UNIVERSITY OF VIRGINIA
HUMAN IMMUNE THERAPY CENTER

MEL 62

A PHASE I/II TRIAL TO EVALUATE THE SAFETY AND IMMUNOGENICITY OF A HELPER PEPTIDE VACCINE PLUS CTLA-4 BLOCKADE IN MELANOMA PATIENTS

Abbreviated title: 6PAC (6 helper Peptides plus Anti-CTLA-4)

ATTACH TO THE FRONT OF EVERY COPY OF THIS PROTOCOL

Signature Page

Sponsor/Sponsor's Representative

Name	Signature	Date

Investigator

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.

UNIVERSITY OF VIRGINIA
HUMAN IMMUNE THERAPY CENTER
MEL 62
A PHASE I/II TRIAL TO EVALUATE THE SAFETY AND IMMUNOGENICITY OF A HELPER PEPTIDE VACCINE PLUS CTLA-4 BLOCKADE IN MELANOMA PATIENTS

Sponsor	University of Virginia Human Immune Therapy Center	
Principal Investigator Director, Human Immune Therapy Center	Elizabeth Gaughan, MD University of Virginia 434-924-7678 emg5x@virginia.edu	
Co-Investigators	Craig L. Slingluff, Jr. 434-924-1730 cls8h@virginia.edu William Grosh, MD 434-924-1904 wwg9u@virginia.edu Lynn Dengel, MD ltd5b@virginia.edu Varinder Kaur, MD vk4q@virginia.edu	Carmel Nail, FNP-C 434-982-4042 cjn2r@virginia.edu Kathleen Haden, ANP-C 434-924-1730 km3s@virginia.edu Kimberly Bullock, PhD 434-924-0180 kb9d@virginia.edu Jennifer Eccles, MPAS, PA-C 434-243-0066 jme5j@virginia.edu
Biostatisticians:	Gina R. Petroni, PhD 434-924-8363 grp4c@virginia.edu	Mark Smolkin, MS 434-982-1032 mes6r@virginia.edu
Clinical Research Coordinators and Data Management	Emily Allred, PhD 434-982-1902 eh4m@virginia.edu Jessica Zareno, MS University of Virginia 434-982-1901 jhz4f@virginia.edu Sarah Lewis, BA University of Virginia 434-924-8709 set5v@virginia.edu	Alex Carney, MPH 434-982-6714 alc6a@virginia.edu Annika Shuali, MS University of Virginia 434-243-7242 ah2ze@virginia.edu
Authors:	Craig L. Slingluff, Jr., MD Kimberly Bullock, PhD	Gina R. Petroni, PhD

Table of Contents

Protocol Synopsis *7*
1.0 BACKGROUND AND SCIENTIFIC RATIONALE *13*
1.1 Introduction 13
1.2 Study Rationale 13
1.3 6MHP Vaccine 14
1.4 Montanide ISA-51 15
1.5 6MHP Administered in Montanide ISA-51 16
1.6 Checkpoint blockade with antibody to CTLA-4 21
1.7 Dosing 24
1.8 Summary 24
2.0 OBJECTIVES *25*
2.1 Primary objectives and endpoints 25
2.2 Secondary objectives and endpoints 25
2.3 Exploratory objectives and endpoints 25
3.0 PARTICIPANT SELECTION CRITERIA *25*
3.1 Inclusion Criteria 25
3.2 Exclusion Criteria 27
3.3 Registration and Randomization 29
4.0 STUDY DRUG (6MHP and MONTANIDE ISA-51) *30*
4.1 Peptide Synthesis 30
4.2 Storage of Individual Peptides 30
4.3 Reconstitution and Vialing of the Vaccine 30
4.4 Vaccine Storage 30
4.5 Lot Testing 30
4.6 Stability testing 30
4.7 Labeling 30
4.8 Montanide ISA-51 31
4.9 Study Drug Accountability 31
5.0 IPILIMUMAB *31*
5.1 Packaging and Labeling 31
5.2 Storage 31
6.0 TREATMENT PLAN *31*

6.1 Management of Participants 31
6.2 Administration of 6MHP 31
6.3 Administration of Ipilimumab 32
6.4 Dose Modifications 32
6.5 Dose Delays 33
6.6 Discontinuation of Therapy 34
6.7 Replacement of Study Participants 35
6.8 Concomitant Medications 35
6.9 Permitted Medications or Treatments 36
6.10 Biopsies 36
7.0 CLINICAL AND LABORATORY EVALUATIONS 36
7.1 Physical Exams and Evaluations 37
7.2 Pathology Review 37
7.3 Performance Status 37
7.4 Clinical Labs 37
7.5 Toxicity Assessments 37
7.6 Research Blood Samples 38
7.7 Tumor Biopsies 38
7.8 Sentinel Immunized Node Biopsy 40
7.9 Assessments 40
7.10 Study Calendar 41
8.0 STATISTICAL CONSIDERATIONS 41
8.1 Design 41
8.2 Evaluation of Sample Populations and Criteria 41
8.3 Sample Size and Accrual 43
8.4 Cohort and post entry classification 43
8.5 Safety Monitoring 43
8.6 Analyses 44
9.0 ADVERSE EVENT DATA COLLECTION AND MONITORING 45
9.1 Definitions 45
9.2 Attribution Assessment 46
9.3 Data collection 48
9.4 Risks and Safety 48

9.5 Adverse Event Classifications 49
9.6 Reporting Adverse Events 50
9.7 Adverse Event Review and Monitoring 52
9.8 Recording Laboratory Values 53
9.9 Dose-limiting Toxicities (DLT) 54
9.10 Management of Toxicity 54
9.11 Data Collection 54
9.12 Monitoring Plan 54
10.0 STUDY CONDUCT AND ETHICAL CONSIDERATIONS 55
10.1 UVA Institutional Review Board for Health Sciences Research 55
10.2 Consent Forms and the Consenting Process 55
10.3 Maintenance of Study Documents 55
11.0 APPENDICES 56
Appendix 1: Study Calendar 57
Appendix 2: AJCC Staging System 59
Appendix 3: ECOG Performance Status 61
Appendix 4: New York Heart Association Disease Classification 62
Appendix 5: RECIST 1.1 Criteria 63
Appendix 6: NCI Common Terminology Criteria for Adverse Events v4 64
Appendix 7: Vaccine Lot Release and Stability Testing 65
Appendix 8: Summary of Changes 67
Reference List 70

Protocol Synopsis

Title
A Phase I/II trial to evaluate the safety and immunogenicity of a helper peptide vaccine plus CTLA-4 blockade in melanoma patients (Mel62; 6PAC)

Investigational Drugs

- 6MHP: a mixture of 6 synthetic melanoma-derived class II MHC-restricted helper peptides
- Montanide ISA-51

Table 1. Peptides used in the 6 Melanoma Helper Peptide (6MHP) vaccine		
Sequence	**Epitope (source protein, residues)**	**Reference**
AQNILLSNAPLGPQFP	Tyrosinase $_{56\text{-}70}$ (alanine added#)	(1)
FLLHHAFVDSIFEQWLQRHRP	Tyrosinase $_{386\text{-}406}$	(2)
RNGYRALMDKSLHVGTQCALTRR	Melan-A/MART-1$_{51\text{-}73}$	(3)
TSYVKVLHHMVKISG	MAGE-3 $_{281\text{-}295}$	(4)
LLKYRAREPVTKAE	MAGE-1,2,3,6 $_{121\text{-}134}$	(5)
WNRQLYPEWTEAQRLD	gp100 $_{44\text{-}59}$	(6,7)

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

FDA-approved agent: Ipilmumab 3 mg/kg IV every 3 weeks for four doses

Objectives and endpoints

Primary:

Safety: To determine whether the combination of 6MHP vaccine plus ipilimumab is safe as evaluated by adverse event assessments, including CTCAE 4.03 and subclassified by irAE categories.

Immunogenicity: To estimate in the blood and in the sentinel immunized node the $CD4^+$ T cell response rate to 6MHP peptides in patients treated with 6MHP vaccine plus ipilimumab.

Secondary:

Induction of epitope-spreading: To estimate the proportion of patients in whom the combination of 6MHP vaccine plus ipilimumab induces epitope-spreading for $CD8^+$ T cells in the blood and in the sentinel immunized node that are reactive to a panel of defined melanoma antigens.

T cell infiltration of the tumor microenvironment (for cohorts 1 and 2): To determine whether the combination of 6MHP vaccine plus ipilimumab increases infiltration of $CD8^+$ and/or $CD4^+FoxP3^{neg}$ T cells into melanoma metastases in at least 50% of patients with tumor evaluable pre- and post-treatment.

Exploratory:

Induction of antibody responses: To estimate the production of serum IgG reactive to 6MHP in patients treated with 6MHP vaccines plus ipilimumab.

Clinical outcome (for cohorts 2 and 3): To obtain preliminary data on risk of recurrence and time to recurrence after resection of tumor to no evidence of disease and administration of the combination of 6MHP vaccine plus ipilimumab.

Associations of immune response and clinical outcome. To obtain preliminary estimates of the associations between T cell and antibody responses to melanoma antigens and survival and with disease control.

Clinical outcome (for cohort 1 only): To obtain preliminary estimates of clinical outcomes, including overall survival, progression-free survival, and clinical response by RECIST 1.1, following administration of the combination of 6MHP vaccine plus ipilimumab.

Design
This is an open-label, phase I/II study.

Treatment Cohorts:

Cohort 1: Unresectable stage III/IV advanced melanoma:

Cohort 2: Neoadjuvant therapy for patients with any of the following clinical presentations, when disease is amenable to complete surgical resection to render the patient clinically free of disease:

a. Primary melanoma with clinically evident lymph node involvement
b. Primary melanoma with clinically evident in transit disease, with or without clinically evident lymph node involvement.
c. In transit recurrent disease amenable to complete excision.
d. Soft tissue or visceral metastatic lesion, amenable to complete surgical excision to render patient free of evident disease

Cohort 3: Adjuvant therapy, to start within 12 weeks of surgery, for:

a. Stage IIA melanoma after complete excision and with class 2 DecisionDx
b. Stage IIB-IV, NED

Treatment Regimen:
All patients will receive 1) the 6MHP vaccine administered in Montanide ISA-51 adjuvant (incomplete Freud's adjuvant) and 2) ipilimumab, an anti-CTLA-4 blocking antibody. Ipilimumab will be administered at 3 mg/kg IV infusion on days 1, 22, 43, and 64. Vaccines will be administered days 1, 8, 15, 43, 64 and 85. On days when both ipilimumab and vaccine are administered, the vaccine may be administered before or after ipilimumab, but preferably before. All peptide vaccines will be administered intradermally and subcutaneously. Blood and biopsy samples of a vaccine-draining node and tumor will be analyzed for immunologic changes. Details are provided in the protocol schema (Figure 1, Figure 2, Figure 3) and in the study calendar (x-page; Appendix 1).

Figure 1: Protocol Schema Cohort 1

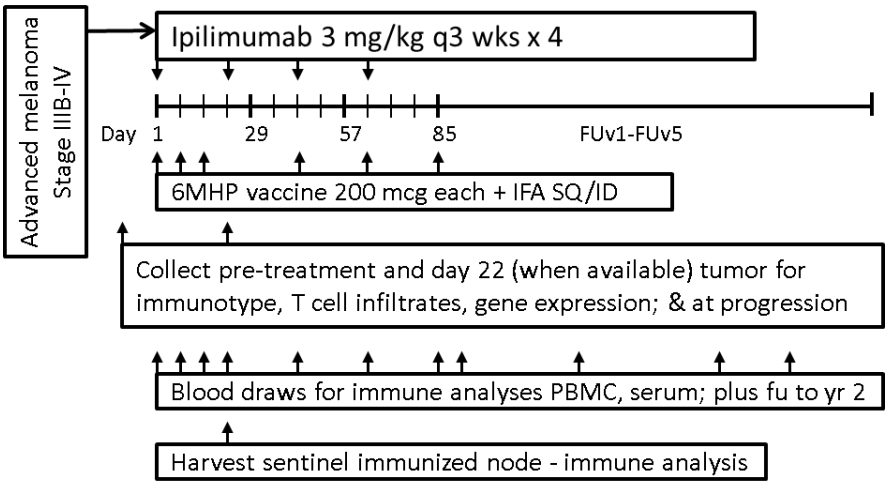


Figure 2: Protocol Schema Cohort 2

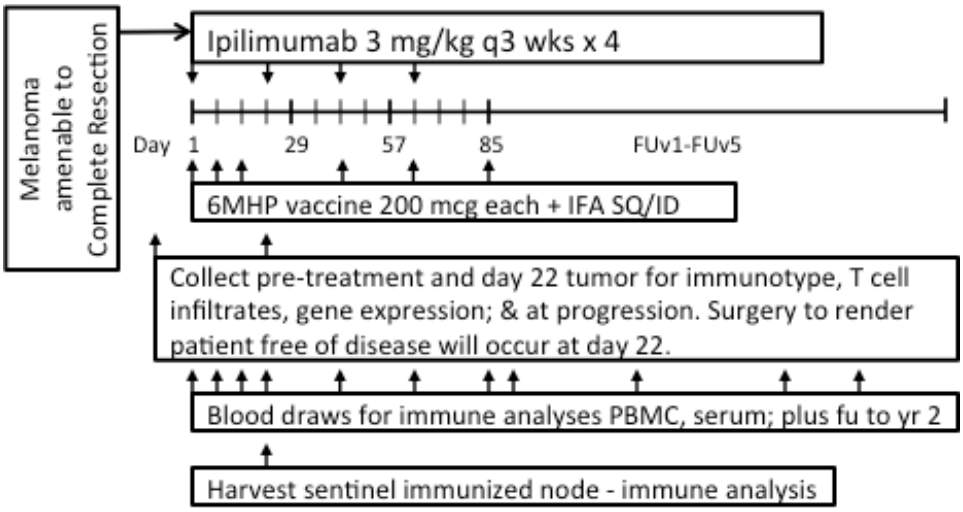
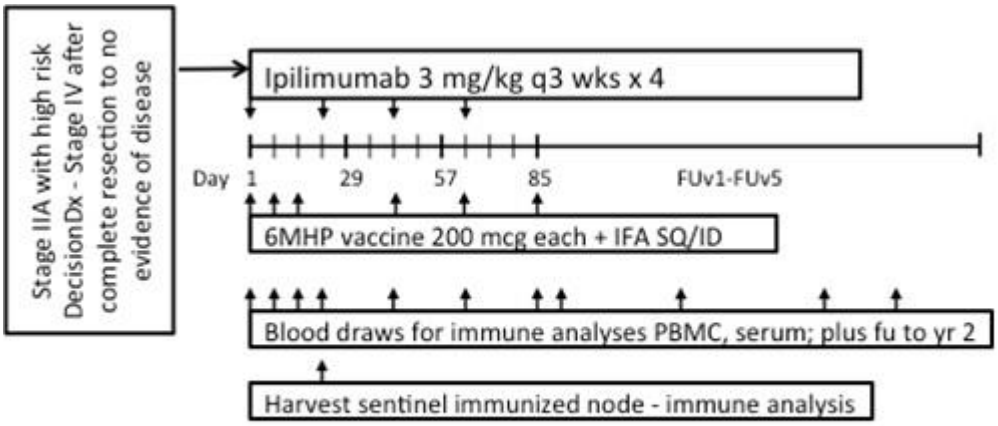


Figure 3: Protocol Schema Cohort 3



Biopsies

Tumor

For participants with metastases safely accessible for biopsy, biopsies will be obtained at baseline and day 22. Such biopsies must be obtained from at least 14 patients in this study. Additional optional biopsies may be performed throughout the study or during follow-up (e.g. at the time of disease progression).

Sentinel Immunized Node

A lymph node draining the 3rd vaccine site (sentinel immunized node (SIN)) will be biopsied on day 22.

