Title: **A Phase Ib study of Neoantigen Vaccine (NeoVax plus Montanide) in combination with Nivolumab and locally administered Ipilimumab in patients with melanoma**

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**Coordinating Center:** **Dana-Farber Cancer Institute**

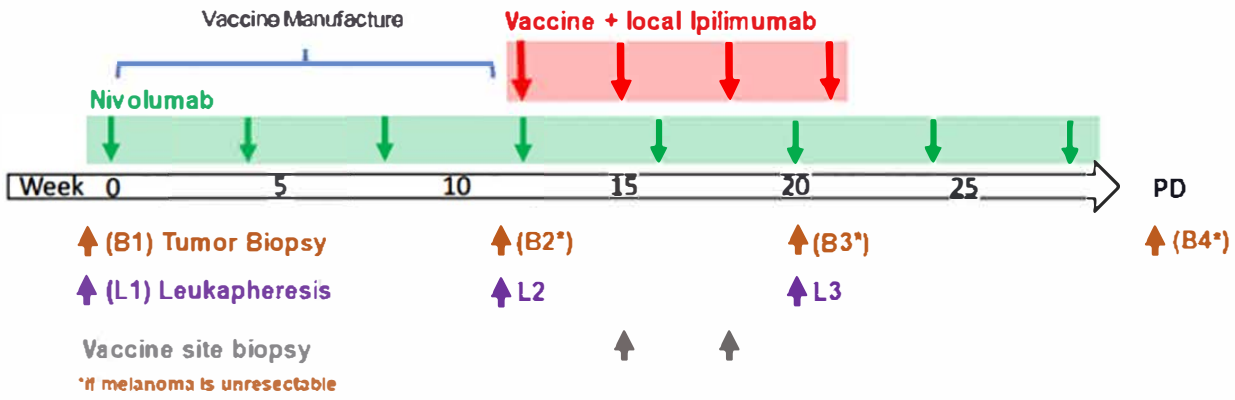
**Principal Investigator (PI):** Patrick A. Ott, MD, PhD;
Dana-Farber Cancer Institute
[redacted]

**Agents:**

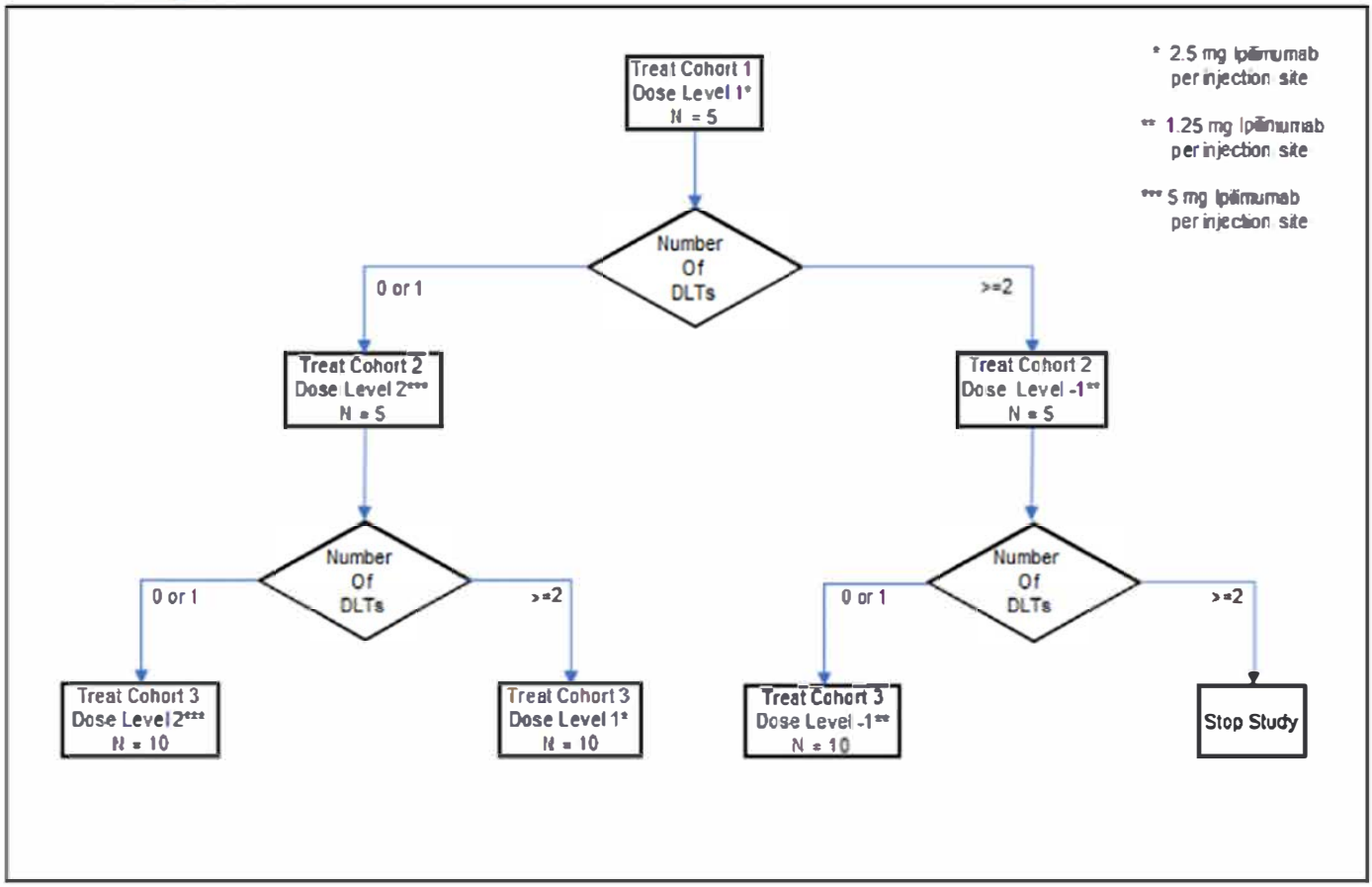
1) Personalized neoantigen peptides IND # 15703 Dana Farber Cancer Institute
2) Poly-ICLC (Hiltonol®) DMF # BB-MF 14,266 and BB-MF 14,302 Oncovir Inc.
3) Montanide® ISA-51 VG, Seppic
4) Nivolumab, Bristol Myers Squibb
5) Ipilimumab, Bristol Myers Squibb

**IND #: 15703**
**IND Sponsor:** Patrick A. Ott, MD PhD
**Protocol Type / Version # / Version Date:** Version 8.0 / November 20, 2023

**SCHEMA A**




**SCHEMA B**



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# 1. OBJECTIVES

## 1.1 Study Design

The study is an open label, phase Ib/II trial in which patients with stage III B/C/D or stage IV melanoma will be treated with NeoVax plus Montanide® ISA-51 VG in combination with Nivolumab and locally administered Ipilimumab. Tumor from a surgical resection specimen or core needle biopsy of a melanoma metastasis will be used to prepare DNA and RNA for sequencing. Patients will begin treatment with Nivolumab and vaccine will be prepared during the initial 12 weeks of Nivolumab therapy. At week 12, patients will begin vaccination with up to 20 neoantigen peptides. These peptides are encoded by non-silent mutations that are identified through DNA and RNA sequencing. Up to 20 peptides ~ 20 amino acids in length will be prepared for each patient and will be administered together with the immune adjuvant poly-ICLC and Montanide® ISA-51 VG, a mineral oil based immune adjuvant analogous to incomplete Freund's adjuvant (IFA).

Concurrently with the vaccine and Nivolumab, Ipilimumab will be delivered via subcutaneous injection in proximity to each vaccination site in order to 1) direct anti-CTLA-4 activity to the vaccine-draining lymph nodes and 2) limit systemic toxic effects. This approach is effective in animal tumor models and is expected to result in significantly reduced levels of systemic Ipilimumab compared to the approved dose/schedule in advanced melanoma (3 mg/kg q3wks for four doses in the metastatic setting). The Ipilimumab dose will be escalated/de-escalated from 2.5 mg flat dose per injection in cohort 1. Serial tumor biopsies and vaccine site biopsies will be performed. (Schema A)

## 1.2 Primary Objective

- To evaluate the safety of administering Neoantigen Vaccine (Neoantigen Peptides plus Hiltonol and Montanide® ISA-51 VG) in combination with Nivolumab and locally administered Ipilimumab

## 1.3 Secondary Objective

- To assess the induction of neoantigen-specific cellular immune responses following administration of NeoVax plus Montanide in combination with Nivolumab and locally administered Ipilimumab
- To estimate rates of disease progression/recurrence depending on whether the patient had all melanoma resected or has measurable disease per RECIST 1.1

# 2. BACKGROUND

## 2.1 Study Disease(s)

Advanced melanoma has the highest per-death loss of years of potential life expectancy except for adult leukemia. The incidence of melanoma continues to rise worldwide at approximately 3% per year. It is estimated that in the United States 100,350 individuals will be diagnosed with melanoma and 6,850 will die from the disease in 2020[1]. According to SEER data, roughly 4% of

melanomas are already metastatic at the time of diagnosis. The tendency for melanoma to spread through the lymphatic system and the bloodstream is in stark contrast to most other skin cancers. Thin melanoma (<1–2 mm) without lymph node involvement is curable in most cases by surgical removal with sufficient margins and adequate staging, which includes assessment of sentinel lymph node involvement if necessary. In contrast, thicker melanomas, ulcerated tumors, or lymph node involvement, although initially mostly amenable to complete surgical resection, confer a much poorer prognosis due to the risk of systemic recurrence, with 5-year survivals ranging from 25% to 75%. Disseminated, locally advanced, or recurrent melanoma is notoriously unresponsive to standard treatment, and is associated with a dismal prognosis, with 5-year survivals of the order of 10–25%. Recent advances with targeted agents that block driver oncogenic mutations such as $BRAF^{V600}$ have shown significant but transient clinical efficacy in patients with advanced melanoma[2-6], whereas immunotherapy using co-stimulatory molecule blockade with monoclonal antibodies such as anti-CTLA-4, PD-1, or PD-L1 can lead to durable responses in 15-40% of melanoma patients [7-10].

### *2.2* Computational and immunological infrastructure for NeoVax plus Montanide

Over the last several years, we at DFCI have developed a computational infrastructure and conceptual framework for translating sequencing information into a therapeutic vaccine through:

(1) *Prediction of mutated peptides that can bind to personal HLA molecules.* Efficiently choosing which particular mutations to utilize as immunogens requires identification of the patient HLA type and the ability to predict which mutated peptides would efficiently bind to the patient's HLA alleles. Recently, neural network based learning approaches with validated binding and non-binding peptides have advanced the accuracy of prediction algorithms for the major HLA-A and -B alleles[11]. In a pilot study, Sidney et al prepared seventy-four 9-mer and sixty-three 10-mer peptides predicted by the algorithm NetMHCpan to have affinities to particular HLA alleles below 1000 nM, with most being below 500 nM. Binding of these peptides to their cognate HLA alleles was experimentally determined using a well-established competitive binding assay[12]. Seventy-five percent of 9-mer peptides predicted to have an affinity below 150 nM were experimentally verified to be below 150 nM and 90% of these peptides were shown to have an affinity below 500 nM, a generally accepted threshold. Fifty percent of 9-mer peptides predicted to have an affinity between 150 and 500 nM were shown to have an affinity below 500 nM. Ten-mer peptide predictions were slightly less precise. For predicted high affinity 10-mer peptides (below 150 nM), 70% were shown to have an affinity below 500 nM. However, only 35% of 10-mer peptides predicted to have an affinity between 150 and 500 nM were shown to have an affinity below 500 nM. Thus, 10-mer peptides with predicted affinities above 150 nM will not be utilized. These predictions provide a reliable tool, when correctly applied, to assist in the selection of potentially immunogenic peptides.

(2) *Evaluating spontaneous human T cell responses to neoantigens.* We have analyzed the predicted HLA-binding properties of 40 different neoepitopes representing both missense mutations and neoORFs identified following an extensive literature review for reports of spontaneous CD8+ T cell responses[13]. This analysis allowed us to conclude that the NetMHCpan algorithm would have accurately predicted each epitope and to determine that a majority of missense epitopes was due to mutations that affected the interaction with the T cell receptor and not the MHC molecule.

(3) *Formulating the drug as a multi-epitope vaccine of long peptides.* For each patient, multiple neoantigens will be targeted. Targeting as many mutated epitopes as practically possible takes advantage of the enormous capacity of the immune system, prevents the opportunity for immunological escape[14] by down-modulation of a particular immune targeted gene product, and compensates for the known inaccuracy of epitope prediction approaches. Synthetic peptides provide a particularly useful means to prepare multiple immunogens efficiently and to rapidly translate identification of mutant epitopes to an effective vaccine. Peptides can be readily synthesized chemically and easily purified utilizing reagents free of contaminating bacteria or animal substances. The small size allows a clear focus on the mutated region of the protein and also reduces irrelevant antigenic competition from other components (unmutated protein or viral vector antigens). "Long" peptides, ~20-30 amino acids in length (as opposed to "short" peptides, ca 8-10 amino acids) have recently been shown to produce a more robust and more durable immune responses[15,16].

(4) *Combination with poly-ICLC, a strong vaccine adjuvant.* Effective vaccines require a strong adjuvant to initiate an immune response. As described in Section 2.3.3, poly-ICLC, an agonist of TLR3 and the RNA helicase domains of MDA5 and RIG3, has shown several desirable properties for a vaccine adjuvant. These properties include the induction of local and systemic activation of immune cells *in vivo*, production of stimulatory chemokines and cytokines, and stimulation of antigen-presentation by DCs. Furthermore, poly-ICLC can induce durable $CD4^+$ and $CD8^+$ responses in humans. Importantly, striking similarities in the upregulation of transcriptional and signal transduction pathways were seen in subjects vaccinated with poly-ICLC and in volunteers who had received the highly effective, replication-competent yellow fever vaccine[17]. Furthermore, >90% of ovarian carcinoma patients immunized with poly-ICLC in combination with a NY-ESO-1 peptide vaccine (in addition to Montanide) showed induction of $CD4^+$ and $CD8^+$ T cell, as well as antibody responses to the peptide in a recent phase 1 study[18]. Moreover, poly-ICLC has been extensively tested in more than 25 clinical trials to date and exhibited a relatively benign toxicity profile.

## 2.3 IND Agents

### 2.3.1 Personalized NeoAntigen Peptides

Sequencing technology has revealed that each tumor contains multiple, patient-specific mutations that alter the protein coding content of a gene[19]. Such mutations create altered proteins, ranging from single amino acid changes (caused by missense mutations) to addition of long regions of novel amino acid sequence due to frame shifts, read-through of termination codons or translation of intron regions (novel open reading frame mutations; neoORFs). These mutated proteins are valuable targets for the host's immune response to the tumor as, unlike native proteins, they are not subject to the immune-dampening effects of self-tolerance. Therefore, mutated proteins are more likely to be immunogenic and are also more specific for the tumor cells compared to normal cells of the patient[20].

There have been several reports indicating that tumors expressing missense mutations or neoORFs can be prevented, and in some cases eradicated, by immunization with peptides corresponding to the mutated protein[21-25]. For example, $CD8^+$ cytotoxic T lymphocytes (CTLs) directed at missense mutations in the RNA helicase protein[24] or the ß2-spectrin protein[26] found in particular murine tumors have been identified. Expression of these mutated proteins by the tumor

was found to correlate with tumor control or progression.

Correspondingly, several human studies of spontaneous regression and long-term survival have shown that powerful CD8+ T cell responses against mutated epitopes correlate with good clinical responses[13]. These studies began with the first identification of human immunogenic neoantigens[27,28], and the seminal study by Lennerz demonstrating the strength and durability of neoantigen response compared to native protein responses[29], and now have included the observations of an increase in neoantigen-specific CD8+ T cells in a patient responding to anti-CTLA4 therapy[30] and tumor regression following infusion of a tumor infiltrating lymphocyte (TIL) population highly enriched in a neoantigen directed CD4+ T cell[31]. These observations were made in multiple cancer types for multiple HLA alleles and have been widely observed in tumor infiltrating T cell populations, suggesting that there may be an association with CD8+ T cell activity and productive clinical responses[29,32-36]. Most of these CD8+ T cell responses show a high degree of specificity toward the mutated missense epitope compared to the native epitope, represent a high proportion of circulating T cells, and result in cells that are more abundant and active than CD8+ T cell responses in the same patients directed toward over-expressed native antigens.

Thus, in animals and in humans, immune responses to both discrete, mutated antigens (such as missense mutations) and expansive novel antigens (neoORFs) are observationally correlated with regression and long-term remission. Extending that correlation among a large set (n=468) of patients found in the Cancer Genome Atlas (TCGA) database, a recent meta-analysis of six tumor types revealed a significant survival advantage (hazard ratio = 0.53; p=0.002) for patients with at least one predicted immunogenic neoepitope compared to patients with no predicted immunogenic epitopes[37].

Four studies in humans have directly assessed the immunotherapeutic potential of mutated antigens. First, because follicular lymphoma is characterized by uncontrolled growth of a B cell expressing rearranged immunoglobulin, many groups purified the rearranged immunoglobulin for use as a vaccine. The induced CD8+ T cells showed reactivity to the rearranged, mutated portion of the immunoglobulin molecule (the idiotype) and not the germline framework[38], and this personalized vaccine approach revealed highly encouraging results in Phase I/II trials. However, only one of three Phase III trials demonstrated prolonged disease-free survival (but this trial failed to achieve its primary clinical end points)[39]. The observed failures have been attributed to flaws in trial design, such as imbalances in standard prognostic scores between treated and control arms as well as enrollment of patients lacking sustained partial or complete remissions. Thus, the value of idiotype vaccines in conjunction with current rituximab-containing regimens remains to be determined. Second, a mix of peptides corresponding to the oncogenic proteins of HPV (a neoORF for humans) has been shown to result in significant remission of premalignant lesions induced by HPV[15,16,40]. Moreover, immunization with a synthetic version of an in-frame junctional deletion variant of the epidermal growth factor receptor (EGFRviii) in glioblastoma patients, a population known to frequently contain this mutation, provided encouraging phase 2 results[41,42]. Importantly, in these glioblastoma studies, evaluation of tumors in patients with tumor recurrence showed that the recurrent tumors almost uniformly (20 of 23) lost expression of EGFRviii. This was interpreted as clear evidence of immunoediting due to immune pressure against an immunogenic neoantigen in humans. Finally, very recently, a personalized dendritic cell-based vaccine targeting melanoma neoantigens

promoted a diverse neoantigen-specific T cell receptor repertoire along with an increase in endogenous neoantigen-specific immune responses in a small Phase I trial[43]. Thus, in animals and in humans, immune responses to both discrete mutated antigens (such as missense mutations) and expansive novel antigen (neo●RF) are observationally correlated with regression and long-term remission and, in two clinical studies, have been shown to control disease following therapeutic vaccination. The direct and comprehensive identification of the many mutated epitopes found in cancer genomes creates the opportunity to use this class of immunogen to improve the immune response and efficacy of cancer vaccines.

Most cancer vaccines employing peptides as immunogens have utilized "short" peptides. These peptides are typically 9 – 10 amino acids in length and capable of direct binding to the HLA molecule on the surface of HLA-expressing cells. "Long" peptides, ~20-30 amino acids in length, have recently been shown to produce a more robust and more durable immune response[15,16]. Long peptides require internalization, processing and cross-presentation in order to bind to HLA molecules; these functions only occur in professional antigen-presenting cells, such as dendritic cells, which can induce strong T cell responses.

Many studies in humans have demonstrated the safety of peptide vaccines. These include studies with multiple short peptides[33] as well as multiple long peptides, including neoORFs. In particular, two studies have been conducted with a mixture of 10 overlapping long peptides derived from p53[28,29] and three separate studies with a mixture of 13 long peptides derived from the oncogenic proteins of HPV[17-19]. In these studies, no toxicity higher than grade 2 was observed and most adverse events were of limited duration and severity. Additionally, many heterologous antigen preparations have been tested in humans. Such preparations include irradiated cell vaccines[44,45] and tumor cell lysates[46]. These heterogeneous vaccines contain mutated antigens, in the form of intact proteins, partially degraded intracellular protein, and peptides found on the surface bound to MHC I. Moreover, they contain over-expressed and selectively-expressed molecules as well as many additional native proteins. In addition, purified heat shock protein (HSP) 96 peptide complexes have been used as antigen[47]; such complexes also contain many mutated peptides. None of these studies have reported significant safety issues directly attributable to the immunogens of the vaccines.

**GMP peptides**

Clinical grade peptides will be prepared by synthetic chemistry, purified UPLC, mixed in small groups (see Sect 5.1.3) and combined with both the immune adjuvant poly-ICLC (a stabilized double-stranded RNA) and Montanide® ISA-51 VG (see Section 5.1.3).

The mixtures of peptides, poly-ICLC, and Montanide® ISA-51 VG will be used for vaccination with the intention to induce cellular immune responses directed at these patient/tumor specific mutations. Each patient will receive the full complement of peptides at each immunization.

### 2.3.2 Poly-ICLC

#### 2.3.2.1 TLR agonists as adjuvants for cancer vaccines

Toll like receptors (TLRs) are important members of the family of pattern recognition receptors

(PRRs) which recognize conserved motifs shared by many micro-organisms, termed "pathogen-associated molecular patterns" (PAMPS). Recognition of these "danger signals" activates multiple elements of the innate and adaptive immune system. TLRs are expressed by cells of the innate and adaptive immune systems such as dendritic cells (DCs), macrophages, T and B cells, mast cells, and granulocytes and are localized in different cellular compartments, such as the plasma membrane, lysosomes, endosomes, and endolysosomes[48]. Different TLRs recognize distinct PAMPS. For example, TLR4 is activated by LPS contained in bacterial cell walls, TLR9 is activated by unmethylated bacterial or viral CpG DNA, and TLR3 is activated by double stranded RNA[49]. TLR ligand binding leads to the activation of one or more intracellular signaling pathways, ultimately resulting in the production of many key molecules associated with inflammation and immunity (particularly the transcription factor NF-κB and the Type-I interferons).

TLR mediated DC activation leads to enhanced DC activation, phagocytosis, upregulation of activation and co-stimulation markers such as CD80, CD83, and CD86, expression of CCR7 allowing migration of DC to draining lymph nodes and facilitating antigen presentation to T cells, as well as increased secretion of cytokines such as type I interferons, IL-12, and IL-6. All of these downstream events are critical for the induction of an adaptive immune response.

Among the most promising cancer vaccine adjuvants currently in clinical development are the TLR9 agonist CpG and the synthetic double-stranded RNA (dsRNA) TLR3 ligand poly-ICLC. In preclinical studies poly-ICLC appears to be the most potent TLR adjuvant when compared to LPS and CpG due to its induction of pro-inflammatory cytokines and lack of stimulation of IL-10, as well as maintenance of high levels of co-stimulatory molecules in DCs[50]. Furthermore, poly-ICLC was recently directly compared to CpG in non-human primates (rhesus macaques) as adjuvant for a protein vaccine consisting of human papillomavirus (HPV)16 capsomers. Poly-ICLC was found to be much more effective in inducing HPV specific Th1 immune responses[51].

#### 2.3.2.2 Poly-ICLC – a synthetic TLR3 agonist with strong vaccine adjuvant properties

Poly-ICLC is a synthetically prepared double-stranded RNA consisting of polyI and polyC strands of average length of about 5000 nucleotides, which has been stabilized to thermal denaturation and hydrolysis by serum nucleases by the addition of polylysine and carboxymethylcellulose. The compound activates TLR3 and the RNA helicase-domains of MDA5 and RIG3, both members of the PAMP family, leading to DC and natural killer (NK) cell activation and production of a "natural mix" of type I interferons, cytokines, and chemokines. Furthermore, poly-ICLC exerts a more direct, broad host-targeted anti-infectious and possibly anti-tumor effect mediated by the two IFN-inducible nuclear enzyme systems, the 2'5'-OAS and the P1/eIF2a kinase, also known as the PKR (4-6), as well as RIG-I helicase and MDA5.

In rodents and non-human primates, poly-ICLC was shown to enhance T cell responses to viral antigens[52-55], cross-priming, and the induction of tumor-, virus-, and autoantigen-specific $CD8^+$ T-cells[56-58]. In a recent study in non-human primates, poly-ICLC was found to be essential for the generation of antibody responses and T-cell immunity to DC targeted or non-targeted HIV Gag p24 protein, emphasizing its effectiveness as a vaccine adjuvant.

In human subjects, transcriptional analysis of serial whole blood samples revealed similar gene expression profiles among 8 healthy human volunteers receiving one single s.c. administration of poly-ICLC and differential expression of up to 212 genes between these 8 subjects versus 4 subjects receiving placebo[17]. Remarkably, comparison of the poly-ICLC gene expression data to previous data from volunteers immunized with the highly effective yellow fever vaccine YF17D[59] showed that a large number of transcriptional and signal transduction canonical pathways, including those of the innate immune system, were similarly upregulated at peak time points.

