collected for Clinical Care

If during the study, participants develop metastases or recurrences, or progress, these may be removed as part of their clinical care, and following receipt by pathology, may be evaluated by the study research team.

7.1.2.8.4 Blood Collection for Research Analyses

Blood should be obtained prior to the vaccine injection if a vaccine is scheduled to be administered. Results of research blood tests are not required prior to administering the vaccine on that date.

The following blood samples for research will be collected and processed by the UVA Biorepository and Tissue Research Facility (BTRF).

- 80 cc -120 cc blood collected in heparinized green top tubes for lymphocytes.
- 20 cc blood collected in red top tubes for serum

Samples will be analyzed for analyses that may include the following (and leftover sample will be banked and stored for future biomedical research):

- Functional assays for T cell responses to melanoma antigens (the 6 melanoma helper peptides of the vaccine, and other melanoma antigens, and control antigens), including ELIspot assays, and multiparameter flow cytometry for cytokine release.
- Multimer assays for T cell receptor specificity
- High throughput T cell receptor sequencing.
- Genetic polymorphisms of immune-related genes
- Gene expression profiling of blood cells
- Production of chemokines, cytokines from circulating lymphocytes and other immune cells.
- Multiparameter flow cytometry for cellular markers, including markers of regulatory T cells, myeloid cells, and T cell maturation.
- Serum antibodies
- Serum cytokines, chemokines or other serum proteins

7.1.3 Laboratory Procedures/Assessments

Laboratory tests for hematology, chemistry, urinalysis, and others are specified in Table 9.

Table 9. Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hematocrit	Albumin	Blood	Urine pregnancy or serum β-human chorionic gonadotropin (β-hCG)†
Hemoglobin	Alkaline phosphatase	Glucose	HgB-A1C
Platelet count	Alanine aminotransferase (ALT)	Protein	PT (INR)
WBC (total and differential)	Aspartate aminotransferase (AST)	Specific gravity	aPTT
Red Blood Cell Count	Lactate dehydrogenase (LDH) (every 6 weeks)	Microscopic exam *(If abnormal)*	Total triiodothyronine (T3)
Absolute Neutrophil Count	Carbon Dioxide *(CO_2 or bicarbonate)*	results are noted	Free thyroxine (T4)
	Creatinine		Thyroid stimulating hormone (TSH)
	Uric Acid		HIV testing
	Calcium		HCV testing
	Chloride		HBV testing
	Glucose		ANA/RF
	Phosphorus		
	Potassium		
	Sodium		
	Magnesium		
	Total Bilirubin		
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*		

Hematology	Chemistry	Urinalysis	Other
	Total protein		
	Blood Urea Nitrogen		
† Perform on women of childbearing potential only. If urine pregnancy results cannot be confirmed as negative, a serum pregnancy test will be required.			

7.1.4 Other Procedures

7.1.4.1 Sentinel Immunized Node (SIN) Biopsy

The SIN biopsy will be performed until the approval of the protocol modification where the requirement for SIN biopsy was removed (through protocol v04-04-17). Thereafter, SIN biopsies will not be performed.

Procedure

The node (sentinel immunized node, SIN) will be identified by radiocolloid (usually technetium 99 sulfur colloid) injection, with or without lymphoscintigraphy imaging, and with use of a handheld gamma probe during the procedure. This will be performed under local anesthesia in the clinic, in conjunction with the vaccine site biopsy, by a qualified surgeon.

Lymphatic mapping will be initiated, usually in the nuclear medicine suite, after intradermal injection with radiocolloid (typically technetium 99-sulfur colloid). The node excision will be performed under local anesthesia (usually lidocaine HCl 1-2%, with or without epinephrine 1:100,000 injection, with or without 8.4% sodium bicarbonate), in the outpatient clinic or comparable procedure room, using sterile technique. A handheld gamma probe will be used.

When possible, the node will be sectioned into 5 sections: a central section (10-20% of the node), leaving two adjacent sections of about 40% each. These latter two sections will be bisected. They will be allocated into various preservation conditions:

- 1 central section will be fixed in formalin, then paraffin-embedded
- (for histology/immunohistology)
- 1 section will be placed in RNA-later. (for RNA/RT PCR)
- 1 section will be quick-frozen (for immunohistology/protein studies)
- 2 sections (40%) will be processed for single cell suspension by mechanical disaggregation, then enzymatic digestion (collagenase, hyaluronidase, DNAase). The resulting suspensions will be cryopreserved in FBS and DMSO (for cellular immune function and flow cytometry).

If there is additional tissue, it may be processed for additional immunologic or angiogenic studies.

The incisions will be sutured closed.

Toxicities related to the biopsies will be recorded.

7.1.4.2 Withdrawal/Discontinuation

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 7.2 - Assessing and Recording

Adverse Events. Subjects who a) attain a CR or b) complete 24 months of treatment with pembrolizumab may discontinue treatment with the option of restarting treatment upon progression. Restarting treatment will be completed as part of a subject's standard clinical care, at the discretion of their treating physician. After discontinuing treatment following assessment of CR, these subjects should return to the site for a Safety Follow-up Visit (described in Section 7.1.5.3.1) and then proceed to the Follow-Up Period of the study (described in Section 7.1.5.4).

7.1.5 Visit Requirements

Visit requirements are outlined in Section 6.0 - Trial Flow Chart. Specific procedure-related details are provided above in Section 7.1 - Trial Procedures.

7.1.5.1 Screening

A member of the study team will explain the purpose of the study and the study-related procedures to potential subjects. Subjects will be asked to provide written informed consent prior to the initiation of any study-related procedures. The results from assessments performed as part of a subject's clinical care prior to receipt of informed consent may be utilized to fulfill a screening requirement, if the assessments were completed within the required window for screening.

7.1.5.2 Treatment Period

The treatment period will begin on Day 1 and will continue until the subject completes the treatment regimen or discontinues treatment.

7.1.5.2.1 End of Treatment Visit

This visit will occur at the time that a subject is discontinued from the study.

7.1.5.2.2 Second Course (Retreatment Period for Post-Complete Remission Relapse Only)

Subjects who stop pembrolizumab with SD or better and who experience disease progression may receive additional doses of pembrolizumab as part of their clinical care following evaluation by their treating physician.

7.1.5.3 Post-Treatment Visits

7.1.5.3.1 Safety Follow-Up Visit

The mandatory Safety Follow-Up Visit should be conducted approximately 30 days after the last dose of trial treatment (pembrolizumab or 6MHP) or before the initiation of a new anti-cancer treatment, whichever comes first. All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Subjects with an AE of Grade > 1 will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-neoplastic therapy, whichever occurs first. SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded.

7.1.5.4 Follow-up Visits

Subjects who discontinue treatment pembrolizumab or both drugs for a reason other than disease progression will move into the Follow-Up Phase and should be assessed every 8 weeks (56 ± 7 days) by radiologic imaging to monitor disease status. Every effort should be made to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression, death, end of the study or if the subject begins retreatment with pembrolizumab as detailed in Section 7.1.5.2.2. Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated.

Follow-up visits every 8 weeks will not be required beyond 2 years from study entry. If a subject is managed locally and is not able to return to UVA every 8 weeks after discontinuation for reasons other than disease progression, because of toxicity or intercurrent health problems, every effort will be made to obtain records that supply data comparable to what is specified in the study calendar.

If a subject discontinues pembrolizumab for toxicity, that subject should be followed every 8 weeks until resolution of the toxicity or until 2 years from study entry, whichever is longer. If the toxicity resolves before 2 years, then the subject should continue with visits requiring research blood draws and tumor biopsies.

Subjects with progressive disease should undergo a second scan at least 4 weeks later to confirm progression and exclude the possibility of a tumor flare reaction, according to irRC guidelines.

7.1.5.4.1 Survival Follow-up

Once a subject experiences confirmed disease progression or starts a new anti-cancer therapy, the subject moves into the survival follow-up phase and should be contacted by telephone annually for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.