Population: The main criteria for inclusion include:

A. Age 18 or older
B. Participants with
    a. Cohort 1 (Advanced Patients): Unresectable stage III or IV melanoma with clinical or radiographic evidence of disease.
    b. Cohort 2 (Neoadjuvant therapy): primary melanoma with clinically apparent lymph node or in transit/satellite lesions with or without lymph node involvement or recurrence amenable to complete resection to no evidence of disease
    c. Cohort 3 (Adjuvant therapy): Stage IIA with class 2 DecisionDx Score, or Stage IIB-IV melanoma resected to no evidence of disease. These patients may have had cutaneous, uveal, mucosal or unknown primary melanoma.
C. Patients may have had cutaneous, uveal, mucosal or unknown primary melanoma
D. Participants must be eligible to be treated with ipilimumab based on clinician judgement
E. ECOG performance status of 0 or 1

Accrual Goal:

To evaluate study endpoints target accrual is estimated at 24 eligible participants, of whom at least 14 must have biopsiable disease. Based upon prior studies, a 10% dropout/ineligibility/lost to follow-up among study participants is possible; therefore maximum accrual to the study is set at 27 participants. The annual accrual rate is projected to be approximately 16 participants; thus, accrual to the study should be completed in less than two years.

List of Abbreviations

Abbreviation	Full text
β–HCG	Beta Human chorionic gonadotropin (pregnancy test)
6MHP	6 melanoma-derived class II MHC-restricted helper peptides
12-MP	12 melanoma-derived class I MHC-restricted peptides
AE	adverse event
AJCC	American Joint Committee on Cancer
ALT	alanine aminotransferase
ANA	Anti-nuclear antibodies
ANC	absolute neutrophil count
APC	antigen presenting cell
AST	aspartate aminotransferase
BRAFi	BRAF inhibitor
CC	Cancer Center
cc	cubic centimeter
CFR	Code of Federal Regulations
cm	centimeter
CR	complete response
CRF	case report form
CT	computed tomography
CTA	cancer-testis antigens
CTCAE	Common Terminology Criteria for Adverse Events
CTL	cytotoxic T lymphocyte
CTO	Clinical Trials Office
Cy	cyclophosphamide
DC	dendritic cells
DCR	disease control rate
dL	deciliter
DLT	dose limiting toxicity
DSMC	Data and Safety Monitoring Committee
ECOG	Eastern Cooperative Oncology Group
ELISA	enzyme linked immunosorbent assay
FACS	fluorescence activated cell sorter
FBS	fetal bovine serum
FDA	Food and Drug Administration
g	Gram
GM-CSF	granulocyte-macrophage stimulating colony
GMP	good manufacturing practice
Hgb	hemoglobin
HGBA1C	hemoglobin a1c
HCV	hepatitis C virus
HITC	Human Immune Therapy Center
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HPLC	high performance liquid chromatography
id	intradermal
IFN	interferon
IL-2	interleukin-2
IML	Immune Monitoring Laboratory
IND	investigational new drug
ip	intraperitoneal
IRB	Institutional Review Board
IU	international unit
IV	intravenous
kg	kilogram
LDH	lactate dehydrogenase
m	meter
mcg	microgram

mcl	microliter
MDP	melanocyte differentiation proteins
mg	milligram
MHC	major histocompatability complex
ml	milliliter
mm	millimeter
MRI	magnetic resonance imaging
NCI	National Cancer Institute
NED	no evidence of disease
NSAID	non-steroidal anti-inflammatory drug
PBL	peripheral blood lymphocytes
PD-1	Programmed-death 1
PET	positron emission tomography
PI	principal investigator
PR	partial response
PRC	protocol review committee
Rf	Rheumatoid factor
SD	stable disease
SQ	subcutaneous
SIN	sentinel immunized node
T_h	$CD4^+$ helper T cells
TIL	tumor infiltrating lymphocytes
TPF	Tissue Procurement Facility
ULN	upper limits of normal
USP	United States Pharmacopeia
UVA	University of Virginia
WBC	white blood cell

1.0 BACKGROUND AND SCIENTIFIC RATIONALE

1.1 Introduction

Cancer immunotherapy for solid tumors is coming of age, with FDA-approved immuno-therapeutics in prostate cancer, melanoma, non-small cell lung cancer and renal cell cancer. Interleukin-2 (IL-2), the CTLA-4 blocking antibody (ipilimumab), and PD-1 blocking antibodies (pembrolizumab and nivolumab) are approved for melanoma; all induce durable clinical regressions. In 2015, the combination of ipilimumab and nivolumab therapy for advanced melanoma was approved based on high response rates and improvement in progression free survival over ipilimumab alone (8). Other antibodies that block PD-1/PD-L1 also induce durable clinical regressions of melanoma, renal cell cancer, and non-small cell lung cancer (NSCLC) (9). Furthermore, antigen-specific adoptive T cell therapy induces clinical regressions that are durable in about 20% of treated patients (10). 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, these 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 3 years, a cancer vaccine has been approved for prostate cancer, based on two randomized trials showing improved survival (11). Also, a randomized prospective trial in melanoma met its primary endpoint, showing that adding a peptide vaccine to high-dose IL-2 significantly prolonged progression-free survival (PFS) when compared to IL-2 alone (12). Thus, after several decades of development and optimization, there is now evidence that 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 induce $CD4^+$ helper T cell (T_H) responses (6 helper peptides, 6MHP), and which has clinical activity in patients with advanced melanoma (13). Goals of the current protocol are to obtain preliminary data on whether the combination of the 6MHP vaccine with immune checkpoint blockade is safe and improves immunologic and clinical outcomes compared to prior experience with each single agent alone. The proposed trial also will incorporate correlative studies of immune responses in blood, skin, lymph nodes, and tumor to obtain a more complete understanding of the host: tumor relationship in the context of peptide and immune checkpoint blockade combination therapies. The proposed study holds promise:

- To extend the life of patients with advanced melanoma,
- To reduce the risk of recurrence of resected melanoma, and
- To obtain preliminary data on whether checkpoint blockade increases helper T cell responses to vaccine and clinical response rate

1.2 Study Rationale

Evidence for the Role of the Immune System in Protecting Against the Development of Solid Tumors

There has long been evidence of immune responses to cancer, but evidence of impact on tumor progression has not been well demonstrated until recently. Convincing evidence of the importance of immune surveillance in preventing the development of solid tumors is provided by work in murine models. In knockout

mice lacking STAT1 and/or IFNγ receptor function, 50-100% developed spontaneous solid tumors (benign and malignant) of various histologies within 12-15 months, whereas normal mice never developed malignancies during the same time period (14). These studies strongly support the role of cellular immune function in the control of cancer progression.

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 depletion of $CD4^+$ T-cells abrogates all or part of protective immune response to vaccines (15). Furthermore, adoptive therapy with $CD4^+$ T-cells has been shown to induce tumor protection in some model systems (16,17). Thus, the protective immunity induced by tumor cell vaccines appears to be mediated both by $CD8^+$ T-cells and by $CD4^+$ T-cells.

Activated T_h cells express CD40L and can activate dendritic cells (DC) through ligation of CD40, for heightened antigen presentation, causing the DC to secrete IL-12 and other cytokines that may help to direct the immune response. Furthermore, strong T_h1 help produces the proper cytokine milieu which is critical to the induction of immune-mediated tumor destruction (17-19). In addition, T_h responses are believed to be involved in the establishment of memory responses.

1.3 6MHP Vaccine

Selection of Class II MHC-restricted Epitopes for Melanoma-reactive T_h Cells

In the current protocol we are including class II MHC-restricted peptides derived from shared melanoma proteins in an effort to generate melanoma-specific T_h responses. The melanoma-specific class II MHC-restricted peptides (Table 1) are derived from melanocytic differentiation proteins (MDP) and cancer-testis antigens (CTA). The peptides were originally reported to bind to HLA-DR1, -DR4, -DR11, -DR13, and/or -DR15, and approximately 90% of the melanoma patient population will express at least one of those class II alleles. Our prior work has demonstrated that these peptides, like other HLA-DR restricted peptides are also presented promiscuously on many other HLA-DR molecules (20); thus, we do not restrict enrollment based on HLA expression.

The melanoma-associated class II MHC-restricted peptides in the 6MHP vaccine include 4 from MDPs (tyrosinase (2), gp100 (1), MelanA/MART-1 (1)), and 2 from CTAs (MAGE proteins). The first report of HLA-DR-restricted peptides recognized by T-cells on melanoma identified $tyrosinase_{56-70}$ and $tyrosinase_{448-462}$ (1). Both peptides require high concentrations to induce T cell responses, but the former peptide has a higher binding affinity for HLA-DR4 than the latter; therefore, $tyrosinase_{56-70}$ (QNILLSNAPLGPQFP) was chosen for use in this study. A DR15-restricted peptide, $tyrosinase_{386-406,}$ was reported also to be an antigen for T_h cells and was selected for use in this study (2). Peptides presented by HLA-DR4 from MART-1/Melan-A have been identified and we have chosen to include Melan-A/$MART\text{-}1_{51-73}$ in this trial. The last MDP represented in the vaccine is gp100, from which a peptide at residues 44-59 has been identified. T-cells sensitized against this peptide can recognize melanoma cells, and this epitope has been demonstrated to be naturally processed and presented in the context of HLA-DR4 (6,7,21).

The peptides from CTA to be included are from MAGE proteins. The peptide $MAGE\text{-}A3_{281-295}$ can stimulate peptide reactive CTL in vitro and is strongly recognized by DR11-restricted MAGE-3 reactive CTL (4). Another MAGE peptide is

homologous with MAGE-1, 2, 3, and 6, and is restricted by DR13. It represents MAGE $_{121-134}$ (5).

Immune Monitoring Assays for $CD4^+$ T-cells

An essential step in the development of effective cancer vaccines is identification of the immune response parameters that effectively measure relevant immunologic endpoints, such as immunogenicity. Ideally, these endpoints will be associated with clinical response. A number of immunologic assays have been evaluated over the years for their ability to serve as sensitive and reliable tools for immune monitoring purposes.

One method for measuring epitope-specific helper T cell responses is the ELIspot assay, which was developed to evaluate functional antigen-specific responses by permitting direct counting of T-cells reacting to antigen by production of IFNγ or other cytokines (22-24). T-cells that are not anergized should secrete IFNγ after exposure to their cognate antigen, especially if they have a memory phenotype (25). ELIspot assays can reproducibly detect functional T cell responses to defined antigens at levels below 0.01% (22,26), and they do not require prolonged *in vitro* culture prior to evaluation.

We have also found measures of proliferation, by incorporation of tritiated thymidine, are effective for detection of helper responses, and that multiparameter flow cytometry enables detection of multifunctional T cells and differentiating $CD4^+$ from $CD8^+$ T cell responses. For the present study, we propose to use flow cytometry as the primary assay, and ELIspot assays as a secondary assay. Characterization of the helper T cell response may aid in detection of differences in the immunologic milieu when the vaccines are administered. This can be achieved by measuring cytokines secreted into the media by $CD4^+$ T cells proliferating in response to antigen, using an ELISA assay (27)or by flow cytometry, with calculation of the $T_h1/T_h2/T_h17$ balance.

1.4 **Montanide ISA-51**

Montanide ISA-51 as a Vaccine Adjuvant

Montanide ISA-51 adjuvant has been effective at inducing immune responses against murine viral antigens when administered with a synthetic peptide epitope (28,29) and is widely used as a vaccine adjuvant in veterinary practices. The product consists of a mineral oil base similar to incomplete Freund's adjuvant (IFA). However, the Arlacel A emulsifying agent of incomplete Freund's, which has caused reactions in the past, has been replaced with a purified manoside monooleate called "montanide", which appears to be safer. The UVA HITC has sponsored studies where peptide-based vaccines in Montanide ISA-51 have been safely administered to more than 600 participants. Immunological responses against the immunizing peptides have been detected in most participants (13,30-34).

Montanide ISA-51 may or may not be an optimal adjuvant; concerns have been raised about its usefulness as an adjuvant with a short peptide vaccine, attributed to changes from a prior formulation to the current one (34), but in our hands, we found that both formulations were very similar in their effectiveness as vaccine adjuvants with peptide vaccines (34). Another concern is that vaccination with short (9-mer) peptides in IFA induces chronic inflammation at the site of vaccination, with retention of antigen-reactive T cells at the vaccine site, both in mice and humans (35,36). However, this does not appear to be the case with longer peptides (35). We believe, thus, that these 14-23 mer peptides in 6MHP are

not likely susceptible to retention at the vaccine site; so that IFA is a good adjuvant to use.

1.5 **6MHP Administered in Montanide ISA-51**

Immunologic and Clinical Results from Studies in Advanced Melanoma: Mel41 and E1602

In two prior advanced melanoma studies, one sponsored by the UVA-HITC (Mel41) and one sponsored by the Eastern Cooperative Oncology Group (E1602), the 6MHP vaccine was administered with the adjuvant Montanide ISA-51 plus GM-CSF (13,30). In the Mel41 trial, 81% of patients developed T cell responses to 6MHP (57% as detected in the blood; 78% in a vaccine-draining node) (13), and in the E1602 trial, helper T cell responses to 6MHP were detected in 40% of patients in the blood (30).

This vaccination strategy was associated also with clinical regressions in 7-12% of patients and with stable disease for an additional 12-29% (mean disease control rate (DCR) 30%, Table 2). In the Mel41 trial, durations of SD and clinical responses have ranged from 1 to 7 years (13).

Table 2. Clinical response rates to 6MHP vaccines in patients 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	42	3	15	7.1%	36%
E1602 Arm C	32	2	8	6.3%	25%
All studies	91	7	27	7.7%	30%
Mel41+Arm D	59	5	19	8.5%	32%

Incorporation of GM-CSF with Peptide-Based Vaccines: Results From Prior Clinical Studies, Mel43 and Mel44.

In a separate peptide-based clinical study for patients with stage IIB-IV resected melanoma (Mel43), we demonstrated that GM-CSF did not increase immunogenicity of a peptide-based vaccine compared to IFA alone. Instead, we found that IFA alone was a better adjuvant than IFA+GM-CSF for induction of $CD4^+$ T cell responses to an intermediate length helper peptide (33). Though GM-CSF continues to be used as a vaccine adjuvant in other settings, studies by us and others (37) provide randomized prospective data on its use in humans in combination with other adjuvants - in both cases, the addition of GM-CSF was associated with lower immunogenicity, worse clinical outcome, or both (33,37). Thus, as a follow-up to the Mel43 study, we evaluated in another trial (Mel44), the immunogenicity of the 6MHP vaccine in Montanide ISA-51, without GM-CSF (32). Mel44 included patients with no clinical evidence of disease (resected stage IIB-IV), and immune responses to 6MHP in the blood were detected in 48% of patients by direct ELISpot assay (32).

Co-administration of the 6MHP vaccine with a peptide vaccine that stimulates $CD8^+$ T cells: Results from Mel44 and E1602

We tested, in two trials (Mel44 and E1602), whether co-administration of the 6MHP vaccine with a peptide vaccine that stimulates $CD8^+$ T cells (12 MHC Class I restricted peptides, 12MP) (31) would induce greater $CD8^+$ T cell reactivity and increased clinical responses than vaccination with 12MP alone. In the Mel44 trial, patients were vaccinated with 12MP+6MHP (MELITAC 12.6) or 12MP+tetanus

helper peptide (MELITAC 12.1), with IFA alone as the adjuvant, in patients with resected stage IIB-IV melanoma. T cell responses were measured by IFNγ ELIspot assay directly *ex vivo*, among $CD8^+$ T cells for 12MP, and among $CD4^+$ T cells for tetanus helper peptide (arms A and B), and 6MHP (arms C and D). The combination of 6MHP with 12MP paradoxically reduced the circulating $CD8^+$ T-cell response rate (Figure 2) (32). Responses to the tetanus helper peptide were detected in 91% of patients vaccinated with this peptide, and the 6MHP induced T_H cell responses in 52% of vaccinated participants (32). Patients on the Mel44 trial also were randomized to pre-treatment with one dose of cyclophosphamide (CY, 300 mg/m^2), which had no effect on $CD8^+$ or $CD4^+$ T cell responses (Figure 2). Early clinical outcome was not altered by adding 6MHP or CY to 12MP.

