NCI Protocol #: CITN-04

Local Protocol #: CITN-04

TITLE: A Phase II Pilot Trial of an Indoleamine2,3, dioxygenase-1 (IDO1) Inhibitor (INCB024360) Plus a Multipeptide Melanoma Vaccine (MELITAC 12.1) in Patients with Advanced Melanoma

(Advanced Melanoma – ICD-10053571)

Coordinating Center: Cancer Immunotherapy Trials Network, Fred Hutchinson Cancer Research Center

***Principal Investigator:** Craig Slingluff, Jr., M.D.
University of Virginia
434-924-1730
434-243-6844
Cls8h@virginia.edu

Co-Investigators: Mark S. Ernstoff, M.D.
Taussig Cancer Institute R-35
Cleveland Clinic Foundation
9500 Euclid Ave
Cleveland, OH 44195
216-444-0888
216-444-9464
ernstom@ccf.org

Brent Hanks, M.D.
Duke University
919-684-1995
919-684-1818
brent.hanks@duke.edu

Keith Delman, M.D.
Emory University
404-778-3303
kdelman@emory.edu

David Lawson, M.D.
Emory University
404-778-1900
dlawson@emory.edu

Participation in the protocol is limited to the institutions and investigators listed above.

Statistician:
Zoe Moodie, Ph.D.
Fred Hutchinson Cancer Research Center
1100 Fairview Ave. N., M2-200
Seattle, WA 98109-1024
206-667-7077
zoe@fhcrc.org

Study Coordinator: N/A

Responsible Research Nurse: N/A

Responsible Data Manager: N/A

NCI Supplied Agent(s): **N/A**

Non-NCI Supplied Agent(s)/Supplier(s):
Indoleamine 2,3, dioxygenase-1 (IDO1) inhibitor (INCB024360), NSC #766086, Incyte Corporation

MELITAC 12.1 vaccine, (12 Melanoma Peptides from MDP and CTA (12MP), NSC #728925, and Peptide-Tet, NSC #728927), University of Virginia

Montanide ISA-51 VG, NSC #737063/, SEPPIC

IND Sponsor: Fred Hutchinson Cancer Research Center, Cancer Immunotherapy Trials Network (CITN) – IND # 15691

Protocol Type / Version # / Version Date: Amendment 8/Version 1.0/ 27 Jan 2016

CANCER TRIALS SUPPORT UNIT (CTSU) ADDRESS AND CONTACT INFORMATION

To submit site registration documents:	**For patient enrollments:**	**Submit study data**
CTSU Regulatory Office 1818 Market Street, Suite 1100 Philadelphia, PA 19103 Phone – 1-866-651-CTSU Fax – 215-569-0206	Please refer to the patient enrollment section for instructions on using the OPEN system.	Data collection for this study will be done exclusively through Medidata Rave. Please see the data submission section of the protocol for further instructions. Do not submit study data or forms to the CTSU Data Operations. Do not copy the CTSU on data submission.
The **study protocol and all related forms and documents** must be downloaded from the protocol-specific Web page of the CTSU Member Web site located at https://www.ctsu.org. Sites must use the current form version and adhere to the instructions and submission schedule outlined in the protocol. CITN sites should follow procedures outlined in the protocol for Site registration, Patient Enrollment, Adverse Event Reporting, Data Submission (including ancillary studies), and Drug Procurement.		
For questions unrelated to patient eligibility, treatment, or data submission contact the CTSU Help Desk by phone or e-mail: CTSU General Information Line – 1-888-823-5923, or ctsucontact@westat.com. All calls and correspondence will be triaged to the appropriate CTSU representative.		
For detailed information on the regulatory and monitoring procedures for CTSU sites please review the CTSU Regulatory and Monitoring Procedures policy located on the CTSU members' website https://www.ctsu.org		
The CTSU Web site is located at https://www.ctsu.org		

SCHEMA

Cohort	INCB024360 Dosing	MELITAC 12.1 Dosing	Blood draws	Tumor biopsies including FNA
A Pts.enrolled >6 wks after failing anti-PD-1/PDL-1	300 mg po bid continuous for 98 days (3 months)	Intradermal/subcutaneous Days 21, 28, 35, 56, 77, 98 (Weeks 3, 4, 5, 8, 11, and 14)	Pretreatment day 0 and on days 21, 42, 98, and 112	Days 0, 21, 42, and (optional day 112) and (optional when coming off study including for progression)
B Pts.enrolled 2-6 wks after failing anti-PD-1/PDL-1	100 mg po bid continuous for 98 days (3 months)	Intradermal/subcutaneous Days 21, 28, 35, 56, 77, 98 (Weeks 3, 4, 5, 8, 11, and 14)	Pretreatment day 0 and on days 21, 42, 98, and 112	Days 0, 21, 42, and (optional day 112) and (optional when coming off study including for progression)
All other pts.	300 mg po bid	Intradermal/subcutaneous Days 21, 28, 35, 56, 77, 98 (Weeks 3, 4, 5, 8, 11, and 14)	Pretreatment day 0 and on days 21, 42, 98, and 112	Days 0, 21, 42, and (optional day 112) and (optional when coming off study including for progression

Abbreviations: bid, twice per day; FNA, fine-needle aspiration; po, orally; pre-tx, pretreatment; SC, subcutaneous
All other patients enrolled who have not been treated with anti-PD1/PDL-1 will receive 300 mg BID INCB024360

TABLE OF CONTENTS

1. OBJECTIVES

INCB024360 is an indoleamine 2,3-dioxygenase 1 (IDO1) inhibitor with the capacity to normalize serum kynurenine/tryptophan (Kyn/Trp) ratios. The goal of this trial is to determine alterations in the tumor microenvironment of melanoma, first using a regimen of INCB024360 that normalizes serum Kyn/Trp ratios and then by adding a multiple peptide melanoma vaccine to INCB024360 treatment. The primary endpoint will be changes in the concentration and number of CD8+ cells, as CD8+ T cell number has been shown to have the best correlation with survival [Erdag 2012], with each patient serving as their own control. If substantial changes at sites of tumor are detected (i.e., changes in CD8+ T cell infiltration) in response to INCB024360 alone or in combination with the vaccine, subsequent iterative trials will be developed to determine the individual contributions of each agent.

1.1 Primary Objectives

Objective 1: To determine the extent to which a regimen of INCB024360 that normalizes serum Kyn/Trp ratios alters the tumor microenvironment of melanoma, including determining the number and character of tumor-infiltrating lymphocytes as determined by examination of serial biopsies with immunohistochemistry (IHC) and gene signatures

Objective 2: To determine the extent to which continued INCB024360 treatment plus the addition of the multipeptide melanoma vaccine, MELITAC 12.1, further alters the tumor microenvironment of melanoma, including determining the number and character of tumor-infiltrating lymphocytes as determined by serial biopsies evaluating IHC and gene signatures

1.2 Secondary Objectives

Objective 3: To determine whether a regimen of INCB024360 that normalizes serum Kyn/Trp ratios plus MELITAC 12.1 vaccine changes the level or character of the vaccine-induced CD8+ and CD4+ T-cell immune responses as measured in peripheral blood, as compared to prior published experience

Objective 4: To evaluate the extent to which INCB024360 plus MELITAC 12.1 vaccine alters the number and character of peripheral blood mononuclear cell (PBMC) populations, including T and natural killer (NK) cells, as evaluated by multiparameter flow cytometry

Objective 5: To evaluate the extent to which INCB024360 plus MELITAC 12.1 vaccine alters the PBMC transcriptome

Objective 6: To assess the safety and tolerability of INCB024360 plus MELITAC 12.1 vaccine

Objective 7: To obtain preliminary data on the tumor response rate of INCB024360 plus MELITAC 12.1 vaccine by objective response rate (ORR), time to tumor progression, and overall survival

Objective 8: To associate any observed changes with the expression of IDO1 protein by IHC in tumor or tumor-infiltrating cells

2. BACKGROUND

2.1 Study Disease(s)

Melanoma represents only 6% of all skin cancer diagnoses, however, it accounts for about 85% of all skin cancer–related deaths [NCI 2012]. The number of new invasive cutaneous melanoma cases projected during 2010 was 68,000—a 23% increase from the 2004 prediction of 55,100 cases. In 2015, the lifetime risk of developing melanoma is estimated to increase to 1 in 50 [Cockerell 2012].

Existing therapy for melanoma consists of adequate surgical resection of the primary tumor and, in some circumstances, surgical resection of isolated metastases. Most of the patients on the current trial will have disseminated disease and be enrolled after failure of standard therapies. "Little consensus exists regarding standard chemotherapy for patients with metastatic melanoma, which most likely reflects the low level of activity of older FDA approved agents" [NCCN 2012]. In general, disseminated disease can be managed by systemic therapy, clinical trial or supportive care. According to NCCN Guidelines, "preferred regimens include ipilumumab, vemurafenib for patients with documented BRAF mutation, treatment in a clinical trial and high-dose IL-2" [NCCN 2012]. "Although approval of ipilumumab and vermurafenib has significantly altered the initial management of patients with stage IV melanoma, each agent has unique limitations" [NCCN 2012] and might not be appropriate for all patients. Other regimens for disseminated disease "include dacarbazine, temozolomide, imatinib for tumors with c-KIT mutations, dacarbazine- or temozolomide-based combination chemotherapy, or biochemotherapy (including cisplatin and vinblastine with or without IL-2, interferon alfa, paclitaxel as monotherapy or in combination with carboplatin [NCCN 2012]. These have demonstrated modest response rates under 20% in first-line and second-line settings."

Some of the patients on the current protocol might have in-transit metastases. According to the NCCN guidelines, "Treatment in the context of a clinical trial is the preferred option" [NCCN 2012].

2.2 CTEP and/or CIP IND Agent(s): N/A

2.3 Other Agents

Indoleamine 2,3-dioxygenase

IDO is an enzyme that catabolizes the essential amino acid tryptophan to kynurenine [Munn 2007; Katz 2008; Löb 2009; Soliman 2010] and IDO activation plays an essential role in the immunological tolerance between mother and fetus. In pregnant women, IDO activation decreases the concentration of tryptophan in the blood and elevates levels of kynurenine, which, in healthy pregnancies, increases with placental development. As a consequence, inducing IDO expression in the trophoblast suppresses T-cell activity against the fetus [Tatsumi 2000]. In murine studies, the administration of the IDO inhibitor 1-methyl-tryptophan caused allogeneic fetal rejection [Munn 1998]. Thus, IDO induction in the trophoblast and placenta is sufficient to inhibit the immune system of the mother and prevent rejection of the fetus.

In addition to its activation in pregnancy, IDO is also expressed by activated immune cells and inflammatory cells, including macrophages and dendritic cells. Expression of IDO dampens the immune responses [Brown 1991; Huang 2002; Mellor 2005; Schroecksnadel 2005]. Upregulation of IDO in inflammatory cells has been shown to provide potent immunosuppression in autoimmune disease, allergies, chronic inflammatory conditions, and chronic infection. Relevant to this protocol, IDO expression is also increased in macrophages and dendritic cells of melanoma metastases. Moreover, enhanced IDO expression in immune cells in the sentinel node is an independent prognostic factor [Speeckaert 2012].

IDO is also overexpressed by some cancer cells. Such overexpression leads to tryptophan depletion and kynurenine excess, leading to T-cell immunosuppression, which can prevent immune recognition and attack on malignant cells, and might afford a tumor-derived mechanism of immune escape [Liu 2009]. Because of this, IDO expression and enzymatic activity correlates strongly with prognosis in multiple cancers. In particular, IDO1 has been observed to be chronically activated and overexpressed in many cancers, including ovarian, colorectal, pancreatic, and prostate cancers, and melanoma. IDO expression and enzymatic activity correlates strongly with prognosis in multiple cancers [Liu 2009]. The expression of IDO1 correlates with more extensive disease and is an independent prognostic factor for reduced overall survival.

In one study, moderate to strong cytoplasmic IDO expression was present in all (15/15) melanoma lymph node metastases in patients with poor survival [Brody 2009]. In other tumor types, increased serum L-kynurenine concentrations predict a poor prognosis in patients with diffuse large B-cell lymphoma treated with R-CHOP (rituximab–cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy [Yoshikawa 2010]. In addition, increased tryptophan degradation and increased IDO activity led to increased Kyn/Trp ratio in patients with ovarian cancer when compared with healthy controls. Furthermore, higher stages of ovarian carcinoma were associated with a higher rate of tryptophan degradation, and Kyn/Trp ratios correlated strongly with concentrations of cytokine interleukin 6 (IL-6), soluble IL-2 receptor alpha, tumor

necrosis factor alpha (TNF-alpha) receptor, and the macrophage marker, neopterin [Schroecksnadel 2005].

Increased serum Kyn/Trp ratios have also been shown to correlate with immunological effects and disease progression in lung cancer and other cancers [Liu 2009]. For example, patients with lung cancer had significantly higher Kyn levels, lower Trp serum levels, and elevated Kyn/Trp ratio compared to normal controls (Table 2.3) [Suzuki 2010]. Although substantial overlap between normal and lung cancer values was demonstrated, patients with more advanced cancer tended to have higher abnormal values.

Table 2.3. Kyn and Trp Levels and Ratios in Patients with Lung Cancer vs Normal Controls

Variable	**Normal controls**	**Lung cancer**	***P***
Kyn serum levels	2.30±0.56 µM	2.82±1.17 µM	0.0036
Trp serum levels	71.1±11.8 µM	62.6±15.8 µM	0.0007
Kyn/Trp ratio	32.9±9	47.1±21.3	<0.0001

Abbreviations: Kyn, kynurenine; Trp, tryptophan.

2.3.1 INCB024360

The novel small-molecule inhibitor of IDO1, INCB024360, is the first IDO1 selective inhibitor to undergo assessment in a human clinical trial. INCB024360 is specific for IDO1 with potency in the nanomolar range (IC_{50}=7.1–12.7 nM) in both human tumor cells and dendritic cells, and reduces tryptophan-to-kynurenine conversion as assessed by biochemical and cellular assays [Koblish 2010; Liu 2010]. It is highly potent in reversing IDO1-mediated suppression of T-cell proliferation in a dose-dependent manner (EC_{50}=17.7 nM) with potency consistent with its inhibition of tryptophan-to-kynurenine conversion. It demonstrates high selectivity and does not inhibit other tryptophan-catabolizing proteins such as tryptophan 2,3-dioxygenase (TDO) or IDO2, nor does it influence tryptophan transporters. It has a greater than 1000-fold selectivity against a number of kinases, ion channels, receptors, and transporters, and does not significantly induce or inhibit cytochrome P450 enzymes. INCB024360 has an excellent preclinical pharmacokinetic profile with an oral bioavailability of 50% in rat and dog, with good tolerability and safety profiles in preclinical rodent and nonrodent studies of 28-day duration.

2.3.1.1 Animal Models

In preclinical murine models, INCB024360 inhibited IDO1 systemically in tumors and in tumor-draining lymph nodes with associated reduced tumor growth and enhanced lymphocyte activity. In mice, plasma Kyn levels decreased within 1 hour of oral INCB024360 administration and remained at minimal levels for as long as plasma levels of INCB024360 exceeded 1.2 µM (corresponding to the cellular IC_{90} value adjusted for plasma protein binding [~8 hours]). Consistent with specific inhibition of IDO1 by INCB024360, no reduction in plasma Kyn levels was observed in IDO1–genetically deficient mice, despite similar compound exposures.

Furthermore, during maximal suppression of IDO1 by INCB024360 in wild-type

mice, the plasma Kyn levels were similar to the baseline plasma levels in IDO1-deficient mice, demonstrating that oral administration of INCB024360 results in efficient and selective IDO1 inhibition in vivo.

Reduced levels of regulatory T cells (Tregs) were demonstrated in the syngeneic tumors in mice that were administered INCB024360, compared with tumors in control mice. As a single agent, INCB024360 inhibited the growth of CT26 colon tumors, which express high levels of endogenous IDO1 when grown in syngeneic BALB/c mice [Koblish 2010]. No effect was observed in tumor models using immunocompromised BALB/c nu/nu mice. Furthermore, the effect of chemotherapy appeared to be enhanced with INCB024360 in preclinical models: in CT26 tumor–bearing mice, tumor growth was reduced by 33% when doxorubicin was administered alone, by 25% when INCB024360 was administered alone, and by 66% when administered in combination.

2.3.1.2 Clinical Study INCB024360-101

INCB024360 is currently being evaluated in an ongoing Phase I study (INCB 24360-101) conducted by Incyte Corporation in subjects with refractory solid tumors [ClinicalTrials.gov Identifier: NCT01195311]. The study has used a 3+3 dose escalation design, with doses of INCB024360 ranging from 50 mg once daily (qd) to 700 mg twice daily (bid). A total of 52 subjects have been treated: 3 initiated treatment at 50 mg qd, 4 at 50 mg bid, 3 at 100 mg bid, 6 at 300 mg bid, 11 at 400 mg bid, 5 at 500 mg bid, and 4 at 700 mg bid. In the food effect expansion cohort, 14 subjects were treated at 600 mg bid.

INCB024360 has generally been well tolerated. The most common adverse events (AEs) (≥20%) include fatigue, nausea, decreased appetite, vomiting, constipation, abdominal pain, diarrhea, dyspnea, back pain, and cough. The most common grade 3 or 4 AEs were abdominal pain, hypokalemia, and fatigue (9.6% each). Despite 1 patient reporting a dose-limiting toxicity (DLT) of radiation pneumonitis at 300 mg bid, additional subjects were enrolled at this dose and no additional DLTs were reported. At 400 mg bid, 1 patient reported a DLT of grade 3 fatigue, with no additional DLTs at this dose level. Another patient at 400 mg bid developed asymptomatic and transient hypopituitarism after 1 cycle of therapy and was able to continue dosing with INCB024360. Among the 18 subjects treated at either 600 or 700 mg bid, no DLTs were observed. Dose escalation was completed based on preliminary pharmacokinetic and pharmacodynamic data, which demonstrated that doses of INCB024360 above 300 mg bid achieved exposures exceeding the IC_{90} determined in nonclinical models and, in pharmacodynamic assays, reached a plateau for Kyn/Trp levels and ratios. In the final cohort of 600 mg bid, the effect of food on INCB024360 absorption was evaluated. No objective responses were reported. At 2 months, stable disease was seen in 15 patients and lasted ≥4 months in 7 patients. The main AE reported in subjects receiving more than 2 cycles of INCB024360 has been grade 1 or 2 fatigue.

2.3.1.3 Clinical Study (INCB024360-201)

INCB 24360-201 is a Phase Ib/II study of INCB024360 in combination with ipilimumab in subjects with advanced melanoma. Between April and August 2012, 8 subjects were enrolled in the Phase Ib portion of the study and were treated with INCB024360 300 mg bid (Cohort 1), 300 mg bid reduced to 100 mg bid (a continuation of Cohort 1 after hepatotoxity was identified), or 100 mg bid (Cohort 2), each in combination with 3 mg/kg of intravenous (IV) ipilimumab every 3 weeks.

The initial 3 subjects treated with 300 mg bid of INCB024360 plus ipilimumab developed grade 3 or 4 elevations of aspartate aminotransferase/alanine aminotransferase (ALT/AST) on study days 76, 41, and 44, respectively. None of these subjects had elevated bilirubin, liver failure, or other evidence of severe liver injury. These subjects were discontinued from therapy and treated with IV methylprednisolone followed by oral prednisone on a tapering schedule until normal liver function recovered. At the time these elevations were reported, 4 additional subjects were already enrolled and receiving INCB024360 at 300 mg bid with 3 mg/kg IV of ipilimumab. All 4 of these subjects were evaluated and were found to have normal liver function tests (LFTs). Yet, in response to the LFT elevations in the first 3 subjects on study and after discussions with the US Food and Drug Administration (FDA), the dose of INCB024360 was reduced to 100 mg bid in these 4 subjects, and screening and enrollment continued to evaluate an initial dose of INCB024360 of 100 mg bid.

After approximately 2–3 weeks of receiving the reduced dose of INCB024360, 2 of the 4 subjects who had a dose reduction developed hepatic toxicity, including 1 subject symptomatic with abdominal discomfort and reported to have grade 4 elevation of ALT and AST with grade 1 elevation of bilirubin and normal coagulation parameters. These 2 subjects improved after treatment with IV methylprednisolone and oral prednisone, until liver function parameters normalized. All 4 subjects received INCB024360 at 300 mg bid for 10, 13, 14, and 52 days, respectively, before having their dose reduced to 100 mg bid for 16–18 days. Among the 4 subjects who had their dose reduced, the 2 subjects who developed hepatic toxicity received INCB024360 at 300 mg bid for 13 and 14 days, respectively. The duration of exposure to INCB024360 at 300 mg bid was not clearly related to the risk of developing hepatic toxicity, since the subject with the longest exposure (52 days) did not develop hepatic toxicity after the dose reduction. Thus, a dose-response relationship might exist for the development of hepatic toxicity.

