October 22, 2018

Martha Kruhm, MS RAC
Head, Protocol and Information Office
Quality Assurance Section
CTEP, DCT, NCI
6130 Executive Blvd, EPN Room 7000
Bethesda, MD 20892

Dear Ms. Kruhm:

Enclosed is Addendum #18 to E1609, *A Phase III Randomized Study of Adjuvant Ipilimumab Anti-CTLA4 Therapy Versus High Dose Interferon a-2b for Resected High Risk Melanoma*

The following revisions to E1609 protocol have been made in this addendum:

	Section	Change
1.	Cover Page	Updated Version Date
2.	Section 9	Added paragraph regarding final statistical analysis time

The following revisions to E1609 Informed Patient Consent Document have been made in this addendum:

	Section	Change
1.	Cover Page	Updated Version Date

The following revisions to E1609 Informed Pediatric Consent Document have been made in this addendum:

	Section	Change
2.	Cover Page	Updated Version Date

If you have any questions regarding this addendum, please contact jdoyle@ecog-acrin.org or 857-504-2900.

We request review and approval of this addendum to E1609 so ECOG-ACRIN may activate it promptly.

Thank you.

Sincerely,

Pamela Cogliano

Protocol Development Manager

28 State Street, Suite 1100, Boston, MA 02109 p: 857.504.2900 f: 617. 589.0914 ecog-acrin.org

Enclosure

CC: Ahmad Tarhini, MD, PhD
F. Stephen Hodi, MD
Sandra Lee, ScD
John Kirkwood, MD
Uma Rao, MD
Lynne Wagner, PhD
Gary Cohen, MD
Matthew Shimizu
Sarah Archambault
Donna Marinucci
Amy Rose, RN
Carol Chami, R.N.
Juanita Andrews
Melinda Flood
Bruce Giantonio, M.D.
Peter O'Dwyer, M.D
Kerry Higgins, MPG
Gayle Ipock
Jean MacDonald, MPH
Colin Burnett
Julie Doyle
Sally Morawski, MPH
Lauren Lambert
Allison Snowhite

ECOG-ACRIN cancer research group
Reshaping the future of patient care

E1609

A Phase III Randomized Study of Adjuvant Ipilimumab Anti-CTLA4 Therapy Versus High Dose Interferon a-2b for Resected High Risk Melanoma

Rev. 2/12

STUDY CHAIR: Ahmad A. Tarhini MD, PhD
STUDY CO-CHAIR: F. Stephen Hodi, MD
STUDY STATISTICIAN: Sandra Lee, ScD
MELANOMA COMMITTEE CHAIR: John M. Kirkwood, MD
PATHOLOGY CO-CHAIR: Uma Rao, MD
PATIENT OUTCOMES AND SURVIVORSHIP CO-CHAIR: Lynne Wagner, PhD
COMMUNITY CO-CHAIR: Gary Cohen, MD
LABORATORY CO-CHAIR: Ahmad A. Tarhini, MD, PhD

Version Date: October 22, 2018
NCI Update Date: September 6, 2017

STUDY PARTICIPANTS

Rev. 4/14

NRG / NRG Oncology Foundation, Inc
ALLIANCE / Alliance for Clinical Trials in Oncology
SWOG / SWOG
NCIC-CTG / NCIC Clinical Trials Group

Rev. 6/11, 9/14

COG / Children's Oncology Group

Rev. 9/14, 3/15

NOTE: As of 9/23/2014, accrual is limited to pediatric/adolescent patients from COG sites only. Please see Section 4.6.5.

ACTIVATION DATE

May 25, 2011
Addendum #1 – Incorporated Prior to Activation
Addendum #2 – Incorporated Prior to Activation
Update #1 – 6/11
Addendum #3 – 10/11
Addendum #4 – 2/12
Addendum #5 – 8/12
Addendum #6 – 2/13
Addendum #7 – 11/13
Addendum #8 – 2/14
Addendum #9 – 4/14
Addendum #10 – 9/14
Addendum #11 - 9/14
Addendum #12 - 9/14
Addendum #13 - 10/14
Addendum #14 - 3/15
Addendum #15 - 5/15
Addendum #16 – 3/16
Update #2 – 9/17
Addendum #17
Addendum #18

Agents	IND #	NSC #	Supply
Ipilimumab		732442	NCI Supplied

Table of Contents

Rev.
Rev. 2/12

STUDY CHAIR

Ahmad A. Tarhini, MD, PhD
Cleveland Clinic Taussig Cancer Institute
Taussig Cancer Center
9500 Euclid Ave, CA6-157
Cleveland, OH 44195
Tel: (216) 636-0200
Fax: (216) 444-0528
E-mail: tarhina1@ccf.org

Rev. 8/12

STUDY CHAIR LIAISON (SCL)

Amy Rose, RN BSN OCN
University of Pittsburgh Cancer Institute
5150 Centre Avenue
Third Floor
Pittsburgh, PA 15232
Phone: (412) 647-8587
Fax: (412) 623-7862
E-mail: kennaj@upmc.edu

Rev. 2/13

Rev. 2/13

STUDY CO-CHAIR

F. Steven Hodi, MD
Dana-Farber Cancer Institute
44 Binney Street
Boston, MA 02115
Tel: (617) 632-5053
Fax: (617) 582-7992
E-mail: stephen_hodi@dfci.harvard.edu

Rev. 8/12, 2/13

Rev. 9/14

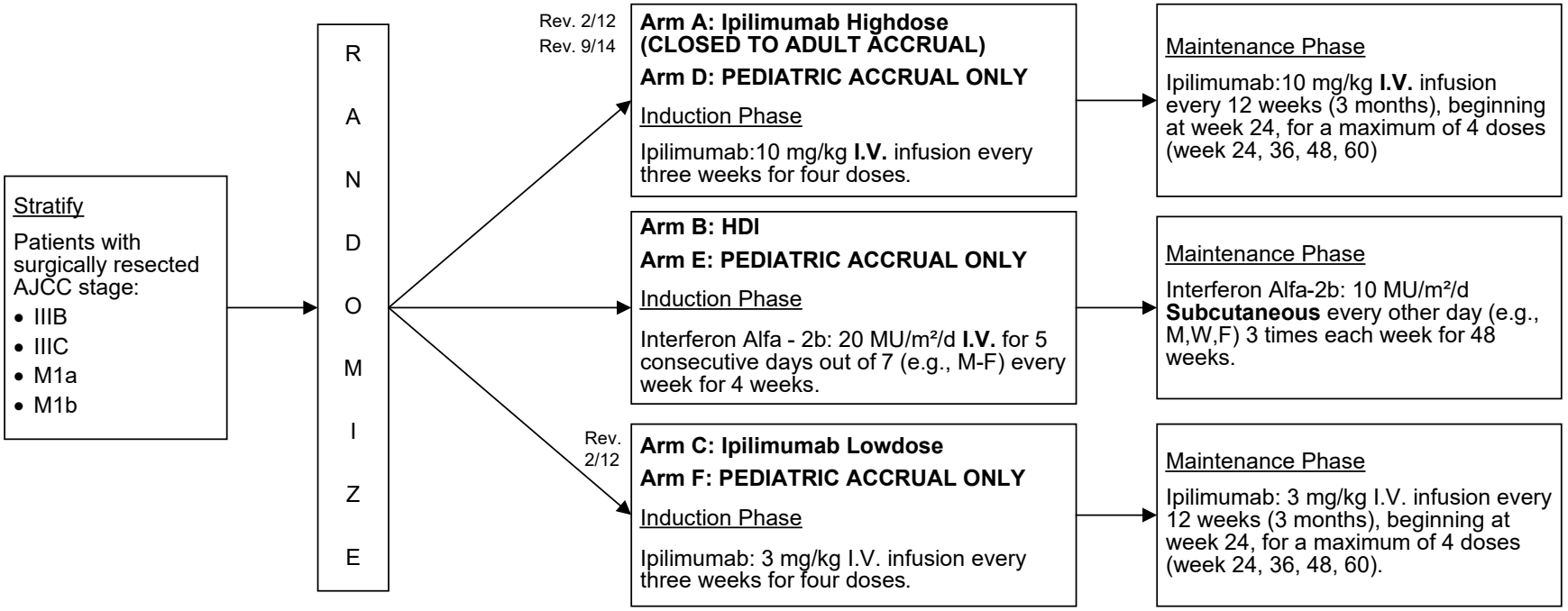
Rev.

CANCER TRIALS SUPPORT UNIT (CTSU) ADDRESS AND CONTACT INFORMATION

To submit site registration documents:	**For patient enrollments:**	**Submit study data directly to the Lead Cooperative Group unless otherwise specified in the protocol:**
Regulatory documentation must be submitted to the CTSU via the Regulatory Submission Portal. Regulatory Submission Portal: (Sign in at www.ctsu.org, and select the Regulatory Submission sub-tab under the Regulatory tab.) Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 to receive further instruction and support. Contact the CTSU Regulatory Help Desk at 1-866-651-2878 for regulatory assistance.	Please refer to the patient enrollment section of the protocol for instructions on using the Oncology Patient Enrollment Network (OPEN) which can be accessed at https://www.ctsu.org/OPEN_SYSTEM/ or https://OPEN.ctsu.org. Contact the CTSU Help Desk with any OPEN-related questions at ctsucontact@westat.com.	ECOG-ACRIN Operations Office - Boston, 28 State St., Suite 1100 Boston, MA 02109 (ATTN: DATA). Phone # 857-504-2900 Fax # 617-589-0914 Data should be sent via postal mail (preferred), however fax is accepted. Do not submit study data or forms to CTSU Data Operations. Do not copy the CTSU on data submissions.
The most current version of the **study protocol and all supporting documents** must be downloaded from the protocol-specific Web page of the CTSU Member Web site located at https://www.ctsu.org. Access to the CTSU members' website is managed through the Cancer Therapy and Evaluation Program – Identity and Access Management (CTEP-IAM) registration system and requires user log on with CTEP-IAM username and password.		
For clinical questions (i.e. patient eligibility or treatment-related questions) Contact the Study PI of the lead protocol organization.		
For non-clinical questions (i.e. unrelated to patient eligibility, treatment, or clinical 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 > education and resources tab > CTSU Operations Information >CTSU Regulatory and Monitoring Policy		
The CTSU Web site is located at https://www.ctsu.org.		

Schema

Rev. 2/15



Rev. 2/12
Rev. 9/14

Accrual ~ 1,500*

Rev. 9/14

*Additional cohort of up to 45 patients aged 12-17 will be part of the study.

NOTE: As of August 15, 2014 adult accrual has completed on Arms A, B, and C. Adolescent patients (ages 12 -17) are randomized to all 3 Study Arms D, E, and F which will follow the same treatment regimen as Arms A, B, and C, respectively.

1. Introduction

1.1 Introduction to Melanoma

Survival of patients with melanoma varies widely by stage, from a highly curable disease when detected in early stages to a disease with dismal prognosis when it reaches advanced, inoperable stages (1). The American Joint Committee on Cancer (AJCC) divides cutaneous melanoma into four stages. In the 2002, 6th edition, stages I and II are assigned to primary tumors confined to the skin and without regional lymph node involvement. These stages are defined on the basis of the thickness (depth) of the tumor, ulceration of the overlying epithelium, or invasion of the reticular dermis or subcutaneous fat (Clark level IV or V). In the 7th edition, the prognostic significance of the mitotic activity (histologically defined as mitoses/mm^2) has been recognized as an important primary tumor prognostic factor. The mitotic rate (equal to or greater than 1/mm^2) has now replaced the level of invasion as a primary criterion for defining the subcategory of T1b in addition to tumor ulceration. Stage III comprises a disease with clinical or pathological evidence of regional lymph node involvement, or the presence of in-transit or satellite metastases. Stage IV disease is defined by the presence of distant metastasis. Patients with stage I melanoma have an excellent prognosis with surgical treatment alone and a cure rate of more than 85%. The 3-5 year post-surgical relapse rate in patients with stages IIA and IIB is 20-30% and 40-55% respectively. Stage III melanoma patients with regional lymph node involvement have a 5-year relapse rate of 40-80%, while stage IV disease has a dismal prognosis with a median survival of only 6 to 9 months (2,3).

1.2 Immunity in melanoma and implications for adjuvant immunotherapy in the high-risk setting of operable disease

Immunity to melanoma appears to be important for disease control in the adjuvant and advanced disease settings. Spontaneous regression of disease has been reported in patients with melanoma, suggesting a role for host immunity, indirectly supported by the pathological evidence for the presence of lymphoid infiltrates at primary melanoma associated with tumor regression. Host cellular immune responses within melanoma have potential prognostic and predictive significance. T-cell infiltrates in primary melanoma have been suggested to be of prognostic significance (4), and T-cell infiltrates within regional nodal metastases predict benefit in patients treated with neoadjuvant IFNα2b therapy (5-7).

The quality of the host immune response has been shown to differ between earlier and more advanced disease settings. While T helper type 1 (Th1)-type CD4+ antitumor T-cell function appears to be critical to the induction and maintenance of antitumor cytotoxic T-lymphocyte (CTL) responses in-vivo, and Th2- or Th3/Tr-type CD4+ T-cell responses may subvert Th1-type cell mediated immunity yielding a microenvironment that facilitates disease progression, patients with active melanoma or renal cell carcinoma have been shown to display strong tumor antigen-specific Th2-type polarization. On the other hand, normal donors and patients who were disease free following therapy demonstrate either weak mixed Th1-/Th2-type or strongly polarized Th1-type responses to the same epitopes (8,9). Therefore, host immune tolerance appears to be an impediment to the therapy of advanced disease. This may be avoidable in the high-risk setting of operable disease, where the host susceptibility to

immunologic interventions may be greater and where IFN-α2b has demonstrated its significant impact upon melanoma relapse and survival.

1.3 Adjuvant interferon (IFN)-α2b for surgically resected melanoma that is at high-risk for recurrence and death

Three national cooperative group studies have evaluated the benefit of high-dose IFN-α2b (HDI) as adjuvant therapy for resectable high-risk cutaneous melanoma. These included patients with regional lymph node metastases and primary localized deep melanomas that have a 5-year post-surgical relapse rate of more than 40-50%. The first and third of these studies both demonstrated significant survival prolongation, compared to observation (E1684; The median relapse-free survival (RFS) was 1.72 years in the HDI arm *versus* 0.98 year in the Obs arm [stratified log-rank one-sided *P* value (P_1) = 0.0023], and the median OS was 3.82 *versus* 2.78 years (P_1 = 0.0237), respectively) (10) and compared to a vaccine (GMK) that was selected as the optimal vaccine candidate at the time (E1694). The results of this trial were reported in 2001 based on a final analysis in June 2000, with a median follow-up interval of 16 months. Among eligible patients in this trial, HDI provided a statistically significant RFS benefit (HR = 1.47; P_1 = 0.0015) and overall survival (OS) benefit (HR = 1.52; P_1 = 0.009) compared with GMK. A similar benefit was observed in the intent-to-treat analysis of RFS (HR = 1.49) and OS (HR = 1.38) (11). The second trial, E1690, conducted in part before and in part after the US FDA approval of HDI, was associated with systematic crossover of patients from the observation-assigned arm (Obs) to treatment at nodal relapse with HDI. This trial showed differences in terms of relapse-free but not overall survival. In the intent-to-treat analysis of RFS, treatment with HDI was associated with a statistically significant benefit compared with observation (HR = 1.28; P_1 = 0.025). In contrast, low-dose IFN-α2b (LDI) was not associated with a significant RFS benefit compared with Obs. Neither HDI nor LDI regimens had any apparent impact on OS compared with observation in this trial. However, a retrospective analysis of salvage therapy demonstrated the occurrence of a disproportionate crossover of patients from the observation arm to HDI therapy post protocol in those patients who developed regional recurrence (stage IIB patients in this trial were not required to undergo lymphadenectomy), which may have confounded the survival analysis (12). The analysis of each of the foregoing studies has been updated in a pooled analysis of survival and relapse-free outcomes to April 2001 (13). The pooled analysis has firmly demonstrated that melanoma relapse has been prevented by IFN to intervals that now approach 20 years, and yet this analysis (that included the observation-controlled trials E1684 and E1690, but not E1694) has not yielded compelling evidence of an impact upon overall survival despite the positive survival results of two randomized US Cooperative Group and Intergroup studies (E1684 and E1694). This may not be surprising, given that the larger of the two observation-controlled trials included in the pooled analysis (E1690) did not show an OS benefit for HDI. As discussed previously, the confounding of the OS analysis of E1690 by the routine crossover to HDI of all but one of 37 patients assigned to observation who had nodal relapse, associated with an unusually prolonged post-relapse survival of those patients in the observation arm treated with HDI, may have been responsible for this outcome variability. Patients treated with HDI in E1694 have not been included in the pooled analysis because the

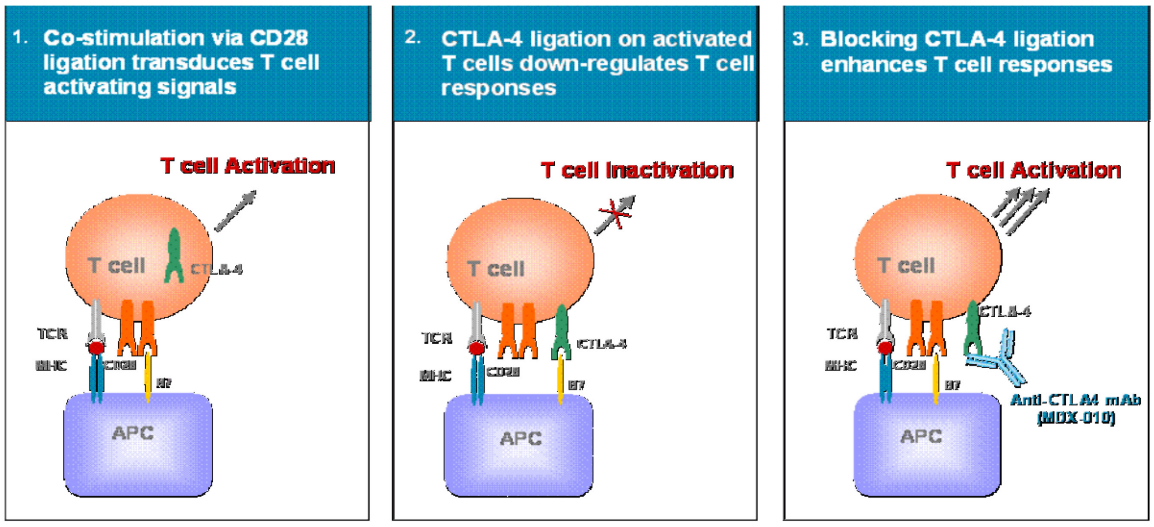
comparator in that trial was the GMK vaccine and not observation as was the case in E1684 and E1690.

Overall, there is wide agreement and evidence of a significant improvement of RFS with HDI that is supported by all 3 US Cooperative Group randomized trials (E1684, E1690, E1694) as well as data from multiple lower-dose IFN trials that have been summarized in 2 meta-analyses by Wheatley and Ives (14,15). Multiple IFN-α2b regimens, that may be categorized as high-dose, intermediate-dose, or low-dose regimens, have been evaluated as adjuvant therapy for intermediate/high-risk (T3-4, lymph node positive) surgically resected melanoma. The only randomized controlled trials that have shown durable relapse-free and overall survival impact have utilized the high-dose IFN-α2b regimen (HDI) (10,12,16). The first meta-analysis of 12 randomized trials of adjuvant IFN-α2b confirmed a highly significant reduction in the odds of recurrence in patients treated with IFN compared to observation. The analysis also demonstrated evidence of increased benefit with increasing IFN dose and a trend for improved benefit with increasing total dose. This meta-analysis did not find a statistically significant overall survival benefit for IFN-α2b. However, the larger second individual patient data meta-analysis of 13 randomized trials showed a significant though small impact of interferon upon overall survival (17,18). In this latter meta-analysis, there was statistically significant benefit for IFN for both event-free survival (EFS) (OR=0.87, CI=0.81-0.93, p=0.00006) and OS (0.9, 0.84-0.97, p=0.008). This survival advantage translates into an absolute benefit of about 3% (CI 1%-5%) at 5 years. This analysis did not, however, clarify whether there is an optimal (high, intermediate or low) dose of IFN (18).

In regards to stage IV melanoma, IFN-α was the first recombinant cytokine to be investigated clinically for the therapy of advanced metastatic melanoma. Initial phase I-II studies yielded overall response rates of about 16% and about one third achieved complete and durable responses. Responses were observed as late as 6 months from initiation of therapy, and up to one third of the responses were durable. However, the median duration of response was only about 4 months (19-22). The use of IFN-α for adjuvant therapy of patients with operable melanoma is based on the hypothesis that micrometastatic disease is the source of future relapse, and is less established in its induction of host tolerance of tumor. While patients with advanced inoperable metastatic melanoma display immunological tolerance, the adjuvant setting may be more susceptible to interventions designed to induce Th1 host-effector mechanisms to eradicate micrometastases. In addition, no other agent has ever been demonstrated to provide similar relapse free or survival benefit for the high-risk adjuvant patient population. Therefore, HDI continues to be the only option available for patients with high-risk surgically resected melanoma outside of a clinical trial.

1.4 CTLA-4 and T cell activation

Figure 1: Mechanism of action of ipilimumab



Advances in the understanding of the mechanisms that regulate T-cell activation have allowed the rational design of new strategies for immunotherapy of tumors, including melanoma. It has been known for some time that engagement of the T-cell antigen receptor by itself is not sufficient for full T-cell activation; a second co-stimulatory signal is required for the induction of IL-2 production, proliferation and differentiation to effector function of naive T cells. The primary source of this costimulation is mediated by engagement of CD28 on the T-cell surface by members of the B7 family on the antigen-presenting cell (APC) (23). (See Figure 1). Expression of B7 has been shown to be limited to “professional” antigen presenting cells; that is, specialized cells of the hematopoietic lineage, including dendritic cells, activated macrophages, and activated B cells. It has been suggested that this sharply-defined restriction of B7 expression is a fail-safe mechanism for maintenance of peripheral T-cell tolerance, insuring that T-cell activation can only be stimulated by appropriate APCs (24). The fact that tumor cells do not express B7 contributes to their poor capacity to elicit immune responses (25,26).

The demonstration that induction of expression of B7 on many tumor cells by transfection, transduction, or other means heightens tumor immunogenicity led to great interest in pursuing this as an approach to tumor immunotherapy. As demonstrated in vivo in murine tumor models, the utility of B7 expression as a vaccination approach is limited by the following factors: (1) B7-expressing tumor cell vaccines are only effective when the tumor cells have a high degree of inherent immunogenicity; (2) while B7-expressing vaccines have been shown in many cases to be effective in inducing protective immune responses, they have demonstrated only limited utility in inducing responses to established tumors; and (3) inactivation of tumor cells by radiation has been shown to destroy the immuno-enhancing activity of the B7 gene product (27,28).

In the past few years it has become apparent that co-stimulation is even more complex than originally thought. After activation, T cells express CTLA-4, a close homologue to CD28. CTLA-4 binds members of the B7 family with a much higher affinity than CD28 (29). Although there was initially some controversy as to the

role of CTLA-4 in regulating T-cell activation, it has become clear that CTLA-4 down-regulates T-cell responses (30). This was initially suggested by the following in vitro observations: (1) blockade of CTLA-4/B7 interactions with antibody enhanced T-cell responses; (2) cross-linking of CTLA-4 with CD3 and CD28 inhibited T-cell responses; and (3) administration of antibodies to CTLA-4 in vivo enhanced the immune response to peptide antigens or superantigens in mice (31-34). Blocking CTLA-4-B7 interaction while preserving signaling via CD28 resulted in enhanced T-cell responses in vitro (32).

Perhaps the most convincing demonstration of the down-regulatory role of CTLA-4 came from examination of mice with a null mutation (35-37). CTLA-4 knockout mice appear to have spontaneously activated T cells evident at approximately 1 week after birth, followed by rampant lymphoproliferation and lymphadenopathy. These mice die at approximately 3 weeks of age, either as a result of polyclonal T-cell expansion and tissue destruction or as a result of toxic shock resulting from lymphokine production by the T cells. Since thymocyte differentiation and selection proceed normally in CTLA-4-deficient mice, the rampant T-cell expansion that occurs in the mice indicates that CTLA-4 plays a critical role in down-regulating T-cell responses in the periphery (34).

1.5 CTLA-4 blockade with Ipilimumab

1.5.1 Pharmacology of Ipilimumab

Ipilimumab is a human immunoglobulin G (IgG1)κ anti-CTLA-4 monoclonal antibody (mAb). In vitro studies were performed with ipilimumab to demonstrate that it is specific for CTLA-4, actively inhibits CTLA-4 interactions with B7.1 and B7.2, does not show any cross-reactivity with human B7.1, B7.2 negative cell lines, and stains the appropriate cells without non-specific cross-reactivity in normal human tissues, as demonstrated by immunohistochemistry. Ipilimumab does cross-react with CTLA-4 in non-human primates including cynomolgus monkeys.

Ipilimumab was originally produced and purified from a hybridoma clone. Subsequently, a transfectoma (CHO cell) has been generated that is capable of producing more ipilimumab on a per cell basis than the hybridoma. Material from the transfectoma will be utilized in this and future ipilimumab clinical studies. Biochemical, immunologic and in vivo preclinical primate assessments demonstrated similarity between hybridoma and transfectoma-derived ipilimumab.

1.5.2 Pre-clinical Toxicology of Ipilimumab

Complete information on the pre-clinical toxicology studies can be found in the Ipilimumab Investigator Brochure (IB). Non-clinical toxicity assessments included in vitro evaluation for the potential of ipilimumab to mediate complement-dependent cellular cytotoxicity (CDCC) or antibody-dependent cellular cytotoxicity (ADCC), and toxicology assessments in cynomolgus monkeys alone and in the presence of vaccines.

The in vitro studies demonstrated that ipilimumab did not mediate CDCC of PHA- or (CD)3-activated human T cells. However, low to moderate ADCC activity was noted at concentrations up to 50 ug/mL.

These data are consistent with the requirement of high levels of antigen expression on the surface of target cells for efficient ADCC or CDCC. Since ipilimumab is a human IgG1, an isotype generally capable of mediating CDCC and ADCC, the lack of these activities is likely due to a very low expression of CTLA-4 on activated T cells. Therefore, these data suggest that ipilimumab treatment would not result in depletion of activated T cells in vivo. Indeed, no depletion of T cells or T-cell subsets were noted in toxicology studies in cynomolgus monkeys.

No mortality or signs of toxicity were observed in three independent 14-day intravenous toxicology studies in cynomolgus monkeys at multiple doses up to 30 mg/kg/dose. Furthermore, ipilimumab was evaluated in sub chronic and chronic toxicology studies in cynomolgus monkeys with and without Hepatitis B (HepB) Vaccine and Melanoma Vaccine. Ipilimumab was well tolerated alone or in combination in all studies. There were no significant changes in clinical signs, body weight values, clinical pathology values or T-cell activation markers. In addition, there were no significant histopathology changes in the stomach or colon.

1.5.3 Human pharmacokinetics and pharmacodynamics of ipilimumab (Ipilimumab IDB)

Pharmacokinetic (PK) profiles for ipilimumab have been analyzed. The primary objective of Protocol MDX-010-015 was to determine the safety and PK profile of single and multiple doses of ipilimumab derived from a transfectoma or hybridoma cell line. This study is still ongoing and data are preliminary. Mean plasma concentrations of ipilimumab administered at dosages of 2.8 mg/kg (transfectoma-derived drug product), 3 mg/kg (hybridoma-derived drug product), 5 mg/kg and 7.5 mg/kg (transfectoma) appear to be dose proportional over time. Preliminary PK analyses reveal that the volume variables were approximately that of plasma volume (range of mean apparent volume of distribution at steady state [Vss] across cohorts 2.8, 3, 5, 7.5, 10, 15, and 20 mg/kg was 57.3 to 82.6 mL/kg), indicating drug distribution was mostly limited to the intravascular space. The clearance (Cl) was, low (range 0.11 to 0.29 mL/h/kg) and reflective of the half-life (range 297 to 414 h). Mean residence time (MRT) was long (range 435 to 538 h), consistent with the long terminal disposition phase of ipilimumab. In general, there was moderate variability in the PK parameters among patients, with coefficient of variation (CV) of 11% to 48% in AUC (0-21d), 20% to 59% in Cl and 17% to 46% in steady state. Future clinical studies, including this study, will utilize the transfectoma-derived product.

The pharmacokinetics of ipilimumab 10 mg/kg in 3 pivotal studies in advanced melanoma (CA184007, CA184008, CA184022) have been summarized. All 3 studies were conducted with transfectoma-derived ipilimumab (the proposed formulation). The mean (± SD) clearance (CL) values after IV administration of 10 mg/kg was 18.3 ± 5.4 mL/hr and the mean (± SD) volume of distribution at steady-state (Vss) values was 5.8 ± 1.7 L.

Exposure-response (E-R) relationships for efficacy and toxicity were characterized with data obtained from 3 Phase II studies in subjects with advanced melanoma (CA184007, CA184008, and CA184022). E-R relationship was assessed for 2 measures of efficacy: best overall response (BOR) defined by mWHO criteria, and immune-related clinical activity (irCA). ir-Clinical Activity includes subjects with ir complete response irCR, or ir partial response (irPR), or late response (irCR or irPR or ir stable disease (irSD) after tumor progression), or subjects who achieved irSD of ≥ 25% reduction in tumor burden from baseline. The safety endpoint employed was the incidence of immune-related adverse events (irAEs). The probability of BOR and irCA increased with increasing minimum plasma concentration (Cmin) of ipilimumab at steady-state (Cminss) over the range of exposures achieved by ipilimumab doses ranging from 0.3 to 10 mg/kg. Likewise, the probability of experiencing Grade ≥ 2 and Grade ≥ 3 irAEs also increased with Cminss of ipilimumab. The majority of these events were medically manageable and resolved within days to weeks following cessation of ipilimumab or treatment with corticosteroids or other anti-inflammatory agents.