Two studies of poly-ICLC in conjunction with long peptides have been published. An immunologic analysis was reported on patients with ovarian, fallopian tube, and primary peritoneal cancer in second or third complete clinical remission who were treated on a phase 1 study of subcutaneous vaccination with synthetic overlapping long peptides (OLP) from the cancer testis antigen NY-ESO-1 alone or with Montanide-ISA-51, or with 1.4 mg poly-ICLC and Montanide. The generation of NY-ESO-1-specific CD4+ and $CD8^+$ T-cell and antibody responses were markedly enhanced with the addition of poly-ICLC and Montanide compared to OLP alone or OLP and Montanide[18]. In a second human study, poly-ICLC was combined with a MUC1 synthetic long peptide in patients with pre-malignant adenomas. Robust antibody responses were detected in nearly half the patients which inversely correlated with the preexisting circulating myeloid derived suppressor cell level[60].

Poly-ICLC has also been utilized as an adjuvant for immunization with minimal epitope-loaded patient-derived dendritic cells. Both CD4+ and CD8+ T cell responses to multiple peptides were observed in the majority of patients[61].

#### 2.3.2.3 Pre-clinical toxicology of poly-ICLC

Complete information on the pre-clinical toxicology studies can be found in the poly-ICLC (Hiltonol®) Investigator Brochure (IB). The results of these toxicology studies are summarized in brief below:

*Single dose toxicity:*

All administrations of poly-ICLC were intravenous (IV).
*Rodents*. The median lethal dose ($LD_{50}$) values for single dose poly-ICLC were approximately 15-18.3 mg/kg in rats and 25-30 mg/kg in mice. No necropsies were conducted.
*Dogs*. In beagle dogs, the lethal dose of single dose poly-ICLC administration was 4.0 mg/kg. Necropsy indicated toxic effects in the gastrointestinal, hepatic, cardiovascular, renal, endocrine, and lymphatic systems. Sublethal doses caused reversible nephrotoxicity and hepatotoxicity. The non-toxic dose for dogs was 0.25 mg/kg.
*Primates*. In rhesus monkeys, single-dose administration of poly-ICLC was non-toxic at a dose of .5 mg/kg, but lethal at 20 mg/kg. Toxic effects were seen in the gastrointestinal, hepatic, cardiovascular, renal, endocrine, and lymphatic systems.

*Repeated dose toxicity:*

*Rodents*. Mice treated with four daily IV doses of up to 3.66 mg/kg poly-ICLC exhibited no toxicity.
*Cats*. One of two cats treated with the same regimen at 3.33 mg/kg poly-ICLC demonstrated

vomiting, an episode of diarrhea, and moderate weight loss.
*Non-human Primates*. Three rhesus monkeys and 6 macaque monkeys were treated with 5 mg/kg IV of poly-ICLC over 2 days and 3 mg/kg 3 times weekly for 16 weeks, respectively; no significant toxicity was observed other than a single instance of emesis and shivering. Necropsy of the macaques showed no drug-related changes by gross or microscopic pathology of all organs. In two additional studies, macaque monkeys were treated daily with up to 3 mg/kg IV for 12 days and chimpanzees were treated daily with 3 mg/kg IV for two 6-day periods, then every other day for a total of 7 weeks. At the 3 mg/kg doses, a decrease in hematocrit was seen in both studies. No change in hematocrit was seen at 0.3 mg/kg. In the chimpanzee study, in addition to a decrease in hematocrit, leukocytosis and elevations in alanine aminotrasferase (ALT) and aspartate aminotrasferase (AST) were observed and normalized after termination of dosing. No other significant toxicity was observed in these studies.

#### 2.3.2.4 Clinical toxicology of poly-ICLC

Poly-ICLC is the TLR3 agonist formulation most extensively tested in patients with infectious diseases and in subjects with a variety of different tumor types.

Prior to the availability of recombinant interferon, poly-ICLC was used clinically at high doses ≥ 6mg/m$^2$ (about 170 µg/kg) in patients with a variety of solid tumors and leukemia[61]. Fever, often above 40°C, was a common adverse event and the primary dose-limiting factor. Other common adverse events were flu-like symptoms (nausea, vomiting, arthralgia, myalgia and fatigue) and hypotension, thrombocytopenia and leukopenia. Once recombinant interferon became clinically available, the need to pursue high dose poly-ICLC was eliminated, and it became recognized that lower doses (10 – 50 µg/kg) were highly effective at stimulating host defense and as an immune adjuvant.

By now, more than 400 patients with malignant gliomas have been entered on 7 clinical trials using low dose (1-2 mg total dose) poly-ICLC either as monotherapy or in conjunction with chemotherapy, radiation, or vaccine (Table 1). Furthermore, patients with various other solid tumors (prostate, colorectal, pancreatic, hepatocellular, breast, and ovarian cancers), in addition to patients with HIV/AIDS and multiple sclerosis have been treated on more than 10 additional clinical phase I and phase II studies. Overall, the drug has been well-tolerated across all studies and spectrum of diseases. The most common adverse events attributed as at least possibly related to poly-ICLC have included:

- bone marrow toxicity (leukopenia, neutropenia, thrombocytopenia, and anemia)
- reversible liver toxicity (AST/ALT elevations, LDH and alkaline phosphatase elevation)
- transient discomfort at the injection site
- transient fatigue/malaise
- transient flu-like symptoms

In a recently published phase I clinical trial, 11 patients with ovarian, fallopian tube, or primary peritoneal cancer were treated with poly-ICLC at a total dose of 1.4 mg in combination with Montanide-ISA-51 and synthetic over-lapping long peptides from NY-ESO-1. The vaccine was generally well-tolerated with grade 1-2 injection site reactions and fatigue as the only adverse

events that were considered possibly or definitely drug-related[18]. No grade 3 or 4 adverse events were reported. In another clinical study which recently completed accrual (ClinicalTrials.gov Identifier NCT01079741) 27 patients with high-risk melanoma received poly-ICLC at a total dose of 1.4 mg in addition to NY-ESO-1 protein ± Montanide-ISA-51 given s.q. every 3 weeks for 4 doses (in addition to 6 patients who received 0.35 mg and 0.70 mg, respectively of poly-ICLC on cohorts 1 and 2 of the dose escalation part of the trial). The vaccine was generally well-tolerated with grade 1 and 2 injection site reactions and transient, grade 1 or 2, flu-like symptoms that resolved after 12-48 hours ([redacted]).

**Selected Clinical Trials with Low-Dose Poly-ICLC**

| Protocol Title | Phase | Protocol Location | Indication | Status | N | Dosing Schedule |
|---|---|---|---|---|---|---|
| Long-term IM poly-ICLC in Malignant Glioma – a Pilot Study | I-II | Walter Reed | Malignant Glioma | Closed | 67 | IM 10-50 µg/kg 1-3 X week |
| Poly-ICLC in Recurrent Malignant Brain Tumors | I-II | MCV | Recurrent Glioma | Closed | 99 | IM 20 µg/kg 3 X week |
| Poly-ICLC in Malignant Pediatric Brain Tumors | I-II | L.A. Childrens Hosp | Pediatric Glioma | Closed | 46 | IM 20 µg/kg 2 X week |
| Poly-ICLC plus Radiation in Glioblastoma | II | NABTC 2001-05 | Glioblastoma | Closed | 31 | IM 20 µg/kg 3 X week |
| Poly-ICLC in Recurrent Anaplastic Glioma | II | NABTC 2001-06 | Recurrent Anaplastic Glioma | Closed | 55 | IM 20 µg/kg 3 X week |
| Poly-ICLC plus Temodar in Newly Diagnosed Glioblastoma | II | NABTT 2005-01 | Glioblastoma | Closed | 97 | IM 20 µg/kg 3 X week |
| Pilot Study of MUC1 Vaccine plus poly-ICLC in Advanced Prostate Cancer | I-II | UPMC 05-086 | Advanced Prostate Cancer | Closed | 25 | IM 25 µg/kg 2 X week |
| Poly-ICLC plus Dendritic Cell vaccine in Recurrent Gliomas | I-II | UPMC | Recurrent gliomas | Closed | 25 | IM 20 mcg/kg |
| Poly-ICLC plus HSP-HPVE7 Vaccine in Cervical Dysplasia | I | Nventa | Cervical Dysplasia | Closed | 24 | .05 – 2 mg |
| IntraTumoral (IT) poly-ICLC plus XRT and TACE in Liver Cancer | I-II | UMDNJ | Hepatoma, Metastatic liver cancers | Closed | 24 | 1 mg IT + 1 mg IM 2xweek |
| A Randomized Controlled Phase I trial of Nasal Hiltonol | I | NIAID, NIH: | Normal volunteers | Closed | 50 | 0.25-4mg IN |
| CDX 1307 vaccine with poly-ICLC in metastatic cancers | I | Various | Metastatic Cancers | Closed | 20 | 2 mg IM |
| Poly-ICLC with glioma associated peptide vaccine | I-II | UPMC | Grade II Gliomas | Closed | 20 | 20 mcg/kg IM 2X Wk |
| MUC1 100-mer and poly-ICLC Vaccine for Colonic Polyposis | I/II | UPMC | Colonic Adenoma | Closed | 45 | .5 mg SC |
| Hiltonol +NYESO1 Protein Vaccine in Ovarian Cancer | I/II | MSKCC, LICR | Ovarian Cancer | Closed | 10 | 1.4 mg SC |
| Intratumoral PICLC and DC in Pancreatic CA | I/II | MUSC | Pancreatic Cancer | Closed | 12 | 1 mg IT + IM |

| Selected Clinical Trials with Low-Dose Poly-ICLC | | | | | | |
|---|---|---|---|---|---|---|
| Intratumoral PICLC and DC in Solid Cancers | I/II | Univ. Navarra Spain | Solid Cancers | Closed | 15 | 1 mg IT |
| Pilot study of intratumoral and IM Poly-ICLC | I/II | Mt. Sinai MC, NY | Solid Cancers | Closed | 8 | 1 mg IT+ IM |
| Poly-ICLC + CDX1401 + FLT3 | I/II | CITN, Celldex | Melanoma | Closed | 60 | 1 mg IT+ IM |
| IMA950 vaccine /Poly-ICLC, Keytruda | I/II | U. Geneva Suisse | Glioblastoma | Open | 20 | 1 mg IM |
| MAGE A3 + Hiltonol in Myeloma | I/II | Univ. of Maryland | Myeloma | Closed | 27 | 1 mg SC |
| DEC205 GAG Protein / Hiltonol | II | Rockefeller Univ. | HIV vaccine | Closed | 40 | 1.5 mg SC |
| WT1 vaccine / Hiltonol / Basiliximab | I/II | U. Chicago | AM Leukemia | Closed | 7 | 1 mg SC |
| In-Situ. IT Autovaccination Hiltonol, FLT3, aPD1 | I/II | Mt. Sinai MC, NY | Lymphoma | Open | 40 | 1 mg |
| In Situ Autovaccination with IT Hiltonol, | II | Multicenter | Solid Tumors | Open | 60 | 1 mg IT + IM |
| GAPVAC Trial, Europe | I | Multicenter | Glioblastoma | Closed | 16 | 1 mg SC |
| A Personalized Cancer Neoantigen vaccine + Poly-ICLC, Nivolumab | I/II | Multicenter | Solid Cancers | Open | 90 | 2 mg SC |
| In situ vaccination with durvalumab ± tremelimumab + IT Hiltonol | I/II | UVA, ISMMS | Solid Cancers | Open | 36 | 1 mg IT |
| Pembrolizumab and poly-ICLC | I/II | Augusta U. | Colon Cancer | Open | 45 | 1 mg IT |
| IM Poly-ICLC in Low grade Glioma | I/II | UCSD, Emory U | Pediatric Low Grade Glioma | Closed | 23 | 20 mcg/kg IM |
| IM Hiltonol + aPD1/L1 | II | Multicenter | Solid Cancers | Open | 60 | 1 mg 2X wk |

### 2.3.3 Montanide IS-51 VG

Montanide ISA-51 VG (Seppic, Inc.) is a commercially available, mineral oil-based immune adjuvant analogous to incomplete Freund's adjuvant (IFA) that enhances the immune response to vaccination through various mechanisms. The depot effect of this water in oil emulsion results in slow release of the antigen from the injection site. The emulsion also protects the antigen from rapid degradation by enzymes, and can modify the electric charge of the antigen leading to enhanced immunogenicity[62]. In addition, the emulsion can create inflammation and stimulate the recruitment and possible activation of antigen presenting cells (APC) and even facilitate antigen uptake by APCs through interactions between the surfactant and cellular membranes. Lymphocyte trapping in draining lymph nodes is an additional mechanism of action of oil adjuvants contributing to prolonged cell-cell interactions in the lymph node. (Seppic Inc., data,

manufacturer of Montanide). Montanide ISA-51 VG must be mixed in a 1:1 ratio with aqueous vaccine preparation to form an emulsion for subcutaneous injection. The compound can be administered in repeat doses ranging from 0.5 ml to 2 ml.[62]

Montanide has been tested in more than 200 clinical phase 1 – 3 cancer vaccine trials targeting melanoma and a number of other solid and hematologic malignancies. The compound has been generally well tolerated and induces transient local reactions such as tenderness, erythema, or edema at the injection site. Transient general reactions including fever, headache, and flu-like symptoms have also been observed. A clinical study of long peptides (~20-mer) derived from the HPV-16 E6 and E7 viral oncoproteins administered in Montanide showed clinical responses in women with vulvar intraepithelial neoplasia; tumor regressions were associated with the generation of HPV-specific, IFN-γ-producing CD4+ and CD8+ T cells[15,16].

Montanide-ISA-51 in combination with poly-ICLC and synthetic over-lapping long peptides from NY-ESO-1 has been administered to 11 patients with ovarian, fallopian tube, or primary peritoneal cancer in a phase I clinical trial. This vaccine was generally well-tolerated with grade 1-2 injection site reactions and fatigue as the only adverse events that were considered possibly or definitely drug-related[18]. No grade 3 or 4 adverse events were reported. In another clinical trial (ClinicalTrials.gov Identifier NCT01079741) 19 patients with high-risk melanoma received Montanide –ISA-51 combined with poly-ICLC and NY-ESO-1 protein given s.q. every 3 weeks for 4 doses. The vaccine was generally well-tolerated with grade 1 and 2 injection site reactions and transient, grade 1 or 2, flu-like symptoms that resolved after 12-48 hours[63].

### 2.3.4 Ipilimumab

Ipilimumab (BMS-734016, MDX010, MDX-CTLA4) is a fully humanized monoclonal immunoglobulin (Ig) G1κ specific for human cytotoxic T lymphocyte antigen 4 (CTLA-4, CD152), which is expressed on a subset of activated T cells. CTLA-4 is a negative regulator of T cell activity. Ipilimumab is a monoclonal antibody that binds to CTLA-4 and blocks the interaction of CTLA-4 with its ligands, CD80/CD86. Blockade of CTLA-4 has been shown to augment T cell activation and proliferation, including the activation and proliferation of tumor infiltrating T-effector cells. Inhibition of CTLA-4 signaling can also reduce T-regulatory cell function, which may contribute to a general increase in T cell responsiveness, including the anti-tumor response.

Yervoy™ (Ipilimumab) has been approved for use in over 47 countries including the United States (US, Mar-2011), the European Union (EU, Jul-2011) and Australia (Jul-2011).

#### 2.3.4.1 Preclinical Toxicology of Ipilimumab

**Complete information on the pre-clinical toxicology studies can be found in the Ipilimumab Investigator Brochure (IB). The results of these toxicology studies are summarized in brief below.**

Ipilimumab binds to human and cynomologous monkey CTLA-4 with high affinity and blocks binding of CD80 (B7.1) and CD86 (B7.2) to CTLA-4. The proposed mechanism of action for Ipilimumab is interference of the interaction of CTLA-4 with its natural ligands, CD80/CD86, expressed on antigen presenting cells, which results in blockade of the inhibitory modulation for

T cell activation. CTLA-4 blockade by Ipilimumab resulted in augmentation of antibody responses to various T cell dependent antigens and suppression of tumor growth in mice.

The cynomologous monkey was selected as the primary toxicology species because Ipilimumab binds specifically to macaque CTLA-4, but not to homologous CTLA-4 in other traditional toxicology species[64]. Administration of Ipilimumab to cynomologous monkeys resulted in marked enhancement of the immune response to a viral antigen (hepatitis B surface antigen), a cell-based vaccine (SK-mel) and to keyhole limpet hemocyanin.

In IV repeat-dose toxicology studies in monkeys, Ipilimumab was tolerated without adverse effects at doses up to 30 mg/kg/day administered every 3 days for 3 doses (peak serum concentrations ≤ 682 µg/mL)[65-67] at 10 mg/kg (equivalent to human dose on a body-weight basis) administered weekly for 1 month[68] (mean area under the concentration-time curve 0-168h) and AUC (0-63days) of 31.6 mg·h/mL and 90.6 mg·h/mL respectively), at 1 mg/kg administered weekly for 10 weeks[69] and at doses up to 10 mg/kg/day administered approximately monthly for up to 6 months[66,68,70,71]. In the pivotal 6-month toxicity study (10 mg/kg administered on days 0,28,56,84 and 140), treatment-related findings were limited to decreases in absolute and relative thyroid (44% to 50%) and testicular (27% to 50%) weights.

In an exploratory pharmacology study, severe colitis occurred after the second dose in 1 of 5 monkeys receiving Ipilimumab at 10 mg/kg approximately monthly in combination with 3 vaccines. The binding of Ipilimumab to CTLA-4 expressed on gut-associated lymphoid tissue was confirmed in human and monkey tissue-binding studies[64,72,73] and suggests that these lymphocytes exist in an activated state, making them susceptible to CTLA-4 blockade. Two gastrointestinal toxicities (diarrhea and colitis) have emerged as significant immune related adverse events in the clinical studies with Ipilimumab (See 2.4.2.2 below).

The effects of Ipilimumab on reproduction and development were studied in an enhanced pre and postnatal development study in cynomologous monkeys[74]. Pregnant monkeys received Ipilimumab every 21 days from the onset of organogenesis through parturition at doses of 2.6 or 7.2 times the clinical exposure at a dose of 3 mg/kg or 0.9 to 2.1 times higher than the clinical exposure at a dose of 10 mg.kg every 21 days of Ipilimumab. Beginning in the third trimester, the Ipilimumab groups experienced increased maternal weight decrements; higher incidences of abortion, stillbirth, premature delivery and higher incidences of infant mortality in a dose-related manner. Infants exposed to 30 mg/kg/q3w had a lower mean body weight at birth that persisted for multiple months. Based on the results for the monkey reproductive system, Ipilimumab is not recommended for use during pregnancy unless the potential benefit justifies the potential risk to the fetus.

In summary, other than the events described above, Ipilimumab did not result in any adverse toxicities in any other monkeys in general toxicity studies when administered IV at doses up to 30 mg/kg for 1 week, 10 mg/kg weekly for 1 month, 1 mg/kg weekly for 10 weeks, or 10 mg/kg monthly for 6 months.

#### 2.3.4.2 Clinical Toxicology of Ipilimumab

Except for pediatric patients, all the clinical studies reported in the Investigator Brochure utilized Ipilimumab delivered intravenously at doses of 3 mg/kg to 10 mg/kg, usually q3weeks X 4. As described in Section 6.1.3, the starting dose level utilized in this protocol results in approximately one thirtieth (1/30) the exposure compared to the 3 mg/kg i.v. dosing. Thus, the safety events observed with Ipilimumab delivered intravenously may be significantly greater than following limited local delivery, a hypothesis of this study.

Complete information on the clinical safety of Ipilimumab delivered intravenously at doses of 3 mg/kg and 10 mg/kg can be found in the Ipilimumab Investigator Brochure (IB) and are summarized in brief below.

Blockade of CTLA-4 by Ipilimumab leads to T cell activation with the potential for clinical inflammatory AEs primarily involving the skin (dermatitis, pruritis), GI tract (diarrhea/colitis), liver (hepatitis), endocrine glands (e.g., hypophysitis, adrenal and thyroid abnormalities) and other less frequent organs (e.g., uveitis/episcleritis). The majority of the inflammatory AEs are reversible with the guidance issued below. In rare cases, these inflammatory AEs may be fatal. Patients should be assessed for signs and symptoms of enterocolitis, dermatitis, neuropathy and endocrinopathy, and clinical chemistries (including liver function and thyroid function tests) should be evaluated at baseline and before each dose of Ipilimumab. GI (diarrhea and colitis) and skin (rash and pruritis)-related toxicities are the most common inflammatory events reported in studies with Ipilimumab. Suggested evaluation procedures for suspected GI, liver, skin, endocrine, neurological and ocular toxicities are described below. Early diagnosis and treatment intervention for inflammatory events can help prevent the occurrence of complications, such as GI perforation.

During evaluation of a suspected inflammatory AE, all efforts should be made to rule out neoplastic, infectious, metabolic, toxin or other etiologic causes. Serological, immunological, imaging, and biopsy with histology (e.g. biopsy-proven lymphocytic) data should be used to support the diagnosis of an immune-mediated toxicity or support an alternative cause of the AE.

In general, for severe inflammatory AEs, Ipilimumab should be permanently discontinued and systemic high-dose corticosteroid therapy should be initiated. For moderate immune-mediated AEs, Ipilimumab should be held or delayed and moderate dose corticosteroids should be considered. Upon improvement, corticosteroids should be tapered gradually over at least 1 month.

Based on limited current clinical experience, corticosteroids do not appear to adversely affect the antitumor response. For example, disease control was maintained in subjects with objective responses who received corticosteroid administration for concomitant serious inflammatory AEs.

The management guidelines for general inflammatory GI, liver, skin, endocrine, and neurological toxicities are provided in detail in Appendix 3 of the Investigators Brochure.

#### 2.3.4.3 Clinical Toxicology of Ipilimumab in combination with a peptide vaccine

A number of clinical trials have evaluated the combination of systemic Ipilimumab with a variety of different vaccines, including peptide vaccines as outlined in the table below. In all cases, no qualitatively or quantitatively different toxicities were observed other than those typically observed with Ipilimumab alone.

| Vaccine | Dose and schedule | Reference |
|---|---|---|
| gp100 peptide vaccine | 3 mg/kg Ipilimumab; iv; q3weeks<br>1 mg peptide; sc;<br>q3weeks (2 peptides) | 2010; Hodi et al[75] |
| GM-CSF transduced allogeneic prostate cancer cells (GVAX- prostate) | 0.3 – 5 mg/kg Ipilimumab; iv; q4weeks<br>GVAX-prostate id; q2weeks for 24 weeks | 2012; Van den Eertwegh et al[76] |
| Prostate-specific antigen and three co-stimulatory molecules (CD58, CD80 and ICAM1) in a poxviral vector (Prostvac) | 1 – 10 mg/kg Ipilimumab; iv; q4weeks<br>Prostvac; sc; q4weeks | 2012; Madan et al[77] |
| GM-CSF transduced allogeneic pancreatic ductal adenocarcinoma cells (GVAX-pancreatic) | 10 mg.kg Ipilimumab; iv; q3weeks<br>GVAX-pancreatic; id; q3weeks | 2013; Le et al[78] |
| gp100, MART-1 and Tyrosinase peptide vaccine | 3 or 10 mg/kg Ipilimumab; iv' q6-8weeks<br>1 mg each peptide; sc; q2weeks x6 then less frequently | 2011; Sarnaik et al[79] |

#### 2.3.4.4 Clinical Efficacy of Ipilimumab as Monotherapy in Advanced Melanoma

In a large, double-blind, double-dummy randomized phase 3 study involving 676 patients, Ipilimumab was delivered at 3 mg/kg in patients who had received 1 or more prior therapies. Patients were randomized (3:1:1) to receive Ipilimumab in combination with a peptide vaccine, Ipilimumab alone or the peptide vaccine alone. All patients were HLA A*02:01. Overall survival was compared in the Ipilimumab + vaccine arm vs the vaccine-alone arm. Treatment with Ipilimumab +vaccine resulted in a 32% reduction of risk of death (HR 0.68; 95% CI 0.55-0.85) versus vaccine alone (p = 0.0004). There was no apparent difference between the Ipilimumab + vaccine arm compared to Ipilimumab alone (HR 1.04; 95% CI 0.83-1.30). The median survival of patients on the Ipilimumab + vaccine arm was improved by 4 months (10 months vs 6 months).

One of the hallmarks of Ipilimumab efficacy is long-term survival. A large meta-analysis of 1861 advanced melanoma subjects treated with Ipilimumab as part of clinical trials demonstrated a plateau in OS Kaplan-Meier curve at Year 3, which remained flat through Year 10[80].

### 2.3.5 Nivolumab

Nivolumab (also referred to as BMS-936558 or MDX-1106) is a fully human monoclonal immunoglobulin G4 (IgG4-S228P) antibody that targets the programmed death–1(PD-1, cluster of differentiation 279 [CD279]) cell surface membrane receptor. PD-1 is a negative regulatory molecule expressed by activated T and B lymphocytes.1 Binding of PD-1 to its ligands, programmed death–ligand 1 (PD-L1) and 2 (PD-L2), results in the down-regulation of lymphocyte activation. Inhibition of the interaction between PD-1 and its ligands promotes immune responses and antigen-specific T-cell responses to both foreign antigens as well as self-

antigens. Nivolumab is expressed in Chinese hamster ovary (CHO) cells and is produced using standard mammalian cell cultivation and chromatographic purification technologies. The clinical study product is a sterile solution for parenteral administration.