7.2 Assessing, Recording, and Reporting Adverse Events

7.2.1 Definitions

Adverse Event (AE): An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the study drugs or protocol-specified procedure is also an adverse event.

Changes resulting from normal growth and development that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events.

Merck product includes any pharmaceutical product, biological product, device, diagnostic agent or protocol-specified procedure, whether investigational (including placebo or active

comparator medication) or marketed, manufactured by, licensed by, provided by or distributed by Merck for human use.

6MHP includes the 6 melanoma helper peptide vaccine.

Adverse events may occur during the course of the use of Merck product and 6MHP in clinical trials or as prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

Progression of the cancer under study is not considered an adverse event.

Unexpected AE – Any adverse event not described in section 7.2.5.

Serious AE: A serious adverse event is any adverse event occurring at any dose during the study that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose;
- Is another important medical event

Refer to Table 10 for additional details regarding each of the above criteria. **Note:** In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable to the Merck in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by Merck for collection purposes:

- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose.

Unanticipated problem - An unanticipated problem is any event/experience that meets ALL 3 criteria below:

- Is unexpected in terms of nature, severity or frequency given the research procedures that are described in the protocol-related documents AND in the characteristics of the subject population being studied.
- Is related or possibly related to participation in research. This means that there is a reasonable possibility that the incident may have been caused by the procedures involved in the research study.
- The incident suggests that the research placed the subject or others at greater risk of harm than was previously known or recognized OR results in actual harm to the subject or others.

Protocol Violation- A protocol violation is defined as any change, deviation, or departure from the study design or procedures of a research project that is NOT approved by the institution's IRB prior to its initiation or implementation, OR deviation from standard operating procedures, Good Clinical Practices (GCPs), federal, state or local regulations. Protocol violations may or may not be under the control of the study team or UVa staff. These protocol violations may be major or minor violations.

Suspected Adverse Reaction (as defined in 21 CFR 312.32 (a))- Any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

7.2.2 Attribution Assessment

Attribution – The determination of whether an adverse event is related to a medical treatment or procedure. The attribution groups are:

Definite – Applies to those adverse events which, the investigator feels are incontrovertibly related to study drug. An adverse event may be assigned an attribution of definitely related if or when (must have all of the following):

- It follows a reasonable temporal sequence from administration of the test drug.
- It could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- It disappears or decreases on cessation or reduction in dose with re-exposure to drug. (Note: This is not to be constructed as requiring re-exposure of the subject; however, the group of definitely related can only be used when a recurrence is observed.)
- It follows a known pattern of response to the test drug.

Probable – Applies to those adverse events for which, after careful consideration at the time they are evaluated, are felt with a high degree of certainty to be related to the test drug. An adverse event may be considered probably related if or when (must have three of the following):

- It follows a reasonable temporal sequence from administration of the test drug.
- It could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- It disappears or decreases on cessation or reduction in dose. There are important exceptions when an adverse event does not disappear upon discontinuation of the drug, yet drug-relatedness clearly exists (e.g. bone marrow depression, fixed drug eruptions, tardive dyskinesia).
- It follows a known pattern of response to the test drug.

Possible – Applies to those adverse events for which, after careful consideration at the time they are evaluated, a connection with the test drug administration appears unlikely but cannot be ruled out with certainty. An adverse event may be considered possibly related if or when (must have two of the following):

- It follows a reasonable temporal sequence from administration of the test drug.
- It could not readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- It follows a known pattern of response to the test drug.

Unlikely – Applies to those adverse events for which, after careful consideration at the time they are evaluated, are judged to be unrelated to the test drug. An adverse event may be considered unlikely if or when (must have two of the following):

- It does not follow a reasonable temporal sequence from administration of the test drug.

- It could readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- It does not follow a known pattern of response to the test drug.
- It does not reappear or worsen when the drug is re-administered.

Unrelated – Applies to those adverse events, which after careful consideration, are clearly and incontrovertibly due to extraneous causes (disease, environment, etc.).

7.2.3 Evaluating Adverse Events

An investigator who is a qualified clinician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 4.0. Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade recorded on the adverse event case report forms/worksheets.

All adverse events regardless of CTCAE grade must also be evaluated for seriousness.

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Table 10. Evaluating Adverse Events
An investigator who is a qualified clinician, will evaluate all adverse events as to:

V4.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of Merck product or 6MHP vaccine that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization for an elective procedure to treat a preexisting condition that has not worsened is not constitute a serious adverse event.. A pre-existing condition is a clinical condition that is diagnosed prior to the use of investigational product and is documented in the patient's medical history.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new cancer; (that is not a condition of the study)**; (**although not serious per ICH definition, is reportable to the Sponsor-Investigator within 24 hours and to Merck within 2 working days to meet certain local requirements); **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event for collection purposes. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours to the Sponsor and to Merck within 2 working days.	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause the Merck product or 6MHP to be discontinued?	
Relationship to investigational drug	Did the Merck product or 6MHP cause the adverse event? The determination of the likelihood that the Merck product or 6MHP caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between the Merck product and 6MHP and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely the Merck product or 6MHP caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to the Merck product or 6MHP such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of the Merck product or 6MHP? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

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Relationship to Merck product or 6MHP (continued)	**The following components are to be used to assess the relationship between the test drug and the AE: (continued)**	
	Dechallenge	Was the Merck product or 6MHP discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Merck product or 6MHP; or (3) the trial is a single-dose drug trial); or (4) Merck product(s) or 6MHP is/are only used one time.)
	Rechallenge	Was the subject re-exposed to the Merck product or 6MHP in this study? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) Merck product(s) or 6MHP is/are used only one time). NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY THE MERCK PRODUCT or 6MHP, OR IF REEXPOSURE TO THE MERCK PRODUCT OR 6MHP POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE U.S. CLINICAL MONITOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding the Merck product or 6MHP drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following	**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of a Merck product or 6MHP relationship).**	
Yes, there is a reasonable possibility of Merck product or 6MHP relationship.	There is evidence of exposure to the Merck product or 6MHP. The temporal sequence of the AE onset relative to the administration of the Merck product or 6MHP is reasonable. The AE is more likely explained by the Merck product or 6MHP than by another cause.	
No, there is not a reasonable possibility of Merck product or 6MHP relationship	Subject did not receive the Merck product or 6MHP OR temporal sequence of the AE onset relative to administration of the Merck product or 6MHP is not reasonable OR the AE is more likely explained by another obvious cause than the Merck product or 6MHP. (Also entered for a subject with overdose without an associated AE.)	

7.2.4 Adverse Event Classifications

Adverse events (AEs) are classified into sections, specified in the CTCAE v4.03. For specific classifications pertaining to the protocol, we specify the following:

Hematologic/Metabolic- Any AE coded under one of the following CTCAE v4.03 categories should be reported under the Hematologic/Metabolic adverse event classification:

Table 11. Hematologic/Metabolic Classifications

Section	AE
Blood and lymphatic	Anemia Leukocytosis
Investigations	**ALL EXCEPT:** Carbon monoxide diffusing capacity decreased Ejection fraction decreased Forced expiratory volume decreased Vital capacity abnormal Weight gain Weight loss
Metabolism and nutrition disorders	**ALL EXCEPT:** Alcohol intolerance Anorexia Dehydration Glucose intolerance Iron overload Obesity Tumor lysis syndrome

Non-hematologic/Non-Metabolic- Any AE not reported under hematologic/metabolic, ocular, or allergic/autoimmune, should be reported under the non-hematologic/non-metabolic adverse event classification.