Recent data (in press; cross study analysis from CA184007, CA184008, CA184022) has been provided by the BMS Ipilimumab Team. In cell based competition assays in vitro, a Cminss (minimum plasma concentration at steady-state) of 20mcg/ml of ipilimumab has been shown to maximally block CTLA-4 binding to B7-1 and B7-2. Analysis of PK data from patients treated with ipilimumab at 0.3 mg/kg (N=47), 3 mg/kg (N=60) and 10 mg/kg (N=311), showed that the target Cminss target threshold of 20 mcg/ml was exceeded in 0% subjects receiving 0.3 mg/kg, 30% subjects receiving 3 mg/kg and 95% subjects receiving 10 mg/kg. In addition the 10 mg/kg dose level has been shown to be more biologically active than 3 or 0.3 mg/kg. The mean absolute lymphocyte count (ALC) increases proportionally with the ipilimumab dose with an ALC slope that is correlated with ipilimumab dose ($p<0.0001$) and with clinical benefit (CR, PR, SD ≥ 6 months) ($p=0.0013$).

1.5.4 Clinical Safety with Ipilimumab

Ipilimumab immunotherapy is currently under investigation in patients with advanced melanoma (unresectable Stage III or Stage IV) to potentially demonstrate improvement in clinical outcome. Ipilimumab has been administered to approximately 2901 patients with different cancers in 25 completed or ongoing clinical trials as of March 31, 2009 with a dose range between 0.3 mg/kg and 20 mg/kg and various combinations. Most experience with ipilimumab exists at the 3 mg/kg and 10 mg/kg dose levels. Patients who received ipilimumab at 3 mg/kg were treated in clinical studies conducted early in the development program and received either a single or multiple injections. Intra-patient dose escalation indicated that patients who were unresponsive at the 3 mg/kg dose level may have responded to 9 mg/kg. Based on preliminary data on the 10 mg/kg dose level of

ipilimumab, the ongoing clinical program investigating ipilimumab in metastatic melanoma utilizes the 10 mg/kg dose level.

The overall summary of safety for the 2901 patients treated with ipilimumab in the completed or ongoing clinical trials and the subset of 658 patients treated at the 10 mg/kg dose level is presented in Table 1.

Table 1: Ipilimumab - Overall Summary of Safety

	Number of Subjects (%)	
	Ipilimumab 0.3 - 20 mg/kg N = 2901	**Ipilimumab 10 mg/kg N = 658**
Any Drug-related AE	2357 (81.2)	561 (85.3)
Grade 1	699 (24.1)	158 (24.0)
Grade 2	889 (30.6)	198 (30.1)
Grade 3	617 (21.3)	163 (24.8)
Grade 4	127 (4.4)	38 (5.8)
Grade 5	20 (0.7)	4 (0.6)
Any Serious Adverse Events	1258 (43.4)	310 (47.1)
Grade 3 - 4	806 (27.8)	179 (27.2)
Any Drug-related Serious Adverse Events	595 (20.5)	179 (27.2)
Grade 3 - 4	469 (16.2)	140 (21.3)

1.5.4.1 Details of Drug-Related Adverse Events

Treatment-emergent adverse events (AEs) considered by the investigator to be related to study drug were reported for 81.2% of all treated subjects and 85.3% of subjects treated with ipilimumab at 10 mg/kg.

Among all treated subjects, the most frequently reported treatment-related AEs of any grade included fatigue (27.8%), diarrhea (27.5%), nausea (23.4%), rash (21.8%), pruritus (19.9%), pyrexia (11.9%), and vomiting (11.7%).

Similarly, among subjects treated with ipilimumab at 10 mg/kg, the most frequently reported treatment-related AEs of any grade included diarrhea (38.1%), fatigue (30.5%), rash (34.5%), pruritus (29.8%), nausea (17.6%), pyrexia (12.3%), vomiting (10.9%). and colitis (10.2%).

1.5.4.2 Drug-related Serious Adverse Events (SAEs)

Among all 2901 treated subjects, SAEs considered possibly, probably, or definitely related to study drug were reported for 20.5% of subjects. Drug-related SAEs reported in at least 1% of the 2901 subjects included diarrhea (5.8%), colitis (4.7%), ALT increased (2.3%), AST increased (2.2%), pyrexia (1.6%), and vomiting (1.3%).

Among the 658 subjects who received ipilimumab at 10 mg/kg, SAEs considered possibly, probably, or definitely related to study drug were reported for 27.2% of subjects. Drug-related SAEs reported in at least 1% of the 658 subjects treated at 10 mg/kg included diarrhea (8.5%), colitis (7.0%), vomiting (2.1%), AST increased (2.1%), ALT increased (2.0%), autoimmune hepatitis (2.0%), pyrexia (1.8%), hypopituitarism (1.7%), dehydration (1.7%), nausea (1.2%), and abdominal pain (1.1%).

1.5.4.3 Immune-Related Adverse Events (irAEs) with Ipilimumab

Many of the adverse events considered related to ipilimumab may be immune in nature and presumably a consequence of the intrinsic biological activity of ipilimumab. An irAE is defined as any adverse event associated with drug exposure and consistent with an immune-mediated event. Disease progression, infections and other etiologic causes are ruled out or deemed unlikely as contributing to the event. Supportive data, such as autoimmune serology tests or biopsies, are helpful but not necessary to deem an event an irAE. Events of unclear etiology which were plausibly “immune-mediated” have been conservatively categorized as irAEs even if serologic or histopathology data are absent. These irAEs likely reflect a loss of tolerance to some self antigens or an unchecked immune response to gut or skin flora. Some breakthrough of immunity may be inseparably linked to the clinical antitumor activity of ipilimumab.

Immune-related AEs predominately involve the GI tract, endocrine glands, liver or skin. Among all 2901 treated subjects, 59.6% (1729/2901) of subjects reported any irAE and 15.2% (441/2901) of subjects reported serious irAEs. Among subjects who received ipilimumab at 10 mg/kg, 21.9% (144/658) of subjects reported serious irAEs. Table 2 summarizes the incidence of serious irAEs among all treated subjects and subjects who received ipilimumab 10 mg/kg.

Table 2: Serious Immune-related Adverse Events Reported for at Least 2% of Subjects in any Event Category

	Number of Subjects (%)	
	Ipilimumab 0.3 - 20 mg/kg N = 2901	Ipilimumab 10 mg/kg N = 658
irAEs[a]		
Any	441 (15.2)	144 (21.9)
Grade 3	298 (10.3)	87 (13.2)
Grade 4	59 (2.0)	25 (3.8)
GI irAE[a]		
Any	236 (8.1)	85 (12.9)
Grade 3	166 (5.7)	58 (8.8)
Grade 4	17 (0.6)	10 (1.5)
Liver irAE[a]		
Any	109 (3.8)	33 (5.0)
Grade 3	72 (2.5)	18 (2.7)
Grade 4	32 (1.1)	13 (2.0)
Endocrine irAE[a]		
Any	61 (2.1)	21 (3.2)
Grade 3	44 (1.5)	12 (1.8)
Grade 4	3 (0.1)	1 (0.2)

[a] Based on treatment-related adverse events retrieved from the clinical database using pre-defined MedDRA terms that were considered potential irAEs.

With few exceptions, irAEs were clinically manageable and reversible with supportive care or corticosteroids. Corticosteroid treatment did not adversely affect antitumor responses in those subjects who had both an irAE requiring steroid therapy and an objective tumor response. Systemic corticosteroids do not appear adversely associated with ipilimumab-induced clinical response when used to manage irAEs in patients with advanced melanoma. Similar results were observed regardless of whether mWHO or the novel irRC criteria were used. Steroids can be used promptly to manage severe irAEs and minimize the risk for serious complications. (42)

In the setting where subjects were enrolled to receive ipilimumab every 3 weeks dosing until progression, irAEs could be reported at any time, with colitis and rash reported most often during the early doses and hypophysitis reported with later doses.

<u>Gastrointestinal irAEs</u>

The most common Grade 3 or greater irAE involved the lower GI tract and clinically manifested as diarrhea or

hematochezia. Diarrhea resulting from treatment with ipilimumab ranged from mild to severe and was life-threatening in some cases. Some cases of diarrhea began as mild and became very severe. Among subjects who received ipilimumab at 10 mg/kg, GI irAEs of any grade were reported for 40.0% (263/658) of subjects, and Grade 3 - 4 GI irAEs were reported for 12.6% (83/658) of subjects. Serious GI irAEs, mostly involving diarrhea or colitis, were reported in 12.9% (85/658) of subjects treated with ipilimumab at 10 mg/kg.

Inflammatory Hepatotoxicity

Immune-related hepatic dysfunction, including hepatitis or abnormal liver function tests (LFT) attributed to ipilimumab therapy, has been reported. Subjects may develop elevations in LFTs in the absence of clinical symptoms. Inflammatory hepatotoxicity includes non-infectious hepatitis (eg, autoimmune hepatitis). Among subjects who received ipilimumab at 10 mg/kg, inflammatory hepatotoxicity of any grade was reported for 9.0% (59/658) of subjects, and Grade 3 - 4 inflammatory hepatotoxicity was reported for 6.4% (42/658). Serious inflammatory hepatotoxicity has been reported in 5.0% (33/658) of subjects who received ipilimumab at 10 mg/kg. Inflammatory hepatotoxicity is usually reversible when immediately treated with high-dose steroids, if applicable, with or without additional immunosuppressants as recommended in the hepatotoxicity management algorithm presented as an appendix in the IB.

Hypophysitis/Hypopituitarism and Other Endocrine Conditions

Hypophysitis/hypopituitarism, clinically manifested by fatigue, has been reported. Most subjects with hypopituitarism presented with nonspecific complaints such as fatigue, confusion, visual disturbance, or impotence. Some had headache as the predominant presentation. The majority of subjects with hypopituitarism demonstrated enlarged pituitary glands based on brain magnetic resonance imaging (MRI). Low adrenocorticotropic hormone (ACTH) and cortisol were the most common biochemical abnormality reported; low thyroid stimulating hormone (TSH), testosterone, or prolactin was also reported in some subjects. (43) Hypophysitis/hypopituitarism was controlled with appropriate hormone-replacement therapy and may be dose related. Among subjects who received ipilimumab at 10 mg/kg, endocrinopathy of any grade was reported for 7.6% (50/658) of subjects, and Grade 3-4 endocrinopathy was reported for 2.4% (16/658) of subjects. Serious drug-related endocrinopathy, such as

hypophysitis/hypopituitarism, was reported in 3.2% (21/658) of subjects who received ipilimumab at 10 mg/kg. The first onset of endocrine irAEs typically occurred between weeks 6 and 12 of treatment. Endocrine events were generally manageable with hormone-replacement therapy, and the majority of subjects were not weaned from steroids.

Rash and Other Skin Conditions

Rash was one of the most common irAEs, and most cases were Grade 1 or 2 in intensity; pruritus has also been reported. (44) When biopsied, pleomorphic infiltrates were noted in the skin. Among subject who received ipilimumab at 10 mg/kg, skin irAEs of any grade were reported for 52.9% (348/658) of subjects, and Grade 3 - 4 skin irAEs were reported for 2.9% (19/658) of subjects. Serious skin irAEs were reported in < 1% (4/658) of subjects who received ipilimumab at 10 mg/kg. Skin irAEs were generally reversible.

Other reported irAEs

Ocular inflammation, manifested as Grade 2 or Grade 3 episcleritis or uveitis, was associated with concomitant diarrhea in a few subjects and occasionally occurred in the absence of clinically apparent GI symptoms. (45) Serious ocular inflammation was reported in 1 of 658 (0.2%) subjects who received ipilimumab at 10 mg/kg (8 [0.3%] of 2901 subjects program-wide reported serious occular inflammation). Preliminary analysis (based on the manual extraction of the SAE data from the internal safety database) indicated that the median time to event onset was approximately 61 days (range: 14 - 114 days). Based on the available data with known outcome, most of the subjects recovered or improved with or without corticosteroid therapy with a median duration of approximately 6 days (range: 5 - 23 days).

Other presumed irAEs reported include, but were not limited to, arthritis/arthralgias, pneumonitis, pancreatitis, autoimmune (aseptic) meningitis, autoimmune nephritis, pure red cell aplasia, noninfective myocarditis, occular inflammation, Guillain-Barre syndrome (GBS), myasthenia gravis, and neuropathy (eg, motor neuropathy, neuritis), of which were individually reported for < 1% of subjects.

Additionally, as of February 2006, there has been observation from a National Cancer Institute (NCI) study of bowel wall perforation in some patients who were administered a high-dose IL-2 following treatment with ipilimumab. Of the 22 patients administered high-dose IL-2, three patients experienced bowel wall perforations. This is a higher rate than would be expected with high-dose IL-2

treatment alone. All three patients had metastatic melanoma and had previously received their last dose of ipilimumab > 77 days before the first dose of IL-2. Two of the patients had clinically significant ipilimumab-related diarrhea or colitis and the symptoms had completely resolved prior to IL-2 administration. One patient did not experience ipilimumab-related diarrhea. It is unknown whether this observation represents a true association or is mechanistically unrelated to prior ipilimumab exposure.

1.5.4.4 Drug-related Deaths

A 32% all-cause mortality frequency has been reported for approximately 3800 subjects treated in the ipilimumab program who had data available in the BMS internal safety database as of June 30, 2009. Study-drug related deaths based on the investigator's assessment were reported in 35 subjects. Therefore, the reporting rate of drug-related deaths from the program-wide studies remained stable at approximately 1% (35/3800; Table 5.6.5 of the IDB), compared with the previous reporting period (28/3000, [1%]). Eleven of the 35 study-drug-related deaths were reported from subjects known to have received ipilimumab at 10 mg/kg multiple doses. While a causal role of ipilimumab in these 35 deaths could not be ruled out, confounding factors could be identified in most of these cases. Study-drug-related deaths reported in studies testing the 10 mg/kg dose are summarized below. For details on all drug-related deaths, refer to the current version of the Ipilimumab Investigator Brochure.

Study CA184008 (10 mg/kg mono ipilimumab; **melanoma**)

- 1 multiorgan failure (with significant liver metastasis as possible cause of death)
- 1 hypovolemic shock (with possible sepsis and evidence of hypopituitarism)
- 1 heart failure secondary to renal failure and disease progression (acute glomerulonephritis)
- 1 liver dysfunction (not treated according to the management algorithm)
- 1 acute myeloid leukemia (one month after one dose with ipilimumab)

Study CA184024 (**Blinded** dacarbazine ± 10 mg/kg ipilimumab; **melanoma**)

- 1 fatal GI bleeding without evidence of colitis
- 1 systemic inflammatory response syndrome (with cause of death listed as pneumonia/unlikely related, septic shock and metastatic melanoma)

CA184004 (one each for 3 & 10 mg/kg ipilimumab; melanoma)

- 2 bowel perforation

CA184041 (**Blinded** paclitaxel/carboplatin ± 10 mg/kg ipilimumab; **lung cancer**)

- 1 toxic epidermal necrolysis; concomitantly with pantoprazole and paclitaxel
- 1 erythema multiforme (cause of death hypoglycemia)
- 1 Guillain-Barre Syndrome (cause of death was aspiration pneumonia)

CA184-042 (10 mg/kg, **brain mets**)

- 1 GI perforation

CA184045 (10 mg/kg, **compassionate use**)

- 1 multiorgan failure
- 1 sepsis
- 1 ARDS (w/ extensive disease in lung and evidence of melanoma infiltration in alveolar spaces)
- 1 GI perforation

1.5.4.5 Safety of 10 mg/kg Multiple Doses

Based on a review of the program-wide SAE data as previously reported, evidence had suggested that ipilimumab-associated irAEs were dose dependent in frequency, and higher irAE rates had been observed at 10 mg/kg than at lower doses of ipilimumab. Subsequently, this dose-dependent effect was further demonstrated in CA184-022 in which three dose levels of ipilimumab were studied, including 0.3 vs 3 vs 10 mg/kg. Table 3 summarizes the overall irAE frequencies by dose from CA184-022 based on safety data from the locked clinical database.

Qualitatively, the safety profile of ipilimumab at 10 mg/kg remains consistent with the low-dose safety profile in that most of the drug-related SAEs are characteristic of immune-related toxicity, and most of the irAEs are reported in the GI, hepatic, and endocrine systems. However, the data presented in Table 3 suggest that the frequency of irAEs in association with 10 mg/kg of ipilimumab at multiple doses is higher compared with the irAE frequency reported for lower doses.

Table 3. Summary of Immune-Related Adverse Events (irAEs) by Treatment Groups - Treated Subjects (CA184-022)

	Number of Subjects (%)		
	Ipilimumab		
	0.3 mg/kg (N=72)	3 mg/kg (N=71)	10 mg/kg (N=71)
Overall irAEs	26.4	64.8	70.4
Grade 3-4	0	7.0	25.4
GI irAEs	16.7	32.4	39.4
Grade 3-4	0	2.8	15.5
Hepatic irAEs	0	0	2.8
Grade 3-4	0	0	2.8
Endocrine irAEs	0	5.6	4.2
Grade 3-4	0	2.8	1.4
Skin irAEs	12.5	45.1	46.5
Grade 3-4	0	1.4	4.2

1.5.4.6 Clinical safety and tolerability data with ipilimumab at 10 mg/kg from 4 phase II studies

For the purpose of this study's concept submission, the BMS ipilimumab team has provided the following safety report that summarizes data from 4 Phase II studies utilizing the 10 mg/kg dose.

1.5.4.6.1 Summary of safety and tolerability data (N=325)

- Drug-related adverse events (AEs) in 84.6% of patients
  - 32.6% were Grade 3/4/5
- Immune-related AEs (irAEs; inflammatory in nature) in 72.3% of patients
  - 25.2% were Grade 3/4 and occurred in 4 main types
    - Gastrointestinal: e.g., colitis, diarrhea (12.3%)
    - Liver: e.g., transaminase elevation (6.8%)
    - Skin: e.g., rash pruritis (2.8 %)
    - Endocrine: e.g., hypophysitis, thyroiditis (2.5%)
- Complications: bowel perforations, liver failure (~1%)
- 5 deaths were considered at least possibly related to treatment

- 4 immune-related: multi-organ failure, abnormal hepatic function, acute glomerulonephritis, and hypovolemic shock
- 1 not immune-related: acute myeloid leukemia

- irAE management algorithms are effective in resolving events and reducing risk of complications

Rev. 11/13

Updated data provided by BMS from a pooled analysis of phase II studies of ipilimumab 10 mg/kg, multiple doses, including studies CA184004, CA184007, CA184008, CA184022 and CA184042, reviewed AEs from 353 patients treated with ipilimumab 10 mg/kg. A total of 10 related deaths were reported and at least 7 were considered irAE. These cases included colon perforation, multiorgan failure/disease progression, Grade 3 hypophysitis/disease progression, acute myeloid leukemia, acute glomerulonephritis, hypovolemic shock, liver dysfunction, Grade 3 AST increase/unknown cause of death, Grade 2 enterocolitis/disease progression, Grade 4 septic shock/Disease. In 3 cases, it was determined that the AE management algorithms were not followed:

- Colon perforation (patient treated with pulse steroids; no high dose steroids and no taper).
- Liver dysfunction (patient received ipilimumab in the setting of severe liver dysfunction).
- Gr2 enterocolitis (treated with prednisone and rapid taper. Patient died of disease progression but the enterocolitis was still ongoing at that time).

1.5.4.6.2 Benefit: Risk Based on Dose. Study CA184-022 (Table 4)

Rev. 2/12

Table 4: Benefit: Risk Based on Dose as reported in Study CA184-022

	CA184022 (N=217)		
	10 mg/kg (N=72)	**3 mg/kg (N=72)**	**0.3 mg/kg (N=73)**
BORR (95% CI)	11.1% (4.9, 20.7)	4.2% (0.9, 11.7)	0
Overall Survival, median months	11.0 (6.9, NR)	8.7 (6.9, 12.1)	8.6 (7.4, 13.0)
Overall irAE rate	70%	65%	26%
High-grade irAE rate	25%	7%	0
Drug-related Deaths (<70 days)	0	0	0

1.5.4.6.3 Time to onset of Grade 2-5 irAEs and time to resolution of Grade 2-4 irAEs (10 mg/kg); (Lebbé, C et al. Perspectives in Melanoma XII 2008; Abstract O-015) (Table 5):

Rev. 2/12

Table 5: Time to onset of Grade 2-5 irAEs and time to resolution of Grade 2-4 irAEs (10mg/kg)

	Time to onset of grade 2-5 irAEs	**Time to Resolution of Grade 2-4 irAEs**
GI	Patients: 76 Median: 6.6 weeks 95% CI: 5.1–8.0	Median: 2.29 weeks
Liver	Patients: 23 Median: 6.7 weeks 95% CI: 6.1–9.3	Median: 4.00 weeks
Endocrine	Patients: 16 Median: 9.2 95% CI: 6.7–11.1	Median: 20.1 weeks
Skin	Patients: 61 Median: 3.6 95% CI: 3.1–4.1	Median: 6.14 weeks

Rev. 2/12

1.5.4.7 Safety of 3 mg/kg as compared to 10 mg/kg multiple doses

Based on a review of the program-wide SAE data as previously reported, evidence had suggested that ipilimumab-associated irAEs were dose dependent, and higher grade irAE rates had been observed at 10 mg/kg than at lower doses of ipilimumab, although overall frequency appeared similar. Subsequently, this dose-dependent effect was further demonstrated in CA184-022 as noted under Section 1.5.4.6.2 in which three dose levels of ipilimumab were studied, including 0.3 vs 3 vs

10 mg/kg.{38} Table 4 in Section 1.5.4.6.2 summarizes the overall irAE frequencies by dose from CA184-022 based on safety data from the locked clinical database.

Qualitatively, the safety profile of ipilimumab at 10 mg/kg remains consistent with the low-dose safety profile in that most of the drug-related SAEs are characteristic of immune-related toxicity, and most of the irAEs are reported in the GI, hepatic, and endocrine systems. However, the data presented in table 4 suggest that the frequency of irAEs in association with 10 mg/kg of ipilimumab at multiple doses is higher compared with the irAE frequency reported for lower doses.

The most common adverse reactions (≥5%) in patients who received ipilimumab at 3 mg/kg in the phase III pivotal trial MDX010-20 testing ipilimumab at 3 mg/kg were fatigue, diarrhea, pruritus, rash, and colitis. Table 6 presents selected adverse reactions from the MDX010-20 phase III trial, which occurred in at least 5% of patients in the ipilimumab-containing arms and with at least 5% increased incidence over the control gp100 arm for all-grade events and at least 1% incidence over the control group for Grade 3–5 events. Table 7 presents the per-patient incidence of severe, life-threatening, or fatal immune-mediated adverse reactions from MDX010-20.

Table 6: Selected Adverse Reactions in MDX010-20

	Percentage (%) of Patients[a]					
	Ipilimumab 3mg/kg n = 131		Ipilimumab 3mg/kg + gp100 n = 380		gp100 n = 132	
System Organ Class/Preferred Term	Any Grade	Grade3-5	Any Grade	Grade3-5	Any Grade	Grade3-5
Gastrointestinal Disorders						
Diarrhea	32	5	37	4	20	1
Colitis	8	5	5	3	2	0
Skin and Subcutaneous Tissue Disorders						
Pruritus	31	0	21	<1	11	0
Rash	29	2	25	2	8	0
General Disorders and Administration Site Conditions						
Fatigue	41	7	34	5	31	3

[a] Incidences presented in this table are based on reports of adverse events regardless of causality.Source: Yervoy Prescribing Information, Bristol-Myers Squibb, March 2011.

Table 7 presents the per-patient incidence of severe, life-threatening, or fatal immune-mediated adverse reactions from MDX010-20.

Table 7: Severe to Fatal Immune-mediated Adverse Reactions in MDX010-20

	Percentage (%) of Patients	
	Ipilimumab 3 mg/kg n = 131	Ipilimumab 3 mg/kg + gp100 n = 380
Any Immune-mediated Adverse Reaction	15	12
Entercolitis [a,b]	7	7
Hepatotoxicity [a]	1	2
Dermatitis [a]	2	3
Neuropathy [a]	1	< 1
Endocrinopathy	4	1
Hypopituitarism	4	1
Adrenal insufficiency	0	1
Other		
Pneumonitis	0	< 1
Meningitis	0	< 1
Nephritis	1	0
Eosinophilia [c]	1	0
Pericarditis [a,c]	0	< 1

[a] Including fatal outcome
[b] Including intestinal perforation
[c] Underlying etiology not established
Source: Yervoy Prescribing Information, Bristol-Myers Squibb, March 2011.

CA184024 phase III pivotal trial evaluated the addition of 10 mg/kg ipilimumab to dacarbazine in patients with previously untreated, metastatic melanoma. A total of 502 patients were randomized to receive up to 8 cycles of dacarbazine 850 mg/m^2 q3w, with either ipilimumab 10 mg or placebo for cycles 1-4 and as maintenance after completion of chemotherapy. Ipilimumab AEs were consistent with previous studies and predominately affected skin, I tract, liver, and the endocrine system. Events were managed with established guidelines and were generally responsive to dose interruption/discontinuation, corticosteroids and/or other immunosuppresants. Select adverse events associated

with the mechanism of action of ipilimumab, regardless of attribution by the investigator) are shown in Table 8.

Table 8: CA184024 Select Adverse Events

	Ipilimumab + DTIC n = 247		Placebo + DTIC n = 251	
	Total	Grade 3 - 4	Total	Grade 3 - 4
	% Patients			
Dermatologic	29.6	2.0	8.8	0
Pruritis	24.7	1.2	6.8	0
Rash				
Gastrointestinal (GI)	36.4	4.0	24.7	0
Diarrhea	4.5	2.0	0.4	0
Colitis	0	0	0	0
GI perforation				
Hepatic	33.2	21.9	5.6	0.8
Increased ALT	29.1	18.2	5.6	1.2
Increased AST				
Endocrine	1.6	0	0.4	0
Hypothyroidism	0.8	0	0	0
Autoimmune thyroiditis	0.4	0	0.4	0
Hyperthyroidism	0	0	0	0
Hypophysitis[a]				

[a]1 (0.4%) hypophysitis was reported on Day 364.

Rev. 9/14

1.5.4.8 A Phase I Trial in Pediatric Patients with Advanced Solid Tumors (NCI 7458)

A pediatric phase I, dose escalation study examined the safety, tolerability, pharmacokinetics, and immunogenicity of ipilimumab administered to patients less than 21 years old with recurrent or progressive solid tumors. Ipilimumab was administered at 1, 3, 5, and 10mg/kg IV in a standard 3 + 3 design with 4 doses of induction therapy every 3 weeks followed by maintenance every 3 months until disease progression or unacceptable toxicity. Twenty six patients (9 with melanoma, 7 osteosarcoma, 7 soft tissue sarcomas, 1 neuroblastoma, 1 renal cell carcinoma and 1 transitional cell carcinoma) were enrolled with 24 considered evaluable for toxicity. The following table summarizes the number of doses administered, the dose limiting toxicities (DLT) at each dose level, and the pattern and percentage of irAEs induced by ipilimumab. At the

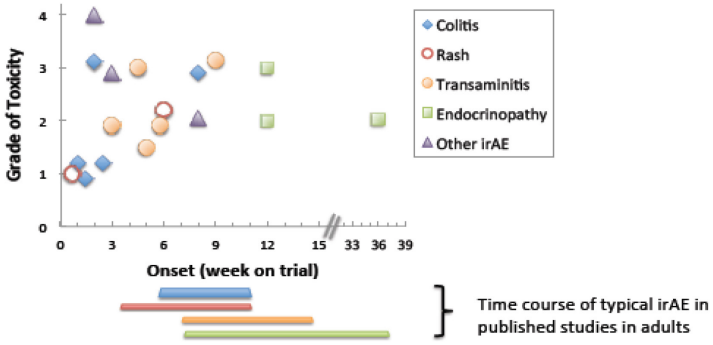
highest dose level of 10mg/kg, two of three patients under the age of 12 developed a DLT (grade 3 colitis and concurrent norovirus in one patient and grade 3 transaminitis in a second patient). One of seven patients between the ages of 12 and 21 developed a DLT in the form of a pleural effusion / pneumonitis in the setting of a progressive pleural based malignancy.

	1mg/kg	3mg/kg	5mg/kg		10mg/kg	
# of patients treated	3	3	8		10	
Total number of doses administered	5	7	31		32	
			<12yo	12-21yo	<12yo	12-21yo
Dose Limiting Toxicity (during cycle 1-2)	0	0	Gr 3 angioedema	Gr 4 Pancreatitis	Gr 3 Colitis Gr 3 ALT/AST	Gr 3 Pleural effusions

	1mg/kg	3mg/kg	5mg/kg	10mg/kg
Immune Related Adverse Events (irAE)	Gr 1 colitis Gr 1 rash	Gr1 Colitis	**Gr 4 Pancreatitis (Week 2)** Gr 2 Transaminitis (Week 2) Gr 3 Hypophysitis (Week 12) Gr 2 Autoimmune Thyroiditis (Week 36) Gr 3 transaminitis (Week 7) Gr 2 rash (Week 6) **Gr 3 Angioedema during infusion (day 1)**	**Gr 3 Colitis (Week 1)** **Gr 3 Transaminitis (Week 4)** Gr 2 Autoimmune Thyroiditis (Week 12) Gr 3 Colitis (Week 19) **Gr 3 pleural effusions (Week 2)** Gr 2 myalgias (Week 6)

	All grades		Grades 3 / 4	
	#	%	#	%
All patients with irAE	14	58%	9	38%
Colitis/Diarrhea	4	17%	3	13%
Rash	2	8%	0	0%
Transaminitis	3	13%	2	8%
Endocrinopathies	2	8%	1	4%
Other irAE	2	8%	2	8%
>1 irAE	1	4%	1	4%

- **Timing of irAE in Children**



1.5.5 Clinical Efficacy of Ipilimumab

Ipilimumab has been administered to over 1654 patients with different cancers in 22 completed or ongoing clinical trials as of March 31, 2007 with a dose range between 0.1 mg/kg and 20 mg/kg. Treatment

with ipilimumab has demonstrated clinically important and durable tumor responses in several malignancies, including melanoma, the most extensively studied tumor type with ipilimumab. Ipilimumab is active in patients with advanced stage melanoma and has demonstrated important disease-control rates. The objective responses observed with ipilimumab are durable and have occurred across a spectrum of doses and schedules. In a study of 217 patients with unresectable stage III/IV melanoma treated with ipilimumab (0.3, 3, 10 mg/kg every 3 weeks X 4; maintenance at week 24 assigned blinded dose; patients with PD could cross over to 10 mg/kg dose), the objective response rate was 15.3%. Disease control rates (DCR: CR+PR+SD) at 0.3, 3, 10 mg/kg dose levels were 13.7%, 26.4%, and 29.2% respectively (38). A Phase II study of patients with unresectable stage III or stage IV melanoma tested ipilimumab at 10 mg/kg every 3 weeks x 4 induction dosing in combination with placebo (Group A) or an oral steroid (budesonide) with minimal systemic exposure used to treat inflammatory bowel disease (Group B). For the 115 patients treated, there was no clinically meaningful difference in the best overall response rate (BORR), disease control rate (DCR), or safety events. BORRs were 15.8% and 12.1%, and DCR were 35.1% and 31% in Groups A and B, respectively (39).