#### 2.3.5.1 Preclinical Toxicology of Nivolumab

**Complete information on the pre-clinical toxicology studies can be found in the Nivolumab Investigator Brochure (IB). The results of these toxicology studies are summarized in brief below:**

Nivolumab has been shown to bind specifically to the human PD-1 receptor and not to related members of the CD28 family such as CD28, inducible co-stimulator (ICOS), cytotoxic T-lymphocyte antigen 4 (CTLA-4) and B and T lymphocyte attenuator (BTLA). Nivolumab inhibits the interaction of PD-1 with its ligands, PD-L1 and PD-L2, resulting in enhanced T-cell proliferation and interferon-gamma (IFN-γ) production in vitro. Fluorescent-activated cell sorter (FACS) analysis confirmed that Nivolumab could bind to transfected CHO and activated human T-cells expressing cell surface PD-1 and to cynomolgous monkey PD-1, but not to rat or rabbit PD-1 molecules. Nivolumab has also been shown to bind to PD-1 on virus-specific CD8+T-cells from chronically infected hepatitis C virus (HCV) patients. PD-1 inhibition in a mixed lymphocyte reaction (MLR) resulted in a reproducible concentration dependent enhancement of IFN-γ release in the MLR up to 50 µg/mL. No effect was observed with a human IgG4 isotype control or CD4+ T-cells and dendritic cell (DC) controls.

The potential of Nivolumab to react with non-target tissues was investigated with cryosections of normal human tissues. Nivolumab demonstrated reactivity with rare to occasional lymphocytes in the majority of tissues at both concentrations. At a concentration of 1 µg/mL, there was no unexpected binding in any tissue. At a concentration of 10 µg/mL, there was moderate to strong cytoplasmic staining of rare to occasional endocrine cells in the adenohypophysis (< 25% of the endocrine cells). The staining of these endocrine cells is considered to be an unexpected cross reactivity because expression of PD-1 has not been reported in this cell type. In addition, the binding can be reduced by the addition of PD-1 to the assay and binding to these cells is not seen with a commercial anti-PD-1 antibody. Although this reactivity is not expected, it is unlikely to result in physiological effects due to the limited exposure of cytoplasmic compartments to Nivolumab in humans. Similar staining patterns were observed in cynomolgus monkey tissues indicating that this is an appropriate animal species to evaluate the potential toxicities of Nivolumab.

In intravenous (IV) repeat-dose toxicology studies in cynomolgus monkeys, Nivolumab was well tolerated at doses up to 50 mg/kg administered weekly for 5 weeks and at doses up to 50 mg/kg, administered twice weekly (Q2W) for 27 doses. Drug-related findings were limited to a reversible decrease of 28% in triiodothyronine (T3) among the females administered 27 doses of 50 mg/kg. No corresponding changes in the level of thyroxin (T4), thyroid stimulating hormone (TSH), or histologic changes in the thyroid were observed. There were no clinical signs of toxicity or effects on body weight, food consumption, blood pressure, heart rate, respiration rate, ophthalmic and electrocardiographic parameters, or clinical or anatomic pathology related to the administration of Nivolumab.

No drug-related findings were observed in standard clinical evaluations of cardiovascular,

respiratory, and neurologic function conducted in cynomolgus monkeys as part of the repeat dose toxicity studies for up to 3 months with Nivolumab. In addition, the potential cardiovascular effect of Nivolumab when administered as a single IV dose was examined in conscious cynomolgus monkeys. The single IV bolus administration of Nivolumab at doses of 10 mg/kg or 50 mg/kg was well tolerated. There were no effects on clinical signs, body weights, body temperatures, mean arterial blood pressures, electrocardiograms or cardiovascular parameters during the study.

While Nivolumab alone was well tolerated in cynomolgus monkeys, combination studies have highlighted the potential for enhanced toxicity when combined with another immunostimulatory agent

#### 2.3.5.2 Clinical Toxicology of Nivolumab

The overall safety experience with Nivolumab, as a monotherapy or in combination with other therapeutics, is based on experience in approximately 1500 subjects treated to date. In general, for monotherapy, the safety profile is similar across tumor types. The one exception is pulmonary inflammation AEs which may be numerically greater in subjects with NSCLC because in some cases it can be difficult to distinguish between Nivolumab-related and unrelated causes of pulmonary symptoms and radiographic changes. The safety profile is generally consistent across completed and ongoing clinical trials with no MTD reached at any dose tested up to 10 mg/kg. There was no pattern in the incidence, severity, or causality of AEs to Nivolumab dose level. Most AEs were low-grade (Grade 1 to Grade 2) with relatively few related high-grade (Grade 3 to Grade 4) AEs. Most high-grade events were manageable with use of corticosteroids or hormone replacement therapy (endocrinopathies). Nivolumab should not be used in subjects with active autoimmune disease given the mechanism of action of the antibody.

A total of 306 subjects with selected recurrent or treatment-refractory malignancies have been treated in an ongoing, Phase I multi-dose study (NCT00730639), respectively[10]. There was no pattern in the incidence, severity, or causality of AEs related to the dose of Nivolumab, between 1 and 10 mg/kg, in MDX1106-03. Of the 306 treated subjects in MDX1106-03, 303 (99.0%) subjects have at least 1 reported AE regardless of causality. The most frequently reported AEs were fatigue (54.9%), decreased appetite (35.0%), diarrhea (34.3%), nausea (30.1%), and cough (29.4%). Treatment-related AEs were reported in 230 (75.2%) of the 306 subjects. The most frequently reported treatment-related AEs were fatigue (28.1%), rash (14.7%), diarrhea (13.4%), and pruritus (10.5%). Most treatment-related AEs were low grade. Treatment-related high grade (Grade 3-4) AEs were reported in 52 (17.0%) of subjects. The most frequently reported treatment-related high-grade AE was fatigue (6.5%).

#### 2.3.5.3 Clinical Toxicology of Nivolumab in combination with a peptide vaccine

In a phase I trial (NCT01176474), 49 HLA-A02:01 positive patients received Nivolumab at doses of 1 mg/kg, 3 mg/kg, and 10 mg/kg in combination with a 4-peptide vaccine consisting of $gp100_{209-217}$ (210M), $gp100_{280-288}$ (288V), $MART\text{-}1_{26-35}$ (27L), and $NY\text{-}ESO\text{-}1_{157-165}$ (165V) emulsified in Montanide ISA 51 VG[81]. One patient who received Nivolumab at 3 mg/kg experienced dose-limiting toxicity (grade-3 bilateral optic neuritis), which resolved with a 60-mg prednisone taper over 4 weeks and topical corticosteroids. Two other patients discontinued

treatment secondary to toxicity beyond the DLT period of 12 weeks: One patient receiving Nivolumab at 1 mg/kg had grade-3 fevers in cycle 2 that required 4 weeks of a prednisone taper from 60 mg for resolution, and one patient receiving Nivolumab at 10 mg/kg had grade-3 pneumonitis after completion of two cycles of therapy requiring a prednisone taper from 120 mg over 2 months for resolution. No dose-limiting immune-related colitis was seen in the study. The rates or severity of AEs in this trial were not different from those seen in trials with Nivolumab alone (such as MDX-1106-03, [NCT00730639]), although the number of patients is smaller.

#### 2.3.5.4 Clinical Efficacy of Nivolumab as Monotherapy in Melanoma

In a large phase I study (NCT00730639), the clinical activity of Nivolumab was demonstrated in a variety of tumor types and across a range of doses (0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, 10 mg/kg)[10]. As of the clinical cut-off date of 05-Mar-2013, a total of 306 subjects with melanoma, RCC, and NSCLC have been treated with Nivolumab. All subjects initiated treatment at least one year prior to analysis. A response of either CR or PR, as determined by investigator assessed tumor evaluations based on modified RECIST 1.0, has been reported at all dose levels. Among 107 patients with advanced melanoma who received Nivolumab, the preliminary objective response rate was 33/107 (31%). Responses occurred at each dose level, with 6/17 (35%), 5/18 (28%), 11/35 (31%), 7/17 (41%), and 4/20 (20%) melanoma subjects responding at 0.1, 0.3, 1, 3, and 10 mg/kg, respectively. Duration of response range from 24.1 to 48.7+, 18.4 to 66.3+, 32.4 to 108.1+, 40.1+ to 115.4+, and 73.9 to 117.0+ months in melanoma subjects treated at 0.1, 0.3, 1, 3, and 10 mg/kg, respectively. An additional 7% of melanoma subjects had stable disease for 24 weeks or longer. Across dose levels, melanoma subjects achieved a median overall survival of 16.8 months (95% CI: 12.5, 31.6), with a 2-year overall survival rate of 43%. Nivolumab demonstrated improved overall survival in patients with advanced melanoma when compared to Ipilimumab and chemotherapy, respectively.[82,83]

Nivolumab was approved by the FDA for adjuvant treatment of patients with melanoma who have undergone complete resection but are at high risk of recurrence based on involvement of regional lymph nodes or metastatic disease. The CheckMate 238 study compared nivolumab 3 mg/kg with ipilimumab 10 mg/kg as adjuvant treatment for patients with high-risk resected stage IIIB-C or stage IV melanoma[100]. At a median follow-up of 19·5 months, nivolumab demonstrated longer recurrence-free survival than did ipilimumab (HR for disease recurrence or death 0·65; 97·56% CI 0·51-0·83; p<0·001), with 12-month recurrence-free survival of 70·5% (95% CI 66·1–74·5) in the nivolumab group versus 60·8% (56·0–65·2) in the ipilimumab group (3-year recurrence-free survival was subsequently reported at 58% *vs* 45%, respectively). Treatment discontinuation due to treatment-related adverse events was reported in 35 (7·7%) of 452 patients who received nivolumab compared with 189 (41·7%) of 453 patients who received ipilimumab. At a minimum follow-up of 4 years, nivolumab demonstrated sustained recurrence-free survival benefit versus ipilimumab in resected stage IIIB–C or IV melanoma[101].

## 2.4 Rationale

### 2.4.1 Melanoma is responsive to Immunotherapy

Multiple immunotherapeutic approaches including cytokines such as interleukin-2 and interferon-alpha, vaccines, adoptive T cell therapy and inhibition of immune checkpoints

(CTLA-4 and PD-1/PD-L1) have shown anti-tumor activity in melanoma. Several immune-modulating therapies have been approved including interleukin-2, interferon-alpha, the anti-CTLA-4 monoclonal antibody Ipilimumab[75], and the anti-PD1 monoclonal antibodies Pembrolizumab and Nivolumab. The combination of Nivolumab and Ipilimumab is also approved by the FDA[84].

Despite the encouraging activity and relative safety of immune checkpoint blockade, many advanced melanoma patients lack responses or do not respond robustly to CTLA-4 or PD-1/PD-L1 inhibition. The objective tumor response rates seen in patients with advanced melanoma treated with concurrent Ipilimumab and Nivolumab suggest that significant improvements are achievable[84], but this regimen is associated with significant immune-related toxicities. For resected stage III or IV melanoma, treatment with nivolumab has demonstrated improved recurrence-free survival over ipilimumab, however a substantial proportion of patients still experience recurrences. **Alternative methods to improve the anti-tumor activity of Nivolumab without increasing toxicity are therefore highly desirable.**

### 2.4.2 Checkpoint blockade may be limited by size and specificity of the existing T cell population

Anti-CTLA-4 and anti-PD-1/LI directed therapy alone may help relieve local immune suppression and overcome T-cell exhaustion or anergy, but may be constrained by the size and specificity of the existing T cell population - T cells arising from the normal physiological presentation of the evolving tumor to the host immune system. The lack of coincident, focused immune stimulation may thus be a critical factor that is limiting the maximal efficacy of checkpoint inhibition therapy - a deficit that may be overcome by an effective vaccine. Indeed, in many animal studies, including the studies supporting the initial development of Ipilimumab, the effect of CTLA4 inhibition alone was dramatically enhanced when combined with a vaccine[85-87] and this improvement has been observed or suggested in several human studies[78,88,89]. In addition, recent preclinical studies in mice have demonstrated the improved efficacy of anti-PD1 treatment in combination with a vaccine[90].

### 2.4.3 NeoVax is a novel personalized cancer vaccine utilizing the exquisitely tumor-specific neoantigens created by the personal mutations found in each patient's tumor[91,92]

While novel, potent immunotherapies such as checkpoint inhibitors unleash T cell killing in a wide variety of cancers, their efficacy is thought to rely upon amplification and reinvigoration of a ***pre-existing*** T cell infiltrate that recognizes tumor neoantigens[93-95]. Thus, resistance to these therapies may be driven by a lack of T cells that recognize tumor neoantigens. A cancer neoantigen vaccine offers the possibility of ***inducing novel*** tumor neoantigen-specific T cell responses as well as expanding pre-existing ones; and thus, improving outcomes for patients who may have not have benefited from other immunotherapeutic approaches.

As described in 2.3, over the last several years, we at DFCI have developed a computational infrastructure and conceptual framework for translating sequencing information into a therapeutic vaccine. Currently, two Phase I studies examining the safety, feasibility and preliminary efficacy of NeoVax (Neoantigen peptides plus Hiltonol) are approved and accruing patients at the Dana-Farber Cancer Institute. DFHCC 13-240 and 14-362 both study NeoVax in the *adjuvant setting* of surgically resectable, advanced melanoma or after adjuvant radiotherapy for resectable

glioblastoma multiforme, respectively.

#### 2.4.4 NeoVax is immunogenic in melanoma and can be effectively combined with anti-PD-1 therapy [96]

On the melanoma trial, we enrolled 12 patients, 8 of whom completed dosing. We have focused our testing of the induction of neoantigen-specific responses on 6 patients with available samples at the time of data cutoff in March of 2016. Across all 6 subjects, we uniformly identified strong, polyfunctional CD4+ and CD8+ T cell responses against multiple neoepitopes. Four of 6 patients remain free of disease while 2 progressed. The 2 patients who progressed were subsequently treated with the anti-PD-1 antibody Pembrolizumab and both achieved a complete radiographic response (CR) after 4 doses of Pembrolizumab which are ongoing, now for > 22 months (CR rate of Pembrolizumab as first-line treatment for metastatic melanoma only 6% [97]. In both patients, **we detected persistence and broadening of neoantigen-reactive T cell responses that were induced by vaccination and then following CPB, consistent with epitope spreading**. In the first patient, we detected CD4+ T cell responses against 25 of 49 assay peptides (with 4 novel responses) and CD8+ T cell responses against 2 of 18 predicted class I epitope peptides. Similarly, for the second patient, CD4+ T cell responses were detected against 15 of 61 assay peptides (with 2 novel responses) and CD8+ T cell responses against 4 of 32 class I epitope peptides (with 2 novel responses) [96]. These data support the hypothesis **that vaccination with neoantigens can both expand pre-existing neoantigen-specific T cell populations and induce a broader repertoire of new T cell specificities in cancer patients.**

### 2.5 Hypothesis

We hypothesize that combining the immune stimulating effects of this neoantigen-targeted vaccine (NeoVax + Montanide® ISA-51 VG) with enhanced priming through the addition of locally administered Ipilimumab and the relief of immune suppression by systemically administered Nivolumab will be safe and will enhance the anti-tumor activity of monotherapy with nivolumab. We propose that this effect occurs by qualitatively broadening the repertoire of T cell targets as well as strengthening the activity of extant and newly-induced neoantigen-specific T cells, both parameters that can be accurately monitored.

### 2.6 Correlative Studies Background

#### 2.6.1 Testing the immunogenicity of NeoVax

In our first trial in patients at high risk for recurrence, testing has focused on the induction of neoantigen-specific responses on the 6 patients enrolled in that study. Per protocol, PBMCs for the melanoma study were collected by leukapheresis (pre-vaccination and at week 16 [4 weeks after 1st boost]), and by monthly venipuncture for the duration of the study. Across all 6 melanoma subjects, we have uniformly identified strong, polyfunctional CD4+ and CD8+ T cell responses against multiple neoepitopes.

As shown in 2 representative examples (Figure 1, Panel A), we have observed high ex vivo responses in PBMCs across multiple peptide pools of overlapping 15-16mers corresponding to the pools of immunizing SLPs. By deconvolution of the peptide pools, we have identified CD4+ and CD8+ T cell responses against 18% (19 of 103) of the immunizing peptides and 19% (16 of

83) predicted epitopes, respectively. The vast majority of T cell lines were specific for the mutated but not corresponding wildtype (Panel B, Figure below).

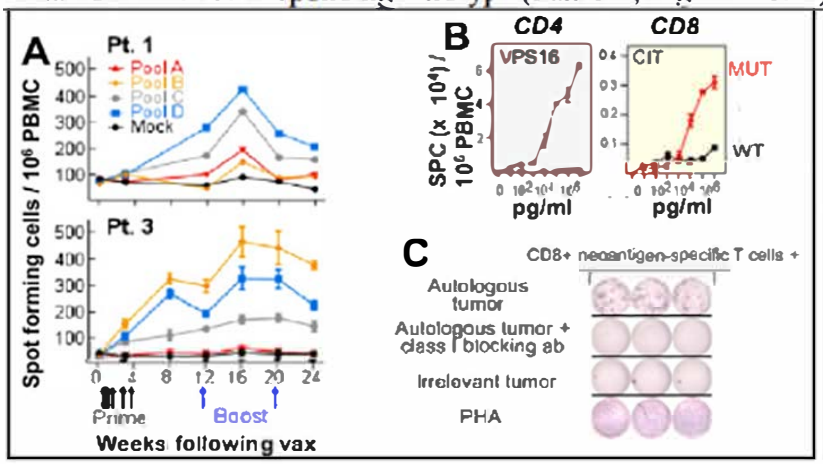



**Figure 1: Detection of immune responses to neoepitopes following NeoVax for melanoma patients.**

A. *Ex vivo* analysis of PBMCs against pools of peptides (A, B, C, D) encompassing the SLPs formulating the personal vaccines across the duration of vaccine delivery. B. Deconvolution against peptide pool responses reveals reactivity against specific mutated but not corresponding wildtype epitopes. C. For some neoantigen-specific T cells, reactivity is directed against autologous tumor but not irrelevant tumor.

### 2.6.2 Sample collection for correlative studies

Peripheral blood as well as tumor and vaccine site biopsy sample collection includes procurement of:

(i) Peripheral blood mononuclear cells (PBMCs): Leukapheresis samples will be obtained from study subjects prior to initiation of Nivolumab (L1), prior to initiation of vaccination (L2) and after the 3rd vaccine at week 20 (L3). PBMCs will also be obtained by venipuncture on weeks 4, 13, 15, 18, 24 and every 4 weeks thereafter until disease progression.

(ii) Tumor samples will be obtained from study subjects with unresectable melanoma prior to initiation of Nivolumab (B1), prior to initiation of vaccination (B2), after the 3rd vaccine at week 20 (B3), and at the time of progression (B4). B2 and B3 biopsies will be performed provided that biopsiable disease is present.

(iii) Vaccine site biopsies will be obtained within 24 hours prior to the 3rd vaccine. One of the 2 vaccines sites will be biopsied as per investigator discretion.

## 3. PARTICIPANT SELECTION

### 3.1 Eligibility Criteria

Eligibility to participate will be assessed at two timepoints: prior to initial core needle/surgical biopsy (Initial Registration) and prior to the first vaccination (Secondary Registration).

#### 3.1.1 Inclusion Criteria

3.1.1.1 Participant is willing and able to give written informed consent

3.1.1.2 Participants must have histologically confirmed stage IIIB/C/D or stage IV cutaneous melanoma (mucosal melanoma or uveal melanoma are excluded) that is surgically resected, is deemed surgically resectable, or is unresectable; tumor tissue for sequence analysis must be available from either previous melanoma resection/biopsy or at least one site of disease must be amenable to surgical or core biopsy

3.1.1.3 Age ≥ 18 years

3.1.1.4 ECOG performance status of 0 or 1

3.1.1.5 Recovered from all toxicities associated with prior treatment, to acceptable baseline status (as to Lab toxicity see below limits for inclusion) or a National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 5.0, Grade of 0 or 1, except for toxicities not considered a safety risk, such as alopecia or vitiligo

3.1.1.6 Participants must have normal organ and marrow function as defined below:

| | |
|---|---|
| – WBC | ≥3,000/μL |
| – ANC | ≥1,500/μL |
| – Platelets | ≥100,000/μL |
| – Hemoglobin | > 9.0 g/dL |
| – Total Bilirubin | ≤ 1.5 x ULN<br>(except subjects with Gilbert Syndrome, who can have total bilirubin < 3.0 mg/dL) |
| – AST(SGOT)/ALT(SGPT) | ≤ 3 x ULN |
| – Creatinine | ≤ 1.5 x ULN<br>OR |
| – Creatinine clearance | ≥40 mL/min/1.73 $m^2$ for participants with creatinine levels above institutional normal (if using the Cockcroft-Gault formula below): |

$$\textit{Female}\ \text{CrCl} = \frac{(140 - \text{age in years}) \times \text{weight in kg} \times 0.85}{72 \times \text{serum creatinine in mg/dL}}$$

$$\textit{Male}\ \text{CrCl} = \frac{(140 - \text{age in years}) \times \text{weight in kg} \times 1.00}{72 \times \text{serum creatinine in mg/dL}}$$

3.1.1.7 Women of childbearing potential (WOCBP) should have a negative serum pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 24 hours prior to the start of Nivolumab, because the effects of NeoVax plus Montanide and Nivolumab on the developing human fetus are unknown

3.1.1.8 Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with study agents, breastfeeding should be discontinued if the mother is treated with Ipilimumab, Nivolumab and NeoVax + Montanide.

3.1.1.9 Female participants enrolled in the study, who are not free from menses for >2 years, post hysterectomy / oophorectomy, or surgically sterilized, should be willing to use either 2 adequate barrier methods *or* a barrier method plus a hormonal method of contraception to prevent pregnancy or to abstain from sexual activity throughout the study, starting with visit 1 through 5 months after the last dose of study therapy. Approved contraceptive methods include for example: intra uterine device, diaphragm with spermicide, cervical cap with spermicide, male condoms, or female condom with spermicide. Spermicides alone are not an acceptable method of contraception. Should a woman become pregnant or suspect she is pregnant while she is participating in this study, she should inform her treating physician immediately.The investigational product will be permanently discontinued in an appropriate manner.

### 3.1.2 Exclusion Criteria

Participants who exhibit any of the following conditions will not be eligible for admission into the study:

3.1.2.1 Prior immunotherapy except for anti-CTLA-4. Patients with unresectable melanoma who have received PD-1 inhibition therapy as adjuvant therapy and stopped receiving PD-1 inhibition for a period of ≥ 6 months before starting treatment with Nivolumab are allowed to participate.

3.1.2.2 > 24 weeks between surgical resection of stage IIIB/C/D or IV melanoma and initiation of study treatment

3.1.2.3 Concomitant therapy with any anti-cancer agents, other investigational anti-cancer therapies, or immunosuppressive agents including but not limited to methotrexate, chloroquine, azathioprine, etc. within six months of study participation

3.1.2.4 Active brain metastases or leptomeningeal metastases

3.1.2.5 Use of a non-oncology vaccine therapy for prevention of infectious diseases (with the exception of vaccination against the SARS-CoV-2 virus for the prevention of COVID-19 disease) during the 4 week period prior to first dose of Nivolumab. Participants may not receive any non-oncology vaccine therapy during the period of Nivolumab or NeoVax plus Montanide administration and until at least 8 weeks after the last dose of study therapy.

Given the severity of the COVID-19 pandemic, vaccination specifically against the SARS-CoV-2 virus for the prevention of COVID-19 is ALLOWED in this study.

3.1.2.6 History of severe allergic reactions attributed to any vaccine therapy for the prevention of infectious diseases.

3.1.2.7 Active, known or suspected autoimmune disease. Subjects are permitted to enroll if they have vitiligo, type I diabetes mellitus, residual hypothyroidism due to autoimmune condition only requiring hormone replacement, psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger.

3.1.2.8 A condition requiring systemic treatment with either corticosteroids (> 10 mg daily prednisone equivalents) or other immunosuppressive medications within 14 days of study drug administration. Inhaled or topical steroids and adrenal replacement doses > 10 mg daily prednisone equivalents are permitted in the absence of active autoimmune disease. Corticosteroids used as pre-medication for imaging studies are allowed.

3.1.2.9 Test positive for hepatitis B virus surface antigen (HBV sAg) or hepatitis C virus ribonucleic acid (HCV antibody) indicating acute or chronic infection.

3.1.2.10 Known history of testing positive for human immunodeficiency virus (HIV) or known acquired immunodeficiency syndrome (AIDS).