After these additional reports of hepatic toxicity, all subjects were removed from study treatment, and screening for the study was suspended. An additional subject had been enrolled in Cohort 2 and received a single dose of INCB024360 at 100 mg at the time the decision was made to stop dosing INCB024360 in all subjects and to suspend enrollment into the study. This subject has continued to receive ipilimumab as part of standard treatment for advanced melanoma, with no hepatic toxicity reported. Among the 7 subjects in Cohort 1, 1 completed all 4 of the planned ipilimumab infusions while in the study. Three of the remaining 6 subjects have gone on to receive

additional doses of ipilimumab, including 1 who had developed hepatic toxicity from the combination of INCB024360 and ipilimumab. No additional hepatic toxicity has been reported in these subjects. The study was recently reopened and is evaluating lower doses of INCB024360 (25–100 mg bid) in combination with ipilimumab. Of note, in the Phase I study (INCB 24360-101), 7 subjects were enrolled with melanoma, including 4 with prior ipilimumab therapy. None of these subjects were reported to have related grade 3 or 4 elevations in LFTs.

Plasma levels of INCB024360 and plasma levels of Kyn were measured in serial samples. Steady-state linear pharmacokinetics were obtained on or before day 8, and appreciable drug accumulation with increases in mean area under the curve (AUC) by 30% between analysis on day 1 and 15 was evident. The half-life was 2.3–4 hours. Pharmacodynamic correlates suggest that significant target inhibition was achieved at bid doses ≥300 mg bid, resulting in steady-state exposures that exceeded the IC_{90}. As a pharmacodynamic effect, blood samples for tryptophan and kynurenine levels show significant target inhibition with doses of 50 mg bid or greater. In patients showing elevations at baseline, ≥300 mg bid doses normalize kynurenine levels. IHC staining of 11 patients demonstrated that IDO1 tumor expression could be observed in tumor cells, infiltrating inflammatory cells, or both, in 10 out of 11 samples, and CD3+ T cells and Foxp3+ Tregs were present in all examined samples. Based on the tolerability and these pharmacokinetic and pharmacodynamic observations, a dose of 600 mg bid has been determined as the recommended Phase-II dose as monotherapy.

In recent preliminary results from a Phase ½ study of INCB024360 combined with ipilimumab in patients with advanced melanoma data showed that in immunotherapy-naïve patients given 25 mg BID or 50 mg BID, 42% of patients demonstrated an objective response and 75% of patients demonstrated disease control by irRC. [Gibney; Hamid 2014] It is therefore proposed that reducing the dose of INCB024360 from 600 mg BID to 300 mg BID in all patients may help prevent adverse events and dose limiting toxicities without limiting biological effect.

In unpublished preliminary data from Incyte, kynurenine concentrations and the Kyn/Trp ratio were higher in the serum of most patients compared to controls and decreased to levels below the median for normal, after treatment with inhibitor when treated with doses that exceeded the IC_{90} (i.e., >300 mg bid [N=19 patients]) (Table 2.4).

Table 2.4. Changes in Kynurenine Concentrations and Kyn/Trp Ratios

Serum Variables	**Control Patients median**	**Cancer Patients median (range)**	**Post-inhibitor median (range)**
All patients (N=19)			
Kynurenine, µM	2.0	2.1 (1.3–4.2)	0.97 (0.5–1.7)
Kyn/Trp ratio, nmol/µmol	29.9	37.4 (17.8–68.9)	18.3 (N/A)
Select patients*			
Kynurenine, µM	2.0	2.7 (2.1–4.2)	1.1 (0.5–1.7)
Kyn/Trp ratio, nmol/µmol	29.9	42.0 (34–69)	18.6 (8–43)

* with Kyn levels >2 µM (n=8) or Kyn/Tyr ratio >30 nmol/µmol (n=14)
Abbreviations: Kyn, kynurenine; N/A, not available; Trp, tryptophan.

2.3.2 MELITAC 12.1 Melanoma Vaccine

MELITAC 12.1 consists of 12 melanoma-associated peptides from melanocytic differentiation proteins (tyrosinase and gp100) and cancer testis antigens (MAGE-A1, MAGE-A3, MAGE-A10, and NY-ESO-1), plus a tetanus peptide that binds promiscuously to HLA-DR molecules and stimulates Th1 dominant helper T cell responses (nonspecific tetanus helper peptide) [Slingluff 2001; Slingluff 2007]. In a Phase II clinical trial of adjuvant therapy in patients with resected stage IIB to IV melanoma, MELITAC 12.1 was found to be more efficacious (i.e., immune response was higher) and demonstrated more complete immunologic responses (i.e., greater cumulative T-cell responses) than vaccines with fewer peptides [Slingluff 2007]. The 12-peptide vaccine was found to induce substantially higher immune response rates: 100% of vaccinated patients had immune responses to at least 1 peptide (detectable in the blood in 83% or in the sentinel immunized lymph nodes in 92%). The immunogenicity of individual peptides was maintained despite competition with additional peptides for binding to major histocompatibility complex (MHC) molecules, with a broader and more robust immune response. Clinical outcome with this peptide vaccine correlated with the immune responses measured in peripheral blood lymphocytes. In subsequent trials, Mel43 and Mel44, when administering the vaccine without granulocyte-macrophage colony-stimulating factor (GM-CSF), immunogenicity was significantly enhanced. In earlier trials with GM-CSF, immune responses were detected after in vitro sensitization. In subsequent trials without GM-CSF, immune responses could be detected directly ex vivo in 70–73% of patients [Slingluff 2011; Slingluff 2009].

This vaccine was well tolerated. In the most recent Mel44 trial (n=170), 8% of patients reported unexpected grade 3 treatment-related AEs, plus 10% with grade 3 injection-site ulceration and 7% with grade 3 injection-site induration; the injection-site reactions were expected and not dose-limiting. One grade 4 toxicity was reported (hypoglycemia, <1%). Other common toxicities were evident for approximately 24 hours and commonly included fatigue, dyspnea, headache, and myalgia. Mild AEs of rigors/chills, anorexia, nausea, sweating, fever, and flushing were commonly experienced [Slingluff 2011].
No concurrent comparator for efficacy was established in the nonrandomized trial of MELITAC 12.1. However, the disease-free survival (DFS) data with this multipeptide vaccine compared favorably to adjuvant trials of high-dose IFN (E1694 clinical trial), favorably with respect to studies E1684 and E1690, and analogously to the Canvaxin trial in patients with resected stage III melanoma. The median DFS was 35 months (2.9 years) with a corresponding 1-year DFS of 76% (95% confidence interval [CI], 60–86%), 2-year DFS of 59% (95% CI, 42–72%), and 3-year DFS of 47% (95% CI, 27–64%). In the more recent Mel43 and Mel44 trials, 3-year DFS was 52% in both trials (95% CI, 43–61%) and overall survival at 3 years was 76% (95% CI, 67–83%) and 79% (95% CI, 71–86%) respectively [Slingluff 2009; Slingluff 2011].

This and similar multipeptide vaccine preparations have been evaluated in prospective randomized Phase II trials with several local adjuvants (e.g., GM-CSF, dendritic cells, incomplete Freund's adjuvant [IFA]), systemic low-dose IL-2, and systemic cyclophosphamide [Slingluff 2004; Slingluff 2007; Slingluff 2009; Slingluff 2011]. Vaccination in IFA was more immunogenic than pulsing peptides on dendritic cells, a finding supported by a separate randomized trial [Slingluff 2003; O'Neill 2009]. No immunologic or clinical benefit was evident by adding low-dose IL-2, GM-CSF, or cyclophosphamide [Slingluff 2004; Slingluff 2009; Slingluff 2011]. Thus, the formulation proposed for this trial is peptide in IFA.

2.4 Rationale

The central thesis of this protocol is that an inhibitor of IDO1 may decrease melanoma-associated immune suppression and immune cell–associated immune suppression and increase the immune response at sites of tumor, specifically increasing both the total number of T cells and the number of melanoma antigen–specific T cells infiltrating the tumor. IDO expression and function arises from 2 potential sources: (1) tumors can overexpress IDO and (2) inflammatory cells can upregulate IDO in response to the IFN-gamma secreted by activated T lymphocytes. Overexpressed and upregulated IDO subsequently depletes tryptophan and increases tryptophan catabolites, including kynurenine, that cause antigen-presenting cells (APCs) and cytotoxic T cells to be ineffective against established tumors. Data from animal experiments [Munn 2012] demonstrate that IDO induction after vaccination serves as a negative feedback loop that dampens the immune response to dendritic cell–based cancer vaccines. An effective IDO inhibitor may reverse this particular form of tumor-associated immune suppression and increase the response to tumor vaccines by augmenting T-cell responses to cancer antigens.

A relatively nontoxic regimen of INCB024360 as monotherapy has been shown to normalize serum Kyn/Trp ratios in patients with cancer. That same regimen is hypothesized to inhibit the effect of IDO expression at sites of tumor deposition and thereby unleash the nascent or vaccine-induced antitumor immune response either at sites of tumor deposition or in the tumor-draining lymph nodes. In addition, the same regimen is hypothesized to increase the systemic immune response to cancer vaccines and/or allow vaccine-induced T cells to replicate and to function better at sites of tumor deposition. Because the melanoma multiple peptide vaccine, MELITAC 12.1, stimulates immune responses, has an excellent safety profile, and is presumed efficacious in the adjuvant setting, treating patients with INCB024360 *before* vaccination with MELITAC 12.1 might increase the immune response to antigens expressed by melanoma cells and allow for an increased antitumor immune response.

2.4.1 IDO Inhibition in Melanoma and T-cell Infiltration into Tumor

IDO is strongly associated with poor prognosis [Gajewski 2005; Brody 2009], and dendritic cells in lymph nodes draining melanomas express IDO [Lee 2003]. High expression of IDO in the sentinel nodes of patients with melanoma is strongly associated

with increased tumor burden and shorter survival [Lee 2011; Speeckaert 2012]. On the other hand, a recent biomarker study found that high IDO expression in tumors before treatment was associated with improved outcome after therapy with ipilimumab, and that increases in T-cell infiltration during treatment were also associated with improved clinical outcome [Hamid 2011].

IDO is induced by IFN-gamma produced by T cells, and thus represents a potent immune regulatory pathway induced when activated T cells infiltrate tumors [Mackler 2003; Zhang 2011; Zhao 2012]. The findings from this recent study emphasize the highly complex interplay of immune activation and suppression in the melanoma microenvironment and its association with T-cell infiltration. They suggest that patients whose tumors express high levels of IDO are those with immune signatures for whom an immune therapy may have a higher chance of success, and that clinical benefit is associated with an increase in T-cell infiltration of melanoma metastases.

2.4.2 Lymphocyte Infiltrates

Patients with brisk lymphocytic infiltrates in primary melanomas have lower risk of metastasis and death than patients with sparse or absent infiltrate [Erdag 2012]. In addition, Slingluff has established that lymphocytic infiltrate in metastatic melanomas correlates with increased survival. Slingluff evaluated the composition and organization of immune cells that infiltrated 183 melanoma metastases in humans. CD45+ cell counts ranged from 0 to 2,142 per mm^2 (Table 2.4.2). The most prevalent immune cells in the first metastases were T cells (53%), of which CD8+ T cells predominated (35%) and CD4+ T cells were less common (19%). B cells (CD20) and plasma cells (CD138) were present at high numbers (25% and 8%, respectively). Macrophage lineage cells (CD163) accounted for 13% of the infiltrates. NK cells (CD56) and mature dendritic cells (DC-LAMP) were rare across all samples, with mean proportions of less than 1% each.

Table 2.4.2. Distribution of Immune Cell Subsets Among 183 Metastases Studied [Erdag 2012]

Subset	N	Mean	Min	25%	Median	75%	Max
CD45	183	246	0	31	117	343	2142
CD138	183	26	0	0	2	23	488
CD20	182	65	0	0	5	47	1796
CD3	183	153	0	20	83	226	1049
CD4	183	52	0	3	16	60	602
CD8	182	103	0	15	71	153	462
CD56	183	3	0	0	0	2	85
PD1	183	18	0	0	2	20	262
Foxp3	182	19	0	0	8	23	175
CD163	182	39	0	2	18	53	360
DC-LAMP		1	0	0	0	1	19

Three histologic patterns of immune cell infiltration were identified. Immunotype A was characterized by no immune cell infiltrate. Immunotype B was characterized by infiltration of immune cells limited only to regions proximal to intratumoral blood vessels. Immunotype C was characterized by a diffuse immune cell infiltrate throughout a metastatic tumor. These

immunotypes represented 29%, 63%, and 8% of metastases with estimated median survival periods of 15, 23, and 130 months, respectively. The best correlates with survival were with CD8+ T cells. Whereas higher densities of CD8+ T cells correlated best with survival, a higher density of CD45+ leukocytes, T cells, and B cells also correlated with increased survival. These reveal how the immune microenvironment can affect outcomes and also provide a method to quantify the effect of the IDO1 inhibitor on immune cell infiltration.

2.4.3 **Data on combination effects of checkpoint blockade antibodies and IDO inhibition.** The success of checkpoint blockade therapy for melanoma has transformed the management of patients with advanced melanoma, but most patients treated with antibodies to CTLA4 or PD-1 will progress despite those therapies. Combined therapy with antibodies to CTLA4 and PD-1 have induced higher response rates, and durable clinical benefit, but at the price of increased toxicity. There is a need for additional combination therapies that may enhance clinical benefit, ideally at lower toxicity than combination of CTLA4 and PD-1 antibodies. IDO and PD-1 can mediate immune escape of melanoma. As effector T cells infiltrate melanoma metastases, they produce interferon-gamma, which in turn can induce both PD-L1 and IDO. Blockade of PD-1/PD-L1 can further increase T cell infiltration; so there is rationale to expect that IDO may mediate progression of cancer on PD-1 antibody therapy. [Spranger 2015], [Gajewski 2015 patent]

Combination of ipilimumab and the IDO inhibitor INCB024360 has induced significant transaminase elevations in 5 of 7 patients, when INCB024360 was administered at 300 mg bid; the study was continued in 8 patients at a much lower dose of INCB024360 (25 mg bid), with disease control observed in 6 of those 8 patients and irPR in 3 (38%). Thus, this small series suggests encouraging clinical outcomes with that combination, but there was unacceptable toxicity with a higher dose of INCB024360. Treatment of patients with another IDO inhibitor (D-1MT) after prior ipilimumab also was associated with dramatic toxicities, including hypophysitis, also raising questions about the toxicity of CTLA-4 blockade plus IDO inhibition.

PD-1 blockade with pembrolizumab or nivolumab has become standard first-line therapy for advanced melanoma (based on data reported in 2015); yet most patients treated with these agents will progress on therapy. Addition of IDO inhibition to these patients offers promise for improving clinical response rates, thus, a cohort has been added to allow addition of INCB024360 early after the last dose of PD-1 antibody (or PD-L1 antibody). If data from this cohort support safety and clinical activity of INCB024360 when added to patients progressing on PD-1 antibody, these data will support future study of adding INCB024360 while continuing PD-1 antibody after failure to respond. Also, biopsies obtained after progression on PD-1 antibody and after adding INCB024360 will provide insight into the role of IDO in mediating failure of PD-1 inhibition and the changes in the tumor microenvironment of adding IDO blockade while PD-1 blockade remains active.

2.4.4 Correlative Studies

To meet the primary objectives, tumors will be biopsied before therapy, after 3 weeks of INCB024360 treatment, and 1 week after the third vaccine plus INCB024360. Because immunotype and immunohistologic characteristics of tumor-infiltrating immune cells are associated with clinical outcome in metastatic melanoma [Erdag 2012], tumors will be assessed by IHC for T-cell infiltration patterns and infiltration with cells expressing CD8. CD8+ T cell infiltration will be reported as cells per mm^2 of cross-sectional tumor area.

Note: Optimally, patients will have tumor approachable for three serial biopsies during the trial. For patients with only one or two tumors approachable for biopsy, available tumor blocks from prior biopsies (without intervening systemic treatment) can serve as the pretreatment sample. The biopsy after 3 weeks of INCB024360 will be considered essential. Biopsy 1 week after the third vaccine plus INCB024360 will be considered optional.

To characterize the immune cell infiltrate further, the tumors will also be assayed for a variety of antigens including CD4, CD8, CD56, CD34, CD45, CD20, CD3, CD8, CD138, CD163, DC-LAMP, FoxP3, and PD-1. IHC staining of tumor biopsies will also be performed to detect IDO1 expression, and the presence and patterns of CD8+ and CD4+ T cell infiltration, Foxp3 protein, and FoxP3 Treg infiltration. Gene signature will be assessed using an Affymetrix chip with focus on IFN signaling, IDO expression, and tumor rejection [Weiss 2011] with each patient serving as their own control. Gene signatures on the tumor will be determined by Ena Wang, PhD, and Franco Marincola MD, Chief, Infectious Disease and Immunogenetics Section in the Department of Transfusion Medicine, Clinical Center of the National Institutes of Health.

A number of other (secondary) correlative studies will be conducted to evaluate whether a regimen of INCB024360 that normalizes serum Kyn/Trp ratios plus MELITAC 12.1 vaccine changes the level or character of the vaccine-induced CD8+ and CD4+ T-cell immune responses as measured in peripheral blood, compared to each patient's baseline. Evaluations will occur at baseline (week 0), again at week 3, week 6, some are done at week 14 and they are repeated optionally at week 16 only if the optional biopsy is done at week 16. Effector 8+ T-cell responses to MELITAC 12.1 will be measured by IFN-gamma enzyme-linked immunosorbent spot assay (ELISpot) and with tetramers/multimers to 8 of the peptides in the vaccine. Effector CD4+ T-cell responses to MELITAC 12.1 will also be tested. Assays will be performed in the CITN Central Laboratory of the Tumor Vaccine Group led by Nora Disis, MD, in collaboration with the immune monitoring laboratory of Craig Slingluff, MD.

The extent to which INCB024360 plus MELITAC 12.1 vaccine alters the number and character of PBMC (including T and NK cells as evaluated by multiparameter flow cytometry) will be calculated by assessing the effect on T lymphocyte number and phenotype (i.e., CD8+, effector memory CD8+, central memory CD8+, CD4+, CD4+ Treg, naïve subsets). The frequency and percentage of PBMC subsets will be determined using multiparameter flow cytometric analysis on whole blood. The markers include

CD3, CD8, CD4, CD56, Foxp3, CD127, CD45RA, CD45RO, CCR7, CD28, CD27, CD25, CD122, CD86, CD14, CD16, CD19, CD123, and CD11c. Changes in myeloid-derived suppressor cell populations will also be assessed by flow cytometry. Assays will be performed in the CITN Central Laboratory of the Tumor Vaccine Group led by Nora Disis, MD, in collaboration with the immune monitoring laboratory of Craig Slingluff, MD.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have malignant melanoma validated by histology or cytology. Patients may have had primary cutaneous, mucosal, or ocular melanoma or metastasis from an unknown primary site.

NOTE: Patients must have measurable disease, defined as at least 1 lesion that can be accurately measured in at least 1 dimension (longest diameter to be recorded for non-nodal lesions and short axis for nodal lesions) as ≥20 mm with conventional chest x-ray or as ≥10 mm with spiral computed tomography (CT) scan, magnetic resonance imaging (MRI), or calipers by clinical exam. See Section 11 for the evaluation of measurable disease.

3.1.2 Unresectable stage III or IV melanoma validated by clinical criteria (including recurrent melanoma), or patients with multiple skin/soft tissue metastases of melanoma that may be resectable but are judged to have a future recurrence risk exceeding 70% (e.g., large adenopathy, distant skin metastases or multiple in-transit melanoma metastases). Tumor deemed amenable to biopsy (excisional, incisional, or core, with at least 100 mm^3 tumor volume per biopsy date) and fine-needle aspiration (FNA) biopsy.

Note: Optimally, patients will have tumor approachable for three serial biopsies during the trial. For patients with only one or two tumors approachable for biopsy, available tumor blocks from prior biopsies can serve as the pretreatment sample, but only if formalin-fixed tumor tissue is available and adequate to provide at least 20 unstained slides with sufficient tumor for analysis.

NOTE: Patients with unresectable advanced stage III or IV melanoma (including recurrent melanoma) are only eligible if they have failed at least one other first-line systemic therapy (other than adjuvant therapy). Exceptions to this requirement are those patients who have refused and/or are ineligible for other systemic therapies.

NOTE: BRAFi should be considered for all ‘unresectable” or metastatic melanoma with BRAFV600E mutation; for low burden in-transit disease patients may enter trial without prior systemic therapy.

3.1.2.1 Stage IV no evidence of disease (NED) is excluded by this criterion

3.1.3 Prior and concurrent therapy

3.1.3.1 Patients may have had prior systemic therapy without constraint on the number of prior treatment regimens except:
a) Patients may not have had >450 mg/m^2 doxorubicin
b) Patients may not have had >3000 cGy to fields encompassing the entire pelvis

3.1.3.2 Patients must not be on any other systemic therapy within the following intervals before study enrollment:
a) 1 week after stereotactic radiosurgery of the brain or comparable technology
b) 4 weeks after cytotoxic chemotherapy or external beam radiation therapy
c) 6 weeks after chemotherapy regimens including BCNU (carmustine) or mitomycin C
d) patients who experience melanoma progression (by RECIST 1.1 criteria) while on or after treatment with PD-1 or PDL-1 antibody may enroll on this study.
NOTE: Patients must be off PD-1/PDL- antibody for at least 2 weeks to assess for delayed toxicity before being enrolled and receiving INCB024360.
Patients who are enrolled 2 weeks and up to 6 weeks after the last dose of PD-/PDL-1 antibody will enroll in cohort B and receive 100mg BID of INCB024360.
Patients enrolled beyond the 6 week period after failing anti-PD-1/PDL-1 will be enrolled in cohort A. Cohort A patients will receive 300mg BID of INCB024360.
Patients must not have active grade 2 autoimmune toxicities attributed to these antibodies at study entry.
e) 8 weeks after ipilimumab, other CTLA-4 antibody or other immunologically active antibody.
NOTE: Patients receiving prior CTLA-4, anti-PD1 antibody or other immunologic therapy must show evidence of normal pituitary function at baseline and must not have active grade 2 autoimmune toxicities attributed to these antibodies at study entry.