Based on a preliminary analysis from a BMS study (MDX010-15) involving ipilimumab 10 mg/kg multiple doses, 34.8% of patients (N=23) were progression free at 6 months and about 17.4% were progression free at 1 year. In comparison, in another BMS study (MDX010-08) involving ipilimumab 3 mg/kg multiple doses, 10.8% patients (N=37) had progression-free survival at 6 months and 8.4% at 1 year.

Rev. 2/12

Two phase III pivotal trials with ipilimumab in advanced inoperable AJCC stage III and stage IV melanoma have been reported. One trial (MDX010-20) tested the combination of ipilimumab with gp100 vaccine versus gp100 vaccine alone and versus ipilimumab monotherapy in the second line setting. The other trial (CA184024) is a first line comparison study of combination therapy of ipilimumab and dacarbazine versus dacarbazine and placebo.

The ipilimumab-Gp100 study (MDX010-20) randomized 676 pretreated patients. (Hodi et. al. NEJM 2010) The vaccine arms meant this second-line trial was restricted to HLA-A2 patients, and ipilimumab induction therapy was given at 3 mg/kg every 3 weeks for four doses without maintenance, with responding patients eligible for re-induction with ipilimumab if they relapsed. The best objective response rate was 5.7% (ipilimumab + gp100), 10.9% (ipilimumab + placebo), 1.5% (gp100 + placebo). The disease control rate (DCR) was 20.1 % (ipilimumab + gp100), 28.5% (ipilimumab + placebo), 11% (gp100 + placebo). Median OS increased from 6.4 months to 10.0 months with the addition of ipilimumab to gp100 vaccine (HR 0.68, $p < 0.0001$) and long-term survival rates improved. DCR improved from 11.0% to 20.1% ($p = 0.02$). The 1year and 2-year survival rates were 44% (ipilimumab + gp100), 46% (ipilimumab +

placebo), 25% (ipilimumab + placebo) and 22% (ipilimumab + gp100), 24% (ipilimumab + placebo), 14% (gp100 + placebo) respectively. Approximately 20% in the group treated with ipilimumab alone were alive at 4 years (46). Based on these results the FDA has granted approval for ipilimumab in March 2011 for the treatment of unresectable or metastatic melanoma. The recommended dose and schedule for ipilimumab is 3 mg/kg as an intravenous infusion every 3 weeks for a total of four doses.

CA184024 evaluated the addition of 10 mg/kg ipilimumab to dacarbazine in patients with previously untreated, metastatic melanoma. (Robert et. al. NEJM. 2011) A total of 502 patients were randomized to receive up to 8 cycles of dacarbazine 850 mg/m^2 q3w, with either ipilimumab 10 mg/kg or placebo cycles 1-4, and as maintenance after completion of chemotherapy. Patients on the ipilimumab arm received a median of 3 ipilimumab induction doses, versus 4 placebo induction doses on the placebo arm. A total of 17.4% and 21.1% of patients continued to receive maintenance ipilimumab or placebo, for a median of 4 and 2 doses, respectively. The number of patients who received all 8 dacarbazine doses was 12.2% in the ipilimumab arm, and 21.5% in the placebo arm. The study met its primary end-point of prolonging overall survival in patients treated with ipilimumab (HR 0.72 (95% CI, 0.59 – 0.87), median OS 11.2 vs 9.1 months, p = 0.0009). One, two and three year survival rates were 47.3%, 28.5% and 20.8% in the ipilimumab arm, and 36.3%, 17.9% and 12.2% in the placebo arm. PFS, a secondary end-point, was also prolonged by the addition ipilimumab, HR 0.76 (95% CI, 0.63 - 0.93). The median PFS was 2.8 months in the ipilimumab and vs 2.6 months in the placebo arm, p = 0.006. BORR was increased from 10.3% in the placebo arm to 15.2% in the ipilimumab arm. More importantly, duration of response was more than twice as long in the ipilimumab arm (19.3 months) than in the placebo arm (8.1 months).

1.5.5.1 Long-term survival benefit from ipilimumab

Rev. 2/12

In addition to the results of the phase III trials, long-term survival benefit from ipilimumab in patients with advanced melanoma has been updated by O'Day et al. in the 2009 ASCO Annual Meeting (40). Data from 3 studies were updated:

- Study CA184-008, a single arm study of ipilimumab 10 mg/kg
- Study CA184-022, a randomized dose-ranging study of ipilimumab 0.3, 3 or 10 mg/kg
- Study CA184-007, a randomized placebo-controlled study of the effect of budesonide on gastrointestinal immune-related adverse events in patients receiving 10 mg/kg

Ipilimumab was given every 3 weeks X4 (induction); eligible patients could continue to receive maintenance ipilimumab every 12 weeks from week 24 in all studies. Median follow up was from 10.1 to 16.3 months, with a range reaching up to 37.5 months:

- 12-month survival rates were >47%
- 18-month survival rates were >34%
- 24-month survival rates were ≥30%
- For previously treated patients, 24-months survival rates ranged from 24% to 33%
- In all 3 studies, a meaningful proportion of patients continued to survive beyond the updated follow-up period
- Long-term survivors included patients with progressive disease according to the modified WHO criteria

Median survival times (months) were as follows:

- CA184-008 (N=155): 10.2, 95% CI (7.6-16.3)
- CA184-022 (N=217 with 214 treated; 10 mg/kg n=72): 11.4, 95% CI(6.9-16.1)
- CA184-007 (N=115)
  - Ipilimumab + placebo (n=57): 19.3, 95% CI (12.0-Not Reached)
  - Ipilimumab + budesonide (n=58): 17.7, 95% CI (6.8-Not Reached)

Rev. 2/12

Overall, these data demonstrate durable clinical antitumor activity of ipilimumab in advanced unresectable melanoma across a spectrum of doses and schedules. Other studies have tested combinations of ipilimumab with vaccines, interleukin-2, and chemotherapy, with relative safety and consistent clinical activity.

1.6 Study Rationale

E1609 targets a patient population that is at a high and unacceptable risk of recurrence and death after standard surgical management.

Patients with resectable high-risk deep cutaneous melanoma, patients with lymph node involvement by melanoma, patients with gross extra-nodal extension of disease, satellites, and/or in-transit lesions, as well as patients with completely resected stage IV disease, have the highest recurrence risk and the poorest disease-free and overall survival rates.

The quality of the host immune response in the earlier adjuvant setting supports a higher likelihood of clinical benefit from immunologic interventions

The quality of the host immune response has been shown to differ between patients with earlier micrometastatic and more advanced measurable disease

settings. While T helper type 1 (Th1)-type CD4+ antitumor T-cell function appears critical to the induction and maintenance of antitumor cytotoxic T-lymphocyte (CTL) responses in vivo, Th2- or Th3/Tr-type CD4+ T-cell responses may subvert Th1-type cell mediated immunity providing a microenvironment conducive to disease progression. Patients with active melanoma or renal cell carcinoma have been shown to display strong tumor antigen specific Th2-type polarization. By contrast, normal donors and patients who are disease free following therapy demonstrate either mixed Th1-/Th2-type or strongly polarized Th1-type responses to the same epitopes (8,9). Therefore, factors of host immune tolerance appear to impede advanced disease therapy, and these may be less pronounced in the high-risk operable setting, where the host may be more susceptible to immunological interventions. This observation is supported by the our clinical experience with HDI, which reduces relapse risk by up to 33% in the adjuvant setting, but induces response in 16% of patients with advanced inoperable disease. This provides support for the evaluation of clinically active immunological agents such as anti-CTLA4 blocking antibodies in the earlier adjuvant disease setting. Taken together with the demonstrable clinical activity of ipilimumab in advanced unresectable melanoma, this also provides support for our current proposal of adjuvant ipilimumab versus HDI for high-risk resected melanoma patients.

HDI is the optimal control arm for the adjuvant study of ipilimumab in patients with high-risk resected melanoma

Three reported national studies have evaluated the benefit of high dose interferon-α2b (HDI) as adjuvant therapy for patients with high-risk stage IIB and III melanoma. The first and third of these studies demonstrated significant overall survival prolongation, compared to observation (E1684) and compared to a vaccine (GMK) that was selected as the optimal vaccine candidate at the time (E1694). The second trial, E1690, conducted in part before and in part after the approval of HDI, did not show evidence of an impact upon survival, but was associated with systematic crossover of patients from the observation-assigned arm to treatment at nodal relapse with HDI. In addition, there is wide agreement and evidence of a significant improvement of relapse-free survival with HDI that is supported by all 3 US Cooperative Group randomized trials (E1684, E1690, E1694) as well as data from multiple lower-dose IFN trials that have also been summarized in 2 meta-analyses of adjuvant IFN-a studies by Wheatley and Ives (14,15). The larger individual patient data meta-analysis of 13 randomized trials has also shown a significant impact of interferon-a up on overall survival. In addition, no other agent has ever been demonstrated to provide similar relapse free or survival benefits for this patient population.

The advantage for patients treated with HDI amounts to a relapse frequency reduction of 24-38% and mortality reduction of 22-32% based upon the hazard ratios for patients treated with HDI or observation, or the vaccine (GMK). Unlike recently presented data on peg-IFN or old studies utilizing low dose IFN regimens, the benefit from HDI has been shown in both the lower and higher tumor burden stage III risk groups. In fact the largest accrual to the pivotal HDI trial occurred in the higher-risk groups with clinically apparent nodal metastasis at presentation or at recurrence.

For stage IV melanoma, IFN-α was the first recombinant cytokine to be investigated clinically for the therapy of advanced metastatic melanoma. Initial

Phase I-II studies yielded overall response rates of about 16% and about one third achieved complete and durable responses. Responses were observed as late as 6 months from initiation of therapy, and up to one third of the responses were durable (19-22). The use of IFN-α for adjuvant therapy of patients with operable melanoma is based on the hypothesis that micrometastatic disease is the source of future relapse, and is less established in its induction of host tolerance of tumor. While patients with advanced inoperable metastatic melanoma display immunological tolerance, the adjuvant setting may be more susceptible to interventions designed to induceTh1 host-effector mechanisms to eradicate micrometastases. In addition, no other agent has ever been demonstrated to provide similar relapse free or survival benefit for the high-risk adjuvant patient population. Therefore, HDI continues to be the only option available for patients with high-risk surgically resected melanoma outside of a clinical trial.

Rev. 2/12

Durable clinical benefits have been demonstrated in the advanced inoperable melanoma setting with ipilimumab, and with relative safety

Ipilimumab inhibits CTLA4, prolonging anti-tumor immune responses and leading to durable anti-tumor effects. Treatment with ipilimumab has demonstrated clinically important and durable tumor responses and disease control rates in patients with unresectable advanced melanoma. Durable objective responses have been reported across a spectrum of doses and schedules, with relative safety in this patient population. Toxicity associated with ipilimumab has not been excessive, and we plan a toxicity-specific, guidelines-driven management of treatment related toxicities based on the vast experience built over the past few years with this new class of agents.

Rev. 2/12

Rationale for testing the 3 mg/kg ipilimumab dose level compared to HDI in E1609 in addition to the testing of 10 mg/kg ipilimumab dose level compared to HDI

The original design of intergroup E1609 was meant to evaluate the safety/efficacy of the 10 mg/kg dose of ipilimumab versus high-dose interferon. This trial was originally designed prior to the report of positive results of 3 mg/kg of ipilimumab in metastatic melanoma, MDX010-20, published in 2010, which resulted in the FDA approval of 3 mg/kg of ipilimumab in treatment of unresectable and metastatic melanoma. (46)

Some data suggest a dose dependant effect of ipilimumab from 0.3 mg/kg to 10 mg/kg (CA184-022, Section 1.5.4.6.2, Section 1.5.4.7), where 10 mg/kg appears to have the greatest efficacy. However the 10 mg/kg ipilimumab dose level also appears to have the greatest frequency of Grade 3-4 adverse events based on CA 184-022, and thus a different safety/efficacy profile compared to 3 mg/kg is possible. However, the magnitude of survival benefit for ipilimumab 10 mg/kg given with dacarbazine in untreated melanoma (CA 184-020, Section 1.5.5) was similar to the magnitude of survival benefit for ipilimumab 3 mg/kg given with vaccine in pretreated melanoma by cross-study comparison (CA184-024: HR 0.72 ($p = 0.0009$), median OS 11.2 vs. 9.1 months; CA 184-020: HR 0.68 ($p < 0.0001$), median OS 6.4 vs. 10.0 months). Given that the risk/benefit ratio for therapies differ in the adjuvant setting compared to the metastatic disease setting, there is increasing need to further understand the safety/efficacy profile of **both** 3 mg/kg and 10 mg/kg dosages of ipilimumab in the adjuvant setting of

melanoma. This is supported by our long experience with IFNα, where despite multiple trials that have demonstrated a reduction in relapse/mortality risk, patients may still refuse treatment. The following points are relevant to the consideration of adding a third arm of treatment to E1609 testing 3mg/kg of ipilimumab at this time:

1. Immunologically, one hypothesis that supports the need to evaluate different doses of ipilimumab in the adjuvant setting is the hypothesis that melanoma patients in the adjuvant setting would mount a qualitatively superior (Th1 polarized) and quantitatively greater immune response than patients with metastatic disease and a compromised host immune response, when given the same dose of ipilimumab or other immunotherapeutic agents.(8,9) This has been demonstrated with HDI in melanoma both clinically and immunologically.(10,11,12,48,7) Therefore, a lower dose of ipilimumab could theoretically have a more optimal safety/efficacy ratio in patients in the adjuvant setting as compared to those with metastatic melanoma.
2. The CA184-022 trial in advanced inoperable melanoma patients suggested a dose-dependant effect of ipilimumab with the highest activity observed in the 10 m/kg dose group (BORR 11.1%, 4.2% and 0% for the 10, 3, and 0.3 mg/kg dose groups, respectively).
3. In addition, the CA184-022 trial suggested that the frequency of grade 3-4 irAEs was different between the 3 mg/kg and 10 mg/kg ipilimumab doses.
4. Given that the frequency of adverse events for 10 mg/kg may be greater than 3 mg/kg (in the metastatic setting) as tested in CA184-022, the 10 mg/kg dose level may not be the optimal dose for adjuvant treatment of melanoma and the 3 mg/kg should therefore be evaluated.
5. The magnitude of survival benefit for ipilimumab 10 mg/kg given with dacarbazine in untreated melanoma (CA 184-020, Section 1.5.5) was similar to the magnitude of survival benefit for ipilimumab 3 mg/kg given with vaccine in pretreated melanoma by cross-study comparison (CA184-024: HR 0.72 ($p = 0.0009$), median OS 11.2 vs. 9.1 months; CA 184-020: HR 0.68 ($p < 0.0001$), median OS 6.4 vs. 10.0 months).
6. At this stage in its conduct, intergroup E1609 trial provides the best opportunity to evaluate the 3 mg/kg ipilimumab dose while evaluating the 10 mg/kg dose.

   In summary, 10 mg/kg ipilimumab was originally chosen to be tested as adjuvant melanoma therapy based on the dose ranging studies supporting the highest likelihood of benefit and also acceptable safety profile in the metastatic melanoma setting. Given that the 3 mg/kg dose of ipilimumab is now commercially available as standard therapy for metastatic melanoma after demonstrating efficacy, adjuvant ipilimumab treatment of melanoma requires further evaluation to determine the optimal dose. E1609 would include study endpoints designed to capture relevant safety and efficacy data and adverse event monitoring for severity, duration, requirement for and duration of corticosteroid and other immunosuppressant use.

Rationale for collecting quality of life (QOL) data and the potential impact of the trial on QOL

An important matter in the adjuvant treatment of patients with high-risk melanoma is how patients weigh the tradeoffs between length of life and QOL. Adjuvant HDI has been demonstrated to provide relapse-free and overall survival benefits for this patient population, but it is also known to be associated with major grade 3/4 toxicities (41). Similarly, patients treated with ipilimumab experience grade 3/4 drug-related, immune-related AEs (irAEs; inflammatory in nature). We therefore plan to collect QOL data utilizing the FACT-G and FACT-BRM health questionnaires and the FACIT-D (diarrhea assessment, one of the most common grade 3/4 irAEs related to ipilimumab) and investigate health-related, quality-of-life outcomes as compared between the HDI and ipilimumab arms. This component of the study will be open to patients enrolled by Community Clinical Oncology Programs (CCOPs).

Rationale for collecting blood samples for prognostic and predictive biomarker studies

As funding is secured, through the banking of serum and PBMC at baseline and multiple time points during and following therapy, this study will also allow us to perform novel prognostic and predictive biomarker evaluations nested within this intergroup trial. Specific and detailed grant proposals to fund these biomarker studies will be submitted separately to the respective funding agencies.

Rev. 9/14

Rationale for expanding the study age eligibility to allow the adolescent population (age 12 - 17 years)

There is a clear medical need to test emerging novel therapeutic agents in the pediatric population and to allow access for these patients to major clinical trials. This would provide additional safety data that may help guide pediatric oncologists caring for pediatric patients with advanced malignancies. Study NCI 7458 has provided phase I study data that supports a relative safety and tolerability profile of ipilmumab at 10 mg/kg in patients with ages 12 - 21 years old. Given the high risk of recurrence and death of patients with stages IIIB, IIIC, M1a and M1b melanoma (E1609 study population), these data support providing these adolescent patients with access to E1609 trial therapeutic options both the standard (HDI) and the experimental (ipilimumab at 3 mg/kg or 10 mg/kg).

Pediatric patients have been allowed on prior ECOG and intergroup adjuvant trials with HDI in collaboration with the Children's Oncology Group (COG) and our clinical experience as well as reports in the literature have shown the feasibility and relative safety of treating pediatric patients with high dose IFNα (Shah et al. J Pediatric Hematology Oncology, 2006 Aug;28(8):496-500; Chao et al. PediatrBlood Cancer. 2005 May;44(5):441-8; Navidet al. Cancer. 2005 Feb 15;103(4):780-7).

1.7 Definition of a Positive Study

1.7.1 The study will be considered a positive study if it meets any one of the following criteria:

1.7.1.1 The first co-primary endpoint of RFS is met

1.7.1.2 The second co-primary endpoint of OS is met

1.7.2 Rationale

Rev. 2/12

A number of new investigational agents have recently been shown to have a significant impact on the clinical outcome of patients with advanced metastatic melanoma. These include B-RAF kinase inhibitors that continue to be tested and show major promise and ipilimumab. For ipilimumab, a recently reported phase III clinical trial has demonstrated a significant overall survival prolongation of patients treated with ipilimumab compared to the control arm of peptide gp100 vaccine (46). This is in addition to the positive survival impact shown in CA184-024 phase III trial. Ipilimumab is currently approved by the FDA for the treatment of advanced/ inoperable metastatic melanoma. Therefore, there is a strong possibility that patients treated on the HDI arm may cross over to be treated with ipilimumab off protocol at disease recurrence which may confound a primary OS endpoint. On the other hand, a positive OS endpoint may be envisioned to also constitute a positive study even in the absence of a positive RFS endpoint, since OS prolongation is the ultimate desired outcome for all patients. In this case, the earlier application of ipilimumab as in E1609 may prove to be superior to delayed treatment in the metastatic unresectable setting given the quality of the host immune response that has been shown to differ between earlier and more advanced disease settings (Tatsumi, Storkus, et al. Introduction, Section 1.2, page 1).

2. Objectives

2.1 Primary Endpoints

Rev. 2/12

2.1.1 First Co-primary Endpoint: To evaluate recurrence-free survival (RFS) between patients randomized to receive post-operative adjuvant ipilimumab given at either 10 mg/kg (high dose ipilimumab; HIP) or 3 mg/kg (low dose ipilimumab: LIP) versus those randomized to receive HDI utilizing a hierarchical design assessing HIP versus HDI first and LIP versus HDI second (if the first comparison is significant).

Rev. 2/12

2.1.2 Second Co-primary Endpoint: To evaluate overall survival (OS) between patients randomized to receive post-operative adjuvant ipilimumab given at either 10 mg/kg (HIP) or 3 mg/kg (LIP) versus those randomized to receive HDI utilizing a hierarchical design assessing HIP versus HDI first and LIP versus HDI second (if the first comparison is significant).

2.2 Secondary Endpoints

Rev. 2/12

2.2.1 To evaluate safety and tolerability of post-operative adjuvant ipilimumab therapy given at either 10 mg/kg (HIP) or 3 mg/kg (LIP).

Rev. 2/12

2.2.2 Among patients enrolled by CCOPs, to compare the global QOL between the ipilimumab arms versus HDI using FACT-G form and to evaluate the effect of treatment-related side effects that may have an impact on the health-related domains of QOL using FACIT-D and FACT-BRM.

3. Selection of Patients

Each of the criteria in the checklist that follows must be met in order for a patient to be considered eligible for this study. Use the checklist to confirm a patient's eligibility. For each patient, this checklist must be photocopied, completed and maintained in the patient's chart.

In calculating days of tests and measurements, the day a test or measurement is done is considered Day 0. Therefore, if a test is done on a Monday, the Monday four weeks later would be considered Day 28.

ECOG-ACRIN Patient No. _____

Patient's Initials (L, F, M) _____

Physician Signature and Date _____

NOTE: All questions regarding eligibility should be directed to the study chair or study chair liaison.

NOTE: Institutions may use the eligibility checklist as source documentation if it has been reviewed, signed, and dated prior to registration/randomization by the treating physician.

NOTE: Only patients with melanoma of a cutaneous origin or unknown primary are eligible for this study. Patients with ocular melanoma or melanoma of mucosal origin are not eligible.

3.1 Eligibility Criteria

Rev. 9/14, 9/14

_____ 3.1.1 Age ≥ 12 years. Age _____

NOTE: Up to 45 patient's ages 12–17 years will be enrolled on this trial.

Rev. 9/14

_____ 3.1.2 All patients must have disease-free status documented by a complete physical examination and imaging studies within 4 weeks prior to randomization. Imaging studies must include a total body PET-CT scan (with or without brain) and brain MRI or CT (if MRI is contraindicated). If PET-CT cannot be done, CT of neck, chest, abdomen, and pelvis should be done.

If for some reason a CT cannot be done, an MRI may be done instead. Any other imaging studies if performed (eg, bone scan) must show no evidence of disease.

Surgically rendered disease-free? Yes _____ No _____

Negative margins? Yes _____ No _____

Complete physical examination? Yes _____ No _____
Date _____

Brain MRI/CT scan (if MRI contraindicated)? Yes _____ No _____
Date _____

Total body PET-CT scan? Yes _____ No _____
Date _____

<u>If PET-CT cannot be done</u>:

CT of neck, chest, abdomen, and pelvis? Yes _____ No _____
Date _____

_____ 3.1.3 Patients must have primary cutaneous melanoma that belong to one of the following AJCC stages (2009 AJCC Melanoma Staging System. Also see Appendix XI):

_____	3.1.3.1	IIIB	T1-4b	N1a	M0	Yes _____	No _____
			T1-4b	N2a	M0	Yes _____	No _____
			T1-4a	N1b	M0	Yes _____	No _____
			T1-4a	N2b	M0	Yes _____	No _____
Rev. 8/12			T1-4a	N2c	M0	Yes _____	No _____
_____	3.1.3.2	IIIC	T1-4b	N1b	M0	Yes _____	No _____
			T1-4b	N2b	M0	Yes _____	No _____
			T1-4b	N2c	M0	Yes _____	No _____
			Any T	N3	M0	Yes _____	No _____
_____	3.1.3.3	IV	M1a			Yes _____	No _____
			M1b			Yes _____	No _____

NOTE: Patients with stage IV melanoma must have normal LDH and either distant skin, subcutaneous, lymph node or lung metastases, but no other visceral metastases in order to be eligible. For patients with resected stage IV melanoma, LDH within the institutional upper limit of normal (ULN) must be documented within 4 weeks prior to randomization.

LDH: _____ ULN: _____ Date _____

Rev. 2/12

_____ 3.1.4 Patients with disease recurrence after adequate surgical excision of the original primary cutaneous/unknown primary melanoma are allowed even if they don't fit the strict staging criteria noted in Section 3.1.3, but only as follows:.

- Recurrence in a regional lymph node basin after a prior complete lymph node dissection. Relapsed disease must be completely surgically resected with free margins.
- Recurrence in the form of in-transit or satellite metastases or distant skin/subcutaneous, nodal or lung metastases that are completely surgically resected with free margins.
- Recurrence in a regional lymph node basin. Relapsed disease must be completely surgically resected with free margins.

Recurrence in a regional lymph node basin. Has relapsed disease been completely surgically resected with free margins?

Yes _____ No _____

Recurrence in a regional lymph node basin after a prior complete lymph node dissection and relapse disease is completely surgically resected with free margins?

Yes____ No _____

Recurrence in the form of in-transit or satellite metastases or distant skin/subcutaneous, nodal or lung metastases that are completely surgically resected with free margins.

Yes _____ No _____

Rev. 2/12

Patients with unknown primary melanoma (Tx) who present with cutaneous, subcutaneous, nodal and/or lung metastases that are completely surgically resected with free margins are allowed. These patients are allowed even if they don't fit the strict staging criteria noted in Section 3.1.3. For stage IV patients LDH within the institutional ULN must be documented within 4 weeks prior to randomization (M1c is not eligible).

Unknown primary melanoma presenting with cutaneous or subcutaneous metastases that are completely surgically resected with free margins?

Yes _____ No _____

Unknown primary melanoma presenting with nodal metastases that are completely surgically resected with free margins?

Yes _____ No _____

Unknown primary melanoma presenting with lung metastases that are completely surgically resected with free margins?

Yes _____ No _____

Rev. 2/12

NOTE: All subjects should be classified as IIIB, IIIC, M1a or M1b including subjects with disease recurrence after adequate surgical excision of the original primary melanoma. That is the treating team/physician investigator should review an overall TNM status (that includes primary tumor presentation and disease recurrence status) and provide a designation of IIIB, IIIC, M1a or M1b. For newly diagnosed primary cutaneous melanoma, refer to section 3.1.3. For subjects with disease recurrence after adequate surgical excision of the original primary melanoma or who present with an unknown primary melanoma, classify as follows (check one):

1=regional nodal and satellite/in-transit disease _____ IIIC
2=regional nodal without satellite/in-transit disease _____ IIIB or _____ IIIC
3=regional satellite/in-transit disease without lymph nodes involvement _____ IIIB or _____ IIIC
4=distant cutaneous/nodal disease _____ M1a
5=distant lung disease _____ M1b

Unknown primary melanoma (newly diagnosed or recurrent) with:

1=cutaneous or nodal disease _____ IIIB or _____ IIIC or _____ M1a

2=lung disease _____ M1b

_____ 3.1.5 Patients must be randomized within 84 days (12 weeks) of surgical resection. If more than one surgical procedure is required to render the patient disease-free, the patient must be randomized within 12 weeks of the last surgery.

Rev. 8/12

NOTE: Patients with clinically positive lymph nodes for melanoma involvement or those with positive lymph nodes identified through lymphoscintigraphic and/or dye lymphographic techniques in the groin, axilla, or neck should have additional lymphadenectomy in those sites. See Section 5.1 and Appendix VII for suggested guidelines on surgical management. The complete lymph node dissection procedure would be considered as the last surgery in counting the 84 days unless a subsequent surgical procedure(s) was clinically required to ensure the disease free status.

Date of Randomization: _____
Date of last surgical resection _____

More than 1 surgery required to render patient disease-free?
Yes ____ No _____

Number of surgeries _____
Dates of surgeries _____, _____, _____, _____

_____ 3.1.6 Patients must not have received any adjuvant treatment (chemotherapy, biotherapy, or limb perfusion) after the resection(s) that make(s) them eligible for this trial.

Rev. 2/12

NOTE: Previous radiation therapy, including after the surgical resection, is allowed as long as 21 days have elapsed between the radiation and initiation of this adjuvant systemic therapy.

Adjuvant treatment (chemotherapy, biotherapy, limb perfusion) received after resection?
Yes _____ No _____

Previous radiation therapy (including after resection)?
Yes _____ No _____ Date of radiation _____

_____ 3.1.7 Prior treatment with anti-CTLA4 monoclonal antibodies or prior CTLA-4 inhibitor or agonist or prior CD137 agonist or prior interferon-α is not allowed.

Other forms of prior treatment for melanoma (e.g., IL-2, anti-tumor vaccine, chemotherapy) are allowed if given before the resection(s) that make(s) the patient eligible for this trial, but these must have been completed at least 4 weeks prior to randomization.

Prior treatment with anti-CTLA4 monoclonal antibodies?
Yes ____ No _____

Prior treatment with CTLA4 inhibitor or agonist?
Yes ____ No _____

Prior treatment with CD137 agonist?
Yes ____ No _____

Prior treatment with interferon-α?
Yes ____ No _____

_____ 3.1.8 Patients must have ECOG performance status of 0-1.
See Appendix VI.

ECOG performance status _____

_____ 3.1.9 Patients must not have an active infection requiring current treatment with parenteral antibiotics.

Active infection requiring current treatment with parenteral antibiotics?
Yes _____ No _____

_____ 3.1.10 Patients must not have other significant medical, surgical, or psychiatric conditions or require any medication or treatment that in the opinion of the investigator may interfere with compliance, make the administration of Ipilimumab or HDI hazardous or obscure the interpretation of AEs, such as a condition associated with frequent diarrhea. Patients with a baseline of frequent diarrhea (e.g. irritable bowel syndrome) are not eligible because of the difficulty in interpreting later expected GI toxicities that may lead to suboptimal management and complications.

Rev. 11/13

Significant medical, surgical or psychiatric conditions as defined above? Yes ____ No _____

Condition requiring any medication or treatment as defined above?
Yes _____ No _____

Condition associated with frequent diarrhea?
Yes _____ No _____

Rev. 2/13

_____ 3.1.11 Given that serious adverse events may occur with ipilimumab and IFNα and that these may impact a patient's wellbeing, it is possible that the occurrence of other SAEs may seriously impact an underlying state of mental depression and lead to suicidal ideation. Therefore, patients should be carefully screened for depression at baseline and if there are indications or a history of depression it is strongly recommended that these patients be closely followed together with behavioral health or psychiatric medical support. Patients with an established diagnosis of depression that, in the assessment of the investigator may make the administration of IFNα or ipilimumab hazardous, should not be enrolled on this protocol. The risks and benefits of being treated with standard adjuvant IFNα should be weighed very carefully in consultation with behavioral health or psychiatry.