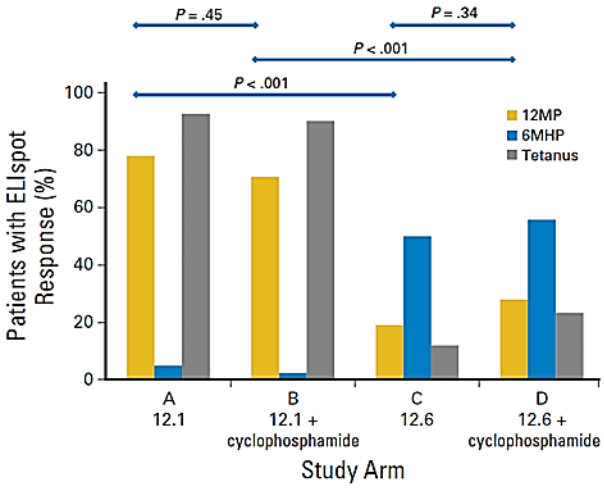


Figure 2. Mel44 trial. Immune response rates for 12MP, 6MHP, and tetanus peptide by ELISpot assay for each study arm, by evaluation of PBMC through day 50. *P* values for the response to 12MP are noted for four pairs of study arms. 12.1 [MELITAC 12.1], vaccines with 12MP plus tetanus peptide AQYIKANSKFIGITEL; 12.6 [MELITAC 12.6], vaccines with 12MP plus 6MHP.

The E1602 trial was a 4-arm, randomized phase II study for patients with advanced measurable melanoma. Study participants randomized to arms A, B, C, and D were vaccinated with 12MP, 12MP+tetanus peptide, 12MP+6MHP, or 6MHP alone, respectively, and with IFA+GM-CSF as a vaccine adjuvant (30). Similar to the finding in the Mel44 trial, the combination of 12MP+6MHP did not induce greater $CD8^+$ T cell responses or better clinical outcome. However, T cell responses were observed in the peripheral blood in about 40% of patients vaccinated with 6MHP (Figure 3), and objective clinical responses were observed in arms C and D: 2 partial responses (PR) in Arm C (6.3%) and 3 in Arm D (7.1%) (Table 2). There is additional evidence of clinical benefit, as the 1 year survival for Arms C and D exceed the 95% confidence interval from a meta- analysis of outcomes from cooperative group trials (Figure 4A). Importantly, there also is a very significant prolongation of survival for patients who had a helper T cell response to the 6MHP vaccines (Arms C+D, p = 0.005, Figure 4B). That difference persisted in a multivariate analysis (p = 0.038, HR 0.5; (30)). The association of survival and immune response to helper peptides was specific for the 6MHP, as no difference in survival was observed with immune response to the tetanus peptide for Arm B (not shown here; (30)).

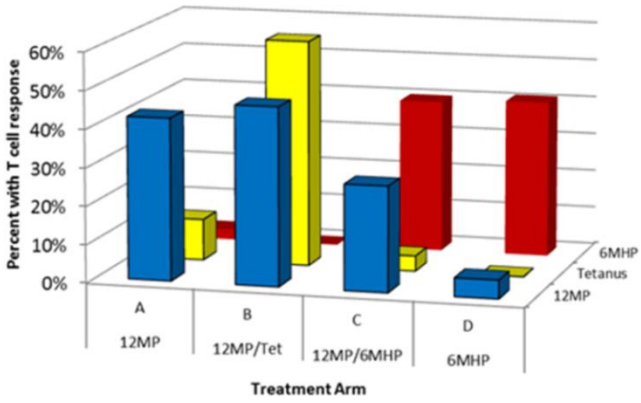


Figure 3. E1602 trial. Immune responses to 12MP, 6MHP, and tetanus peptide. The proportions of analyzable patients with CTL response to 12MP (blue bars), helper T cell response to tetanus peptide (yellow bars), and helper T cell response to 6MHP (maroon bars) are shown for each study arm.

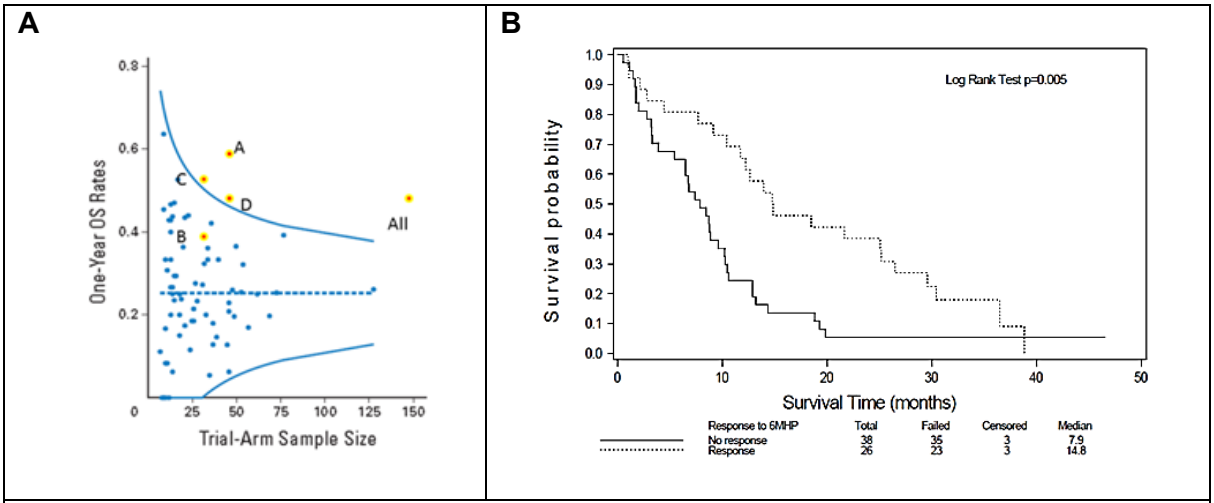


Figure 4. Overall survival on E1602. **(A)** 1 year survival for each treatment arm and for the full study population is shown on the plot of 1 year OS for all melanoma trials as previously published by Korn et al. (39), and **(B)** Overall survival by helper T cell response to 6MHP in analyzable patients on arms C and D of E1602 trial (n = 64).

Summary of preliminary immunogenicity data with 6-MHP vaccines

A consistent finding from these 3 trials is that 6MHP vaccines are immunogenic, with 40-50% immune response rate, when measured in peripheral blood, and 81% when measured in vaccine-draining nodes. Also, in the two trials that enrolled patients with measureable disease, there were durable clinical responses (13), and there was clinical activity with an 8% RECIST-defined response rate, and 30% disease control rate (Table 2), similar to those of ipilimumab (RR 11%, DCR 29%) (38) and durable clinical responses. Importantly, in the E1602 trial, there was a strong and specific association between immune response to the 6MHP and survival, supporting the clinical relevance of immune responses induced to the 6MHP vaccine. Thus, the current proposal will test the 6MHP

vaccine approach in combination therapy of melanoma based on immunogenicity, safety, and association between immune response and survival.

Safety of Peptide-based Vaccines

Peptide-based vaccines have been administered safely to humans in many clinical trials, with toxicity limited usually to local injection site reactions and transient grade I-II systemic reactions. In our experience, other systemic toxicities are attributable to cytokines added as adjuvants, but the peptides themselves appear to be very well-tolerated (40). In previous studies conducted by the UVA-HITC and by the Eastern Cooperative Oncology Group (ECOG), vaccines containing 6MHP have been administered to about 200 patients and have been well-tolerated (13,30,32). In fact, injection site reactions have been lower with 6MHP than with Class I MHC associated peptides (41).

The peptides used in peptide-based vaccines are identical or similar to a portion of a normal protein. Thus, risks of autoimmunity in humans are important to evaluate. There is no murine system that adequately models the human immune response to these peptides. The most meaningful evaluation of this peptide vaccine mixture is in patients with melanoma. This trial will include participants with advanced melanoma (stage IIIB/C, or IV melanoma with measurable disease). These individuals face a high risk (> 70-90%) of premature death, and the anticipated risk of short-term or long-term toxicity of this vaccine preparation is minimal, while the vaccine may delay or decrease the risk of morbidity and mortality due to melanoma in these patients (13,30).

The potential implications of autoimmunity against cells of melanocytic lineage are illustrated by reported cases of vitiligo occurring coincident with regressions of melanoma (42). Most of these are limited, often occurring in skin surrounding the regressing melanoma, but occasionally occurring systemically. While pathogenesis of this phenomenon can only be hypothesized, it is reasonable to consider this a worst-case scenario. The loss of skin pigment and hair pigment can be striking in these cases, but is not a cause of morbidity or mortality. Of greater potential concern is the theoretical risk of damage to the retinal pigment epithelium; however, visual loss has not been observed with these peptide vaccines, in over 200 patients treated, despite observing vitiligo in up to 10% of patients (13,30,32). Depigmentation of the retinal pigment epithelium has been observed in a small number of patients vaccinated with dendritic cells pulsed with MDP-derived peptides; however, this change was asymptomatic and was not associated with loss of visual acuity (personal communication – Frank Haluska). A careful study of the retinal pigment epithelium using monobenzyl ether of hydroquinone to induce pigment cell destruction on a biochemical basis suggests the safety of pigment cell destruction and supports immunotherapy directed against MDP as a strategy for melanoma therapy (personal communication, JM Kirkwood).

Toxicities Previously Reported for Participants Receiving the 6-MHP Vaccine Administered in Montanide ISA-51 adjuvant, with or without GM-CSF.

In Mel41, Mel44, and E1602 trials combined, vaccines containing 6MHP have been administered to about 207 patients and have been well-tolerated (13,30,32). In fact, injection site reactions have been lower with 6MHP than with Class I MHC associated peptides (41). Toxicities were graded using the NCI Common Terminology Criteria for Adverse Events v3.0. Treatment-related adverse events

experienced by at least 4% of participants and treatment-related toxicities with grade 3 or higher severity in any patient are listed in Table 3.

Table 3. 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%	
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%	

Treatment related adverse events of a given grade are considered expected toxicities if they have been observed in 4% of patients at that grade or higher. These are listed in Table 4 for 6MHP.

Table 4. Expected toxicities for 6MHP vaccines

Toxicity	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

1.6 **Checkpoint blockade with antibody to CTLA-4**

Checkpoint blockade

Cancer immunotherapy for solid tumors is coming of age, especially with checkpoint blockade providing durable clinical benefit in about 30-50% of patients. The anti-CTLA-4 blocking antibody, ipilimumab, is approved for use in melanoma and has been shown to induce durable clinical regressions and to prolong survival in patients with stage IV disease (38,43). 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) (9,44). Despite the effectiveness of checkpoint blockade therapies, about 50-70% of patients go on to develop progressive disease following treatment. Thus, there is a need for new combination approaches that build on the demonstrated clinical value of immune therapy.

Ipilimumab at 3 mg/kg is approved by the FDA for the treatment of patients with advanced melanoma (Stage IV and unresectable stage IIIB/C). A pooled analysis of the survival data for ipilimumab in the advanced setting demonstrated a 22% three year survival rate for all patients and a plateau in the survival curve identified around three years after treatment (45). This agent is now widely used in combination with nivolumab for the management of advanced melanoma and as single agent therapy in the salvage setting.

In 2015, based on the results of the EORTC 18072, high dose ipilimumab (10 mg/kg) was FDA-approved in the adjuvant setting. Patients eligible for this trial included those with stage IIIA melanoma with at least one metastasis measuring >1 mm in greatest dimension in the involved node, and stage IIIB/IIIC were included if there were no in-transit metastases (46). The treatment was administered every three weeks for four doses followed by every three months for up to three years. Survival analysis from this trial revealed an 11% improvement in overall survival with the use of ipilimumab versus placebo (47). Fifty-four percent (54%) of patients treated with ipilimumab experienced a grade 3 or 4 adverse event including 41.6% with an immune-related adverse event. There were also five deaths in the ipilimumab group attributed to immune-related toxicity.

In the metastatic setting, Ipilimumab at 10 mg/kg has a higher response rate than 3 mg/kg (11.1% vs 4.2%, p = 0.0015), but whether this benefit extends to the adjuvant setting is not known (48). Preliminary results of a phase III study of

advanced melanoma patients randomized to 10 mg/kg versus 3 mg/kg ipilimumab were presented at the 2016 ESMO conference, demonstrating a statistically-significant improvement in 3 year overall survival with the 10 mg/kg dose versus the lower dose (49). Association of higher rates of toxicity with the 10 mg/kg dose of ipilimumab has been consistently identified across studies. At this time, a trial randomizing patients to Ipilimumab 3 mg/kg, Ipilimumab 10 mg/kg, and standard Interferon (ECOG 1609) in the adjuvant setting has completed accrual but results are not expected until 2018. The potential of serious and life-threateining toxicity of ipilimumab 10 mg/kg administered in the adjuvant setting has limited enthusiasm for this treatment. At this time, the adjuvant therapy options for most melanoma patients remains compromised by toxicity and low efficacy, and many patients opt for surveillance.

In 2010, Hodi et al reported the results of a phase III trial combining ipilimumab with the gp100 vaccine which incorporates HLA-A*0201-restricted peptides designed to stimulate CD8+ cells (38). There were three treatment groups including ipilimumab alone, ipilimumab with gp100 vaccine and gp100 vaccine alone. The overall survival for the ipilimumab alone arm was 10.0 months versus 10.1 months for the ipilimumab with gp100 versus 6.4 months for the gp100 vaccine alone. There was no difference in overall survival between the two ipilimumab arms and there was no major differences in toxicity profile with the addition of the vaccine. Despite some promise for vaccines incorporating short peptides designed to stimulate CD8 T cells, overall clinical impact of these vaccines has been disappointing (12,31,50). In contrast, we have found that vaccination with a mixture of longer peptides (14-23 amino acids) designed to induce helper T cell responses (6MHP) has induced durable clinical activity in a subset of patients (30,51) and very encouraging overall survival for stage IV melanoma patients (13,30,52). This vaccine induces Th1-dominant helper T cells, which is expected to synergize with the effects of CTLA-4 blockade with ipilimumab, since ipilimumab's antitumor effects are mediated in large part through effects on CD4 T cells (53). Thus, the present protocol explores combination therapy with 6MHP vaccine plus ipilimumab in several relevant clinical settings.

In our study, we plan to include a broader patient population than the EORTC 18072 study with a wider risk profile. To balance the outcomes for our patients, we aim to augment the benefit of ipilimumab at 3 mg/kg with the addition of the 6MHP vaccine while limiting the risk to these patients with the lower dose of anti-CTLA-4 antibody and the low toxicity profile of this vaccine. The primary endpoints of our study are the safety and immunogenicity of this combination. Our goal is to utilize this study to provide the foundation for future, more definitive investigations with this combination therapy.

Toxicities Previously Reported for Participants Receiving ipillimumab.

Ipilimumab is approved by the FDA for the treatment of patients with advanced melanoma (Stage IV and unresectable stage IIIB/C). Adverse events have been reported, and include a range of toxicities, including some that appear to be immune-mediated and some of which have been life-threatening or fatal. The package insert lists the most common toxicities as fatigue, diarrhea, itching and rash. It also lists the potential serious toxicities as colitis, hepatitis, toxic epidermal necrolysis, neuritis, hypophysitis, and inflammation of the eyes. Toxicity data reported for 380 patients treated with ipilimumab plus a gp100 peptide vaccine and 131 treated with ipilimumab alone (total 511 participants) are summarized below in Table 5.