Ocular – Any AE coded under one of the following CTCAE v4.03 Adverse Event Terms should be reported under the Ocular adverse event classification:

- Eye Disorders: Night blindness (nyctalopia)
- Eye Disorders: Papilledema
- Eye Disorders: Retinopathy
- Eye Disorders: Blurred vision
- Eye Disorders: Flashing lights
- Eye Disorders: Floaters

Allergic/Autoimmune – Only AEs coded as Immune System Disorder: Allergic reaction, autoimmune disorder, or anaphylaxis should be reported under the Allergic/Autoimmune adverse event classification. Other AEs coded under Immune System Disorder should be reported under Non-hematologic/Non-metabolic

7.2.5 Agent-Specific Expected Adverse Events List

7.2.5.1 6MHP

The following toxicities are those greater than grade 1 that are to be considered expected:

Table 102. Expected Toxicities for 6MHP vaccines

	Grade 2	Grade 3
Injection site reaction	+	+[a]
Ulceration	+	
Fatigue	+	

[a] Note: Injection site reaction with ulceration ≤2 cm is expected. This is consistent with the CTCAE v4.0 coding for ulceration (Grade 2). Other grade 3 injection site reactions are not expected.

7.2.5.2 Pembrolizumab

Treatment-related adverse reactions are described in Section 7 of the Investigator's Brochure.

7.2.5.3 Tumor Biopsies, Vaccine Site Biopsies, and Sentinel Immunized Node Biopsies

The following are toxicities that are greater than grade 1 and that are to be considered expected (up to grade 2):

- Bleeding
- Bruising
- Pain
- Infection
- Lymphedema
- Delayed wound healing
- Scarring
- Numbness

7.2.6 Dose-Limiting Toxicities

6MHP
A DLT of 6MHP vaccine is defined as any Grade 3 or greater hematologic or non-hematologic toxicity that is definitely, probably, or possibly related to the administration of the vaccine. Small ulcerations of the skin at vaccine sites are expected in a subset of subjects and are not considered DLTs. Ulcerations will be considered DLTs only if the ulcers are > 2 cm in diameter, require antibiotics or surgical debridement.

Pembrolizumab

A DLT of pembrolizumab is defined as any toxicity requiring permanent discontinuation per Table 6: Dose Modification Guidelines for Drug-Related Adverse Events.

Pembrolizumab and/or 6MHP
≥ Grade 2 ocular adverse events as defined below, regardless of the agent to which the event may be attributed:

1) A single ≥ Grade 2 treatment-related experience of the following adverse events will be classified as a DLT:
   - Eye Disorders: Night blindness (nyctalopia)
   - Eye Disorders: Papilledema
   - Eye Disorders: Retinopathy

Subjects will be referred for an ophthalmologic exam if any of these ocular adverse events occur.

2) For ≥ Grade 2 of the following, treatment will be held and subjects will be referred for an ophthalmologic exam. Evidence of eye inflammation will be a basis for discontinuation of pembrolizumab and 6MHP. If no inflammation is noted and subjects improve to ≤ Grade 1 by the next scheduled visit, subjects can resume treatment with both agents.
   - Eye Disorders: Blurred vision
   - Eye Disorders: Flashing lights
   - Eye Disorders: Floaters

7.2.7 Recording and Reporting Adverse Events

7.2.7.1 Time Span for Reporting Adverse Events

Reporting of AEs will begin when the subject is administered the study drug or has a study related biopsy. Reporting will continue through 30 days following cessation of treatment and at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described below. The investigator will make every attempt to follow all subjects with non-serious adverse events for outcome.

All adverse events that occur after the consent form is signed, but before treatment allocation must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Adverse events will not be collected for subjects during the pre-screening period (for determination of archival tissue status) as long as that subject has not undergone any protocol-specified procedure or intervention. If the subject requires a blood draw, fresh tumor biopsy etc., the subject is first required to provide consent to the main study and AEs will be captured according to guidelines for standard AE reporting.

7.2.7.2 Process for Recording Adverse Events

Dose-limiting toxicities

DLTs will be entered into OnCore within 5 calendar days of the study team learning of the event. DLT's that are deemed serious and unexpected will be submitted to the IRB per institutional guidelines (see below).

Other AEs

AEs must be recorded into the University of Virginia Cancer Center OnCore database per the guidelines in Table 113.

Table 113. AE reporting

High Risk Studies
Reporting requirements for AEs that occur within 30 days of the last dose of protocol specified treatment

	Grade 1	Grade 2		Grade 3				Grade 4 & 5
	Expected and unexpected	Expected	Unexpected	Expected		Unexpected		Expected and Unexpected
				Without hospitalization	With hospitalization	Without hospitalization	With hospitalization	
Unrelated Unlikely	OnCore 30 days[a]	OnCore 30 days	OnCore 30 days	OnCore 30 days	OnCore 15 days	OnCore 30 days	OnCore 15 days	OnCore 7 days
Possible Probable Definite	OnCore 30 days[a]	OnCore 30 days	OnCore 15 days	OnCore 30 days	OnCore 15 days	OnCore 7 days	OnCore 7 days	OnCore (24-hrs)* 7 days

*Enter into OnCore database within 24 hours if unexpected and definitely related to protocol specified treatment
Hospitalization defined as an inpatient hospital stay or prolongation of a hospital stay equal to or greater than 24 hours
[a] Grade 1 unexpected or expected hematologic/metabolic events will be recorded in the Cancer Center Database; however, regardless of attribution, these events do not have to be reported.

7.2.7.3 Recording Laboratory Values

The following laboratory values will be recorded in the UVA Cancer Center database, graded using the CTCAE v4.03 (if a grading category exists), and reported as described in Section 7.2.8.3.

1. Alk Phosphatase
2. ALT (SGPT)
3. ANA
4. AST (SGOT)
5. Bilirubin, total
6. Creatinine
7. Eosinophil #
8. Hepatitis B virus
9. Hepatitis C virus
10. beta-HCG
11. Hgb
12. HgbA1C
13. HIV
14. HLA type
15. LDH
16. Potassium
17. RF
18. Urinalysis
19. WBC
20. T3
21. TSH
22. Free T4
23. PT/INR and aPTT

Any abnormal laboratory values captured which are not included in the above list, but are considered to be pertinent positive clinical signs/symptoms, and laboratory results obtained as part of routine care of patients will be recorded in the UVA Cancer Center database and reported. If there is any doubt on the part of study personnel concerning what constitutes a pertinent positive finding, sponsor-investigator will be consulted.

7.2.7.4 UVA IRB Reporting Requirements

The University of Virginia is responsible for reporting to the UVA IRB-HSR per the following guidelines:

Table 124. UVA IRB-HSR Reporting Guidelines

Type of Event	To whom will it be reported:	Time Frame for Reporting	How reported?
Any internal event resulting in death that is deemed DEFINITELY related to (caused by) study participation (Note: An internal event is one that occurs in a subject enrolled in a UVa protocol.)	IRB-HSR	Within 24 hours	IRB Online and phone call www.irb.virginia.edu/
Internal, Serious, Unexpected adverse event.	IRB-HSR	Within 7 calendar days from the time the study team received knowledge of the event. *Timeline includes submission of signed hardcopy of AE form.*	IRB Online www.irb.virginia.edu/
Unanticipated Problems that are not adverse events or protocol violations This would include a Data Breach.	IRB-HSR	Within 7 calendar days from the time the study team received knowledge of the event.	Unanticipated Problem report form. *http://www.virginia.edu/vprgs/irb/HSR_docs/Forms/Reporting_Requirements-Unanticipated_Problems.doc)*
Protocol Violations (*The IRB-HSR only requires that MAJOR violation be reported, unless otherwise required by your sponsor, if applicable.)* Or Enrollment Exceptions	IRB-HSR	Within 7 calendar days from the time the study team received knowledge of the event.	Protocol Violation and Enrollment Exception Reporting Form *http://www.virginia.edu/vprgs/irb/hsr_forms.html*
Data Breach	The UVa Corporate Compliance and Privacy Office and ITC: if breach involves electronic data- UVa Police if breach includes such things as stolen computers.	As soon as possible and no later than 24 hours from the time the incident is identified. As soon as possible and no later than 24 hours from the time the incident is identified. IMMEDIATELY.	UVa Corporate Compliance and Privacy Office- Phone 924-9741 ITC: Information Security Incident Reporting procedure, http://www.itc.virginia.edu/security/reporting.html Ph(434) 924-7166

7.2.7.5 Reporting to the FDA

The Sponsor-Investigator for the study (the UVA PI or designee) is responsible for providing safety updates to the FDA per the following guidelines. The reporting times refer to the time the study team received knowledge of the AE.