3.1.4 Age ≥18 years

[**NOTE**: Because insufficient dosing AE data are currently available on the use of INCB024360 in combination with MELITAC 12.1 in patients <18 years of age, children are excluded from this study, but will be eligible for future pediatric trials.]

3.1.5 ECOG performance status 0–1 (Karnofsky ≥70%, see Appendix A).

3.1.6 Life expectancy of at least 6 months

3.1.7 Patients must have normal organ and marrow function as defined below:

– leukocytes	≥3,000/mcL
– absolute neutrophil count	≥1,500/mcL
– platelets	≥75,000/mcL
– hemoglobin	>9g/dL
– total bilirubin	<1.5 × institutional upper limit of normal [bilirubin <3 × institutional upper limit of normal for Gilbert's syndrome]
– AST(SGOT)/ALT(SGPT)	Up to 2.5 times ULN
– creatinine	<1.5 × institutional upper limit of normal OR
– creatinine clearance	≥60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal.
– PT, INR	≤1.5 × institutional ULN unless patient is therapeutically anticoagulated. If on anticoagulants, PT/INR need to be within appropriate anticoagulation limits for the clinical indication. Patients who are receiving anticoagulants may participate in the trial if their anticoagulation can be stopped safely for several days at the time of each biopsy.
– TSH,	Up to 4 times ULN if T4 is normal
– T4	Within normal limits. If abnormal and patient is receiving thyroid replacement therapy, the thyroid medication may be adjusted and the T4 may be re-tested.

3.1.8 Patients must express HLA-A1+, -A2+, or -A3+ (80% of patients)

3.1.9 LDH <5 × upper limits of normal

[**NOTE**: These criteria will select against patients with bulky disease and will select for patients with less disease and earlier disease.]

3.1.10 Participants must not have had prior autoimmune disorders requiring cytotoxic or immunosuppressive therapy, or autoimmune disorders with visceral involvement. Participants must not have an active or inactive autoimmune disorders (e.g., rheumatoid arthritis, moderate or severe psoriasis, multiple sclerosis, inflammatory bowel disease, etc.) Participants who are receiving therapy for an autoimmune or inflammatory disease requiring these therapies are also excluded.

3.1.11 The following will not be exclusionary:
- Resolved ipilimumab associated inflammatory disease
- The presence of laboratory evidence of autoimmune disease (e.g., positive antinuclear antibody [ANA] titer) without associated symptoms
- Subjects with vitiligo, thyroiditis, or atopic dermatitis, but otherwise not meeting this criterion may be enrolled. Individual cases can be discussed with the sponsor.

3.1.12 Not likely curable with surgery alone

3.1.13 Not currently receiving therapy

3.1.14 Females of childbearing potential must have a negative pregnancy test within 48 hours before initiating protocol therapy.

NOTE: The effects of INCB024360 and MELITAC 12.1 on the developing human fetus are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control or abstinence) before study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception before the study, for the duration of study participation, and 4 months after completing INCB024360 and MELITAC 12.1 administration.
NOTE: Subjects are considered not of child bearing potential if they are surgically sterile, have undergone a hysterectomy, bilateral tubal ligation, or bilateral oophorectomy, or are postmenopausal. Menopause is the age associated with complete cessation of menstrual cycles and menses, and implies the loss of reproductive potential. By a practical definition, the term assumes menopause after 1 year without menses with an appropriate clinical profile at the appropriate age.

3.1.15 Ability to understand and willingness to sign a written informed consent document

3.2 Exclusion Criteria

3.2.1 Patients who have had cytotoxic chemotherapy, radiotherapy, IFN, immunosuppressive therapy, or steroids within 4 weeks (6 weeks for nitrosoureas or mitomycin C) before entering the study or those who have not recovered from AEs due to agents administered more than 4 weeks earlier

3.2.2 Ipilimumab, or other immunologically active therapy within 8 weeks of enrollment.
NOTE: Patients who experience melanoma progression (by RECIST 1.1 criteria) while on or after treatment with PD-1 or PDL-1 antibody may enroll on this study (per section 3.1.3.2 above)

3.2.3 Active immunosuppressive therapy, including concurrent systemic immunosuppressive therapy or steroid therapy with more than 7 consecutive days of steroids within the prior 4 weeks
- The use of prednisone or equivalent <0.125 mg/kg/day (absolute maximum of 10 mg/day) as replacement therapy is permitted.
- Inhaled corticosteroids are permitted.

3.2.4 Cardiovascular disease that meets one of the following: congestive heart failure (New York Heart Association Class III or IV), active angina pectoris, or recent myocardial infarction or acute coronary syndrome (within the last 6 months)

3.2.5 History of peripheral vascular disease (PVD) that has required surgical or percutaneous intervention or documented PVD that requires medical management with medications such as ASA + Clopidogrel. Patients with Diabetes that is not well controlled are excluded from participation. Not well controlled is defined as a Hgb A1C of greater than 7.5%.

3.2.6 Current or history of systemic autoimmune disease requiring systemic therapy, including significant autoimmunity associated with prior ipilimumab therapy or therapy with antibodies to PD-1 or PD-L1

3.2.7 Cirrhosis, chronic hepatitis C virus positivity, or chronic hepatitis B infection. Subjects who may not tolerate immune-mediated hepatitis due to compromised hepatic reserve are also excluded from participation including: 1) Subjects with extensive liver metastasis (as judged by the investigator) 2) Subjects who drink more than two standard alcoholic beverages per day on a regular basis 3) Subjects who consume more than 2 grams of Acetaminophen per day on a regular basis.

- A positive hepatitis B serology indicative of previous immunization (i.e., HBsAb-positive and HBcAb-negative), or a fully resolved acute hepatitis B infection is not an exclusion criterion.

3.2.8 Patients who are receiving any other investigational agents for melanoma

3.2.9 Patients who have had a grade one or grade two gastrointestinal adverse event during or after receiving anti-CTLA-4, anti-PD1 or anti-PD, without a colonoscopy verifying complete resolution of the adverse event.

3.2.10 Patients who have experienced bowel perforation, neurologic involvement, Guillain Barré syndrome, Myasthenia Gravis, Steven Johnson syndrome and other intractable events or grade 4 non-laboratory toxicity.

3.2.11 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements

3.2.12 Pregnancy, nursing, or unwilling to take adequate birth control during therapy

NOTE: Pregnant women are excluded from this study because INCB024360 and MELITAC 12.1 have the potential for teratogenic or abortifacient effects. Because of unknown but potential risks for AEs in nursing infants secondary to treatment of the mother with INCB024360 and MELITAC 12.1, breastfeeding should be discontinued if the mother is treated with INCB024360 and MELITAC 12.1 These potential risks may

also apply to other agents used in this study.

3.2.13 Known HIV or other history of immunodeficiency disorder

NOTE: HIV-positive patients taking combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with INCB024360 and MELITAC 12.1. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in patients receiving combination antiretroviral therapy when indicated.

3.2.14 Extensive active brain disease, including symptomatic brain metastases or the presence of leptomeningeal disease

- Patients with brain metastasis, after definitive therapy with surgery or stereotactic radiation and stable off steroids for >4 weeks, are eligible.

3.2.15 Any malignancy that has not been in complete remission for at least 3 years

NOTE: Patients with cured basal or squamous cell skin cancer are not excluded. Patients with a history of excised in situ cancers, including breast, cervical, colon, superficial bladder, prostate or other body system are not excluded. Study entry will be allowed at the discretion of the Principal Investigator.

NOTE: Recurrence of the in situ cancer or tumor at the time of study entry would be exclusionary.

3.2.16 Monoamine oxidase (MAO) inhibitor use within the past 3 weeks or prior evidence of serotonin syndrome

3.2.17 History of allergic reactions attributed to compounds of similar chemical or biologic composition to INCB024360, MELITAC 12.1, or other vaccine components

3.2.18 Prior organ allograft or allogeneic transplantation, if the transplanted tissue is still in place

3.2.19 Medical or psychiatric illness that would, in the opinion of the investigator, preclude participation in the study or the ability of patients to provide informed consent for themselves

3.2.20 History of pulmonary disease such as emphysema or chronic obstructive pulmonary disease (COPD), (FEV1>60% of predicted for height and age). Pulmonary function tests (PFTs) are required in patients with prolonged smoking history or symptoms of respiratory dysfunction.

3.2.21 Use of any UGT1A9 inhibitor including: acitretin, amitriptyline, androsterone, cyclosporine, dasatinib, diclofenac, diflunisal, efavirenz, erlotinib, estradiol (17-beta), flutamide, geftinib, gemfibrozil, glycyrrhetinic acid, glycyrrhizin, imatinib, imipramine, ketoconazole, lineoleic acid, mefenamic acid, mycophenolic acid, niflumic acid, nilotinib, phenobarbital, phenylbutazone, phenytoin, probenecid propofol, quinidine, ritonavir, Sorafenib, sulfinpyrazone, valproic acid and verapamil from screening through follow-up period.

3.2.22 Low-dose Coumadin (1 mg) is acceptable; however, doses that increase INR are not permitted. If an alternative to Coumadin-based anticoagulants cannot be used, the INR should be monitored weekly after initiation of therapy and upon discontinuation of INCB024360, until INR normalization.

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial (Table 3.3).

Table 3.3. Accrual Targets by Race, Ethnicity, and Sex/Gender.

Accrual Targets					
	Sex/Gender				
Ethnic Category	**Females**		**Males**		**Total**
Hispanic or Latino	2	+	2	=	4
Not Hispanic or Latino	3	+	5	=	8
Ethnic Category: Total of all subjects	5 (A1)	+	7 (B1)	=	12 (C1)
Racial Category					
American Indian or Alaskan Native		+	1	=	1
Asian	1	+	1	=	2
Black or African American		+		=	
Native Hawaiian or other Pacific Islander		+		=	
White	4	+	5	=	9
Racial Category: Total of all subjects	5 (A2)	+	7 (B2)	=	12 (C2)
	(A1=A2)		(B1=B2)		(C1=C2)

4. REGISTRATION PROCEDURES

Following registration, patients should begin protocol treatment within 5 days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

4.1 Registration Process

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

Prior to the recruitment of a patient for this study, investigators must be registered members of a Cooperative Group. Each investigator must have an NCI investigator number and must maintain an "active" investigator registration status through the annual

submission of a complete investigator registration packet (FDA Form 1572 with original signature, current CV, Supplemental Investigator Data Form with signature, and Financial Disclosure Form with original signature) to the Pharmaceutical Management Branch, CTEP, DCTD, NCI. These forms are available on the CTSU Web site (enter credentials at https://www.ctsu.org; then click on the Register tab) or by calling the PMB at 301-496-5725 Monday through Friday between 8:30 a.m. and 4:30 p.m. Eastern time.

Each investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can enroll patients. Study centers can check the status of their registration packets by querying the Regulatory Support System (RSS) site registration status page of the CTSU member web site by entering credentials at https://www.ctsu.org.

Requirements for protocol number site registration:

- CTSU IRB Certification
- CTSU IRB/Regulatory Approval Transmittal Sheet
- IRB-approved consent form (if applicable)
- CTSU RT Facilities Inventory Form (If applicable)

NOTE: Per NCI policy all institutions that participate on protocols with a radiation therapy component must participate in the Radiological Physics Center (RPC) monitoring program. If this form has been previously submitted to CTSU it does not need to be resubmitted unless updates have occurred at the RT facility.

Patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). OPEN is a web-based registration system available on a 24/7 basis.

All site staff will use OPEN to enroll patients to this study. It is integrated with the CTSU Enterprise System for regulatory and roster data and, upon enrollment, initializes the patient position in the Rave database.
OPEN can be accessed at https://open.ctsu.org or from the OPEN tab on the CTSU members' side of the website at https://www.ctsu.org.

Prior to accessing OPEN site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes. Site staff should use the registration forms provided on the CITN or CTSU web site as a tool to verify eligibility.
- All patients have signed an appropriate consent form and HIPAA authorization form (if applicable).

Access requirements for OPEN:

- Site staff will need to be registered with CTEP and have a valid and active CTEP-IAM account. This is the same account (user id and password) used for the CTSU members' web site.
- To perform registrations, the site user must have been assigned the 'Registrar' role on the CITN roster.

Note: The OPEN system will provide the site with a printable confirmation of registration and treatment information. Please print this confirmation for your records.

Further instructional information is provided on the OPEN tab of the CTSU members' side of the CTSU website at https://www.ctsu.org or at https://open.ctsu.org. For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

Data collection for this study will be done exclusively through Medidata Rave. Access to the trial in Rave is granted through the iMedidata application to all persons with the appropriate roles in RSS. To access iMedidata/Rave the site user must have an active CTEP IAM account (https://eapps-ctep.nci.nih.gov/iam). In addition, site users that are a member of the CITN must have the Rave roles (Rave CRA, Read-Only, Site Investigator) in RSS at the enrolling site.

Upon initial site registration approval for the study in RSS, all persons with Rave roles assigned on the CITN roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site users must log into the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM user name and password, and click on the "accept" link in the upper right corner of the iMedidata page. Please note, site users will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be listed in the upper right pane of the iMedidata screen.

Users who have not previously activated their iMedidata/Rave accounts will receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website, Rave tab under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU website under the Rave tab at www.ctsu.org/RAVE/ or by contacting the CTSU Help Desk at 1-888-823-5923 or by e-mail at ctsucontact@westat.com.

5. TREATMENT PLAN

5.1 Agent Administration

Patients with surgically incurable stage III or IV melanoma and tissue amenable to serial biopsy will undergo pretreatment tumor biopsy and receive a 3-month course of INCB024360 administered as a twice daily continuous dosing oral regimen. The

MELITAC 12.1 vaccine has 3 components: 12-MP peptides, Peptide-tet, and Montanide ISA-51 VG vaccine adjuvant, which will be prepared together as an emulsion for administration. Three (3) mL of emulsion will be prepared. Two (2) mL of this emulsion will be administered, with 1 mL at each of 2 vaccine sites. At each site, half of each dose (0.5 mL) will be injected subcutaneously and half (0.5 mL) will be injected intradermally. The remaining 1 mL of the prepared emulsion will be discarded.

On day 21, a week 3 tumor biopsy will be collected and the effects of inhibitor alone on the tumor microenvironment will be evaluated by comparing the pretreatment tumor biopsy to the day 21 tumor biopsy, assessing changes in IHC and gene signatures.

Note: The biopsy on day 21 is mandatory. For patients with only one or two tumors approachable for biopsy, available tumor blocks from prior biopsies can serve as the pretreatment sample.

Also on day 21, a vaccination regimen with MELITAC 12.1 multipeptide vaccine will begin, with vaccines administered on days 21, 28, 35, 56, 77, and 98 (Weeks 3, 4, 5, 8, 11, and 14. MELITAC 12.1, will be administered intradermally/subcutaneously at a fixed dose of 2.0 ml for 6 vaccinations. On day 42, tumor will be biopsied and the effect of continued INCB024360 plus vaccine on the tumor microenvironment will be evaluated by comparing the week 3 tumor biopsy to the week 6 tumor biopsy, assessing changes in IHC and gene signatures.

Patients will receive 3 weeks of INCB024360, followed by continued INCB024360 plus 6 vaccinations with MELITAC 12.1. Patients who have disease progression while receiving anti-PD1 or anti-PDL-1 antibody treatment will be allowed to enroll and receive INCB024360 within 2-6 weeks of last antibody treatment administration. Patients enrolled within the 6 week window will be enrolled in Cohort B and administered INCB024360 orally at a dose of 100 mg twice daily continuously up to day 98 (3 months) plus vaccines. Patients who enroll in the study after confirmed disease progression on anti-PD1/PDL-1 after 6 weeks of receiving the last dose of antibody will be enrolled in Cohort A and receive 300 mg BID of INCB024360 plus vaccines.

Patients that show response at 3 months will be considered for additional INCB024360.

As a lead-in to ensure safety and tolerability of the combination of INCB024360 and MELITAC 12.1, the first 3 patients enrolled in the study will be at the dose of 300 mg bid staggering each patient's enrollment by at least 2 weeks so that the first patient will receive 2 doses of the vaccine in combination with INCB024360 before the second patient starts the combination of agents. Enrollment will be halted after the first 3 patients are enrolled to allow for observation of any toxicity before opening further enrollment. The next nine patients will receive either 300 mg bid of INCB024360 or 100 mg bid (Table 5.1). For patient safety, if a Dose Limiting Toxicity (DLT) occurs in the first three patients treated at the 300 mg BID dose level of INCB024360 + MELITAC 12.1 vaccine, the subsequent 9 patients will be treated with the dose of either 300 mg BID or 100 mg of INCB024360 + MELITAC 12.1. If two DLTs occur in the first 6 patients treated at the

300 mg dose of INCB024360 + MELITAC 12.1 the study will be stopped.

Treatment will be administered on an outpatient basis. Reported AEs and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy. The patient will be requested to maintain a medication diary of each dose of medication. The medication diary will be returned to clinic staff at the end of each course.

Table 5.1. Agent Administration and Timing of Assessments

Cohort	INCB024360 Dosing	MELITAC 12.1 Dosing	Blood draws	Tumor biopsies including FNA
A Pts.enrolled >6 wks after failing anti-PD-1/PDL-1	300 mg po bid continuous for 98 days (3 months)	Intradermal/subcutaneous Days 21, 28, 35, 56, 77, 98 (Weeks 3, 4, 5, 8, 11, and 14)	Pretreatment day 0 and on days 21, 42, 98, and 112	Days 0, 21, 42, and (optional day 112) and (optional when coming off study including for progression)
B Pts.enrolled 2-6 wks after failing anti-PD-1/PDL-1	100 mg po bid continuous for 98 days (3 months)	Intradermal/subcutaneous Days 21, 28, 35, 56, 77, 98 (Weeks 3, 4, 5, 8, 11, and 14)	Pretreatment day 0 and on days 21, 42, 98, and 112	Days 0, 21, 42, and (optional day 112) and (optional when coming off study including for progression)

Abbreviations: bid, twice per day; FNA, fine-needle aspiration; po, orally; pre-tx, pretreatment; SC, subcutaneous

Enrollment will be staggered so that only 3 patients will initially be treated at the 300-mg bid dose in order to observe their response to the INCB024360-MELITAC 12.1 combination. After the first patient is started on INCB024360, treatment will proceed for 2 weeks before the second patient is started on INCB024360 monotherapy. Before the second patient starts the combination of INCB024360 and MELITAC 12, the first patient will have already received the second vaccination of MELITAC 12.1 in combination with INCB024360 and observed for any toxicity. The third patient will start INCB024360 monotherapy 2 weeks after patient number 2 has received the combination of INCB024360 and MELITAC 12.1 and been observed for toxicities. After the first 3 patients are treated at the 300-mg bid dose of INCB024360 in combination with the MELITAC 12.1 and found to tolerate the combination well, the dose of INCB024360 will be 300 mg BID for subsequent patients. Those patients treated with anti-PD-1/PDL1antibody who have failed therapy and enrolled in the study 6 weeks or longer after progression of disease will be enrolled in Cohort A and receive 300 mg BID of INCB024360. Patients who fail anti-PD-1/PDL-1 therapy and enroll within 2-6 weeks of progression of disease will receive100 mg BID in Cohort B. All other patients enrolled in the study will receive INCB024360 300 mg BID.

A total of 12 patients will be enrolled in the study. Assignment to Cohort A or B is for dose assignment only.

5.1.1 CTEP IND Agent(s*):* N/A

5.1.2 Other Agent(s): **INCB024360**

Combining INCB024360 with the multipeptide vaccine MELITAC 12.1 is a novel approach. Although no adverse findings in toxicology or safety pharmacology assessments have been described herein, and the most common grade 1 or 2 AEs at the recommended Phase-II dose (600 mg) include fatigue, nausea and vomiting, constipation, and anorexia; to decrease the possibility of unanticipated toxicities with the combination, the dose of INCB024360 in the first 3 patients treated in the study will be at the dose of 300 mg bid.

Weak cross-reactivity of INCB024360 to the human vasopressin 1a receptor, dopamine receptor, and carbonic anhydrase II may lead to changes in blood pressure or blood electrolytes. In 28-day toxicology studies, C_{max} values have exceeded the IC_{50} for the IDO1 enzyme (72 nM) by up to 37-fold and the IC_{50} for the vasopressin 1a receptor by up to 4-fold (13-fold in single-dose studies) in the absence of any toxicity, so the risk of unintended pharmacological activity is expected to be low.