Table 5. Expected toxicities in 511 patients with ipilimumab 3 mg/kg IV, with (n=380) or without (n=131) vaccine (adapted from (38))

Toxicity	All grades	Grade 3	Grade 4
ADVERSE EVENTS IN > 15% OF PARTICIPANTS			
Diarrhea	189 (37%)	23 (5%)	1 (<1%)
Nausea	175 (34%)	8 (2%)	1 (<1%)
Constipation	108 (21%)	6 (1%)	.
Vomiting	106 (21%)	9 (2%)	1 (<1%)
Abdominal pain	87 (17%)	8 (2%)	.
Fatigue	179 (35%)	28 (5%)	.
Anorexia	123 (24%)	7 (1%)	1 (<1%)
Fever	94 (18%)	2 (<1%)	.
Headache	84 (16%)	7 (1%)	.
Cough	76 (15%)	1 (<1%)	.
Dyspnea	65 (13%)	16 (3%)	3 (<1%)
Anemia	56 (11%)	15 (3%)	.
IMMUNE RELATED ADVERSE EVENTS			
Pruritis	99 (19%)	1 (<1%)	.
Rash	92 (18%)	6 (1%)	.
Vitiligo: (skin hypopigmentation)	17 (3%)	.	.
Diarrhea	151 (30%)	20 (4%)	.
Colitis: (GI disorder or autoimmune disorder)	30 (6%)	18 (4%)	1 (<1%)
Hypothyroid	8 (2%)	1 (<1%)	.
Hypopituitarism: increased serum thyrotopin and decreased serum corticotropin (autoimmune disorder)	6 (1%)	3 (<1%)	1 (<1%)
Hypophysitis: increased serum thyrotopin and decreased serum corticotropin (autoimmune disorder)	4 (<1%)	4 (<1%)	.
Adrenal insufficiency	5 (1%)	2 (<1%)	.
Increased serum thyrotropin	3 (<1%)	.	.
Decreased serum corticotropin	2 (<1%)	.	1 (<1%)
Increased ALT (alanine aminotransferase increased)	5 (1%)	2 (<1%)	.
Increased AST (aspartate aminotransferase increased)	5 (1%)	1 (<1%)	.
Hepatitis (autoimmune disorder)	3 (<1%)	1 (<1%)	
Other	18 (4%)	7 (1%)	1 (<1%)

Treatment related adverse events of a given grade are considered expected toxicities if they have been observed in 4% of patients at that grade or higher. These are listed in Table 6 for ipilimumab.

Table 6. Expected toxicities for IV ipilimumab 3 mg/kg x 4

Toxicity	Grades 1 - 2	Grade 3
Diarrhea	+	+
Nausea	+	.
Constipation	+	.
Vomiting	+	.
Abdominal pain	+	.
Fatigue	+	+
Anorexia	+	.
Fever	+	.
Headache	+	.
Cough	+	.
Dyspnea	+	.
Anemia	+	.
Pruritis	+	.
Rash	+	.
Colitis (GI disorder or autoimmune disorder)	+	+
Increased transaminases (alanine aminotransferase increased) (aspartate aminotransferase increased)	+	.

This list includes toxicities observed in >4% of participants.

1.7 **Dosing**

6 MHP

The peptide vaccine is sequestered locally, and the immune response occurs primarily locally and in the draining lymph nodes. Thus, the dose of the vaccine does not need to be scaled up proportionately to the size (by weight or body surface area) of the recipient, as might be done for a drug whose effect is related to its distribution in body fluid. Because direct toxicity of the peptide is not expected, dose escalation is not as meaningful as it would be with a drug with a narrow therapeutic index.

Ipilimumab

Dosing for the ipilimumab is based on the current FDA approved dose and schedule.

1.8 **Summary**

We have developed a vaccine incorporating 6 intermediate-length peptides that induce $CD4^+$ helper T cell (T_H) responses (6MHP), and have previously shown that the vaccine has clinical activity in patients with advanced melanoma. The current proposal is to obtain preliminary data on whether combination of the 6MHP vaccine with CTLA-4 blockade will be safe and will result in a CD4+ T cell response to 6MHP peptides. We intend to use this combination in three clinical cohorts: advanced disease, neoadjuvant therapy and adjuvant therapy. This trial will provide preliminary information on the disease control rate of the combination in the advanced setting as well as the recurrence risk and time to recurrence in the neoadjuvant and adjuvant settings. The trial incorporates correlative studies of immune responses in the blood, sentinel immunized lymph node, and tumor to obtain a more complete understanding of the host:tumor relationship in the context of these combination therapies.

2.0 OBJECTIVES

2.1 Primary objectives and endpoints

Safety

- To determine whether the combination of 6MHP vaccine plus ipilimumab is safe as evaluated by adverse event assessments, including CTCAE 4.03 and subclassified by irAE categories (9).

Immunogenicity

- To estimate in the blood and in the sentinel immunized node the CD4+ T cell response rate to 6MHP peptides in patients treated with 6MHP vaccine plus ipilimumab

2.2 Secondary objectives and endpoints

Induction of epitope-spreading

- To estimate the proportion of patients in whom the combination of 6MHP vaccine plus ipilimumab induces epitope-spreading CD8+ T cells in the blood and in the sentinel immunized node that are reactive to a panel of defined melanoma antigens.

2.3 Exploratory objectives and endpoints

Induction of antibody responses

- To estimate the production of serum IgG reactive to 6MHP in patients treated with 6MHP vaccines plus ipilimumab

Clinical outcome

- For cohort 1: To obtain preliminary estimates of clinical outcomes including overall survival, progression-free survival, and clinical response by irRC criteria following administration of the combination of 6MHP vaccine plus ipilimumab.

- For cohorts 2 and 3: To obtain preliminary data on risk of recurrence and time to recurrence after resection of tumor to no evidence of disease and administration of the combination of 6MHP vaccine plus ipilimumab.

Associations of immune response and clinical outcome

- To obtain preliminary estimates of the associations between T cell and antibody responses to melanoma antigens and survival and with disease control.

T cell infiltration of the tumor microenvironment

- To determine whether the combination of 6MHP vaccine plus ipilimumab increases infiltration of CD8+ and/or CD4+FoxP3neg T cells into melanoma metastases in at least 50% of patients with tumor evaluable pre- and post-treatment.

3.0 PARTICIPANT SELECTION CRITERIA

3.1 Inclusion Criteria

3.1.1 Participants with stage IIA (with class 2 DecisionDx Score) through IV

melanoma in cohorts defined below. These participants may have had cutaneous, uveal, mucosal primary melanoma, or an unknown primary melanoma. The diagnosis of melanoma must be confirmed by cytological or histological examination. Staging of cutaneous melanoma will be based on the revised AJCC staging system (Appendix 2) (54). Participants must be eligible to be treated with ipilimumab based on clinician judgment within standard of care.

- Cohort 1 (Advanced Patients): unresectable stage III or IV melanoma that have clinical or radiographic evidence of disease.
- Cohort 2 (Neoadjuvant therapy): primary melanoma with clinically apparent lymph node or in transit/satellite lesions with or without lymph node involvement, in transit recurrence or metastatic recurrence amenable to complete resection to no evidence of disease
- Cohort 3 (Adjuvant therapy): Stage IIA (with class 2 DecisionDx Score), IIB-IV melanoma resected to no evidence of disease.

3.1.2 Participants 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.

3.1.3 Participants who have melanoma available for biopsy pre-treatment and on day 22 must consent to having those biopsies.

Melanoma lesions may be in nodes, skin, soft tissue, liver, or other sites that can be accessed by core needle biopsy, or incisional or excisional biopsy, with or without image guidance.
The lesion(s) must be large enough to enable biopsy of at least 0.1 cm^3 of tumor tissue (ideally 0.3 cm^3 or more) in 5 core biopsies (ideally 14-16 gauge, but 18 gauge is acceptable) or incisional/excisional biopsies at both time points. Biopsies may be taken from a single lesion or multiple lesions at each of the time points depending on the size of each lesion. Different lesions may be sampled at each time point. It is acceptable to perform a biopsy pretreatment, and then to perform an excision at day 22, even under general anesthesia if needed.
The lesions to be biopsied must be specified at study enrollment and not included as target lesions for RECIST calculations. There must be measurable disease in addition to the lesion(s) to be biopsied for cohort 1.
Up to 15 participants whose metastases are not available for biopsy may be enrolled in the first stage of enrollment, and up to 17 participants whose metastases are not available for biopsy may be enrolled in the second stage of enrollment.

3.1.4 Participants who have had brain metastases will be eligible if all of the following are true:

3.1.4.1 Each brain metastasis must have been treated by surgical removal, stereotactic radiosurgery or managed to complete resolution with immunotherapy. Patients with brain lesions managed by immunotherapy without excision or radiosurgery are included provided that the brain metastases have completely resolved after systemic therapy and there are no neurologic symptoms or need for systemic therapy to control CNS-disease related symptoms.
3.1.4.2 There has been no evident growth of any brain metastasis since the most recent treatment. If a patient has been managed by immunotherapy alone, the prior lesions must be completely resolved.
3.1.4.3 No brain metastasis is > 2 cm in diameter at the time of registration
3.1.4.4 The most recent surgical resections or gamma-knife therapy for malignant melanoma must have been completed ≥ 1 week prior to registration.

3.1.5 ECOG performance status of 0 or 1 (Appendix 3) (55)

3.1.6 Ability and willingness to give informed consent

3.1.7 Laboratory parameters as follows:

3.1.7.1 ANC ≥ 1000/mm^3
3.1.7.2 Platelets ≥ 100,000/mm^3
3.1.7.3 Hgb ≥ 9 g/dL
3.1.7.4 HgB-A1C ≤ 7.5%
3.1.7.5 AST and ALT ≤ 2.5 x upper limits of normal (ULN)
3.1.7.6 Bilirubin ≤ 1.5 x ULN (except in patients with Gilbert's disease, where bilirubin to 4x ULN is allowed).
3.1.7.7 Alkaline phosphatase ≤ 2.5 x ULN
3.1.7.8 Creatinine ≤ 1.5 x ULN

3.1.8 Age 18 years or older at registration.

3.1.9 Participants must have at least two intact (undissected) axillary and/or inguinal lymph node basins

3.2 **Exclusion Criteria**

3.2.1 Participants who have received the following medications or treatments at any time within 4 weeks of registration:

- Chemotherapy
- Interferon (e.g. Intron-A®)
- Radiation therapy (Stereotactic radiotherapy, such as gamma knife, can be used ≥ 1 week prior to registration)
- Allergy desensitization injections
- High doses of systemic corticosteroids, with the following qualifications and exceptions:
    - Daily doses of 10 mg predisone (or equivalent) per day administered parenterally or orally are not allowed in patients

with normal adrenal and pituitary function.
    - In patients with adrenal or pituitary insufficiency replacement steroid doses are allowed.
    - Inhaled steroids (e.g.: Advair®, Flovent®, Azmacort®) are permitted at low doses (less than 500 mcg fluticasone per day, or equivalent) (56,57).
    - Topical and nasal corticosteroids are acceptable.
- Growth factors (e.g. Procrit®, Aranesp®, Neulasta®)
- Interleukins (e.g. Proleukin®)
- Any investigational medication
- Targeted therapies specific for mutated BRAF or for MEK

3.2.2 HIV positivity or evidence of active Hepatitis C virus (testing to be done within 6 months of study entry).

3.2.3 Participants who are currently receiving nitrosoureas or who have received this therapy within the preceding 6 weeks

3.2.4 Participants who are currently receiving a checkpoint molecule blockade therapy, or who have received this therapy within the preceding 6 weeks,

with the following exception:

3.2.4.1 Participants who have received a PD-1 blocking antibody (eg: pembrolizumab or nivolumab) may be enrolled 3 weeks after receiving the last dose of that antibody.

3.2.4.2 Participants who have been treated previously with a CTLA-4 blocking antibody either as monotherapy or as part of combination CTLA-4/PD-1 blockade will be ineligible if CTLA-4 therapy:

1) was discontinued early for toxicity, or
2) did not induce stable disease or objective clinical response (by RECIST or irRC criteria) lasting 6 months or more.

Note: Patients may be eligible if they have experienced progression after a period of stable disease (6 months or more) or an objective clinical response (by irRC or RECIST) (6 months or more) induced by CTLA-4 blockade or combination CTLA-4/PD-1 blockade

Note: Similar guidelines will apply for tremelimumab or other CTLA-4 blocking antibodies.

3.2.5 Participants with known or suspected allergies to any component of the vaccine.

3.2.6 Participants may not have been vaccinated previously with any of the synthetic peptides included in this protocol. Participants who have received vaccinations containing agents other than the synthetic peptides included in this protocol and have recurred during or after administration of the vaccine will be eligible to enroll 12 weeks following their last vaccination.

3.2.7 Pregnancy. Female participants of childbearing potential must have a negative pregnancy test (urinary or serum beta-HCG) obtained within 2

weeks prior to registration. Males and females must agree, in the consent form, to use effective birth control methods during the course of vaccination. This is consistent with existing standards of practice for vaccine and chemotherapy protocols.

3.2.8 Female participants must not be breastfeeding

3.2.9 Participants in whom there is a medical contraindication or potential problem in complying with the requirements of the protocol in the opinion of the investigator.

3.2.10 Participants classified according to the New York Heart Association classification as having Class III or IV heart disease (Appendix 4).

3.2.11 Participants with uncontrolled diabetes, defined as having a HgB-A1C > 7.5%.

3.2.12 Participants must not have had prior autoimmune disorders requiring cytotoxic or immunosuppressive therapy, or autoimmune disorders with visceral involvement. Participants with an active autoimmune disorder requiring these therapies are also excluded. The following will not be exclusionary:

- The presence of laboratory evidence of autoimmune disease (e.g. positive ANA titer) without symptoms
- Clinical evidence of vitiligo
- Other forms of depigmenting illness
- Mild arthritis requiring NSAID medications
- A history of immune-related adverse events with immune therapy, if they have resolved completely.

3.2.13 Participants who have another cancer diagnosis, except that the following diagnoses will be allowed:

- squamous cell cancer of the skin without known metastasis
- basal cell cancer of the skin without known metastasis
- carcinoma in situ of the breast (DCIS or LCIS)
- carcinoma in situ of the cervix
- any cancer without distant metastasis that has been treated successfully, without evidence of recurrence or metastasis for over 2 years

3.2.14 Participants with known addiction to alcohol or drugs who are actively taking those agents, or participants with recent (within 1 year) or ongoing illicit IV drug use.

3.2.15 Body weight < 110 pounds at registration, due to the amount and frequency with which blood will be drawn.

3.3 **Registration and Randomization**

All participants must sign the consent form prior to determination of eligibility for this study. All participants who meet the inclusion/exclusion criteria may be registered. Registration will occur following verification of eligibility by the treating physician.

Participants should receive their first study treatment within 2 weeks of registration.

No randomization is required; all participants will receive the same vaccine regimen.

4.0 STUDY DRUG (6MHP and MONTANIDE ISA-51)

4.1 Peptide Synthesis

The vaccine drug product (6MHP) to be administered consists of 6 peptides. All peptides were synthesized directly from amino acids by Multiple Peptide Systems (now Polypeptide Group, San Diego, CA) under GMP conditions. Recombinant vectors in bacteria or viruses were not used. The synthetic peptides were purified by HPLC. The identity of the synthetic peptides has been confirmed by verifying their mass and amino acid sequences by mass spectrometry. Details of the synthesis, certificates of analysis, and technical summaries are included in the IND application.

4.2 Storage of Individual Peptides

Each bulk peptide was supplied to the HITC as lyophilized powder without excipients and stored at a temperature ≤ -70°C and protected from light.

4.3 Reconstitution and Vialing of the Vaccine

Lyophilized peptides were reconstituted, mixed and vialed under GMP conditions by Clinalfa (Merck Biosciences AG, Laufelfingen, Switzerland). Lyophilized peptides were supplied to the HITC as individual use vials. Lot release testing of the final vialed peptide has also been completed by Clinalfa in accord with FDA guidelines. Details of the vialing are included in the IND application.