3.1.2.11 Known sensitivity or allergy to Nivolumab and/or Ipilimumab.

3.1.2.12 Uncontrolled intercurrent illness including, but not limited to ongoing or active infection requiring treatment, symptomatic.

3.1.2.13 Any underlying medical condition, psychiatric condition or social situation that in the opinion of the investigator would compromise study administration as per protocol or compromise the assessment of AEs.

3.1.2.14 Planned major surgery (excluding surgery for resection of melanoma if applicable).

3.1.2.15 Pregnant women are excluded from this study because Nivolumab, personalized neoantigen peptides and poly-ICLC are agents with unknown risks to the developing fetus. Because there is an unknown but potential risk of adverse events in nursing infants secondary to treatment of the mother with Nivolumab, personalized neoantigen peptides and poly-ICLC, nursing women are excluded from this study.

3.1.2.16 Individuals with a history of an invasive malignancy are ineligible except for the following circumstances: a) individuals with a history of invasive malignancy are eligible if they have been disease-free for at least 3 years and are deemed by the investigator to be at low risk for recurrence of that malignancy; b) individuals with the following cancers are eligible if diagnosed and treated - carcinoma *in situ* of the breast, oral cavity or cervix, localized prostate cancer, basal cell or squamous cell carcinoma of the skin.

3.1.2.17 Prisoners, or subjects who are compulsory detained are not eligible to participate.

### 3.2 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

## 4. REGISTRATION PROCEDURES

Eligibility will be confirmed prior to the initial biopsy to obtain tumor for sequencing. Prior to initial biopsy, the patient is screened for eligibility using the eligibility checklist. Once overall trial initial eligibility is confirmed, the patient is registered, undergoes core needle or surgical biopsy and preparation of the participant's vaccine is initiated (while the patient is receiving Nivolumab).

Before the first dose of the vaccine plus ipilimumab the patient will be registered to the correspondent dose level vaccine plus ipilimumab .

### 4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

For registration, an investigator will confirm eligibility criteria and a member of the study team will complete the appropriate protocol-specific eligibility checklist.

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. Registration cancellations must be made in OnCore as soon as possible.

### 4.2 Registration Process for DF/HCC Institutions

**The Registration procedures are as follows:**

To be eligible for registration to the study, the participant must meet each inclusion and exclusion criteria listed in protocol section 3.

Reminder: Confirm eligibility for ancillary studies at the same time as eligibility for the treatment study. Registration to both treatment and ancillary studies will not be completed if eligibility requirements are not met for all studies.

1. Confirm written informed consent from the participant has been obtained prior to the performance of any protocol-specific screening procedures or assessments.
2. Complete the *Eligibility Checklist*
3. Perform subject registration per DF/HCC SOP REGIST-101
4. Verify the success of the subject registration in all DF/HCC systems.

## 5. TREATMENT PLAN

### Overall Treatment administration

Treatment will be administered on an outpatient basis. Expected toxicities and potential risks as well as dose modifications for Nivolumab and NeoVax plus Montanide and locally administered Ipilimumab, respectively, are described in Section 6 (Expected Toxicities and Dosing Delays/Dose Modification). No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Participants will be identified and enrolled on the study if they meet all eligibility criteria. After confirmation of adequate tumor content in the surgical resection/core biopsy specimen (at least 30% tumor in specimen), each participant will start receiving Nivolumab as described in section Section 5.2.1, while vaccine is being prepared. If there is no adequate tumor content in the specimen, the patient will be removed from protocol and replaced. Treatment with NeoVax plus Montanide plus local Ipilimumab will begin within 12 weeks after initiation of Nivolumab. Days will not be counted continuously on protocol therapy. If a toxicity results in a hold of study vaccine administration, counting of days will hold until treatment resumes (e.g. should a patient not be able to receive his/her second vaccine at Week 15 due to a toxicity, counting is held; if the toxicity has resolved a week later, the administration of that second vaccine becomes the participant's new Week 15). Following a dose delay, those day's assessments should be repeated in order to restart participant's vaccinations.

## 5.1 Preparation of NeoVax plus Montanide

### 5.1.1 Tumor and normal tissue harvest

Patients with resectable or unresectable melanoma will undergo surgical resection, surgical biopsy, or core needle biopsy of a melanoma metastasis. For patients with melanoma that bas already been completely resected, archival tissue can be used for assessment of tumor content by a staff pathologist at Brigham and Women's Hospital (in an H&E stained tumor specimen) as well as wbole-exome and transcriptome sequencing. Dr. Patrick Ott, the principal investigator ([redacted]) should be contacted at the time of the initial consent to ensure that ancillary studies are coordinated.

For patients with resectable or unresectable melanoma who undergo surgical resection, surgical biopsy, or core needle biopsy of a melanoma metastasis, the study investigators and members of the study team will coordinate tissue acquisition with the interventional radiology or surgical staff of the Brigham and Women's Hospital and the staff of the BWH-SHL, and TIGL. After adequate tumor for pathological assessment has been harvested as deemed by the interventional radiologist or surgeon, some or all of the remaining tumor tissue will be placed in sterile media in a sterile container and transferred to the Specialized Histopathology Core at BWH. A staff pathologist at Brigham and Women's Hospital will assess H&E stained tumor specimen for tumor content. Portions of the tumor tissue will be used for whole-exome and transcriptome sequencing. Cell line and tumor infiltrating lymphocyte generation (if adequate tissue) will be performed in the Translational Immunogenomics Laboratory (TIGL).

In the event that sequence analysis yields no results or sub-optimal results, tumor cell line cells or paraffin embedded tumor sections from the recently resected tumor (if available) may be used to prepare additional nucleic acid for sequencing. No saved samples will be used to prepare cellular products for future clinical use. Peripheral blood mononuclear cells from a blood draw will be utilized for a normal tissue sample.

### 5.1.2 DNA/RNA Sequencing

Nucleic acid will be extracted from the tissue samples and sequencing will be conducted at either the CLIA-certified laboratory at Broad Institute/Clinical Research Sequencing Platform (CRSP) [redacted] Samples will be de-identified before being shipped to either the Clinical Research Sequencing Platform (CRSP) and Broad Genomics Platform research lab at the Broad Institute [redacted].

**Sequencing at the Broad Institute**
Whole-exome sequencing will be conducted in the Clinical Research Sequencing Platform, LLC (CRSP) at the Broad Institute. CRSP is CLIA licensed ([redacted]) and CAP accredited [redacted] and one of tests offered by CRSP is Somatic Whole Exome sequencing, which will be utilized for these trials. CRSP will extract total DNA from blood and tumor tissue using standardized procedures. The DNA samples will be processed through the CLIA Somatic WES test. For tumor and normal DNA samples, whole exome capture will be conducted prior to sequencing on Illumina HiSeq or NovaSeq. RNA sequencing is completed at the non-CLIA Broad Genomics Platform research lab. For tumor RNA, a cDNA library will be prepared and

may be enriched by transcriptome capture prior to sequencing on Illumina HiSeq or NovaSeq. If the quantity or quality of DNA or RNA isolated from the tissue sample is inadequate for exome or cDNA library preparation and sequencing, then DNA or RNA may be extracted from the patient-specific tumor cell line (if generated).

### 5.1.3 Preparation of personalized neoantigen peptides

[redacted]

#### 5.1.3.1 Peptide synthesis

[redacted]

#### 5.1.3.2 Preparation of Neoantigen peptide pools

[redacted]

[redacted]

[redacted]

## 5.2 Agent Administration

| Agent | Treatment phase | Schedule** | Allowable Treatment Admin Window | Dose | Route |
|---|---|---|---|---|---|
| Nivolumab | Entire Duration | Every 4 weeks | ± 3 days | 480 mg flat dose | i.v. |
| Neoantigen Vaccine (peptides + poly-ICLC + Montanide® ISA-51 VG)*, *** | Vaccination Phase | Week 12, 15, 18, and 21 | ± 3 days | Poly-ICLC: 2 x 0.5 mg (0.25ml each)<br><br>Peptides: 2 x 300µg per peptide/0.75ml<br><br>Montanide® ISA-51 VG: 2 x 1ml<br><br>Total Neoantigen Vaccine Volume*: up to 2 x 1.6 ml | Neoantigen Vaccine<br><br>s.c. injections into up to 2 different anatomic sites |
| Ipilimumab | Vaccination Phase | Week 12, 15, 18, and 21 | = 3 days | 2 x 1.25 mg (0.25ml), 2.5 mg (0.5 ml) or 5 mg (1.0 ml) depending on the cohort | s.c injection within 1 cm of each Neoantigen Vaccine injection |

***During NeoVax (personalized neoantigen peptides + Hiltonol) and Montanide mixing preparation there is a loss of 0.4 mL**
****Please refer to section 5.2.1.2 regarding delays on vaccination therapy start**
*****If emulsion of either pool A or pool B or both pools A and B is unsuccessful after two attempts, the neoantigen vaccine will be prepared without Montanide as stated in section 8.6.3.**

If one of the initial vaccine doses is not successfully emulsified with montanide after two attempts, the vaccine will be prepared without the Montanide (see table below and section 8.6.3).

During the vaccination treatment period if any of the two vaccine doses is not successfully emulsified with montanide after two attempts, the vaccine will be prepared without the Montanide.

**Treatment plan if emulsion NeoVax – Montanide fails:**

| Agent | Treatment phase | Schedule** | Allowable Treatment Admin Window | Dose | Route |
|---|---|---|---|---|---|
| Nivolumab | Entire Duration | Every 4 weeks | ± 3 days | 480 mg flat dose | i.v. |
| NeoVax (peptides + poly-ICLC)*** | Vaccination Phase | Week 12, 15, 18, and 21 | ± 3 days | Poly-ICLC: 2 x 0.5 mg (0.25ml each)<br><br>Peptides: 2 x 300µg per peptide/0.75ml<br><br>Total NeoVax Volume: up to 1.0 ml (+/- 0.1 ml) | NeoVax<br><br>s.c. injections into up to 2 different anatomic sites |
| Ipilimumab | Vaccination Phase | Week 12, 15, 18, and 21 | ± 3 days | 2 x 1.25 mg (0.25ml), 2.5 mg (0.5 ml) or 5 mg (1.0 ml) depending on the cohort | s.c injection within 1 cm of each NeoVax injection |

*****If emulsion of either pool A or pool B or both pools A and B is unsuccessful after two attempts the vaccine will be prepared without Montanide as stated in section 8.6.3**

### 5.2.1 Nivolumab

#### 5.2.1.1 Nivolumab Run-in Period

Run in period will begin within 2 weeks of metastatic tissue biopsy or within 4 weeks of enrollment if the patient has resected melanoma, once the following criteria have been met:

1) Histopathologic confirmation of melanoma
2) Confirmation of adequate tumor content in resection specimen for DNA sequencing

Patients will receive Nivolumab at a flat dose of 480 mg as a 30-minutes (+/- 10 min.) i.v. infusion every 4 weeks (28 days).

#### 5.2.1.2 Nivolumab plus neoantigen vaccine (NeoVax plus Montanide) / Ipilimumab Period

Patients will begin therapy with neoantigen vaccine (NeoVax plus Montanide) /Ipilimumab once the vaccine has been prepared and testing completed at week 12. Patients will receive Nivolumab at a flat dose of 480 mg as a 30 minutes (+/- 10 min.) i.v. infusion every 4 weeks (28 days). Nivolumab will be given 60 minutes (+/- 10 min) following local Ipilimumab administration.

If therapy with neoantigen vaccine (NeoVax plus Montanide) / Ipilimumab gets delayed, vaccintation timepoints will be modified as follows:

Neoantigen vaccine (NeoVax plus Montanide) / Ipilimumab vaccination timepoints #2 (week 15), #3 (week 18), and #4 (week 21) will be moved accordingly so there is a window of three weeks (+/- 3 days) between each vaccination timepoint. If any of the vaccine adminstrations coincide with Nivolumab treatment, neoantigen vaccine (NeoVax plus Montanide) / local Ipilimumab will be administrated first and Nivolumab will be given 60 minutes (+/- 10 min) following local Ipilimumab administration.

#### 5.2.1.3 Nivolumab Continuation Period

After neoantigen vaccine (NeoVax plus Montanide) / Ipilimumab treatment has been completed, patients with unresectable stage IIIB/C/D or IV melanoma will continue to receive Nivolumab alone at a flat dose of 480 mg as a 30 minutes (+/- 10 min.) i.v. infusion every 4 weeks (28 days), until disease recurrence or progression, unacceptable toxicity, or withdrawal of consent, for a maximum duration of up to 2 years from the first study treatment in the Run in Period. Participants with completely resected melanoma will receive nivolumab for a maximum period of one year (13 doses)

### 5.2.2 Neoantigen Vaccine (NeoVax + Montanide® ISA-51 VG)

#### 5.2.2.1 Injections

##### 5.2.3.1.1 Neoantigen Vaccine (NeoVax + Montanide® ISA-51 VG)

Each of the Neoantigen Vaccine syringes will be assigned to one of two extremities. At each immunization, each NeoVax plus Montanide syringe will be administered s.c. to the assigned extremity (i.e., NeoVax + Montanide A will be injected into left arm week 12, 15 etc.; NeoVax + Montanide B will be injected into right arm week 12, 15). For participants who have undergone a complete axillary lymph node dissection, alternative anatomical locations are the left and/or right thigh or left and/or right midriff. The arms or left and/or right midriff can also be used for participants who have undergone a complete inguinal/femoral lymph node dissection (or other contraindications that prevent injections to a particular extremity). The personalized neoantigen vaccine (peptide + poly-ICLC + Montanide® ISA-51 VG) should be administered within 6 hours after preparing the peptide + poly-ICLC + Montanide® ISA-51 VG mixture, and injection sites may be shifted in order to avoid administering injections to areas with evidence of persistent local reaction.

NOTE: If emulsion of either pool A or pool B or both pools A and B is unsuccessful after two attempts, the vaccine will be prepared without Montanide (please see section 8.6.3).

##### 5.2.3.1.2 Ipilimumab

Within 30 minutes (+/- 10 min) following NeoVax plus Montanide administration, Ipilimumab will be injected within 1 cm of each neoantigen vaccine administration.

#### 5.2.2.2 Treatment window

Neoantigen vaccine (NeoVax plus Montanide) and Ipilimumab may be administered within 3 days of the scheduled administration date. (See Section 10; Study Calendar).

Days will not be counted continuously on protocol therapy. If a toxicity results in a hold of study vaccine and nivolumab administration, counting of days will hold until treatment resumes. (e.g. should patient not be able to receive his/her second vaccine at Week 15 due to a toxicity, counting is held; if the toxicity has resolved a week later, the administration of that second vaccine becomes the participant's new Week 15) Following a dose delay, those day's

assessments should be repeated in order to restart participant's vaccinations.

#### 5.2.2.3 Observation post vaccination

Participants should be observed in the clinic for at least 60 minutes after the last of the 4 injections and vital signs should be checked at 30 minutes (+/- 10 min) and 60 minutes (+/- 10 min) after the last injection for each clinic visit. Monitoring for immediate adverse events should include attention to possible injections site reaction or a systemic reaction. In the absence of the occurrence of an adverse event, participants will be discharged from the outpatient clinic at DFCI.

## 5.3 Pretreatment criteria

Preparation of the final Nivolumab, Ipilimumab, and/or Neoantigen vaccine product will begin upon confirmation that the participant is medically cleared to undergo treatment (confirmed arrival in the clinic, stable vital signs, no new acute medical issues or laboratory abnormalities potentially interfering with vaccine or Nivolumab administration).

### 5.3.1 Day 1

Laboratory parameters must be reviewed prior to administration of the study agents. No specific prophylactic or supportive care is necessary prior to administration of the study agents. Participants who have developed flu-like symptoms after NeoVax plus Montanide that are controlled with acetaminophen or non-steroidal anti-inflammatory drugs during previous treatments may be pre-treated with acetaminophen (650 mg PO) or ibuprofen (400-600mg PO) as per the investigator's discretion.

### 5.3.2 Subsequent treatment days

The following must be reviewed and confirmed within **three days** prior to subsequent treatment administrations:

1. Stable vital signs
2. ECOG performance status
3. No new acute medical issues that would require a dose hold or delay as noted in dose modification section 6.3

No specific prophylactic or supportive care is necessary prior to administration of the study agents.

## 5.4 Dose schema and staggering of participants

### 5.4.1 DLT and expansion cohorts

Adverse events classified as dose-limiting toxicities (DLT) and the assessment time for DLTs are summarized in Section 5.5. Five patients will be treated with NeoVax plus Montanide plus local Ipilimumab plus Nivolumab (starting at week 12) for the initial safety evaluation (Cohort 1). We have chosen dose escalation cohorts of 5 patients, rather than a more traditional 3+3 design,

because the determination of whether to treat at a different dose in this trial is made over a longer period of time due to the time required for vaccine preparation and the extended period for safety evaluation. These larger cohort sizes help us avoid having to pause and then restart enrollment to a dosing cohort several months later.

If none or only 1 patient in Cohort 1 experiences a dose limiting toxicity (DLT) during the first 7 weeks of treatment (NeoVax plus Montanide plus local Ipilimumab plus Nivolumab) of Cohort 1, 5 patients will be treated as Cohort 2 Dose Level 2. If two or more patients in Cohort 1 experience a DLT during the first 7 weeks of treatment, then 5 patients will be treated as Cohort 2, Dose Level -1.

If none or only 1 patient experiences a DLT on Cohort 2 Dose Level 2, then Dose Level 2 will be the maximum tolerated dose (MTD) and an additional 10 patients will be treated at that dose level (Cohort 3, Dose Level 2) to increase the likelihood of detecting serious toxicities, to complete biologic correlative endpoints and to gain preliminary experience with clinical tumor activity. If two or more patients in Cohort 2 Dose Level 2 experience dose limiting toxicity (DLT), then Dose Level 1 will be the MTD and an additional 10 patients will be treated in an expansion cohort at this dose (Cohort 3 Dose Level 1).

If none or only 1 patient experiences a DLT on Cohort 2 Dose Level -1, then Dose Level-1 will be the maximum tolerated dose (MTD) and an additional 10 patients will be treated in an expansion cohort at that dose level (Cohort 3 Dose Level-1). If two or more patients in Cohort 2 Dose Level -1 experience dose limiting toxicity (DLT), then the study will be stopped.

| Dose Escalation schedule | | |
|---|---|---|
| | Dose Level | Dose of Ipilimumab |
| | -1 | 1.25 mg (0.25 mL) |
| Starting Dose --> | 1 | 2.5 mg (0.5 mL) |
| | 2 | 5 mg (1.0 mL) |

### 5.4.2 Staggering of patients

The first three patients will be enrolled in a staggered fashion: Patients 2 and 3 will not start treatment until the previous patient has completed 4 weeks after the first dose of the NeoVax vaccine.

### 5.4.3 Definition of evaluable participants

All participants receiving at least one dose of Nivolumab will be evaluable for toxicity. Participants will be evaluable for immunologic activity if they have received all vaccinations during the priming phase and the first boost vaccination.

Participants with unresectable and measurable disease who have received at least one dose of study vaccine therapy will be considered evaluable for determination of tumor response.

A minimum of 2 vaccinations and absence of DLTs are required for a participant to complete the

6-week DLT observation period successfully.

### 5.5 Definition of Dose-Limiting Toxicity

Dose-limiting toxicity (DLT) is based on the CTEP Active Version (version 5.0) of the NCI Common Terminology Criteria for Adverse Events (CTCAE). DLT refers to toxicities experienced within 42 days (6 weeks of Neoantigen Vaccine treatment initiation i.e. 2 vaccinations "DLT window"). If treatment has to be delayed because of a toxicity that does not fulfill the criteria of a DLT, the DLT window should be extended by that time period of delay. A DLT will be defined as follows:

1. Grade 3 or 4 toxicity that is definitely, probably, or possibly related to the administration of vaccine, excluding:
   - Transient (≤ 72 hours) flu-like symptoms
   - Grade 3 nausea, vomiting, diarrhea, or constipation that returns to grade 2 (or lower) level within 48 hours
   - Any grade 3 rash that resolves to grade 2 or grade 1 within ≤ 14 days
   - Any grade 3 endocrine abnormality that is corrected with hormonal therapy within 4 weeks
2. Grade 3 or 4 abnormal laboratory value that is definitely, probably, or possibly related to the administration of vaccine if it persists for more than 7 days, or requires hospitalization or medical intervention excluding:
   -Any grade 3 electrolyte abnormality that:
   - lasts ≤ 72 hours,
   - is not clinically complicated,
   - and resolves spontaneously or responds to conventional medical intervention
3. Any grade 3 or grade 4 toxicity that is considered, in the opinion of the Principal Investigator, to be dose-limiting.
4. Any death related to study treatment.

Management and dose modifications associated with the above adverse events are outlined in Section 6 (Expected Toxicities and Dosing Delays/Dose Modifications).

### 5.6 General Concomitant Medication and Supportive Care Guidelines

Participants who have developed flu-like symptoms that are controlled with acetaminophen or non-steroidal anti-inflammatory drugs during previous treatments may be pre-treated with acetaminophen (650 mg PO) or ibuprofen (400-600mg PO) as per the investigator's discretion.

Participants may not receive any non-oncology vaccine therapy (with the exception of vaccination against the SARS-CoV-2 virus for the prevention of COVID-19 disease) during the 4-week period to first dose of Nivolumab and until at least 8 weeks after the last dose of Neoantigen Vaccine administration.

Given the severity of the COVID-19 pandemic, vaccination specifically against the SARS-CoV-2 virus for the prevention of COVID-19 is ALLOWED in this study.

Investigators may prescribe all other concomitant medications or treatments deemed necessary to provide adequate patient care.

Management of more serious toxicities will be directed by a study investigator following discussion with study PI and will be in accordance with standard of care clinical practice.

### 5.7 Duration of Therapy / Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will depend on tolerability of the immunizations and evidence of disease recurrence or progression as judged by the treating investigator. In the absence of treatment delays due to adverse events, treatment will be given for two years in patients with unresectable melanoma and for one year (13 doses) for participants with stage III/B/C/D or IV completely resected melanoma, respectively, or until one of the following criteria applies:

- Disease progression or recurrence, if it is deemed by the treating investigator to be in the best interest of the participant to discontinue study treatment. If there is clinical or radiographic disease progression or recurrence prior to initiation of vaccine at week 12 or at the week 12 scan, treatment as per protocol (Nivolumab plus NeoVax + Montanide + local Ipilimumab) should be given unless deemed not in the best interest of the patient by the investigator.
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Participant demonstrates an inability or unwillingness to comply with protocol requirements
- Participant decides to withdraw from the study, or
- General or specific changes in the participant's condition which render the participant unacceptable for further treatment in the opinion of the treating investigator.

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). Alternative care options will be discussed with the participant.

When a participant is removed from protocol therapy and/or is off of the study, the relevant Off-Treatment/Off-Study information will be updated in OnCore.

In the event of unusual or life-threatening complications, participating investigators must immediately notify the Principal Investigator, Patrick Ott, MD, PhD at DFCI [redacted].

### 5.8 Duration of Follow Up

Participants removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. All participants will be followed until resolution or stabilization of any serious adverse events occurring during treatment or starting within 100 days of last study drug.

Accrual duration is expected to be around 2 years. The overall study duration will be approximately 6 years.

Active Follow-Up: Participants removed from active study treatment will enter active follow-up and will be followed at the following schedule until initiation of a new therapy for progressive disease, withdrawal of consent, or death (whichever comes first):

- At least every 3 months (+/- 2 week) for the first 2 years after initiation of study therapy
- At least every 4 months (+/- 2 weeks) for the third year after study therapy initiation
- And at least every 6 months (+/- 4 weeks) thereafter, until 5 years have passed since study therapy initiation

NOTE: When progression is noted during follow-up, the respective measurements documenting progression will be recorded on the appropriate CRF.

Long-Term Follow-Up: Participants removed from active follow-up for progression/initiation of a new therapy will enter long-term follow-up, where they will be followed via medical record review until death for survival. All patients will be followed for survival. Updates to the CRFs are to be made roughly every six months.

### 5.9 Nivolumab treatment beyond progression

Accumulating evidence indicates a minority of subjects with unresectable melanoma treated with immunotherapy may derive clinical benefit despite initial evidence of progressive disease (PD). Subjects with unresectable stage IIIB/C/D or IV melanoma who show initial evidence of progression (as defined by modified RECIST 1.1) after or during vaccination will be permitted to continue or resume treatment with Nivolumab if documented, as long as they meet all of the following criteria:

- Investigator-assessed clinical benefit
- Subject has tolerated prior Nivolumab
- Stable or improved performance status
- Treatment beyond progression will not delay an intervention to prevent imminent serious complications of disease progression (e.g, CNS metastases)

Subject will be required to sign written informed consent prior to receiving additional Nivolumab treatment, using an ICF describing any reasonably foreseeable risks or discomforts and other alternative treatment options. The assessment of clinical benefit should take into account whether the subject is clinically deteriorating and unlikely to receive further benefit from continued Nivolumab treatment.