_____ 3.1.12 Patients must not have a documented history of inflammatory bowel disease (including ulcerative colitis and Crohn's disease) or diverticulitis (history of diverticulosis is allowed).

Documented history of inflammatory bowel disease?
Yes _____ No _____

History of diverticulitis?
Yes _____ No _____

_____ 3.1.13 Patients must not have autoimmune disorders or conditions of immunosuppression that require current ongoing treatment with systemic corticosteroids (or other systemic immunosuppressants), including oral steroids (i.e., prednisone, dexamethasone) or continuous use of topical steroid creams or ointments or ophthalmologic steroids. A history of occasional (but not continuous) use of steroid inhalers is allowed. Replacement doses of steroids for patients with adrenal insufficiency are allowed. Patients who discontinue use of these classes of medication for at least 2 weeks prior to randomization are eligible if, in the judgment of the treating physician investigator, the patient is not likely to require resumption of treatment with these classes of drugs during the study.

Exclusion from this study also includes patients with a history of symptomatic autoimmune disease (e.g., rheumatoid arthritis, systemic progressive sclerosis [scleroderma], systemic lupus erythematosus, Sjögren's syndrome, autoimmune vasculitis [e.g., Wegener's Granulomatosis]); motor neuropathy considered of autoimmune origin (e.g., Guillain-Barre Syndrome and Myasthenia Gravis); other CNS autoimmune disease (e.g., poliomyelitis, Multiple sclerosis).

Patients with autoimmune hypothyroid disease or type I diabetes on replacement treatment are eligible.

Treatment with systemic corticosteroids (including oral steroids)?
Yes _____ No _____

Continuous use of topical steroid creams/ointments?
Yes _____ No _____

Continuous use of steroid containing inhalers?
Yes _____ No _____

Adrenal insufficiency?
Yes _____ No _____

Date of last dose of steroid containing medicines _____

_____ 3.1.14 Due to the possible effect of treatment with ipilimumab on the immunologic response to infectious disease vaccines, patients must not have had any infectious disease vaccination (e.g., standard influenza, H1N1 influenza, pneumococcal, meningococcal, tetanus toxoid) within 4 weeks prior to randomization.

Has the patient had an infectious disease vaccine within the past four weeks?

Yes _____ No _____ Date of vaccine _____

_____ 3.1.15 Patients must not be prisoners or subjects who are compulsorily detained (involuntarily incarcerated) for treatment of either a psychiatric or physical (e.g., infectious) illness. This is due to concerns about subject safety and compliance with study procedures.

Is the patient a prisoner or involuntarily incarcerated?
Yes _____ No _____

_____ 3.1.16 Patients who have other current malignancies are not eligible. Patients with other malignancies are eligible if they have been continuously disease free for > 5 years prior to the time of randomization. Patients with prior history at any time of any in situ cancer, lobular carcinoma of the breast in situ, cervical cancer in situ, atypical melanocytic hyperplasia or melanoma in situ are eligible. Patients with prior history of basal or squamous skin cancer are eligible. Patients who have had multiple primary melanomas are eligible.

Other current malignancies? Yes ___ No _____

Type of malignancy _____

Other previous malignancies? Yes ____ No ____

Type of malignancy _____

Disease-free? Yes _____ No _____

Date of last treatment _____

_____ 3.1.17 Women must not be pregnant or breast-feeding due to the unknown effects of ipilimumab and HDI on conception and the fetus. All females of childbearing potential must have a blood test or urine study during screening to rule out pregnancy. Please see Section 7.1.5 for required pregnancy testing prior to and during treatment.

Female? _____ (Yes or No)
Date of blood test or urine study: _____

NOTE: A woman of childbearing potential (WOCBP) is any woman, regardless of sexual orientation or whether they have undergone tubal ligation, who meets the following criteria: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).

Post-menopause is defined as:

Amenorrhea ≥ 12 consecutive months without another cause, or

For women with irregular menstrual periods and taking hormone replacement therapy (HRT), a documented serum follicle stimulating hormone (FSH) level ≥ 35 mIU/mL.

Rev. 2/12

WOCBP must be using an adequate method of contraception to avoid pregnancy throughout the study and for up to 26 weeks after the last dose of ipilimumab or HDI, in such a manner that the risk of

pregnancy is minimized. Women who are using oral contraceptives, other hormonal contraceptives (vaginal products, skin patches, or implanted or injectable products), or mechanical products such as an intrauterine device or barrier methods (diaphragm, condoms, spermicides) to prevent pregnancy, or are practicing abstinence or where their partner is sterile (e.g., vasectomy) should be considered to be of childbearing potential.

Men of fathering potential and WOCBP must be using an adequate method of contraception to avoid conception/pregnancy throughout the study and for up to 26 weeks after the last dose of ipilimumab or HDI in such a manner that the risk of pregnancy is minimized. Men or WOCBP who are unwilling or unable to strictly follow this requirement are not eligible.

WOCBP are not eligible if they satisfy any of the following:

- A positive pregnancy test at baseline

Pregnant or breastfeeding

_____ 3.1.18 Patients must have the following required values for initial laboratory tests obtained within 4 weeks prior to randomization (ULN: institutional upper limit of normal):

- WBC $\geq$ 3000/uL — WBC count: _____
  Date _____
- ANC $\geq$ 1500/uL — ANC count: _____
  Date _____
- Platelets $\geq$ 100 x 10^3/uL — Platelet count: _____
  Date _____
- Hemoglobin $\geq$ 10 g/dL — Hemoglobin: _____
  Date _____

Rev. 11/13

- Serum creatinine $\leq$ 1.5 mg/dl — Serum creatinine: _____
  Date _____
- AST/ALT $\leq$ 2.5 x ULN
  AST: _____ Date _____
  ULN: _____
  ALT: _____ Date _____
  ULN: _____

Rev. 11/13, 4/14

- Serum bilirubin ≤ 1.5 X ULN, (except patients with Gilbert's Syndrome, who must have a total bilirubin less than 3.0 mg/dL)
  Serum bilirubin: _____ Date _____ ULN: _____

_____ 3.1.19 No active or chronic infection with HIV, Hepatitis B, or Hepatitis C due to the unknown effects of ipilimumab. Patients must have negative testing for HIV, HBV, HCV within 4 weeks prior to randomization.

Negative HIV serology? Yes _____ No _____
Date _____

Rev. 8/12

Negative Hepatitis B antigen testing? Yes _____ No _____
Date _____

Negative HCV serology? Yes _____ No _____
Date _____

_____ _____

Physician Signature Date

OPTIONAL: This signature line is provided for use by institutions wishing to use the eligibility checklist as source documentation.

Rev. 9/14

4. Registration Procedures

Rev. 3/15

NOTE: As of August 15, 2014, adult accrual to Arms A, B, and C has completed. Adolescent patients (ages 12 -17) will be randomized to study arms D, E, or F.

Rev. 9/14

Rev.

CTEP Registration Procedures

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account (https://ctepcore.nci.nih.gov/iam/). In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) (i.e., clinical site staff requiring write access to OPEN, RAVE, or TRIAD or acting as a primary site contact) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) (https://ctepcore.nci.nih.gov/rcr). Documentation requirements per registration type are outlined in the table below.

Documentation Required	IVR	NPIVR	AP	A
FDA Form 1572	✔	✔		
Financial Disclosure Form	✔	✔	✔	
NCI Biosketch (education, training, employment, license, and certification)	✔	✔	✔	
HSP/GCP training	✔	✔	✔	
Agent Shipment Form (if applicable)	✔			
CV (optional)	✔	✔	✔	

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and IRBs covering their practice sites on the FDA Form 1572 in RCR to allow the following:

Added to a site roster

Assigned the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN

Act as the site-protocol PI on the IRB approval

Additional information can be found on the CTEP website at <https://ctep.cancer.gov/investigatorResources/default.htm>.

For questions, please contact the RCR ***Help Desk*** by email at <RCRHelpDesk@nih.gov>.

CTSU Registration Procedures

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

Rev.

IRB Approval:

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 be approved to enroll patients. Assignment of site

registration status in the CTSU Regulatory Support System (RSS) uses extensive data to make a determination of whether a site has fulfilled all regulatory criteria including but not limited to the following:

- An active Federal Wide Assurance (FWA) number
- An active roster affiliation with the Lead Network or a participating organization
- A valid IRB approval
- Compliance with all protocol specific requirements.

In addition, the site-protocol Principal Investigator (PI) must meet the following criteria:

- Active registration status
- The IRB number of the site IRB of record listed on their Form FDA 1572
- An active status on a participating roster at the registering site.

Sites participating on the NCI CIRB initiative that are approved by the CIRB for this study are not required to submit IRB approval documentation to the CTSU Regulatory Office. For sites using the CIRB, IRB approval information is received from the CIRB and applied to the RSS in an automated process. Signatory Institutions must submit a Study Specific Worksheet for Local Context (SSW) to the CIRB via IRB Manager to indicate their intent to open the study locally. The CIRB's approval of the SSW is then communicated to the CTSU Regulatory Office. In order for the SSW approval to be processed, the Signatory Institution must inform the CTSU which CIRB-approved institutions aligned with the Signatory Institution are participating in the study.

Rev.

Downloading Site Registration Documents:

Site registration forms may be downloaded from the **E1609** protocol page located on the CTSU members' website.

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Protocols tab in the upper left of your screen
- Either enter the protocol # in the search field at the top of the protocol tree, or
- Click on the By Lead Organization folder to expand
- Click on the **ECOG-ACRIN** link to expand, then select trial protocol **E1609**
- Click on LPO Documents, select the Site Registration documents link, and download and complete the forms provided.

Rev.

Requirements for E1609 site registration:

- IRB approval (For sites not participating via the NCI CIRB; local IRB documentation, an IRB-signed CTSU IRB Certification Form, Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form, or combination is accepted)
- Ipilimumab Investigator Training (please see Section 4.6.4)

Submitting Regulatory Documents

Submit required forms and documents to the CTSU Regulatory Office via the Regulatory Submission Portal, where they will be entered and tracked in the CTSU RSS.

Regulatory Submission Portal: www.ctsu.org (members' area) → Regulatory Tab → Regulatory Submission

When applicable, original documents should be mailed to:

CTSU Regulatory Office
1818 Market Street, Suite 3000
Philadelphia, PA 19103

Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support.

Rev.

Required Protocol Specific Regulatory Documents

1. **Copy of IRB Informed Consent Document.**

   NOTE: Any deletion or substantive modification of information concerning risks or alternative procedures contained in the sample informed consent document must be justified in writing by the investigator and approved by the IRB.

2. A. CTSU IRB Certification Form.
   Or
   B. Signed HHS OMB No. 0990-0263 (replaces Form 310).
   Or
   C. IRB Approval Letter

NOTE: **The above submissions must include the following details:**

- Indicate all sites approved for the protocol under an assurance number.
- OHRP assurance number of reviewing IRB
- Full protocol title and number
- Version Date
- Type of review (full board vs. expedited)
- Date of review.
- Signature of IRB official

Rev.

Checking Your Site's Registration Status:

You can verify your site registration status on the members' section of the CTSU website

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Regulatory tab
- Click on the Site Registration tab
- Enter your 5-character CTEP Institution Code and click on Go

NOTE: The status given only reflects compliance with IRB documentation and institutional compliance with protocol-specific requirements outlined by the Lead Network. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with the NCI or their affiliated networks.

Rev.

Patient Enrollment:

Patients must not start protocol treatment prior to registration.

Rev. 2/12

Treatment should start within 7 working days after registration.

Patient registration can occur only after pre-treatment evaluation is complete, eligibility criteria have been met, and the study site is listed as 'approved' in the CTSU RSS. Patients must have signed and dated all applicable consents and authorization forms.

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. To access OPEN, the site user must have an active CTEP-IAM account (check at <https://ctepcore.nci.nih.gov/iam/>) and a 'Registrar' role on either the LPO or participating organization roster. Registrars must hold a minimum of an AP registration type.

All site staff (Lead Group and CTSU Sites) will use OPEN to enroll patients to this study. It is integrated with the CTSU Enterprise System for regulatory and roster data. 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. To assign an IVR or NPIVR as the treating, crediting, consenting, drug shipment (IVR only), or investigator receiving a transfer in OPEN, the IVR or NPIVR must list on their Form FDA 1572 in RCR the IRB number used on the site's IRB approval.

Prior to accessing OPEN site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes.
- All patients have signed an appropriate consent form and HIPAA authorization form (if applicable).

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.

The following information will be requested

4.1 Protocol Number

4.2 Investigator Identification

- 4.2.1 Institution and affiliate name (Institution CTEP ID)
- 4.2.2 Investigator's name (NCI number)
- 4.2.3 Cooperative Group Credit
- 4.2.4 Credit Investigator
- 4.2.5 Protocol specific contact information

4.3 Patient Identification

- 4.3.1 Patient's initials (first and last)
- 4.3.2 Patient's Hospital ID and/or Social Security number
- 4.3.3 Patient demographics
  - 4.3.3.1 Gender
  - 4.3.3.2 Birth date

4.3.3.3 Race

4.3.3.4 Ethnicity

4.3.3.5 Nine-digit ZIP code

4.3.3.6 Method of payment

4.3.3.7 Country of residence

4.4 Eligibility Verification

Patients must meet all of the eligibility requirements listed in Section 3. An eligibility checklist has been appended to the protocol. A confirmation of registration will be forwarded by the ECOG-ACRIN Operations Office - Boston.

4.5 Stratification Factors

Patients with surgically resected AJCC stage:

- IIIB
- IIIC
- M1a
- M1b

Rev. 9/14, 9/14

4.6 Additional Requirements

4.6.1 Patients must provide a signed and dated, written informed consent form.

Rev. 9/14

NOTE: CCOP institutions are required to present the patient with the Quality of Life questionnaires. The CCOP and non CCOP versions of the model consent are posted as separate documents on the ECOG website.

NOTE: Copies of the consent are not collected by the ECOG-ACRIN Operations Office - Boston.

Rev. 9/14

4.6.2 Pathological materials are to be submitted as indicated in Section 10 for central diagnostic review (required), and banking (including use for research in accordance with patient consent).

4.6.3 Blood samples are to be submitted for banking, including use for research per patient consent as indicated in Section 11.

Rev. 9/14

NOTE: ECOG-ACRIN requires that biological samples submitted from patients participating in E1609 be entered and tracked via the online ECOG-ACRIN Sample Tracking System (STS). See Section 10.4.

NOTE: Institutions outside of the United States and Canada must confer with the receiving laboratory and the ECOG-ACRIN Operations Office - Boston regarding logistics for submission of fresh samples.

4.6.4 Additional Registration Training Requirement

Mandatory Investigator Training Course

ECOG-ACRIN has developed a training course to provide additional information to enrolling investigators on the toxicity profile of ipilimumab. Each investigator is required to review the slide deck

titled: ***E1609 Ipilimumab Immune Related Adverse Events: Summary and Recommended Management***, prior to their first patient enrollment by accessing the following URL:

Rev. 2/14

http://coccg.mindflash.com/PublicCoursePage.aspx?CourseId=555542873P

Patient enrollments will be blocked via the OPEN system if the enrolling investigator ***has not*** completed the required training. If your site has a patient waiting and the enrolling INV completed the training after the hours of 9am – 5:30pmET Monday through Friday, please email the ECOG-ACRIN Ipi Education Team at ECOGIpi@ecogchair.org for after hours assistance.

Rev. 9/14

4.6.5 Patients must be at least 12 years of age. Patients 12-17 years of age must be registered and treated at a COG institution and be presented with the "Sample Research Information Consent/Parental Permission Form."

Rev. 3/15

4.7 Instructions for Patients who Do Not Start Assigned Protocol Treatment

If a patient does not receive any assigned protocol treatment, baseline and follow-up data will still be collected and must be submitted according to the instructions in the E1609 Forms Packet. Document the reason for not starting protocol treatment on the Off Treatment form. Also report the date and type of the first non-protocol treatment that the patient receives.

5. Treatment Plan

5.1 Surgical Considerations

Refer to Appendix VII for suggested guidelines on surgical management and techniques for lymphadenectomy. These are important standard of care surgical guidelines and all investigators are strongly urged to consider them in the management of their patients.

5.2 Administration Schedule

Rev. 9/14, 9/14
Rev. 3/15

NOTE: As of August 15, 2014, adult accrual to Arms A, B, and C has completed. Adolescent patients (ages 12 -17) will be randomized to study arms D, E, or F.

Rev. 2/12

Patients will be randomized to either Arms A or D (ipilimumab at 10 mg/kg) or Arms C or F (ipilimumab at 3 mg/kg) or Arms B or E (HDI).

5.2.1 ARMS A,C, D, or F: Patients Receiving Ipilimumab

5.2.1.1 Patients must receive their first infusion of ipilimumab within 7 days of randomization, and only after complete wound healing from surgery.

5.2.1.2 Use actual weight when calculating the dose.

Rev. 2/12

5.2.1.3 Ipilimumab at 10 mg/kg IV (Arms A, D) or 3 mg/kg (Arm C, F) infusion administered as follows:

Induction Phase

Rev. 2/12

Ipilimumab 10 mg/kg (Arms A, D) or 3 mg/kg (Arms C, F), administered by IV infusion every 3 weeks for a total of four doses.

Maintenance Phase

Rev. 2/12

Ipilimumab 10 mg/kg (Arms A, D) or 3 mg/kg (Arms C, F), administered by IV infusion every 12 weeks (3 months), beginning at week 24, then at weeks 36, 48, and 60.

Dose delays are allowed as per the dosing criteria described later in this section. Infusions should be given over 90 minutes (not bolus or IV push).

5.2.1.4 Dose Calculations: Calculate **Total Dose** as follows:

Rev. 2/12

Patient body weight in kg x [*10 mg or 3 mg/kg*] = total dose in mg

Calculate **Total Infusion Volume** as follows:

Total dose in mg ÷ 5 mg/mL = infusion volume in mL

Calculate **Rate of Infusion** as follows:

Infusion volume in mL ÷ 90 minutes = rate of infusion in mL/min.

Rev. 2/12

For example, a patient on Arms A or D (10 mg/kg) weighing 114 kg (250 lb) would be administered 1140 mg of ipilimumab (114 kg x 10 mg/kg = 1140 mg) with an infusion volume of 228 mL (1140 mg ÷ 5 mg/mL = 228 mL) at a rate of approximately 2.5 mL/min (228 mL ÷ 90 minutes) in 90 minutes.

5.2.2 ARMS B and E: Patients receiving Interferon Alfa - 2b

5.2.2.1 Patients must receive their first injection of IFN Alfa - 2b within 7 working days of randomization.

Rev. 2/13

5.2.2.2 Use actual weight when calculating body surface area.

5.2.2.3 **Induction Phase**

Interferon Alfa - 2b, 20 MU/m²/d (rounded to the nearest 1.0 million unit) administered **IV** x 5 consecutive days out of 7 (e.g., M-F) every week x 4 weeks.

Maintenance Phase

Interferon Alfa - 2b, 10 MU/m²/d (rounded to the nearest 1.0 million unit) subcutaneous every other day (e.g., M,W,F) three times each week x 48 wks.

5.2.2.4 Days 2-5 of interferon administration for weeks 1-4 may be administered at an institution other than the registering institution provided that the registering physician still retains primary oversight responsibility for the patient's treatment. Day 1 of each week should be administered at the registering institution. Documentation concerning all drugs administered, side effects and tests performed must be forwarded to the registering institution. The registering institution must document any care given at an outside institution. Home Health Agencies (HHA) may be used to treat patients, if the HHA is under the supervision of a Theradex approved institution and the nurse treating the patient is supervised by an approved doctor.

Rev. 2/12

5.2.2.5 Self Administration of Subcutaneous Doses

Patients who are deemed competent to self administer the subcutaneous maintenance doses of IFN Alfa - 2b may do so following the first 4 weeks of treatment. Interferon Alfa-2b should be prescribed in 10 million unit vials with instructions to reconstitute with 1 ml of diluent to reach a final concentration of 10:1. See Appendix X for directions and patient IFN information guide. Patients must complete the E1609 Patient Diary -Interferon (Appendix IV).

5.2.2.6 Corticosteroids and other immunosuppressive medications are contraindicated during IFN Alfa-2b therapy because of immune suppressive effects. No systemic treatment with steroids (including creams) is permitted. Exception: patients may receive antihistamines and inhaled steroids may be permitted at dosages that are not systematically

Rev. 2/14

immunosuppressive if there is no alternative medication. Also, steroids (topical, inhaled or systemic) may be used to treat adverse events or toxicities if there is a clear clinical indication.

5.2.3 Treatment at Recurrence

Rev. 8/12

A patient on E1609 ipilimumab treatment arms who experiences disease recurrence but was still considered surgically operable and can be rendered disease free by surgery should be reported as disease recurrence. However, in the absence of limiting toxicities per protocol criteria, such a patient may be offered additional ipilimumab therapy on protocol that must be captured on the usual forms (including treatment forms, toxicity forms, long term follow up for subsequent recurrence). In such a case the patient would re-start ipilimumab treatment at the next scheduled dose when medically cleared by his treating physician investigator.

Rev. 4/14

5.3 Adverse Event Reporting Requirements

5.3.1 **Purpose**

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial (please refer to the E1609 Forms Packet for the list of forms with directions for routine adverse event reporting). Additionally, certain adverse events must be reported in an expedited manner for more timely monitoring of patient safety and care. The following sections provide information about expedited reporting.

5.3.2 **Determination of reporting requirements**

Reporting requirements may include the following considerations: 1) whether the patient has received an investigational or commercial agent; 2) the characteristics of the adverse event including the grade (severity), the relationship to the study therapy (attribution), and the prior experience (expectedness) of the adverse event; 3) the phase (1, 2, or 3) of the trial; and 4) whether or not hospitalization or prolongation of hospitalization was associated with the event.

An investigational agent is a protocol drug administered under an Investigational New Drug Application (IND). In some instances, the investigational agent may be available commercially, but is actually being tested for indications not included in the approved package label.

Commercial agents are those agents not provided under an IND but obtained instead from a commercial source. The NCI, rather than a commercial distributor, may on some occasions distribute commercial agents for a trial.

Steps to determine if an adverse event is to be reported in an expedited manner:

Step 1: *Identify the type of event:* The descriptions and grading scales found in the Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. The CTEP Active Version of the CTCAE is identified and located on the CTEP website at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. All appropriate treatment areas should have access to a copy of the CTEP Active Version of CTCAE.

Step 2: *Grade the event using the Active Version of the NCI CTCAE.*

Step 3: *Determine whether the adverse event is related to the protocol therapy (investigational or commercial).* Attribution categories are as follows: Unrelated, Unlikely, Possible, Probable, and Definite.

Step 4: *Determine the prior experience of the adverse event. Expected* events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered *unexpected*, for expedited reporting purposes only, when either the type of event or the severity of the event is NOT listed in:

Rev. 10/11, 2/12 9/14

- **Arms A and D (ipilimumab at 10 mg/kg) and Arms C and F (ipilimumab at 3 mg/kg)** – the current NCI Specific Protocol Exceptions to Expedited Reporting (SPEER)
- **Arms B and E** – the drug package insert or protocol

Rev. 10/11, 2/12

NOTE: The NCI Specific Protocol Exceptions to Expedited Reporting (SPEER) is included in section 5.4 of the protocol. The SPEER is presented in the last column of the CAEPR and identified with bold and italicized text. FOR THIS PROTOCOL, events listed in the SPEER column should be considered EXPECTED if the grade being reported is the same or lower than the grade noted in the parentheses next to the AE in the SPEER. Events listed in the SPEER column should be considered UNEXPECTED if the grade being reported exceeds the grade noted in parentheses next to the AE in the SPEER."

Step 5: *Review the "Additional instructions, requirements, and exceptions for protocol E1609" table in section 5.3.6 for protocol and/or ECOG-ACRIN specific requirements for expedited reporting of specific adverse events that require special monitoring.*

NOTE: For general questions regarding expedited reporting requirements, please contact the CTEP-AERS Help Desk: 301-897-7497.

Rev. 2/12

5.3.3 Reporting procedures

This study requires that expedited adverse event reporting use CTEP's Adverse Event Reporting System (CTEP-AERS). CTEP's guidelines for CTEP-AERS can be found at http://ctep.cancer.gov. A CTEP-AERS report must be submitted electronically to ECOG-ACRIN and the appropriate regulatory agencies via the CTEP-AERS Web-based application located at http://ctep.cancer.gov.

In the rare event when Internet connectivity is disrupted a 24-hour notification is to be made by telephone to

- the AE Team at ECOG-ACRIN (617-632-3610) for Arms A, B, C, D, E, and F

Rev. 2/13, 9/14

- the NCI (301-897-7497) for Arms A and D, and Arms C and F

Rev. 2/13

- the FDA (800-332-1088) for Arms B and E

An electronic report MUST be submitted immediately upon re-establishment of internet connection.

Supporting and follow up data: Any supporting or follow up documentation must be faxed to ECOG-ACRIN (617-632-2990), Attention: AE within 48-72 hours. In addition, supporting or follow up documentation must be faxed to the NCI (301- 230-0159) for Arms A, C, D and F and FDA (800-332-0178) for Arms B and E in the same timeframe.

NCI Technical Help Desk: For any technical questions or system problems regarding the use of the CTEP-AERS application, please contact the NCI Technical Help Desk at ncictephelp@ctep.nci.nih.gov or by phone at 1-888-283-7457.

Rev. 2/12

5.3.4 When to report an event in an expedited manner

Some adverse events require 24-hour notification (refer to Section 5.3.6). Please complete a 24-Hour Notification Report via CTEP's website (http://ctep.cancer.gov) within 24 hours of learning of the event. The full CTEP-AERS report must be completed and submitted via CTEP-AERS within 5 calendar days.

Rev. 9/14

If the CTEP-AERS system is down, a 24-hour notification call must be made to ECOG-ACRIN (617-632-3610) and to NCI (301-897-7497). Once the system is restored, a 24-hour Notification Report must be entered into the CTEP-AERS system by the original submitter of the report at the site.

When an adverse event requires expedited reporting, submit a full CTEP-AERS report within the timeframes outlined in Section 5.3.6.

NOTE: Adverse events that meet the reporting requirements in Section 5.3.6 and occur within 30 days of the last dose of protocol treatment must be reported on an expedited adverse event report form (using CTEP-AERS). For any adverse events that occur more than 30 days after the last dose of treatment, only those that have an attribution of possibly, probably, or definitely AND meet the reporting

requirements in Section 5.3.6 must be reported on an expedited adverse event report form (using CTEP-AERS).

5.3.5 Other recipients of adverse event reports

DCTD/NCI will notify ECOG-ACRIN/pharmaceutical collaborator(s) of all AEs reported to FDA. Any additional written AE information requested by ECOG-ACRIN MUST be submitted to BOTH the NCI and ECOG-ACRIN.

Adverse events determined to be reportable must also be reported by the institution, according to the local policy and procedures, to the Institutional Review Board responsible for oversight of the patient.

5.3.6 Expedited reporting for investigational agents

NOTE: Although Arms B and E contain only commercially available agents, all arms will follow the investigational adverse event reporting requirements for this protocol.

Rev. 2/12, 9/14

Phase 2 and 3 Trials Utilizing an Agent under a CTEP IND: CTEP-AERS Expedited Reporting Requirements for Adverse Events That Occur Within 30 Days[1] of the Last Dose of Investigational Agent [Ipilimumab] in this Study (Arms A, C, D, or F) OR Within 30 Days of the Last Dose of Any Protocol Treatment (Arms A, B, C, D, E, or F).

Attribution	Grade 1	Grade 2	Grade 2	Grade 3		Grade 3		Grades 4 & 5[2]	Grades 4 & 5[2]
				Unexpected		Expected			
	Unexpected and Expected	Unexpected	Expected	with Hospitali-zation	without Hospitali-zation	with Hospitali-zation	without Hospitali-zation	Unexpected	Expected
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days	10 Calendar Days

[1] **Adverse events with attribution of possible, probable, or definite that occur greater than 30 days after the last dose of treatment with an agent under a CTEP IND require reporting as follows:**

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

- Grade 4 and Grade 5 unexpected events

CTEP-AERS 10 calendar day report:

- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 5 expected events

[2] Although a CTEP-AERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.

Please see additional information below under section entitled "Additional instructions, requirements, and exceptions for protocol E1609"

March 2005

NOTE: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause should be provided.

- **Expedited AE reporting timelines:**
  - **24 Hours; 5 calendar days** – The investigator must initially report the AE via CTEP-AERS within 24 hours of learning of the event followed by a complete CTEP-AERS report within 5 calendar days of the initial 24-hour report.
  - **10 calendar days** – A complete CTEP-AERS report on the AE must be submitted within 10 calendar days of the investigator learning of the event.

- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates **hospitalization* (or prolongation of existing hospitalization)** must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.
- Any event that results in **persistent or significant disability/incapacity, congenital anomaly, or birth defect** must be reported via CTEP-AERS if the event occurs following treatment with an agent under a CTEP IND
- Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.

* Hospitalizations are defined as lasting 24 hours or longer and these events must be reported via CTEP-AERS.

Additional instructions, requirements and exceptions for protocol E1609

1. Additional Instructions:

- With respect to determining the specific day by which the event must be reported, the day the reporter learns of the adverse event constitutes "Day 0"
- For instructions on how to specifically report events that result in persistent or significant disability/incapacity, congenital anomaly, or birth defect events via CTEP-AERS, please contact the AEMD Help Desk at 301-897-7497 or aemd@tech-res.com.

2. ECOG-ACRIN and Protocol Specific expedited reporting requirements:

The adverse events listed below also require expedited reporting for this trial:

ECOG-ACRIN specific expedited reporting requirements:

- **Hospitalizations:** Any grade 1 or 2 adverse event with precipitates a hospitalization lasting ≥ 24 hours (or prolongs hospitalization) must be reported via CTEP-AERS within 10 calendar days of learning of the event regardless of the attribution and designation as expected or unexpected.

Protocol specific expedited reporting requirements:

- **Bowel Perforation:** Any grade 3 or higher bowel perforation must be reported via CTEP-AERS within 10 calendar days of learning of the event regardless of the attribution and designation as expected or unexpected.