4.4 Vaccine Storage

The vials of lyophilized peptide are stored by the HITC at a temperature ≤ -70°C and protected from light. Once thawed, the vial(s) must be used for preparation of the vaccine within 24 hours.

4.5 Lot Testing

Each lot of peptide vaccine is evaluated as required by the FDA for identity, sterility, general safety, purity, and pyrogenicity. The details of these tests are outlined in the IND application.

4.6 Stability testing

The peptide vaccine will undergo stability testing as described in Appendix 7.

4.7 Labeling

Each vial of lyophilized peptide is labeled with the following information:

- Short name of the product
- Product number
- Proper name of the product
- Name and address of the vialing facility
- Lot number
- Date of manufacture (the date of vialing the reconstituted peptides)

- Serial number
- Quantity of each peptide per vial
- Vial contains no preservative, store at ≤ -70°C
- "Caution: New Drug – Limited by US Federal law to investigational use"

4.8 Montanide ISA-51

Montanide ISA-51 is available from Seppic, Inc. (Fairfield, NJ). A drug master file for Montanide ISA-51 is filed with the FDA and is cross-referenced in the IND application.

Class II MHC-restricted melanoma peptides (6-MHP; 200 mcg) in aqueous solution are mixed 1/1 with Montanide ISA-51 to form water-in-oil emulsions.

4.9 Study Drug Accountability

The investigational drug will be stored in accord with directions specified in Section 4 of the protocol in a secure area with the UVA-HITC laboratories. Study drug accountability is maintained using the InvestMed database.

5.0 IPILIMUMAB

5.1 Packaging and Labeling

Ipilimumab (YERVOY®) is an FDA approved drug for usefor the treatment of patients with unresectable or metastatic melanoma. The study drug is manufactured by Bristol Myers-Squibb and is packaged and labeled in accord with the package insert. It is provided as a sterile aqueous liquid at 5 mg/ml in single-use vials with either of two volumes (10 ml or 40 ml).

5.2 Storage

Ipilimumab is stored refrigerated at 2°C– 8°C (36°F–46°F). Vials are to be protected from light and should not be frozen

6.0 TREATMENT PLAN

6.1 Management of Participants

This study will be conducted on an outpatient basis, with participants scheduled to be evaluated as needed for clinical care, and as specified in the study calendar (Appendix 1), through 24 months (or more often if needed for testing or medical reasons). Participants will be off treatment follow-up at the end of 24 months, or when another therapy is initiated, whichever occurs first. Once off treatment follow-up, participants will be followed yearly for progression-free survival and overall survival.

6.2 Administration of 6MHP

6.2.1 Overview

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 1), emulsified in Montanide ISA-51 adjuvant (2 ml total will be administered). Each of vaccines 1-3 will be administered subcutaneously (50%) and intradermally (50%) at one skin site. If the site of vaccines 1 or 2 has severe inflammation or ulceration, the next vaccine may be placed near the original site. Vaccines 4-6 will be administered at a different site than vaccines 1-3, but vaccines 4-6 will all be

administered at one site. If the site of vaccines 4 or 5 has severe inflammation or ulceration after 1-2 vaccines, the next vaccine may be placed near the site designated for vaccines 4-6.

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

6.2.2 Dose Calculations

At each designated time-point, 200 mcg each of the 6 peptides (Table 1), emulsified in Montanide ISA-51 adjuvant (2 ml total) will be administered.

6.2.3 Pre-Medications

None required.

6.2.4 Preparation of Study Drug

Directions on how to prepare the investigational drug will be provided. The prepared peptide vaccines, containing 2 ml of peptide emulsion, 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.

6.2.5 Post-Vaccination Observation

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

6.3 **Administration of Ipilimumab**

6.3.1 Overview

Ipilimumab will be administered in accord with the official prescribing information: 3 mg/kg intravenously once every 3 weeks, for 4 doses.

6.3.2 Pre-Medications

None required.

6.4 **Dose Modifications**

6.4.1 6MHP

There will be no dose modifications of the vaccine components

6.4.2 Ipilimumab

In the event of treatment related toxicity, the dose of ipilimumab should be modified in accord with current practice guidelines. Recommendations are also provided in the package insert. Immune-related adverse events should be managed per standard clinical practice and in accord with the package

insert for YERVOY® (ipilimumab) and the Immune-mediated Adverse Reaction Management Guide, available from Bristol-Myers Squibb. (https://www.hcp.yervoy.com/pages/rems.aspx)

6.5 Dose Delays

Protocol treatment includes both the 6MHP vaccine and ipilimumab. Either or both may be delayed for any of the following reasons:

6.5.1 Dose Delays Due to Toxicity

- 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.
- Dose delays for ipilimumab will follow standard clinical guidelines.

6.5.2 Delayed Visits for Reasons Other Than Toxicity

A schedule for return visits should be established at the first visit. If a participant misses a treatment, the missed treatment will be administered as soon as possible, so that subsequent treatments will be given in the appropriate intervals. Treatment may be continued for an additional time period, if needed. Participants who are treated 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 participant 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	Participant Treatment	Protocol Deviation
*Vaccine 1**/Ipilimumab 1			
Day 1	± 2 days	Vaccine, Labs, Ipilimumab	No
	± 3 to 7 days	Vaccine, Labs, Ipilimumab	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/Ipilimumab 2			
Day 22	± 2 days	Labs, Biopsies, Ipilimumab	No
	± 3 to 7 days	Labs, Biopsies, Ipilimumab	Yes
	± 8 or more days	Labs	Yes
*Vaccine 4**/Ipilimumab 3			

Days 43	± 2 days	Vaccine, Labs, Ipilimumab	No
	± 3 to 7 days	Vaccine, Labs, Ipilimumab	Yes
	± 8 or more days	Labs	Yes
Assessment			
Day 57	± 7 days	Scans**	No
	± 8 to 14 days	Scans	Yes
	± 15 or more days	Scans	Yes
*Vaccine 5**/Ipilimumab 4			
Day 64	± 7 days	Vaccine, Labs, Ipilimumab	No
	± 8 to 14 days	Vaccine, Labs, Ipilimumab	Yes
	± 15 or more days	Labs	Yes
*Vaccine 6**			
Day 85	± 7 days	Vaccine, Labs, Scans**	No
	± 8 to 14 days	Vaccine, Labs, Scans**	Yes
	± 15 or more days	Labs/Scans	Yes
Assessment			
Day 92	± 7 days	Labs	No
	± 8 to 14 days	Labs	Yes
	± 15 or more days	Labs	Yes
Follow-up			
FUv1 and FUv2	± 14 days	Labs/Scans**	No
	± 15 or more days	Labs/Scans	Yes
Follow-up			
FUv3, FUv4, FUV5	± 28 days	Labs/Scans**	No
	± 29 or more days	Labs/Scans	Yes

* A participant 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, 85, FUv1, FUv2, FUv3, FUv4, FUv5 should also be completed within the specified time limits. The scan at day 57 is to measure response, with confirmatory scans at day 85 only if needed. Followup scans beyond day 85 may be performed earlier or more frequently than specified in the protocol if indicated as part of clinical care. If earlier or more frequent scans have been performed for clinical care, it is not a violation to omit scans on followup dates if they have been performed within 3 months prior.

6.6 **Discontinuation of Therapy**

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

Protocol treatment will be discontinued for a participant for any of the following reasons:

- Any dose-limiting toxicity (DLT), as defined in Section 9 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. However, management of toxicities attributed to ipilimumab

should follow current standard practice guidelines, including use of steroids when indicated.

- 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 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.
- Initiation of cytotoxic chemotherapy, radiation therapy, or targeted therapy for melanoma or other cancer.
- Initiation of non-permitted medication, or other systemic immunosuppressive therapy, except that steroid or immunosuppressive therapy for management of toxicities of ipilimumab 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.
- Noncompliance with the requirements of the study.
- Therapy may be discontinued at the participant's request.
- Therapy may be discontinued at the discretion of an Investigator.
- Pregnancy. Pregnant participants will continue to be followed for the duration of the pregnancy.
- Treatment delays as specified in section 6.5.1.

6.6.1 Safety follow-up Visits

In the event of an AE, appropriate action will be taken to ensure adequate care for the patient. If the patient is still on protocol, treatment delay or withdrawal from the protocol will be considered according to the protocol guidelines (Section 6.6).

Subjects who discontinue receiving investigational product will begin study-specific follow-up as outlined in Appendix 1. Exceptions for the timing of the follow-up visits include the following:

- Safety follow-up visits may occur prior to the designated time frames for subjects who will begin another cancer therapy and/or for subjects who withdraw consent at the time of discontinuation.

6.7 **Replacement of Study Participants**

A participant who is enrolled but who does not receive study drug or any of the study related procedures may be replaced. Every attempt will be made to evaluate any data from these participants for endpoint assessment.

6.8 **Concomitant Medications**

Medications taken within 30 days prior to registration will be recorded on the baseline case report form. This includes prescription medications, over-the-counter medications, injected medications, biological products, blood products, imported drugs, or street drugs. Participants should be maintained on drugs that they were taking prior to entry unless a change in regimen is medically indicated.

The following are non-permitted medications or treatments

- Cytotoxic chemotherapy
- Interferon therapy (e.g. Intron-A®)
- Radiation therapy
- Nitrosoureas
- Allergy desensitization injections
- Corticosteroids, as detailed in section 3.2.1.
- Growth factors (e.g. Procrit®, Aranesp®, Neulasta®)
- Interleukins (e.g. Proleukin®)
- Antibodies to PD-1 or other immune checkpoint blockade therapies (e.g. Keytruda®)
- Other investigational medications
- Street drugs

6.9 **Permitted Medications or Treatments**

- Nonsteroidal anti-inflammatory agents
- Anti-histamines (e.g. Claritin®, Allegra®)
- Topical corticosteroids or steroids for the reasons cited in section 3.2.1 above.
- Short-term therapy for acute conditions not specifically related to melanoma
- Chronic medications except those listed in section 6.8
- Influenza vaccines are permitted, but should be administered at least 2 weeks prior to or at least 2 weeks after a study vaccine.

6.10 **Biopsies**

For participants with biopsy accessible disease, biopsies (incisional, core or excisional biopsies) of cutaneous, subcutaneous, soft tissue or visceral metastatic melanoma will be obtained:

- at baseline: may be collected previously according to specifications below, during screening or once enrolled, prior to treatment on day 1.
- on day 22: 1 week after the third vaccine.

Biopsies collected previously as part of standard treatment or other protocols may be used as the baseline biopsy provided they meet all of the following:

a. Sufficient tissue is available
b. Tissue was collected within 3 months of Day 1
c. There were no intervening treatments in between the biopsy and the first protocol treatment (dietary supplements are permitted)

Cohort 2 only: oncologic surgery to render patient free of disease on day 22 will provide tissue for day 22 biopsy.

Additional optional biopsies may be performed during active treatment or during follow-up, e.g. for new lesions.

7.0 CLINICAL AND LABORATORY EVALUATIONS

The following evaluations will be performed on an outpatient basis. Please refer to the study calendar (Appendix 1) for scheduling.

7.1 Physical Exams and Evaluations

- Medical History
- Complete Physical Exam (includes vital signs, weight, performance status, medication review, neurologic function-general)
- Assessment of skin and nodal basins for evidence of disease recurrence or metastasis
- Assessment of skin for vitiligo
- Assessment of hair and eye color
- Designation of vaccination sites (at screening only)
- Visual acuity (Snellen chart) (baseline only)
- Color vision exam (Ishihara eye chart) (baseline only)
- Assessment of baseline symptoms (baseline only)

7.2 Pathology Review

- Review of pathology at the University of Virginia

7.3 Performance Status

- ECOG performance status criteria will be used in the evaluations (Appendix 3).

7.4 Clinical Labs

- CBC with differential, including automated lymphocyte count (0.3 ml)
- Comprehensive chemistry panel to include sodium, potassium, creatinine, glucose, calcium total bilirubin, AST, ALT, and alkaline phosphatase. NOTE: fasting blood sugars, when required, may be evaluated 4 hours or more after last eating. (0.9 ml)
- Lactate dehydrogenase (LDH) (0.3 ml)
- Urinalysis
- β-HCG for women of childbearing potential (2 ml)
- Cortisol (3 ml; combined with TSH and Free T4)
- TSH
- Free T4
- HgB-A1C (3 ml)
- HIV screening (antibody screen); if the antibody screen is positive, follow-up testing by RNA analysis may be completed to determine whether active disease is present. (3 ml)
- HCV screening (antibody screen); if the antibody screen is positive, follow-up testing by RNA analysis may be completed to determine whether active disease is present. (combined with HIV)
- Anti-nuclear antibody and rheumatoid factor (4 ml)

7.5 Toxicity Assessments

- Assessment of adverse events. The NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be used for the characterization and grading of adverse events.
- Toxicity diaries will be distributed to participants and reviewed by study personnel.

7.6 Research Blood Samples

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

7.7 Tumor Biopsies

Tumor biopsies will be completed in participants with adequate and accessible metastatic tumor in addition to measurable disease.

7.7.1 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 0.1 cm^3 and up to about 0.3 cm^3 of 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.

7.7.2 Sampling

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

7.7.3 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 when possible:

a. 20% (1/5 of single biopsy or 1 core biopsy) will be fixed in formalin, then paraffin-embedded (for histology/immunohistology)
b. 20% (1/5 of single biopsy or 1 core biopsy) will be processed for protein studies

c. 40% (2/5 of single biopsy or 2 core biopsies) 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).
d. 20% (1/5 of single biopsy or 1 core biopsy) will be placed in RNA-later (for RNA/RT PCR)
e. 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.

7.7.4 Evaluations

a. 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.
b. Tumor escape mechanisms may also be evaluated.
c. 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.
d. Specimens may be used to study the immunologic aspects of the tumor microenvironment or as targets or controls in laboratory assays.
e. Specimens may be used to establish cell lines for long-term studies.
f. 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.
g. HLA typing (Class I and Class II) will be analyzed as part of the immunologic endpoints. (8 ml)

7.7.5 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 62 participant is no longer participating in the Mel 62 study and the participant consents to allow their tissue to be collected under the IRB #10598 tissue banking study to be analyzed for Mel 62, the tissue may be analyzed as part of the Mel 62 analysis.

Optional biopsy samples may be evaluated as described in section 7.7.4.

7.7.6 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.

7.8 **Sentinel Immunized Node Biopsy**

7.8.1 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:

a. 1 central section will be fixed in formalin, then paraffin-embedded (for histology/immunohistology)
b. 1 section will be placed in RNA-later. (for RNA/RT PCR)
c. 1 section will be quick-frozen (for immunohistology/protein studies)
d. 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).

7.9 **Assessments**

7.9.1 Anti-tumor Activity

Anti-tumor activity will be assessed by the following:

<u>Tumor Imaging</u>
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. For each participant, the same method of assessment will be used to evaluate tumor burden at baseline and throughout the course of the study.

<u>Tumor Measurments</u>

RECIST 1.1 Criteria will be used to evaluate tumor burden. These are summarized in Appendix 5 (58)

7.9.2 Immunologic Assessments

Assessments of T cell function may include, but are not limited to the following:

- ELIspot assays
- ELISAs
- Chromium-release assays
- Proliferation assays
- Intracellular cytokine staining
- T cell receptor sequencing.
- Cytokine bead array
- Flow cytometry
- HLA typing

Characterization of cellular populations may include, but are not limited to the following:

- Immunohistochemistry
- Gene expression analysis
- Flow cytometry
- ELISAs
- Western-blot analysis
- Intracellular cytokine staining
- Cytokine bead array

7.10 **Study Calendar**

(See Appendix 1)

8.0 STATISTICAL CONSIDERATIONS

8.1 **Design**

This is an early phase trial to evaluate whether the combination of the 6MHP vaccine with CTLA-4 blockade ipilimumab will be safe and will result in a CD4+ T cell response to 6MHP peptides. The combination will be assessed in three clinical cohorts: advanced disease, neoadjuvant therapy and adjuvant therapy. The trial should provide preliminary information on the disease control rate of the combination in the advanced setting as well as the recurrence risk and time to recurrence in the neoadjuvant and adjuvant settings. The primary goals are to assess safety and to determine if magnitude of immune response to the 6MHP vaccine can be improved by the addition of anti-CTLA-4.