A radiographic tumor assessment must be performed 4 to 8 weeks after the assessment documenting initial PD. Subjects must discontinue study treatment upon evidence of further progression on the next scheduled tumor assessment, defined as an additional 10% or greater increase in tumor burden from the time of initial PD (including all target lesions and new measurable lesions). If further progression is not documented on this next tumor assessment, then study treatment may continue until there is evidence of further progression on any subsequent tumor assessment.

New lesions are considered measurable at the time of initial PD documented if the longest diameter is at least 10 mm (except for pathological lymph nodes, which must have a short axis of at least 15 mm). Any new lesion considered non-measurable at the time of initial PD may become measurable and therefore included in the tumor burden measurement if the longest diameter increases to at least 10 mm (except for pathological lymph nodes, which must have an increase in short axis to at least 15 mm).

For statistical analyses that include the investigator-assessed progression date, subjects who continue Nivolumab treatment beyond initial investigator-assessed progression, as defined by modified RECIST 1.1, will be considered to have investigator-assessed progressive disease at the time of the initial progression event.

### 5.10 Criteria for Taking a Participant Off Study

Participants will be removed from study when any of the following criteria apply:

- Dose-limiting toxicity (DLT) as defined in protocol section 5.5
- Grade 3 or greater skin toxicity (ulcer )
- Lost to follow-up
- Failure to produce an adequate supply of acceptable vaccine
- Withdrawal of consent for data submission
- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF). In addition, the study team will ensure Off Treatment/Off Study information is updated in OnCore in accordance with DF/HCC policy REGIST-101.

In the event of unusual or life-threatening complications, participating investigators must immediately notify the Principal Investigator, Patrick Ott, MD, PhD at DFCI [redacted].

# 6. EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made as indicated in the following table(s). The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

If possible, symptoms should be managed as needed. In the case of toxicity, appropriate medical treatment should be used.

All adverse events experienced by participants will be collected from the time of the first dose of study treatment (first Nivolumab administration), through the study and until the final study visit. Participants continuing to experience toxicity at the end of treatment visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

## 6.1 Anticipated Toxicities

A list of the adverse events and potential risks associated with the agents administered in this study appears on section 7.1 and will determine whether dose delays and modifications will be made or whether the event requires expedited reporting **in addition** to routine reporting. If one drug is held due to an AE all the 3 drugs will be held until original AE is resolved.

## 6.2 Toxicity Management

If treatment needs to be held because of toxicity, all study drug will be held until resolution of the adverse event.

### 6.2.1 Nivolumab

#### 6.2.1.1 Dose Modification and Guidelines for Management of Nivolumab-related Adverse Events

Management algorithms (listed in Appendix B) have been developed to assist investigators in assessing and managing the following groups of AEs:

- Gastrointestinal
- Renal
- Pulmonary
- Hepatic
- Endocrinopathies
- Skin
- Neurological

#### 6.2.1.2 Treatment of Nivolumab Related Infusion Reactions

Since Nivolumab contains only human immunoglobulin protein sequences, it is unlikely to be immunogenic and induce infusion or hypersensitivity reactions. However, if such a reaction were

to occur, it might manifest with fever, chills, rigors, headache, rash, pruritis, arthralgias, hypo- or hypertension, bronchospasm, or other symptoms.

All Grade 3 or 4 infusion reactions should be reported as an SAE if criteria are met. Infusion reactions should be graded according to NCI CTCAE (CTEP Active Version (version 5.0) of the NCI Common Terminology Criteria for Adverse Events (CTCAE) which is identified and located on the CTEP website at:
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm guidelines.

Treatment recommendations are provided below and may be modified based on local treatment standards and guidelines as appropriate:

**For Grade 1 symptoms:** (Mild reaction; infusion interruption not indicated; intervention not indicated) Remain at bedside and monitor subject until recovery from symptoms. The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) at least 30 minutes before additional Nivolumab administrations.

**For Grade 2 symptoms**: (Moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [eg, antihistamines, non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; prophylactic medications indicated for 24 hours).

Stop the Nivolumab infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen); remain at bedside and monitor subject until resolution of symptoms. Corticosteroid or bronchodilator therapy may also be administered as appropriate. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor subject closely. If symptoms recur then no further Nivolumab will be administered at that visit. Administer diphenbydramine 50 mg IV, and remain at bedside and monitor the subject until resolution of symptoms. The amount of study drug infused must be recorded on the electronic case report form (eCRF). The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) should be administered at least 30 minutes before additional Nivolumab administrations. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.

**For Grade 3 or Grade 4 symptoms:** (Severe reaction, Grade 3: prolonged [ie, not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae [eg, renal impairment, pulmonary infiltrates]). Grade 4: (life threatening; pressor or ventilatory support indicated).

Immediately discontinue infusion of Nivolumab. Begin an IV infusion of normal saline, and treat the subject as follows. Recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly

for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Subject should be monitored until the investigator is comfortable that the symptoms will not recur. Nivolumab will be permanently discontinued. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery from symptoms. In the case of late-occurring hypersensitivity symptoms (eg, appearance of a localized or generalized pruritis within 1 week after treatment), symptomatic treatment may be given (eg, oral antihistamine, or corticosteroids).

### 6.2.2 NeoVax plus Montanide

#### 6.2.2.1 Localized Reaction

Treatment with corticosteroids and antihistamines are not recommended for initial treatment, but may be instituted at the discretion of the treating physician if clinically necessary. Local applications of cold compresses and moisturizing creams are preferable over systemic agents for the management of local reactions. The use of topical or systemic steroids should be reserved for severe injection site reactions. Topical or systemic non-steroidal anti-inflammatory or antihistamine medications may be used as an alternative, however routine use to prevent injection site reactions should be avoided, and doses should be the minimum necessary for appropriate clinical management. Neoantigen Vaccine injection sites may be shifted in order to avoid administering injections to areas with evidence of persistent local reaction. The injection will occur on the same extremity (or flank).

#### 6.2.2.2 Systemic Reactions

Flu-like symptoms: Participants who develop flu-like symptoms that are controlled with acetaminophen or non-steroidal anti-inflammatory drugs given at the discretion of the investigator may be pre-treated with acetaminophen (650 mg PO) or ibuprofen (400-600 mg PO).

Hypersensitivity reactions: Participants who develop grade 1 or 2 allergic reactions may continue to receive NeoVax plus Montanide study injections following discussion with the study PI as long as:

1) The participant is made aware of the potential risk for future reactions, including potentially life-threatening reactions, and consents to continue treatment

2) The participant is pre-medicated with an anti-histamine and H-2 blocker. Premedication with corticosteroids may also be considered, but should be used judiciously in order to decrease potential ability of these agents to diminish the vaccine's effectiveness

3) At the next dosing visit, the patient is observed for at least 4 hours after the vaccination

Participants who develop grade 3 or 4 allergic reactions attributable to NeoVax plus Montanide administration should not receive further vaccination therapy and should discontinue study therapy.

## 6.3 Dose Modifications/Delays

### 6.3.1 Nivolumab

Dose Modifications

Dose reductions or dose escalations are not permitted.

Dose Delay Criteria

**Because of the potential for clinically meaningful Nivolumab-related AEs requiring early recognition and prompt intervention, management algorithms have been developed for suspected AEs of selected categories (listed in Appendix B).**

- Gastrointestinal
- Renal
- Pulmonary
- Hepatic
- Endocrinopathies
- Skin
- Neurological

Dose delay criteria apply for all drug-related adverse events (regardless of whether or not the event is attributed to Nivolumab). All study drugs must be delayed until treatment can resume.

Dose delay criteria apply for all drug-related AEs. Nivolumab must be delayed until treatment can resume

**Nivolumab administration should be delayed for the following:**

Any Grade ≥ 2 non-skin, drug-related AE, with the following exceptions:

- Grade 2 drug-related fatigue or laboratory abnormalities do not require a treatment delay
- Any Grade 3 skin, drug-related AE

Any Grade 3 drug-related laboratory abnormality, with the following exceptions for lymphopenia, leukopenia, AST, ALT, total bilirubin, or asymptomatic amylase or lipase:

- Grade 3 lymphopenia or leukopenia does not require dose delay.
- If a subject has a baseline AST, ALT, or total bilirubin that is within normal limits, delay dosing for drug-related Grade ≥ 2 toxicity.
- If a subject has baseline AST, ALT, or total bilirubin within the Grade 1 toxicity range, delay dosing for drug-related Grade ≥ 3 toxicity.
- Asymptomatic amylase or lipase do not require a treatment delay

Any AE, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, warrants delaying the dose of study medication.

**Criteria to Resume Treatment**

Subjects may resume treatment with Nivolumab when the drug-related AE(s) resolve to Grade ≤1 or baseline value, with the following exceptions:

- Subjects may resume treatment in the presence of Grade 2 fatigue
- Subjects who have not experienced a Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin toxicity
- Subjects with baseline Grade 1 AST/ALT or total bilirubin who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of Grade 2 AST/ALT OR total bilirubin
- Subjects with combined Grade 2 AST/ALT AND total bilirubin values meeting discontinuation parameters should have treatment permanently discontinued
- Drug-related pulmonary toxicity, diarrhea, or colitis, must have resolved to baseline before treatment is resumed
- Drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment

If the criteria to resume treatment are met, the subject should restart treatment at the next scheduled timepoint per protocol. However, if the treatment is delayed past the next scheduled timepoint per protocol, the next scheduled timepoint will be delayed until dosing resumes.

If treatment is delayed > 6 weeks, the subject must be permanently discontinued from study therapy, except as specified in discontinuation section.

**Discontinuation Criteria**

Treatment should be permanently discontinued for the following:

- Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment
- Any Grade 3 non-skin, drug-related adverse event lasting > 7 days, with the following exceptions for drug-related laboratory abnormalities, uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic adverse event, hypersensitivity reactions, and infusion reactions
    - Grade 3 drug-related uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic adverse event, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation
    - Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except those noted below
        - Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding requires discontinuation
        - Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:

- AST or ALT > 8 x ULN
- Total bilirubin > 5 x ULN
- Concurrent AST or ALT > 3 x ULN and total bilirubin > 2 x ULN

- Any Grade 4 drug-related adverse event or laboratory abnormality, except for the following events which do not require discontinuation:
  - Isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis and decrease to < Grade 4 within 1 week of onset.
  - Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset
- Any dosing interruption lasting > 6 weeks with the following exceptions:
  - Dosing interruptions to allow for prolonged steroid tapers to manage drug-related adverse events are allowed. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the Principal Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted
  - Dosing interruptions > 6 weeks that occur for non-drug-related reasons may be allowed if approved by the Principal Investigator. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the Principal Investigator, presents a substantial clinical risk to the subject with continued Nivolumab dosing

### 6.3.2 NeoVax plus Montanide

6.3.2.1 If a participant develops grade 3 toxicity attributable to the vaccine, additional treatment will be withheld until the toxicity has resolved or improved to grade 1. If the toxicity constitutes a DLT as defined in 5.6, the participant will be removed from the study. If the toxicity does not fulfill the criteria of a DLT, the next vaccine administration may be delayed for a maximum duration of 14 days. If vaccine dosing has to be delayed more than 2 times because of an attributable toxicity that is not considered a DLT, the participant will be removed from the study

6.3.2.2 If a participant develops grade 4 toxicity attributable to the vaccine s/he will be removed from treatment

6.3.2.3 If two or more grade 4 or greater toxicities attributable to the vaccine are observed, the trial will be suspended to investigate the causes of these (unexpected) toxicities

6.3.2.4 Vaccine dosing may be delayed in the event of unrelated adverse events if it is felt to be in the medical best interest of the patient following discussion by the treating investigator with the study Principal Investigator or designee. Vaccine dosing may be resumed once the treating investigator deems it is medically safe to do so following discussion with the study Principal Investigator or designee as long as the participant has not developed progressive disease

### 6.3.3 Ipilimumab

#### 6.3.3.1 Treatment of Ipilimumab Related Infusion Reactions

Since Ipilimumab contains only human immunoglobulin protein sequences, it is unlikely to be immunogenic and induce infusion or hypersensitivity reactions. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash, pruritis, arthralgias, hypo- or hypertension, bronchospasm, or other symptoms.
All Grade 3 or 4 injection reactions should be reported as an SAE if criteria are met. Injection reactions should be graded according to NCI CTCAE (CTEP Active Version (version 5.0) of the NCI Common Terminology Criteria for Adverse Events (CTCAE) which is identified and located on the CTEP website at:
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm guidelines).

Treatment recommendations are provided below and may be modified based on local treatment standards and guidelines as appropriate:

**For Grade 1 symptoms:** (Mild reaction; intervention not indicated) Remain at bedside and monitor subject until recovery from symptoms. The following prophylactic premedications are recommended for future injections: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) at least 30 minutes before additional Ipilimumab administrations.

**For Grade 2 symptoms**: (Moderate reaction requires therapy or injection interruption but responds promptly to symptomatic treatment [eg, antihistamines, non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; prophylactic medications indicated for 24 hours).

Stop Ipilimumab injections, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen); remain at bedside and monitor subject until resolution of symptoms. Corticosteroid or bronchodilator therapy may also be administered as appropriate. The following prophylactic premedications are recommended for future injections: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) should be administered at least 30 minutes before additional Ipilimumab administrations. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.

**For Grade 3 or Grade 4 symptoms:** (Severe reaction, Grade 3: prolonged [ie, not rapidly responsive to symptomatic medication; recurrence of symptoms following initial improvement;

hospitalization indicated for other clinical sequelae [eg, renal impairment, pulmonary infiltrates]). Grade 4: (life threatening; pressor or ventilatory support indicated).

Stop Ipilimumab injections. Begin an IV infusion of normal saline and treat the subject as follows. Recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Subject should be monitored until the investigator is comfortable that the symptoms will not recur. Ipilimumab will be permanently discontinued. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery from symptoms. In the case of late-occurring hypersensitivity symptoms (eg, appearance of a localized or generalized pruritis within 1 week after treatment), symptomatic treatment may be given (eg, oral antihistamine, or corticosteroids).

#### 6.3.3.2 Ipilimumab should be delayed for the following:

Any Grade ≥ 2 non-skin, drug-related AE, with the following exceptions:

- Grade 2 drug-related fatigue or laboratory abnormalities do not require a treatment delay
- Any Grade 3 skin, drug-related AE

Any Grade 3 drug-related laboratory abnormality, with the following exceptions for lymphopenia, leukopenia, AST, ALT, total bilirubin, or asymptomatic amylase or lipase:

- Grade 3 lymphopenia or leukopenia does not require dose delay.
- If a subject has a baseline AST, ALT, or total bilirubin that is within normal limits, delay dosing for drug-related Grade ≥ 2 toxicity.
- If a subject has baseline AST, ALT, or total bilirubin within the Grade 1 toxicity range, delay dosing for drug-related Grade ≥ 3 toxicity.
- Asymptomatic amylase or lipase do not require a treatment delay

Any AE, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, warrants delaying the dose of study medication.

### 6.4 Criteria to Resume Treatment

Subjects may resume treatment with Ipilimumab and NeoVax plus Montanide when the drug-related AE(s) resolve to Grade ≤1 or baseline value, with the following exceptions:

- Subjects may resume treatment in the presence of Grade 2 fatigue
- Subjects who have not experienced a Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin toxicity
- Subjects with baseline Grade 1 AST/ALT or total bilirubin who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of Grade 2 AST/ALT OR total bilirubin

- Subjects with combined Grade 2 AST/ALT AND total bilirubin values meeting discontinuation parameters should have treatment permanently discontinued
- Drug-related pulmonary toxicity, diarrhea, or colitis, must have resolved to baseline before treatment is resumed
- Drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment

If the criteria to resume treatment are met, the subject should restart treatment at the next scheduled timepoint per protocol. However, if the treatment is delayed past the next scheduled timepoint per protocol, the next scheduled timepoint will be delayed until dosing resumes.

If treatment is delayed > 6 weeks, the subject must be permanently discontinued from study therapy, except as specified in discontinuation section.

### 6.5 Discontinuation criteria

Treatment should be permanently discontinued for the following:

- Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment
- Any Grade 3 non-skin, drug-related adverse event lasting > 7 days, with the following exceptions for drug-related laboratory abnormalities, uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic adverse event, hypersensitivity reactions, and injection reactions
    - Grade 3 drug-related uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic adverse event, hypersensitivity reaction, or injection reaction of any duration requires discontinuation
    - Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except those noted below
        - Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding requires discontinuation
        - Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:
            - AST or ALT > 8 x ULN
            - Total bilirubin > 5 x ULN
            - Concurrent AST or ALT > 3 x ULN and total bilirubin > 2 x ULN
- Any Grade 4 drug-related adverse event or laboratory abnormality, except for the following events which do not require discontinuation:
    - Isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis and decrease to < Grade 4 within 1 week of onset.

- Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset

- Any dosing interruption lasting > 6 weeks with the following exceptions:
  - Dosing interruptions to allow for prolonged steroid tapers to manage drug-related adverse events are allowed. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the Principal Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted
  - Dosing interruptions > 6 weeks that occur for non-drug-related reasons may be allowed if approved by the Principal Investigator. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted
  - Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the Principal Investigator, presents a substantial clinical risk to the subject with continued Ipilimumab dosing

# 7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

## 7.1 Adverse Event Definition and Characteristics

### 7.1.1 Definitions

**ADVERSE EVENTS**

An Adverse Event (AE) is defined as any new untoward medical occurrence or worsening of a preexisting medical condition in a clinical investigation participant administered study drug and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (such as an abnormal laboratory finding), symptom, or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product.

A ***non-serious adverse event*** is an AE not classified as serious.

**SERIOUS ADVERSE EVENTS**

A ***Serious Adverse Event (SAE)*** is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening (defined as an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)

- requires inpatient hospitalization or causes prolongation of existing hospitalization
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [eg, medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)
- Suspected transmission of an infectious agent (eg, pathogenic or nonpathogenic) via the study drug is an SAE.

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs (Section 7.9) and the characteristics of an observed AE (Section 7.1) will determine whether the event requires expedited reporting **in addition** to routine reporting.

### 7.1.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent(s) that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.
  - Other AEs for the protocol that do not require expedited reporting are outlined in the next section (Expedited Adverse Event Reporting) under the sub-heading of Protocol-Specific Expedited Adverse Event Reporting Exclusions.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

## 7.2 AE and SAE Recording and Reporting

The study must be conducted in compliance with FDA regulations, local safety reporting requirements, and reporting requirements of the principal investigator.

All Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must <u>also</u> be reported in routine study data submissions.**

It is the responsibility of each participating investigator to report serious adverse events to the study sponsor and/or others as described below.

### 7.2.1 Non-Serious Adverse Events

Non-serious adverse events will be reported to the DF/HCC Overall Principal Investigator on the toxicity Case Report Forms.

**Reporting Non-Serious Adverse Events to BMS**

- Non-serious Adverse Events (AE) are to be provided to BMS in aggregate via interim or final study reports as specified in the agreement or, if a regulatory requirement [eg, IND US trial] as part of an annual reporting requirement.
- Non-serious AE information should also be collected from following the subject's written consent to participate in the study.

The collection of non-serious AE information should begin following the subject's written consent to participate in the study. All non-serious adverse events (not only those deemed to be treatment-related) should be collected continuously during the treatment period and for a minimum of 100 days following the last dose of study treatment.

Non-serious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up is also required for non-serious AEs that cause interruption or discontinuation of study drug and for those present at the end of study treatment as appropriate.

**Laboratory Test Abnormalities**

All laboratory test results captured as part of the study should be recorded following institutional procedures. Test results that constitute SAEs should be documented and reported to BMS as such.

The following laboratory abnormalities should be documented and reported appropriately:

- any laboratory test result that is clinically significant or meets the definition of an SAE
- any laboratory abnormality that required the participant to have study drug discontinued or interrupted
- any laboratory abnormality that required the subject to receive specific corrective therapy.

It is expected that wherever possible, the clinical rather than laboratory term would be used by the reporting investigator (eg, anemia versus low hemoglobin value).

### 7.2.2 Serious Adverse Events

All serious adverse events that occur after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment must be reported to the DF/HCC Overall Principal Investigator on the local institutional SAE form. This includes events meeting the criteria outlined in Section 11.1.2, as well as the following:

- Grade 2 (moderate) and Grade 3 (severe) Events – that are *Unexpected* and there is a *Reasonable Possibility* that the Adverse Event is related to the study intervention
- All Grade 4 (life-threatening or disabling) Events – Report all events that are Unexpected. Events that are expected and listed within the protocol and /or current consent form do not need to be reported to the DFCI IRB. Please not, an event that present at a higher severity than what is currently listed within protocol and/or current consent as expected would be considered unexpected and reportable.
- All Grade 5 (fatal) Events – When the patient is enrolled and actively participating in the trial OR when the event occurs within 30 days of the last study intervention.
  Note: If the patient is in long term follow up, report the death at the time of continuing review.
- Although pregnancy and potential drug-induced liver injury (DILI), are not always serious by regulatory definition, however, these events must be reported within the SAEs timeline.
  - Potential drug induced liver injury is defined as:

    1. AT (ALT or AST) elevation > 3 times upper limit of normal (ULN)

    **AND**

    2. Total bilirubin > 2 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase)

    **AND**

    3. No other immediately apparent possible causes of AT elevation and hyperbilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drug(s) known to be hepatotoxic.

- Any component of a study endpoint that is considered related to study therapy should be reported as an SAE (eg, death is an endpoint, if death occurred due to anaphylaxis, anaphylaxis must be reported).
- Any significant worsening noted during interim or final physical examinations, electrocardiograms, X-rays, and any other potential safety assessments, whether or not these procedures are required by the protocol, should also be recorded as a non-serious or serious AE, as appropriate, and reported accordingly.

### 7.2.3 DF/HCC Reportable Adverse Events

- In the event of an unanticipated problem or life-threatening complications treating investigators must immediately notify the Overall PI.
- Investigators **must** report to the Overall PI any adverse event (AE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of

treatment on the local institutional SAE form.

- Adverse Events Reporting Guidelines
  All participating sites will report AEs to the Sponsor-Investigator per DF/HCC requirements, and the IRB of record for each site as applicable per IRB policies. The table below indicates which events must be reported to the DF/HCC Sponsor-Investigator:

| Attribution | DF/HCC Reportable Adverse Events(AEs) | | | | |
|---|---|---|---|---|---|
| | Gr. 2 & 3 AE Expected | Gr. 2 & 3 AE Unexpected | Gr. 4 AE Expected | Gr. 4 AE Unexpected | Gr. 5 AE Expected or Unexpected |
| Unrelated Unlikely | Not required | Not required | 5 calendar days# | 5 calendar days | 24 hours* |
| Possible Probable Definite | Not required | 5 calendar days | 5 calendar days# | 5 calendar days | 24 hours* |
| # If listed in protocol as expected and not requiring expedited reporting, event does not need to be reported. | | | | | |
| * For participants enrolled and actively participating in the study *or* for AEs occurring within 30 days of the last intervention, events must be reported within 1 business day of learning of the event. | | | | | |

The following information regarding injection site reaction(s) will be collected during the visits belonging to the vaccine treatment period for both peptide pools and ipilimumab injections:

- Erythema/redness: yes/no. If yes length /width will be provided
- Induration/swelling: yes/no. If yes length /width will be provided
- Warmth: yes/no
- Pruritus: yes/no
- Other (if applicable)

### 7.3 Reporting to the Food and Drug Administration (FDA)

The Overall PI, as study sponsor, will be responsible for all communications with the FDA. The Overall PI will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

### 7.4 Reporting to Study Sponsor

Participating investigators must report each serious adverse event to the DF/HCC Overall Principal Investigator and Study Sponsor (Dr. Patrick Ott, DFCI) within 24 hours of learning of the occurrence using the Medwatch 3500A. The form can be found here: https://www.fda.gov/safety/medical-product-safety-information/medwatch-forms-fda-safety-reporting. In the event that the participating investigator does not become aware of the serious adverse event immediately (e.g., patient sought treatment elsewhere), the participating investigator is to report the event within 24 hours after learning of it and document the time of his or her first awareness of the adverse event. Report serious adverse events by telephone, email or facsimile to:

**DF/HCC Overall PI / Study Sponsor**
Patrick Ott, MD, PhD
[redacted]

Within the following 24-48 hours, the participating investigator must provide follow-up information on the serious adverse event. Follow-up information should describe whether the event has resolved or continues, if and how the event was treated, and whether the patient will continue or discontinue study participation.

**Oncovir, Inc**. and **SEPPIC** will be provided with a simultaneous copy of all adverse events filed with the FDA.

### 7.5 Reporting SAEs to BMS

All SAEs that occur following the subject's written consent to participate in the study through 100 days of discontinuation of dosing must be reported to **BMS** Worldwide Safety, whether related or not related to study drug, including those thought to be associated with protocol-specified procedures (eg, a follow-up skin biopsy) within **24 hours \ 1 business day** to comply with regulatory requirements. MedWatch 3500A form should be completed and submitted to BMS to report the SAEs.