Rev. 2/14

- **Immune Related Adverse Events:** Any grade 2 or higher immune related adverse event (see section 5.5.1.3 for definition), *excluding skin reactions*, that occurs within 70 days of the last day of protocol treatment must be reported via CTEP-AERS within 10 calendar days of learning of the event regardless of the attribution and designation as expected or unexpected. Please submit any supporting data as well (ie: autoimmune serology tests or biopsy reports). In addition, any grade 2 or higher IRAE, excluding grade 2 or 3 skin reactions, that occurs greater than 70 days after the last dose of protocol treatment with an attribution of possible, probable, or definite must also be reported via CTEP-AERS within 10 calendar days of learning of the event. Any questions regarding if an event qualifies as an IRAE can be directed to the study chair.

Rev. 2/12, 4/14

5.3.7 Reporting second primary cancers

All cases of second primary cancers, including acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), that occur following treatment on NCI-sponsored trials must be reported to ECOG-ACRIN:

- **A second malignancy is a cancer that is UNRELATED to any prior anti-cancer treatment (including the treatment on this protocol). Second malignancies require ONLY routine reporting as follows:**
  1. Submit a completed Second Primary Form within 30 days to ECOG-ACRIN at

     ECOG-ACRIN Operations Office - Boston
     FSTRF
     900 Commonwealth Avenue
     Boston, MA 02215
  2. Submit a copy of the pathology report to ECOG-ACRIN confirming the diagnosis.
  3. If the patient has been diagnosed with AML/MDS, submit a copy of the cytogenetics report (if available) to ECOG-ACRIN
- **A secondary malignancy is a cancer CAUSED BY any prior anti-cancer treatment (including the treatment on this protocol). Secondary malignancies require both routine and expedited reporting as follows:**
  1. Submit a completed Second Primary Form within 30 days to ECOG-ACRIN at

     ECOG-ACRIN Operations Office - Boston
     FSTRF
     900 Commonwealth Avenue
     Boston, MA 02215
  2. Report the diagnosis via CTEP-AERS at http://ctep.cancer.gov
     Report under a.) leukemia secondary to oncology chemotherapy, b.) myelodysplastic syndrome, or c.) treatment related secondary malignancy
  3. Submit a copy of the pathology report to ECOG-ACRIN and NCI/CTEP confirming the diagnosis.
  4. If the patient has been diagnosed with AML/MDS, submit a copy of the cytogenetics report (if available) to ECOG-ACRIN and NCI/CTEP.

NOTE: The Second Primary Form and the CTEP-AERS report should not be used to report recurrence or development of metastatic disease.

NOTE: If a patient has been enrolled in more than one NCI-sponsored study, the Second Primary Form must be submitted for the most recent trial. ECOG-ACRIN must be provided with a copy of the form and the associated pathology report and cytogenetics report (if available) even if ECOG-ACRIN was not the patient's most recent trial.

NOTE: Once data regarding survival and remission status are no longer required by the protocol, no follow-up data should be submitted via CTEP-AERS or by the Second Primary Form.

Rev. 10/11, 11/13, 3/16

5.4 Comprehensive Adverse Events and Potential Risks List

The Comprehensive Adverse Events 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. Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 2678 patients.* Below is the CAEPR for Ipilimumab (MDX-010).

Rev. 2/12

Rev. 2/12

NOTE: FOR THIS PROTOCOL, events listed in the SPEER column should be considered EXPECTED if the grade being reported is the same or lower than the grade noted in the parentheses next to the AE in the SPEER. Events listed in the SPEER column should be considered UNEXPECTED if the grade being reported exceeds the grade noted in parentheses next to the AE in the SPEER.

Rev. 10/14

Version 2.7, June 28, 2015[1]

Adverse Events with Possible Relationship to Ipilimumab (MDX-010) (CTCAE 4.0 Term) [n= 2678]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
		Blood and lymphatic system disorders - Other (acquired hemophilia)	
CARDIAC DISORDERS			
	Atrial fibrillation		
		Myocarditis[2]	
EAR AND LABYRINTH DISORDERS			
	Hearing impaired		
ENDOCRINE DISORDERS			
	Adrenal insufficiency[2]		
	Endocrine disorders - Other (hypopituitarism/hypophysitis)[2]		
	Endocrine disorders - Other (testosterone deficiency)[2]		
	Hyperthyroidism[2]		
	Hypothyroidism[2]		

Adverse Events with Possible Relationship to Ipilimumab (MDX-010) (CTCAE 4.0 Term) [n= 2678]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
EYE DISORDERS			
	Eye disorders - Other (episcleritis)[2]		
	Uveitis[2]		
GASTROINTESTINAL DISORDERS			
	Abdominal pain		
	Colitis[2]		***Colitis (Gr 3)***
		Colonic perforation[3]	
	Constipation		
Diarrhea			***Diarrhea (Gr 3)***
	Enterocolitis		
	Esophagitis		
		Ileus	
Nausea			***Nausea (Gr 3)***
	Pancreatitis[2]		
	Vomiting		
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills		
Fatigue			***Fatigue (Gr 3)***
	Fever		***Fever (Gr 2)***
	Infusion related reaction		
		Multi-organ failure	
HEPATOBILIARY DISORDERS			
	Hepatobiliary disorders - Other (hepatitis)[2]		
IMMUNE SYSTEM DISORDERS			
	Autoimmune disorder[2]		
		Immune system disorders - Other (GVHD in the setting of allotransplant)	
INFECTIONS AND INFESTATIONS			
	Infections and infestations - Other (aseptic meningitis)[2]		
INVESTIGATIONS			
	Alanine aminotransferase increased		
	Aspartate aminotransferase increased		
	Neutrophil count decreased		

Adverse Events with Possible Relationship to Ipilimumab (MDX-010) (CTCAE 4.0 Term) [n= 2678]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		
	Dehydration		
	Hyperglycemia		
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		
	Arthritis		
	Musculoskeletal and connective tissue disorder - Other (polymyositis)[2]		
NERVOUS SYSTEM DISORDERS			
	Facial nerve disorder		
	Headache		
	Nervous system disorders - Other (Guillain-Barre syndrome)[2]		
	Nervous system disorders - Other (myasthenia gravis)[2]		
	Trigeminal nerve disorder		
RENAL AND URINARY DISORDERS			
	Acute kidney injury		
	Renal and urinary disorders - Other (granulomatous tubulointerstitial nephritis)		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Pneumonitis		
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
		Erythema multiforme	
	Pruritus		***Pruritus (Gr 3)***
Rash maculo-papular			***Rash maculo-papular (Gr 3)***
	Skin and subcutaneous disorders - Other (Sweet's Syndrome)		
		Stevens-Johnson syndrome	
		Toxic epidermal necrolysis	
	Urticaria		
VASCULAR DISORDERS			
	Hypotension		

[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] Ipilimumab can result in severe and fatal immune-mediated adverse events probably due to T-cell activation and proliferation. These can include (but are not limited to) autoimmune hemolytic anemia, acquired anti-factor VIII immune response, autoimmune aseptic meningitis, autoimmune hepatitis, autoimmune thyroiditis, hepatic failure, pure red cell aplasia, pancreatitis, ulcerative and hemorrhagic colitis, endocrine disorders (e.g., autoimmune thyroiditis, hyperthyroidism, hypothyroidism, autoimmune hypophysitis/hypopituitarism, and adrenal insufficiency), ocular manifestations (e.g., uveitis, iritis, conjunctivitis, blepharitis, and episcleritis), sarcoid granuloma, myasthenia gravis, polymyositis, and Guillain-Barre syndrome. The majority of these reactions manifested early during treatment; however, a minority occurred weeks to months after discontinuation of ipilimumab especially with the initiation of additional treatments.

[3] Late bowel perforations have been noted in patients receiving MDX-010 (ipilimumab) in association with subsequent IL-2 therapy.

[4] In rare cases diplopia (double vision) has occurred as a result of muscle weakness (Myasthenia gravis).

[5] Gastrointestinal hemorrhage includes Anal hemorrhage, Cecal hemorrhage, Colonic hemorrhage, Duodenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage, Hemorrhoidal hemorrhage, Ileal hemorrhage, Intra-abdominal hemorrhage, Jejunal hemorrhage, Lower gastrointestinal hemorrhage, Oral hemorrhage, Pancreatic hemorrhage, Rectal hemorrhage, Retroperitoneal hemorrhage, and Upper gastrointestinal hemorrhage under the GASTROINTESTINAL DISORDERS SOC

[6] Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

Adverse events reported on Ipilimumab (MDX-010) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that Ipilimumab (MDX-010) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Anemia; Blood and lymphatic system disorders - Other (pure red cell aplasia)[2]; Febrile neutropenia

CARDIAC DISORDERS - Conduction disorder; Restrictive cardiomyopathy

EYE DISORDERS - Extraocular muscle paresis[4]; Eye disorders - Other (retinal pigment changes)

GASTROINTESTINAL DISORDERS - Dyspepsia; Dysphagia; Gastrointestinal hemorrhage[5]

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Flu like symptoms; Non-cardiac chest pain

HEPATOBILIARY DISORDERS - Hepatic failure[2]

IMMUNE SYSTEM DISORDERS - Allergic reaction

INFECTIONS AND INFESTATIONS - Infection[6]

INVESTIGATIONS - Creatinine increased; Investigations - Other (rheumatoid factor); Lipase increased; Platelet count decreased; Serum amylase increased; Weight loss; White blood cell decreased

METABOLISM AND NUTRITION DISORDERS - Metabolism and nutrition disorders - Other (exacerbation of pre-existing diabetes mellitus)

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Back pain; Joint range of motion decreased; Myalgia; Pain in extremity

NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Tumor pain

NERVOUS SYSTEM DISORDERS - Dizziness; Dysphasia; Ischemia cerebrovascular; Peripheral motor neuropathy; Peripheral sensory neuropathy; Seizure

PSYCHIATRIC DISORDERS - Anxiety; Confusion; Depression; Insomnia

RENAL AND URINARY DISORDERS - Proteinuria

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Allergic rhinitis; Cough; Dyspnea; Laryngospasm

SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia, Dry skin; Hyperhidrosis; Skin hypopigmentation

VASCULAR DISORDERS - Flushing; Hypertension; Vascular disorders - Other (temporal arteritis)

NOTE: Ipilimumab (MDX-010) 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.

5.5 Dose Modifications

All toxicities should be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

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).

Rev. 11/13 9/14

NOTE: Per Addendum #7, the dose modification plan for Arms A and D (ipilimumab 10 mg/kg) has been modified to become stricter due to a higher incidence of severe toxicities observed on Arms A and D than seen in Arms C and F. Arms C and F dose modification guidelines continue to be the same, with minor changes.

Rev. 2/12, 11/13 9/14

5.5.1 ARMS A and D and Arms C and F: Patients Receiving Ipilimumab

5.5.1.1 Dose and Schedule Modifications for Arms A and D (Ipilimumab 10 mg/kg)

There will be no dose reductions for ipilimumab. The dose of ipilimumab will either be given or delayed/discontinued. Patients may develop study drug-related toxicities that may require skipping doses or dose discontinuation. Some of these adverse events may be consistent with potentially drug-related immune-mediated phenomena; termed IRAEs (Appendix XIII-Appendix XVII). Details of how to dose study medication in the presence of adverse drug reactions that may or may not be IRAEs are addressed below.

Patients will delay or discontinue treatment with ipilimumab if they experience at least one adverse event, specified below, considered by the investigator to be **certainly**, **probably**, or **possibly** related to ipilimumab treatment unless otherwise specified. The following criteria will be used to determine dosing delay, restarting doses, or discontinuing ipilimumab. For an adverse event, review the following criteria in a stepwise manner: First, assess the dose delay criteria and decide whether a scheduled dose should be delayed. Second, determine whether the permanent discontinuation criteria apply to the adverse event in question as well.

NOTE: Due to the possible effect of treatment with ipilimumab on the immunologic response to infectious disease vaccines, patients must not have had any infectious disease vaccination (e.g., standard influenza, H1N1 influenza, pneumococcal, meningococcal, tetanus toxoid) 4 weeks before or after any dose of ipilimumab.

5.5.1.1.1 Criteria to delay/skip one dose of ipilimumab on Arms A or D (ipilimumab 10 mg/kg)

Delay ipilimumab dosing for any of the following certainly, probably, or possibly treatment related adverse events, unless otherwise specified:

- Grade ≥ 1 diarrhea and/or colitis **regardless of attribution**. Grade ≥ 2 diarrhea and/or colitis, certainly, probably, or possibly related to ipilimumab, requires permanent discontinuation (Section 5.5.1.1.3).
- Any other ≥ Grade 2 non-skin related adverse event (including IRAEs) except for laboratory abnormalities.
- Grade ≥ 2 laboratory abnormalities that are secondary to an immune-related adverse event or autoimmune phenomenon (e.g., Grade ≥ 2 TSH associated with a CTCAE v.4 grade 2 thyroid dysfunction induced by ipilimumab, anemia, neutropenia, amylase, lipase, CPK, hyperglycemia, or elevated LFTs) should also lead to an ipilimumab dosing delay/skipping.
- Any other ≥ Grade 3 laboratory abnormality.
- Any ≥ Grade 3 skin-related adverse event (including IRAEs) **regardless of attribution**.

5.5.1.1.2 Criteria to resume ipilimumab treatment on Arms A or D (ipilimumab 10mg/kg)

Ipilimumab **may not** be restarted while the patient is being treated with oral or intravenous corticosteriods for the management of immune related adverse events except for patients on stable doses of hormone replacement therapy for adrenal insufficiency such as hydrocortisone. In addition, patients must be off and have no requirement for oral/I.V. corticosteroids for at least 1 week and meet the other criteria for retreatment as outlined below.

Restart ipilimumab dosing if/when the adverse event(s) resolve(s) to ≤ Grade 1

severity or returns to baseline within 3 weeks of last dose administration:

- If the adverse event has resolved (to ≤ Grade 1 severity or returns to baseline), restart ipilimumab dosing at the next scheduled time point per protocol.
- If the adverse event has not resolved in the protocol-specified dosing window (3 weeks [+/- 3 days], the next scheduled dose will be omitted.

Rev. 2/14

- Patients with Grade 1 diarrhea and/or colitis who require steroid therapy must have resolution to grade 0 (baseline) before resuming dosing with ipilimumab. Patients with Grade ≥ 2 related diarrhea and/or colitis must have ipilimumab permanently discontinued (Section 5.5.1.1.3).

5.5.1.1.3 Criteria for permanent discontinuation of ipilimumab for Related Adverse Events on Arms A or D (ipilimumab 10mg/kg)

Ipilimumab administration must be permanently discontinued for any of the following certainly, probably, or possibly treatment related adverse events, unless otherwise specified:

- Any ≥ Grade 2 eye pain or reduction of visual acuity that does not respond to topical therapy and does not improve to ≤ Grade 1 severity within 2 weeks of starting therapy, OR, requires systemic treatment.
- Any ≥ Grade 2 diarrhea and/or colitis related to ipilimumab. Any ≥ Grade 2 diarrhea/colitis should be considered RELATED unless immune related colitis is definitely ruled out (including by endoscopy and biopsy.)
- Any ≥ Grade 2 hypophysitis, pneumonitis, nephritis, and/or sarcoid-like lesions.
- Any new motor or sensory neurologic toxicity ≥ Grade 2 regardless of attribution (including Guillain-Barré syndrome and myasthenia gravis).
- Any ≥ Grade 3 bronchospasm or other hypersensitivity reaction.

- Any other ≥ Grade 3 non-skin adverse event with the exception of events listed under "Exceptions to Permanent Discontinuation" (Section 5.5.1.1.4).
- AST or ALT > 5 x ULN.
- Total Bilirubin > 3 x ULN.
- Any other ≥ Grade 4 laboratory abnormalities except for specified exceptions (Section 5.5.1.1.4).
- Any other ≥ Grade 4 adverse event.
- Stevens-Johnson syndrome, toxic epidermal necrolysis, or rash complicated by full thickness dermal ulceration or necrotic, bullous, or hemorrhagic manifestations.
- Any adverse event, laboratory abnormality or intercurrent illness which, in the judgment of the investigator, presents a substantial clinical risk to the patient with continued dosing.
- Patients who require high dose steroids, other immune suppressants or anti-TNF drug therapy for the management of immune related adverse events as described in the Toxicity Management Guidelines/Algorithms should have ipilimumab permanently discontinued.

5.5.1.1.4 Exceptions to permanent discontinuation of ipilimumab on Arms A and D (ipilimumab 10mg/kg)

Ipilimumab administration may be resumed in the following cases:

- Potentially reversible inflammation (< Grade 4), attributable to a local anti-tumor reaction and a potential therapeutic response. This includes inflammatory reactions at sites of tumor resections or in draining lymph nodes, or at sites suspicious for, but not diagnostic of metastasis.
- Laboratory abnormalities that are rapidly reversible, not life threatening, do not reflect underlying organ system dysfunction, and are not related to the study treatment, such as transient elevations of uric acid, hypocalcaemia, hypophosphatemia.

- Hospitalization for ≤ Grade 2 adverse events (not including Grade 2 events that require permanent discontinuation as listed under Section 5.5.1.1.3) where the primary reason for hospitalization is to expedite the clinical work-up.
- Patients with the following conditions where in the investigator's opinion continuing study drug administration is justified:
  - Ocular toxicity that has responded to topical therapy and has improved to ≤ Grade 1 severity within 2 weeks of starting therapy.
  - Patients without a diagnosis of hypophysitis who have Grade 2 hypothyroidism, Grade 2 low testosterone or Grade 2 adrenal insufficiency where clinical symptoms are controlled with appropriate hormone replacement therapy.

Rev. 11/13, 9/14

5.5.1.2 Dose and Schedule Modifications for **Arms C and F (Ipilimumab 3 mg/kg)**

There will be no dose reductions for ipilimumab. The dose of ipilimumab will either be given or delayed/discontinued. Patients may develop study drug-related toxicities that may require skipping doses or dose discontinuation. Some of these adverse events may be consistent with potentially drug-related immune-mediated phenomena; termed IRAEs (Appendix XIII-Appendix XVII). Details of how to dose study medication in the presence of adverse drug reactions that may or may not be IRAEs are addressed below.

Rev. 8/12

Patients will delay or discontinue treatment with ipilimumab if they experience at least one adverse event, specified below, considered by the investigator to be **certainly**, **probably**, or **possibly** related to ipilimumab treatment unless otherwise specified. The following criteria will be used to determine dosing delay, restarting doses, or discontinuing ipilimumab. For an adverse event, review the following criteria in a stepwise manner: First, assess the dose delay criteria and decide whether a scheduled dose should be delayed. Second, determine whether the permanent discontinuation criteria apply to the adverse event in question as well.

NOTE: Due to the possible effect of treatment with ipilimumab on the immunologic response to infectious disease vaccines, patients must not

have had any infectious disease vaccination (e.g., standard influenza, H1N1 influenza, pneumococcal, meningococcal, tetanus toxoid) 4 weeks before or after any dose of ipilimumab.

Rev. 9/14

5.5.1.2.1 **Criteria to delay/skip one dose of ipilimumab on Arms C and F (ipilimumab 3 mg/kg)**

Delay ipilimumab dosing for the following certainly, probably, or possibly treatment related adverse events, unless otherwise specified:

- Any ≥ Grade 2 non-skin related adverse event (including IRAEs) except for laboratory abnormalities.
- Grade ≥ 2 laboratory abnormalities that are secondary to an immune-related adverse event or autoimmune phenomenon (e.g., Grade ≥ 2 TSH associated with a CTCAE v.4 grade 2 thyroid dysfunction induced by ipilimumab, anemia, neutropenia, amylase, lipase, CPK, hyperglycemia, or elevated LFTs) should also lead to an ipilimumab dosing delay/skipping.
- Any other ≥ Grade 3 laboratory abnormality.
- Any ≥ Grade 3 skin-related adverse event (including IRAEs) regardless of attribution.

Rev. 2/12, 9/14

5.5.1.2.2 **Criteria to resume ipilimumab treatment on Arms C and F (ipilimumab 3mg/kg)**

Ipilimumab may not be restarted while the patient is being treated with oral or intravenous corticosteriods for the management of immune related adverse events except for patients on stable doses of hormone replacement therapy for adrenal insufficiency such as hydrocortisone. In addition, patients must be off and have no requirement for oral/I.V. corticosteroids for at least 1 week and meet the other criteria for retreatment as outlined below.

Restart ipilimumab dosing if/when the adverse event(s) resolve(s) to ≤ Grade 1 severity or returns to baseline within 3 weeks of initial dose administration:

- If the adverse event has resolved (to ≤ Grade 1 severity or returns to baseline), restart ipilimumab dosing at the next scheduled time point per protocol.
- If the adverse event has not resolved in the protocol-specified dosing window (3 weeks [+/- 3 days], the next scheduled dose will be omitted.
- Patients with Grade 2 diarrhea/colitis must have resolution to Grade 0 (baseline) severity and must have a follow-up colonoscopy to document endoscopic (with or without pathologic) resolution of inflammation before resuming dosing with ipilimumab.

5.5.1.2.3 **Criteria for permanent discontinuation of ipilimumab for Related Adverse Events**

Ipilimumab administration must be permanently discontinued if any of the following Related Adverse Events occurs:

- Any ≥ Grade 2 eye pain or reduction of visual acuity that does not respond to topical therapy and does not improve to ≤ Grade 1 severity within 2 weeks of starting therapy, OR, requires systemic treatment.
- Any ≥ Grade 2 hypophysitis.
- Any ≥ Grade 3 bronchospasm or other hypersensitivity reaction.
- Any other ≥ Grade 3 non-skin related adverse event with the exception of events listed under “Exceptions to Permanent Discontinuation” (Section 5.5.1.2.4).
- Any ≥ Grade 4 laboratory abnormalities, except for specified exceptions (Section 5.5.1.2.4).
- For AST, ALT, or Total Bilirubin, then as follows:
  - AST or ALT > 5 x ULN.
  - Total Bilirubin > 3 x ULN.
- Any other ≥ Grade 4 adverse event except for specified exceptions (Section 5.5.1.2.4).
- Stevens-Johnson syndrome, toxic epidermal necrolysis, or rash

complicated by full thickness dermal ulceration or necrotic, bullous, or hemorrhagic manifestations.

- Any adverse event, laboratory abnormality or intercurrent illness which, in the judgment of the investigator, presents a substantial clinical risk to the patient with continued dosing.
- Any motor or sensory neurologic toxicity ≥ Grade 3 regardless of attribution.
- Diagnosis with Guillain-Barré syndrome or myasthenia gravis (any grade).
- Patients who require high dose steroids, other immune suppressants or anti-TNF drug therapy for the management of immune related adverse events as described in the Toxicity Management Guidelines/Algorithms should have ipilimumab permanently discontinued. Treatment with oral budesonide or moderate dose steroids for grade 2 colitis or grade 2 or lower skin rash or higher dose IV steroids for grade 3 skin rash are criteria for ipilimumab dose delay but not permanent discontinuation.

Rev. 9/14

5.5.1.2.4 **Exceptions to permanent discontinuation of ipilimumab on Arms C and F (ipilimumab 3mg/kg)**

Ipilimumab administration may be resumed in the following cases:

- Potentially reversible inflammation (< Grade 4), attributable to a local anti-tumor reaction and a potential therapeutic response. This includes inflammatory reactions at sites of tumor resections or in draining lymph nodes, or at sites suspicious for, but not diagnostic of metastasis.
- Laboratory abnormalities that are rapidly reversible, not life threatening, do not reflect underlying organ system dysfunction, and are not related to the study treatment, such as transient elevations of uric acid, hypocalcaemia, hypophosphatemia.
- Hospitalization for ≤ Grade 2 adverse events (not including Grade 2 events

that require permanent discontinuation as listed under Section 5.5.1.2.3) where the primary reason for hospitalization is to expedite the clinical work-up.

- Patients with the following conditions where in the investigator's opinion continuing study drug administration is justified:
  - Ocular toxicity that has responded to topical therapy and has improved to ≤ Grade 1 severity within 2 weeks of starting therapy.
  - Patients without a diagnosis of hypophysitis who have Grade 2 hypothyroidism, Grade 2 low testosterone or Grade 2 adrenal insufficiency where clinical symptoms are controlled with appropriate hormone replacement therapy.

Rev. 8/12

Rev. 9/14

NOTE: A patient on E1609 ipilimumab treatment arms **(Arm A and Arm C or Arm D and Arm F, respectively)** who experiences disease recurrence but was still considered surgically operable and can be rendered disease free by surgery should be reported as disease recurrence. However, in the absence of limiting toxicities per protocol criteria, such a patient may be offered additional ipilimumab therapy on protocol that must be captured on the usual forms (including treatment forms, toxicity forms, long term follow up for subsequent recurrence). In such a case the patient would re-start ipilimumab treatment at the next scheduled dose when medically cleared by his treating physician investigator.

5.5.1.3 Immune-Related Adverse Events (irAEs): Definition, Monitoring, and Treatment

Blocking CTLA-4 function may permit the emergence of auto-reactive T cells and resultant clinical autoimmunity. Rash/vitiligo, diarrhea/colitis, uveitis/episcleritis, hepatitis,

and hypopituitarism were drug-related, presumptive autoimmune events, now termed irAEs, noted in previous ipilimumab studies.

For the purposes of this study, an irAE is defined as an AE of unknown etiology associated with drug exposure and consistent with an immune phenomenon. Efforts should be made to rule out neoplastic, infectious, metabolic, toxin or other etiologic causes prior to labeling an AE an irAE. Serological, immunological, and histological (biopsy) data should be used to support the diagnosis of an immune-mediated toxicity. Suspected irAEs must be documented on an AE or SAE form.

Patients should be informed of and carefully monitored for evidence of clinically significant systemic irAE (e.g., systemic lupus erythematosus-like diseases) or organ-specific irAE (e.g., rash, colitis, uveitis, hepatitis or thyroid disease). If an irAE is noted, appropriate work-up (including biopsy if possible) should be performed, and steroid therapy may be considered if clinically necessary. See Appendix XIII for suggested work-up and treatment of irAEs.

It is unknown if systemic corticosteroid therapy has an attenuating effect on ipilimumab activity. However, clinical anti-tumor responses have been maintained in patients treated with corticosteroids and discontinued from ipilimumab. If utilized, corticosteroid therapy should be individualized for each patient.

Corticosteroid replacement therapy is not allowed except for patients who develop endocrinopathies during this study that require corticosteroid replacement therapy (such as hydrocortisone) at stable doses.

Rev. 11/13

Prior experience suggests that diarrhea and/or colitis that is persistent or severe requires corticosteroid treatment. See Appendix XIV for GI Management Algorithm for additional details.

5.5.1.4 Supportive care considerations for ipilimumab administration

5.5.1.4.1 Treatment of infusion reactions associated with ipilimumab

Since ipilimumab contains only human protein sequences, it is less likely that any allergic reaction will be seen in patients. However, it is possible that infusion of ipilimumab will induce a cytokine release syndrome that could be evidenced by fever, chills, rigors, rash, pruritus, hypotension, hypertension, bronchospasm, or other

symptoms. No prophylactic pre-medication will be given unless indicated by previous experience in an individual patient. Reactions should be treated based upon the following recommendations.

- For mild symptoms (e.g., localized cutaneous reactions such as mild pruritus, flushing, rash):
  - Decrease the rate of infusion until recovery from symptoms, remain at bedside and monitor patient.
  - Complete the ipilimumab infusion at the initial planned rate.
  - Diphenhydramine 50 mg IV may be administered at the discretion of the treating physician and patients may receive additional doses with close monitoring.
  - Premedication with diphenhydramine may be given at the discretion of the investigator for subsequent doses of ipilimumab.
- For moderate symptoms (any symptom not listed above [mild symptoms] or below [severe symptoms] such as generalized pruritus, flushing, rash, dyspnea, hypotension with systolic BP > 80 mmHg):
  - Interrupt ipilimumab.
  - Administer diphenhydramine 50 mg IV.
  - Monitor patient closely until resolution of symptoms.
  - Corticosteroids may abrogate any beneficial immunologic effect, but may be administered at the discretion of the treating physician.
  - Resume ipilimumab infusion after recovery of symptoms.
  - At the discretion of the treating physician, ipilimumab infusion may be resumed at one half the initial infusion rate, then increased incrementally to the initial infusion rate.
  - If symptoms develop after resumption of the infusion, the infusion should be discontinued and

no additional ipilimumab should be administered that day.

- The next dose of ipilimumab will be administered at its next scheduled time and may be given with pre-medication (diphenhydramine and acetaminophen) and careful monitoring, following the same treatment guidelines outlined above.
- At the discretion of the treating physician additional oral or IV antihistamine may be administered prior to dosing with ipilimumab.

- For severe symptoms (e.g., any reaction such as bronchospasm, generalized urticaria, systolic blood pressure < 80 mm Hg, or angioedema):
  - Immediately discontinue infusion of ipilimumab, and disconnect infusion tubing from the subject.
  - Consider bronchodilators, epinephrine 1 mg IV or subcutaneously, and/or diphenhydramine 50 mg IV, with solumedrol 100 mg IV, as needed.
  - Patients should be monitored until the investigator is comfortable that the symptoms will not recur.
  - No further ipilimumab will be administered.
- In case of late-occurring hypersensitivity symptoms (e.g., appearance within one week after treatment of a localized or generalized pruritus), symptomatic treatment may be given (e.g., oral antihistamine, or corticosteroids).

5.5.1.4.2 **Treatment of Ipilimumab-Related Isolated Drug Fever**

In the event of isolated drug fever, the investigator must use clinical judgment to determine if the fever is related to the ipilimumab or to an infectious etiology. If a patient experiences isolated drug fever, for the next dose, pre-treatment with acetaminophen or non-steroidal anti-inflammatory agent (investigator discretion) should be instituted and a repeated antipyretic dose at 6 and 12 hours after

ipilimumab infusion, should be administered. The infusion rate will remain unchanged for future doses. If a patient experiences recurrent isolated drug fever following premedication and post dosing with an appropriate antipyretic, the infusion rate for subsequent dosing should be decreased to 50% of the previous rate. If fever recurs following infusion rate change, the investigator should assess the patient's level of discomfort with the event and use clinical judgment to determine if the patient should receive further ipilimumab.