Table 135. FDA Reporting Requirements

UVa PI HELD IND			
Type of Event	**To whom will it be reported:**	**Time Frame for Reporting**	**How reported?**
Life-threatening and/or fatal unexpected events related or possibly related to the use of the investigational agent.	FDA	Within 7 calendar days of the study team learning of the event	Form FDA 3500A (MedWatch) or narrative
Serious, unexpected and related or possibly related adverse events	FDA	Within 15 calendar days after the study team receives knowledge of the event	Form FDA 3500A (MedWatch) or narrative
All adverse events	FDA	Annually	IND annual report

7.2.7.6 Reporting of Pregnancy and Lactation to the Sponsor-Investigator and to Merck

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them), including the pregnancy of a male subject's female partner that occurs during the trial.

Pregnancies and lactations that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Pregnancies and lactations that occur from the time of treatment allocation/randomization through 120 days following cessation of Sponsor's product, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, must be reported by the investigator. All reported pregnancies must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor-Investigator and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-661-6229)

7.2.7.7 Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor-Investigator and to Merck

For purposes of this trial, an overdose will be defined as any dose exceeding the prescribed dose for pembrolizumab by 20% over the prescribed dose. No specific information is available on the treatment of overdose of pembrolizumab. In the event of overdose, pembrolizumab should be discontinued and the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event(s) is associated with ("results from") the overdose of a Merck product, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of Merck's product meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."

All reports of overdose with and without an adverse event must be reported within 24 hours to the Sponsor-Investigator and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-661-6229)

7.2.7.8 Immediate Reporting of Adverse Events to the Sponsor-Investigator and to Merck

Serious Adverse Events

For the time period beginning when the consent form is signed until treatment allocation/randomization, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study (see section 7.2.8.8) that occurs to any subject must be reported within 24 hours to the Sponsor-Investigator and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study whether or not related to the Merck product, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety.

Non-serious Events of Clinical Interest related to the Merck product will be forwarded to Merck Global Safety and will be handled in the same manner as SAEs.

Additionally, any serious adverse event, considered by an investigator who is a qualified clinician to be related to Merck product that is brought to the attention of the investigator at any time following consent through the end of the specified safety follow-up period specified in the paragraph above, or at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor-Investigator and to Merck Global Safety.

All subjects with serious adverse events must be followed up for outcome.

SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: +1-215-661-6229

A copy of all 15 Day Reports and Annual Progress Reports is submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators. Investigators will cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally investigators will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215-661-6229) at the time of submission to FDA.

All subjects with serious adverse events must be followed up for outcome.

7.2.7.9 Protocol-Specific Exceptions to Serious Adverse Event Reporting

Efficacy endpoints as outlined in this section will not be reported to Merck as described in Section 7.2.7.8.- Immediate Reporting of Adverse Events to the Sponsor and to Merck, unless there is evidence suggesting a causal relationship between the drug and the event. Any such event will be submitted to the Sponsor within 24 hours and to Merck Global Safety within 2 working days either by electronic or paper media.

Specifically, the suspected/actual events covered in this exception include any event that is disease progression of the cancer under study.

The Sponsor will monitor unblinded aggregated efficacy endpoint events and safety data to ensure the safety of the subjects in the trial. Any suspected endpoint which upon review is not progression of the cancer under study will be forwarded to Merck Global Safety as a SAE within 2 working days of determination that the event is not progression of the cancer under study

Hospitalization related to convenience (e.g.transportation issues etc.) will not be considered an SAE.

7.2.7.10 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be recorded as such on the Adverse Event case report forms/worksheets and reported within 24 hours to the Sponsor-Investigator and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-661-6229)

For the time period beginning when the consent form is signed until treatment allocation/randomization, any ECI, or follow up to an ECI, that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to Merck product, must be reported within 24 hours to the Sponsor and within 24 hours to Merck Global Safety.

Events of clinical interest for this trial include:

1. An overdose of Merck product, as defined in Section 7.2.7.7 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor-Investigator, that is not associated with clinical symptoms or abnormal laboratory results.

2. An elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

   *Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology.

7.2.8 Sponsor Responsibility for Reporting Adverse Events

All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations.

8.0 STATISTICAL CONSIDERATIONS

8.1 Design

This is an early phase trial to assess the safety and immunogenicity of the combination therapy of a helper peptide vaccine (6MHP) plus PD-1 blockade (pembrolizumab) in patients with advanced melanoma. In addition, exploratory analysis of clinical benefit will be described.

8.2 Evaluation of Sample Populations and Criteria

8.2.1 Safety

All participants receiving any protocol treatment will be evaluated for safety. The 6MHP vaccine has been safe, with lower toxicities than with class I peptides, in particular with lower autoimmune or hyperimmune toxicities. Based upon reported results in the patient population treated with pembrolizumab (51), drug-related serious adverse events occurred in 7/89 (7.9%, 95% CI(3.2, 15.5%)) of participants treated at the 2mg/kg dose every 3 weeks. These results are assumed to be reflective of the safety profile for the 200 mg study dose, as described in section 4.3.2. Thus, this trial will be designed with stopping rules for observed toxicities beyond those expected with pembrolizumab alone. Specifically, formal safety bounds based upon monitoring dose-limiting toxicities (DLT) attributed to 6MHP or pembrolizumab will guide decisions about early stopping due to potential safety concerns.

DLTs will be defined based on the agent to which they are attributed, as specified in section 7.2.6. If a stopping bound is crossed then accrual to the study will be suspended until the study PI, co-investigators and the DSMC can review the data, and determine if the study should continue, be amended or be closed to further accrual.

8.2.2 Immunogenicity

All eligible participants receiving any protocol treatment will be evaluated for the following immune response parameters.

Primary:

CD4+ T cell response to 6MHP peptides
CD4+ T cell responses to the 6MHP peptides will be evaluated in the blood using the direct ELIspot assay. In order to be classified as achieving a high durable immune response in the blood, at least two 5-fold increases over baseline must be observed in the blood.

Secondary:
Tumor Microenvironment (in the subset of participants for which tumor biopsies are obtained)
Infiltration of CD4+ and CD8+ T cells in the tumor microenvironment and Th1-dominant immune signatures will be evaluated by immunohistochemistry/immunofluorescence and/or by flow cytometry.

Evaluated in the blood and in the SIN (in the subset of participants for which SINs are obtained):
CD4+ T cell responses to the 6MHP peptides in the SIN.
Epitope-spreading for CD8+ T cells reactive to a panel of defined melanoma antigens will be evaluated by ELIspot assay.
IgG antibody responses to 6MHP will be evaluated by ELISA.

8.2.3 Clinical efficacy

All participants receiving any protocol treatment will be evaluated for clinical benefit to treatment as defined by the following measures.

- Clinical response by RECIST 1.1
- Clinical response by irRC
- Progression-free survival (PFS), defined as the time from the date of start of treatment to the date of progression or death from any cause, whichever occurs first. A participant who dies without a reported progression will be considered an event on the date of death. Participants who have neither progressed nor died will be censored on the date of last evaluable tumor assessment.
- Overall survival (OS), defined as the time from the date of start of treatment to the date of death from any cause. Participants who do not experience an event (death) will be censored at date of last follow-up/contact.

8.3 Sample Size and Accrual

Conditional on safety bounds not being crossed, maximum target sample size is based upon having sufficient information to obtain preliminary information on whether combined treatment with 6MHP vaccine plus pembrolizumab increases the immunogenicity of 6MHP alone in the entire study population. Data from Mel44 on immune response as measured by direct ELIspot in blood through month 12 for arms C and D (which included vaccination with 6MHP) resulted in a high durable response rate in the blood (at least two 5-fold increases) of 20% with an upper bound 90% CI of 26.7%. Lower responses (at least two 2-fold increases) occurred in 32% of subjects (upper bound 90% CI of 39.1%). Based upon these prior results, for this study we would be interested in detecting an improvement in the percent of high durable responders in the blood from a null rate of 20% to 45% (above the low responder upper limit) using a using a fixed sample design with approximate type I and type II error rates of 10% ($\alpha=\beta=0.10$). Note, Mel44 did not include assessments in the SIN thus sample size determination is based upon prior results for the blood only.