5.1.3 Other Agent (s): **MELITAC 12.1**

MELITAC 12.1 vaccine comprises 100 µg each of the 12 melanoma peptides plus 190 µg tetanus toxoid peptide (Peptide-tet). Peptides are synthesized under good manufacturing practice (GMP) conditions by PolyPeptide Group (San Diego, CA; formerly Multiple Peptide Systems), reconstituted, mixed, sterile-filtered, and vialed under GMP conditions by Merck (Germany). The 12–melanoma peptide mixture and tetanus peptide preparation are made as separate sterile aqueous solutions to be administered in an emulsion with Montanide ISA-51 VG adjuvant (1 mL), using the 2-syringe method after emulsion stability and quality is verified. MELITAC 12.1 stimulated immune responses with an excellent safety profile and with presumptive evidence of efficacy in the adjuvant setting.

At this writing, the 12-MP has been administered in combination with Peptide-tet and GM-CSF in Montanide ISA-51 VG adjuvant to more than 200 participants enrolled in 3 studies run through the University of Virginia Human Immune Therapy Center (HITC) in collaboration with other centers (Mel39, Mel43, E1602) [Slingluff 2007; Slingluff 2009; Slingluff 2010] and 12-MP has been administered in Montanide ISA-51 VG adjuvant, with or without Peptide-tet in about 300 participants (Mel43, Mel44, E1602) [Slingluff 2010; Slingluff 2011; Slingluff 2009]. No data are available regarding the distribution and excretion of the vaccine preparation. In vitro studies have indicated the half-life of synthetic peptides in the presence of serum peptidases is short (approximately 22 seconds) [Brinckerhoff 1999]. However, in vivo data describing the metabolism of the vaccine are not available.

In the first 51 participants vaccinated with the 12-MP and Peptide-tet with GM-CSF in Montanide ISA-51 VG adjuvant, the majority of toxicities described have been of minimal severity. The most frequent AEs for participants receiving the 12-MP in

combination with Peptide-tet include maximum grade 1 fatigue (49%), maximum grade 1 chills (49%), maximum grade 1 headache (42%), and maximum grade 2 injection site reactions (69%). Other common AEs include maximum grade 1 sweating (27%), maximum grade 1 hyperglycemia (33%), maximum grade 1 myalgia (24%), and maximum grade 3 injection site reaction (27%). At this writing, at least 1 case of vitiligo and no cases of visual changes related to the vaccine after vaccination with the 12-MP and Peptide-tet administered with GM-CSF in Montanide ISA-51 VG adjuvant have been reported.

5.1.4 Other Modalities or Procedures: N/A

5.1.5 Investigational Imaging Agent Administration: N/A

5.2 Definition of Dose-Limiting Toxicity:

Dose-limiting toxicities (DLTs) include all Grade 3 AEs regardless of investigator attribution or relatedness except for those listed below.
Based on investigator determination the following Grade 3 toxicities can be deemed non-DLT:

- adverse event with a clear alternative explanation (e.g. due to disease progression)
- transient toxicities (≤72 hours)
- injection site reactions that are expected and do not require any surgical intervention or repair
- abnormal laboratory values without associated clinically significant signs or symptoms

There are two exceptions to the above. Grade 1 or greater ocular toxicities will be considered a DLT. Grade 2 LFT elevations will be considered a DLT.

In the event of a DLT, it will be determined by the safety team whether both the INCB024360 AND the MELITAC 12.1 will be permanently discontinued. In cases of vaccine injection site reaction or other vaccine related adverse events the INCB024360 may be continued if the vaccine related adverse event continues to show improvement. Management and dose modifications associated with the above adverse events are further outlined in Section 6.

5.3 General Concomitant Medication and Supportive Care Guidelines

5.3.1 INCB024360

INCB024360 is neither a substrate nor an inhibitor or inducer of cytochrome P450 enzymes. Analysis of human plasma samples indicated that INCB024360 is metabolized by 2 major pathways: O-glucuronidation and reduction. The identification of the 2 major pathways and the enzymes responsible for those pathways has enabled the refinement of the inclusion/exclusion criteria around concomitant medications. However for safety purposes, the case report form must capture the concurrent use of all other drugs, over-

the-counter medications, or alternative therapies.
Hypothetically, inhibition of IDO could cause an increase in serotonin levels that could precipitate a cluster of AEs termed serotonin syndrome. This rare syndrome has been associated with some MAO inhibitors and with combinations of serotonergic drugs. Symptom onset is usually rapid, often occurring within minutes, and encompasses a wide range of clinical findings:

- Mild symptoms may only consist of increased heart rate, shivering, sweating, dilated pupils, myoclonus, and over responsive reflexes.
- Moderate symptoms include additional abnormalities such as hyperactive bowel sounds, high blood pressure, and hyperthermia. A temperature as high as 40°C (104°F) is common in moderate intoxication. Mental status changes include hypervigilance and agitation.
- Severe symptoms include severe increases in heart rate and blood pressure that may lead to shock. In life-threatening cases, temperature may rise to above 41.1°C (106.0°F). Other abnormalities include metabolic acidosis, rhabdomyolysis, seizures, renal failure, and disseminated intravascular coagulation; these effects usually arise as a consequence of hyperthermia.

At this writing, no laboratory test exists for serotonin syndrome; therefore diagnosis is by symptom observation. Management is based primarily on stopping the usage of the precipitating drugs, the administration of serotonin antagonists such as cyproheptadine, and supportive care including the control of agitation, autonomic instability, and hyperthermia. Additionally, those who ingest large doses serotonergic agents may benefit from gastrointestinal decontamination with activated charcoal if it can be administered within an hour of overdose. The intensity of therapy depends on the severity of symptoms. If the symptoms are mild, treatment may only consist of discontinuation of the offending medication or medications, offering supportive measures, giving benzodiazepines for myoclonus, and waiting for the symptoms to resolve. Moderate cases should have all thermal and cardiorespiratory abnormalities corrected and can benefit from serotonin antagonists. As mentioned above, the serotonin antagonist cyproheptadine is the recommended initial therapy. Additional pharmacological treatment for severe case includes administering atypical antipsychotic drugs with serotonin antagonist activity (e.g., olanzapine). Critically ill patients should receive the above therapies as well as sedation and/or neuromuscular paralysis. Patients who have autonomic instability such as low blood pressure require treatment with direct-acting sympathomimetics, such as epinephrine, norepinephrine, or phenylephrine. Conversely, hypertension or tachycardia can be treated with short-acting antihypertensive drugs such as nitroprusside or esmolol; longer-acting drugs such as propranolol should be avoided as they may lead to hypotension and shock.

As mentioned above, weak cross-reactivity of INCB024360 to the human vasopressin 1a receptor, dopamine receptor, and carbonic anhydrase II may lead to changes in blood pressure or blood electrolytes. In 28-day toxicology studies, C_{max} values have exceeded the IC_{50} for the IDO1 enzyme (72 nM) by up to 37-fold and the IC_{50} for the vasopressin 1a receptor by up to 4-fold (13-fold in single-dose studies) in the absence of any toxicity, so the risk of unintended pharmacological activity is expected to be low.

Studies imply that IDO1 may be a negative inhibition pathway to control autoimmune diseases associated with inflammation. Recent studies with the anti-CTLA-4 antibody ipilimumab have shown dysregulation of mucosal immunity and gastrointestinal toxicity, which manifests as diarrhea. For these reasons, subjects with a history of autoimmune disease should be excluded from participation in this study, and study participants should be closely monitored for signs or symptoms of developing autoimmune or inflammatory disease.

Analysis of human plasma samples indicated that INCB024360 is metabolized by 2 major pathways: O-glucuronidation and reduction. The O-glucuronide (M9) was formed by UGT1A9 and the reduced metabolite (M11) appears to be catalyzed by intestinal microflora. Metabolite M11 is also further metabolized to M12, a product formed by oxidative cleavage catalyzed by CYPs. The identification of the 2 major pathways and the enzymes responsible for those pathways has enabled the refinement of the inclusion/exclusion criteria around concomitant medications. It should be noted that all three major metabolites (M9, M11 & M12) are inactive against IDO1 enzyme.

Use of any UGT1A9 inhibitor including: acitretin, amitriptyline, androsterone, cyclosporine, dasatinib, diclofenac, diflunisal, efavirenz, erlotinib, estradiol (17-beta), flutamide, geftinib, gemfibrozil, glycyrrhetinic acid glycyrrhizin, imatinib, imipramine, ketoconazole, linoleic acid, mefenamic acid, mycophenolic acid, niflumic acid, nilotinib, phenobartital, phenylbutazone, phenytoin, and probenecid propofol, quinidine, ritonavir, Sorafenib, sulfinpyrazone, valproic acid, and verapamil should be avoided from screening through follow-up period.

Low-dose Coumadin (1 mg) is acceptable; however, doses that increase INR are not permitted. If an alternative to Coumadin-based anticoagulants cannot be used, the INR should be monitored weekly after initiation of therapy and upon discontinuation of INCB024360, until INR normalization.

5.3.2 MELITAC 12.1

In several studies, the vaccine has been well tolerated. In the most recent study, in which the adjuvant was Montanide ISA-51 VG alone, Mel44 (n=170), 8% of patients reported unexpected grade 3 treatment-related AEs, plus 10% of patients with grade 3 injection-site ulceration and 7% with grade 3 injection-site induration; the injection-site reactions were expected and not dose-limiting. One grade 4 toxicity was reported (hypoglycemia, <1%). Other common toxicities were evident for approximately 24 hours included fatigue, dyspnea, headache, and myalgia. Mild AEs of rigors/chills, anorexia, nausea, sweating, fever, and flushing were commonly experienced [Slingluff 2011].

5.4 Duration of Therapy

Patients with surgically incurable stage III or IV melanoma and tissue amenable to serial biopsy will receive the sequential therapy with INCB024360 alone for 3 weeks followed

by continued INCB024360 plus 6 MELITAC 12.1 vaccines administered intradermally or subcutaneously on days 21, 28, 35, 56, 77, and 98 (weeks 3, 4, 5, 8, 11, and 14).

Serial biopsies will be obtained up to 14 days before starting INCB024360, on day 21 (before the first vaccine), and on day 42 (a week after the third vaccine). When feasible a biopsy will be obtained at week 16 (2 weeks after the sixth vaccination). If possible, tumors will be biopsied from patients coming off study, including for progression of disease.

In the absence of treatment delays due to AE(s), treatment may continue for up to 3 additional cycles of INCB024360 (a total of 4 cycles) after consultation with the trial Principal Investigator, CTEP, and the CITN, or until one of the following criteria applies:

- Disease progression by Response Evaluation Criteria In Solid Tumors (RECIST) or Immune-Related Response Criteria (irRC)
- Intercurrent illness that prevents further administration of treatment
- Unacceptable AE(s)
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition rendering the patient unacceptable for further treatment in the judgment of the investigator

If additional cycles of INCB024360 are deemed appropriate for the patient, the INCB024360 will be given without the administration of the MELITAC 12.1 vaccines.

Only the end of treatment biopsy will be obtained if additional cycles of INCB024360 are approved for each patient.

In patients who are approved to receive additional cycles of INCB024360, a reduced volume of blood will be drawn for specific assays. (Study Calendar, footnote ***)

A delay of up to 60 days between treatment cycles will be allowed.

In the event of unexpected serious AEs related to either component of the combination, treatment of the affected participant with INCB024360 and/or MELITAC 12.1 will be discontinued after analysis by the DSMC, Incyte, and CITN.

5.5 Duration of Follow-up

Patients will be followed for 1 year after removal from study or until death, whichever occurs first. Patients removed from study for unacceptable AE(s) will be followed until resolution or stabilization of the AE. Unacceptable toxicity is defined as any of the following:

1) grade 3 or 4 adverse event requiring the patient to be removed from study treatment,
2) DLT's of grade 1 treatment related ocular toxicity, grade 2 allergic reactions,
3) >2 cm ulceration at the vaccination site or a vaccination adverse event requiring antibiotic treatment, debridement or narcotic management,

NOTE: Chronic inflammatory reactions at the vaccine sites are expected to occur in all participants who receive Montanide ISA-51. Induration may persist for days to months, but is not expected to require additional therapy. Sterile abscesses may occur in some participants. These are not a basis for discontinuation of the vaccines. However, if the inflammatory reactions develop significant ulceration of the skin (> 2 cm), then the patient would be taken off the study, however, this would not be considered a dose-limiting toxicity.
4) any unexpected grade 3 or greater non-hematologic or hematologic toxicity.

5.6 Criteria for Removal from Study

Patients will be removed from study when any of the criteria listed in Section 5.4 applies. The reason for study removal and the date the patient was removed must be documented in the case report form. The patient should be followed per protocol requirements.

AEs will be described and coded based upon the National Cancer Institute Common Terminology Criteria for Adverse Events v4.0 (NCI CTCAE v4.0). Safety will be evaluated by assessing and chronicling treatment-related AEs.

6. DOSING DELAYS/DOSE MODIFICATIONS

All toxicity associated with prior treatment must resolve to grade 1 or less off steroid treatment for at least 2 weeks. Patients who have discontinued treatment for pulmonary, renal or neurologic toxicity or who have experienced grade 4 toxicity should not be enrolled in the study.

All related toxicities will be reviewed by the protocol P.I., participating site P.I.s, CTEP and the CITN Coordinating Center for an indication to change the dosing schedule.

The selected doses of INCB024360 and MELITAC 12.1 have been established as having excellent safety profiles. Dose escalation is not planned or necessary. However, when used together, unexpected toxicities might occur. Thus, rules for stopping will be incorporated in the event that dose-limiting AEs are observed.

Toxicities for each agent will be monitored continuously and will be graded by the NCI CTCAE v4.0 with stopping rules for AEs. At the discretion of the investigators, treatment may be held for up to 14 days to permit the evaluation or resolution of symptoms that may be unrelated to treatment but may affect patient safety (e.g., influenza, minor trauma).

DLT's include all Grade 3 AEs regardless of investigator attribution or relatedness except for expected grade 3 injection site reactions. (section 5.5)
Based on investigator determination the following Grade 3 toxicities can be deemed non-DLT:

- adverse event with a clear alternative explanation (e.g. due to disease progression)
- transient toxicities (≤72 hours)
- injection site reactions that are expected and do not require any surgical intervention or repair

- abnormal laboratory values without associated clinically significant signs or symptoms

There are two exceptions to the above: Grade 1 or greater ocular toxicities will be considered a DLT. Grade 2 LFT elevations will be considered a DLT.

In the event of a DLT, it will be determined by the safety team whether both the INCB024360 AND the MELITAC 12.1 will be permanently discontinued.

Vitiligo will not be considered a DLT.

Pages 33, 34 and 35 include tables for some of the more common AE's including instructions for handling. For any AE's not listed in these tables, proceed in the following manner:
Grade 1 and Grade 2: Continue to monitor, follow and grade these adverse events.
Grade 3: Stop Protocol Therapy including both INCB024360 and MELITAC 12.1
Grade 4: Stop Protocol Therapy including both INCB024360 and MELITAC 12.1

In addition, treatment will be discontinued for (a) Any DLT, (b) disease progression requiring other therapy, (c) noncompliance with study requirements, (d) other safety considerations in the judgment of the investigator, or (e) patient request.

The treatment regimen will be considered safe as long as the number of participants experiencing a treatment-related AE (DLT) does not exceed 33%. If more than 33% of patients accrued are known to have experienced a treatment-related DLT, the study will be closed to further accrual, except that 1 treatment related DLT observed out of the first 3 patients accrued will be acceptable and will not result in the closing of the study.

Nausea	**Management/Next Dose for INCB024360**	**Management/Next Dose for MELITAC 12.1**
≤ Grade 1	No change in dose	No change in dose
Grade 2	No change in dose	No change in dose
Grade 3	Off Protocol Therapy	Off Protocol Therapy
Grade 4	Off protocol therapy	Off protocol therapy
Recommended management: antiemetics		

Vomiting	**Management/Next Dose for INCB024360**	**Management/Next Dose for MELITAC 12.1**
≤ Grade 1	No change in dose	No change in dose
Grade 2	No change in dose	No change in dose
Grade 3	Off Protocol Therapy	Off Protocol Therapy
Grade 4	Off protocol therapy	Off protocol therapy
Recommended management: antiemetics		

Investigations (Liver Function Tests ALT + AST)	**Management/Next Dose for INCB024360**	**Management/Next Dose for MELITAC 12.1**
< Grade 1	No change in dose	No change in dose
Grade 1*	No change in dose	No change in dose

Investigations (Liver Function Tests ALT + AST)	**Management/Next Dose for INCB024360**	**Management/Next Dose for MELITAC 12.1**
Grade 2	Off Protocol Therapy	Off Protocol Therapy
Grade 3 or 4	Off Protocol Therapy	Off Protocol Therapy
***For Grade 1 LFT Elevations, increase LFT monitoring to twice weekly.**		

Diarrhea	**Management/Next Dose for INCB024360**	**Management/Next Dose for MELITAC 12.1**
≤ Grade 1	No change in dose	No change in dose
Grade 2	No change in dose	No change in dose
Grade 3	Off Protocol Therapy	Off Protocol Therapy
Grade 4	Off protocol therapy	Off protocol therapy
Recommended management: physician discretion		

Neutropenia	**Management/Next Dose for INCB024360**	**Management/Next Dose for MELITAC 12.1**
≤ Grade 1	No change in dose	No change in dose
Grade 2	No change in dose	No change in dose
Grade 3	Off Protocol Therapy	Off Protocol Therapy
Grade 4	Off protocol therapy	Off protocol therapy
Recommended management: treatment of infection as appropriate		

Thrombocytopenia	**Management/Next Dose for INCB024360**	**Management/Next Dose for MELITAC 12.1**
≤ Grade 1	No change in dose	No change in dose
Grade 2	No change in dose	No change in dose
Grade 3	Off protocol therapy	Off protocol therapy
Grade 4	Off protocol therapy	Off protocol therapy
Recommended management: platelet transfusions if indicated		

Fatigue	**Management/Next Dose for INCB024360**	**Management/Next Dose for MELITAC 12.1**
≤ Grade 1	No change in dose	No change in dose
Grade 2	No change in dose	No change in dose
Grade 3 or Grade 4	Off Protocol Therapy	Off Protocol Therapy
Recommended management: physician discretion		

Ocular Toxicity	**Management/Next Dose for INCB024360**	**Management/Next Dose for MELITAC 12.1**
≤ Grade 1	Off protocol therapy*	Off protocol therapy
Grade 2	Off protocol therapy	Off protocol therapy
Grade 3	Off protocol therapy	Off protocol therapy
Grade 4	Off protocol therapy	Off protocol therapy
*Defined as treatment-related grade 1 ocular toxicities		

Chills/fever/malaise following immunization	**Management/Next Dose for INCB024360**	**Management/Next Dose for MELITAC 12.1**
≤ Grade 1	No change in dose	No change in dose
Grade 2	No change in dose	No change in dose
Grade 3	Off protocol therapy if fails to resolve by the next scheduled immunization	Off protocol therapy if fails to resolve by the next scheduled immunization
Grade 4	Off protocol therapy	Off protocol therapy

Injection site reaction	**Management/Next Dose for INCB024360**	**Management/Next Dose for MELITAC 12.1**
≤ Grade 1	No change in dose	No change in dose
Grade 2	No change in dose	No change in dose
Grade 3	No change in dose*	No change in dose**
Grade 4	Off protocol therapy	Off protocol therapy

*See section 5.2
** MELITAC 12.1 vaccine may be given if the grade 3 injection site reaction combined area of skin ulceration is <2 cm

6.1 Delayed Visits for Reasons Other Than Toxicity

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

Table 6.1 defines what constitutes a delayed visit for reasons other than toxicity, whether the participant should continue to be treated, and whether a protocol violation should be reported and recorded. The range of days is counted from the original scheduled date.

Table 6.1. Delayed Visit for Reasons Other than Toxicity

Treatment Period	**Range of Days**	**Participant Treatment**	**Protocol Deviation**
Biopsies *Day 0*	Days -14 to 0*	Biopsies/Labs	
*Vaccine 1, Biopsies*** Day 21 (week 3)	± 2 days ± 3 to 7 days ± 8 or more days	Vaccine/Labs Vaccine/Labs Labs	No Yes Yes
Vaccines 2 Day 28 (week 4)	± 2 days ± 3 to 7 days ± 8 or more days	Vaccine/ /Biopsy Vaccine/ /Biopsy Labs	No Yes Yes
Vaccine 3 Day 35 (week 5)	± 2 days	Vaccine/	No

Treatment Period	Range of Days	Participant Treatment	Protocol Deviation
	± 3 to 7 days	Vaccine/	Yes
	± 8 or more days	Labs	Yes
*Biopsies***			
Day 42 (week 6)	± 2 days	Labs	No
	± 3 to 7 days	Labs	Yes
	± 8 or more days	Labs	Yes
*Vaccines 4–6***			
Days 56, 77, 98	± 2 days	Vaccine/Labs***	No
(weeks 8, 11, 14)	± 3 to 7 days	Vaccine/Labs***	Yes
	± 8 or more days	Labs	Yes
*Biopsies (Optional)***			
Day 112 (Week 16)	± 7 days	Labs	No
	± 8 to 14 days	Labs	Yes
	± 15 or more days	Labs	Yes
Follow-up			
Week 26	± 14 days	Scans/Labs	No
	± 15 days or more	Scans/Labs	Yes

* The pretreatment biopsies should be performed before the first treatment with INCB024360, within 14 days of that start date. For patients with only one or two tumors approachable for biopsy, available tumor blocks from prior biopsies can serve as the pretreatment sample provided at least 20 unstained slides are available.

**A participant will be taken off protocol treatment if more than 1 vaccination is delayed [± 3 to 7 days] during the treatment period.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting (via CTEP-AERS) **in addition** to routine reporting.