5.5.1.5 Suggested evaluation and treatment for Immune Related Adverse Events (irAEs) associated with ipilimumab

NOTE: This information has been summarized from the Ipilimumab Investigator Brochure (IB). Please refer to the current version of the IB for more details on the Suggested Work-up and Treatment for irAEs and Management Algorithms. Although these are suggested guidelines that take into consideration potential variations that may be required based on a specific clinical situation, these guidelines are strongly recommended.

Management algorithms for the early detection and treatment of ipilimumab associated toxicities are provided in Appendices Appendix XII-Appendix XVII.

Gastrointestinal (diarrhea) and skin (rash)-related toxicities are the most common irAEs reported in previous studies with ipilimumab. Suggested evaluation procedures for suspected irAEs of the GI tract, liver, skin, eye, pituitary, and adrenal gland are described in the current version of the IB. When symptomatic therapy is inadequate or inappropriate, an irAE should be treated with systemic corticosteroids followed by a gradual taper.

5.5.1.5.1 Gastrointestinal Tract

Diarrhea (defined as either first watery stool, or increase in frequency 50% above baseline with urgency or nocturnal bowel movement, or bloody stool) should be further evaluated and infectious or alternate etiologies ruled out. Subjects should be advised to inform the investigator if any diarrhea occurs, even if it is mild. An algorithm for managing subjects with

diarrhea or suspected colitis is provided in Appendix XIV and the IB.

Corticosteroid therapy is strongly recommended for ipilimumab related ≥ Grade 3 diarrhea/colitis and should be slowly tapered according to symptomatic response over at least 1 month. Subjects with ipilimumab related Grade 2 diarrhea/colitis may be initially treated conservatively, but should be immediately switched to corticosteroids if symptoms persist or worsen. For more severe symptoms in patients with grade 1 or 2, prednisone 60 mg or equivalent may be required to control initial symptoms, and the dose should be gradually tapered over at least a 1-month duration. Lower doses of prednisone may be considered for less severe cases of colitis. It is suggested that prednisone (for oral administration) or solumedrol (for IV administration) be the corticosteroids of choice in the treatment of colitis.

Rev. 11/13

If the diarrhea is prolonged or severe or is associated with signs of systemic inflammation or acute phase reactants (e.g., increased CRP or platelet count; or bandemia), it is recommended that sigmoidoscopy (or colonoscopy, if appropriate) with colonic biopsy of 3 to 5 specimens for standard paraffin block be performed. It is strongly recommended that these patients be hospitalized for I.V. corticosteroids and inpatient work-up. All subjects with confirmed colitis should also have an ophthalmologic examination, and a slit-lamp exam should be considered, to rule out uveitis. Also consider testing for stool calprotectin and stool WBCs as outlined in Appendix XIV. Negative stool testing for calprotectin or WBCs does not rule out autoimmune colitis.

Rev. 2/12

Rev. 11/13

Infrequently, subjects will appear refractory to corticosteroids or will flare following taper of corticosteroids. In these subjects, unless contraindicated (i.e., sepsis and other serious infections, or perforation), a single dose of infliximab at 5 mg/kg may provide benefit.

5.5.1.5.2 **Hepatotoxicity**

Liver function tests should always be performed and reviewed prior to administration of all ipilimumab doses. In addition, subjects presenting with right upper quadrant abdominal pain, unexplained nausea, or vomiting should have LFTs performed immediately and reviewed before administering the next dose of study drug. A Hepatotoxicity Management Algorithm is provided in Appendix XV and the current IB.

LFTs ≥ Grade 2 (for subjects with normal baseline LFT) or LFT ≥ 2 times baseline values (for subjects with baseline LFT of Grade 1 or 2) should prompt treating physicians to: (1) increase frequency of monitoring LFTs to at least every 3 days until LFT have stabilized or improved; (2) investigate to rule out non-irAE etiologies; and (3) initiate an autoimmunity evaluation. Disease progression, other malignancies, concurrent medications, viral hepatitis, and toxic etiologies should be considered and addressed, as appropriate. Imaging of the liver, gall bladder, and bile ducts should be considered to rule out neoplastic or other non-irAE-related causes for the increased LFTs. An ANA, perinuclear anti-neutrophil cytoplasmic antibody (pANCA), and anti-smooth muscle antibody test should be performed if an autoimmune etiology is considered. Consultation with a hepatologist is appropriate for a suspected liver IRAE and a biopsy should be considered.

Rev. 2/13

For rising LFTs, ipilimumab dosing should be held according to the dose delay/permanent discontinuation guidelines.

Rev. 2/13

For AST/ALT > 5x the ULN or total bilirubin > 3x the ULN, the following should be done: (1) ipilimumab should not be administered; (2) LFTs should be repeated every 24 hours until stabilization or improvement; and (3) therapeutic intervention with high dose steroids should be strongly considered (e.g., methylprednisolone 1-2 mg/kg once or twice daily or equivalent). If symptoms or LFT elevations are controlled, the corticosteroid dose should be gradually tapered over a

period of at least 1 month. Flare in LFTs during this taper may be treated with an increase in the dose of steroid and a slower taper. The most current experience with immune-related hepatitis has allowed further development of this management algorithm (see flow chart in Appendix XV and current IB) to include recommendations for treatment.

Rev. 2/13, 11/13

For rising LFTs with AST/ALT> 5X ULN or total bilirubin >3X the ULN or suspected immune-mediated hepatitis:

a. Admit subject to hospital for evaluation and close monitoring.

Rev. 2/13

b. Stop any further ipilimumab dosing per protocol (Section 5.5.1.2.3 of protocol).

Rev. 2/13

c. Start at least 2 mg/kg methylprednisolone sodium succinate per day, given IV as a single or divided dose.

d. Check liver laboratory test values (LFTs, T-bilirubin) daily until stable or showing signs of improvement for at least 3 consecutive days.

e. If no decrease in LFTs after 3 days or rebound hepatitis occurs despite treatment with corticosteroids, then add mycophenolate mofetil 1g BID per institutional guidelines for immunosuppression of liver transplants (supportive treatment as required, including prophylaxis for opportunistic infections per institutional guidelines).

f. If no improvement after 5 to 7 days, consider adding 0.10 to 0.15 mg/kg/day of tacrolimus (trough level 5-20 ng/mL)

Rev. 2/13

g. If target trough level is achieved with tacrolimus but no improvement is observed after 5 to 7 days, consider other immunosuppressive therapy per institutional guidelines and in consultation with hepatology.

h. Continue to check LFTs daily for at least 2 weeks to monitor sustained response to treatment.

5.5.1.5.3 Pancreas

Symptoms of abdominal pain associated with elevations of amylase and lipase, suggestive of pancreatitis, may rarely be associated with anti-CTLA-4 monoclonal antibody administration. The differential diagnosis of acute abdominal pain should include pancreatitis. Appropriate workup should include serum amylase and lipase tests.

5.5.1.5.4 Skin

A dermatologist should evaluate persistent or severe rash or pruritus. A biopsy should be performed if appropriate and if possible, photos of the rash should also be obtained. Any non-protocol drugs that could contribute to a drug reaction should be stopped if possible pending evaluation. Patients with low-grade ipilimumab-mediated skin toxicity (Grade 1 or 2) may remain on therapy and could be treated with symptomatic therapy (e.g., antihistamines). Low-grade symptoms persisting for 1 to 2 weeks and relapsing should be treated with topical or moderate dose oral corticosteroid therapy (e.g., prednisone 1 mg/kg once daily or equivalent). High-grade (persistent Grade 3 despite moderate dose oral corticosteroid such as prednisone 1 mg/kg once daily or equivalent or any Grade 4) symptoms require high-dose IV corticosteroid therapy (e.g., methylprednisolone 2 mg/kg once or twice per day or equivalent) to control initial symptoms. A skin biopsy should be performed if appropriate. Once rash or pruritis is controlled, the initiation of corticosteroid taper should be based on clinical judgment; however, the corticosteroid dose should be gradually tapered over a period of at least 1 month.

Patients with any high-grade skin related toxicity (Grade 3 regardless of causality) have to skip ipilimumab and may only continue treatment with ipilimumab if the initial symptoms have improved to ≤ Grade 1, while patients with grade 4 skin toxicities have to permanently discontinue ipilimumab.

5.5.1.5.5 Endocrine

Subjects with unexplained symptoms such as fatigue, myalgias, impotence, mental status changes, or constipation should be investigated for the presence of thyroid, pituitary, or adrenal endocrinopathies. An endocrinologist should be consulted if an endocrinopathy is suspected. If there are any signs of adrenal crisis such as severe dehydration, hypotension, or shock, intravenous corticosteroids with mineralocorticoid activity (e.g., methylprednisolone) should be initiated immediately. If the patient's symptoms are suggestive of an endocrinopathy but the patient is not in adrenal crisis, endocrine laboratory results should be evaluated before corticosteroid therapy is initiated.

Endocrine work up should include at least Thyroid stimulating hormone and free T4 levels to determine if thyroid abnormalities are present. TSH, prolactin, and a morning cortisol level will help to differentiate primary adrenal insufficiency from primary pituitary insufficiency. Radiographic imaging (e.g., MRI) with pituitary cuts should be performed. If the pituitary scan and/or endocrine laboratory tests are abnormal and suggestive of pituitary endocrinopathy, a short course of high dose corticosteroids (e.g., dexamethasone 4 mg every 6 hours or equivalent) should be strongly considered in an attempt to treat the presumed pituitary inflammation, but it is currently unknown if this will reverse the pituitary dysfunction. Abrupt discontinuation of corticosteroids should be avoided due to possible prolonged adrenal suppression. Once symptoms or laboratory abnormalities are controlled, and overall patient improvement is evident, the initiation of steroid taper should be based on clinical judgment; however the corticosteroid dose should be gradually tapered over a period of at least 1 month. Appropriate hormone replacement therapy should be instituted if an endocrinopathy is documented, and it is possible that subjects may require life-long hormone replacement.

Patients diagnosed with hypophysitis should be permanently discontinued from additional ipilimumab therapy.

Please see Appendix XVI and the current IB for the Endocrinopathy Management Algorithm.

5.5.1.5.6 **Eye**

Ocular inflammation (episcleritis or uveitis), usually in association with colitis, was reported in a few subjects. These conditions responded to topical corticosteroid therapy. An ophthalmologist should evaluate visual complaints with examination of the conjunctiva, anterior and posterior chambers and retina; visual field testing and an electroretinogram should also be performed. Patients with ipilimumab related uveitis or episcleritis have been treated with topical corticosteroid eye drops. On this study, the last dose of ipilimumab will be at approximately week 60. Ophthalmologic examination is strongly recommended at 6 and 18 months after start of treatment, to be performed by an ophthalmologist. If melanoma recurrence occurs before these time points, the ophthalmological examination should still be done if clinically indicated.

Rev. 2/13

5.5.1.5.7 **Neuropathies**

Isolated cases of motor neuropathy of an autoimmune origin have been reported among patients treated with ipilimumab. Three cases have been diagnosed as Guillain-Barre syndrome (GBS), two of which were considered study related. In both cases, the GBS was atypical in nature and more clinically resembled polyneuritis. As of 30 June 2009, 27 cases of neuropathy SAEs have been reported.

Of these, 22 were assessed as unrelated to study therapy because alternative etiologies, including brain metastases, spinal cord compression, arterial thrombosis, or platinum-based chemotherapy were identified in almost every case. Please see Appendix XVII and the current IB for the Neuropathy Management Algorithm.

Rev. 9/14

5.5.2 ARMS B AND E: Patients receiving Interferon Alfa - 2b

Induction therapy by the IV route in the first 4 weeks shall be evaluated separately from Maintenance in weeks 5-52; a patient requiring dose modification(s) in the first month will therefore commence month 2 without prejudice, at full dosage. Doses missed during treatment due to toxicity, patient compliance, holiday, etc. should not be made up.

Overview

If a patient requires a dose modification (details are listed below), treatment must first be held. The first modification then requires a 33% reduction of dosage (to 13.3 MU/m²) and the second modification requires a 66% reduction of dosage (to 6.6 MU/m²). A patient who requires a third dose modification will be removed from treatment. Dose re-escalation will not be attempted following resolution of toxicity that required dose interruption or attenuation. Please see the table below.

	Full Treatment	Dose Mod 1	Dose Mod 2	Dose Mod 3
Arm B or E Induction (Weeks 1-4): High Dose IFN Alfa - 2b	20 MU/m²	13.3 MU/m²	6.6 MU/m²	Off
Arm B or E Maintenance (Weeks 5-52): High Dose IFN Alfa - 2b	10 MU/m²	6.6 MU/m²	3.3 MU/m²	Off

PLEASE NOTE: If a patient experiences any of the toxicities listed below, the patient must have a dose modification as follows: treatment must be held until the toxicity returns to institution's normal limits, patient's baseline, or normal limits per Common Toxicity Criteria or as listed in the table under Section 5.5.2.8, then reduced per above.

Rev. 2/12
Rev. 8/12

EXCEPTIONS: For Grade 3 proteinuria without Creatinine or BUN elevation, dose should be held until return to Grade 2 toxicity. For Grade 2 weight loss observed over a period of one month, dose should be held until weight gain or stabilization.

The following are the most common toxicities observed with interferon. Guidelines for HDI Dose Modification and Discontinuation are also provided. These toxicities and their management guidelines are based on experience from previous studies (E1684, E1690, E1694) and the following 2 publications that may also serve as additional resources on the toxicities associated with HDI and their management:

Kirkwood JM, Bender C, Agarwala S, Tarhini A, Shipe-Spotloe J, Smelko B, Donnelly S, Stover L. Mechanisms and management of toxicities associated with high-dose interferon alfa-2b therapy. *J Clin Oncol*. 2002 Sep 1;20(17):3703-18. Review.

Hauschild A, Gogas H, Tarhini A, Middleton MR, Testori A, Dréno B, Kirkwood JM. Practical guidelines for the management of interferon-alpha-2b side effects in patients receiving adjuvant treatment for melanoma: expert opinion. *Cancer*. 2008 Mar 1;112(5):982-94.

NOTE: Dose modifications may be done for toxicities related to HDI therapy even if they don't fit these guidelines if it is determined by the treating physician that it is in the best interest of the patient for safety reasons taking into consideration the overall clinical status of the subject. A note has to be made of such a modification.

Rev. 2/12

5.5.2.1 **Constitutional Toxicity**

Fever, chill, myalgia/arthralgia, fatigue, headache: these constitutional toxicities of the interferons and other biologics are observed in the first weeks of treatment. Fatigue may worsen during the course of therapy and require a dose modification. In general, dose modifications are done for grade 3 or higher toxicities.

5.5.2.2 **Myelotoxicity**

Leukopenia, anemia and thrombocytopenia are observed with acute and chronic interferon therapy. Dose modifications are indicated for grade 4 granulocytopenia and grade 3 thrombocytopenia.

5.5.2.3 **Hepatotoxicity**

Fatal hepatotoxicity has been observed in patients receiving sustained intensive high dosages of interferon, and close attention to the occurrence of hepatocellular enzyme abnormalities is warranted. Grade 3 or greater elevations of bilirubin, AST, ALT or alkaline phosphatase require dosage modification. Please refer to table under Section 5.5.2.8.

Rev. 8/12

5.5.2.4 **Nephrotoxicity**

Rev. 8/12

Acute kidney injury of Grade 1 (CTCAE v.4) is an indication for dose modification and elevations of Grade 2 require withdrawal of therapy.

5.5.2.5 Neurotoxicity, including Neuropsychiatric, Neurosensory, and Neuromotor

Mood alterations and cognitive dysfunction have been reported as toxicities of the interferons, especially in the elderly and those with underlying disorders. Grade 3 or greater Neurotoxicity is a dose modification criterion, and if neuropsychiatric toxicity is encountered this ought to be evaluated by a capable specialist (psychiatrist, and/or psychologist).

5.5.2.6 **Cardiopulmonary**

Cardiac arrhythmia has been reported as a complication of interferon therapy, and the appearance of signs or

symptoms of cardiac arrhythmia, or other cardiopulmonary toxicity of Grade 2 dictates dose modification, and resumption of treatment only after normalization and, in general, formal cardiologic evaluation and clearance. Grade 3 toxicity at any time requires withdrawal of therapy.

5.5.2.7 Gastrointestinal

Nausea, vomiting, and/or diarrhea are infrequent but well-defined toxicities of interferon, which may abate with supportive care but require dose modification if observed at Grade 3, at any time, or if persistent for more than 2 weeks at Grade 2. Weight loss of Grade 2 is likewise a criterion for dose modification if observed over a period of one month.

5.5.2.8 HDI Dose Modification and Discontinuation Guidelines (please also see sections 5.5.2.1-5.5.2.7)

Toxicity	1. Threshold for Reduction 2. % Dose Reduction from Starting Dose 3. Threshold for Resumption		
	First Dose Reduction	Second Dose Reduction	Treatment Discontinuation
Autoimmune Reactions			
Hypothyroidism	None	None	Discontinue if thyroid function is unstable with treatment.
Psoriasis	1. ≥ grade 2 skin rash (localized desquamation or other lesions covering <50% of body surface area) 2. 33% 3. Grade 1 (erythema without associated symptoms)	1. ≥ grade 2 skin rash (localized desquamation or other lesions covering <50% of body surface area) 2. 66% 3. Grade 1 (erythema without associated symptoms)	1. ≥ grade 2 skin rash (localized desquamation or other lesions covering <50% of body surface area) 2. Discontinue
Hematologic Toxicity			
Granulocytopenia	1. < 500 cells/mm^3 (Grade 4) 2. 33% 3. ≥1000 cells/mm^3	1. < 500 cells/mm^3 (Grade 4) 2. 66% 3. ≥1000 cells/mm^3	1. < 500 cells/mm^3 (Grade 4) 2. Discontinue
Thrombocytopenia	1. < 50,000 cells/mm^3 (Grade 3) 2. 33% 3. >75,000 cells/mm^3 (Grade 1)	1. < 50,000 cells/mm^3 (Grade 3) 2. 66% 3. >75,000 cells/mm^3 (Grade 1)	1. <50,000 cells/mm^3 (Grade 3) 2. Discontinue

Other			
Anorexia	1. Grade 3 or 4 2. 33% 3. Grade 2	1. Grade 3 or 4 2. 66% 3. Grade 2	1. Grade 3 or 4 2. Discontinue
Cardiotoxicity	1. Grade 2 2. 33% 3. Normalization and, consider formal cardiologic evaluation and clearance	1. Grade 2 2. 66% 3. Normalization and, consider formal cardiologic evaluation and clearance	1. Grade 2 2. Discontinue. 3. Grade 3 toxicity at any time requires withdrawal of therapy
Creatine Kinase (CPK)	1. > 5 times normal (in the absence of a clinical suspicion of rhabdomyolysis) or any elevation and a clinical suspicion of rhabdomyolysis 2. 33% 3. ≤ 2 times normal	1. > 5 times normal (in the absence of a clinical suspicion of rhabdomyolysis) or any elevation and a clinical suspicion of rhabdomyolysis 2. 66% 3. ≤ 2 times normal	1. > 5 times normal 2. Discontinue. 3. Discontinue at any time if diagnosed with rhabdomyolysis.
Depression	1. Grade 3 or 4 2. 33% 3. Grade 1	1. Grade 3 or 4 2. 66% 3. Grade 1	1. Grade 3 or 4 2. Discontinue
Diarrhea	1. Grade 3 or 4 or if persistent for more than 2 weeks at Grade 2 2. 33% 3. Grade 1	1. Grade 3 or 4 or if persistent for more than 2 weeks at Grade 2 2. 66% 3. Grade 1	1. Grade 3 or 4 or if persistent for more than 2 weeks at Grade 2 2. Discontinue
Fatigue	1. Grade 3 or 4 2. 33% 3. Grade 1	1. Grade 3 or 4 2. 66% 3. Grade 1	1. Grade 3 or 4 2. Discontinue
Flu-like Symptoms-Constitutional Symptoms	1. Grade 3 or 4 2. 33% 3. Grade 1	1. Grade 3 or 4 2. 66% 3. Grade 1	1. Grade 3 or 4 2. Discontinue
Hepatotoxicity (SGPT [ALT] SGPT [AST])	1. > 5 times normal (Grade 3 or 4) 2. 33% 3. Grade 1	1. > 5 times normal (Grade 3 or 4) 2. 66% 3. Grade 1	1. > 5 times normal (Grade 3 or 4) 2. Discontinue
Nausea	1. Grade 3 or 4 or if persistent for more than 2 weeks at Grade 2 2. 33% 3. Grade 1	1. Grade 3 or 4 or if persistent for more than 2 weeks at Grade 2 2. 66% 3. Grade 1	1. Grade 3 or 4 or if persistent for more than 2 weeks at Grade 2 2. Discontinue
Ocular toxicity	None	None	Discontinue for any ocular toxicity

Rev. 2/13

Renal	1. Grade 1 2. 33% 3. normal	1. Grade 1 2. 66% 3. normal	1. Grade 1 2. Discontinue 3. Elevations of Grade 2 or higher at anytime require withdrawal of therapy
Vomiting	1. Grade 3 or 4 or if persistent for more than 2 weeks at Grade 2 2. 33% 3. Grade 1	1. Grade 3 or 4 or if persistent for more than 2 weeks at Grade 2 2. 66% 3. Grade 1	1. Grade 3 or 4 or if persistent for more than 2 weeks at Grade 2 2. Discontinue
Weight loss	1. Grade 2 if observed over a period of one month and is unintentional 2. 33% 3. Continue at the reduced dose if no other limiting adverse events	1. Grade 2 if observed over a period of one month and is unintentional 2. 66% 3. Continue at the reduced dose if no other limiting adverse events	1. Grade 2 is if observed over a period of one month and is unintentional 2. Discontinue.

Rev. 2/13

Rev. 2/13

5.6 Supportive Care

5.6.1 All supportive measures consistent with optimal patient care will be given throughout the study.

5.6.2 Patients requiring chemotherapy or radiation therapy will be taken off study treatment. Any exceptions must be discussed with the study chair.

5.6.3 Additional Prohibited and Restricted Therapies During the Study

5.6.3.1 **Prohibited Therapies**

Patients in this study may not use vaccines for the treatment of cancer or prevention of disease (including those for common medical conditions) for up to one month pre and post dosing with ipilimumab. Concomitant systemic or local anti-cancer medications or treatments are prohibited in this study while receiving ipilimumab or HDI treatments.

Patients may not use any of the following therapies during the study:

- Any non-study anti-cancer agent (investigational or non-investigational)
- Any other investigational agents
- Any other CTLA-4 inhibitors or agonists
- CD137 agonists
- Immunosuppressive agents

- Chronic systemic corticosteroids
- Any non-oncology vaccine therapies used for the prevention of infectious diseases (for up to 30 days prior to or after any dose of study drug).

5.6.3.2 **Precautions**

Caution is advised when considering treatment with high-dose IL-2 in patients who have previously been administered ipilimumab, particularly in patients who experienced ipilimumab-related diarrhea/colitis.

Colonoscopy or sigmoidoscopy with biopsy may be advisable prior to IL-2 administration once the patient is no longer receiving ipilimumab.

5.6.4 **Please see Section 5.5.1.4 that provides additional supportive care considerations for ipilimumab administration.**

5.7 Duration of Therapy

Rev. 2/12, 9/14

5.7.1 The duration of therapy for Arms A and D and Arms C and F (ipilimumab) patients is approximately 60 weeks (See Section 5.5.1 for dose and schedule delay criteria). For Arms B and E (HDI) patients the duration of therapy is 52 weeks.

Rev. 2/14

5.7.2 Patients who develop any recurrent melanoma will be removed from study treatment, unless they fit the criteria in Section 5.2.3. They will, however, continue to be followed for survival. In addition, data on salvage patterns post recurrence will be collected.

5.7.3 Patients will be discontinued from treatment because of excessive or unexpected toxicity (see Section 5.5), pregnancy, change in medical condition or noncompliance with the study protocol that in the opinion of the investigator, necessitates removal of patient from treatment.

5.7.4 Withdrawal of consent will automatically remove a patient from study treatment.

5.8 Duration of Follow-up

Rev. 2/12

For this protocol, all patients, including those who discontinue protocol therapy early, will be followed for response until progression and for survival for 20 years from the date of registration. All patients must also be followed through completion of all protocol therapy.

5.9 Quality of Life Evaluations

Rev. 2/14

Aspects of quality of life (QOL) will be measured for adult patients enrolled by CCOPS to arms A, B, and C utilizing the FACT-G and FACT-BRM health questionnaires and the FACIT-D (Diarrhea assessment). QOL data will be collected at baseline, every 12 weeks (+/- 2 weeks) while on treatment, at completion of treatment, and every 24 weeks (+/- 2 weeks) while off-treatment for one year. Patients who go off treatment early due to excessive toxicity or progressive disease should complete QOL measures at the specified time points. The calendar should not be reset. Baseline measures are necessary to draw

conclusions about the impact of therapy despite the fact that they will have already been altered from predisease levels. They will serve as a comparison for each patient's responses after therapy. The follow-up assessments are included at regular intervals to observe changes in quality of life as treatment unfolds and to determine whether quality of life returns to baseline following the completion of treatment.

5.9.1 FACT-G has four areas of measurements—physical well-being, social/family well-being, emotional well-being, and functional well-being—all on a scale of 0 to 4. FACT-BRM has two additional sections addressing physical and mental QOL aspects.

5.9.2 Since colitis/diarrhea is one of the main toxicities of ipilimumab, FACIT-D form will be used to assess QOL- related from colitis/diarrhea. FACIT-D form has five areas on physical well-being, social/family well-being, emotional well-being, functional well-being, and additional concerns with a scale of 0 to 4. The last section on additional concerns has detailed questions pertaining to diarrhea. The data items collected in this section will be carefully reviewed. The changes between before and after treatment (for each area and summary of all five areas) will be compared between the two treatment arms.

5.9.3 The main comparison will be the change before and after the treatment between the two treatment arms. Longitudinal regression models will also be used to address the overall change over time during which the QOL data is collected.

5.9.4 Every study patient enrolled by a CCOP will be expected to complete the QOL assessment. All patients will be informed at the time of enrollment that they will be asked to complete the quality of life assessment at the specified timepoints. This will also be noted on the Informed Consent. Patient calendars will reflect the dates of the quality of life assessment and will be given to patients at the time of enrollment.

5.9.5 All quality of life measures will be administered to the patient as self-report assessments. CRAs will inform patients that the quality of life assessment will require about 5 to 15 minutes to complete, and the CRA will provide the patient a quiet place to complete the questionnaires. Patients will be instructed to read all instructions at the top of the quality of life assessment. After the patient's understanding of the instructions has been confirmed, he/she should be instructed to complete every item in order without skipping any items on any measure, except where directed. If family members prefer to remain with the patients while completing the quality of life assessment, CRAs will encourage patients to answer the questions on their own (i.e., without the influence of family members).

5.9.6 If patients are unable to complete any of the quality of life measures independently, because language, literacy or physical condition is a barrier, the measures should be administered by a trained staff member. Any time that additional assistance is provided, it should be

documented on the patient form or the Assessment Compliance Form before submitting it to the ECOG-ACRIN Operations Office - Boston.

5.9.7 If patients are unable to come to the clinic, the quality of life measures will be mailed to them with a request to complete and return them to the appropriate person (institutional quality of life coordinator, CRA or physician) using a self-addressed stamped envelope. This request should be followed by a telephone call by the CRA to assist the patient and further completion.

5.9.8 Patients should be encouraged to respond to all of the items on all measures even those that may not seem pertinent to their situation. While patients are still in the clinic, the nurse must check each quality of life measure for completeness and encourage the patient to complete any uncompleted items. However, patients have the option to refuse and the CRA should note the reasons in the margin why an item was left unanswered.

5.9.9 If a patient fails to complete an assessment for any reason, it will be documented on the Assessment Compliance Form. The patient will still be asked to complete the remaining quality of life assessments.

5.9.10 Each CCOP institution will designate an individual who will ensure the timely completion of the quality of life assessments.

6. Measurement of Effect

6.1 Local, Regional Recurrence

The development of a local or regional recurrence of cancer.

6.2 Distant Recurrence

The development of a distant recurrence of cancer.

6.3 Recurrence-Free Survival

Date of randomization to the date of first treatment failure (recurrence or death before recurrence).

6.4 Survival

Date of randomization to date of death.

6.5 Histological or cytological evidence of recurrence should be attempted in all cases except for brain metastases. Strong consideration should be given to resection of solitary brain lesions in the absence of other systemic disease.

6.6 Documentation of recurrence will require specification of all sites involved to establish the pattern of recurrence.

6.7 The following criteria of treatment failure constitute the only acceptable evidence of disease recurrence.

6.7.1 Lung

Positive cytology or biopsy in the presence of a single new lesion or the appearance of multiple lesions consistent with metastatic disease.

6.7.2 Liver

Positive cytology or biopsy in the presence of a single new lesion or the appearance of multiple lesions consistent with metastatic disease.

6.7.3 Central Nervous System

A positive brain CT or MRI scan or CSF cytology.

6.7.4 Cutaneous, Subcutaneous and Lymph Node Recurrence

Positive cytology or biopsy in the presence of a single new lesion or the appearance of multiple lesions consistent with metastatic disease.

6.7.5 Bone and Other Organs

Positive cytology or biopsy in the presence of a single new lesion or the appearance of multiple lesions consistent with metastatic disease identified on two different radiologic studies: i.e., positive nuclear bone scan or PET scan and contrast GI series or ultrasound, X-ray or CT of abdomen for abdominal disease.

7. Study Parameters

Rev. 2/12
Rev. 9/14

7.1 Therapeutic Parameters for all arms

7.1.1 All patients must have disease-free status documented by a complete physical examination and imaging studies within 4 weeks prior to randomization.

Rev. 2/13

7.1.2 Pre-study CBC (with differential and platelet count), chemistries and other laboratory studies required in Section 3 (Selection of Patients) should be done within 4 weeks before randomization. On-study procedures (both safety and research related) including laboratory studies should be followed per the following study calendars. This applies also to patients who go off study treatment for toxicity or other reasons but have not had disease recurrence. Patients who have disease recurrence should have research blood collected at that time point then followed for safety, survival and post-progression salvage therapy patterns and other requested follow up data per protocol and study forms.

Rev. 2/12, 9/14

7.1.3 For WOCBP, pregnancy test (b-HCG test; serum or urine, minimum sensitivity 25 IU/L or equivalent units of b-HCG) must be done during screening within four weeks prior to randomization. Following randomization, serum or urine pregnancy test must be done within 72 hours prior to each dose on the ipilimumab arms (Arms A and D, and Arms C and F). It should also be done monthly (-/+ 72 hours) on the HDI arm (Arms B and E) starting within 72 hours of the first dose of HDI and ending on the first day of month 12 (-/+ 72 hours) or with the discontinuation of HDI if earlier than month 12.