Accrual of a total of 22 eligible participants provides 90% power to test for a null high immune response rate of 20% vs an alternative high immune response rate of 45% with a one-side 10% level binomial test. At study conclusion the null hypothesis will be rejected and the treatment

regimen considered worthy of further study if at least 8/22 (36%) eligible participants experience a high durable immune response.

Maximum target sample size is estimated to be 25 participants, which assumes a 10% dropout/ineligibility/lost to follow-up adjustment. Accrual is estimated at approximately 12 participants per year. Considering enrollment as of May 2018, accrual to the study should be completed in June 2019.

8.4 Stratification

Participants will be monitored for post entry stratification by whether or not biopsies are obtained at baseline and day 22; (yes/no). Target sample size for participants for whom biopsies are obtained is set at a minimum of 12 eligible participants. If increased infiltration of $CD4^+$ response on day 22 compared to day 1 is observed in the tumor in 9/12 participants then the lower limit of a one-sided 90% confidence interval exceeds 50% and this would be considered a promising result and supportive of further study of the treatment regimen. Similarly, if increased infiltration of $CD8^+$ response on day 22 compared to day 1 is observed in the tumor in 9/12 participants then this would be considered a promising result and supportive of further study of the treatment regimen.

8.5 Safety Monitoring

Toxicities will be monitored using CTCAE 4.03 criteria. Stopping rules will be based on the serious adverse events rate of 7.9% (95% CI(3.2, 15.5%)) reported by Roberts, 2014. Significant increases over these rates are not expected but would be a reason for early discontinuation. Thus, formal safety bounds based upon the observed number of participants who experience a DLT related to 6MHP or pembrolizumab as defined in Section 7.2.6 will guide decisions about early stopping due to potential safety concerns. The rate of DLTs for early stopping will include assessment up to the day 43 treatment. DLTs will be monitored up to 30 days after the last study treatment and late observed rates that cross the bound would be reason to suspend accrual for a safety review by the Sponsor-Investigator and the DSMC. A sequential probability ratio test (SPRT) based upon a binomial test of proportions for DLTs will be used. Only the upper boundary will be used for monitoring to protect against excessive failure rates. The stopping boundary are for a SPRT contrasting a 15% versus 30% DLT rate, with nominal type I and II errors of 10% and 10%, respectively. The slope of the parallel lines for monitoring is 0.219 and the intercept for the upper bound is 2.476.

Table 146. Stopping guideline for DLTs

Number of participants	Boundary
4-6	≥ 4
7-11	≥ 5
12-15	≥ 6
16-20	≥ 7
21-25	≥ 8

8.6 Analyses

Study populations and evaluation criteria are noted in Section 8.2. Adverse events will be summarized by frequency and magnitude of event. Incidence of DLTs will be monitored against the safety bounds above. The bounds are non-binding, and provide a guideline that may result in study modification or closure

Previous methods used to measure responses to 6MHP include ELIspot assays, proliferation assays, and flow cytometry (Mel41, Mel44, E1602 trials). In the proposed trial, the study hypothesis will be tested using the direct ELIspot assay results from the blood. The criteria for defining immune responses have been reported for ELIspot assays (54). The circulating $CD4^+$ T cell response rate to 6MHP peptides in participants treated with 6MHP vaccine plus PD-1 will be assessed at each time noted in the study calendar. Hypothesis testing will be based upon the number of participants that satisfy the criteria of high durable immune response in the blood. The proportion of participants with high immune response overall as measured in the SIN or blood will be calculated along with 90% confidence intervals.

Secondary measures will also be used to evaluate 6MHP specific $CD4^+$ T cell responses. Flow cytometry has value in defining the responding cell population and the function of responding cells using multi-parameter staining. CyTOF mass cytometry also is evolving as a valuable method. Additional methods may be incorporated over time as new technologies are developed and validated; however, for the purposes of testing the study hypothesis, the direct ELIspot assay will used as the primary measure. A secondary endpoint will also be to assess the CD4+ T cell response to 6MHP in the sentinel immunized node, using methods used for assessment of circulating responses.

Our prior measures of immune response in the blood to the 6MHP resulted in high durable immune response rates of 20%. An observed high immune response rate of 36% overall would support further study of the treatment regimen. Other measures assessed over time in the blood include a) epitope-spreading for circulating $CD8^+$ T cells reactive to a panel of defined melanoma antigens by ELispot assay, and b) production of serum IgG reactive to 6MHP, by ELISA assay.

Assessments in the tumor microenvironment include assessment of CD4+ and $CD8^+$ T cells into melanoma by immunohistochemistry/immunofluorescence and/or by flow cytometry. Each participant will be assessed for the endpoints of interest and the proportion of participants experiencing an event (immune response, IgG antibody response to 6MHP, presence of $CD8^+$ T cell infiltrates into the tumor (a subset), etc) will be estimated along with 95% confidence intervals. Repeated measure models will be used to estimate changes over time in interval measurements in the blood and tissue.

Participants will be assessed for clinical response and will be classified by best overall response by week 52 and at final assessment. ORR will be estimated along with a 95% confidence interval around the point estimate.

TTR, PFS, DFS and OS distributions will be estimated by the product limit method of Kaplan and Meier. Other summary measures for time to event distributions will include estimates and 95% CIs for median and 1-year estimates to assess relative to published data. Exploratory proportional hazard models will be used to obtain preliminary estimates of potential associations with T cell and antibody responses to melanoma antigens and survival.

9.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

9.1 Investigational Product

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

Clinical Supplies will be provided by Merck and UVA-HITC as summarized in Table 15. The peptide vaccine will undergo stability testing as described in Appendix 12.6.

Table 158. Product Descriptions

Product Name & Potency	Dosage Form	Supplier
pembrolizumab 100 mg/ 4mL	Solution for Injection	Merck
6MHP	Lyophilized Powder for Injection	UVA
Montanide ISA-51	Solution for Injection	Seppic, Inc. (purchased by UVA)

9.2 Packaging and Labeling Information

Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

9.3 Clinical Supplies Disclosure

This trial is open-label; therefore, the subject, the trial site personnel, the Sponsor-Investigator and/or designee are not blinded to treatment. Drug identity (name, strength) is included in the label text; random code/disclosure envelopes or lists are not provided.

9.4 Storage and Handling Requirements

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label.

Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site.

Clinical supplies may not be used for any purpose other than that stated in the protocol.

9.5 Returns and Reconciliation

The investigator is responsible for keeping accurate records of the clinical supplies received from Merck or designee, the amount dispensed to and returned by the subjects and the amount remaining at the conclusion of the trial.

Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

10.0 ADMINISTRATIVE AND REGULATORY DETAILS

10.1 Study Conduct and Ethical Considerations

This study will be conducted in accordance with the standards of Good Clinical Practice, all applicable federal, state, and local laws, and in accord with the ethical principles that originated in the Declaration of Helsinki. The PI will ensure that staff are trained and carry out the study in accord with the protocol specifications. The PI will ensure that all study site personnel are aware that the study protocol and all data generated are confidential and should not be

disclosed to third parties (with the exception of local and national regulatory bodies which require access for oversight purposes).

10.2 UVA Institutional Review Board for Health Sciences Research

The UVA Institutional Review Board for Health Sciences Research (UVA IRB-HSR) will approve all aspects of this study, including the clinical trial protocol, informed consent documents, and patient materials. Modifications to the protocol or consent form will be reviewed and approved by the UVA IRB-HSR prior to implementation, except when necessary to eliminate apparent immediate hazards to the study subjects. The study will undergo continuing IRB review based on the level of risk as assessed by the IRB. This review will take place no less than annually. Reporting to the UVA IRB-HSR will occur as specified in Section 7.2.