7.1 Comprehensive Adverse Events and Potential Risks List(s) (CAEPRs)

7.1.1 **CAEPRs for CTEP IND Agent(s):** N/A

7.1.2 **CAEPRs for INCB024360** (NSC 766086)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI via CTEP-AERS (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements'

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. The CAEPR does not provide frequency data; refer to the Investigator's Brochure for this information. Below is the CAEPR for INCB024360.

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Adverse Events with Possible Relationship to INCB024360 (CTCAE 4.0 Term) **Version 1.1, October 15, 2015**	**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
GASTROINTESTINAL DISORDERS	
Abdominal distension	
Abdominal Pain	***Abdominal pain (Gr 2)***
Constipation	***Constipation (Gr 2)***
Diarrhea	***Diarrhea (Gr 2)***
Nausea	***Nausea (Gr 2)***
Vomiting	***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	
Edema limbs	***Edema limbs (Gr 2)***
Fatigue	***Fatigue (Gr 2)***
Fever	***Fever (Gr 1)***
Pain	***Pain (Gr 2)***
IMMUNE SYSTEM DISORDERS	
Autoimmune disorder[2]	
INVESTIGATIONS	
Alanine aminotransferase increased	***Alanine aminotransferase increased (Gr 1)***
Aspartate aminotransferase increased	***Aspartate aminotransferase increased (Gr 1)***
Weight loss	***Weight loss (Gr 2)***
METABOLISM AND NUTRITION DISORDERS	

Anorexia	***Anorexia (Gr 2)***
Dehydration	***Dehydration (Gr 2)***
Hypokalemia	***Hypokalemia (Gr 2)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	
Arthralgia	
Back Pain	***Back pain (Gr 2)***
NERVOUS SYSTEM DISORDERS	
Dizziness	
Dysgeusia	
Headache	
Peripheral sensory neuropathy	***Peripheral sensory neuropathy (Gr 2)***
PSYCHIATRIC DISORDERS	
Insomnia	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	
Cough	***Cough (Gr 1)***
Dyspnea	***Dyspnea (Gr 1)***
Hypoxia	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	
Rash maculo-papular	***Rash maculo-papular (GR 2)***

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]INCB024360 can result in severe and potentially fatal immune-mediated adverse events. These include (but are not limited to) autoimmune hepatitis and autoimmune hypophysitis.

Adverse events reported on INCB024360 trials, but for which there is insufficient eveidence to suggest that there was a reasonable possibility that INCB024360 caused the adverse event:

Investigations – GGT increased

Metabolism and nutrition disorders – Hypernatremia
Respiratory, thoracic and mediastinal disorders – Pneumonitis
Vascular disorders - Hypertension

Note: INCB024360 in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

No AEs have been reported consistent with the serotonin syndrome.

7.1.2.1 Suspected, Unexpected Serious Adverse Reactions

A single suspected, unexpected, serious adverse reaction (SUSAR) reported in any ongoing clinical study of INCB024360 up to the clinical cutoff date for data inclusion in the Investigator Brochure (03 December 2013) is listed in Table 7.1.2.1.

Table 7.1.2.1. Adverse Events Occurring in ≥20% of Subjects

MedDRA System Organ Class	Worst Seriousness Criteria	Preferred Term	Study Drug
Injury, poisoning, and procedural complications	Hospitalized	Radiation pneumonitis	INCB024360
General disorders and administration site conditions	Hospitalized	Pyrexia	INCB024360
General disorders and administration site conditions	Hospitalized	Pyrexia	INCB024360
General disorders and administration site conditions	Hospitalized	Abdominal pain	INCB024360
Infections and infestations	Hospitalized	Pneumonia	INCB024360
Infections and infestations	Hospitalized	Colitis	INCB024360+ ipilimumab
Infections and infestations	Medically significant	Infection	INCB024360 + ipilimumab
Investigations	Medically significant, hospitalized	Alanine aminotransferase increased	INCB024360 + ipilimumab
Investigations	Medically significant, hospitalized	Aspartate aminotransferase increased	INCB024360 + ipilimumab
Investigations	Medically significant	Alanine aminotransferase increased	INCB024360 + ipilimumab
Investigations	Medically significant	Aspartate aminotransferase increased	INCB024360 + ipilimumab

Abbreviations: MedDRA, Medical Dictionary for Regulatory Activities.

7.1.3 CAEPRs for MELITAC 12.1

7.1.3.1 12-MP and Peptide-tet vaccine

There is no reason to expect direct toxicity of the melanoma peptides, as they are not directly cytotoxic in vitro. On the other hand, because some of these peptides are identical or similar to a portion of a normal protein, risks of autoimmunity in humans are important to evaluate. Because no murine system adequately models the human immune response to these peptides, the most meaningful evaluation of this peptide vaccine mixture is in participants with melanoma. We will focus this study on participants with advanced melanoma (i.e., unresectable stage III or stage IV melanoma). These individuals face a high risk (>90%) of premature death, and the anticipated risk of short-term or long-term toxicity of this vaccine preparation is minimal (Table 7.1.4.1), although it might delay or decrease the risk of morbidity and mortality due to melanoma in these participants. The potential implications of autoimmunity against cells of melanocytic lineage are illustrated by reported cases of vitiligo occurring coincident with regressions of melanoma. Most of these are limited, often occurring in skin surrounding the regressing melanoma, but occasionally occurring systemically. While pathogenesis of this phenomenon can only be hypothesized, it is reasonable to consider this a worst-case scenario.

****All participants should be closely observed for AEs for at least 20 minutes after each vaccination. Any time thereafter, participants should report any AEs to their treating physician.**

Table 7.1.4.1. Toxicity Data for Mel 39 (Arm B) and Mel 43 (Arms B and D)

			Grade, n		
Description	**N**	**%**	**1**	**2**	**3**
Overall number of Participants	51	100	1	35	15
Allergy/Immunology	6	12	5	1	.
Allergic rhinitis	4	8	3	1	.
Autoimmune reaction	2	4	2	.	.
Blood/Bone Marrow	8	16	7	1	.
Hemoglobin	4	8	4	.	.
Hemoglobin	2	4	2	.	.
Lymphopenia	2	4	1	1	.
Cardiovascular (General)	2	4	2	.	.
Edema	2	4	2	.	.
Constitutional Symptoms	43	84	27	12	4
Constitutional symptoms, other	1	2	1	.	.
Fatigue (lethargy, malaise, asthenia)	42	82	27	11	4
Fever (in the absence of neutropenia)	14	27	11	3	.
Rigors, chills	27	53	25	2	.
Sweating (diaphoresis)	16	31	16	.	.
Dermatology/Skin	51	100	3	35	13
Alopecia	1	2	1	.	.
Bruising	1	2	1	.	.
Dermatology/Skin, other	4	8	3	1	.
Flushing	10	20	10	.	.

			Grade, n		
Description	**N**	**%**	**1**	**2**	**3**
Hand-foot syndrome	1	2	1	.	.
Injection site reaction	51	100	3	35	13
Pigmentation changes (e.g., vitiligo)	1	2	1	.	.
Pruritus	3	6	3	.	.
Rash	2	4	1	1	.
Rash/desquamation	1	2	1	.	.
Wound, noninfectious	4	8	4	.	.
Endocrine	2	4		.	.
Endocrine, other	1	2	1	.	.
Hyperthyroidism	1	2	1	.	.
Gastrointestinal	26	51	23	3	.
Anorexia	11	22	9	2	.
Constipation	1	2	1	.	
Diarrhea patients without colostomy	5	10	5	.	.
Flatulence	1	2	1	.	.
Gastrointestinal, other	1	2	1	.	.
Heartburn	2	4	2	.	.
Mouth dryness	1	2	1	.	.
Mucositis, oral cavity	2	4	2	.	.
Nausea	14	27	12	2	.
Stomatitis/pharyngitis	3	6	3	.	.
Vomiting	3	6	3	.	.
Hemorrhage	2	4	2	.	.
Hemorrhage pulmonary, nose	1	2	1	.	.
Rectal bleeding/hematochezia	1	2	1	.	.
Hepatic	7	14	6	1	.
Alkaline phosphatase	4	8	4	.	.
SGOT (AST)	3	6	3	.	.
SGPT (ALT)	2	4	1	1	.
Infection/Febrile Neutropenia	2	4	.	2	.
Infection, cellulitis	1	2	.	1	.
Infection without neutropenia	1	2	.	1	.
Lymphatics	6	12	6	.	.
Edema: limb	4	8	4	.	.
Lymphatics	1	2	1	.	.
Lymphatics, other	3	6	3	.	.
Metabolic/Laboratory	27	53	21	6	.
Creatinine	2	4	.	2	.
GFR	1	2	1	.	.
Hyperglycemia	19	37	16	3	.
Hyperkalemia	1	2	1	.	.
Hypernatremia	1	2	1	.	.
Hypocalcemia	2	4	2	.	.
Hypoglycemia	4	8	4	.	.
Hypokalemia	2	4	2	.	.
Hypomagnesemia	3	6	3	.	.
Hyponatremia	1	2	1	.	.
Hypophosphatemia	1	2	.	1	.

			Grade, n		
Description	**N**	**%**	**1**	**2**	**3**
Metabolic/Lab, other	1	2	1	.	.
SGPT (ALT)	1	2	1	.	.
Musculoskeletal	5	10	4	1	.
Musculoskeletal, other	5	10	4	1	.
Neurology	13	25	10	3	.
CNS ischemia	1	2	.	1	.
Confusion	1	2	1	.	.
Dizziness	5	10	4	1	.
Dizziness/lightheadedness	3	6	3	.	.
Memory loss	1	2	1	.	.
Mood alteration, agitation	1	2	1	.	.
Mood alteration, anxiety	1	2	.	1	.
Mood alteration, depression	1	2	1	.	.
Neuropathy, sensory	2	4	2	.	.
Syncope (fainting)	2	4	2	.	.
Ocular/Visual	3	6	3	.	.
Dry eye	1	2	1	.	.
Ocular, other	2	4	2	.	.
Ocular surface disease	1	2	1	.	.
Pain	40	78	27	13	.
Arthralgia (joint pain)	17	33	14	3	.
Headache	24	47	21	3	.
Myalgia (muscle pain)	24	47	15	9	.
Pain, abdomen NOS	1	2	1	.	.
Pain, back	3	6	3	.	.
Pain extremity, limb	2	4	2	.	.
Pain, throat/pharynx/larynx	1	2	1	.	.
Pain, other	5	10	4	1	.
Pulmonary	12	24	6	4	2
Cough	7	14	5	2	.
Dyspnea (shortness of breath)	6	12	3	1	2
Pneumonitis	1	2	.	1	.
Pulmonary, other	2	4	2	.	.
Voice changes/strider/larynx	3	6	3	.	.
Renal/Genitourinary	3	6	3	.	.
Creatinine	2	4	2	.	.
Renal, other	1	2	1	.	.
Sexual/Reproductive Function	1	2	.	1	.
Vaginitis	1	2	.	1	.

Montanide ISA-51 VG

The adjuvant Montanide ISA-51 VG has been used in a number of clinical trials with little toxicity observed. The possible side effects of receiving Montanide ISA-51 VG adjuvant include local vaccine-site skin reaction. In addition, a theoretical risk exists for anaphylaxis in response to one of the ingredients in Montanide ISA-51 VG. Such a

reaction might include hives, rash, generalized swelling of the body, hypotension, dyspnea, and abdominal pain, and possibly death.

7.1.4 **CAEPRs for Commercial Agent:** N/A

7.1.5 Adverse Event List(s) for CIP (e.g., Study-Specific) Commercial Imaging Agents: N/A

7.2 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 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 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 that are ***bold and italicized*** in the CAEPR (*i.e.*, those listed in the SPEER column, Section 7.1.1) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.
  - Other AEs for the protocol that do not require expedited reporting are outlined in Section 7.3.4.

- **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.

7.3 Expedited Adverse Event Reporting

7.3.1 Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP Web site (http://ctep.cancer.gov). The reporting procedures to be followed are presented in the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" which can be downloaded from the CTEP Web site (http://ctep.cancer.gov). These requirements are briefly outlined in the tables below (Section 7.3.3).

In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.

7.3.2 CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Study Coordinator of the Lead Organization, Principal Investigator, and the local treating physician. CTEP-AERS provides a copy feature for other e-mail recipients.

7.3.3 **Expedited Reporting Guidelines**

Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.

Death due to progressive disease should be reported as **Grade 5 "Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (Progressive Disease)"** under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (e.g., radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Early Phase 2 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria MUST be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3–5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days	24-Hour 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR.

Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" - The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" - A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:

Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 3, 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

[2]For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

7.3.4 Additional Protocol-Specific Expedited Adverse Event Reporting Exclusions*:* N/A

7.4 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions. **AEs reported through CTEP-AERS must <u>also</u> be reported in routine study data submissions.**

7.5 Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (e.g., acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

7.6 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine reporting via CDUS unless otherwise specified.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational agent, vaccine and adjuvant administered in this study can be found in Section 7.1.

8.1 CTEP IND Agent(s): N/A

8.2 INCB024360, Inhibitor of Indoleamine 2,3-dioxygenase (IDO, NSC # 766086)

8.2.1 Product Description

The INCB024360 drug substance is a white to off-white powder. INCB024360 is formulated as an immediate-release tablet in 3 strengths (25 mg, 100 mg, and 300 mg). The tablets contain the active drug (INCB024360) along with commonly used compendial excipients. The chemical name of INCB024360 is (*Z*)-*N*-(3-Bromo-4-fluorophenyl)-*N*′-hydroxy-4-(2-(sulfamoylamino) ethylamino)-1,2,5-oxadiazole-3-carboximidamide. INCB024360 has a molecular formula of C11H13BrFN7O4S and a molecular weight of 438.23.

The predicted clinical plasma Cmax is ≤10 μM for a pharmacologically active dose of INCB024360.

8.2.2 Storage Conditions

The INCB024360 drug product should be stored at ambient conditions (15°–30°C or 59°-86°F).

8.2.3 Stability

The INCB024360 drug substance has been shown to be stable for at least 6 months at accelerated ICH conditions of 40°C/75% relative humidity and at least 24 months at long-term storage conditions of 25°C/60% relative humidity. INCB024360 tablets (25 mg and 100 mg) are stable for at least 6 months at accelerated ICH conditions and for at least 12 months at long-term storage conditions. INCB024360 300-mg tablets are stable for at least 1 month at accelerated ICH conditions and long-term storage conditions. Stability of INCB024360 drug substance and tablets will be monitored to support ongoing clinical trials.

8.2.4 Route of Administration

Administration of INCB024360 is by mouth twice daily, taken on an empty stomach at least one hour prior or two hours after a meal.

8.2.5 **Overdosage**

No clinical experience with overdosage of INCB024360 has been reported. Treatment of overdosage should consist of general supportive measures.

8.2.6 **Intended Indications**

The intended indication for INCB024360 is for the treatment of malignant diseases.

8.2.7 **Contraindications**

INCB024360 is contraindicated in subjects with clinically significant hypersensitivity to any component of its formulation.

8.2.8 **Carcinogenesis, Mutagenesis, and Impairment of Fertility Studies**

INCB024360 was not genotoxic in a non-GLP (good laboratory practice) *Salmonella* Plate Incorporation Assay. Fertility studies and carcinogenicity studies have not been performed.

8.2.9 **Usage in Pregnancy**

INCB024360 should not be used by pregnant women. Studies to evaluate the potential for embryo toxicity and teratogenicity have not been performed.

8.2.10 **Nursing Mothers**

It is not known whether INCB024360 passes into human milk. INCB024360 should not be taken by nursing mothers.

8.2.11 **Availability**

INCB024360 is an investigational agent supplied to investigators by Incyte Corporation (Wilmington, DE).
Incyte Corporation will provide a limited stock supply of INCB024360 to each institution participating in protocol CITN04 prior to the first participant being enrolled.

8.2.12 **Potential Drug Interactions:**

Metabolism of INCB024360 occurs via UGT1A9 isoenzymes and via glucuronidation. Concomitant use of potent UGT1A9 inducers or inhibitors are prohibited. In vitro data suggests INCB024360 is a substrate and weak inhibitor of P-glycoprotein. Interaction studies have not been performed, but INCB024360 is believed to have low potential to cause drug-drug interactions through P-gp interaction. INCB024360 may cause an increase in serotonin levels which has the potential to precipitate serotonin syndrome when administered in combination with monoamine oxidase (MAO) inhibitors or

serotonergic agents. Concomitant use of MAO inhibitors is prohibited.

Use of coumadin is discouraged. Low-dose Coumadin (1 mg) is acceptable; however, doses that increase the INR are discouraged and will require dose modification. If an alternative to coumarin-based anticoagulants cannot be used, dose adjustment of the Coumadin may be needed. Based on the observed magnitude of epacadostat (INCB024360)/warfarin PK interaction and PK/PD modeling results, for an epacadostat dose of 300 mg BID, the dose of warfarin should be reduced by approximately one-third after initiation of epacadostat (INCB024360) administration based on approximately 30% to 40% reduction in S- and R-warfarin oral clearance values. Close INR monitoring is recommended for patients on a stable dose of warfarin who are starting treatment with epacadostat. Based on PK/PD modeling, recommendations for warfarin dose modifications for subjects receiving other epacadostat doses, are summarized in the table below based on the INR prior to starting epacadostat. Refer to protocol eligibility criteria for additional information.

Stable Baseline INR	**Epacadostat (INCB024360) Dose**		
	< 100 mg BID	**200 mg BID**	**300 mg BID**
INR ≤	Close INR monitoring	Close INR monitoring	Reduce warfarin by ~33% and monitor INR
INR ≥	Close INR monitoring	Reduce warfarin by 20-25% and monitor INR	Reduce warfarin by ~ 33% and monitor INR

8.3 MELITAC 12.1

8.3.1 Product Description

The MELITAC 12.1 vaccine has 3 components: 12-MP peptides, Peptide-tet, and Montanide ISA-51 VG vaccine adjuvant, which will be prepared together as an emulsion for administration.

12-MP is a multipeptide mixture containing 12 class I MHC-restricted peptides derived from melanocyte differentiation proteins (MDP) and cancer testis antigens (CTA). The 12 peptides included in the vaccine are restricted to HLA-A1, -A2, or -A3 (Table 8.3.1.1).

Table 8.3.1.1. 12-MP Peptides

Allele	**Sequence**	**Epitope**
HLA-A1	DAEKSDICTDEY	Tyrosinase $_{240-251}$ *
	SSDYVIPIGTY	Tyrosinase $_{146-56}$
	EADPTGHSY	MAGE-A1 $_{161-169}$
	EVDPIGHLY	MAGE-A3 $_{168-176}$
HLA-A2	YMDGTMSQV	Tyrosinase $_{369-377}$ ♦
	IMDQVPFSV	gp100 $_{209-217}$ #
	YLEPGPVTA	gp100 $_{280-288}$

	GLYDGMEHL	MAGE-A10 $_{254-262}$
HLA-A3	ALLAVGATK	gp100 $_{17-25}$
	LIYRRRLMK	gp100 $_{614-622}$
	SLFRAVITK	MAGE-A1 $_{96-104}$
	ASGPGGGAPR	NY-ESO-1 $_{53-62}$

***** (substitution of S for C at residue 244)
♦ (post-translational change of N to D at residue 371)
(209-2M, substitution of M for T at position 210)

Peptide-tet is a slightly modified form of a 15 residue peptide p2 of tetanus toxoid (QYIKANSKFIGITEL), representing amino acid residues 830-844. This peptide binds to HLA-DR1, DRw15(2), DRw18(3), DR4Dw4, DRw11(5), DRw13(w6), DR7, DRw8, DR9, DRw52a, and DRw52b, which account for 80–90% of the patient population. Because glutamine (Q) at the N-terminus can cyclize, giving rise to a slight change in molecular structure, we have prepared this peptide as a 16-residue species, where an alanine residue (A) has been added to the N-terminus. Thus, the sequence of the tetanus peptide used is AQYIKANSKFIGITEL (Table 8.3.1.2).

Table 8.3.1.2. Tetanus Toxoid-derived Helper Peptide

Allele	**Sequence**	**Epitope**
Binds to multiple class II alleles	AQYIKANSKFIGITEL	$p2_{830-844}$**

All peptide preparations are lyophilized and stored in sterile vials sealed with a rubber stopper. The peptides are stored at ≤-70^{o}C and protected from light. Each lot of peptide has met the lot release criteria for identity, purity, trifluoroacetic acid (TFA) content, potency, pyrogenicity, general safety, and sterility. Human studies investigating the pharmacokinetics and metabolism of the 12-MP and Peptide-tet vaccines have not been conducted.

8.3.2 **Agent Supply**

8.3.2.1 12-MP

The 12-MP mixture is supplied in sterile single-use vials of lyophilized white powder containing a mixture of the 12 peptides and salts to buffer the solution once reconstituted. Each vial of 12-MP contains 150 μg each of the 12 melanoma peptides. All peptides are synthesized under GMP conditions by Multiple Peptide Systems (San Diego, CA). The peptides were vialed under GMP conditions by Clinalfa Line (Merck Biosciences AG, Laufelfingen, Switzerland). These 12-MP vials are provided by the University of Virginia. The University of Virginia will be the distributor of the agent to the research sites.