7.1.4 The follow-up imaging schedule in this protocol is identical for both study arms.

7.1.4.1 Total body PET-CT and Brain MRI (or CT brain with contrast if MRI cannot be done) at baseline. If PET-CT cannot be done, CT of neck, chest, abdomen and pelvis should be done. If for some reason a CT cannot be done, an MRI may be done instead. Any other imaging studies if performed (e.g., bone scan) must show no evidence of disease.

7.1.4.2 Follow up imaging studies on both arms, will be done every 3 months (-/+ 2 weeks) if patient is < 2 years from study entry, then every 6 months (-/+4 weeks) if patient is 2-5 years from study entry, and every 12 months (-/+4 weeks) if patient is > 5 years.

7.1.4.3 Follow-up imaging studies will consist of CT (or MRI if CT cannot be done) neck/chest/abdomen/pelvis at follow up assessment time points for all patients (A PET-CT is not required, but is acceptable if done). MRI/CT brain or other imaging studies may be done as clinically indicated but are not required in the absence of clinical indications.

7.1.5 Pathologic/cytologic confirmation for melanoma recurrence:

7.1.5.1 Required for recurrence in (a) solitary or in doubtful lesions, (b) cutaneous, (c) subcutaneous, (d) lymph node lesions.

7.1.5.2 Should be attempted in all cases (if possible) except for brain metastases.

7.1.5.3 Date of relapse is the date of first documented recurrence (by imaging or physical examination).

7.1.6 Radiologic follow up imaging of recurrence is required at least 4 weeks following first documentation of recurrence and before initiation of subsequent salvage therapy (given the known mechanism of action and reported progression/response patterns of ipilimumab).

NOTE: A 4-week "wash out" period is expected to be required in most cases on subsequent salvage clinical trials, but it will be important to capture any subsequent disease regression on E1609, which would be an indication to hold salvage therapy and either observe or resume E1609 therapy. Physician investigators are encouraged to resume E1609 therapy in patients who experience disease regression at follow up imaging following first documented melanoma recurrence.

Rev. 2/13

7.1.7 During treatment, patients should be closely monitored for the onset of neuropsychiatric symptoms. Specific questions should be incorporated into the routine evaluation conducted at each patient visit so as to allow for early detection of neuropsychiatric symptoms.

Rev. 2/12, 9/14

7.2 Arms A,C, D, and F: Patients Treated with Ipilimumab at 10 mg/kg (Arm A and D) or 3 mg/kg (Arm C and F)

Test/ Assessment	Within 4 weeks (28 days) of starting study drugs, unless stated otherwise	Induction Phase[a]				Week 12 ± 2 weeks	Maintenance Phase[a] (Until 12 weeks after the last ipilimumab dose or until relapse)		End of Study Assessment[n]	Follow Up[l]
		Day 1 ± 3 days (Week 1)	Day 22 ± 3 days (Week 4)	Day 43 ± 3 days (Week 7)	Day 64 ± 3 days (Week 10)		Week 24, 36, 48, 60 ± 2 Weeks	Every 6 Weeks ±1 Week		
Informed Consent	X									
History/Physical/ECOG PS	X	X	X	X	X	X	X	X	X	X
Weight/Vital Signs[b]	X	X	X	X	X	X	X	X	X	X
Rev. 2/12 Corticosteroid, immunosuppressant, hormone meds Log		X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	
Hematology labs[c]	X	X	X	X	X	X	X	X	X	X
Chemistry labs[d]	X	X	X	X	X	X	X	X	X	X
Immunologic labs[o]	See Footnote o									
Urinalysis[e]	See Footnote e									
Pregnancy test (b-HCG)[f]	X	X	X	X	X		X			
HIV, HBV, HCV[g]	X									
ECG	X									
Ophthalmologic examination[h]	X[h]						X[h]			X[h]
Ipilimumab infusion		X	X	X	X		X			
Rev. 11/13 Hormonal studies[i]		X	X	X	X	X	X		X	
Adverse events assessment[j]		X	X	X	X	X	X	X	X	X
Rev. 2/12 Imaging studies[k]	X[k]					X[k]	X[k]			X[k]
Rev. 2/13, 2/14 QOL evaluations[m]	X					X	X		X	X
Biological sample submissions	See Section 7.4									

Rev. 2/12
Rev.2/13

a. All Study procedures, blood samples collected for pretreatment laboratory tests may be collected and analyzed no more than 3 days prior to dosing. Chemistry results must be reviewed and confirm that subject's liver function tests and other safety labs still meet inclusion criteria prior to administration of ipilimumab dose. Baseline pregnancy exam must be performed within 3 days of beginning ipilimumab dosing and determined to be negative. During the maintenance phase, in addition to the evaluations done on the dosing days every 12 weeks, patients will be seen and evaluated as noted in the study calendar every 6 weeks (-/+ 1 week), starting at week 18. They should be seen and evaluated more often if clinically indicated for the management of toxicities, at the discretion of the treating physician investigator. Hormonal studies and immunologic labs are required for monitoring at the specified time points and as clinically indicated. The results of these tests (hormonal studies and immunologic labs) are not required for dosing unless there are clinical indications and/or associated adverse events as described under Section 5.5.1.1 Dose and Schedule Modifications for Ipilimumab and Section 5.5.2.8 HDI Dose Modification and Discontinuation Guidelines.

b. For first infusion only, vital signs to be collected prior to dosing, every 15 minutes during dosing and 30 - 60 minutes after dosing until vital signs normalize or return to baseline. For subsequent infusions, vital signs should be collected prior to dosing, every 30 minutes during dosing, and 1 hour post dosing.

c. Hematology labs to include hemoglobin, hematocrit, red blood cell count, white blood cell count, platelets (direct platelet count), as well as total and differential CBC counts. The CBC differential includes enumeration of neutrophils, lymphocytes, eosinophils, monocytes, basophils and any abnormal blood cells. These labs must be done and reviewed before ipilimumab infusion. These labs are required to be done throughout follow-up regardless if the patient goes off treatment early for anything other than recurrence. Once recurrence occurs, these labs are no longer required to be completed unless clinically indicated for safety purposes as per the standard of care for these patients.

Rev. 2/13

Rev. 8/12

d. Chemistry laboratory analysis includes albumin, amylase, lipase, urea or BUN, creatinine, ALT, AST, LDH, serum alkaline phosphatase, direct and total bilirubin, glucose, total protein, sodium, potassium, chloride, HCO_3 (CO2; venous blood), calcium, phosphorous. In follow up (after completion of ipilimumab treatment), amylase and lipase will be done only if clinically indicated. These labs must be done and reviewed before ipilimumab infusion. These labs are required to be done throughout follow-up regardless if the patient goes off treatment early for anything other than recurrence. Once recurrence occurs, these labs are no longer required to be completed unless clinically indicated for safety purposes as per the standard of care for these patients.

Rev. 2/13

e. Urinalysis will be done as clinically indicated. Urinalysis tests to include gross examination including specific gravity, protein, glucose and blood. A microscopic evaluation will also be performed, as clinically indicated, to include WBC/HPF, RBC/HPF and any additional findings.

f. WOCBP must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) at screening. Serum or urine pregnancy test must be done within 72 hours prior to each dose of ipilimumab.

Rev. 8/12

At screening, testing should be performed for HIV antibody, hepatitis C antibody, and HBsAg (hepatitis B antigen) utilizing local standard informed consent procedures prior to this laboratory collection. These tests could be repeated later during the course of the study if clinically indicated.

h. Ophthalmologic examination will be done at baseline only if clinically indicated. On this study, the last dose of ipilimumab will be at approximately week 60. Ophthalmological examination is strongly recommended at 6 and 18 months (± 4 weeks) after start of treatment especially in patients who experienced diarrhea or colitis, to be performed by an ophthalmologist. If melanoma recurrence occurs before these time points, the Ophthalmological examination should still be done if clinically indicated. It should also be performed at other timepoints if clincially indicated.

Rev. 2/13

i. Hormonal studies: To be done at the indicated visits and when clinically indicated. These include TSH, free T4, morning ACTH, morning cortisol. For Men: testosterone. For WOCBP: prolactin, LH, FSH, and estradiol that will be done only if a WOCBP is experiencing amenorrhea while on protocol treatment.

Rev. 8/12 j. Until 70 days after the last study drug administration. All adverse events must be collected whether they occur on treatment or non-treatment weeks and must be submitted utilizing the corresponding E1609 Adverse Event Forms, covering all time periods specified on the forms.

k. **Baseline imaging studies:** should be done within 4 weeks prior to randomization. These must include a total body PET-CT scan (with or without brain) and brain MRI or CT (if MRI is contraindicated). If PET-CT cannot be done, CT of neck, chest, abdomen and pelvis should be done. If for some reason a CT cannot be done, an MRI may be done instead. Any other imaging studies if performed (e.g., bone scan) must show no evidence of disease. **During treatment and on follow-up:** CT (or MRI if CT cannot be done) neck/chest/abdomen/pelvis will be done every 3 months (-/+ 2 weeks) if patient is < 2 years from study entry, then every 6 months (+/- 4 weeks) if patient is 2-5 years from study entry, and every 12 months (+/- 4 weeks) if patient is > 5 years from study entry for up to 20 years. A PET-CT is not required, but is acceptable if done. Brain MRI/CT or other imaging studies may be done as clinically indicated but are not required in the absence of clinical indications.

Rev. 8/12

Rev. 2/12, 2/13 l. Follow up period will start 12 weeks after the last ipilimumab dose for patients who have no disease progression. These patients should be seen at 6 weeks (-/+ 1 week) after the last dose then 12 weeks (-/+ 1 week) after the last dose and later evaluated per the standard ECOG-ACRIN follow-up schedule: Every 3 months (+/- 2 weeks) if patient is < 2 years from study entry, every 6 months (+/- 4 weeks) if patient is 2-5 years from study entry, and every 12 months (+/- 4 weeks) if patient is > 5 years from study entry for up to 20 years. However, patients with ongoing toxicities should be seen more often as clinically indicated. Patients who develop recurrent melanoma will be followed for survival and for information on salvage patterns. The schedule of clinical follow up for these patients will be at the discretion of the treating physicians and according to established Standard of Care. Adverse Events Assessment on the study will continue for all patients until 70 days after the last study drug administration.

Rev. 2/13, 9/14 m. QOL data will be collected for adult patients enrolled by CCOPs at baseline, every 12 weeks (+/- 2 weeks) while on treatment, at completion of treatment, and then every 24 weeks (+/- 2 weeks) while off-treatment for one year. Pediatric patients will not participate in the QOL evaluations.

Rev. 2/12, 2/13 n. End of Study Assessment will be done within 6 weeks (-/+ 1 week) of the discontinuation of study treatment.

Rev. 8/12 o. The following labs are to be done at baseline (within 4 weeks of starting the study drug) and at 12, 24, 42, 60, and 96 weeks, and at melanoma relapse (+/- 2 weeks for all time points except the 96 week time point, a +/- 4 weeks window is allowed). These labs must be completed even if the patient goes off treatment early for any reason other than recurrence. If melanoma relapse occurs before any of these time points, no additional testing needs to be done after the relapse time point: C-reactive protein, Antinuclear antibody (ANA) Screen, Thyroid Stimulating Immunoglobulin (TSI), Antithyroglobulin antibody (ATGAB), Antithyroperoxidase Antibody (ATPOAB), Anticardiolipin (TOTAL).

Rev. 2/12, 2/13

Rev. 2/12

Rev. 9/14

7.3 Arm B and E: Patients treated with interferon-α2b:

Test/ Assessment	Within 4 weeks (28 days) of starting study drugs, unless stated otherwise (eg. randomization)	Induction Phase[a]				Day 29 (Start of maintenance)	Maintenance Phase[a] (Until 4 Weeks after the last IFN-α2b dose or until relapse)		End of Study Assessment[l]	Follow Up[J]
		Day 1 (Week 1)	Day 8 (Week 2)	Day 15 (Week 3)	Day 22 (Week 4)		Every 4 Weeks ±1 Week for 2 months	Every 6 Weeks ±1 Week (after 2 months)		
Informed Consent	X									
History/Physical/ECOG PS	X	X	X	X	X	X	X	X	X	X
Weight/Vital Signs	X	X	X	X	X	X	X	X	X	X
Rev. 2/12 Corticosteroid immunosuppressant, hormone Meds Log		X			X		X	X	X	X
Concomitant medications	X	X			X		X	X	X	
Hematology Labs[b]	X	X	X	X	X	X	X	X	X	X
Chemistry Labs[c]	X	X	X	X	X	X	X	X	X	X
Immunologic labs[m]	See Footnote m									
Urinalysis[d]	See Footnote d									
Pregnancy Test (b-HCG)[e]	X	See Footnote e								
HIV, HBV, HCV[f]	X									
ECG	X									
Hormonal studies[g]		X				X	X		X	
Adverse Events Assessment[h]		X	X	X	X	X	X	X	X	X
Rev. 2/12 Imaging studies[i]	X[i]						X[i]			X[i]
Rev. 2/13, 2/14 QOL evaluations[k]	X								X	X
Biological Sample Submissions	See Section 7.4									

a. All Study procedures, blood samples collected for pretreatment laboratory tests may be collected and analyzed no more than 3 days prior to dosing. Baseline pregnancy exam must be performed within 3 days of beginning IFN-α2b treatment and determined to be negative. NOTE: for Days 8, 15, 22 and 29 if IFN-α2b was delayed per the dose delay/scheduling criteria or if a visit had to be delayed due to major circumstances (such as a health emergency, family/personal emergency, transportation difficulties, scheduling difficulties; a visit date falls on a holiday), the study assessments scheduled for these dates will be delayed. Hormonal studies and immunologic labs are required for monitoring at the specified time points and as clinically indicated. The results of these tests (hormonal studies and immunologic labs) are not required for dosing unless there are clinical indications and/or associated adverse events as described under Section 5.5.1.1 Dose and Schedule Modifications for Ipilimumab and Section 5.5.2.8 HDI Dose Modification and Discontinuation Guidelines.

b. Hematology labs to include hemoglobin, hematocrit, red blood cell count, white blood cell count, platelets (direct platelet count), as well as total and differential CBC counts. The CBC differential includes enumeration of neutrophils, lymphocytes, eosinophils, monocytes, basophils and any abnormal blood cells. These labs are required to be done throughout follow-up regardless if the patient goes off treatment early for anything other than recurrence. Once recurrence occurs, these labs are no longer required to be completed unless clinically indicated for safety purposes as per the standard of care for these patients.

Rev. 2/13

Rev. 8/12

c. Chemistry laboratory analysis includes albumin, creatine kinase (CPK), urea or BUN, creatinine, ALT, AST, LDH, serum alkaline phosphatase, direct and total bilirubin, glucose, total protein, sodium, potassium, chloride, HCO3 (CO_2; venous blood), calcium, phosphorous. These labs are required to be done throughout follow-up regardless if the patient goes off treatment early for anything other than recurrence. Once recurrence occurs, these labs are no longer required to be completed unless clinically indicated for safety purposes as per the standard of care for these patients.

Rev. 2/13

d. Urinalysis will be done as clinically indicated. Urinalysis tests to include gross examination including specific gravity, protein, glucose and blood. A microscopic evaluation will also be performed, as clinically indicated, to include WBC/HPF, RBC/HPF and any additional findings.

e. WOCBP must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) at screening. Serum or urine pregnancy test should also be done within 72 hours of the first dose of HDI, then during HDI therapy every 4 weeks x3 (-/+ 1 week), then every 6 weeks (-/+ 1 week) and ending with the discontinuation of HDI. At HDI discontinuation, a negative pregnancy test within the preceding 6 weeks is sufficient. A pregnancy test should also be done at anytime if there are clinical concerns for possible pregnancy.

Rev. 8/12

f. At screening, testing should be performed for HIV antibody, hepatitis C antibody, and HBsAg (hepatitis B antigen) utilizing local standard informed consent procedures prior to this laboratory collection. These tests could be repeated later during the course of the study if clinically indicated.

g. Hormonal studies: To be done at the indicated visits and when clinically indicated. These include TSH, free T4.

Rev. 8/12

h. Until 70 days after the last study drug administration. All adverse events must be collected whether they occur on treatment or non-treatment weeks and must be submitted utilizing the corresponding E1609 Adverse Event forms, covering all time points specified on the forms.

i. **Baseline imaging studies:** should be done within 4 weeks prior to randomization. These must include a total body PET-CT scan (with or without brain) and brain MRI or CT (if MRI is contraindicated). If PET-CT cannot be done, CT of neck, chest, abdomen and pelvis should be done. If for some reason a CT cannot be done, an MRI may be done instead. Any other imaging studies if performed (e.g., bone scan) must show no evidence of disease. **During treatment and on follow-up:** CT (or MRI if CT cannot be done) neck/chest/abdomen/pelvis will be done every 3 months (-/+ 2 weeks) if patient is < 2 years from study entry, then every 6 months (+/- 4 weeks) if patient is 2-5 years from study entry, and every 12 months (+/- 4 weeks) if patient is > 5 years from study entry for up to 20 years. A PET-CT is not required, but is acceptable if done. Brain MRI/CT or other imaging studies may be done as clinically indicated but are not required in the absence of clinical indications.

Rev. 2/12, 2/13

j. Follow up period will start 12 weeks after the last interferon dose for patients who have no disease progression. These patients should be seen at 6 weeks (-/+ 1 week) after the last dose then 12 weeks (-/+ 1 week) after the last dose and later evaluated per the standard ECOG-ACRIN follow-up schedule. Every 3 months (+/- 2 weeks) if patient is < 2 years from study entry, every 6 months (+/- 4 weeks) if patient is 2-5 years from study entry, and every 12 months (+/- 4 weeks) if patient is > 5 years from study entry for up to 20 years. However, patients with ongoing toxicities should be seen more often as clinically indicated. Patients who develop recurrent melanoma will be followed for survival and for information on salvage patterns. The schedule of clinical follow up for these patients will be at the discretion of the treating physicians and according to established Standard of Care. Adverse Events Assessment on the study will continue for all patients until 70 days after the last study drug administration.

Rev. 9/14

k. QOL data will be collected for patients enrolled by CCOPs at baseline, every 12 weeks (-/+ 2 weeks) while on treatment, at completion of treatment, and then every 24 weeks (-/+ 2 weeks) while off-treatment for one year. Pediatric patients will not participate in the QOL evaluations.

Rev. 2/13

Rev. 2/12, 2/13

l. End of Study Assessment will be done within 6 weeks (-/+ 1 week) of discontinuation of study treatment.

Rev. 2/12, 8/12

Rev. 2/13

m. The following labs are to be done at baseline (within 4 weeks of starting the study drug) and at 12, 24, 42, 60, and 96 weeks, and at melanoma relapse (+/- 2 weeks for all time points except the 96 week time point, a +/- 4 weeks window is allowed). These labs must be completed even if the patient goes off treatment early for any reason other than recurrence. If melanoma relapse occurs before any of these time points, no additional testing needs to be done after the relapse time point: C-reactive protein, Antinuclear antibody (ANA) Screen, Thyroid Stimulating Immunoglobulin (TSI), Antithyroglobulin antibody (ATGAB), Antithyroperoxidase Antibody (ATPOAB), Anticardiolipin (TOTAL).

7.4 Biological Sample Submissions

1. Submission of pathology samples at baseline for diagnostic review is mandatory in order for the patient to be considered evaluable. See Section 10 (Pathology Review) and Appendix II (Pathology Submission Guidelines).
2. Pathology samples for banking should be submitted as outlined in Section 10 per patient consent.
3. Blood samples for banking should be submitted as outlined in Section 11 per patient consent.

Rev. 9/14

NOTE: It is required that biological sample submissions be logged into the ECOG-ACRIN Sample Tracking System (STS) (see Section 10.4) for purposes of monitoring compliance.

NOTE: An informed consent must be signed prior to the submission of any samples, including mandatory diagnostic reviews and banking for research in accordance with the patient consent. Samples for banking should be submitted only from patients who have given written consent for the use of their samples for these purposes.

NOTE: Institutions outside of the United States and Canada must confer with the receiving laboratory and the ECOG-ACRIN Operations Office - Boston regarding logistics for submission of fresh samples.

Rev. 2/12, 8/12, 9/14

Biological Materials	Baseline[1]	After 3 weeks (Arms A/D, and C/F) / 4 weeks (Arms B/E) of Initiating Treatment[12]	After 12 weeks of Initiating Treatment[2]	48 weeks[2]	Progression/ Relapse[8]
MANDATORY for Central Diagnostic Review					
Pathology Samples[6]	X[9]				X[8]
From patients who answer *"YES"* to "I agree to provide additional blood for research."					
Peripheral Blood (ten 10cc green top heparin tubes)[4,5,7]	X	X	X	X	X[2,3]
Peripheral Blood (three 10cc red top tubes)[4,5,7]	X	X	X	X	X[2,3]
PAXgene RNA Tube (1)[4,5,7]	X	X	X		
Peripheral Blood, (one 10cc yellow ACD top tube)[4,5,7]	X				

1. Prior to start of treatment.
2. ± 2 weeks.

Rev. 2/12, 8/12

3. If relapse occurs before the 48 week time point, no additional blood samples are required after the relapse blood sample is sent.
4. Kits are being provided for the collection and shipment of the blood samples. Please refer to Section 11.1.2.1 for instructions.
5. **Please completely fill all blood tubes as full as possible.**

Rev. 3/15

6. Submit to ECOG-ACRIN Central Biorepository and Pathology Facility.
7. Submit to ECOG-ACRIN Immunologic Monitoring and Cellular Products Laboratory.
8. Submit from patients who have consented to have tissue submitted for research.
9. Mandatory submission for central review.
10. [Deleted in Addendum #5]
11. [Deleted in Addendum #5]

Rev. 8/12, 9/14

12. On the day of the scheduled administration of the second dose of ipilimumab (day 22 -/+ 3 days) and **before administering the second dose** of ipilimumab on Arms A, C, D, and F (up to one week prior to the scheduled administration of the second dose is acceptable if not possible on the same day). On the day of initiation of maintenance subcutaneous IFN (day 29 -/+ 3days) on Arm B or E (up to one week prior to initiation of maintenance subcutaneous IFN is acceptable if not possible on the same day). If the study treatment was delayed or discontinued for that day due to adverse events the blood specimen should still be collected.

8. Drug Formulation and Procurement

This information has been prepared by the ECOG-ACRIN Pharmacy and Nursing Committees.

Availability

Drug Ordering: Bristol-Myers-Squibb is supplying ipilimumab, through the Division of Cancer Treatment and Diagnosis, NCI, for this protocol. Maintenance of NCI drug accountability records is required. Ipilimumab (NSC 732442) (IND) may be requested by the Principal Investigator (or their authorized designees) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that drug be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained – see general information). Completed Clinical Drug Requests (NIH-986) should be submitted to the
Rev. 2/14 PMB by fax 240-276-7893 or mailed to the Pharmaceutical Management Branch, CTEP, DCTDC, NCI, 9000 Rockville Pike, EPN, Rm. 7179, Bethesda, M.D. 20892. The NCI Clinical Drug Request form is available on the NCI home page (http://ctep.info.nih.gov)
Rev. 2/14 or by calling the PMB at 240-276-6575.

NCI Supplied Agent(s) – General Information

NOTE: Under no circumstances can commercially supplied ipilimumab be used or substituted for the NCI-supplied ipilimumab.

Rev. 2/14 **Questions about drug orders, transfers, returns, or accountability should be addressed to the PMB by calling 240-276-6575 Monday through Friday between 8:30 AM and 4:30 PM Eastern Time.**

Drug Returns: All unused drug supplies must be returned to the PMB. When it is necessary to return study drug (e.g., sealed vials remaining when a patient permanently discontinues protocol treatment, expired vials recalled by the PMB), investigators must
Rev. 2/14 return the study drug to the PMB using the NCI Return Agent Form available on the NCI home page (http://ctep.cancer.gov) or by calling the PMB at 240-276-6575.

Drug Accountability: The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, disposition, and return of all drugs received from the PMB using the NCI Investigational Agent Accountability Record
Rev. 2/14 available on the NCI home page (http://ctep.cancer.gov) or by calling the PMB at 240-276-6575.

8.1 Ipilimumab

In this study, the investigational product is ipilimumab.

8.1.1 Drug Name

Ipilimumab (NSC 732442)

8.1.2 Other Names

Anti-CTLA-4 monoclonal antibody, MDX-010 (MDX-CTLA4, Transfectoma-derived)

8.1.3 Classification

Human monoclonal antibody, IgG1 subclass

M.W.: 147, 991 Daltons

Ipilimumab has two manufacturing processes- ongoing trials have been using substances manufactured using Process B. This trial, E1609, uses ipilimumab that is manufactured by Process C. The Process C has been developed using a higher producing sub-clone of the current Master Cell Bank, and modified cell culture and purification steps.

8.1.4 Mode of Action

Ipilimumab is specific for the CTLA4 antigen expressed on a subset of activated Tcells. CTLA4 interaction with the B7 molecule, one of its ligands expressed on professional antigen presenting cells, can down-regulate T-cell response. Ipilimumab is thought to act by blocking the interaction of CTLA4 with the B7 ligand, resulting in a blockade of the inhibitory effect of T-cell activation. The CTLA4/B7 creates the interaction.

8.1.5 Storage and Stability

Ipilimumab is available in 5 mg/mL single-use vials (40 mL). The sterile solution in the vial is clear and colorless. Ipilimumab is administered via intravenous infusion only. Ipilimumab must be stored in a secure area according to local regulations. The investigator must ensure that it is stored at a temperature ≥ 2°C and ≤ 8°C.

Ipilimumab is given undiluted or further diluted in 0.9% NaCl Injection, USP or 5% Dextrose Injection, USP to a concentration between 1 mg/mL and 4 mg/mL. Undiluted or diluted ipilimumab solution is stable in a polyvinyl chloride (PVC), non- PVC/non DEHP (di-(2-ethylhexul) phthalate) IV bag or glass container up to 24 hours refrigerated at (2°C to 8°C) or at room temperature/ room light.

Shelf-life surveillance of the intact vials is ongoing.

CAUTION: Ipilimumab does not contain antibacterial preservatives. Use prepared IV solution immediately. Discard partially used vials.

Each vial is a Type I flint glass vial with gray butyl stoppers and sealed with aluminum seals.

	Process B		**Process C**	
Component	**50 mg/ vial[a]**	**200 mg/ vial[b]**	**50 mg/ vial[a]**	**200 mg/ vial[b]**
Ipilimumab	53.5 mg	213 mg	53.5 mg	213 mg
Sodium Chloride, USP	62.6 mg	249 mg	62.6 mg	249 mg
TRIS-hydrochloride	33.7 mg	134.3 mg	33.7 mg	134.3 mg
Diethylenetriamine pentacetic acid	0.42 mg	1.67 mg	0.42 mg	1.67 mg
Mannitol, USP	107 mg	426 mg	107 mg	426 mg
Polysorbate 80 (plant-derived)	1.07 mg	4.26 mg	1.18 mg	4.69 mg
Sodium Hydroxide	QS to pH 7			
Hydrochloric acid	QS to pH 7			
Water for Injection	QS: 10.7 mL	QS: 42.6 mL	QS: 10.7 mL	QS: 42.6 mL
Nitrogen[c]	Processing agent			

[a] Includes 0.7 overfill; [b] Includes 2.6 mL overfill.

[c] Nitrogen is used to transfer the bulk solution through the pre-filled and sterilizing filters into the aseptic area.

8.1.6 Dose Specifics

Induction Phase:

Rev. 2/12, 9/14

Ipilimumab 10 mg/kg (Arms A and D) or 3 mg/kg (Arms C and F), administered by IV infusion every three weeks for a total of four doses, until local recurrence or distant progression, unacceptable toxicity or withdrawal of consent.

Maintenance Phase

Rev. 2/12, 9/14

Ipilimumab 10 mg/kg (Arms A and D) or 3 mg/kg (Arms C and F), administered by IV infusion every 12 weeks (3 months), beginning at Week 24, then at weeks 36, 48, 60, until disease recurrence, unacceptable toxicity or withdrawal of consent for a maximum of 4 maintenance doses.

Dose delays are allowed as per the dosing criteria. Infusions should be given over 90 minutes (not bolus or IV push).

8.1.6.1 Dose Calculations: Calculate **Total Dose** as follows:

Rev. 2/12

Patient body weight in kg x [*10 mg or 3 mg/kg*] = total dose in mg

Calculate **Total Infusion Volume** as follows:

Total dose in mg ÷ 5 mg/mL = infusion volume in mL

Calculate **Rate of Infusion** as follows:

Infusion volume in mL ÷ 90 minutes = rate of infusion in mL/min.

Rev. 2/12, 9/14

For example, a patient on Arms A and D weighing 114 kg (250 lb) would be administered 1140 mg of ipilimumab (114 kg x 10 mg/kg = 1140 mg) with an infusion volume of 228 mL (1140 mg ÷ 5 mg/mL = 228 mL) at a rate of approximately 2.5 mL/min (228 mL ÷ 90 minutes) in 90 minutes.

Rev. 2/12

8.1.7 Preparation

The supplies needed for Ipilimumab preparation and administration include calibrated syringes and infusion containers. Ipilimumab is to be administered as an intravenous infusion using an in-line filter (pore size of 0.2 micrometer to 1.2 micrometer) and a volumetric pump, at the 10 mg/kg dose or the 3mg/kg dose, to complete the infusion in 90 minutes, with a 10-mL normal saline flush at the completion of the infusion.

- As ipilimumab is stored at refrigerated temperatures (2-8ºC), allow the appropriate number of vials of ipilimumab to stand at room temperature for approximately five minutes.
- Aseptically withdraw the required volume of ipilimumab solution into a syringe. Insert the needle at an angle into the ipilimumab vial by placing the needle – bevel side down – against the glass, with the tip touching the neck of the vial. The initial solution

concentration is 5 mg/mL. [Note: A sufficient excess of ipilimumab is incorporated into each vial to account for withdrawal losses].