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. Ipilimumab causes toxicities related to unmasking autoimmunity, some of which can be severe, but are managed well with steroids. We do not

anticipate increased autoimmune toxicities by combining 6MHP vaccine with ipilimumab, but relatively high rates of hypophysitis have been reported by combining ipilimumab with each of two other vaccines. Thus, this trial will be designed with stopping rules for toxicities beyond those expected with ipilimumab alone. Formal safety bounds (Section 8.5) based upon monitoring dose limiting toxicities (DLT) will guide decisions about early stopping due to potential safety concerns. 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. The proposed safety bound will be applied overall and within cohort.

8.2.2 Immunogenicity

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

i. Primary:
   Circulating $CD4^+$ T cell response to 6MHP peptides where the primary endpoint is the proportion of participants with at least two consecutive 10-fold over baseline responses to the pool of 6MHP peptides.
   $CD4^+$ T cell response to 6MHP peptides in the SIN.

ii. Secondary:
   Evaluated in the blood and in the SIN
   Induction of epitope-spreading for $CD8^+$ T cells reactive to a panel of defined melanoma antigens.
   Evaluated in the tumor microenvironment (In the subset of participants for which tumor biopsies are obtained pre- and post- treatment)
   T cell infiltration of $CD8^+$ and/or $CD4^+FoxP3^{neg}$ T cells.

iii. Exploratory:
   Induction of antibody responses
   IgG antibody responses to 6MHP peptides.

8.2.3 Clinical efficacy

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

i. Exploratory:
   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 on the date of last follow-up/contact.
   (Cohort 1)
   Disease control as assessed by the RECIST 1.1 (CR, PR, SD at 12 weeks) criteria.
   Clinical response by irRC criteria.
   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.

(Cohort 2 and 3)
Disease-free survival (DFS), 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.

8.3 **Sample Size and Accrual**

Target sample size is based upon having sufficient information to assess immunogenicity overall cohort conditional on safety bounds not being crossed. Observed immune response from arm D from our most current 6MHP protocol Mel63, provides the basis for sample size determination. Define immune response as 'at least two consecutive 10 fold' responses for 6MHP pool assessed in the blood. Results for Mel63 arm D indicate a immune response rate of 55% (12/22) with 95%CI(32, 76%) satisfy this definition. Accrual of a total of 24 eligible participants provides 80% power to test for a null immune response rate of 55% vs an alternative immune response rate of 80% with a one-side 5% level binomial test. The alternative immune response rate of 80% was chosen to be above the upper limit of the CI to determine that the addition of ipilimumab improved immune response. At study conclusion the null hypothesis will be rejected and the treatment considered worthy of further study if at least 18/24 (75%) eligible participants experience an immune response.

Maximum target sample size is estimated to be 27 participants, which assumes a 10% dropout/ineligibility/lost to follow-up adjustment. Accrual to cohort 3 is expected to accumulate the most participants, at approximately 10 per year, followed by cohort 2 at 3-5 per year, and cohort 1 at 1-3 per year. Therefore, total accrual is estimated at approximately 16 participants per year, therefore, accrual to the study should be completed in less that two years.

8.4 **Cohort and post entry classification**

At study entery participants will be stratified by cohort. In addition. Participants will be monitored for post entry classification by whether or not biopsies are obtained at baseline and one week after the third vaccine; (yes/no). Target sample size for participants for whom biopsies are obtained is set at a minimum of 14 eligible participants. If increased infiltration of $CD8^{+}$ T cell response from day 1 compared to day 22 tumor is observed in 10/14 participants then the lower limit of a one-sided 90% confidence interval exceeds 50% and would be considered to support further study of the treatment regimen. Similar criteria apply for $CD4^{+}FoxP3^{neg}$ T cells response.

8.5 **Safety Monitoring**

Toxicities will be monitored using CTCAE 4.03 criteria, with a focus on recording immune-related adverse events (irAE) (9). Stopping rules will be based on an expected irAE rate of 15-20%, where 10-15% had grade 3-4 irAE in one study (38) and in another 19% had grade 3, and 3.2% grade 4 irAEs (59). 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 as defined in Section 9.9 will guide decisions about early stopping due to potential safety concerns (Table 8). The definition of

DLT considers both irAEs and previously reported DLTs related to vaccine. 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. The rate of DLTs will be monitored up to 30 days after the last vaccination. 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 8: Stopping Guidelines for DLTs

Number of participants	Boundary
4-6	≥ 4
7-11	≥ 5
12-15	≥ 6
16-20	≥ 7
21-25	≥ 8
26-27	≥ 9

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. Participants in cohorts 1 and 2 will be assessed for clinical response and will be classified by disease control by week 12 and at final assessement. DCR will be estimated along with a 95% confidence interval around the point estimate.

Immune response will be measured based upon the methods in Section 7.9. The criteria for defining immune responses have been reported for ELIspot assays (34). The circulating $CD4^+$ T cell response rate to 6MHP peptides in participants treated with 6MHP vaccine plus ipilimumab will be assessed at each time noted in the study calendar. Maximum response over all time periods will be used to estimate the overall $CD4^+$ T cell response. Other measures assessed over time in the blood include epitope-spreading for circulating $CD8^+$ T cells reactive to a panel of defined melanoma antigens, and production of serum IgG reactive to 6MHP. Assessments in the tumor microenvironment include assessment for of $CD8^+$ and/or $CD4^+FoxP3^{neg}$ T cells into melanoma. 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 explored to estimate changes over time in interval measurements in the blood and tissue.

Additionally, for cohort 3 only, estimates of immune response within the cohort will be estimated. We expect accrual of at least 15 eligible participants to cohort 3. At study conclusion, if 12/15 (80%) eligible pariticipants satisfy the definition of immune response in cohort 3 then a 90%CI for immune response rate of 80% is (56%, 94%) which would support that immune response rate has improved in that cohort specifically.

PFS, DFS (within appropriate cohorts) and OS distributions will be estimated by the product limit method of Kaplan and Meier. Other summary measures for PFS, DFS and OS will include estimates and 95% CIs for median and 1-year estimates of PFS, DFS and OS to assess relative to published data. Clinical response by irRC criteria will be tabulated and cross classified by DCR. We acknowledge that participants who progress by RECIST criteria and then go onto other therapies may result in an underestimate of response by irRC criteria. However, if deemed appropriate some of these participants may be followed for a longer period before start of other treatment given that they received immune therapy and their response by irRC may be captured. 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 ADVERSE EVENT DATA COLLECTION AND MONITORING

9.1 Definitions

9.1.1 **Adverse event (AE)** – Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure (attribution of unrelated, unlikely, possible, probable, or definite). Medical conditions or diseases present before starting the investigational drug will be considered as treatment-related AEs if they worsen after starting study treatment.

9.1.2 **Unexpected AE** – Any adverse event not listed in Tables 3-6.

9.1.3 **Serious AE –** Any adverse drug experience occurring at any dose that results in any of the following outcomes:

- death;
- a life-threatening adverse drug experience;
- inpatient hospitalization, or prolongation of existing hospitalization (as defined below in this section);
- a persistent or significant disability/incapacity; or a congenital anomaly/birth defect.
- Important medical events that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.
- Hospitalization for expedited AE reporting purposes is defined as an inpatient hospital stay equal to or greater than 24 hours. Hospitalization is used as an indicator of the seriousness of the adverse event and should be reserved for situations where the adverse event truly fits this definition and not for hospitalizations associated with less serious events. For example, the following are not considered serious adverse events:
    - a hospital visit where a patient is admitted for observation or minor treatment (e.g. hydration) and released in less than 24 hours

- hospitalization for pharmacokinetic sampling
- admission to hospice
- hospitalizations planned before entry into the clinical study
- hospitalization for elective treatments
- hospitalizations to work up Grade 1 adverse events

9.1.4 **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 participant 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 participant or others at greater risk of harm than was previously known or recognized OR results in actual harm to the participant or others.

9.1.5 **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.

9.1.6 **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.

9.2 **Attribution Assessment**

9.2.1 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 participant's clinical state, environmental or toxic factors, or other modes of therapy administered to the participant.
- 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 participant; 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 participant's clinical state, environmental or toxic factors, or other modes of therapy administered to the participant.
- 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 participant's clinical state, environmental or toxic factors, or other modes of therapy administered to the participant.
- 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 participant's clinical state, environmental or toxic factors, or other modes of therapy administered to the participant.
- 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.).

9.3 **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**.

9.4 **Risks and Safety**

9.4.1 Adverse Event Descriptions and Grading Scales

The NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be used for the characterization and grading of adverse events.

9.4.2 Time Span for Reporting Adverse Events

Reporting of AEs will begin when the participant is administered the study drug or has a study related biopsy. Events occurring through 30 days after administration of the last dose of 6MHP vaccine or ipilimumab, regardless of attribution, will be reported. AEs should be followed to resolution or stabilization. If an AE worsens and becomes an SAE, it should be reported as serious per the guidelines specified for SAE reporting.

AEs that are possibly, probably, or definitely related to any of the study drugs will be recorded until the participant completes treatment follow-up. If, during treatment follow-up, the participant receives an alternative anti-cancer treatment, participants will be off treatment follow-up and will be followed yearly for disease progression and survival.

9.4.3 Agent-Specific Expected Adverse Events List:

Any AE not in this list will be considered an unexpected AE.

Expected toxicities related to 6MHP
The list of expected AEs for the 6MHP vaccine is based on prior data from the UVA-Mel41 and Mel44 clinical trials, plus the ECOG trial E1602; from these trials, aggregated data are available from over 200 patients vaccinated with the 6MHP vaccine in an emulsion with Montanide ISA-51 adjuvant, with or without GM-CSF. These data are summarized in Table 3 and Table 4 in section 1.

Expected toxicities related to ipilimumab
Adverse events reported for treatment of patients with the CTLA-4 antibody ipilimumab have been reported and are summarized in section 1 and Table 5 and Table 6. Interactions with the toxicities of the 6MHP vaccine are not expected, but toxicities will be tracked.

Adverse events expected from tumor and node biopsies.
Below is a list of expected AEs related to tumor and SIN biopsies:

- bleeding
- bruising
- pain

- infection
- lymphedema
- delayed wound healing
- scarring
- numbness

9.5 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 9: 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 Disordersl: Retinopathy
- Eye Disorders: Blurred vision
- Eye Disorders: Flashing lights
- Eye Disorders: Floaters

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

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 adverse event classification.

9.6 Reporting Adverse Events

9.6.1 Process for Reporting AEs:

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 following guidelines (Table 10)

Table 10: 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.

Pregnant-Partner Outcomes
If a male has been exposed to the investigational agent prior to or around the time of conception, this will not be considered an SAE. The HITC will ask permission of the pregnant partner to be followed until term.

Pregnancy

If a female has been exposed to the investigational agent prior to or around the time of conception, this will not be considered an SAE. The HITC will follow the pregnancy until term.

9.6.2 IRB Reporting Requirements

The University of Virginia is responsible for reporting to the UVA IRB-HSR per the following guidelines (Table 11):

Table 11: UVA IRB-HSR reporting

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 Phone- (434) 924-7166

9.6.3 Additional Reporting Requirements for the Sponsor (UVA)

Reporting to the FDA

- Serious and unexpected suspected adverse reactions will be reported to the FDA no later than 15 calendar days after the sponsor determines that the requirements for an IND safety report have been met. The FDA will be notified using an FDA Form 3500a.
- Unexpected fatal or life-threatening suspected adverse reactions will be reported to the FDA no later than 7 calendar days after the Sponsor receives the initial information of the event. The FDA will be notified using an FDA Form 3500a.
- Other adverse event information will be sent to the FDA in the IND annual report.

9.6.4 Reporting of Participant Withdrawals/Dropouts Prior to Study Completion

Participants who withdraw consent and those dropping out of the study secondary to an AE will be reported to the UVA IRB yearly on the IRB continuation form.

9.7 Adverse Event Review and Monitoring

9.7.1 Capturing Adverse Events

In addition to clinic notes, adverse events will be initially captured using study-specific tools and participant toxicity diaries.
Each participant will be evaluated by a licensed clinician. The following will be performed as designated in the protocol: routine disease-directed physical exam including performance status and blood collection for clinical labs.

Participants should keep a daily diary of toxicities until FUv1. The diaries will be reviewed by a research clinician prior to the next scheduled visit. During clinic visits, participants will also be asked about subjective symptoms including headache, malaise, fatigue, dyspnea, nausea, rash, diarrhea, abdominal discomfort, peripheral nerve pain, visual changes, appetite, tremors, night sweats, and ability to concentrate. Additional toxicities will be captured from laboratory tests.

After administration of each 6MHP vaccine, participants will be observed for AEs for at least 20 minutes. Follow-up phone calls will be made per the judgment of the research clinicians with regard to individual participant need. Participants will be instructed on how to reach their provider should they have any questions and/or problems during the study.

9.7.2 Review of Adverse Events by the Study Team

Individual AEs will be reviewed by the treating physician, principal investigator, and the clinical research coordinator(s) (CRC). Other staff on the research team may also review AEs.

SAEs will be reviewed about once per month by the PI and Sponsor during the UVA Melanoma Team Meeting. This meeting will occur at least 20 times in a calendar year. Those present at the meeting may include the sponsor/overall study PI, sub-investigators, protocol development staff, biostatisticians, research nurses, research coordinators, laboratory specialists, and laboratory research managers. These meetings also include the review of individual participants to assess whether they are protocol candidates, whether AEs warrant discontinuation, and whether existing protocols should be continued or closed.

9.8 **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 9.6:

1. Alk Phosphatase
2. ALT (SGPT)
3. ANA
4. AST (SGOT)
5. Bilirubin, total
6. Creatinine
7. Eosinophil #
8. Hepatitis C serology or virus measures
9. beta-HCG
10. Hgb
11. HIV
12. HLA type
13. LDH
14. Potassium
15. RF
16. Urinalysis
17. WBC
18. Cortisol
19. TSH
20. Free T4

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 as described in Section 9.6. If there is any doubt on the part of study personnel concerning what constitutes a pertinent positive finding, the PI and sponsor will be consulted.

9.9 Dose-limiting Toxicities (DLT)

6MHP

A DLT of the 6MHP vaccine is defined as any unexpected 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. Thus, ulcerations will be considered DLTs only if the ulcers are ≥2 cm in diameter, require antibiotics or surgical debridement.

Ipilimumab

A DLT of ipilimumab is defined as any toxicity that is definitely, probably, or possibly related to the administration of ipilimumab and requires permanent discontinuation of ipilimumab.

If a DLT occurs and cannot be attributed to either the 6MHP or ipilimumab, then a DLT will be attributed to both.

9.10 Management of Toxicity

The study will be monitored continuously for treatment-related adverse events. Expected treatment-related toxicities of ipilimumab will be managed per standard clinical practice and in accord with the package insert for YERVOY® (ipilimumab).

9.11 Data Collection

9.11.1 Endpoint Data

- Endpoint data will be collected using HITC IML data forms, participant-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.

9.12 Monitoring Plan

9.12.1 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).

9.12.2 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

9.12.3 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 (and if appropriate to the PRC and IRB) and a formal response from the

sponsor is requested. Per the UVA Cancer Center NIH approved institutional plan, this study will be audited approximately every 6 months.

10.0 STUDY CONDUCT AND ETHICAL CONSIDERATIONS

This study will be conducted in compliance with ICH Good Clinical Practice (GCP) Guidelines and in accord with the ethical principles that originated in the Declaration of Helsinki. In addition, all local laws and regulations will apply. The PI will ensure that staff are trained and carry out the study in accord with the protocol specifications.

10.1 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 participants. 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 9.6.