**Note: Please include the BMS Protocol number on the SAE form or on the cover sheet with the SAE form transmission**

An SAE report should be completed for any event where doubt exists regarding its seriousness; if the investigator believes that an SAE is not related to study drug, but is potentially related to the conditions of the study (such as withdrawal of previous therapy or a complication of a study procedure), the relationship should be specified in the narrative section of the SAE Report Form.

Investigators should report to the responsible regulatory authority as appropriate.

All SAEs must be reported by confirmed facsimile (fax) transmission or reported via electronic mail to the below email address and the BMS Protocol number must be included on the SAE form or on the cover sheet with the SAE form transmission

**SAE Email Address:** [redacted]

**SAE Facsimile Number:** [redacted]

If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported.)

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours \ 1 Business Day to BMS using the same procedure used for transmitting the initial S. AEreport.

All SAEs should be followed to resolution or stabilization.

The causal relationship to study drug is determined by a physician and should be used to assess all adverse events (AE). The casual relationship can be one of the following:

- Related: There is a reasonable causal relationship between study drug administration and the AE.
- Not related: There is not a reasonable causal relationship between study drug administration and the AE.

The term "reasonable causal relationship" means there is evidence to suggest a causal relationship.

Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. (In order to prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more AEs.)

The Sponsor-Investigator will reconcile the clinical database AE cases (**case level only**) transmitted to BMS Global Pharmacovigilance [redacted].

- The Investigator will request from BMS GPV&E, [redacted] the SAE reconciliation report and include the BMS protocol number every 3 months and prior to data base lock or final data summary
- GPV&E will send the investigator the report to verify and confirm all AE and SAEs have been transmitted to BMS GPV&E.
- The data elements listed on the GPV&E reconciliation report will be used for case identification purposes. If the Investigator determines a case was not transmitted to BMS GPV&E, the case should be sent immediately to BMS ([redacted].

In addition to the Sponsor Investigator's responsibility to report events to their local HA, suspected serious adverse reactions (whether expected or unexpected) shall be reported by BMS to the relevant competent health authorities in all concerned countries according to local regulations (either as expedited and/or in aggregate reports).

In accordance with local regulations, BMS will notify sponsor investigators of all reported SAEs that are suspected (related to the investigational product) and unexpected (ie, not previously described in the IB). An event meeting these criteria is termed a Suspected, Unexpected Serious Adverse Reaction (SUSAR). Sponsor investigator notification of these events will be in the form of either a SUSAR Report or a Semi-Annual SUSAR Report.

- Other important findings which may be reported by BMS as an Expedited Safety Report (ESR) include: increased frequency of a clinically significant expected SAE, an SAE considered associated with study procedures that could modify the conduct of the study, lack of efficacy that poses significant hazard to study subjects, clinically significant safety finding from a nonclinical (eg, animal) study, important safety recommendations

from a study data monitoring committee, or sponsor or BMS decision to end or temporarily halt a clinical study for safety reasons.

- Upon receiving an ESR from BMS, the investigator must review and retain the ESR with the IB. Where required by local regulations or when there is a central IRB/IEC for the study, the sponsor will submit the ESR to the appropriate IRB/IEC. The investigator and IRB/IEC will determine if the informed consent requires revision. The investigator should also comply with the IRB/IEC procedures for reporting any other safety information.

### 7.5.1 Reporting Pregnancy event to BMS

If it is discovered a patient is pregnant or may have been pregnant at the time of exposure to the BMS product associated with this study, including during at least 5 half-lives after product administration, the pregnancy, AEs associated with maternal exposure and pregnancy outcomes must be reported and submitted to BMS on a BMS Pregnancy Surveillance Form or the MedWatch or approved site SAE form, and reported to BMS within 24 hours/1 business day by confirmed fax or reported via electronic mail to [redacted] If only limited information is initially available, follow up reports may be required. Your original forms are to remain on site. Protocol-required procedures for study discontinuation and follow-up must be performed on the participant.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the MedWatch, BMS Pregnancy Surveillance Form, **or** approved site SAE form. A BMS Pregnancy Surveillance Form may be provided upon request.

## 7.6 Reporting to the Institutional Review Board (IRB)

Investigative sites within DF/HCC will report all serious adverse events directly to the DFCI Office for Human Research Studies (OHRS).

## 7.7 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports, sentinel events or unanticipated problems that require reporting per institutional policy.

## 7.8 Expected Toxicities

A list of the adverse events and potential risks associated with the agents administered in this study appears below and will determine whether dose delays and modifications will be made or whether the event requires expedited reporting **in addition** to routine reporting.

### 7.8.1 Adverse Events List

#### 7.8.1.1 Adverse Event List for poly-ICLC

Poly-ICLC is an investigational agent; however there has been significant experience in previous clinical trials as discussed in 2.3. The safety profile has been acceptable.These reactions have been generally mild to moderate and transient for most patients in previous studies.

Localized reactions:

- Transient (<24 h) injection fluid accumulation
- Erythema
- Bruising
- Induration
- Pruritus
- Edema
- Localized rash
- Perioral numbness

Systemic reactions:
Flu-like symptoms, including:

- Malaise
- Fatigue
- Myalgia/arthralgia
- Headache
- Fever
- Chills
- Dizziness
- Elevated blood pressure
- Elevated pulse rate
- Shortness of breath

#### 7.8.1.2 Adverse Event List for Personalized NeoAntigen Peptides

Localized reactions or systemic reactions are expected but they are anticipated to be mild to moderate in the majority of cases:

Localized reactions:

- Erythema
- Bruising
- Induration
- Pruritus
- Edema
- Localized rash
- Perioral numbness

Systemic reactions:
Flu-like symptoms, including:

- Malaise
- Fatigue
- Myalgia/arthralgia
- Headache
- Fever
- Chills
- Arthralgia
- Dizziness
- Flushing
- Elevated blood pressure
- Elevated pulse rate
- Shortness of breath

Hypersensitivity reactions represent allergic reactions. Hypersensitivity reactions typically occur in an immediate fashion (i.e. within the same day as the vaccination) although they may be delayed with onset of symptoms from one day to weeks after vaccination. Symptoms typically include mild to moderate pruritus, erythema, flushing, rash, perioral numbness, lightheadedness, shortness of breath, myalgia and increased pulse, blood pressure and temperature. Precautions should be taken to prepare for the possibility of hypersensitivity reactions after NeoVax plus Montanide administration. Patients should be observed for a minimum of one hour following vaccination in order to evaluate and treat any immediate hypersensitivity reactions. The use of all routine supportive medication is permitted for hypersensitivity reactions, although routine use of prophylactic antihistamines, non-steroidal anti-inflammatory agents and concomitant corticosteroids are to be avoided if at all possible. If used, doses of corticosteroids should be the minimum necessary for appropriate clinical management. If any new administration or increased dose of systemic corticosteroids is necessary, every effort should be taken to taper, and preferably discontinue treatment with them as quickly as clinically feasible.

Autoimmune diseases: In principle, the mechanisms that allow the immune system to recognize tumor antigens could also lead to breakdown of tolerance to native or self-antigens, generating an autoimmune reaction. Thus, symptoms that mimic autoimmune diseases or other autoimmune reactions are a theoretical possibility for which participants will be monitored.

#### 7.8.1.3 Adverse Event List for Montanide

Monatnide has been generally well tolerated and induces transient local reactions such as tenderness, erythema, or edema at the injection site. Transient general reactions including fever, headache, and flu-like symptoms have also been observed.

#### 7.8.1.4 Adverse Event List for Nivolumab

Immuno-oncology agents such as Nivolumab are associated with AEs that can differ in severity and duration from AEs caused by other therapeutic classes. Early recognition and management of AEs associated with Nivolumab may mitigate severe toxicity. Immune related AEs have been

observed in the following organ systems:

- Gastrointestinal
- Renal
- Pulmonary
- Hepatic
- Endocrinopathies
- Skin
- Neurological

In a previous large phase I study, the most frequently reported AEs were fatigue (54.9%), decreased appetite (35.0%), diarrhea (34.3%), nausea (30.1%), and cough (29.4%)[10]. Treatment-related AEs were reported in 230 (75.2%) of the 306 subjects. The most frequently reported treatment-related AEs were fatigue (28.1%), rash (14.7%), diarrhea (13.4%), and pruritus (10.5%)[10]. Most treatment-related AEs were low grade. Treatment-related high grade (Grade 3-4) AEs were reported in 52 (17.0%) of subjects. The most frequently reported treatment-related high-grade AE was fatigue (6.5%).

#### 7.8.1.5 Adverse Events for Ipilimumab

Localized reactions:

- Erythema
- Induration
- Pruritus
- Edema
- Localized rash

The systemic exposure of patients to Ipilimumab will be significantly lower than the exposure according to approved dosing regimen for melanoma (3 mg/kg q3weeks x4) as shown in the Table below.

**Table 1. Dose Level Description and Comparison to Approved Ipilimumab Dosing Regimen***

| | Vaccination Phase | | |
|---|---|---|---|
| **Cohort Dose Level** | **Localized Ipilimumab**** | **Approved Ipilimumab***** | **Relative Exposure******** |
| **Cohort -1 Dose Level-1** | 0.25 ml per injection site<br>10 mg over first 63 days | 630 mg over<br>63 days | 1/60 |
| **Cohort 1 Dose Level 1** | 0.5 ml per injection site<br>20 mg over first 63 days | 630 mg over<br>63 days | 1/30 |
| **Cohort 2 Dose Level 2** | 1 ml per injection site<br>40 mg over first 22 days | 630 mg over<br>63 days | 1/15 |

*The Neoantigen Vaccine dose is identical for all injections: 300 µg of each of up to 10 peptides per injection site + 0.5 mg Poly-ICLC (Hiltonol®) + 1 mL Montanide per injection site
** Localized Ipilimumab dosing: 5 mg/ml dosage form; 4 injection sites/day; 5 injection days
***Approved Ipilimumab dosing: 3 mg/kg, q3weeks X4
****Relative exposure is calculated as the mass of Ipilimumab delivered subcutaneously divided by that delivered systemically

The dose of Ipilimumab will vary between 1.25 and 5 mg per injection site during the MTD determination phase. At the highest dose of Ipilimumab planned (5 mg Ipilimumab at each vaccination site with the vaccine), the maximal exposure would be 140 mg over 20 weeks, about 11-fold lower than the approved regimen [840 mg for a 70 kg patient given over 10 – 16 weeks]. Thus, systemic reactions are not expected, but may occur:

- Gastrointestinal
- Renal
- Pulmonary
- Hepatic
- Endocrinopathies
- Skin
- Neurological

## 8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or other agents administered in this study can be found in Section 7.1.

### 8.1 Nivolumab

#### 8.1.1 Description
Nivolumab is a fully human monoclonal immunoglobulin G4 (IgG4-S228P) antibody that targets the programmed death (PD-1) cell surface membrane receptor.

#### 8.1.2 Form
Nivolumab Injection Drug product is a sterile, non-pyrogenic, single-use, isotonic aqueous solution formulated at 10 mg/ml. The solution is a clear to opalescent, colorless to pale liquid and may contain particles. It is supplied in 100mg, 10mg/ml vials stoppered with butyl stoppers and sealed with aluminum seals.

#### 8.1.3 Storage and Stability
Nivolumab must be stored at 2°-8°C (36°-46°F) and protected from light and freezing. IV bags containing undiluted and diluted solutions of Nivolumab prepared for dosing may be stored up to 20 hours in a refrigerator at 2°-8°C (36°-46°F) and used within 8 hours at room temperature and under room light. The maximum 8-hour period under room temperature and room light conditions for undiluted and diluted solutions of Nivolumab injection in the IV bag should be inclusive of the product administration period.

#### 8.1.4 Compatibility
Nivolumab will not be mixed with any other study agents.

#### 8.1.5 Availability
Nivolumab is an FDA-approved drug for the treatment of metastatic melanoma and will be supplied by Bristol Myers Squibb to the participating institution's pharmacy.

At the end of the study period, Bristol-Myers Squibb Company will not continue to supply Nivolumab to subjects/investigators unless the Sponsor-Investigator chooses to extend their study. The investigator is responsible to ensure that you receive appropriate standard of care or other appropriate treatment in the independent medical judgement of the Investigator to treat the condition under study.

#### 8.1.6 Handling and Preparation

Nivolumab injection is to be administered as an IV infusion through a 0.2-micron to 1.2-micron pore size, low-protein binding (eg, polyethersulfone membrane) in-line filter at the protocol-specified doses and infusion times. It is not to be administered as an IV push or bolus injection. When the dose is based on patient weight (ie, mg/kg), nivolumab injection can be infused undiluted (10 mg/mL) or diluted with 0.9% sodium chloride injection or 5% dextrose injection to protein concentrations as low as 0.35 mg/mL. When the dose is fixed (eg, 240 mg, 360 mg, or 480 mg flat dose), nivolumab injection can be infused undiluted or diluted so as not to exceed a total infusion volume of 160 mL. For patients weighing less than 40 kg, the total volume of infusion must not exceed 4 mL per kg of patient weight.

During drug product preparation and handling, vigorous mixing or shaking is to be avoided. Care must be taken to ensure sterility of the prepared solution as the product does not contain any antimicrobial preservative or bacteriostatic agent. Nivolumab infusions are compatible with polyvinyl chloride or polyolefin containers and infusion sets, and glass bottles.

#### 8.1.7 Administration

Nivolumab injection is to be administered as an IV infusion, using a volumetric pump with a 0.2 / 0.22 micron ore size, low-protein binding polyethersulfone membrane in-line filter at the protocol-specified doses.

#### 8.1.8 Ordering

Nivolumab will be obtained from Bristol Myers Squibb (BMS) as an Investigator Initiated Study. Nivolumab can be ordered from BMS by providing a completed Drug Shipment Form.

#### 8.1.9 Accountability

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

#### 8.1.10 Destruction and Return

At the end of the study, unused supplies of Nivolumab should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

### *8.2* Ipilimumab

#### 8.2.1 Description

Ipilimumab is a fully human monoclonal immunoglobulin G1κ antibody that targets the human cytotoxic T lymphocyte antigen 4 (CTLA-4) cell surface membrane receptor.

### 8.2.2 Form
Ipilimumab Injection Drug product is a sterile, non-pyrogenic, single-use, isotonic aqueous solution formulated at 5 mg/ml (0.9% sodium chloride injection; USP, or 5% dextrose injection). The solution is a clear to slightly opalescent colorless to pale yellow liquid and may contain a small amount of translucent-to-white particles. BMS will provide 50mg/10ml or 200mg/40mL vials for this study.

### 8.2.3 Storage and Stability
Ipilimumab must be stored at 2°-8°C (36°-46°F) and protected from light and freezing. Partially used vials should be discarded after single use.

### 8.2.4 Compatibility
Ipilimumab will not be mixed with any other study agents.

### 8.2.5 Handling
Vials containing Ipilimumab should not be shaken and should be discarded if cloudy.

### 8.2.6 Availability
In this protocol, Ipilimumab is an investigational product and will be supplied by Bristol Myers Squibb to the participating institution's pharmacy.

At the end of the study period, Bristol-Myers Squibb Company will not continue to supply Ipilimumab to subjects/investigators unless the Sponsor-Investigator chooses to extend their study. The investigator is responsible to ensure that you receive appropriate standard of care or other appropriate treatment in the independent medical judgement of the Investigator to treat the condition under study.

### 8.2.7 Preparation
Ipilimumab will be used directly from the supplied vial; no further dilution will be required.

### 8.2.8 Administration
The specified volume of Ipilimumab will be drawn directly into a syringe from the supplied vial. The needle for withdrawal from the vial will be removed and replaced with a needle for subcutaneous injection. Filled syringes should be used for injection as soon as possible after preparation.

### 8.2.9 Ordering
Ipilimumab will be obtained from Bristol Myers Squibb (BMS) as an Investigator Initiated Study. Ipilimumab can be ordered from BMS by providing a completed Drug Shipment Form.

### 8.2.10 Accountability
The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

#### 8.2.11 Destruction and Return

At the end of the study, unused supplies of Ipilimumab should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

### 8.3 Poly-ICLC (Hiltonol®)

#### 8.3.1 Description

[redacted]

#### 8.3.2 Form

Poly-ICLC is supplied by Oncovir in single-dose vials containing 1 mL of 1.8 mg/mL opalescent white suspension. Each mililiter of poly-ICLC for injection contains 1.8 mg/mL poly-IC, 1.5 mg/mL poly-L-lysine, and 5 mg/mL sodium carboxymethylcellulose in 0.9% sodium chloride solution, adjusted to pH 6-8 with sodium hydroxide.

#### 8.3.3 Storage and Stability

Poly-ICLC is normally shipped or stored refrigerated at about 40°F (2-8°C) but should not be frozen. Based on extended formal stability data at +5°C ± 3°C and at +25°C ± 2°C, it is justified to accept temperature excursions during transport, shipment and storage of Hiltonol® at clinical sites up to +27°C for up to 5 days. Likewise, temperature deviations between 0°C and +2°C are acceptable and have no effect on product quality.

#### 8.3.4 Compatibility

Poly-ICLC will be mixed with personalized neoantigen peptides just prior to use to prepare the final product (NeoVax plus Montanide).

#### 8.3.5 Handling

There are no specific handling instructions for poly-ICLC.

#### 8.3.6 Availability

Poly-ICLC is an investigational agent and has been purchased from Oncovir, Inc. under a Clinical Trials Agreement between DFCI and Oncovir, Inc, dated May 2012. Under that agreement Oncovir, Inc will grant access to the Drug Master File for poly-ICLC for regulatory purposes and provides DFCI information necessary to pursue the clinical use of poly-ICLC. Vials of poly-ICLC sufficient for this clinical trial will be shipped from the Hiltonol® storage facility (Bellwyck Packaging, Mississauga, Ontario, Canada) to the DFCI Pharmacy. Poly-ICLC can be ordered directly from Oncovir, Inc, by submitting a purchase order to:

**Oncovir, Inc.**

[redacted]

### 8.3.7 Preparation

Poly-ICLC is supplied as an opalescent white suspension. It will be directly withdrawn from the vial under sterile conditions and mixed with the personalized neoantigen peptides (see Section 8.4.7).

Upon allowing the vial to reach room temperature (approximately 1-2 hours), the vial should be gently inverted about 10 times to insure suspension of the formulation. Gently tap the vial to gather the liquid suspension to the bottom of the vial to aid in extracting the required volume for dose preparation. If visible particulates or aggregates are present when measured against a black background and are not resuspendable or the settled material fails to re-suspend, the vial should be placed in quarantine and not used for clinical administration. Obtain a new vial of Poly-ICLC for dosage preparation.

### 8.3.8 Administration

Following mixing with the personalized neoantigen peptides, the vaccine (peptide + poly-ICLC + Montanide) is to be administered subcutaneously.

### 8.3.9 Accountability

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

### 8.3.10 Destruction and Return

At the end of the study, unused supplies of poly-ICLC will be held by the IND holder for future use until they are out of date at which point they will be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

## 8.4 Personalized neoantigen peptides

### 8.4.1 Description

Personalized neoantigen peptides are comprised of up to 20 distinct peptides unique to each participant. Each peptide is a linear polymer of ~20 - ~30 L-amino acids joined by standard peptide bonds. The amino terminus is a primary amine ($NH_2$-) and the carboxy terminus is a carbonyl group (-COOH). Only the standard 20 amino acids commonly found in mammalian cells are utilized (alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine). The molecular weight of each peptide varies based on its length and sequence and is calculated for each peptide.

### 8.4.2 Form

The two peptide pools, each containing up to 10 peptides, will each be provided in one bulk aliquot for use in Drug Product preparation; they will be packaged with a tamper evident seal, labelled and shipped under appropriate temperature controlled conditions to DFCI Pharmacy dept.

Bulk aliquots for up to 2 different peptide pools will identified with the patient's Study ID number (i.e. 18279-101). Final sterile filtration will be performed by the Pharmacy department prior to aliquoting into vials. The vials containing peptide pools are to be stored at -70°C or colder until use.

### 8.4.3 Storage and Stability

Personalized neoantigen peptides are to be stored at the DFCI Pharmacy at a temperature of -70°C or colder. The thawed vials and the final mixture of personalized neoantigen peptides, poly-ICLC, and Montanide can be kept at room temperature but should be used as soon as possible after preparing the peptide + poly-ICLC + Montanide mixture.

### 8.4.4 Compatibility

Personalized neoantigen peptides will be mixed with poly-ICLC just prior to use to prepare the final product (NeoVax plus Montanide).

### 8.4.5 Handling

There are no specific instructions for handling personalized neoantigen peptides.

### 8.4.6 Availability

The personalized neoantigen peptides are prepared by a GMP facility under contract with DFCI and will be supplied directly to the DFCI. No other institutions will be involved in delivering personalized neoantigen peptides under this protocol.

### 8.4.7 Preparation

### 8.4.8 Administration

Following mixing with personalized neoantigen peptides, the personalized neoantigen vaccine is to be administered subcutaneously.

### 8.4.9 Ordering

The order for personalized neoantigen peptides to the peptide vendor will be placed by the DFCI separately for each individual participant. Delivery time is anticipated to be 4 – 8 weeks.

### 8.4.10 Accountability

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

### 8.4.11 Destruction and Return

At the end of the study, after a patient finishes treatment, or after a patient discontinues treatment early, unused supplies of personalized neoantigen peptides will be released to the sponsor for further research or destroyed according to institutional policies. If unused personalized neoantigen peptides are destroyed, this will be documented in the Drug Accountability Record Form.

## 8.5 Montanide

### 8.5.1 Description

[redacted]

[redacted]

**8.5.2 Form**

Montanide ISA-51 VG is supplied by Seppic in 2R3 amber vials filled with 3 ml containing Montanide ISA-51 VG.

**8.5.3 Storage and Stability**

Montanide ISA-51 SG is stable at room temperature for 3 years from the date of manufacture. It is preferable to keep unused vials in the original styrofoam box at standard room temperature.

**8.5.4 Compatibility**

Montanide ISA-51 SG will be mixed with personalized neoantigen peptides and poly-ICLC just prior to use to prepare the final product (neoantigen vaccine).

**8.5.5 Handling**

There are no specific handling instructions for Montanide ISA-51 VG.

**8.5.6 Availability**

Montanide ISA 51 VG is an investigational agent and will be sourced as commercially available product from Seppic.

**8.5.7 Required Supplies**

[redacted]

I-connectors (luer lock adapters) used to facilitate emulsion preparation will also be supplied by the study.

**8.5.8 Administration**

Following mixing with the personalized neoantigen peptides, the vaccine (peptide + poly-ICLC + Montanide® ISA-51 VG) is to be administered subcutaneously.

**8.5.9 Accountability**

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's

Handbook for Procedures for Drug Accountability and Storage.)

### 8.5.10 Destruction and Return

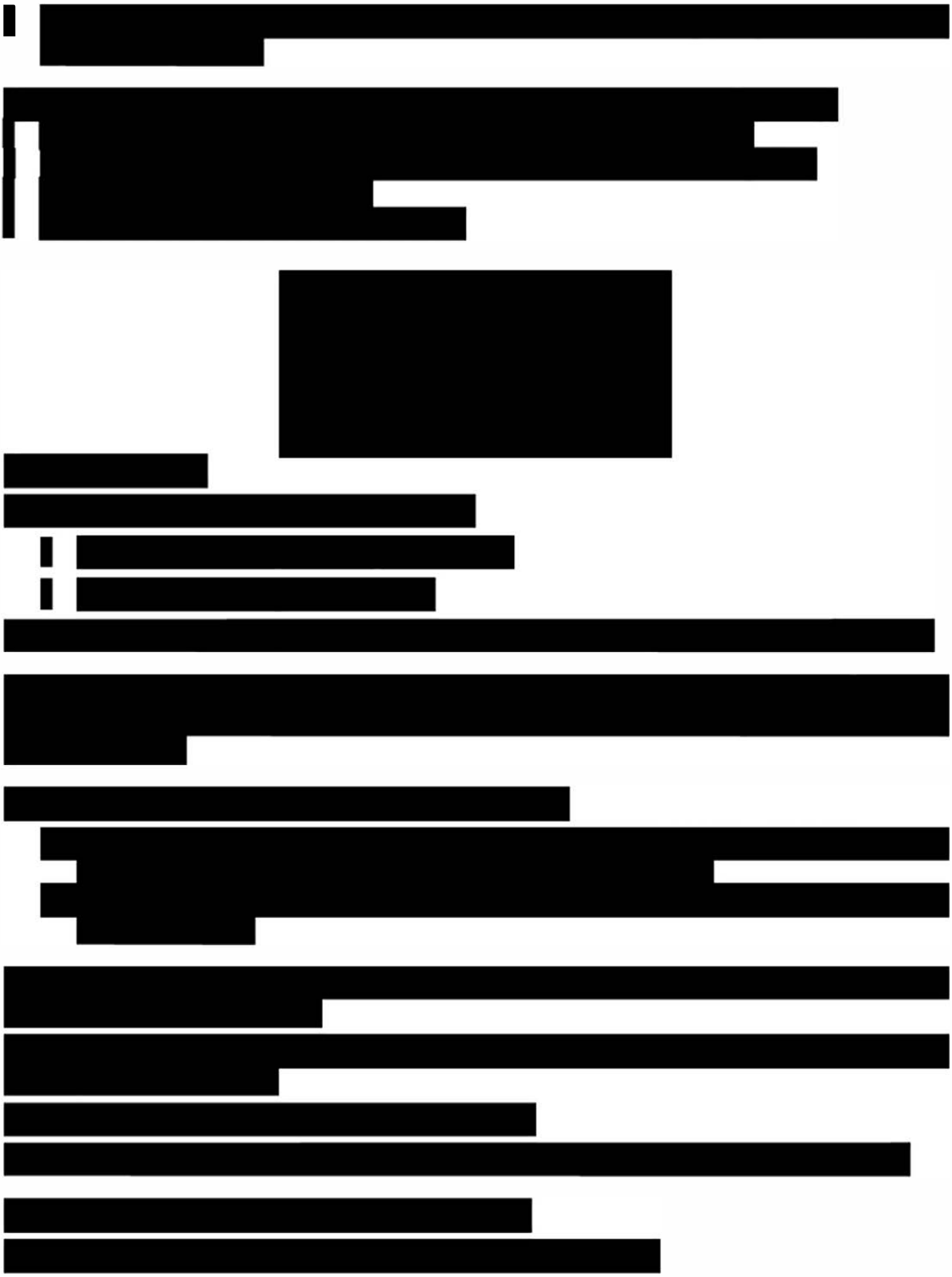
At the end of the study, unused supplies of Montanide will be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

## 8.6 Preparation of NeoVax + Montanide

[redacted]

### 8.6.1 Preparing NeoVax A + Montanide vaccine

[redacted]



[redacted]

### 8.6.2 Preparing Pool B NeoVax + Montanide vaccine

[redacted]

[redacted]

### 8.6.3 NeoVax vaccine preparation without using Montanide

[redacted]

[redacted]

## 9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

### 9.1 Pharmacodynamic Studies

Immune monitoring will be performed in a step-wise fashion as outlined below to characterize the intensity and quality of the elicited immune responses. Peripheral blood and tumor will be collected serially at different time points as specified in the study calendar.