8.3.2.2 Peptide-tet

Peptide-tet is supplied in sterile single-use vials of white powder. Each vial of Peptide-tet contains 300 μg of tetanus peptide. The peptide was synthesized under GMP conditions by Multiple Peptide Systems (San Diego, CA). The peptides were vialed under GMP conditions by Clinalfa Line (Merck Biosciences AG, Laufelfingen, Switzerland). The agent is provided by the University of Virginia. The University of Virginia will be the distributor of the agent to the research sites.

8.3.3 Solution Preparation

All vaccines are to be made in a certified, sterile laminar flow hood. The emulsion will be prepared as 3-mL quantities: 2 mL of this emulsion will be administered, with 1 mL of emulsion administered at each of 2 vaccine sites. At each site, half of each dose (0.5 mL) will be injected subcutaneously and half (0.5 mL) will be injected intradermally. The remaining 1 mL of the prepared emulsion is to be discarded.

The 2-mL dose of each vaccine contains 100 μg each of the 12 peptides listed in Table 1 of Appendix E and 200 μg of tetanus peptide (Table 2 of Appendix E), emulsified in 1 mL of Montanide ISA-51 VG adjuvant. See Appendix E for complete solution preparation instructions.

8.3.4 Storage Requirements

Vials of lyophilized peptides should be stored at ≤-70°C and protected from light. All peptide preparations are lyophilized and stored in sterile vials sealed with a rubber stopper. Each lot of peptide has met the lot release criteria for identity, purity, TFA content, potency, pyrogenicity, general safety, and sterility.

Ideally, vials of the 12-MP and Peptide-tet should be thawed just before preparing a vaccine (i.e., thawed at room temperature for 1–2 minutes before reconstitution). Repeated freeze-thaws should be avoided. Vials that are frozen and thawed >2 times should not be used. Vials should not be left at room temperature for more than 24 hours.

8.3.5 Stability

All peptide preparations will be assayed for stability at months 3, 6, 12, 24, and 36. High-performance liquid chromatography will be performed to confirm purity and sterility testing will be conducted in accord with FDA guidelines in 21 CFR, part 610.12. In addition, 1 vial of peptide will be submitted to either the Clinical Microbiology Laboratory at the University of Virginia or Microbiology Research Associates, Inc. (Acton, MA).

MELITAC 12.1 vaccine comprises 100 μg each of the 12-MP mixture plus 190 μg of Peptide-tet. Peptides are synthesized under GMP conditions by PolyPeptide Group (formerly Multiple Peptide Systems), reconstituted, mixed, sterile-filtered, and vialed under GMP conditions by Merck. The 12–melanoma peptide mixture and tetanus peptide preparation are made as separate sterile aqueous solutions to be administered in an emulsion with Montanide ISA-51 VG adjuvant (1 mL), using the 2-syringe method after emulsion stability and quality is verified.
After a peptide-based vaccine is mixed, the prepared vaccine should be refrigerated until just before administration. Ideally, the vaccine should be administered within 1–2 hours after mixing. The vaccine must be administered within 4 hours of mixing. If the vaccine is not administered within 4 hours of mixing, it should be discarded.

8.3.6 Route of Administration

All participants will receive 6 vaccination times over 12 weeks with MELITAC 12.1 (100 μg of each of 12 Class I MHC restricted melanoma peptides) (Table 1 of Appendix E) and 200 μg of a tetanus helper peptide (Table 2 of Appendix E) emulsified in Montanide ISA-51 VG adjuvant. In a given patient, half of the vaccine solution will be administered subcutaneously and half intradermally, once each week (vaccines 1–3), then once every 3 weeks (vaccines 4–6), in rotating skin locations on extremities clinically uninvolved with melanoma. For each vaccine, a total of 2 mL of emulsion will be delivered, which will consist of 1 mL of Montanide ISA-51 VG and the MELITAC 12.1 peptides. The vaccine dose will be divided and administered into 2 extremity sites uninvolved with melanoma (typically upper outer arm and/or upper lateral thigh).

At each injection site, a single needle puncture is to be performed, with delivery of 0.5 mL into the dermis and 0.5 mL into the subcutaneous tissue. This usually will require advancing the needle into the dermis, parallel with the skin surface, to deliver the first 0.5 mL, then pulling the needle back to near the skin puncture site, and advancing it into the subcutaneous tissue for delivery of the remaining 0.5 mL. For the dermal injections, it is acceptable to make several passes of the needle as the emulsion is delivered, where those needle passes are oriented radially from the needle puncture site.

8.4 Montanide ISA-51 VG

8.4.1 Product Description

The adjuvant Montanide ISA-51 VG has been used in a number of clinical trials with little toxicity observed. The possible side effects of receiving Montanide VG adjuvant include anaphylaxis in response to one of the ingredients (rare). Such a reaction might include hives, rash, generalized swelling of the body, hypotension, dyspnea, and abdominal pain, and possibly death.

8.4.2 How Supplied

Montanide ISA-51 VG is supplied in a glass container as a sterile, clear, pale yellow liquid by Seppic, Inc. (Fairfield, NJ). It is commercially available from Seppic, Inc., and will be distributed by the University of Virginia.

8.4.3 Solution Preparation

The vaccine adjuvant Montanide ISA-51 VG is prepared as a GMP-grade agent, in single-use glass vials or ampules. Refer to Appendix E for complete vaccine preparation instructions.

8.4.4 Storage Conditions

Montanide ISA-51 VG should be stored at room temperature.

8.4.5 **Stability**

An expiration date for Montanide ISA-51 VG is provided by Seppic, Inc., and the drug should not be used beyond this date.

8.4.6 **Route of Administration**

Refer to Appendix E for complete vaccine preparation instructions.

8.5 Commerical Agent: N/A

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Biomarker Studies

9.1.1 **Collection of Specimens**

9.1.1.1 Fine-Needle Aspiration (FNA) Biopsy of Tumor
FNA biopsy will be prepared as detailed below and stored frozen in liquid nitrogen.

9.1.1.2 Tumor Biopsies
Biopsy specimens must include at least 100 mm^3 of tumor tissue and ideally 1 cm^3 or more. The specimens may be obtained by excisional biopsy, incisional biopsy, or core biopsy. Each surgical specimen or set of core biopsies will be divided into 5 portions: $1/4^{th}$ will be placed in RPMI and will be processed to yield cryopreserved viable cells for flow cytometric analysis, $1/4^{th}$ will be fixed in formalin and then embedded in paraffin for histology, $1/6^{th}$ will be flash frozen in OCT for histology, $1/6^{th}$ will be flash frozen without medium for Kyn/Trp ratio and $1/6^{th}$ will be flash frozen in RNA Later for gene expression. (See Laboratory Manual for additional information).

If an archived pre-treatment biopsy is obtained from a formalin-fixed paraffin-embedded (FFPE) tissue block, there must be at least 20 slides available for study.

9.1.1.3 Blood
Blood will be collected for serum and PBMC isolation as designated below.

9.1.2 **Processing and Shipping of Specimen(s)**

Tumor specimens will be collected as described above, at the bedside when tumor is biopsied. They will then be processed and shipped as specified below.

9.1.2.1 Portion for single-cell suspensions
This will be submitted to mechanical dissociation then, as appropriate, enzymatic digestion (typically collagenase, DNAase, and hyaluronidase). The resulting cells will be collected by centrifugation and cryopreserved viably in 10% dimethyl sulfoxide (DMSO) and either 12.5% HSA (Human serum albumin or 12.5% FCS (Fetal calf serum) using a

controlled-rate freezer. Samples will be stored at -80°C and then batch-shipped on dry ice to the CITN Central Laboratory.

9.1.2.2 Portion for Formalin Fixation

These will be placed in formalin, and shipped (ambient) overnight to the CITN Central Laboratory. After adequate fixation, samples will be embedded in paraffin and formalin-fixed paraffin embedded (FFPE) blocks stored at room temperature.

9.1.2.3 Portion for Quick-Freezing in OCT

This will be placed in OCT at the patient bedside and placed immediately onto dry-ice for hardening. Samples will be stored at -80°C and then batch-shipped on dry ice to the CITN Central Laboratory.

9.1.2.4 Portion for Quick-Freezing Without Media

This will be placed in a cryovial and placed immediately into liquid nitrogen for flash freezing. Samples will be stored at -80°C or in liquid nitrogen and then batch-shipped on dry ice to the CITN Central Laboratory.

9.1.2.5 Blood Samples

A) Blood in red-top (or comparable) tubes for serum isolation and green-top tubes for plasma isolation, and CPT tubes (for RNA isolation) will be processed locally, aliquoted into cryovials, and frozen at -80°C. They will be batch-shipped on dry ice to the CITN Central Laboratory.

B) Blood in green-top (or comparable) tubes for PBMC isolation and CellSave Preservation tubes will be shipped at ambient temperature in insulated containers to the CITN central laboratory at the University of Washington.

9.1.3 **Shipping of Specimens**

Fresh blood collected for PBMC isolation will be shipped at ambient temperature overnight in insulated containers to the CITN central laboratory. Biopsy specimens in formalin will be shipped with the green top tubes to the CITN Central Laboratory for paraffin embedding as described above. (FFPE) tumor biopsies will be stored at room temperature, then shipped in batch periodically to the University of Virginia (Slingluff laboratory) for analysis. FNA biopsies will be stored in liquid nitrogen or at -80^{o}C, then shipped in batch to the CITN Central Laboratory for later batch shipment to the laboratory of Drs. Marincola and Wang for analyses. Frozen serum, plasma and viably frozen tumor cells and frozen tumor tissue will be shipped in batch to the CITN Central Laboratory on dry ice for same day or overnight delivery.

9.2 Laboratory Correlative Studies

9.2.1 **Immunohistochemical Evaluation of Tumor Biopsies - Laboratory Correlative Study #1**

Tumors will be assessed by IHC for pattern of T-cell distribution and infiltration of cells expressing a variety of antigens including CD3, CD4, CD8, CD56, CD34, CD45, CD20, CD138, CD163, DC-LAMP, FoxP3, PD-1, and IDO1, as well as PD-L1 on tumors. The primary endpoint of the trial will be a change in the number of CD8+ cells infiltrating tumor (per mm^2) comparing pre- and post-treatment biopsies for each patient. We will use the assessment of total CD3+ T cells and total CD45+ lymphocytes as confirmatory assays. IHC staining will also be performed to detect other key parameters including IDO1 expression, and presence and patterns of CD4+ T cell and FoxP3+ Treg infiltration. These antibodies all work in FFPE tissue sections.

Tissue sections will be processed with heat-induced antigen retrieval and stained as described [Erdag 2012], using antibodies to CD4, CD8, NKp46, CD31, CD45, CD20, CD3, CD138, CD163, DC-LAMP, FoxP3, PD-1, and IDO1. EnVision™ System (enzyme-conjugated polymer backbone coupled to secondary antibodies) and 3,30-diaminobenzidine chromogen (Dako North America, Inc., Carpinteria, CA) will be applied to develop the staining. Positive controls for lymphocyte antibodies will include lymph node and for CD34 antibody, placenta. Negative control slides will use phosphate buffered saline instead of primary antibody, with other conditions held constant.

If the tumor specimen is too small for evaluation of all antigens, the priority will be CD8 > CD3 > IDO > CD31 > CD45 > FoxP3 > remainder. Stained sections will be reviewed for type, density, and location of tumor-infiltrating immune cells, tumor cell type, necrosis, hemorrhage, and melanin pigment. At least 5 high-powered fields will be examined. Fields with no tumor tissue, more than 50% necrosis, or hemorrhage will be excluded. Stained cells will be enumerated by a single pathologist on each core in a high-power microscope field, excluding cells within blood vessels or in hemorrhagic or necrotic areas. Cell counts will be normalized to per mm^2. Alternatively, when automated image analysis software is available and validated, it may be used for quantification of the staining. Change in the number of tumor-infiltrating lymphocytes will be analyzed using standard descriptive statistical analyses described below.

Immunotypes will be defined by staining for immune cells (CD45) and for intratumoral blood vessels (CD31) as described [Erdag 2012].

Additional histologic studies may be performed as appropriate to elucidate the immunologic changes in the tumor specimens. If IHC studies are needed using antibodies that do not work well in FFPE sections, or for immunofluorescence studies, one of the quick-frozen tissue samples will be used for those purposes.

9.2.1.1 Site(s) Performing Correlative Study

The primary endpoints (IHC for CD8 and CD3) will be evaluated on FFPE slides of tumor tissue at Phenopath Laboratories, Seattle, WA. All other IHC will be performed at the University of Virginia.

9.2.2 Gene Expression Analyses of Tumor Biopsies, Fine Needle Aspirates of Metastases, and Peripheral Blood—Laboratory Correlative Study #2

Tumor biopsies will be obtained as described above for IHC analyses. FNA of melanoma metastases will be performed at each biopsy date, before the excisional/incisional/core biopsy; both tissues will be evaluated for gene signatures. Gene signatures will also be performed on PBMCs obtained before therapy and on days 21 and 42, the same day of the tumor biopsies. PBMC gene signatures may be compared to data of tumor biopsy, immunologic response, and clinical response.

9.2.2.1 Collection of Specimen(s)

For FNA a 22- or 23-gauge hypodermic needle will be inserted into the tumor and multiple passes with changing angles of insertion will be made through all quadrants of the tumor, while under suction. Tissue from the needle will be suspended in 10 mL of cold RPMI. For PBMC, a CPT tube will be drawn and processed as described below.

9.2.2.2 Handling of Specimen(s)

Specimens will be collected at the clinical sites as described above and centrifuged at 1500 rpm for 5 minutes at 4°C. The cell pellet will then be harvested, red blood cells lysed as per the technique described by Weiss et al [Weiss 2011]. Cells will be isolated by centrifugation and resuspended in Qiazol to obtain total RNA. Samples will be stored at -80°C and then batch-shipped as described below.

9.2.2.3 Shipping of Specimen(s)

All RNA samples will be batched and shipped on dry ice using FedEx® overnight delivery to the CITN Central Laboratory. The CITN Central Laboratory will hold specimens for shipment to the laboratory of Dr. Marincola and Wang for gene expression array studies. Any leftover sample will be returned to the CITN Central Laboratory for storage and future ancillary studies.

9.2.2.4 Site(s) Performing Correlative Study

Gene expression studies, including analyses of the data, will be conducted in the laboratory of Dr. Francesco Marincola, Chief Research Officer of Sidra Medical and Research Center, Qatar.

9.2.3 CD8+ T-cell IFN-gamma Responses to the 12-peptide Mixture, and CD4+ T-cell Responses to Tetanus Peptide—Laboratory Correlative Study #3

PBMC (and lymphocytes isolated from tumor biopsies, when available) will be analyzed by IFN-gamma ELISpot assay for responses to the 12-peptide mixture. Assessment of immunologic response will be based on a fold-increase measure from baseline as well as using a positivity threshold. Since 11 of the 12 melanoma-associated immunizing peptides are 9- and 10-mers, it is safely presumed that IFN-gamma ELISpot responses among PBMC will derive from $CD8^+$ T cells. No depletion of $CD4^+$ T cells is planned for the ELISpot testing. The frequency of CD8+ T cells specific to 8 of the 12 immunizing peptides will be confirmed using tetramers and flow cytometric analysis. CD8+ T cell

responses will be assayed directly ex vivo (using viably cryopreserved specimens), after stimulation with the 12-peptide mixture (10 μg/mL each) at baseline (i.e., before initiating INCB024360 treatment), at week 3 (before the first immunization with MELITAC12.1), at weeks 6, and optionally at week 16 (2 weeks after the last dose of MELITAC12.1).

Similarly, these assays will include the tetanus peptide and multiple controls (CEF peptide, negative controls, etc.). Responses to the tetanus peptide will be interpreted as helper (CD4+) T-cell responses.

9.2.3.1 Collection of Specimen(s)

Whole blood will be collected into heparinized (green top) tubes.

9.2.3.2 Handling of Specimen(s)

Samples will be collected at room temperature and shipped to the CITN Central Laboratory the same day as the blood draw, so that the sample is received at the central laboratory within 24 hours of blood draw. PBMC will be isolated and cryopreserved before testing.

9.2.3.3 Shipping of Specimen(s)

Samples will be shipped at ambient temperature for overnight FedEx delivery. Clinical research sites will utilize a web-based specimen tracking system (BSI-II) to communicate with the CITN central laboratory.

9.2.3.4 Site(s) Performing Correlative Study

The IFN-gamma ELISpot assay will be conducted at the CITN Central Laboratory at the University of Washington Immunologic Monitoring Laboratory in Seattle, in collaboration with the laboratory of Dr. Slingluff at the University of Virginia. Flow cytometry for tetramer-positive cells will be conducted in the laboratory of Dr. Slingluff.

9.2.4 **Quantification of PBMC and T-cell Subsets—Laboratory Correlative Study #4**

The effect of treatment on T lymphocyte number and phenotype (i.e., CD8+, effector memory CD8+, central memory CD8+, CD4+, CD4+ Treg, naïve subsets) will be assessed. The frequency and percentage of PBMC subsets will be determined using multiparameter flow cytometric analysis of whole blood. The markers include CD3, CD8, CD4, CD39, CD56, Foxp3, CD127, CD45RA, CD45RO, CCR7, CD28, CD27, CD25, CD122, CD86, CD14, CD16, CD19, CD123, and CD11c. Changes in myeloid-derived suppressor cell populations will also be assessed by flow cytometry.

The effect of INCB024360 and MELITAC 12.1 on lymphocyte number and phenotype will be assessed at baseline (i.e., before initiating INCB024360 treatment), week 3 (before first immunization with MELITAC12.1), week 6 (before the second set of MELITAC12.1 vaccines), week 14 (just before the last dose of MELITAC12.1), and, if possible, week 16 (2 weeks after the last dose of MELITAC12.1).

9.2.4.1 Collection of Specimen(s)
Whole blood will be collected into heparinized tubes.

9.2.4.2 Handling of Specimen(s)
Samples will be collected at room temperature and shipped to the CITN Central Laboratory the same day as the blood draw, so that the sample is received at the central laboratory within 24 hours of blood draw.

9.2.4.3 Shipping of Specimen(s)
Samples will be shipped at ambient temperature for overnight FedEx delivery. Clinical research sites will utilize a web-based specimen tracking system (BSI-II) to communicate with the CITN central laboratory.

9.2.4.4 Sites Performing Correlative Study
The immunophenotyping assays will be conducted at the CITN Central Laboratory.

9.2.5 **Intratumor and Whole Blood Kyn/Trp Ratios—Laboratory Correlative Study #5**

Kyn/Trp ratios will be assayed in patients receiving a regimen of INCB024360 plus MELITAC 12.1 vaccine as measured in peripheral blood and in intra-tumoral specimens at baseline and on specimens obtained at subsequent tumor biopsy timepoints.

Evaluations will occur at baseline (week 0) and after each vaccination.

9.2.5.1 Collection of Specimens
Whole blood will be collected into heparinized tubes.

Biopsy specimens will be collected as described in Section 9.1.1

9.2.5.2 Handling of Specimen(s)
Blood samples will be processed locally, and frozen aliquots shipped to the CITN Central Laboratory. The Central Laboratory will distribute aliquots to Incyte where testing will be done in a blinded fashion.

Biopsy specimens will be collected and stored at -80°C (if frozen) at the collection site.

9.2.5.3 Shipping of Specimens
Frozen plasma and flash frozen biopsy specimens will be batch shipped to the CITN Central Laboratory. The CITN Central Laboratory will ship specimens to Incyte Corporation by FedEx overnight delivery.

9.2.5.4 Site(s) Performing Correlative Study
The Kyn/Trp analysis will be conducted at Incyte Corporation.

9.2.6 **Other Immunologic Assessments—Laboratory Correlative Study #6**

9.2.6.1 Collection of Specimens

At baseline, week 3, week 6 and optionally at week 16, 10 mL of serum will be collected in red-top tubes for serum storage, and 10 mL will be collected in green-top tubes for plasma storage.

9.2.6.2 Handling of Specimen(s)

Samples will be collected at room temperature. Blood for serum, plasma, and the cell pellet into RNA will be processed at the Local Lab within 4 hours at the collection site, aliquoted according to protocol, and frozen at -80°C.

9.2.6.3 Shipping of Specimen(s)

Serum, plasma and cell pellet into RNA will be processed at each clinical site and batch-shipped on dry ice overnight to the CITN central laboratory. Clinical research sites will utilize a web-based specimen tracking system (BSI-II) to communicate with the CITN central laboratory. Frozen samples will be batch-shipped to the CITN central laboratory/repository on dry ice.

9.2.6.4 Site(s) Performing Correlative Study

It is anticipated that a number of CITN investigators with laboratories may propose ancillary studies to be conducted on CITN repository specimens, but the specific sites have yet to be determined.

9.3 Special Studies: Cytokine and INCB024360 Blood Levels

9.3.1 Collection of Specimens

10 mL of serum will be collected in red-top tubes for determining the INCB024360 level, and cytokine levels.

9.3.2 Handling of Specimen(s)

Samples will be collected at room temperature and processed in the local laboratory within 4 hours of blood draw, aliquoted according to protocol, and frozen at -80°C.

9.3.3 Shipping of Specimen(s)

Frozen aliquots of serum and plasma will be batch-shipped to the CITN Central Laboratory. The Central Laboratory will distribute aliquots to Incyte. Clinical research sites will utilize a web-based specimen tracking system (BSI-II) to communicate with the CITN Central Laboratory.