10.2 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. Participants will be given a consent form to review and a member of the study team will be available to answer any questions. Informed consent will be obtained from each participant prior to conducting any study-specific procedures or administering study drug.

10.3 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.

11.0 APPENDICES

Appendix 1: Study Calendar
Appendix 2: AJCC Staging System
Appendix 3: ECOG Performance Status
Appendix 4: New York Heart Association Disease Classification
Appendix 5: RECIST 1.1 Criteria
Appendix 6: NCI Common Terminology Criteria for Adverse Events v4
Appendix 7: Vaccine Lot Release and Stability Testing
Appendix 8: Summary of Changes

Appendix 1: Study Calendar

Studies & Tests	At Screening	Day	Active Treatment									Follow-up[m]				
			1	8	15	22	43	57	64	85	92	FU v1	FU v2	FU v3	FU v4	FU v5
		Week	0	1	2	3	6	8	9	12	13	18	24	27	52	104
Informed consent	X[a]															
Pathology review	X[a]															
CBC with differential	X[b]		X[f]			X	X		X	X					X	X
Comprehensive chemistry	X[b,e]		X[f]			X	X		X	X					X	X
HGB-A1C	X[b]															
LDH	X[b]															
Cortisol			X			X	X		X							
Free T4			X			X	X		X							
TSH			X			X	X		X							
Urinalysis	X[b]															
β-HCG	X[c]															
HIV / Hepatitis C	X[d]															
CT chest/abdomen/pelvis or PET-CT	X[b]							X		X[h]		X[i]		X	X[j]	X[j]
Head MRI / CT	X[b]							X[l]		X[h]		X[i]		X	X[j]	X[j]
History & physical	X[b]		X[f]	X	X	X	X		X	X	X	X	X	X	X	X
Medication Review	X[b]		X	X	X	X	X		X	X	X	X[n]	X[n]	X[n]	X[n]	X[n]
Record baseline symptoms			X													
AE assessment				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]									X				X	X	X
Assessment of skin for vitiligo			X			X				X				X	X	X
Assessment of hair and eye color			X			X				X				X	X	X
Visual acuity exam/ color vision			X													
120cc green top tubes			X[k]													
80cc green top tubes				X	X	X	X		X	X	X	X	X	X	X	X

Studies & Tests			Active Treatment									Follow-up[m]				
	At Screening	Day	1	8	15	22	43	57	64	85	92	FU v1	FU v2	FU v3	FU v4	FU v5
		Week	0	1	2	3	6	8	9	12	13	18	24	27	52	104
20cc red top tubes			X	X	X	X	X		X	X	X	X	X	X	X	X
Anti-nuclear antibody / Rf factor			X						X						X	X
Vaccination with 6MHP			X	X	X		X		X	X						
Tumor biopsy[g]			X			X										
Sentinel immunized node biopsy						X										
Participant diary reviewed and/or distributed			X	X	X	X	X		X	X	X	X				
ipilimumab: 3 mg/kg IV			X			X	X		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 scheduled for Day 1 are not required if prestudy assessments were within 10 calendar days of day 1. However, hair and eye color assessment and assessment of skin for vitiligo should be performed if theses assessments were not completed as part of the pre-study visit.
[g]For patients who have tumor accessible to biopsy, biopsies will be completed 1) during screening or, once enrolled, prior to treatment on Day 1, and 2) at day 22, for subjects who have melanoma available for biopsy. Additional optional biopsies may be performed for research purposes during the study or during follow-up if participants are removed from the study or experience progression. If surgical resection or biopsies are completed for clinical care, the tumor tissue may be collected for study related analyses.
[h] Tumor imaging at day 85 should be performed only when clinically needed to verify objective clinical responses at day 57.
[i]If scans were completed at day 85, the scans do not have to be repeated at this visit.
[j] Scans do not need to be repeated for the protocol if they have been performed recently (typically within 3 months).
[k]Blood for HLA typing is included in the research bloods.
[l] Required only for subjects with brain metastases.
[m]Follow-up visits in the initial follow-up phase will occur as follows: FUv1: 42 days from the last dose of 6MHP; FUv2 = 24 weeks from the first dose of 6MHP; FUv3 = 27 weeks from the first dose of 6MHP; FUv4 = 1 year from the first dose of 6MHP; FUv5 = 2 years from the first dose of 6MHP.
[n]Only information about anticancer therapy will be collected

Appendix 2: 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 metastatsis	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

Appendix 3: ECOG Performance Status

ECOG PERFORMANCE STATUS (55)	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

Appendix 4: 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

Appendix 5: RECIST 1.1 Criteria

Please refer to the following publication for evaluation of clinical response by RECIST 1.1 (58).

Appendix 6: NCI Common Terminology Criteria for Adverse Events v4

http://evs.nci.nih.gov/ftp1/CTCAE/About.html

Appendix 7: 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.

Appendix 8: Summary of Changes

10-10-17	1) Updated personnel list. 2) Protocol Synopsis: clarified additional biopsies are optional, removed reference to baseline tumor collection in Figure 3 and biopsy details addressed in section 6.10. 3) Section 6.10: clarified biopsy timepoints. 4) Section 7.7.3: added clarifier "when possible" and shifted RNA later to the least priority. 5) Section 7.7.5: added section describing optional biopsies. 6) Section 7.7.6: added section to address tissue removed as part of clinical care. 7) Section 9.7.1: removed reference to vital signs being collected after 20 minute observation period. 8) Section 9.10: removed non-functional link and reference to the Immune-mediated Adverse Reaction Management Guide. 9) Appendix 1: clarified that additional biopsies are optional and may be collected for research purposes or as part of clinical care in footnote "g". 10) Corrected version date in summary of changes for v05-18-17.
05-18-17	1) Revised study title from "A Phase I/II Trial to Evaluate the Safety, Immunogenicity and Clinical Activity of a Helper Peptide Vaccine plus CTLA-4 Blockade in Advanced Melanoma" to "A Phase I/II Trial to Evaluate the Safety and Immunogenicity of a Helper Peptide Vaccine plus CTLA-4 Blockade in Melanoma Patients" 2) Updated personnel list 3) Updated Table of Contents 4) Updated List of Abbreviations 5) Editorial and formatting changes made throughout document 6) Protocol Synopsis – added information about FDA approved dosing for Ipilimumab 7) Protocol Synopsis, Section 1.1, Section 2.0, and Section 8.2—revised study objectives and endpoints 8) Protocol Synopsis, Section 1.8, Section 3.1.1, and Section 8.1 -- changed the study design to include 3 cohorts: Unresectable stage III/IV advanced melanoma (Cohort 1); Neoadjuvant therapy (Cohort 2); and Adjuvant therapy (Cohort 3) 9) Protocol Synopsis and Section 8.3 changed maximum target sample size from 51 to 27 and the target accrual from 46 to 24 eligible participants 10) Section 1.1 Added additional background information on combination of ipilimumab and nivolumab 11) Section 1.5 Added footnote a to Table 4 to indicate that injection site reaction with ulceration <2cm is expected 12) Section 1.6 revised clinical data for checkpoint blockade with CTLA-4 antibody 13) Section 3.1.3 and Section 6.10 Clarified that biopsies will be done on Day 22 for Cohorts 1 and 2 only 14) Section 3.1.4 Revised inclusion criteria for subjects who have had brain metastases 15) Section 3.2.4.2 Revised inclusion criteria to specify that subjects may have previously received CTLA-4 blocking antibody either as

	monotherapy or as part of combination CTLA-4/PD-1 blockade 16) Section 3.2.13 Revised the exclusion criteria to include an exception for participants who have another cancer diagnosis for any cancer without distant metastasis that has been treated successfully, without evidence of recurrence or metastasis for over 2 years instead of over 3 years. 17) Section 8.4 changed section name from "Stratification" to "Cohort and Post Entry Classification" 18) Appendix 1 Study Calendar by deleting Comprehensive Chemistry and CBC with differential from Day 8 and Day 15 19) Updated Reference list
05-06-16	1) Updated signature page 2) Updated personnel list. 3) Updated table of contents 4) Editorial corrections made throughout document. 5) Section 3.2.13: revised to change 5 years to 3 years for exclusion criterion related to other cancer diagnoses. 6) Schema, Table 7, Section 9.7.1 Appendix 1; replaced Days 127, 269, 190, 365, 730 with FUv1, FUv2, FUv3, FUv4, and FUv5, respectively. 7) Section 6.2.1: Revised wording to specify that vaccines 4-6 will be administered at a separate site than vaccines 1-3 and that vaccines 4-6 will be administered at the same site and not rotated. 8) Section 6.2.4: revised to specify that the vaccine should be administered within 1-2 hours after mixing. 9) Section 6.6.1: this section has been added to clarify the procedures for safety follow-up visits. 10) Section 9.7.1: revised section and moved corrected text to section 6.6.1. 11) Appendix 1: Added CBC and comp chemistry labs to Days 8 and 15. 12) Revised the date ordering of summary of changes so that the most recent changes are at the top of the list.
09-10-15	1) Updated Investigator's Agreement to match updated protocol template. 2) Updated Study Personnel 3) Updated Header and Table of Contents 4) Section 3.2.4.1: Updated exclusion criteria to allow subjects who have received a prior PD-1 blocking antibody to be enrolled 3 weeks after receiving the last dose of that antibody. This decrease in the waiting period from 6 weeks to 3 weeks following the last dose of PD-1 blocking antibody is considered a sufficient wash-out period by the clinicians. 5) Section 3.2.4.2: Updated exclusion criteria to specify in which instances prior treatment with a CTLA-4 blocking antibody is considered to be exclusionary. The rationale for the change is to broaden the entry criteria and is based on the potential synergistic effects of peptide plus CTLA-4 therapy. 6) Section 9.4.3: Updated expected toxicity list for SIN and tumor biopsies to be consistent with risks described in the consent form. 7) Section 9.5: Removed Grade 1 ocular toxicities as DLTs as Gr1 immune-mediated ocular disease is described in the package insert for ipilimumab. 8) Editorial changes throughout the document are noted.
04-04-15	1) Investigator's statement has been updated. 2) Updated Investigator list

	3) Updated Table of Contents 4) Section 6.4: Clarified that dose modifications of immune-related adverse events for ipilimumab will be managed in accord with standard practice, the package insert for ipilimumab and the Immune-mediated Adverse Reaction Management Guide, which is available from BMS. 5) Preicis, Section 6.10: clarified timing for the baseline biopsy 6) Tables 5 and 6: added CTCAE adverse event terms for clarification 7) Section 8.5: reference has been corrected; title of Table 8 has been corrected. 8) Section 8.6: Clarification of measurements of response by irRC and how progression evaluated by RECIST will impact evaluation of response by irRC. 9) Section 9.9: deleted reference to package insert. 10) Section 9.10: added the package insert for ipilimumab and the link to the Immune-mediated Adverse Reaction Management Guide 11) Editorial changes made throughout document. 12) Appendix 1: a) Clarified completion of hair and eye color assessments at baseline b) Clarified timing for the biopsies. c) Clarified that the Head MRI/CT at day 57 is required only for subjects with brain metastases.
01-22-15	1) Table of Contents and header: Updated 2) Investigator's Statement has been added 3) Section 4.6: Reference to manual was removed. Information is included in Appendix 7. 4) Section 4.8: Reference to manual was removed. Information on preparation will be supplied on vaccine mixing forms. 5) Section 6.2.4: Reference to manual was removed. 6) Section 11: List of appendices was updated. 7) Appendix 1: editorial change. 8) Appendix 6: Appendix and link to NCI CTCAE v4 were added. 9) Appendix 7: Appendix for vaccine lot release and stability testing was added. 10) Formatting changes throughout document.
01-06-15	1) List of personnel: Elizabeth Gaughan's role was changed to PI. Craig Slingluff's role was changed to co-investigator. 2) Section 6.2.1: volume of vaccine was changed to 2 ml 3) Section 6.2.2: volume of vaccine was changed to 2 ml 4) Section 6.2.4: volume of vaccine was changed to 2 ml 5) Section 7.10: editorial change, corrected hyperlink

Reference List

1. Topalian SL, Gonzales MI, Parkhurst M, Li YF, Southwood S, Sette A, Rosenberg SA, Robbins PF. Melanoma-specific CD4+ T cells recognize nonmutated HLA-DR-restricted tyrosinase epitopes. *J Exp Med.* 1996;183(5):1965-1971.
2. Kobayashi H, Kokubo T, Sato K, Kimura S, Asano K, Takahashi H, Iizuka H, Miyokawa N, Katagiri M. CD4+ T cells from peripheral blood of a melanoma patient recognize peptides derived from nonmutated tyrosinase. *Cancer Res.* 1998;58(2):296-301.
3. Zarour HM, Kirkwood JM, Kierstead LS, Herr W, Brusic V, Slingluff CL, Jr., Sidney J, Sette A, Storkus WJ. Melan-A/MART-1(51-73) represents an immunogenic HLA-DR4-restricted epitope recognized by melanoma-reactive CD4(+) T cells. *Proc Natl Acad Sci U S A.* 2000;97(1):400-405.
4. Manici S, Sturniolo T, Imro MA, Hammer J, Sinigaglia F, Noppen C, Spagnoli G, Mazzi B, Bellone M, Dellabona P, Protti MP. Melanoma cells present a MAGE-3 epitope to CD4(+) cytotoxic T cells in association with histocompatibility leukocyte antigen DR11. *J Exp Med.* 1999;189(5):871-876.
5. Chaux P, Vantomme V, Stroobant V, Thielemans K, Corthals J, Luiten R, Eggermont AM, Boon T, van der Bruggen P. Identification of MAGE-3 epitopes presented by HLA-DR molecules to CD4(+) T lymphocytes. *J Exp Med.* 1999;189(5):767-778.
6. Halder T, Pawelec G, Kirkin AF, Zeuthen J, Meyer HE, Kun L, Kalbacher H. Isolation of novel HLA-DR restricted potential tumor-associated antigens from the melanoma cell line FM3. *Cancer Res.* 1997;57(15):3238-3244.
7. Li K, Adibzadeh M, Halder T, Kalbacher H, Heinzel S, Muller C, Zeuthen J, Pawelec G. Tumour-specific MHC-class-II-restricted responses after in vitro sensitization to synthetic peptides corresponding to gp100 and Annexin II eluted from melanoma cells. *Cancer Immunol Immunother.* 1998;47(1):32-38.
8. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, Schadendorf D, Dummer R, Smylie M, Rutkowski P, Ferrucci PF, Hill A, Wagstaff J, Carlino MS, Haanen JB, Maio M, Marquez-Rodas I, McArthur GA, Ascierto PA, Long GV, Callahan MK, Postow MA, Grossmann K, Sznol M, Dreno B, Bastholt L, Yang A, Rollin LM, Horak C, Hodi FS, Wolchok JD. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. *N Engl J Med.* 2015;373(1):23-34.
9. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM, Sznol M. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med.* 2012;366(26):2443-2454.
10. Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes MS, Phan GQ, Citrin DE, Restifo NP, Robbins PF, Wunderlich JR, Morton KE, Laurencot CM, Steinberg SM, White DE, Dudley ME. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2011;17(13):4550-4557.
11. Higano CS, Schellhammer PF, Small EJ, Burch PA, Nemunaitis J, Yuh L, Provost N, Frohlich MW. Integrated data from 2 randomized, double-blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer. *Cancer.* 2009;115(16):3670-3679.