If sufficient tumor tissue is available, a portion of the tumor will be used to develop autologous melanoma cell lines for use in cytotoxic T-cell assays.

#### 9.1.1 Screening *ex vivo* IFN-γ ELISPOT

For each participant, a set of screening peptides will be synthesized. The screening peptides will be 15-17 amino acids in length, overlapping by 11 amino acids and covering the entire length of each peptide or the entire length of the neoORF for neoORF-derived peptides. The set of participant-specific screening peptides will be pooled together (either as a single pool or in four sub-pools corresponding to the immunizing peptide pools) at approximately equal concentration and a portion of each peptide will also be stored individually. In addition, the predicted 9mer or 10mer epitope for each immunizing peptide will be synthesized and pooled. Purity of the peptide pools will be ascertained by testing PBMC from 5 healthy donors with established low background in ex vivo IFN-γ ELISPOTs. Initially, PBMC obtained prior to Nivolumab, prior to Vaccination, and at week 20 (the primary immunological endpoint) will be stimulated for 18 hours with the overlapping 15-17mer peptides (11 amino acids overlap) and with the 9mer/10mer epitopes to examine the global response to the peptide vaccine. Subsequent assays may utilize PBMC collected at other time points as indicated. If no response is identified at the primary immunological endpoint using the ex vivo IFN-γ ELISPOT assay, PBMC may be stimulated *in vitro* with the peptide pool for a longer time period (up to 10 days) and re-analyzed.

#### 9.1.2 Deconvolution of epitopes in follow-up *ex vivo* IFN-γ ELISPOT assays

Once an IFN-γ ELISPOT response elicited by an overlapping or epitope peptide pool is observed (defined as at least 55 spot forming units / $10^6$ PBMC or increased at least 3 times over baseline), the particular immunogenic peptide eliciting this response will be identified by de-convoluting the peptide pool into sub-pools based on the immunizing peptides and repeating the *ex vivo* IFN-γ ELISPOT assays.

In some cases, it may be necessary to synthesize additional peptides to precisely characterize the stimulating epitope.

### 9.1.3 Additional assays to be conducted on a case-by case basis for appropriate samples

- The entire 15-17mer pool or sub-pools will be used as stimulating peptides for intracellular cytokine staining assays to identify and quantify antigen-specific $CD4^+$, $CD8^+$, central memory and effector memory populations.
- Similarly, these pools will be used to evaluate the pattern of cytokines secreted by these cells to determine the $T_H1$ vs $T_H2$ phenotype.
- Fluorescent-labeled tetramers may be prepared for direct flow-cytmoetric characterization of responding CD8+ T cells to directly track and measure the size of T cell populations responding to neoantigens.
- If a melanoma cell line is successfully established from a responding participant and the activating epitope can be identified, T-cell cytotoxicity assays will be conducted using the mutant and corresponding wild type peptide reactive T cells against the autologous tumor cell line.
- Single cell RNA-Seq analysis from pre- and post-treatment tumor samples to assess the activation states of T cells and other immune cells.
- Single cell sequencing of both TCR Vα and Vβ subunits in serially collected peripheral blood and tumor samples collected before and after Nivolumab and vaccination, to characterize the global changes in TCR repertoire following vaccination as well as to track individual T cell clones over time in relation to vaccination.

### 9.1.4 Immunohistochemistry

Immunohistochemistry of tumor samples will be conducted to assess PD-L1 expression by tumor cells and tumor infiltrating immune cells and to quantify immune infiltrates such as CD4+, CD8+, MDSC, and Treg infiltrating populations.

## 9.2 Future Analyses

We may amend this protocol at a future date should we decide to conduct additional analyses utilizing future-generated samples from study participants (e.g. additional surgeries and autopsy tissue). Patients consenting to this trial agree to allow access to these future-generated samples.

## 10. STUDY CALENDAR

| Procedure[1] | Screen[2] | Pre-Treatment[3] | Pre-Vaccination Treatment | | | | Vaccination Treatment | | | | | | | | | Post Vaccination | | Follow Up[14] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Week* | N/A | | 0 | 4 | 8 | 10 | 12 | 13 | 15 | 16 | 18 | 19 | 20 | 21 | 22 | 24 | Q4 weeks | |
| Informed Consent | X | | | | | | | | | | | | | | | | | |
| Med Hx | X | | | | | | | | | | | | | | | | | |
| Concomitant Medication | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| PE/Vitals/Pulse Ox | X | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| ECOG PS | X | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Staging scans[3] | X | | | | | | X | | | | | | | | | X | | X |
| CBC w/ diff | X | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Serum Chem[4] | X | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| 12-lead EKG | X | | | | | | | | | | | | | | | | | |
| Pregnancy Testing[5] | X | | X | X | | X | X | | X | | X | | | | | | | |
| Thyroid Stimulating Hormone[6] | X | | | | | | | | | | | | | | | | | |
| Infectious Panel[7] | X | | | | | | | | | | | | | | | | | |
| Urinalysis | X | | | | | | | | | | | | | | | | | |
| Blood draw for vaccine manufacturing[8] | X | | | | | | | | | | | | | | | | | |
| HLA class I and II typing[9] | | X | | | | | | | | | | | | | | | | |
| Nivolumab administration[10] | | | X | X | X | | X | | | X | | | X | | | X | X | X |
| Ipilimumab administration* | | | | | | | *please see Vaccine + Ipilimumab administration and vaccine-related assessments table below | | | | | | | | | | | |
| Neoantigen vaccine Administration* | | | | | | | | | | | | | | | | | | |
| Tumor biopsy[11,*] | | X[11] | | | | | | | | | | | | | | | X[11] | |
| Leukapheresis[12,*] | | X[12] | | | | | | | | | | | | | | | X[12] | |
| Blood draw for immune response monitoring[13,*] | | | | X[13] | | X[13] | | | | | | | | | | X | X | |
| Vaccine biopsy* | | | | | | | | | | | | | | | | | | |
| Adverse Events | | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |

1 All study procedures should be performed within +/- 3 day window unless specified otherwise

2 Screening assessments must be completed within 30 days of, and prior to the first dose of Nivolumab on Week 0 Day 1. An additional 15 days is allowed for a repeat biopsy, if needed, to obtain adequate tissue for sequencing. This initial biopsy sample should have approximately 30% tumor cellularity and a minimum of 5-8 cores or 100 mm3 in order for sequencing to be completed

3 +/- 14 day window for staging scans. CT or MRI of other areas of disease (e.g. neck) should be obtained as clinically indicated. If IV contrast is contraindicated, a non-contrast CT and MRI may be used to evaluate sites of disease where a CT without contrast is not adequate. The imaging modality should remain consistent throughout the study and should be the same modality used at Screening. Photographs should be taken of one non-biopsied representative skin lesion, if feasible, on the same day that radiographic scans are obtained. The 100-day CT/MRI can be obtained within a ±30-day window. If a patient has undergone a procedure (CT or MRI) that is within the allotted timeframe for evaluation, then the patient does not have to undergo an additional procedure. Clinical assessments must be based on RECIST v1.1. After the week 24 timepoint, staging scans should be performed every 12 weeks

4 Glucose, urea nitrogen, creatinine, sodium, potassium, calcium, total and direct bilirubin, AST, ALT, alkaline phosphatase

5 Women of childbearing potential (WOCBP) must have a negative serum or urine β-HCG pregnancy test; Urine or serum pregnancy test should be at screening, within 24 hours prior to receiving the first dose of study medication and then every 4 weeks while on treatment.

6 Thyroid stimulating hormone with reflex T4

7 HBsAg, HBcAb, HCV, HIV I/II Ab

8 At least two 10mL EDTA tubes will be collected for vaccine production purposes. The tubes will be kept frozen at the CRL until the day of shipment for DNA sequencing

9 Molecular HLA typing to be performed by Tissue Typing Laboratory (HLA-A, HLA-B, HLA-C, and HLA-DR)

10 Nivolumab treatment will start within 2 weeks of tumor biopsy and once histopathology report confirms melanoma and the biopsy sample is confirmed to have adequate tumor content for DNA sequencing. Nivolumab dosing every 4 weeks (+/- 3 days) until disease progression or intolerable toxicity (minimum 26 days between dosing). Nivolumab will be administered until intolerable toxicity, disease progression (unless treatment beyond progression is deemed to be beneficial for the patient) for a maximum duration of 24 months

11 Tumor biopsies will be collected in patients with unresectable melanoma who have metastatic sites accessible to serial tumor biopsies within two weeks prior to Nivolumab treatment initiation. Tumor biopsy must contain ≥30% of tumor cells .
At a minimum, 5 core biopsies or a surgical tumor specimen of 100mm3 will be obtained. Skin punch biopsies of a skin lesion are also allowed. The collection of tumor biopsies for research purposes do not apply for patients with stage III melanoma

12 Leukapheresis procedure will be performed within 14 days before starting Nivolumab
At each Leukapheresis collection timepoint 2x10 mL EDTA tubes will be collected. These tubes should be dropped off at the IAL upon collection
If Leukapheresis cannot be performed a total of 200mL of blood should be drawn. If starting of vaccine administration gets delayed, week 20 leukapheresis will be collected before vaccination timepoint #4.
A Leukapheresis during the Post-Vaccination treatment/ Followu up period may be performed

13 Unless otherwise specified, 80 ml of blood will be drawn for immune monitoring.

14 Active Follow-Up: Participants removed from active study treatment will be followed at the following schedule until initiation of a new therapy for recurrent disease, withdrawal of consent, or death (whichever comes first):
- At least every 3 months (+/- 2 week) for the first 2 years after initiation of study therapy,
- At least every 4 months (+/- 2 weeks) for the third year after study therapy initiation,
- At least every 6 months (+/- 4 weeks) thereafter, until 5 years have passed since study therapy initiation

Long-Term Follow-Up: Participants removed from active follow-up for progression/initiation of a new therapy will enter long-term follow-up, where they will be followed via medical record review until death for survival. Updates to the CRFs are to be made approximately every six months

• Please see table below

## Neoantigen vaccine + Ipilimumab administration calendar and assessments related to vaccination timepoints

| Procedure[1] | Pre-vaccine assessments | Neoantigen Vaccine Dose #1[c] | Post vaccine dose 1 visit | Neoantigen Vaccine Dose #2[c] | Post vaccine dose 2 visit | Neoantigen Vaccine Dose #3[c] | Post vaccine dose 3 visit | Neoantigen Vaccine Dose #4[c] | Post vaccine dose 4 visit |
|---|---|---|---|---|---|---|---|---|---|
| PE/vitals Concomitant Medications ECOG, CBC w/ diff, Serum Chemistry, | | X | X | X | X | X | X | X | X |
| Leukapheresis | X[a] | | | | | | | X[a] | |
| Tumor biopsy | X[b] | | | | | | | X[b] | |
| Blood draw for immune response monitoring | | | X[d] | X[d] | | X[d] | | | X[d] |
| Neoantigen vaccine administration | | X | | | | X | | X | |
| Ipilimlumab administration | | X | | | | X | | X | |
| Vaccine site skin biopsy | | | | | | X[e] | | | |

a Leukapheresis procedure will be performed at the following timepoints:
- within 14 days before first vaccination timepoint #1
- within 7 days before vaccination timepoint #4

At each Leukapheresis collection timepoint 2x10 mL EDTA tubes will be collected. These tubes should be dropped off at the IAL upon collection
NOTE: If Leukapheresis cannot be performed a total of 200mL of blood should be drawn. If starting of vaccine administration gets delayed, week 20 leukapheresis will be collected before vaccination timepoint #4.

b Tumor biopsies will be collected at the following timepoints:
- within two weeks before starting the neoantigen vaccine Dose #1
- within two weeks prior to neoantigen vaccine Dose #4
- at the time of progression

At a minimum, 5 core biopsies or a surgical tumor specimen of 100mm3 will be obtained. Skin punch biopsies of a skin lesion are also allowed

c Expected neoantigen vaccine administration timepoints (see below). If there is a delay in vaccine preparation, Dose #1 should be administered as soon as the neoantigen vaccine is available. Doses #2, #3, and #4 should be scheduled every 3 weeks after Dose #1:
- neoantigen vaccine Dose #1: Week 12
- neoantigen vaccine Dose #2: Week 15
- neoantigen vaccine Dose #3: Week 18
- neoantigen vaccine Dose #4: Week 21.

If neoantigen vaccine administration coincides with nivolumab administration, neoantigen vaccine + Ipilimumab will be administered before Nivolumab

d Unless otherwise specified, 80 ml of blood will be drawn for immune monitoring. Blood collections will be performed at the following timepoints:
- one week (+/- 3 days) after neoantigen vaccine Dose #1
- before neoantigen vaccine Dose #2
- before neoantigen vaccine Dose #3
- within 3 weeks (+/- 3 days) after neoantigen vaccine Dose #4
- before each nivolumab administration during the post vaccination period

e Skin biopsies near vaccine injection sites (two 5 mm punch biopsies) should be performed at the following timepoints:
- prior to neoantigen vaccine Dose #3,
- between 48-72 hours after neoantigen vaccine Dose #3

Additionally, if a different vaccine site appears to clinically have a higher degree of inflammation, additional punch biopsy (two 5mm) of that site may be taken.

## 11. MEASUREMENT OF EFFECT

Although response is not the primary endpoint of this trial, participants with measurable disease will be assessed by standard criteria. For the purposes of this study, participants should be re-evaluated every 12 weeks. In addition to a baseline scan, confirmatory scans will also be obtained 4 weeks following initial documentation of an objective response.

### 11.1 Antitumor Effect – Solid Tumors

For the purposes of this study, participants should be re-evaluated for response every 8 weeks. In addition to a baseline scan, confirmatory scans should also be obtained 4 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

#### 11.1.1 Definitions

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

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

#### 11.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm by chest x-ray or ≥10 mm with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable

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

the short axis will be measured and followed.

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

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same participant, these are preferred for selection as target lesions.

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

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

### 11.1.3 Methods for Evaluation of Disease

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

The same method of assessment and the same technique should be used to characterize

each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

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

Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung; however, CT is preferable.

Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT thickness is 5mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size of a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

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

(a) Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
(b) No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

(c) FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

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

PET-CT. At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

### 11.1.4 Response Criteria

#### 11.1.4.1 Evaluation of Target Lesions

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

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

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

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

#### 11.1.4.2 Evaluation of Non-Target Lesions

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

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.
Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

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

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

#### 11.1.4.3 Evaluation of New Lesions

The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

#### 11.1.4.4 Evaluation of Best Overall Response

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

**For Participants with Measurable Disease (*i.e.*, Target Disease)**

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

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Participants with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration.*" Every effort should be made to document the objective progression even after discontinuation of treatment.

**For Participants with Non-Measurable Disease (*i.e.*, Non-Target Disease)**

| Non-Target Lesions | New Lesions | Overall Response |
|---|---|---|
| CR | No | CR |
| Non-CR/non-PD | No | Non-CR/non-PD* |
| Not all evaluated | No | not evaluated |
| Unequivocal PD | Yes or No | PD |
| Any | Yes | PD |

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

### 11.1.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started, or death due to any cause. Participants without events reported are censored at the last disease evaluation).

Duration of overall complete response: The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented, or death due to any cause. Participants without events reported are censored at the last disease evaluation.

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

### 11.1.6 Progression-Free Survival

Overall Survival: Overall Survival (OS) is defined as the time from randomization (or registration) to death due to any cause, or censored at date last known alive.

Progression-Free Survival: Progression-Free Survival (PFS) is defined as the time from randomization (or registration) to the earlier of progression or death due to any cause. Participants alive without disease progression are censored at date of last disease evaluation.

Time to Progression: Time to Progression (TTP) is defined as the time from randomization (or registration) to progression or censored at date of last disease evaluation for those without progression reported.

## 11.2 Other Response Parameters

Immune related Response Criteria

New end point definitions for trials of immunologic agents have been proposed based on novel patterns of clinical activity in malignant melanoma[98,99] and are described in detail in Appendix B. These alternative definitions allow time for immunologically mediated effectors to develop that may result in late tumor responses even after initial progression by RECIST. Also, in some patients, tumors necrosis and inflammation may increase tumor size radiographically prior to response. Changing the definitions of OR and PD may alter (increase) the number of patients achieving responses and the duration of PFS.

On a subject by subject basis, we would consider allowing study treatment to continue during initial progression up to the 12-16 weeks assessment to allow time for responses to be observed, if the patient is clinically stable, there is no deterioration in PS, and there is no need for

immediate additional treatment. While maintaining standard definitions of progression and response, we would allow new lesions and some progression beyond 20% increases in tumor measurements during the initial treatment period to allow time for responses to develop (these delayed tumor responses may be seen in 10-20% of melanoma patients who initially progress during the initial treatment cycles and evaluation). We do not have experience with response patterns with combination therapy nor in diseases other than melanoma. Please use standard response definitions as the primary end point in these studies.

Note that the proposed irRC may be incorporated as secondary end points to compare to standard criteria and evaluate alternative patterns of response in various disease setting and treatment regimens. A copy of the proposed criteria is presented in Appendix B.

Patients who demonstrate mixed responses, stable disease, or objective responses by standard RECIST following initial progression may be identified separately as "delayed SD, PR, or CR".

**Overall Risk/Benefit Assessment**

The unique immune-based mechanism of action is reflected in the clinical patterns of anti-cancer activity in some patients. Immune checkpoint blockade affects tumor cells indirectly, and measurable clinical effects emerge after the immunological effects. Tumor infiltration with lymphocytes and the associated inflammation (documented by biopsy in some subjects) is likely the cornerstone of the effect of immune checkpoint inhibition and can manifest in various patterns of clinical activity leading to tumor control. In some cases, response may be preceded by an apparent increase in initial tumor volume and/or the appearance of new lesions, which may be mistaken for tumor progression on radiological evaluations. Therefore, in subjects who are not experiencing rapid clinical deterioration, confirmation of progression is recommended, at the investigator's discretion, to better understand the prognosis as well as to avoid unnecessarily initiating potentially toxic alternative therapies in subjects who might be benefitting from treatment. Immune-related (ir) response criteria were developed based on these observations to systematically categorize novel patterns of clinical activity and are currently being prospectively evaluated in clinical studies.

In metastatic diseases, stabilization is more common than response, and in some instances is associated with slow, steady decline in tumor burden over many months, sometimes improving to partial and/or complete responses. Thus, the immune-based mechanism of action of Ipilimumab results in durable disease control, sometimes with novel patterns of response, which contribute to its improvement in OS.

The immune-based mechanism of action is also reflected in the safety profile. The most common drug-related AEs are immune-mediated, consistent with the mechanism of action of the drug and generally medically manageable with topical and/or systemic immunosuppressants. As previously discussed, the immune-mediated adverse reactions primarily involve the GI tract, skin, liver, endocrine glands, and nervous system.

The early diagnosis of immune-mediated adverse reactions is important to initiate therapy and minimize complications. Immune-mediated adverse reactions are generally manageable using

symptomatic or immunosuppressive therapy as recommended through detailed diagnosis and management guidelines, as described fully in the current IB. The management guidelines for general immune-mediated adverse reactions and Ipilimumab-related GI toxicities, hepatotoxicity, endocrinopathy, and neuropathy are provided in the appendices of the current IB.

## 12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

### 12.1 Data Reporting

**12.1.1** Method

The Office of Data Quality (ODQ) will collect, manage, and perform quality checks on the data for this study.

**12.1.2** Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the Office of Data Quality (ODQ) in accordance with DF/HCC policies.

### 12.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of medical oncologists, research nurses, pharmacists and biostatisticians with direct experience in cancer clinical research. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

The DSMC will review each protocol up to four times a year with the frequency determined by the outcome of previous reviews. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

## 13. STATISTICAL CONSIDERATIONS

### 13.1 Study Design/Endpoints

The primary goal of this Phase I trial is to assess safety of the vaccine (neoantigen peptides and poly-ICLC) and locally-delivered Ipilimumab and determine the recommended maximum tolerated dose (MTD).

**Primary Endpoint: Rate of DLT(s)**

Adverse events classified as dose-limiting toxicities (DLT) and the assessment time for DLTs are summarized in Section 5.5. Five patients will be treated for the initial safety evaluation (Cohort 1). We have chosen dose escalation cohorts of 5 patients, rather than a more traditional 3+3 design, because the determination of whether to treat at a different dose in this trial is made over a longer period of time due to the time required for vaccine preparation and the extended period for safety evaluation. These larger cohort sizes help us avoid having to pause and then restart enrollment to a dosing cohort several months later.

If none or only 1 patient in Cohort 1 Dose Level 1 experiences a dose limiting toxicity (DLT) during the first 7 weeks of treatment of Cohort 1, 5 patients will be treated as Cohort 2 dose Level 2. If two or more patients in Cohort 1 experience a DLT during the first 7 weeks of treatment, then 5 patients will be treated as Cohort 2 Dose Level -1.

If none or only 1 patient experiences a DLT on Cohort 2 Dose Level 2, then Dose Level 2 will be the maximum tolerated dose (MTD) and an additional 10 patients will be treated at that dose level (Cohort 3 Dose Level 2) to increase the likelihood of detecting serious toxicities, to complete biologic correlative endpoints and to gain preliminary experience with clinical tumor activity. If two or more patients in Cohort 2 Dose Level 2 experience dose limiting toxicity (DLT), then Dose Level 1 will be the MTD and an additional 10 patients will be treated in an expansion cohort (Cohort # Dose Level 1) at this dose.

If none or only 1 patient experiences a DLT on Cohort 2 Dose Level -1, then Dose Level-1 will be the maximum tolerated dose (MTD) and an additional 10 patients will be treated in an expansion cohort (Cohort 3 Dose Level -1) at that dose level. If two or more patients in Cohort 2 Dose Level -1 experience dose limiting toxicity (DLT), then the study will be stopped.

The table below shows the probability of moving to the dose expansion phase (0 or 1 patients with DLT out of 5 patients at the MTD) for various possible values of the true, but unknown, toxicity rate.

| True rate of DLT (%) | Probability of Dose Expansion (%) |
|---|---|
| 10 | 92 |
| 20 | 74 |
| 30 | 53 |
| 40 | 34 |
| 50 | 19 |
| 60 | 9 |

If the true probability of DLT is 10% or less, then the probability of proceeding to dose expansion is at least 92%. The probability of dose expansion is less than 50% if the true rate of dose-limiting toxicities is 32% or greater.

**Minimum vaccination requirement to pass DLT observation period:** A minimum of 2 vaccinations and absence of DLTs are required for a patient to complete the 7-week DLT observation period successfully.

**Expansion Cohort:** When the recommended dose has been determined, an expansion cohort of 10 patients will be treated to assess the performance of the vaccine and to gain additional information about toxicities. Because of the long lead time between patient registration and first vaccination, patients in the expansion cohort may be registered to the trial while safety in the initial cohort(s) is being assessed. Patients in the expansion cohort will not be treated until the safety evaluation of the initial cohorts is complete.