9.3.4 Site(s) Performing Correlative Study

The INCB024360 drug levels will be conducted at Incyte Corporation.

10. STUDY CALENDAR

All baseline evaluations are to be conducted within 1 week before start of protocol therapy. Scans and x-rays must be done ≤4 weeks before the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours before initiating the next cycle of therapy.

	Pre-Study -30 day	Pre-Study -7 day	Wk 0 Day 0	Wk 1 Day 7 +/- 2 d	Wk 2 Day 14 +/- 2d	Wk 3 Day 21 +/- 2d	Wk 4 Day 28 +/- 2d	Wk 5 Day 35 +/- 2d	Wk 6 Day 42 +/- 2d	Wk 8 Day 56 +/- 2d	Wk 11 Day 77 +/- 2d	Wk 14 Day 98 +/-2d	Wk 16 Day 112 +/-2d	Repeat [b] Cycles Wks [c] +/-2d	10 Wk Post-Tx F/U‡ F/U +/-14d	36 Wk Post-Tx FU F/U +/-30d	Off Study
INCB024360 daily			X	X	X	X	X	X	X	X	X	X	→	X			
MELITAC 12.1 vaccine**						X	X	X		X	X	X					
Informed consent	X																
Demographics	X																
Medical history	X																
Concomitant medication		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical exam		X	X			X		X		X	X	X	X	X	X	X	X
Vital signs		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height		X												X			
Weight		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Performance status		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Tumor Biopsy[1,2 *]			X[a]			X			X[3]				X[3]				X[1]
FNA[4,5]			X[a]			X			X				X[3]				X[1]
CBC w/diff, plts (local lab)		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serum chemistry[6] (local lab)		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
B-HCG [7] (local lab)			X[7]														
PBMC[5] and T-cell subsets			X			X			X			X	X[8]				
IFN-gamma ELISpot***			X			X			X				X	X			
Confirmatory Tetramer staining			X			X			X				X				

	Pre-Study -30 day	Pre-Study -7 day	Wk 0 Day 0	Wk 1 Day 7 +/- 2 d	Wk 2 Day 14 +/- 2d	Wk 3 Day 21 +/- 2d	Wk 4 Day 28 +/- 2d	Wk 5 Day 35 +/- 2d	Wk 6 Day 42 +/- 2d	Wk 8 Day 56 +/- 2d	Wk 11 Day 77 +/- 2d	Wk 14 Day 98 +/-2d	Wk 16 Day 112 +/-2d	Repeat [b] Cycles Wks [c] +/-2d	10 Wk Post-Tx F/U‡ F/U +/-14d	36 Wk Post-Tx FU F/U +/-30d	Off Study
Kyn/Trp ratio			X			X			X				X*				
RNA Cell Pellet for gene expression analysis			X			X			X				X				
Serum Cytokines			X			X			X				X				
INCB024360 level						X			X				X				
Circulating tumor cells			X										X				
PBMC storage***			X			X			X				X[8]	X			
Serum storage			X			X			X				X[8]				
EKG (pre-study and as indicated)	X																
Adverse event evaluation	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Tumor measurements	X			Tumor measurements are repeated every 12 weeks *or* per institutional standard of care. Documentation (radiologic) must be provided for patients removed from study for progressive disease.													
Radiologic evaluation[9]	X		.							X[9]			X[9]		X[9]		

[a]Tumor biopsy and FNA may be obtained up to 14 days prior to starting therapy. For patients with only one or two tumors approachable for biopsy, available tumor blocks from prior biopsies can serve as the pretreatment sample provided at least 20 unstained slides are available.

[b] Patients who are eligible to receive additional cycles of IDO inhibitor without vaccinations will maintain the same visit schedule as cycle 1

NOTE: If additional cycles of IDO inhibitor are given only the end of treatment biopsy is to be obtained when possible.

Note: If additional cycles of IDO inhibitor are given a reduced volume of blood will be drawn for correlative assays. See footnote *** below for details.

[c] A delay of up to 60 days between treatment cycles will be allowed.

[1]Tumor biopsies will be assessed by immunohistochemistry (IHC)

[2]If optional biopsy performed

[3]Optional biopsy

[4]FNA (Fine Needle Aspirate): before the excisional/incisional/core biopsy

[5]Gene Expression Analyses of tumor biopsies, FNA, and PBMC

[6]Serum chemistries include: albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.

[7]Serum Pregnancy test done 48 hours before start of therapy

[8]Optional blood draw

[9]CT, MRI, or PET within 0 days (14 days preferred) is acceptable for imaging assessment purposes

[9] Radiologic evaluations done at weeks 8 and 16 +/-7 days. If a clinical response is seen at week 8 a confirmatory scan is to be repeated at least 4 weeks later (week 12), therefore the week 16 scan can be delayed an additional 4 weeks and repeated at week 20. After week 26 scans are to be done every 12 weeks in patients without progression through year 5 of follow-up.

* Pretreatment biopsies should be performed before the first treatment with IDO inhibitor, within 14 days of that start date.

**A participant will be taken off protocol treatment if >1 vaccination is delayed [± 3 to 7 days] during the treatment period.

***For cycles beyond cycle 1, correlative science blood draws will be limited to only IFN-gamma ELISpot/PBMC Storage.

11. MEASUREMENT OF EFFECT

In all patients, assessments for toxicology, response, and pharmacodynamics for immunologic effect will be performed. Effects of IDO inhibition on systemic vaccine-induced immunity will be assessed by evaluating peripheral blood immune responses to the MELITAC 12.1 vaccine. The comparator for immune responses will be published experience with similar patients treated with the same vaccine regimen but without INCB024360 [Slingluff, 2011; Slingluff 2009]. If substantial changes at sites of tumor are detected in response to INCB024360 alone or in combination with vaccine, subsequent iterative trials will determine the individual contributions of INCB024360 and the vaccine. From these data, we will endeavor to associate any observed changes with the expression of IDO1 protein by IHC in tumor or tumor-infiltrating cells.

The primary endpoint will be changes in the concentration and number of CD8+ cells infiltrating tumor before and after treatment with each patient serving as their own control. We will use the assessment of total CD3 T cells and total CD45 lymphocytes as confirmatory assays. The histologic pattern of T-cell infiltration and changes in pattern will be concurrently assessed and chronicled. Whether to expect a small or large effect on the tumor microenvironment from the therapy remains unknown, thus the endpoint objectives are exploratory and the trial is sized accordingly. A prior study of changes in the tumor microenvironment with systemic immune therapy (IL-2) identified significant changes in the tumor microenvironment with a sample size of 13 patients. The sample size of 12 patients is a reasonable number for this initial study to determine whether INCB024360 plus vaccine has an appreciable effect on the tumor microenvironment.

Each patient's tumor(s) will be biopsied before therapy and during therapy to assess the extent to which INCB024360 plus MELITAC 12.1 alters the tumor as evaluated by IHC and transcriptome analysis. In addition, the effect on immune responses in peripheral blood and on PBMC phenotype and character will be assessed (1) before therapy, (2) on day 21, before the first vaccine, (3) on day 42, a week after the third vaccine and coinciding with the day 42 biopsy, and (4) at week 16, 2 weeks after the sixth vaccine.

Therapeutic response will be assessed by serial scans and measures of circulating tumor cells. Overall clinical response rate and tumor progression will be assessed by evaluating nonbiopsied lesions if present, using standard methods (RECIST and irRC), including physical exam and CT scans performed before therapy, at weeks 8, 16, 26 and every 3 months through 1 year according to standard practice. If a clinical response is seen at week 8 a confirmatory scan is to be repeated at least 4 weeks later (week 12), therefore obtaining the week 16 scan may be delayed an additional 4 weeks and repeated at week 20. After week 26 scans are to be done every 12 weeks in patients without progression. Both systems will be used for reporting response. Patients may be managed in accordance with irRC guidelines as published by Wolchok [Wolchok 2009]. The irRC criteria allow for reporting as "response" patients that respond after a period of progression. The irRC criteria accordingly do not demand immediate cessation of experimental therapy with the first sign of progression.

In addition, the safety and tolerability of INCB024360 with MELITAC 12.1 will be assessed in this study utilizing the National Cancer Institute Common Terminology Criteria for Adverse Events v4.0 (NCI CTCAE v4.0).

11.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be evaluated for response according to standard practice at weeks 8, 16, 26 and every 3 months thereafter through year 1 according to standard practice. In addition to a baseline scan, confirmatory scans should also be obtained at least 4 weeks after the initial documentation of objective response (i.e., week 12). A 4 week delay in obtaining the week 16 scans, (i.e., obtaining scans at week 20), would be acceptable due to the timing of obtaining confirmatory response scans.

.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [Eisenhauer 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. Results will be reported by RECIST and irRC guidelines.

11.1.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with INCB024360 followed by MELITAC 12.1 vaccine.

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

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

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 as ≥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, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

NOTE: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor nonmeasurable) 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 patient, 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 Measurable Disease

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

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions Clinical lesions will only be considered measurable when they are

superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

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 slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g., for body scans).

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.

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.

Ultrasound Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [Bubley 1999; Rustin 2004; Scher 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [Vergote 2000].

Cytology, Histology These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

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.

11.1.4 Response Criteria

11.1.4.1 Evaluation of Target Lesions by RECIST

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or no-ntarget) 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 by RECIST

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 Target Lesions by irRC

Complete Response (irCR): Complete disappearance of all lesions (whether measurable or not, and no new lesions) confirmation by a repeat, consecutive assessment no less than 4 weeks from the date first documented.

Partial Response (irPR): A ≥50% decrease in tumor burden compared with baseline in 2 observations at least 4 weeks apart. (**NOTE**: For irRC, index and measurable lesions are taken into account. At the baseline tumor assessment, the sum of the products of the 2 largest perpendicular diameters (SPD) of all index lesions (5 lesions per organ, up to 10 visceral lesions and 5 cutaneous index lesions) is calculated. At each subsequent tumor assessment, the SPD of the index lesions and of the new, measurable lesions (≥5 × 5mm; up to 5 new lesions per organ: 5 new cutaneous lesions and 10 visceral lesions)

are added together to provide the total tumor burden:

$$\text{Tumor Burden} = SPD_{\text{index lesions}} + SPD_{\text{new, measurable lesions}}$$

Progressive Disease (irPD): At least 25% increase in tumor burden compared with nadir (at any single time point) in 2 consecutive observations at least 4 weeks apart. (**NOTE**: The appearance of 1 or more new lesions does not define progression but does preclude irCR).

Stable Disease (irSD): A 50% decrease in tumor burden compared with baseline cannot be established, nor can a 25% increase compared with nadir.

11.1.4.4 Evaluation of Best Overall Response (RECIST)

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

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

Target Lesions	**Nontarget 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 nonrandomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in nontarget lesions may be accepted as disease progression.

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

For Patients with Non-measurable Disease (*i.e.*, Non-target Disease)

Nontarget 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 nontarget 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).

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

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

11.1.6 Progression-Free Survival – N/A

11.1.7 Response Review

No review of response rate is contemplated. Investigator determined responses will be chronicled.

11.2 Antitumor Effect – Hematologic Tumors - N/A

11.3 Other Response Parameters

11.3.1 Circulating Tumor Cells

Circulating tumor cells will be enumerated by the approved CELLSEARCH® assay (Veridex LLC, Raritan, NJ) as exploratory analysis.

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

This study will be monitored by the Clinical Data Update System (CDUS) Version 3.0. Cumulative protocol- and patient-specific CDUS data will be submitted electronically to CTEP on a quarterly basis, either by FTP burst of data or via the CDS web application. Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting data using the CDUS can be found on the CTEP Web site: (http://ctep.cancer.gov/reporting/cdus.html)

Note: This study has been assigned to CDUS-Abbreviated reporting. No adverse event reporting (routine or expedited) is required to be reported via CDUS.

12.1.2 Responsibility for Data Submission

The CITN COSC is responsible for compiling and submitting CDUS data for all participants. The Coordinating Center will submit the data quarterly. The date for submission to the CITN will be set by them. When setting the dates, allow time for Coordinating Center compilation, Principal Investigator review, and timely submission to CTEP by the quarterly deadlines (see Section 12.1.1).

The CITN COSC is responsible for compiling and submitting CDUS data to CTEP for all participants and for providing the data to the Principal Investigator for review.

12.2 CTEP Multicenter Guidelines

This protocol will adhere to the policies and requirements of the CTEP Multicenter Guidelines. The specific responsibilities of the Principal Investigator and the Coordinating Center (Study Coordinator) and the procedures for auditing are presented in Appendix B.

- The CITN COSC is responsible for distributing all IND Action Letters or Safety Reports received from CTEP to all participating institutions for submission to their individual IRBs for action as required.

- Agent will be supplied by Incyte Corporation. Agent distribution to a participating site will occur only after all required regulatory documents have been submitted to the appropriate institution at the direction of the CITN Coordinating Center.

12.3 Collaborative Agreements Language – N/A

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

The proposed study is a Phase II, open label, multicenter study of the administration of INCB024360, an IDO1 inhibitor, plus a multipeptide melanoma vaccine, MELITAC 12.1, in patients with advanced (i.e., surgically incurable stage III or IV) melanoma having tissue amenable to serial biopsy.

The first 3 patients will be administered the INCB024360 in a reduced amount of 300 mg bid and enrolled in a staggered design with a 2-week waiting period between each of the first 3 patients (Table 5.1). This is a safety precaution due to unknown effects of the combination of INCB024360 with the multipeptide melanoma vaccine. Although both agents have excellent safety profiles, unexpected toxicities could occur when used together.

Each patient's tumor(s) will be biopsied before therapy and during therapy to assess the effects of INCB024360 and vaccine by IHC and transcriptome analysis (Table 5.1).

INCB024360 is expected to normalize serum Kyn/Trp ratios, in turn altering the tumor microenvironment, which includes the number and character of tumor-infiltrating T cells. The addition of MELITAC 12.1, a multipeptide melanoma vaccine, will be used to compare INCB024360 alone and with the vaccine. Effects of IDO inhibition on systemic vaccine-induced immunity will be assessed by evaluating peripheral blood immune responses to the MELITAC 12.1 vaccine. The comparator for immune responses will be taken from published experience with similar patients treated with the same vaccine regimen but without INCB024360 [Slingluff 2011; Slingluff 2009].

13.2 Primary Endpoint

Published data on immune cell infiltration of melanoma metastases showed that amongst 148 metastases, CD8 T cells ranged from 0 to 592 per mm2. The mean was 137, with standard deviation of 143; median 94 per mm2.[Erdag, Cancer Research 2012]

The primary endpoint will be changes in the concentration and number of CD8+ cells infiltrating tumor pre- and post-treatment with each patient serving as their own control so that relative increases within each patient will be assessed. We will use the assessment of total CD3 T cells and total CD45 lymphocytes as confirmatory assays. Twelve patients should enable adequate power to discern large changes (e.g. increases of about 1 SD of the original data set.)

13.3 Sample Size/Accrual Rate

The study plans to enroll a total of 12 patients at 3 or 4 clinical research sites. Each patient will act as their own control. Patients without adequate sampling for immune responses will be replaced.
Accrual rate of patients is expected to be approximately 3 patients per month.

13.4 Stratification Factors - None

13.5 Analysis of Secondary Endpoints

13.5.1 To determine whether a regimen of INCB024360 that normalizes serum Kyn/Trp ratios plus MELITAC 12.1 vaccine changes the level or character of the vaccine-induced CD8+ and CD4+ specific T-cell immune responses as measured in peripheral blood, as compared to prior published experience.

13.5.2 To evaluate the extent to which INCB024360 plus MELITAC 12.1 vaccine alters the number and character of PBMC populations, including T and NK cells, as evaluated by multiparameter flow cytometry.

13.5.3 To evaluate the extent to which INCB024360 plus MELITAC 12.1 vaccine alters the PBMC transcriptome.

13.5.4 To assess the safety and tolerability of INCB024360 plus MELITAC 12.1 vaccine.

13.5.5 To obtain preliminary data on the tumor response rate of INCB024360 plus MELITAC 12.1 vaccine by ORR, time to tumor progression, and overall survival.

13.5.6 To associate any observed changes with the expression of IDO1 protein by IHC in tumor or tumor-infiltrating cells.

13.6 Reporting and Exclusions

13.6.1 Evaluation of Toxicity

All patients will be evaluable for toxicity from the time of their first treatment with INCB024360.

13.6.2 Evaluation of Response

All patients included in the study will be assessed for response to treatment, even if major deviations from protocol treatment occur or if they are ineligible. Each patient will be assigned one of the following categories: (1) complete response,(2) partial response, (3) stable disease, (4) progressive disease, (5) early death from malignant disease, (6) early death from toxicity, (7) early death because of other cause, or (9) unknown (not assessable, insufficient data).

[**NOTE**: By arbitrary convention, category 9 usually designates the “unknown” status of any type of data in a clinical database.]

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) will be included in the main analysis of the response rate. Patients in response categories 4–9 will be considered to have a treatment failure (i.e., disease progression). Thus, an incorrect treatment schedule or drug administration does not

result in exclusion from the analysis of the response rate.

All conclusions will be based on all eligible patients. Subanalyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these subanalyses will not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis will be clearly reported. The 95% confidence intervals will also be provided.

REFERENCES

Brinckerhoff, L. H., V. V. Kalashnikov, et al. (1999). Terminal modifications inhibit proteolytic degradation of an immunogenic MART-1(27-35) peptide: implications for peptide vaccines. Int J Cancer 83(3): 326-334.

Brody, J. R., C. L. Costantino, et al. (2009). Expression of indoleamine 2,3-dioxygenase in metastatic malignant melanoma recruits regulatory T cells to avoid immune detection and affects survival. Cell Cycle 8(12): 1930-1934.

Brown, R. R., Y. Ozaki, et al. (1991). Implications of interferon-induced tryptophan catabolism in cancer, auto-immune diseases and AIDS. Adv Exp Med Biol 294: 425-435.

Bubley, G. J., M. Carducci, et al. (1999). Eligibility and response guidelines for phase II clinical trials in androgen-independent prostate cancer: recommendations from the Prostate-Specific Antigen Working Group. J Clin Oncol 17(11): 3461-3467.

Cockerell, C. J. (2012). The pathology of melanoma. Dermatol Clin 30(3): 445-468.

Eisenhauer, E. A., P. Therasse, et al. (2009). New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 45(2): 228-247.

Erdag, G., J. T. Schaefer, et al. (2012). Immunotype and immunohistologic characteristics of tumor-infiltrating immune cells are associated with clinical outcome in metastatic melanoma. Cancer Res 72(5): 1070-1080.

Gajewski, T. F., A. G. Peterson, et al. (2005). Reciprocal expression of indoleamine-2,3-dioxygenase (IDO) and arginase-I in metastatic melanoma tumors [abstract]. J Clin Oncol 23(16S Part I of II (June 1 Supplement)): 7523.

Gibney, G. T., O. Hamid, et al. (2014). Preliminary results from a Phase I/2 study of INCB024360 combined with ipilimumab in patients with melanoma. Presented at: Annual Meeting of the American Society of Clinical Oncology; 2014 May 30-June 3, Chicago, IL.

Hamid, O., H. Schmidt, et al. (2011). A prospective phase II trial exploring the association between tumor microenvironment biomarkers and clinical activity of ipilimumab in advanced melanoma. J Transl Med 9: 204.

Huang, A., D. Fuchs, et al. (2002). Serum tryptophan decrease correlates with immune activation and impaired quality of life in colorectal cancer. Br J Cancer 86(11): 1691-1696.

Katz, J. B., A. J. Muller, et al. (2008). Indoleamine 2,3-dioxygenase in T-cell tolerance and tumoral immune escape.Immunol Rev 222: 206-221.

Koblish, H. K., M. J. Hansbury, et al. (2010). Hydroxyamidine inhibitors of indoleamine-2,3-dioxygenase potently suppress systemic tryptophan catabolism and the growth of IDO-expressing tumors. Mol Cancer Ther **9**(2): 489-498.

Lee, J. H., Y. Chen, et al. (2011). Molecular analysis of melanoma-induced sentinel lymph node immune dysfunction. Cancer Immunol Immunother 60(5): 685-692.

Lee, J. R., R. R. Dalton, et al. (2003). Pattern of recruitment of immunoregulatory antigen-presenting cells in malignant melanoma. Lab Invest **83**(10): 1457-1466.

Liu, X., R. C. Newton, et al. (2009). Indoleamine 2,3-dioxygenase, an emerging target for anti-cancer therapy. Curr Cancer Drug Targets 9(8): 938-952.

Liu, X., N. Shin, et al. (2010). Selective inhibition of IDO1 effectively regulates mediators of antitumor immunity. Blood 115(17): 3520-3530.

Löb, S., A. Konigsrainer, et al. (2009). Inhibitors of indoleamine-2,3-dioxygenase for cancer therapy: can we see the wood for the trees? Nat Rev Cancer 9(6): 445-452.

Mackler, A. M., E. M. Barber, et al. (2003). Indoleamine 2,3-dioxygenase is regulated by IFN-gamma in the mouse placenta during Listeria monocytogenes infection. J Immunol 170(2): 823-830.

Mellor, A. L., B. Baban, et al. (2005). Cutting edge: CpG oligonucleotides induce splenic CD19+ dendritic cells to acquire potent indoleamine 2,3-dioxygenase-dependent T cell regulatory functions via IFN Type 1 signaling. J Immunol 175(9): 5601-5605.