10.3 Consent Forms and the Consenting Process

Consent forms will be written in accord with 21 CFR 50 and will be reviewed and approved by the UVA IRB-HSR prior to use. Subjects will be given an approved consent form to review. A member of the study team will explain the study and study procedures to each subject and will be available to answer questions. Informed consent will be obtained from each subject prior to conducting any study-specific procedures or administering study drug.

10.4 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the Sponsor of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

10.5 Maintenance of Study Documents

Signed consent forms and other research records will be retained in a confidential manner. Study records will be kept for at least 6 years after completion of the study.

10.6 Data Collection

Data will be collected using a centralized electronic case report form called **ON**-line **C**linical **O**ncology **R**esearch **E**nvironment = **Oncore**.

10.6.1 Endpoint Data

- Endpoint data will be collected using HITC IML data forms, subject-specific binders, and the HITC laboratory database.

- The HITC laboratory database, which has password-restricted access, is stored on the UVA Health System Computing Services secured server.

10.7 Monitoring Plan

The University of Virginia Cancer Center Data and Safety Monitoring Committee (CC DSMC) will provide oversight of the conduct of this study. The CC DSMC will report to the UVA Protocol Review Committee (PRC).

The UVA CC DSMC will review the following:

- All adverse events
- Audit results
- Application of study designed stopping/decision rules
- Whether the study accrual pattern warrants continuation/action
- Protocol violations

10.7.1 Monitoring Schedule

The UVA CC DSMC will meet every month for aggregate review of data. Tracking reports of the meetings are available to the PI for review. Issues of immediate concern by the DSMC are brought to the attention of the Sponsor-Investigator (and if appropriate to the PRC and IRB) and a formal response from the Sponsor-Investigator is requested. Per the UVA Cancer Center NIH approved institutional plan, this study will be audited approximately every 6 months. The audit may include direct access to source data/documents.

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12.0 APPENDICES

12.1 ECOG Performance Status

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.
* As published in Am. J. Clin. Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.* The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.	

12.2 Common Terminology Criteria for Adverse Events V4.0 (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for adverse event reporting. (http://ctep.cancer.gov/reporting/ctc.html)

12.3 Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 Criteria for Evaluating Response in Solid Tumors

RECIST version 1.1* will be used in this study for assessment of tumor response. While either CT or MRI may be utilized, as per RECIST 1.1, CT is the preferred imaging technique in this study.

* As published in the European Journal of Cancer:

E.A. Eisenhauer, P. Therasse, J. Bogaerts, L.H. Schwartz, D. Sargent, R. Ford, J. Dancey, S. Arbuck, S. Gwyther, M. Mooney, L. Rubinstein, L. Shankar, L. Dodd, R. Kaplan, D. Lacombe, J. Verweij. New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1). Eur J Cancer. 2009 Jan;45(2):228-47. [4]

12.4 AJCC Staging System

Melanoma TNM Classification

T Classification	Thickness	Ulceration Status
T1	≤ 1.0 mm	a: without ulceration or mitoses
		b: with ulceration or mitoses ≥ 1
T2	1.01 – 2.0 mm	a: without ulceration
		b: with ulceration
T3	2.01 – 4.0 mm	a: without ulceration
		b: with ulceration
T4	> 4.0 mm	a: without ulceration
		b: with ulceration

N Classification	# of Metastatic Nodes	Nodal Metastatic Mass
N1	1 node	a: micrometastasis*
		b: macrometastasis†
N2	2 – 3 nodes	a: micrometastasis*
		b: macrometastasis†
		c: in transit met(s)/satellite(s) without metastatic nodes
N3	4 or more metastatic nodes, or matted nodes, or in transit met(s)/satellites(s) with metastatic node(s)	

M Classification	Site	Serum Lactate Dehydrogenase
M1a	Distant skin, subcutaneous or nodal mets	Normal
M1b	Lung metastases	Normal
M1c	All other visceral metastases	Normal
	Any distant metastasis	Elevated

* Micrometastases are diagnosed after sentinel or elective lymphadenectomy.
† Macrometastases are defined as clinically detectable nodal metastases confirmed by therapeutic lymphadenectomy or when nodal metastasis exhibits gross extracapsular extension.

Stage Groupings for Cutaneous Melanoma

	Clinical Staging			Pathologic Staging		
	T	**N**	**M**	**T**	**N**	**M**
0	Tis	N0	M0	Tis	N0	M0
IA	T1a	N0	M0	T1a	N0	M0
IB	T1b	N0	M0	T1b	N0	M0
	T2a	N0	M0	T2a	N0	M0
IIA	T2b	N0	M0	T2b	N0	M0
	T3a	N0	M0	T3a	N0	M0
IIB	T3b	N0	M0	T3b	N0	M0
	T4a	N0	M0	T4a	N0	M0
IIC	T4b	N0	M0	T4b	N0	M0
III‡	Any T	N1-3	M0			
IIIA				T1-4a	N1a	M0
				T1-4a	N2a	M0
IIIB				T1-4b	N1a	M0
				T1-4b	N2a	M0
				T1-4a	N1b	M0
				T1-4a	N2b	M0
				T1-4a/b	N2c	M0
IIIC				T1-4b	N1b	M0
				T1-4b	N2b	M0
				Any T	N3	M0
IV	Any T	Any N	Any M1	Any T	Any N	Any M1

* Clinical staging includes microstaging of the primary melanoma and clinical/radiologic evaluation for metastases. By convention, it should be used after complete excision of the primary melanoma with clinical assessment for regional and distant metastases.

† Pathologic staging includes microstaging of the primary melanoma and pathologic information about the regional lymph nodes after partial or complete lymphadenectomy. Pathology stage 0 or stage 1A patients are the exception; they do not require pathologic evaluation of their lymph nodes.

‡ There are no stage III subgroups for clinical staging.

Staging for Mucosal Melanomas
This system is based on the staging of cutaneous melanomas.

Stage IIB: Clinically localized primary melanoma > 4mm thick
Stage III: Lymph node metastases
Stage IV: Distant metastases

12.5 New York Heart Association Disease Classification

Functional Capacity	Objective Assessment
Class I. Patients with cardiac disease but without resulting limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.	No objective evidence of cardiovascular disease.
Class II. Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of minimal cardiovascular disease
Class III. Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of moderately severe cardiovascular disease.
Class IV. Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.	Objective evidence of severe cardiovascular disease.

* The Criteria Committee of the New York Heart Association. Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels. 9th ed. Boston, Mass: Little, Brown & Co; 1994:253-256

12.6 Vaccine Lot Release and Stability Testing

A. Preparation of the synthetic melanoma and tetanus peptides
All peptides were synthesized under GMP conditions by Multiple Peptide Systems (San Diego, CA).

Peptide preparation and vialing were performed under GMP conditions by Clinalfa (Merck Biosciences AG, Laufelfingen, Switzerland). Documentation relating to the procedures used to prepare and vial the peptides were included in the Chemistry and Manufacturing Section of prior IND application applications (10825 and 12191).

B. Quality Assurance Testing

Prepared peptides were subjected to the following tests:

1. Identity. Identity was confirmed by structural studies. The individual peptides were tested for identity by mass spectrometry (to define molecular mass and amino acid sequence) and HPLC (to confirm purity) in a GMP laboratory (Polypeptide Group).

2. Purity. Purity was assessed before and after vialing the peptide mixtures. Before vialing the peptide mixtures, each synthetic peptide was evaluated for the presence of a single dominant species by high pressure liquid chromatography (HPLC) in a GMP laboratory (Polypeptide Group). Purity of each peptide component exceeded 90%. Variants of the original peptide may have included incomplete products of synthesis, minor degradation products due to oxidation of methionine residues, and dimerization of cysteine-containing peptides. After vialing the peptide mixture, purity was reconfirmed by HPLC in a GMP laboratory (Clinalfa).

3. Trifluoroacetic acid (TFA). The amount of total fluorine in each peptide preparation was less than 0.5% or 5000 ppm as determined by Multiple Peptide Systems.