**Stopping rule for Expansion Cohort:** If four or more patients in the expansion cohort experience dose limiting toxicity (DLT) during the first 7 weeks of treatment, then the dose expansion cohort will be determined to have unacceptable toxicity and the study will be stopped prior to completion of enrollment of the expansion cohort. The table below summarizes the probability of observing 4 or more DLTs within the 10 patients of the expansion cohort for varying underlying rates of toxicity.

| | | True, but unknown, toxicity rate | | | | |
|---|---|---|---|---|---|---|
| | 5% | 10% | 20% | 30% | 36% | 40% |
| Probability of observing 4 or more patients with DLT | 0.001 | 0.013 | 0.121 | 0.350 | 0.513 | 0.618 |

If the true probability of DLT in the expansion cohort is higher than 35%, then the probability is at least 50% of observing 4 or more patients with DLT within the 10 patients of the expansion cohort.

**Total Sample Size:** The final sample size for this trial will depend upon the number of dose-escalation cohorts; however, the number of patients treated will be between 4 and 20. The minimum number of patients to be treated is based on observing DLTs after the first dose of Neoantigen Vaccine in the first two patients of Cohort 1 and similarly in Cohort 2. The maximum would occur if two dose-escalation cohorts were treated, with an additional 10 patients at the MTD.

**Timelines:** We expect to enroll one patient per month. We expect FPFV to occur by September 2020. LPFV is expected for July 2022 and overall duration of the study will be 4 years.

**Analysis of Secondary Endpoints**

Secondary endpoint in this clinical trial will: (a) assess the induction of IFN-γ T-cell response or tetramer staining to the assay peptides, and (b) to estimate rates of disease progression or recurrence (RECIST 1.1) depending on whether the patient is resected or has measurable disease.Analyses of the secondary endpoint will be based on 15 patients treated at the MTD.

The induction of IFN-γ T-cell response will be based on ELISPOT assessments taken prior to vaccine administration and at week 16. The proportion of patients who achieve more than 55 SFU/$10^6$ PBMC *or* 3 times their baseline level will be presented with a 90% exact binomial

confidence interval. Based on a cohort of size 15, the confidence interval will be no wider than 0.46.

The proportions of patients with disease progression or recurrence, according to melanoma stage, will be presented with 90% exact binomial confidence intervals.

**Analysis of Correlative Endpoints**

Longitudinal analyses of PBMC immune response kinetics to the peptide pool and to individual epitopes will be presented graphically and descriptively at each time point. Changes in the magnitude of the response relative to pre-treatment at several different time points after vaccination will be summarized descriptively. Changes in response between pre-treatment and 16 weeks post-vaccination, which is the time of the primary immunologic endpoint, will be assessed using the Wilcoxon signed-rank test.

Immune studies of T cell subpopulations ($CD4^+$, $CD8^+$, central/effector memory cells, Th1/Th2 cells, T-regs, etc.), myeloid-derived suppressor cells (MDSC), CTL response, and T cell activation status will be summarized using descriptive statistics.

## 14. PUBLICATION PLAN

This study is intended for publication, even if terminated prematurely. Publication may include any or all of the following: posting of a synopsis online, abstract and/or presentation at a scientific conference, or publication of a full manuscript. The Sponsor/Principal Investigator will work with all authors to submit a manuscript describing study results within 24 months after the last data become available, which may take up to several months after the last participant visit.

This is a NIH-funded research. De-identified genomic information obtained from participants on this study will be shared with NIH's dbGaP.

**REFERENCES**

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA: a cancer journal for clinicians 2014;64:9-29.
2. Chapman PB, Hauschild A, Robert C, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. The New England journal of medicine 2011;364:2507-16.
3. Flaherty KT, Puzanov I, Kim KB, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. The New England journal of medicine 2010;363:809-19.
4. Sosman JA, Kim KB, Schuchter L, et al. Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. The New England journal of medicine 2012;366:707-14.
5. Flaherty KT, Infante JR, Daud A, et al. Combined BRAF and MEK Inhibition in Melanoma with BRAF V600 Mutations. The New England journal of medicine 2012.
6. Flaherty KT, Robert C, Hersey P, et al. Improved Survival with MEK Inhibition in BRAF-Mutated Melanoma. The New England journal of medicine 2012.
7. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. The New England journal of medicine 2010;363:711-23.
8. Robert C, Thomas L, Bondarenko I, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. The New England journal of medicine 2011;364:2517-26.
9. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. The New England journal of medicine 2012;366:2455-65.
10. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. The New England journal of medicine 2012;366:2443-54.
11. Lundegaard C, Lund O, Nielsen M. Prediction of epitopes using neural network based methods. J Immunol Methods 2011;374:26-34.
12. Sidney J, Southwood S, Oseroff C, del Guercio MF, Sette A, Grey HM. Measurement of MHC/peptide interactions by gel filtration. Current protocols in immunology / edited by John E Coligan [et al] 2001;Chapter 18:Unit 18 3.
13. Fritsch EF, Rajasagi M, Ott PA, Brusic V, Hacohen N, Wu CJ. HLA-binding properties of tumor neoepitopes in humans. Cancer immunology research 2014;2:522-9.
14. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science 2011;331:1565-70.
15. Welters MJ, Kenter GG, Piersma SJ, et al. Induction of tumor-specific CD4+ and CD8+ T-cell immunity in cervical cancer patients by a human papillomavirus type 16 E6 and E7 long peptides vaccine. Clinical cancer research : an official journal of the American Association for Cancer Research 2008;14:178-87.
16. Kenter GG, Welters MJ, Valentijn AR, et al. Phase I immunotherapeutic trial with long peptides spanning the E6 and E7 sequences of high-risk human papillomavirus 16 in end-stage cervical cancer patients shows low toxicity and robust immunogenicity. Clinical cancer research : an official journal of the American Association for Cancer Research 2008;14:169-77.
17. Caskey M, Lefebvre F, Filali-Mouhim A, et al. Synthetic double-stranded RNA induces innate immune responses similar to a live viral vaccine in humans. The Journal of experimental medicine 2011;208:2357-66.
18. Sabbatini P, Tsuji T, Ferran L, et al. Phase I trial of overlapping long peptides from a tumor self-antigen and poly-ICLC shows rapid induction of integrated immune response in ovarian cancer patients. Clin Cancer Res 2012;18:6497-508.

19. Wood LD, Parsons DW, Jones S, et al. The genomic landscapes of human breast and colorectal cancers. Science 2007;318:1108-13.
20. Hacohen N FE, Carter TA, Lander ES, and Wu CJ. Getting Personal with Neoantigen-Based Therapeutic Cancer Vaccines Cancer Immunol Res 2013;Published OnlineFirst April 7, 2013; doi:10.1158/2326-6066.CIR-13-0022
21. Mandelboim O, Vadai E, Fridkin M, et al. Regression of established murine carcinoma metastases following vaccination with tumour-associated antigen peptides. Nat Med 1995;1:1179-83.
22. Zwaveling S, Ferreira Mota SC, Nouta J, et al. Established human papillomavirus type 16-expressing tumors are effectively eradicated following vaccination with long peptides. J Immunol 2002;169:350-8.
23. Castle JC, Kreiter S, Diekmann J, et al. Exploiting the mutanome for tumor vaccination. Cancer Res 2012;72:1081-91.
24. Dubey P, Hendrickson RC, Meredith SC, et al. The immunodominant antigen of an ultraviolet-induced regressor tumor is generated by a somatic point mutation in the DEAD box helicase p68. The Journal of experimental medicine 1997;185:695-705.
25. Sensi M, Anichini A. Unique tumor antigens: evidence for immune control of genome integrity and immunogenic targets for T cell-mediated patient-specific immunotherapy. Clinical cancer research : an official journal of the American Association for Cancer Research 2006;12:5023-32.
26. Matsushita H, Vesely MD, Koboldt DC, et al. Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. Nature 2012;482:400-4.
27. Wolfel T, Hauer M, Schneider J, et al. A p16INK4a-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. Science 1995;269:1281-4.
28. Coulie PG, Lehmann F, Lethe B, et al. A mutated intron sequence codes for an antigenic peptide recognized by cytolytic T lymphocytes on a human melanoma. Proceedings of the National Academy of Sciences of the United States of America 1995;92:7976-80.
29. Lennerz V, Fatho M, Gentilini C, et al. The response of autologous T cells to a human melanoma is dominated by mutated neoantigens. Proceedings of the National Academy of Sciences of the United States of America 2005;102:16013-8.
30. van Rooij N, van Buuren MM, Philips D, et al. Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2013;31:e439-42.
31. Tran E, Turcotte S, Gros A, et al. Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer. Science 2014;344:641-5.
32. Baurain JF, Colau D, van Baren N, et al. High frequency of autologous anti-melanoma CTL directed against an antigen generated by a point mutation in a new helicase gene. J Immunol 2000;164:6057-66.
33. Chiari R, Foury F, De Plaen E, Baurain JF, Thonnard J, Coulie PG. Two antigens recognized by autologous cytolytic T lymphocytes on a melanoma result from a single point mutation in an essential housekeeping gene. Cancer Res 1999;59:5785-92.
34. Huang J, El-Gamil M, Dudley ME, Li YF, Rosenberg SA, Robbins PF. T cells associated with tumor regression recognize frameshifted products of the CDKN2A tumor suppressor gene locus and a mutated HLA class I gene product. J Immunol 2004;172:6057-64.

35. Karanikas V, Colau D, Baurain JF, et al. High frequency of cytolytic T lymphocytes directed against a tumor-specific mutated antigen detectable with HLA tetramers in the blood of a lung carcinoma patient with long survival. Cancer Res 2001;61:3718-24.
36. Zorn E, Hercend T. A natural cytotoxic T cell response in a spontaneously regressing human melanoma targets a neoantigen resulting from a somatic point mutation. European journal of immunology 1999;29:592-601.
37. Brown SD, Warren RL, Gibb EA, et al. Neo-antigens predicted by tumor genome meta-analysis correlate with increased patient survival. Genome research 2014;24:743-50.
38. Baskar S, Kobrin CB, Kwak LW. Autologous lymphoma vaccines induce human T cell responses against multiple, unique epitopes. J Clin Invest 2004;113:1498-510.
39. Schuster SJ, Neelapu SS, Gause BL, et al. Vaccination with patient-specific tumor-derived antigen in first remission improves disease-free survival in follicular lymphoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2011;29:2787-94.
40. Kenter GG, Welters MJ, Valentijn AR, et al. Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. The New England journal of medicine 2009;361:1838-47.
41. Sampson JH, Aldape KD, Archer GE, et al. Greater chemotherapy-induced lymphopenia enhances tumor-specific immune responses that eliminate EGFRvIII-expressing tumor cells in patients with glioblastoma. Neuro-oncology 2011;13:324-33.
42. Sampson JH, Heimberger AB, Archer GE, et al. Immunologic escape after prolonged progression-free survival with epidermal growth factor receptor variant III peptide vaccination in patients with newly diagnosed glioblastoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2010;28:4722-9.
43. Carreno BM, Magrini V, Becker-Hapak M, et al. Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. Science 2015;348:803-8.
44. Morton DL, Hsueh EC, Essner R, et al. Prolonged survival of patients receiving active immunotherapy with Canvaxin therapeutic polyvalent vaccine after complete resection of melanoma metastatic to regional lymph nodes. Annals of surgery 2002;236:438-48; discussion 48-9.
45. Soiffer R, Hodi FS, Haluska F, et al. Vaccination with irradiated, autologous melanoma cells engineered to secrete granulocyte-macrophage colony-stimulating factor by adenoviral-mediated gene transfer augments antitumor immunity in patients with metastatic melanoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2003;21:3343-50.
46. Vaishampayan U, Abrams J, Darrah D, Jones V, Mitchell MS. Active immunotherapy of metastatic melanoma with allogeneic melanoma lysates and interferon alpha. Clinical cancer research : an official journal of the American Association for Cancer Research 2002;8:3696-701.
47. Testori A, Richards J, Whitman E, et al. Phase III comparison of vitespen, an autologous tumor-derived heat shock protein gp96 peptide complex vaccine, with physician's choice of treatment for stage IV melanoma: the C-100-21 Study Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2008;26:955-62.
48. Kawai T, Akira S. TLR signaling. Seminars in immunology 2007;19:24-32.
49. Adams S. Toll-like receptor agonists in cancer therapy. Immunotherapy 2009;1:949-64.

50. Bogunovic D, Manches O, Godefroy E, et al. TLR4 engagement during TLR3-induced proinflammatory signaling in dendritic cells promotes IL-10-mediated suppression of antitumor immunity. Cancer Res 2011;71:5467-76.
51. Stahl-Hennig C, Eisenblatter M, Jasny E, et al. Synthetic double-stranded RNAs are adjuvants for the induction of T helper 1 and humoral immune responses to human papillomavirus in rhesus macaques. PLoS pathogens 2009;5:e1000373.
52. Boscardin SB, Hafalla JC, Masilamani RF, et al. Antigen targeting to dendritic cells elicits long-lived T cell help for antibody responses. The Journal of experimental medicine 2006;203:599-606.
53. Soares H, Waechter H, Glaichenhaus N, et al. A subset of dendritic cells induces CD4+ T cells to produce IFN-gamma by an IL-12-independent but CD70-dependent mechanism in vivo. The Journal of experimental medicine 2007;204:1095-106.
54. Trumpfheller C, Caskey M, Nchinda G, et al. The microbial mimic poly IC induces durable and protective CD4+ T cell immunity together with a dendritic cell targeted vaccine. Proc Natl Acad Sci U S A 2008;105:2574-9.
55. Trumpfheller C, Finke JS, Lopez CB, et al. Intensified and protective CD4+ T cell immunity in mice with anti-dendritic cell HIV gag fusion antibody vaccine. The Journal of experimental medicine 2006;203:607-17.
56. Salem ML, Kadima AN, Cole DJ, Gillanders WE. Defining the antigen-specific T-cell response to vaccination and poly(I:C)/TLR3 signaling: evidence of enhanced primary and memory CD8 T-cell responses and antitumor immunity. J Immunother 2005;28:220-8.
57. Tough DF, Borrow P, Sprent J. Induction of bystander T cell proliferation by viruses and type I interferon in vivo. Science 1996;272:1947-50.
58. Zhu X, Nishimura F, Sasaki K, et al. Toll like receptor-3 ligand poly-ICLC promotes the efficacy of peripheral vaccinations with tumor antigen-derived peptide epitopes in murine CNS tumor models. Journal of translational medicine 2007;5:10.
59. Gaucher D, Therrien R, Kettaf N, et al. Yellow fever vaccine induces integrated multilineage and polyfunctional immune responses. The Journal of experimental medicine 2008;205:3119-31.
60. Okada H, Kalinski P, Ueda R, et al. Induction of CD8+ T-cell responses against novel glioma-associated antigen peptides and clinical activity by vaccinations with {alpha}-type 1 polarized dendritic cells and polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose in patients with recurrent malignant glioma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2011;29:330-6.
61. Robinson RA, DeVita VT, Levy HB, Baron S, Hubbard SP, Levine AS. A phase I-II trial of multiple-dose polyriboinosic-polyribocytidylic acid in patieonts with leukemia or solid tumors. Journal of the National Cancer Institute 1976;57:599-602.
62. Aucouturier J, Dupuis L, Deville S, Ascarateil S, Ganne V. Montanide ISA 720 and 51: a new generation of water in oil emulsions as adjuvants for human vaccines. Expert review of vaccines 2002;1:111-8.
63. Rachel Lubong Sabado ACP, Sacha Gnjatic, Farah Hasan, Meredith Spadaccia, Bike Su Oner, Hanqing Dong, Rose Marie Holman, Isabelita Vengco, Caroline Muren, Crystal Escano, Ethel Yepes, Claire Stein, Achim A Jungbluth, Linda S. Pan, Ralph Rudolph Venhaus, Andres M. Salazar, Patrick Alexander Ott, Nina Bhardwaj. Poly-ICLC as an adjuvant for NY-ESO-1 protein vaccination with or without Montanide ISA-51 VG in patients with melanoma.

Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2016;33:suppl; abstr e14034.
64. IB BMSR. BMS-734016 (MDX-010) Comparative Tissue-Binding Study with Mouse, Rat, Rabbit, Monkey and Human Tissues (Study DS05067), Report2005.
65. IB BMSR. Study SBi 0919-128: A 2-week toxicity study of human anti-CTLA4 administered by intravenous injection to cynomologous monkeys.2000.
66. IB BMSR. Study SBi 0992-128: An immunogenic study following combined intravenous/intramuscular administration of MAb10D1/HBsAg, respectively, to cynomologous monkeys.2001.
67. IB BMSDR. Covance Study No. 7114-100: 14 day intravenous toxicity study with mAB 10D1 in cynomologous monkeys.2000.
68. Document BMS. Study No. DS0604: BMS-663513 and BMS-734016 (MDX-010): One-month intravenous combination toxicity in monkeys2007.
69. IB BMSR. Study SBi 1416-128: A repeat dose toxicology and effficacy study MDX-010 or MDX-010-HYB in combination with HBsAg and SKMel vaccines in cynomologous monkeys2003.
70. IB BMSDR. Repeated-dose (q3d x 3, 14-day) toxiciyt study of anti-CTLA4/10D1 administered via intravenous injection to cynomologous monkeys (Study 126-002). Report Redfield Laboratories2000.
71. Document BMS. Study 01-3460: MDX-CTLA4: A 6-Month intravenous toxicology study in cynomologous monkeys2002.
72. IB BMSDR. Cross-reactivity study of fluoresceinated human monoclonal antibody 10D1 with normal human tissues (Study IM578) Report Pathology Associates International 2000.
73. IB BMSDR. Cross-reactivity study of fluoresceinated MDX-010 with limited normal human tissues (Study IM933), Report Pathology Associates International2003.
74. IB BMSDR. Ipilimumab (BMS-734016): Intravenous study of pre- and postnatal development in cynomologous monkeys with a 6-month postnatal evaluation.2011.
75. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363:711-23.
76. van den Eertwegh AJ, Versluis J, van den Berg HP, et al. Combined immunotherapy with granulocyte-macrophage colony-stimulating factor-transduced allogeneic prostate cancer cells and ipilimumab in patients with metastatic castration-resistant prostate cancer: a phase 1 dose-escalation trial. Lancet Oncol 2012;13:509-17.
77. Madan RA, Mohebtash M, Arlen PM, et al. Ipilimumab and a poxviral vaccine targeting prostate-specific antigen in metastatic castration-resistant prostate cancer: a phase 1 dose-escalation trial. Lancet Oncol 2012;13:501-8.
78. Le DT, Lutz E, Uram JN, et al. Evaluation of ipilimumab in combination with allogeneic pancreatic tumor cells transfected with a GM-CSF gene in previously treated pancreatic cancer. J Immunother 2013;36:382-9.
79. Sarnaik AA, Yu B, Yu D, et al. Extended dose ipilimumab with a peptide vaccine: immune correlates associated with clinical benefit in patients with resected high-risk stage IIIc/IV melanoma. Clin Cancer Res 2011;17:896-906.
80. Schadendorf D, Hodi FS, Robert C, et al. Pooled Analysis of Long-Term Survival Data From Phase II and Phase III Trials of Ipilimumab in Unresectable or Metastatic Melanoma.

Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2015;33:1889-94.
81. Weber JS, Kudchadkar RR, Yu B, et al. Safety, efficacy, and biomarkers of nivolumab with vaccine in ipilimumab-refractory or -naive melanoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2013;31:4311-8.
82. Robert C, Long GV, Brady B, et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med 2015;372:320-30.
83. Weber JS, D'Angelo SP, Minor D, et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. Lancet Oncol 2015;16:375-84.
84. Wolchok JD, Kluger H, Callahan MK, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med 2013;369:122-33.
85. Quezada SA, Peggs KS, Curran MA, Allison JP. CTLA4 blockade and GM-CSF combination immunotherapy alters the intratumor balance of effector and regulatory T cells. J Clin Invest 2006;116:1935-45.
86. Simpson TR, Li F, Montalvo-Ortiz W, et al. Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma. J Exp Med 2013;210:1695-710.
87. Son CH, Bae JH, Shin DY, et al. Ctla-4 blockade enhances antitumor immunity of intratumoral injection of immature dendritic cells into irradiated tumor in a mouse colon cancer model. J Immunother 2014;37:1-7.
88. Hodi FS, Butler M, Oble DA, et al. Immunologic and clinical effects of antibody blockade of cytotoxic T lymphocyte-associated antigen 4 in previously vaccinated cancer patients. Proc Natl Acad Sci USA 2008;105:3005-10.
89. Hodi FS, Mihm MC, Soiffer RJ, et al. Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. Proc Natl Acad Sci U S A 2003;100:4712-7.
90. Karyampudi L, Lamichhane P, Scheid AD, et al. Accumulation of Memory Precursor CD8 T Cells in Regressing Tumors Following Combination Therapy with Vaccine and Anti-PD-1 Antibody. Cancer Res 2014.
91. Hacohen N, Fritsch EF, Carter TA, Lander ES, Wu CJ. Getting personal with neoantigen-based therapeutic cancer vaccines. Cancer Immunology Research (Cancer Immunology at the Crossroads) 2013;1:11-5.
92. Heemskerk B, Kvistborg P, Schumacher TN. The cancer antigenome. EMBO J 2013;32:194-203.
93. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. Science 2015;348:69-74.
94. Tumeh PC, Harview CL, Yearley JH, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 2014;515:568-71.
95. Rizvi NA, Hellmann MD, Snyder A, et al. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 2015.
96. Ott PA, Hu Z, Keskin DB, et al. An immunogenic personal neoantigen vaccine for patients with melanoma. Nature 2017;547:217-21.
97. Robert C, Schachter J, Long GV, et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. N Engl J Med 2015;372:2521-32.

98. Hoos A, Eggermont AM, Janetzki S, et al. Improved endpoints for cancer immunotherapy trials. Journal of the National Cancer Institute 2010;102:1388-97.
99. Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clinical cancer research : an official journal of the American Association for Cancer Research 2009;15:7412-20.
100. Weber J, Mandala M, DelVecchio M, et al. Adjuvant Nivolumab versus Ipilimumab in Resected Stage III or IV Melanoma. N Engl J Med 2017; 377:1824-1835.
101. Ascierto PA, Del Vecchio M, Mandalá M, Gogas H, Arance AM, Dalle S, Cowey CL, et al. Adjuvant nivolumab versus ipilimumab in resected stage IIIB-C and stage IV melanoma (CheckMate 238): 4-year results from a multicentre, double-blind, randomised, controlled, phase 3 trial. Lancet Oncol. 2020 Nov;21(11):1465-1477.

**APPENDIX A PERFORMANCE STATUS CRITERIA**

| ECOG Performance Status Scale | | Karnofsky Performance Scale | |
|---|---|---|---|
| Grade | Descriptions | Percent | Description |
| 0 | Normal activity. Fully active, able to carry on all pre-disease performance without restriction. | 100 | Normal, no complaints, no evidence of disease. |
| | | 90 | Able to carry on normal activity; minor signs or symptoms of disease. |
| 1 | Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work). | 80 | Normal activity with effort; some signs or symptoms of disease. |
| | | 70 | Cares for self, unable to carry on normal activity or to do active work. |
| 2 | In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours. | 60 | Requires occasional assistance, but is able to care for most of his/her needs. |
| | | 50 | Requires considerable assistance and frequent medical care. |
| 3 | In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours. | 40 | Disabled, requires special care and assistance. |
| | | 30 | Severely disabled, hospitalization indicated. Death not imminent. |
| 4 | 100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair. | 20 | Very sick, hospitalization indicated. Death not imminent. |
| | | 10 | Moribund, fatal processes progressing rapidly. |
| 5 | Dead. | 0 | Dead. |

**APPENDIX B ADVERSE EVENT MANAGEMENT**

**Appendix B1:**

**Appendix B2:**

**Appendix B3:**

**Appendix B4:**

**Appendix B5:**

**Appendix B6:**

**Appendix B7:**

**Appendix B8:**

## APPENDIX C REGULATORY CONSIDERATIONS

Dana-Farber/Harvard Cancer Center (DF/HCC) follows the International Conference on Harmonisation Guidelines for Good Clinical Practice (ICH GCP) to the extent those guidelines reflect the regulations and guidance set forth by the Food and Drug Administration (FDA) regulations. Where the ICH GCP guidelines include recommendations or requirements that go beyond those set forth under the FDA regulations, DF/HCC may or may not choose to institute those additional recommendations or requirements.

The study will be conducted in compliance with the protocol. The protocol and any amendments and the subject informed consent will receive Institutional Review Board/Independent Ethics Committee (IRB/IEC) approval/favorable opinion prior to initiation of the study.

All potential serious breaches must be reported to the study PI, study sponsor (Dr. Patrick Ott, Md PhD), and DF/HCC IRB.

A serious breach is a breach of the conditions and principles of GCP in connection with the study or the protocol, which is likely to affect, to a significant degree, the safety or physical or mental integrity of the subjects of the study or the scientific value of the study.

Personnel involved in conducting this study will be qualified by education, training, and experience to perform their respective tasks. This study will not use the services of study personnel where sanctions have been invoked or where there has been scientific misconduct or fraud (e.g., loss of medical licensure, debarment).