Munn, D. H. (2012). Blocking IDO activity to enhance anti-tumor immunity. Front Biosci (Elite Ed) 4: 734-745.

Munn, D. H. and A. L. Mellor (2007). Indoleamine 2,3-dioxygenase and tumor-induced tolerance. J Clin Invest 117(5): 1147-1154.

Munn, D. H., M. Zhou, et al. (1998). Prevention of allogeneic fetal rejection by tryptophan catabolism. Science 281(5380): 1191-1193.

National Cancer Institute. (2012). Skin Cancer. Cancer Topics Retrieved February 25, 2013, from http://www.cancer.gov/cancertopics/types/skin.

National Comprehensive Cancer Network (NCCN). (2012). Clinical practice guidelines in oncology: melanoma v. 2.2013. Retrieved March 14, 2013, from www.nccn.org.

O'Neill, D. W., S. Adams, et al. (2009). Comparison of the immunogenicity of Montanide ISA 51 adjuvant and cytokine-matured dendritic cells in a randomized controlled clinical trial of melanoma vaccines. J Clin Oncol. 27: (abstract 3002)

Rustin, G. J., M. Quinn, et al. (2004). Re: New guidelines to evaluate the response to treatment in solid tumors (ovarian cancer). J Natl Cancer Inst 96(6): 487-488.

Scher, H. I., S. Halabi, et al. (2008). Design and end points of clinical trials for patients with

progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group. J Clin Oncol 26(7): 1148-1159.
Schroecksnadel, K., C. Winkler, et al. (2005). Tryptophan degradation in patients with gynecological cancer correlates with immune activation. Cancer Lett 223(2): 323-329.

Slingluff, C. L., Jr., S. J. Lee, et al. (2010). First report of a randomized phase II trial of multi-epitope vaccination with melanoma peptides for cytotoxic T cells and helper T cells in patients with metastatic melanoma: An Eastern Cooperative Oncology Group Study (E1602) [abstract]. J Clin Oncol 28(15s): 8508.

Slingluff, C. L., Jr., G. R. Petroni, et al. (2007). Immunologic and clinical outcomes of a randomized phase II trial of two multipeptide vaccines for melanoma in the adjuvant setting. Clin Cancer Res 13(21): 6386-6395.

Slingluff, C. L., Jr., G. R. Petroni, et al. (2011). Randomized multicenter trial of the effects of melanoma-associated helper peptides and cyclophosphamide on the immunogenicity of a multipeptide melanoma vaccine. J Clin Oncol 29(21): 2924-2932.

Slingluff, C. L., Jr., G. R. Petroni, et al. (2009). Effect of granulocyte/macrophage colony-stimulating factor on circulating CD8+ and CD4+ T-cell responses to a multipeptide melanoma vaccine: outcome of a multicenter randomized trial. Clin Cancer Res 15(22): 7036-7044.

Slingluff, C. L., Jr., G. R. Petroni, et al. (2003). Clinical and immunologic results of a randomized phase II trial of vaccination using four melanoma peptides either administered in granulocyte-macrophage colony-stimulating factor in adjuvant or pulsed on dendritic cells. J Clin Oncol 21(21): 4016-4026.

Slingluff, C. L., Jr., G. R. Petroni, et al. (2004). Immunologic and clinical outcomes of vaccination with a multiepitope melanoma peptide vaccine plus low-dose interleukin-2 administered either concurrently or on a delayed schedule. J Clin Oncol 22(22): 4474-4485.

Slingluff, C. L., Jr., G. Yamshchikov, et al. (2001). Phase I trial of a melanoma vaccine with gp100(280-288) peptide and tetanus helper peptide in adjuvant: immunologic and clinical outcomes. Clin Cancer Res 7(10): 3012-3024.

Soliman, H., M. Mediavilla-Varela, et al. (2010). Indoleamine 2,3-dioxygenase: is it an immune suppressor? Cancer J 16(4): 354-359.

Speeckaert, R., K. Vermaelen, et al. (2012). Indoleamine 2,3-dioxygenase, a new prognostic marker in sentinel lymph nodes of melanoma patients. Eur J Cancer 48(13): 2004-2011.

Suzuki, Y., T. Suda, et al. (2010). Increased serum kynurenine/tryptophan ratio correlates with disease progression in lung cancer. Lung Cancer 67(3): 361-365.

Spranger, S., Bao, R., Gajewski, TF, (2015). Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity. Nature 523(7559):231-5

Gajewski, TF, Spranger, S., (2014) Synergistic combination of immunologic inhibitors for the treatment of cancer. US Patent 201466834 A1

Tatsumi, K., T. Higuchi, et al. (2000). Induction of tryptophan 2,3-dioxygenase in the mouse endometrium during implantation. Biochem Biophys Res Commun 274(1): 166-170.

Vergote, I., G. J. Rustin, et al. (2000). Re: new guidelines to evaluate the response to treatment in solid tumors [ovarian cancer]. Gynecologic Cancer Intergroup. J Natl Cancer Inst 92(18): 1534-1535.

Weiss, G. R., W. W. Grosh, et al. (2011). Molecular insights on the peripheral and intratumoral effects of systemic high-dose rIL-2 (aldesleukin) administration for the treatment of metastatic melanoma. Clin Cancer Res 17(23): 7440-7450.

Wolchok, J. D., A. Hoos, et al. (2009). Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res 15(23): 7412-7420.

Yoshikawa, T., T. Hara, et al. (2010). Serum concentration of L-kynurenine predicts the clinical outcome of patients with diffuse large B-cell lymphoma treated with R-CHOP. Eur J Haematol 84(4): 304-309.

Zhang, G., W. L. Liu, et al. (2011). Involvement of indoleamine 2,3-dioxygenase in impairing tumor-infiltrating CD8 T-cell functions in esophageal squamous cell carcinoma. Clin Dev Immunol 2011: 384726 (epub).

Zhao, Q., D. M. Kuang, et al. (2012). Activated CD69+ T cells foster immune privilege by regulating IDO expression in tumor-associated macrophages. J Immunol 188(3): 1117-1124.

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 CTEP MULTICENTER GUIDELINES

If an institution wishes to collaborate with other participating institutions in performing a CTEP sponsored research protocol, then the following guidelines must be followed.

Responsibility of the Protocol Chair

- The Protocol Chair will be the single liaison with the CTEP Protocol and Information Office (PIO). The Protocol Chair is responsible for the coordination, development, submission, and approval of the protocol as well as its subsequent amendments. The protocol must not be rewritten or modified by anyone other than the Protocol Chair. There will be only one version of the protocol, and each participating institution will use that document. The Protocol Chair is responsible for assuring that all participating institutions are using the correct version of the protocol.
- The Protocol Chair is responsible for the overall conduct of the study at all participating institutions and for monitoring its progress. All reporting requirements to CTEP are the responsibility of the Protocol Chair in conjunction with the CITN Coordinating Center.
- The Protocol Chair is responsible for the timely review of Adverse Events (AE) to ensure safety of the patients.
- The Protocol Chair will be responsible for the review of and timely submission of data for study analysis.

Responsibilities of the CITN Coordinating Center (Central Operations and Statistical Center, COSC)

- Each participating institution will have an appropriate assurance on file with the Office for Human Research Protection (OHRP), NIH. The Coordinating Center is responsible for assuring that each participating institution has an OHRP assurance and verifies with CTSU, Regulatory Support System, (RSS) that each institution has obtained IRB approval prior to site activation.
- Prior to the activation of the protocol at each participating institution, an OHRP form 310 (documentation of IRB approval) must be submitted to CTSU, (RSS).
- The Cancer Trials Support Unit is responsible for central patient registration through the OPEN registration system. The Coordinating Center is responsible for assuring that IRB approval has been obtained at each participating site before the first patient registration from that site.
- The Coordinating Center is responsible for the preparation of all submitted data for review by the Protocol Chair.
- The Coordinating Center will maintain documentation of AE reports. Participating institutions will report to the Coordinating Center who in turn report to CTEP or the FDA as appropriate. The Coordinating Center will submit AE reports to the Protocol Chair for timely review.
- Audits may be accomplished by selecting a percentage of patient records on-site at participating sites. If the NCI chooses to have an audit at the Coordinating Center, then the Coordinating Center is responsible for having all source documents, research records, all IRB approval documents, NCI Drug Accountability Record forms, patient registration lists, response assessments scans, x-rays, etc. available for the audit.

Inclusion of Multicenter Guidelines in the Protocol

- The protocol will include the following minimum information:
  - The title page must include the name and address of each participating institution and the name, telephone number and e-mail address of the responsible investigator at each participating institution.
  - The Coordinating Center must be designated on the title page.
  - Central registration of patients is required. The procedures for registration must be stated in the protocol.
  - Data collection forms should be of a common format. Sample forms should be submitted with the protocol. The frequency and timing of data submission forms to the Coordinating Center should be stated.
  - Describe how AEs will be reported from the participating institutions, either directly to CTEP or through the Coordinating Center.
  - Describe how Safety Reports and Action Letters from CTEP will be distributed to participating institutions.

APPENDIX C INFORMATION ON POSSIBLE DRUG INTERACTIONS

Information on Possible Interactions with Other Agents for Patients and Their Caregivers and Non-Study Healthcare Team

The patient _____ is enrolled on a clinical trial using the experimental agent **INCB024360 (an IDO Inhibitor).** *This clinical trial is sponsored by the National Cancer Institute. This form is addressed to the patient, but includes important information for others who care for this patient.*

INCB024360 interacts with many drugs that are processed by your liver; it is very important to tell your study doctors about all of your medicine before you start this study. It is also very important to tell them if you stop taking any regular medicine, or if you start taking a new medicine while you take part in this study. When you talk about your medicine with your study doctor, include medicine you buy without a prescription at the drug store (over-the-counter remedy) or herbal supplements.

Many healthcare prescribers can write prescriptions. You must also tell your other prescribers (doctors, physicians' assistants or nurse practitioners) that you are taking part in a clinical trial. **Bring this paper with you and keep the attached information card in your wallet**. These are the things that you and they need to know:

INCB024360 interacts with (a) certain specific enzyme(s) in your liver.

- The enzyme(s) in question is UGT1A9. Hypothetically, inhibition of IDO could cause an increase in serotonin levels that could precipitate a cluster of adverse events termed serotonin syndrome. This rare syndrome has been associated with some MAO inhibitors and with combinations of serotonergic drugs. Symptom onset is usually rapid, often occurring within minutes, and encompasses a wide range of clinical findings:
    - Mild symptoms may only consist of increased heart rate, shivering, sweating, dilated pupils, and over-responsive reflexes.
    - Moderate symptoms may include additional abnormalities such as hyperactive bowel sounds, high blood pressure, and hyperthermia. A temperature as high as 40°C (104°F) is common in moderate intoxication. Mental status changes include hypervigilance and agitation.
    - Severe symptoms include severe increases in heart rate and blood pressure that may lead to shock. In life-threatening cases, temperature may rise to above 41.1°C (106.0°F). Other abnormalities include metabolic acidosis, rhabdomyolysis, seizures, renal failure, and disseminated intravascular coagulation; these effects usually arise as a consequence of hyperthermia.

- **INCB024360** must be used very carefully with other medicines that need these liver enzymes to be effective or to be cleared from your system.
- You and healthcare providers who prescribe drugs for you must be careful about adding or removing any drug in this category.
- Before you start the study, your study doctor will work with your regular prescriber to

switch any medicines that are considered "strong inducers/inhibitors of UGT1A9 " Inhibitors include acitretin, amitriptyline, androsterone, cyclosporine, dasatinib, diclofenac, diflunisal, efavirenz, erlotinib, estradiol (17-beta), flutamide, geftinib, gemfibrozil, glycyrrhetinic acid glycyrrhizin, imatinib, imipramine, ketoconazole, linoleic acid, mefenamic acid, mycophenolic acid, niflumic acid, nilotinib, phenobartital, phenylbutazone, phenytoin, and probenecid propofol, quinidine, ritonavir, Sorafenib, sulfinpyrazone, valproic acid, and verapmil

- Several patients on INCB024360 and coumadin (an anticoagulant blood thinner) have had elevated INRs, possibly from interactions with the CYP enzyme system. Thus, patients on coumadin should not be entered onto trial. If coumadin needs to be administrated during or after the trial, INR needs to be monitored closely.
- Your prescriber should consult a medical reference to see if any medicine they want to prescribe is on a list of drugs to avoid.
- Please be very careful! Over-the-counter drugs have a brand name on the label—it's usually big and catches your eye. They also have a generic name—it's usually small and located above or below the brand name, and printed in the ingredient list. Find the generic name and determine, with the pharmacist's help, whether there could be an adverse interaction.
- Be careful:
    - If you take acetaminophen regularly: You should not take more than 2 grams per day on a regular or ongoing basis. This is equal to approximately 6 regular strength tablets or 4 extra strength tablets per day. Read labels carefully! Acetaminophen is an ingredient in many medicines for pain, flu, and cold.
    - You should not be on anticoagulants (blood thinners) such as Coumadin.

Other medicines can be a problem with your study drugs.

- You should check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.

- Your regular prescriber should check a medical reference or call your study doctor before prescribing any new medicine for you.

Your study doctor's name is:

_____

and he or she can be contacted at

_____

INFORMATION ON POSSIBLE DRUG INTERACTIONS	
You are enrolled on a clinical trial using the experimental agent **INCB024360.** This clinical trial is sponsored by the NCI. **INCB024360** interacts with drugs that are processed by your liver. Because of this, it is very important to: ➢ Tell your doctors if you stop taking regular medicine or if you start taking a new medicine. ➢ Tell all of your prescribers (doctor, physicians' assistant, nurse practitioner, pharmacist) that you are taking part in a clinical trial. ➢ Check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.	**INCB024360** might interact with a specific liver enzyme called **UGT1A9**, and must be used very carefully with other medicines that interact with this enzyme. ➢ Before you start the study, your study doctor will work with your regular prescriber to switch any medicines that are considered "strong inducers/inhibitors or substrates of **UGT1A9**." ➢ Before prescribing new medicines, your regular prescribers consult a medical reference to see if any medicine they want to prescribe is on a list of drugs to avoid or contact your study doctor. ➢ Your study doctor's name is _____ and can be contacted at _____.

APPENDIX D BIOASSAY TEMPLATES

APPENDIX E VACCINE PREPARATION

12-MP + Tetanus Peptide Vaccine + Montanide ISA-51 VG

Three (3) mL of emulsion will be prepared. Two (2) mL of this emulsion will be administered, with 1 mL administered at each of 2 vaccine sites. At each site, half of each dose (0.5 mL) will be injected subcutaneously and half (0.5 mL) will be injected intradermally. The remaining 1 mL of the prepared emulsion will be discarded.

The 2 mL dose of each vaccine contains 100 μg each of the 12 peptides listed in Table 1 and 200 μg of tetanus peptide (Table 2), emulsified in 1 mL Montanide ISA-51 VG adjuvant.

Vaccine composition: The peptides described in Tables 1–2 will be used in the vaccines.

Table 1: 12 melanoma peptides (12-MP)

Allele	Sequence	Epitope
HLA-A1	DAEKSDICTDEY	Tyrosinase $_{240-251}$ *
	SSDYVIPIGTY	Tyrosinase $_{146-156}$
	EADPTGHSY	MAGE-A1 $_{161-169}$
	EVDPIGHLY	MAGE-A3 $_{168-176}$
HLA-A2	YMDGTMSQV	Tyrosinase $_{369-377}$ ♦
	IMDQVPFSV	gp100 $_{209-217}$ #
	YLEPGPVTA	gp100 $_{280-288}$
	GLYDGMEHL	MAGE-A10 $_{254-262}$
HLA-A3/A11	ALLAVGATK	gp100 $_{17-25}$
	LIYRRRLMK	gp100 $_{614-622}$
	SLFRAVITK	MAGE-A1 $_{96-104}$
	ASGPGGGAPR	NY-ESO-1 $_{53-62}$

*(substitution of S for C at residue 244)
♦(post-translational change of N to D at residue 371)
#(209-2M, substitution of M for T at residue 210)

Table 2: Tetanus toxoid-derived helper peptide

Allele	Sequence	Epitope
Binds to multiple class II alleles	AQYIKANSKFIGITEL	$p2_{830-844}$**

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

Materials
Gloves (sterile)
2–3 sterile gauze packets
70% ethanol
1 – 12-MP – "12 Melanoma Peptides from MDP and CTA" [150 mcg each peptide per vial]
1 – Tet – "Peptide-tet" [300 mcg peptide per vial]
1 – Sterile water for injection
1 – Sterile field
1 - I connector
3 – Alcohol swabs, 70%
2 – 5-mL plastic syringe (Latex and silicon oil free and rubber tip free plunger)
1 – 25-gauge 5/8" needle. A 27 gauge needle may be used for injection if desired.
1 – 3-mL vial of Montanide ISA-51 VG (or larger)

2 – 19 gauge 1 ½ "needle
1 – Cap from 15 ml conical tube filled with de-ionized water
1 -- Ziploc bag
3 -- Information labels

General Preparation

1. Remove 12-MP and Tet vials from the freezer. Each type of vial contains sterile lyophilized peptide. Thaw at room temperature (1–2 minutes).
2. Turn on blower in hood.
3. While wearing gloves, clean the hood with sterile gauze pads and 70% ethanol.
4. Lay out the sterile field in the hood.
5. Open the packages of sterile gauze packets. Allow gauze to fall onto sterile field.
6. Lay all other required items inside the hood (not on the sterile field).
7. Remove the protective covers to expose the stoppers on the sterile water, 12-MP and Tet vials.
8. Wipe the rubber stopper of the 12-MP, Tet and sterile water using a new alcohol swab for each vial.
9. Place a 19 gauge needle on a plastic 5 mL syringe. Uncap the needle and withdraw 1.5 ml of sterile water.
10. Inject the 1.5 ml of sterile water into the 12-MP vial. Swirl until dissolved. This solution is now at 100 mcg/ml.
11. Using the same 5 mL plastic syringe and needle, withdraw all of the 12-MP solution (1.5 ml).
12. Inject the 1.5 ml of the 12-MP solution into the Tet vial. Swirl until dissolved. This now contains 100 mcg/ml of each 12MP plus 200 mcg/ml of tetanus peptide.
13. Using the same 5 mL plastic syringe and needle, withdraw all of the peptide solution (1.5 ml). Remove any air from the syringe and remove the needle.
14. Remove a cap from the I-connector. Attach the syringe containing the peptide solution to the I-connector.
15. Lay the 5 mL syringe containing the peptide solution and I-connector on the sterile field.

Montanide Preparation –

16. Wipe the top of the vial of Montanide ISA-51 with an alcohol swab.
17. Place a new 19 gauge needle on the second 5 mL syringe. Uncap the needle and withdraw 1.5 ml of Montanide ISA-51.
18. Remove the other cap from the I-connector. Attach the syringe containing the Montanide ISA-51 to the I-connector.

Mixing and Testing

19. Hold the syringe/connector/syringe system firmly to guarantee a constant connection. Thumbs will be used to push the plungers apart. (To avoid any leak, do not push with both of the thumbs simultaneously).
20. Push completely on the plunger of one of the syringes in order to get both phases in one syringe.
21. Start to emulsify by transferring alternatively the formulation from one syringe to the other very slowly.

22. The first 20 cycles (one cycle corresponds to the passage of the entire formulation from one container to the other through the connector and back) are done at slow rhythm. A complete cycle requires an average of 4 seconds. The 20 cycles should take 1 minute and a half.
23. Once 20 cycles are complete, the speed is dramatically increased for 40 remaining cycles. This speed is as fast as possible. A timer can be set for 40 seconds to avoid losing count.
24. Transfer the entire emulsion into one syringe.
25. Disconnect the syringe from the connector.
26. Twist a sterile 25 gauge needle onto the full syringe and drop 1-2 drops of the emulsion to a container of de-ionized water. If the drops disperse in the water, mix for an additional 40 seconds and recheck as described. This test may be repeated twice.

Preparing the vaccine for injection

27. Inject excess emulsion (down to 2 ml in syringe) onto a sterile gauze pad. Tap on the syringe to void any bubbles. This 2 ml now contains 100 mcg of each of the 12MP plus 200 mcg of tetanus peptide plus 1 ml Montanide ISA-51 VG.
28. Recap the needle and pull back on the piston slightly.
29. Affix the patient information label to the syringe and add an additional label inside the Ziploc bag.
30. Place the syringe into the Ziploc bag for transport and attach an additional label to the bag for identification

Documentation & Clean-up

31. Dispose of the syringes, needles, and vials in a contaminated materials container. Dispose of all other packaging in a regular trash can.
32. Complete paperwork.

The remaining vaccine should be discarded.

The prepared vaccine should be refrigerated until just before administration. Ideally, the vaccine should be administered within 1–2 hours after mixing. The vaccine must be administered within 4 hours of mixing. If the vaccine is not administered within 4 hours after mixing, it should be discarded.

APPENDIX F PATIENT MEDICATION DIARY

Patient ID: _____ Site ID: _____

Cycle: _____

STUDY MEDICATION: INCB024360

Date	Time A.M.	Time P.M.	Dose	Pt. Initials	Staff Initials

Patient Signature: _____ Date: _____

Staff Signature: _____ Date: _____