4. Potency. Peptides are synthesized under GMP conditions and the net peptide content calculated for each. The amounts of each peptide (mcg quantities) added to the vaccine vials are calculated based on the net peptide content of the original stock of lyophilized peptides.

5. Pyrogenicity. Pyrogenicity testing was conducted by Clinalfa in accordance with USP guidelines.

6. General Safety. General safety testing was conducted by Clinalfa in accordance with USP guidelines.

7. Sterility. Sterility testing was conducted by Clinalfa in accordance with USP guidelines.
8. Stability. The peptide preparations were assayed for stability at months 3, 6, 12, 24, and 36 and were shown to be stable. The peptides will continue to be assessed yearly for stability while subjects are on active treatment. The following analyses will be performed to confirm stability.
    a. HPLC: HPLC will be performed to confirm purity. An optical comparison to previous HPLC data will be performed. Ideally, the purity of each peptide component will exceed 90% (94%-98%). Variants of the original peptide may include incomplete products of synthesis, minor degradation products due to oxidation of methionine residues, and dimerization of cysteine-containing peptides. Such minor variants will be tolerated as long as the peptide represents at least 75% of the intended peptide species. Because measures of peptide quantity are subject to variability, a peptide lot will be rejected only if two sequential measures fail to meet the criterion stated above

b. Sterility. One vial of peptide will be submitted to the Clinical Microbiology Laboratory at the University of Virginia or Microbiology Research Associates, Inc. (Acton, MA) for sterility testing.

12.7 Summary of Changes

Version Date	Description of Changes
7-13-18	1. Section 5.2.1.3: removed text "or baseline" from the general instructions in Table 6. Dose Modification and Toxicity Management Guidelines for Immune-related AEs Associated with Pembrolizumab.
6-14-18	1. Updated Merck safety fax number through the protocol document. 2. Removed references for draft text not used in version 6-01-18.
6-01-18	1. Study team updated to reflect personnel changes. 2. Signature page edited to reflect Sponsor-Investigator role. 3. Updated table of contents pages numbers. 4. Updated hyperlinks and corrected minor typographical errors throughout protocol document. 5. Sections 1, 2.1, 8: target enrollment reduced from 47 to 25 (22 eligible). 6. Sections 2, 3.1, 4.3.3.1, 6.1, 7.1.4.1, 7, 8.2.2: requirement for sentinel immunized node removed. 7. Sections 2.1, 5.2.1.4, 6: tumor imaging schedule was revised to match standard clinical care: -required scans at baseline, days 57 and 127 (with a window of ± 14 Days) and per standard care thereafter, -head scans at baseline and as clinically indicated thereafter. 8. Sections 3, 4.3.3.1: study endpoints modified to focus more on immune response and effects of therapy on the tumor microenvironment. 9. Section 4.3.1: added statement regarding factors associated with increased risk of recurrence. 10. Section 4.3.2: added statement regarding 2 year treatment duration. 11. Section 4.3.3.1: added text regarding measurement of T cell response, epitope spreading and the identification of neo antigens. 12. Section 5.1.1: clarified staging timing, use of AJCC v7, and that subjects should be eligible to receive pembrolizumab per clinician judgement. 13. Section 5.1.3: exclusion criteria 20 revised to indicate HGBA1C >8.5% as exclusionary. 14. Section 5.2.1.3: replaced Table 6 with revised Merck provided table "Dose Modification and Toxicity Management Guidelines for Immune-related AEs Associated with Pembrolizumab" 15. Section 5.2.1.4: edited table 7 to include biopsy and to indicate that scans ± 8 to 14 days from day 57 would not be considered a protocol violation. Section 5.4 16. Section 7.1.3: removed bulleted list of labs and updated table 9 for consistency. Removed redundant Table 10 and renumbered later tables. 17. Section 8: reduced number of subjects required to have biopsiable disease from 14 to 12.
04-04-17	1. Section 5.1.3: corrected exclusion criteria 4 regarding immunosuppressive therapy to within 7 days of registration to match inclusion criteria 6. 2. Section 5.8.: referenced section 7.1.5.3.1 and 7.2.7.8 regarding adverse event monitoring after study treatment discontinuation. 3. Section 6.1: removed comprehensive chemistry and CBC on days 8 & 15, and medication review at follow up visits after the safety follow up. Clarified biopsies may be collected at progression for research purposes or as part of clinical care in footnote "n". 4. Section 7.1.2.8.2: added section describing optional biopsies.

	5. Section 7.1.2.8.3: added section to address tissue removed as part of clinicanl care. 6. Section 7.2.5.1: clarified that an ulceration of 2 cm or less is considered an expected toxicity for the 6MHP.
10-24-16	1. Updated study personnel 2. Updated Table of Contents 3. Section 5.1.2 #3a: revised to clarify the entry criteria for patients who had previously received a PD-1/PD-L1 antibody and progressed during or after that therapy. Section 5.1.2 #3b: revised to broaden the patient population to include patients who have not experienced an objective clinical response after anti-PD1 antibody or anti-CTLA4/anti-PD1 combination therapy, and who are candidates to receive pembrolizumab therapy. 4. Section 5.1.3 #5: revised treatment window for prior monoclonal antibody treatment from 4 weeks to 3 weeks. 5. Section 7.1.3.1: clarified that LDH would be tested every 6 weeks.
09-07-16	1. Section 5.1.3: updated exclusion criteria of pneumonitis per Merck investigator letter dated 7/25/16. 2. Section 5.2.1.2: under 6MHP added “within” to clarify that the vaccine should be administered within 1-2 hours after mixing. 3. Section 5.2.1.4: removed extra “2 years” in table 7 next to last row. 4. Section 5.8: removed “up to one year of”, “study” and incorrect section reference, clarifying possible extended pembrolizumab treatment as part of clinical care. 5. Section 6.1: added safety labs on days 8 & 15, indicated concomitant medication review at all clinic visits, and removed LDH from end of treatment/post-treatment table as it is included in the comprehensive chemistry panel. 6. Section 7.1.5.2.2: removed “Phase” from heading as the second course treatment is not considered part of the study.
03-21-16	1. Editorial changes and typographical and formatting corrections were made throughout the study document. 2. Updated investigator list. 3. Section 1.0: added estimated average length of treatment per patient to the trial summary table in accordance with Merck updates to the protocol template. 4. Section 3.0: revised the objectives based on the expansion of the patient population to include both patients who are naïve for or received ipilimumab or other anti-CTLA-4 antibody in the adjuvant setting or in the therapeutic setting for advanced melanoma. The primary objectives have been changed to include safety and immunogenicity. Exploratory objectives have been revised to include estimates of clinical response. 5. Section 4.1: revised background to include text to address the immunologic objectives and removed text related to the clinical objectives that were previously listed as primary objectives. 6. Section 4.3.1: revised rationale to include text to address the new primary objective of immunogenicity. 7. Section 4.3.3.1: added the ELIspot assay, proliferation assay, and/or flow cytometry as techniques that will be used to measure immune response. 8. Section 4.3.3: added a phrase clarifying that this study is the first evaluation of and PD-1/PD-L1 antibody in combination with a helper peptide vaccine. 9. Section 5.1.2: revised (#3) to broaden the patient population. Subjects may be naïve for immunotherapy agents or have received interferon-alpha,