12. Schwartzentruber DJ, Lawson DH, Richards JM, Conry RM, Miller DM, Treisman J, Gailani F, Riley L, Conlon K, Pockaj B, Kendra KL, White RL, Gonzalez R, Kuzel TM, Curti B, Leming PD, Whitman ED, Balkissoon J, Reintgen DS, Kaufman H, Marincola FM, Merino MJ, Rosenberg SA, Choyke P, Vena D, Hwu P. gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. *N Engl J Med.* 2011;364(22):2119-2127.
13. Slingluff CL, Jr., Petroni GR, Olson W, Czarkowski A, Grosh WW, Smolkin M, Chianese-Bullock KA, Neese PY, Deacon DH, Nail C, Merrill P, Fink R, Patterson JW, Rehm PK. Helper T-cell responses and clinical activity of a melanoma vaccine with multiple peptides from MAGE and melanocytic differentiation antigens. *J Clin Oncol.* 2008;26(30):4973-4980.
14. Shankaran V, Ikeda H, Bruce AT, White JM, Swanson PE, Old LJ, Schreiber RD. IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature.* 2001;410(6832):1107-1111.
15. Kayaga J, Souberbielle BE, Sheikh N, Morrow WJ, Scott-Taylor T, Vile R, Chong H, Dalgleish AG. Anti-tumour activity against B16-F10 melanoma with a GM-CSF secreting allogeneic tumour cell vaccine. *Gene Ther.* 1999;6(8):1475-1481.
16. Hunder NN, Wallen H, Cao J, Hendricks DW, Reilly JZ, Rodmyre R, Jungbluth A, Gnjatic S, Thompson JA, Yee C. Treatment of metastatic melanoma with autologous CD4+ T cells against NY-ESO-1. *N Engl J Med.* 2008;358(25):2698-2703.
17. Kahn M, Sugawara H, McGowan P, Okuno K, Nagoya S, Hellstrom KE, Hellstrom I, Greenberg P. CD4+ T cell clones specific for the human p97 melanoma-associated antigen can eradicate pulmonary metastases from a murine tumor expressing the p97 antigen. *J Immunol.* 1991;146(9):3235-3241.
18. Hung K, Hayashi R, Lafond-Walker A, Lowenstein C, Pardoll D, Levitsky H. The central role of CD4(+) T cells in the antitumor immune response. *J Exp Med.* 1998;188(12):2357-2368.
19. Matsui S, Ahlers JD, Vortmeyer AO, Terabe M, Tsukui T, Carbone DP, Liotta LA, Berzofsky JA. A model for CD8+ CTL tumor immunosurveillance and regulation of tumor escape by CD4 T cells through an effect on quality of CTL. *J Immunol.* 1999;163(1):184-193.
20. Hu Y, Petroni GR, Olson WC, Czarkowski A, Smolkin ME, Grosh WW, Chianese-Bullock KA, Slingluff CL, Jr. Immunologic hierarchy, class II MHC promiscuity, and epitope spreading of a melanoma helper peptide vaccine. *Cancer Immunol Immunother.* 2014;63(8):779-786.
21. Touloukian CE, Leitner WW, Topalian SL, Li YF, Robbins PF, Rosenberg SA, Restifo NP. Identification of a MHC class II-restricted human gp100 epitope using DR4-IE transgenic mice. *J Immunol.* 2000;164(7):3535-3542.
22. Herr W, Schneider J, Lohse AW, Meyer zum Buschenfelde KH, Wolfel T. Detection and quantification of blood-derived CD8+ T lymphocytes secreting tumor necrosis factor alpha in response to HLA-A2.1-binding melanoma and viral peptide antigens. *J Immunol Methods.* 1996;191(2):131-142.
23. Scheibenbogen C, Lee KH, Mayer S, Stevanovic S, Moebius U, Herr W, Rammensee HG, Keilholz U. A sensitive ELISPOT assay for detection of CD8+ T lymphocytes specific for HLA class I-binding peptide epitopes derived from influenza proteins in the blood of healthy donors and melanoma patients. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 1997;3(2):221-226.
24. Lalvani A, Brookes R, Hambleton S, Britton WJ, Hill AV, McMichael AJ. Rapid effector function in CD8+ memory T cells. *J Exp Med.* 1997;186(6):859-865.

25. Tanchot C, Guillaume S, Delon J, Bourgeois C, Franzke A, Sarukhan A, Trautmann A, Rocha B. Modifications of CD8+ T cell function during in vivo memory or tolerance induction. *Immunity.* 1998;8(5):581-590.
26. Reynolds SR, Oratz R, Shapiro RL, Hao P, Yun Z, Fotino M, Vukmanovic S, Bystryn JC. Stimulation of CD8+ T cell responses to MAGE-3 and Melan A/MART-1 by immunization to a polyvalent melanoma vaccine. *Int J Cancer.* 1997;72(6):972-976.
27. Slingluff CL, Jr., Yamshchikov G, Neese P, Galavotti H, Eastham S, Engelhard VH, Kittlesen D, Deacon D, Hibbitts S, Grosh WW, Petroni G, Cohen R, Wiernasz C, Patterson JW, Conway BP, Ross WG. Phase I trial of a melanoma vaccine with gp100(280-288) peptide and tetanus helper peptide in adjuvant: immunologic and clinical outcomes. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2001;7(10):3012-3024.
28. Fernandez IM, Snijders A, Benaissa-Trouw BJ, Harmsen M, Snippe H, Kraaijeveld CA. Influence of epitope polarity and adjuvants on the immunogenicity and efficacy of a synthetic peptide vaccine against Semliki Forest virus. *J Virol.* 1993;67(10):5843-5848.
29. Scalzo AA, Elliott SL, Cox J, Gardner J, Moss DJ, Suhrbier A. Induction of protective cytotoxic T cells to murine cytomegalovirus by using a nonapeptide and a human-compatible adjuvant (Montanide ISA 720). *J Virol.* 1995;69(2):1306-1309.
30. Slingluff CL, Jr., Lee S, Zhao F, Chianese-Bullock KA, Olson WC, Butterfield LH, Whiteside TL, Leming PD, Kirkwood JM. A randomized phase II trial of multiepitope vaccination with melanoma peptides for cytotoxic T cells and helper T cells for patients with metastatic melanoma (E1602). *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2013;19(15):4228-4238.
31. Slingluff CL, Jr., Petroni GR, Chianese-Bullock KA, Smolkin ME, Hibbitts S, Murphy C, Johansen N, Grosh WW, Yamshchikov GV, Neese PY, Patterson JW, Fink R, Rehm PK. Immunologic and clinical outcomes of a randomized phase II trial of two multipeptide vaccines for melanoma in the adjuvant setting. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2007;13(21):6386-6395.
32. Slingluff CL, Jr., Petroni GR, Chianese-Bullock KA, Smolkin ME, Ross MI, Haas NB, von Mehren M, Grosh WW. Randomized multicenter trial of the effects of melanoma-associated helper peptides and cyclophosphamide on the immunogenicity of a multipeptide melanoma vaccine. *J Clin Oncol.* 2011;29(21):2924-2932.
33. Slingluff CL, Jr., Petroni GR, Olson WC, Smolkin ME, Ross MI, Haas NB, Grosh WW, Boisvert ME, Kirkwood JM, Chianese-Bullock KA. Effect of granulocyte/macrophage colony-stimulating factor on circulating CD8+ and CD4+ T-cell responses to a multipeptide melanoma vaccine: outcome of a multicenter randomized trial. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2009;15(22):7036-7044.
34. Slingluff CL, Petroni GR, Smolkin ME, Chianese-Bullock KA, Smith K, Murphy C, Galeassi N, Neese PY, Grosh WW, Nail CJ, Ross M, von Mehren M, Haas N, Boisvert ME, Kirkwood JM. Immunogenicity for CD8+ and CD4+ T cells of 2 formulations of an incomplete freund's adjuvant for multipeptide melanoma vaccines. *J Immunother.* 2010;33(6):630-638.
35. Hailemichael Y, Dai Z, Jaffarzad N, Ye Y, Medina MA, Huang XF, Dorta-Estremera SM, Greeley NR, Nitti G, Peng W, Liu C, Lou Y, Wang Z, Ma W, Rabinovich B, Sowell RT, Schluns KS, Davis RE, Hwu P, Overwijk WW. Persistent antigen at

vaccination sites induces tumor-specific CD8(+) T cell sequestration, dysfunction and deletion. *Nat Med.* 2013;19(4):465-472.
36. Salerno EP, Shea SM, Olson WC, Petroni GR, Smolkin ME, McSkimming C, Chianese-Bullock KA, Slingluff CL, Jr. Activation, dysfunction and retention of T cells in vaccine sites after injection of incomplete Freund's adjuvant, with or without peptide. *Cancer Immunol Immunother.* 2013;62(7):1149-1159.
37. Faries MB, Hsueh EC, Ye X, Hoban M, Morton DL. Effect of granulocyte/macrophage colony-stimulating factor on vaccination with an allogeneic whole-cell melanoma vaccine. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2009;15(22):7029-7035.
38. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJ, Lutzky J, Lorigan P, Vaubel JM, Linette GP, Hogg D, Ottensmeier CH, Lebbe C, Peschel C, Quirt I, Clark JI, Wolchok JD, Weber JS, Tian J, Yellin MJ, Nichol GM, Hoos A, Urba WJ. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med.* 2010;363(8):711-723.
39. Korn EL, Liu PY, Lee SJ, Chapman JA, Niedzwiecki D, Suman VJ, Moon J, Sondak VK, Atkins MB, Eisenhauer EA, Parulekar W, Markovic SN, Saxman S, Kirkwood JM. Meta-analysis of phase II cooperative group trials in metastatic stage IV melanoma to determine progression-free and overall survival benchmarks for future phase II trials. *J Clin Oncol.* 2008;26(4):527-534.
40. Woodson EM, Chianese-Bullock KA, Wiernasz CJ, Bissonette EA, Grosh WW, Neese PY, Merrill PK, Barnd DL, Petroni GR, Slingluff CL, Jr. Assessment of the toxicities of systemic low-dose interleukin-2 administered in conjunction with a melanoma peptide vaccine. *J Immunother.* 2004;27(5):380-388.
41. Hu Y, Smolkin ME, White EJ, Petroni GR, Neese PY, Slingluff CL, Jr. Inflammatory adverse events are associated with disease-free survival after vaccine therapy among patients with melanoma. *Ann Surg Oncol.* 2014;21(12):3978-3984.
42. Kawakami Y, Rosenberg SA. Immunobiology of human melanoma antigens MART-1 and gp100 and their use for immuno-gene therapy. *Int Rev Immunol.* 1997;14(2-3):173-192.
43. Robert C, Thomas L, Bondarenko I, O'Day S, Weber J, Garbe C, Lebbe C, Baurain JF, Testori A, Grob JJ, Davidson N, Richards J, Maio M, Hauschild A, Miller WH, Jr., Gascon P, Lotem M, Harmankaya K, Ibrahim R, Francis S, Chen TT, Humphrey R, Hoos A, Wolchok JD. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med.* 2011;364(26):2517-2526.
44. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, Pitot HC, Hamid O, Bhatia S, Martins R, Eaton K, Chen S, Salay TM, Alaparthy S, Grosso JF, Korman AJ, Parker SM, Agrawal S, Goldberg SM, Pardoll DM, Gupta A, Wigginton JM. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med.* 2012;366(26):2455-2465.
45. Schadendorf D, Hodi FS, Robert C, Weber JS, Margolin K, Hamid O, Patt D, Chen TT, Berman DM, Wolchok JD. Pooled Analysis of Long-Term Survival Data From Phase II and Phase III Trials of Ipilimumab in Unresectable or Metastatic Melanoma. *J Clin Oncol.* 2015;33(17):1889-1894.
46. Eggermont AM, Chiarion-Sileni V, Grob JJ, Dummer R, Wolchok JD, Schmidt H, Hamid O, Robert C, Ascierto PA, Richards JM, Lebbe C, Ferraresi V, Smylie M, Weber JS, Maio M, Konto C, Hoos A, de Pril V, Gurunath RK, de Schaetzen G, Suciu S, Testori A. Adjuvant ipilimumab versus placebo after complete resection of

high-risk stage III melanoma (EORTC 18071): a randomised, double-blind, phase 3 trial. *Lancet Oncol.* 2015;16(5):522-530.

47. Eggermont AM, Chiarion-Sileni V, Grob JJ, Dummer R, Wolchok JD, Schmidt H, Hamid O, Robert C, Ascierto PA, Richards JM, Lebbe C, Ferraresi V, Smylie M, Weber JS, Maio M, Bastholt L, Mortier L, Thomas L, Tahir S, Hauschild A, Hassel JC, Hodi FS, Taitt C, de Pril V, de Schaetzen G, Suciu S, Testori A. Prolonged Survival in Stage III Melanoma with Ipilimumab Adjuvant Therapy. *N Engl J Med.* 2016;375(19):1845-1855.
48. Wolchok JD, Neyns B, Linette G, Negrier S, Lutzky J, Thomas L, Waterfield W, Schadendorf D, Smylie M, Guthrie T, Jr., Grob JJ, Chesney J, Chin K, Chen K, Hoos A, O'Day SJ, Lebbe C. Ipilimumab monotherapy in patients with pretreated advanced melanoma: a randomised, double-blind, multicentre, phase 2, dose-ranging study. *Lancet Oncol.* 2010;11(2):155-164.
49. Ascierto PA, Del Vecchio M, Robert C, Mackiewicz A, Chiarion-Sileni V, Arance A, Lebbe C, Bastholt L, Hamid O, Rutkowski P, McNeil C, Garbe C, Loquai C, Dreno B, Thomas L, Grob JJ, Liszkay G, Nyakas M, Gutzmer R, Pikiel J, Grange F, Hoeller C, Ferraresi V, Smylie M, Schadendorf D, Mortier L, Svane IM, Hennicken D, Qureshi A, Maio M. Ipilimumab 10 mg/kg versus ipilimumab 3 mg/kg in patients with unresectable or metastatic melanoma: a randomised, double-blind, multicentre, phase 3 trial. *Lancet Oncol.* 2017.
50. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. *Nat Med.* 2004;10(9):909-915.
51. Slingluff CL, Jr., Petroni GR, Olson W, Czarkowski AR, Grosh WW, Smolkin M, Chianese-Bullock KA, P.Y. N, Deacon DH, Nail CJ, Merrill P, Fink R, Rehm PK. Helper T cell responses and clinical activity of a melanoma vaccine with multiple peptides from MAGE and melanocytic differentiation antigens. *Journal of Clinical Oncology.* 2008;26(30):4973-4980.
52. Hu Y, Kim H, Blackwell CM, Slingluff CL, Jr. Long-term outcomes of helper peptide vaccination for metastatic melanoma. *Ann Surg.* 2015;262(3):456-464; discussion 462-454.
53. Chambers CA, Sullivan TJ, Allison JP. Lymphoproliferation in CTLA-4-deficient mice is mediated by costimulation-dependent activation of CD4+ T cells. *Immunity.* 1997;7(6):885-895.
54. Balch CM, Gershenwald JE, Soong SJ, Thompson JF, Atkins MB, Byrd DR, Buzaid AC, Cochran AJ, Coit DG, Ding S, Eggermont AM, Flaherty KT, Gimotty PA, Kirkwood JM, McMasters KM, Mihm MC, Jr., Morton DL, Ross MI, Sober AJ, Sondak VK. Final version of 2009 AJCC melanoma staging and classification. *J Clin Oncol.* 2009;27(36):6199-6206.
55. Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, Carbone PP. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol.* 1982;5(6):649-655.
56. Ernst P, Suissa S. Systemic effects of inhaled corticosteroids. *Curr Opin Pulm Med.* 2012;18(1):85-89.
57. Lapi F, Kezouh A, Suissa S, Ernst P. The use of inhaled corticosteroids and the risk of adrenal insufficiency. *Eur Respir J.* 2013;42(1):79-86.
58. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer.* 2009;45(2):228-247.
59. O'Day SJ, Maio M, Chiarion-Sileni V, Gajewski TF, Pehamberger H, Bondarenko IN, Queirolo P, Lundgren L, Mikhailov S, Roman L, Verschraegen C, Humphrey R,

Ibrahim R, de Pril V, Hoos A, Wolchok JD. Efficacy and safety of ipilimumab monotherapy in patients with pretreated advanced melanoma: a multicenter single-arm phase II study. *Ann Oncol.* 2010;21(8):1712-1717.