	ipilimumab or other CTLA-4 antibody, PD-1 antibody (or anti-PD-L1 antibody), interleukin-2, or prior cancer vaccines other than the 6MHP vaccine; however, patients who have received a PD-1/PD-L1 antibody must have experienced progression of melanoma after that therapy. 10. Section 5.1.2: revised (#5) to clarify that biopsies will be completed on sites that can be accessed safely. 11. Section 5.1.2: revised (#6). Combined exclusion criterion #8 with inclusion criterion #6. Added the following 3 bullet points to inclusion criterion #6: • Neurologic symptoms have returned to baseline, • There is no evidence of new or enlarging brain metastases, • Subjects are not using steroids for at least 7 days prior to registration Removed the following bullet point from entry criteria (#6) • The total number of brain metastases ever ≤ 3. 12. Section 5.1.2: revised (#11) to specify that female subjects of childbearing potential must use an adequate method of contraception. This change was based on updates to the Merck protocol template. 13. Section 5.1.2: revised (#12) to specify that male subjects of childbearing potential must use adequate method of contraception. A note clarifying that abstinence is acceptable has been added. This change was based on updates to the Merck protocol template. 14. Section 5.1.3: deleted exclusion criteria for anti-PD-1, anti-PD-L1, anti-PD-L2, or anti-CD137 (or any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathway, with the exception of ipilimumab). 15. Section 5.2.13: added the revised dose modification guidelines for drug-related adverse events. This change was based on updates to the Merck protocol template. 16. Section 5.2.1.4: corrected Table 7 a. to specify "safety" follow-up and to remove scans at the safety follow-up visit. b. Correct description of the timing of the follow-up visits 17. Section 5.2.1.4: corrected 18. Section 5.5.2: clarified that investigational agents (with the exception of pembrolizumab and 6MHP during active treatment) are not permitted. 19. Section 5.6: added a statement to clarify utilization of the treatment guidance (section 5.2.1). 20. Section 5.7.2: updated the contraception section of the protocol to clarify the criteria for non-reproductive potential, the conditions for using contraception and the acceptable methods of contraception. These changes were based on updates to the Merck protocol template. 21. Section 5.8: deleted the note referring to section 5.2.2 for unconfirmed disease progression. 22. Section 5.8: Clarified the length of time of treatment with pembrolizumab for the purposes of discontinuing from the study protocol. 23. Section 6.1: Deleted footnote "f" PT/INR and aPTT and removed PT/INR and aPTT from footnote "f" as these labs are not completed as part of the day 1 assessment. 24. Revised footnote "l" to specify that the safety follow-up visit for subjects who discontinue 6MHP will occur at the time of their next infusion of pembrolizumab. 25. Section 7.1.2.1: removed reference to the separate ECI guidance

	document. This document is no longer required (per Merck) as the criteria for capturing events of clinical interest have been modified (see section 7.2.7.10). This change was based on updates to the Merck protocol template. 26. Section 7.1.3.1: added LDH to the clinical lab list to be consistent with Table 9. 27. Section 7.2: revised the ordering of this section to improve readability. 28. Section 7.2.1: added a statement to clarify that progression of the cancer is not considered to be an adverse event. 29. Section 7.2.1: Revised description of collection of adverse events during pre-allocation baseline period and moved this paragraph to section 7.2.7.1. 30. Section 7.2.1: Added adverse events that are reportable to Merck within the same timeframe as SAEs, according to the Merck protocol template. 31. Section 7.2.3,Table 11: a. Added a statement to define pre-existing condition. b. Clarified reporting guidelines for the development of new cancers. c. Clarified reporting guidelines for an overdose. d. Added the term "investigational" when describing the relationship to the drug. e. Clarified the description of how to determine whether there is a reasonable possibility of Merck product or 6MHP relationship to an adverse event. 32. Section 7.2.6: Reordered this section to improve readability a. Revised the DLT definition for pembrolizumab to be consistent with guidelines for discontinuing treatment as outlined in table 6. b. Revised definition for ocular DLTs. c. Removed Grade 1 ocular toxicities as DLTs 33. Section 7.2.7.1: Added the second paragraph to describe the requirements for adverse event reporting after the consent form is signed, but before treatemt allocation/randomization occurs. This change was based on the language provided in the Merck template. 34. Section 7.2.7.6: Revised to describe the requirements for reporting pregnancies and lactation. Changes were based on the updated language provided in the Merck template. 35. Section 7.2.7.8: Revised the description for reporting SAEs to the Sponsor-Investigator and to Merck. These changes were based on the updated language provided in the Merck template. 36. Section 7.2.7.9: This section has been added. These changes were based on the updated language provided in the Merck template. 37. Section 8: The statistical section was revised based on the changes made to the study objectives. 38. Summary of changes: reordered list beginning with the most recent changes. 39. Summary of changes: corrected version date from 07-29-15 to 07-30-15.
07-30-15	1. Updated study personnel. 2. Updated Table of Contents 3. Updated Table Numbers. 4. Editorial changes throughout the document. 5. Added IND # to the protocol document. 6. Section 3.3: removed durable clinical response and durable disease control as exploratory objectives as these objectives were not meant to be included as part of the study design. 7. Section 5.1.2 (#7): deleted "and ≤ 6 months" as this time frame was incorrect for the intended population.

	8. Section 5.1.3 (#3): Added wording to clarify the window for receiving nitrosureas. 9. Section 5.1.6 (#6): Deleted reference to gamma knife administration as the timing of gamma knife administration is covered in the inclusion criteria. 10. Section 5.2.1.2: Revised to specify that vaccines 4-6 will be administered at the same site. 11. Section 6.1: Added footnotes "u" and "v" to the study flow chart to clarify reference to timing of the pregnancy test and to refer the reader to Table 9 for details on the comprehensive chemistry panel. 12. Section 6.1: Revised footnote "f" to include clarification for timing of PT/INR and aPTT 13. Section 6.1: Removed LDH as a separate line item as LDH is included as part of the chemistry panel (see Table 9). 14. Section 7.1.2.8.1: Added a statement to clarify that image guidance may be used during the biopsy procedures. This is consistent with section 5.1.2(#5). 15. Section 7.1.2.8.1: Revised the number of core biopsies from five to six. The intent was to include six core biopsies as described under the "Procedure" section of this portion of the protocol. 16. Section 7.2.5: Revised this section to move the language describing ocular dose-limiting toxicities to section 8.2.1.1 of the protocol. 17. Section 7.2.7.2: Changed the cross-reference for reporting from protocol section 9.6 to section 7.2.7.3. 18. Section 8.2.1.1: Revised the DLT section to : a) Clarify that small ulcerations are expected in a subset of subjects and are not considered DLTs. b) Reformat DLT list and the "Exceptions to DLT" list for pembrolizumab to include information in a table, per the suggestion from the IRB. c) Added a category of "Other drug-related toxicities not specified in 8.2.1.1" to the DLT Table 17. d) Moved the definition of ocular DLTs from section 7.2.5 to this section of the protocol. 19. Section 8.5: Added a statement to the Safety Monitoring section to clarify the timing of DLT monitoring.
05-20-15	1. Revised Table of Contents 2. Section 7.2.5: Added definitions for adverse event classifications for clarification. 3. Section 7.2.6.2: Revised this section to refer the reader to the tables in the investigator brochure for a description of treatment-related adverse events. 4. Section 7.2.6.3: Added delayed wound healing, scarring, and numbness to the list of expected toxicities for the biopsies. 5. Section 8.2.1.1: Revised the definitions of dose-limiting toxicities for 6MHP and pembrolizumab. The pembrolizumab definition has been based on unacceptable toxicity requiring discontinuation, per table 6. 6. Editorial changes have been made throughout the study.
05-18-2015	7. Updated header 8. Updated study personnel 9. Added the Investigator Statement page 10. Updated Table of Contents 11. Section 5.1.1: entry criteria revised for clarification of disease stage in response to PRC review. 12. Section 5.1.2 (#3): revised for clarification for subjects who fail ipilimumab in response to PRC review. 13. Section 5.1.3 (bullet point 8): revised for clarification of brain metastases

	in response to PRC review 14. Section 5.5.1: revised to include nasal corticosteroids and to be consistent with entry criteria. 15. Section 5.5.2: 1) revised to specify 6MHP and 2) revised to allow inhaled steroids at low doses and to be consistent with entry criteria. 16. Section 6.1: removed ECGs as part of the required testing as ECGs are not required with pembrolizumab treatment. 17. Section 7.2.2: 1) added "study drugs or protocol-specified procedures" for clarification of the definition of adverse event, 2) clarified the definition of serious AE to include events that occur during the study. 18. Section 7.2.6.2: added this section to specify the lab values that are required to be recorded in the UVA Cancer Center database. 19. Editorial comments were made throughout the study protocol.