- Ensure that the ipilimumab solution is clear colorless, essentially free from particulate matter on visual inspection. If multiple vials are needed for a subject, it is important to use a separate sterile syringe and needle for each vial to prevent problems such as dulling of needle tip, stopper coring, repeated friction of plunger against syringe barrel wall, etc.
- Inject ipilimumab solution withdrawn into an appropriate size evacuated infusion bag to produce a final infusion volume that has been calculated from the weight of the patient. For example, if preparing a 10mg/kg treatment for a 65 kg patient you will use 4 vials of the 200 mg vial size (or 650 mg).
- If the total dose calculates to less than 90 mL of solution then the total dose needed should be diluted to a total volume of 90 mL in 0.9% sodium chloride.
- Mix by GENTLY inverting several times. DO NOT shake.
- Visually inspect the final solution. If the initial diluted solution or final dilution for infusion is not clear or contents appear to contain precipitate, the solution should be discarded.
- Do not draw into each vial more than once. Any partial vials should be safely discarded and should not be stored for reuse.

8.1.8 Route of Administration

Ipilimumab is administered as an IV infusion only. Infusions should be given over 90 minutes (not bolus or IV push). Ipilimumab should be administered under the supervision of a physician experienced in the use of intravenous (IV) agents.

8.1.9 Incompatibilities

No compatability information is available.

8.1.10 Availability

Bristol-Myers-Squibb is supplying ipilimumab, through the Division of Cancer Treatment and Diagnosis, NCI, for this protocol.

8.1.11 Side Effects

See Section 5.4(CAEPR).

8.1.12 Nursing/Patient Implications

Monitor patients for immune-related adverse events, e.g., rash/vitiligo, diarrhea/colitis, uveitis/episcleritis, hepatitis and hypothyroidism. If you suspect toxicity, refer to the protocol guidelines for ruling out other causes.

Ipilimumab may be excreted in milk or cross the placenta; therefore, nursing womenand women with known or suspected pregnancy should not take ipilumumab.

Closely monitor patients who are on narcotics during the treatment with ipilumumab. Narcotics may mask GI signs and symptoms such as diarrhea or abdominal pain, which are relevant complications of a bowel perforation. Minor diarrhea can be a potential sign of colitis and require immediate attention.

8.1.13 Handling and Disposal

As with all injectable drugs, care should be taken when handling and preparing ipilimumab. Whenever possible, ipilimumab should be prepared in a laminar flow hood or safety cabinet using standard precautions for the safe handling of intravenous agents applying aseptic technique. Latex gloves are required. If ipilimumab concentrate or solution comes in contact with skin or mucosa, immediately and thoroughly wash with soap and water. After final drug reconciliation, unused ipilimumab solution should be disposed at the site following procedures for the disposal of anticancer drugs.

8.1.14 Ipilimumab Destruction

Partial vials can be destroyed on site per institution policy. Intact vials of the expired drug, recalled, or when protocol is closed to treatment can not be destroyed on site without the PMB/NCI approval. If ipilimumab is to be destroyed on site, it is the investigator's responsibility to ensure that arrangements have been made for disposal and that procedures for proper disposal have been established according to applicable regulations, guidelines, and institutional procedures. Appropriate records of the disposal must be maintained.

8.2 Alfa Interferon

8.2.1 Other Names

Interferon Alfa - 2b, Intron A, IFN-alpha 2b, NSC #377523

8.2.2 Classification

Biological response modifier.

8.2.3 Mode of Action

Interferon Alfa - 2b has antiviral, antiproliferative (cytostatic) and immunomodulatory properties. Its direct antiproliferative properties (e.g., inhibition of cell growth) may explain its activity in certain malignancies.

8.2.4 Storage and Stability

Interferon Alfa - 2b is provided as a lyophilized powder which must be reconstituted prior to administration. Unreconstituted drug vials bear expiration dates and are stored under refrigeration.

Reconstituted solutions of interferon Alfa - 2b are stable for 1 month at refrigeration temperatures and for 2 weeks at room temperature when reconstituted as directed by the manufacturer. Reconstituted solutions of interferon Alfa - 2b, left in the original vials, are stable for 1 month in the freezer and through 4 freeze-thaw cycles.

In plastic or glass syringes the drug is stable for 1 month frozen and through 2 freeze-thaw cycles. When interferon Alfa - 2b is reconstituted to a concentration ≥100,000 u/ml in normal saline, it is stable for only 24 hours at room or refrigeration temperatures.

NOTE: Intron A Solution for Injection is not recommended for intravenous administration and should not be used for the induction phase of malignant melanoma.

8.2.5 Dose Specifics

Induction Phase

Interferon Alfa - 2b, 20 MU/m²/d (rounded to the nearest 1.0 million unit) administered **IV** x 5 consecutive days out of 7 (e.g., M-F) every week x 4 weeks.

Maintenance Phase

Interferon Alfa - 2b, 10 MU/m²/d (rounded to the nearest 1.0 million unit) **subcutaneous** every other day (e.g., M,W,F) three times each week x 48 wks.

8.2.6 Preparation

The lyophilized product is reconstituted as directed by the manufacturer.

For intravenous injection, it is recommended that interferon Alfa - 2b be administered as a 100,000 U/mL solution to minimize adsorption of the drug to glass and plastic containers.

8.2.7 Administration

It is recommended that Interferon Alfa-2b be prescribed in 10 million unit vials (with instructions to reconstitute with 1 mL of diluent to reach a final concentration of 10:1).

However, other standard vial strengths are acceptable. Subcutaneous administration utilizes standard technique. The vial strength prescribed and final dose volume for each injection should be recorded in the comments section of the patient diary if self-administered. Intravenous dose should be diluted in sodium chloride 0.9% / 100 mL and given over 20 minutes.

8.2.8 Incompatibilities

Interferon Alfa - 2b is incompatible with 5% dextrose in water and with a component of the Travenol Infusor.

8.2.9 Compatibilities

Information regarding the compatibility, stability, etc. of drugs is constantly changing Consult your pharmacist to obtain the most up to date information. Interferon Alfa - 2b is compatible in normal saline, Ringer's injection, Lactated Ringers' and 5% sodium bicarbonate injection.

8.2.10 Availability

Interferon Alfa - 2b is a commercial product.

8.2.11 Side Effects

Flu-like symptoms: Fever, chills, diaphoresis and rigors occur universally regardless of dose, route or schedule. Usual onset is in 1-2 hours with peak in 4-8 hours and duration less than 18 hours. Symptoms tend to lessen with continued dosing.

Constitutional Symptoms: Fatigue, malaise, anorexia, weight loss, Raynaud's phenomenon, muscle pain, arthralgias, headaches; may be doselimiting, usually occurring during the first or second week of treatment.

Hematologic: Leukopenia, thrombocytopenia and anemia (uncommon).

Hepatic: Increased bilirubin, increased alkaline phosphatase, increased transaminases occasionally, hepatitis (uncommon).

Cardiovascular: Hypotension, hypertension, dizziness, syncope, arrhythmia (atrial or ventricular), tachycardia, congestive heart failure and myocardial infarction have been reported.

Neurologic: Somnolence, confusion with high doses; numbness, paresthesia, neuropathy; depression, personality disorder, psychomotor retardation, acute paranoid reactions, hallucinations, inability to concentrate, agitation, anxiety; visual disturbances, eye pain, hemianopsia, retinal infarction with vision loss (1 patient); sleep disturbances, insomnia; tremor, seizures, acute aphasia, coma, cerebral edema.

Gastrointestinal: Mild nausea, vomiting, diarrhea, dysphagia, anorexia, taste change, flatulence, constipation, abdominal pain and gastric distress have been reported.

Dermatologic: Alopecia, rash, pain at injection site, dry skin, flushing, urticaria, epidermal necrosis.

Renal: Proteinuria, microscopic hematuria, pyuria, azotemia, acute renal failure, nephrotic syndrome (1 patient), glycosuria, albuminuria, polyuria.

Pulmonary: Orthopnea, dyspnea, bronchospasm, cough, pulmonary edema/acute respiratory distress syndrome, pharyngitis.

Metabolic: Hyperglycemia, hypertriglyceridemia, hypothyroidism.

Coagulation: Increased PT, PTT.

8.2.12 Nursing Implications

Inform patient of expected side effects and reassure him/her that symptoms are not necessarily related to tumor progression.

Instruct patient in keeping a daily record of temperature, symptoms and activity level (Refer to E1609 Patient Diary - Interferon, Appendix IV).

Review and document type, character and duration of side effects.

Fever, rigors and other flu-like symptoms: Acetaminophen administered prior to treatments and every four hours following the initial injections may decrease severity of symptoms. Nonsteroidal or steroidal anti-inflammatory agents should be avoided since their effect on the immune system is not known.

Fatigue and CNS toxicity: Patient performance status and mental status should be assessed regularly and appropriate dose adjustments made per protocol. Instruct patient to arrange most important activities in the morning and allow for frequent rest periods.

Anorexia: Monitor weight regularly. Encourage patient to maintain adequate fluid, caloric and protein intake. Antiemetics as needed.

Granulocytopenia, thrombocytopenia, liver enzyme elevations: Monitor blood counts closely and modify dosage per protocol. Advise patient of special precautions regarding infection and/or bleeding when appropriate.

8.2.13 References

Kirkwood JM, et al. Kirkwood JM, Strawderman MH, Ernstoff MS, et al. Interferon alfa-2b adjuvant therapy of high-risk resected cutaneous melanoma: the Eastern Cooperative Oncology Group Trial EST 1684. *J Clin Oncol.* 1996; 14(1):7–17.

Kirkwood JM, Ibrahim JG, Sondak VK, et al. High- and low-dose interferon alfa-2b in high-risk melanoma: first analysis of intergroup trial E1690/S9111/C9190. *J Clin Oncol.* 2000; 18(12):2444–2458.

Kirkwood JM, Ibrahim JG, Sosman JA, et al. High-dose interferon alfa-2b significantly prolongs relapse-free and overall survival compared with the GM2-KLH/QS-21 vaccine in patients with resected stage IIB–III melanoma: results of intergroup trial E1694/S9512/C509801. *J Clin Oncol.* 2001; 19(9):2370–2380.

Kirkwood JM, Bender C, Agarwala S, Tarhini A, Shipe-Spotloe J, Smelko B, Donnelly S, Stover L. Mechanisms and management of toxicities associated with high-dose interferon alfa-2b therapy. *J Clin Oncol*. 2002 Sep 1;20(17):3703-18. Review.

Hauschild A, Gogas H, Tarhini A, Middleton MR, Testori A, Dréno B, Kirkwood JM. Practical guidelines for the management of interferon-alpha-2b side effects in patients receiving adjuvant treatment for melanoma: expert opinion. *Cancer*. 2008 Mar 1;112(5):982-94.

Kirkwood JM, Tarhini AA, Panelli MC, Moschos SJ, Zarour HM, Butterfield LH, Gogas HJ. Next generation of immunotherapy for melanoma. J Clin Oncol. 2008 Jul 10;26(20):3445-55.

Rev. 2/12
Rev. 5/15
Rev. Add18

9 Statistical Considerations

Updated Final Analysis Plan:

As information for the primary endpoint was accumulating slower than anticipated, the pattern of information-time accumulation was reviewed by the study team from ECOG-ACRIN, CTEP and BMS on July 13, 2018. Dr. Edward Korn (NCI CTEP) and Dr. Catalano (independent ECOG-ACRIN statistician) concluded that it is not likely to obtain the required number of events with a longer follow-up time. This conclusion was obtained using the pattern of information-time accumulation, the survival-curve assumptions in the protocol, and the published survival results with ipilimumab in a similar setting (Eggermont, et al. Lancet Oncol. 2015 May;16(5):522-30). The vast majority of the deaths were expected to occur in the first 4.5 years. Dr. Korn and Dr. Catalano recommended that the study team conduct the final analysis 4.5 years after the date the last patient was accrued on study arms A, B, or C, (which was August 15, 2014). Therefore, the recommended date of the final analysis would be February 15, 2019. The Z-value for declaring statistical significance at this final analysis can be calculated (in the usual way) from the remaining type I error not already spent from the interim analyses prior to February 15, 2019, using the correlation between the interim-analyses test statistics and the final analysis test statistic. The regularly scheduled interim analysis for the DMSC in the Fall 2018 was recommended to proceed as usual, with the trial amended for the new final analysis date soon after the Fall DSMC meeting. A total of four interim analyses have been conducted as of September 2018 and following this recommendation, the final analysis will be conducted using the data as of February 15, 2018. This will alter the interim analysis plan stated in section 9.6. The analysis based on the data as of February 15, 2019 will be the planned final analysis for all comparisons and the hierarchical testing plan described in sections 9.4 and 9.5 will still be followed.

9.1 Previous Study Design

Initially this study was designed to compare the high-dose (10mg/kg) ipilimumab (Ipi) vs. HDI. The study was activated on May 25, 2011. Subsequently the importance of the low-dose Ipi (3mg/kg) in this patient population was discussed based on the results of the MDX010-20 trial and the FDA approval of ipilimumab at 3 mg/kg in the treatment of metastatic melanoma, leading to the addition of the 3 mg/kg arm to this trial as of January 2012. Since then patients are randomized to three treatment arms: High dose interferon-alfa (HDI), high dose ipilimumab (HIP) and low dose ipilimumab (LIP).

With the addition of low dose ipilimumab (LIP) arm, a two-step hierarchical approach was adopted. In the first step, the high-dose Ipi was to be compared with HDI. If the high-dose Ipi is significantly better than HDI, then the low-dose Ipi was to be compared with HDI as a second step. However, the order of the comparison has been revised to the low-dose Ipi vs. HDI first then to the high-dose Ipi vs. HDI in February 2015. This revision was made since the low-dose Ipi has become the standard of care in the treatment of metastatic melanoma. Since no formal interim analysis had been conducted prior to this revision, this change did not affect the overall type I error rate of the study design.

9.2 Primary Objective

This is a randomized phase III study of high-dose Ipi (HIP) vs. low-dose Ipi (LIP) vs. HDI in patients with resected high-risk melanoma. Approximately 1500 patients will be randomized equally among the three treatment arms, stratified by the AJCC stage (IIIB, IIIC, M1a, M1b). The stratified randomization will be based on the permuted block method.

Rev. 9/14

This study has a two-step hierarchical approach. In the first step, the low-dose Ipi will be compared with HDI. If the low-dose Ipi is significantly better than HDI, then the high-dose Ipi will be compared with HDI as a second step. When comparing the two investigational treatment groups, the primary comparison will be an intent to treat (ITT-defining groups by assigned treatment) analysis of recurrence free survival (RFS**; First Co-primary Endpoint**) and overall survival (OS**; Second Co-primary Endpoint**) in all patients compared to HDI. Both of these endpoints will be used as co-primary endpoints in the sense that the study will be considered a positive study if it meets either one of these 2 endpoints (see Section 1.7.1). That is, in the first step of comparing the low-dose Ipi vs. HDI, it will be a positive study if the low-dose Ipi has better RFS or OS outcome. Then the comparison will proceed to the second step where the high-dose Ipi will be compared to HDI. In each comparison, patients concurrently randomized to the two arms being compared will be included in the analysis. . If high-dose Ipi leads to a better RFS or OS outcome than the HDI, it will be considered as a positive study with respect to the second comparison.

9.3 Sample Size and Accrual

Approximately 1500 patients will be enrolled over 3.3 years. Accrual rate is expected to be 38 per month. Note that the low-dose Ipi arm will be added in January 2012 and the low-dose Ipi arm will need a concurrent HDI arm as control group. Each group has a sample size of about 500 cases. After the low-dose ipi is added, patients will be still randomized equally to three treatment arms. Accrual to high-dose Ipi arm will be suspended when it reaches 500 cases. For the other two arms (low-dose Ipi and HDI), accrual will continue until each arm has 500 cases since the time of adding the low-dose Ipi arm. This will lead to a slightly larger sample size (> 500) for the HDI arm. However, it is expected that the overall accrual period necessary for completion of the 3mg/kg dose arm and concurrent HDI accrual will not be more than several months due to this adjustment. Every effort will be made to shorten the accrual duration with active intergroup participations. With additional follow up time, a total duration of study is expected to be less than 6 years. The primary comparisons of RFS and OS will be performed using the log-rank test stratified on AJCC stage.

Rev. 9/14

Per amendment #10, approximately 45 adolescent patients (ages 12-17) will be enrolled. If accrual to the adult population reaches its goal before 45 adolescent patients enroll to this study, the study will remain open only to the adolescent population until 2016. If the adolescent population reaches its accrual goal of 45 before the adult population reaches its accrual goal, adolescent accrual will be closed. With the data from a small group (45 or less) of adolescent population, analysis will be mostly descriptive and exploratory.

Rev. 3/15

As of August 15, 2014, adult accrual to Arms A, B, and C has completed. Adolescent patients (ages 12 -17) will be randomized to study arms D, E, and F.

Rev. 9/14

9.4 First Step: Comparison of Low-dose Ipi vs. HDI

9.4.1 RFS

The recurrence-free survival (RFS) is defined from the time from randomization to the time of disease recurrence or death from any cause.

Since a moderate percentage of long-term cures have been observed in melanoma patients on previous melanoma study, E4697, we have used a model proposed by Berkson and Gage (1952) and Goldman (1984). This model specifies that the target population is a mixture, with proportion p who will be cured, and (1 – p) who will fail according to an exponential distribution with rate β. The RFS function D(t) for the HDI arm (A) and the Ipi arm (B) is expressed as:

$$D_A(t) = p + (1 - p) \exp(- \beta t)$$

$$D_B(t) = \{p + (1 - p) \exp(- \beta t)\}^{\theta}$$

where ɵ represents a hazard ratio under the proportional hazards alternative. For RFS, it was assumed that the cure rate is 29% and the median survival time for those not cured is 0.487 years in patients randomized to the HDI arm. Under the proportional hazards alternative, we hypothesize that Ipi will reduce the hazard by 25% (hazard ratio ɵ=0.75). The full information is 655 events in high-dose Ipi and HDI groups. This design provides at least 80% power at a one-sided type I error rate of 0.003. An ITT analysis using the stratified log-rank test will be performed.

9.4.2 OS

Based on E4697 data, the cure rate model is appropriate for this endpoint. This model specifies that the target population is a mixture, with proportion p who will be cured, and (1 – p) who will fail according to an exponential distribution with rate β. The survival function S(t) for the HDI arm (A) and the Ipi arm (B) is expressed as:

$$S_A(t) = p + (1 - p) \exp(- \beta t)$$

$$S_B(t) = \{p + (1 - p) \exp(- \beta t)\}^{\theta}$$

where ɵ represents a hazard ratio under the proportional hazards alternative. For the primary endpoint, OS, it was assumed that the cure rate is 35% and the median survival time for those not cured is 2.44 years in patients randomized to the HDI arm. Under the proportional hazards alternative, we hypothesize that Ipi will reduce the hazard by 25% (hazard ratio ɵ=0.75). This design requires a total of 1000 patients. The full information corresponds to 416 deaths in low-dose Ipi and HDI groups. This design will provide 80% power to detect the above difference at a one-sided type I error rate of 0.022. An ITT analysis using the stratified log-rank test will be performed.

9.5 Second Step: Comparison of High-dose Ipi vs. HDI

The second step comparison will only occur if the first step comparison described in Section 9.4 is significant for either RFS or OS endpoint. In such case, the high-dose Ipi will be compared to HDI. In these comparisons, only the HDI

patients enrolled after the low dose ipi arm is added will be included. The same design considerations in Section 9.4 will be applied in the second step.

9.6 Interim Analysis

First with respect to the first step comparison of low-dose Ipi vs. HDI, interim analyses of RFS and OS will be performed for all semi-annual DMC meetings beginning when approximately 50% of the planned full information (416 deaths among patients treated with low-dose Ipi and HDI) has occurred. The data from both of these co-primary endpoints will be reviewed, however an early stopping of the study will be considered only based on the OS endpoint. It is expected to have 6 interim analyses at .5, .62, .72, .81, .89, .96 information time and 1 final analysis. To preserve the overall type I error rate, critical values at the interim analyses will be determined using a truncated version of the Lan-DeMets spending function corresponding to the O'Brien-Fleming boundary. Boundary values at a nominal significance less than 0.0005 will be truncated at 0.0005, with the boundary also adjusted to preserve the overall one-sided type I error rate of 0.022 for the OS endpoint. This study will also be monitored for early stopping in favor of the null hypothesis. In particular, at each scheduled interim analysis, we will compute the conditional power of rejecting the null hypothesis at full information. If the conditional power is less than 10%, an early termination of the study will be considered.

For the RFS endpoint, interim analysis will begin when the OS endpoint has reached 50% information time and will be repeated every 6 months. The expected information at the first interim analysis is about .83 for the RFS endpoint. Interim analysis of RFS will be presented to the DSMC at the time of OS interim analyses, but the study does not have stopping rules based on the RFS endpoint. The primary analysis for RFS will take place when the full information (655 events) is reached. If a treatment benefit is indicated in the RFS endpoint at this time, this study will be considered as a positive study regardless of the OS result at this time.

The interim analyses for the second step comparison (high-dose ipi vs. HDI) will begin only when the interim analysis for OS or full-information analysis for RFS indicates significantly better result in the low-dose Ipi arm in comparison to the HDI arm.

To preserve overall type I error rates for both RFS and OS endpoints, the hierarchical testing will be implemented separately for RFS and OS endpoints. Interim analyses of low dose Ipi vs. HDI for RFS will begin once low dose Ipi vs. HDI is significant for RFS (and only if it is) and interim analyses of high-dose Ipi vs. HDi for OS will begin once low dose Ipi vs. HDI is significant for OS (and only if it is).

Similarly during the final analysis, if low dose Ipi vs. HDI is significant for RFS at the 0.3% level, then we will compare RFS between high dose Ipi vs. HDI at the 0.3% level. If low dose Ipi vs. HDI is significant for OS at the 2.2% level, then we will compare high dose Ipi vs. HDI at the 2.2% level. These two decisions will be made separately and if only RFS is significant for low dose Ipi vs. HDI, then only RFS will be compared for high dose Ipi vs. HDI. Similarly if only OS is significant to low dose ipi vs. HDI, then only OS would be compared for the high dose arm.

The futility analysis based on the OS will start at the same time as the first step OS comparison and will continue. At each scheduled interim analysis, we will compute the conditional power of rejecting the null hypothesis at full information, If the conditional power is less than 10%, an early termination of the study will be considered. If the low-dose Ipi arm is suspended due to the futility analysis, the high-dose Ipi vs. HDI comparison will still continue.

9.7 Secondary Objectives

9.7.1 Toxicity Monitoring

The population for the safety analysis will be comprised of all patients who received at least one dose of study medication. Patients will be monitored for adverse events using the National Cancer Institute's (NCI) v.4.0 of the Common Terminology Criteria for Adverse Events (CTCAE). All treatment-emergent and baseline adverse events and hematological/biochemical toxicities based on laboratory measurements, as well as drug related AE's, will be summarized by treatment group and NCI CTCAE worst grade.

Expected toxicity data for ipilimumab treated patients, based on the BMS four phase II studies utilizing the 10 mg/kg dose with 325 patients, is as follows. Overall 32.6% experienced the worst treatment-related AEs of grade 3/4/5. Immune-related AEs were experienced in 72.3% of patients. Of these, 25.2% were Grade 3/4 and occurred in the following 4 main types: i) Gastrointestinal: e.g., colitis, diarrhea (12.3%), ii) Liver: e.g., transaminase elevation (6.8%), iii) Skin: e.g., rash pruritis (2.8 %), iv) Endocrine: e.g., hypophysitis, thyroiditis (2.5%). Other complications, i.e., bowel perforations, liver failure were minor (~1%). In addition there were 5 treatment-related deaths: 4 immune-related: multi-organ failure, abnormal hepatic function, and acute glomerulonephritis, hypovolemic shock and 1 not immune-related: acute myeloid leukemia. The toxicity data from all treated patients will be reviewed carefully by the ECOG-ACRIN DMC. In the early part of the study, when 50 patients and then 100 patients are treated with each of the ipilimumab doses, toxicity incidence data will be summarized and presented to DMC. All treatment-related toxicity data will be presented along with a summary on the worst grade. A particular attention will be given to the specific toxicity types mentioned above. Toxicity incidence rate and 95% confidence interval for each type will be presented. Upon DMC's review, if there are major issues, this will be communicated with the CTEP.

In the MDX010-20 trial (ipilimumab 3 mg/kg), 97-98% patients experienced any adverse event and approximately 46% experienced grade 3/4/5 adverse events. (Hodi et al. NEJM 2010) The most common adverse events related to the study drugs were immune-related events, which occurred in approximately 60% of the patients treated with ipilimumab. The frequency of grade 3 or 4 immune related adverse events was 10 to 15% in the ipilimumab groups. The most common immune-related adverse event was diarrhea, which occurred at any grade in 27 to 31% of the patients in the ipilimumab groups. The frequency of organ specific grade 3 and 4 adverse events

in the ipilimumab/gp100 and ipilimumab/placebo arms respectively was as follows: dermatologic (2.4% and 1.5% of patients), GI (5.8% and 7.6%), endocrine (1.1% and 3.8%), hepatic (1.1% and 0%). Death due to irAE occurred in 1.3% and 1.5% respectively.

Interim toxicity monitoring and analysis will be performed separately for the high dose ipilimumab (HIP) and low dose ipilimumab (LIP) arms. If one arm meets criteria for discontinuation due to toxicity the other arm will continue enrolling patients and continue to be monitored per protocol monitoring criteria. For example, if HIP arm was discontinued due to toxicity, LIP arm will continue per protocol unless LIP meats criteria for discontinuation as well.

9.7.2 Quality of Life

General hypothesis: There are no clinically relevant differences between the two arms using global QOL scale at the assessed intervals (every 3 months while on treatment and every 6 months off-treatment for one year). There may be a later advantage to the ipilimumab arm if disease progression occurs, thereby leading to a better global QOL.

Primary hypothesis (for sample size calculation): Ipilimumab is superior to HDI using global QOL scale at 12 weeks following the initiation of therapy (after completion of the ipilimumab induction phase).

The primary objective for the QOL study is to evaluate if patients treated with ipilimumab will have a better global QOL. This component of the protocol will be open to patients enrolled by Community Clinical Oncology Programs (CCOPs).

FACT-G will be collected at baseline, every 3 months while on treatment and every 6 months off-treatment for one year. The main time point for the comparison of global QOL between the two treatment arms will be at 3 months following the initiation of therapy (after completion of the ipilimumab induction phase).

FACT-G has four areas of measurements (physical well being, social/family well-being, emotional well-being and functional well-being) with a scale of 0-4. The changes between before and after treatment for each area and summary of all four areas will be compared between the two treatment arms. We do not have the preliminary QOL data to justify the sample size calculation. We plan to perform the QOL study in all patients enrolled by CCOPs, projected to be about a third of the patients (450 total patients, 150 per arm). We will use the baseline and 3 months data to compare the change before and after the treatment between the two treatment arms (high-dose Ipi vs. HDI and low-dose Ipi vs. HDI). If the standardized difference (difference in means / sd) is at least 0.4, this analysis will have at least 80% power for each comparison. Given the exploratory nature of this analysis, no adjustments for the multiple comparisons will be made.

This is based on one-sided type I error rate of 0.025. While the study is open to accrual, the proportion of patients from CCOP institutions will be monitored. If the QOL accrual target cannot be met by CCOPs alone, a plan to expand the QOL component to other institution types will be considered.

The secondary objective of QOL study is to evaluate the effect of adjuvant ipilimumab and HDI on the various symptoms and functioning scales as treatment related side effects that may have an impact on the health-related domains of QOL. FACT-BRM and FACIT-D forms will also be collected on the same schedule as the FACT-G form. FACT-BRM has two additional sections addressing physical and mental QOL aspects. FACIT-D has a section addressing colitis and diarrhea related issues.

For both QOL objectives, ITT analysis in all patients (from two treatments) will be conducted. The changes between before and after treatment will be compared between the two treatment arms. The two-sample t-test will be used to compare continuous measurements and Chi-square test will be used to compare the categorical outcomes between the two treatment groups. Longitudinal regression models will also be used to address the overall change over time during which the QOL data is collected.

9.8 Statistical Analysis Plan

9.8.1 Primary analysis

RFS is defined as the time from randomization to the time of disease recurrence or death from any cause. OS is defined as the time from randomization to the time of death due to any cause. Both RFS and OS will serve as co-primary endpoints in this study. When 50% information time (208 deaths among patients treated with low dose Ipi or HDI) is reached for OS, the first interim analysis will begin. To preserve overall type I error rates for both RFS and OS endpoints, the hierarchical testing will be implemented separately for RFS and OS endpoints. Interim analyses of high dose Ipi vs. HDI for RFS will begin once low dose Ipi vs. HDI is significant for RFS (and only if it is) and interim analyses of high-dose Ipi vs. HDi for OS will begin once low dose Ipi vs. HDI is significant for OS (and only if it is).

Similarly during the final analysis, if low dose Ipi vs. HDI is significant for RFS at the 0.3% level, then we will compare RFS between high dose Ipi vs. HDI at the 0.3% level. If low dose Ipi vs. HDI is significant for OS at the 2.2% level, then we will compare high dose Ipi vs. HDI at the 2.2% level. These two decisions will be made separately and if only RFS is significant for low dose Ipi vs. HDI, then only RFS will be compared for high dose Ipi vs. HDI. Similarly if only OS is significant to low dose ipi vs. HDI, then only OS would be compared for the high dose arm.

The primary comparison will be an ITT analysis including all cases as randomized using the stratified (by AJCC stages: IIIB, IIIC, M1a, M1b)

log-rank test. Kaplan-Meir plot will be generated and two-sided p-values will be reported.

During the interim analysis, data available as of the data cut-off date for a specified interim analysis will be included. Specific guidelines outlined in section 9.6 will be used. Briefly, for the efficacy analysis, the type I error rate will be allocated per truncated O'Brien-Fleming boundary (equivalent to O'Brien-Fleming boundary in our case since the first analysis will not begin until 50% information time). For the futility analysis, the conditional power will be calculated. Hazard ratio will be estimated and repeated confidence intervals will be generated for OS and RFS.

At the final analysis, all cases will be included. If the proportional hazard assumption is appropriate, multivariate Cox proportional hazard models for RFS and OS will be developed at the final analysis. Prognostic factors such as age, gender, stage, ulceration, etc will be included. For the stratified log-rank test and Cox proportional hazards regression model, sensitivity analysis will be conducted at the final analysis based on analyzable(eligible and treated) patients only.

At the final analysis, if the low-dose Ipi vs. HDI is significant and high-dose Ipi vs. HDI is significant for OS then OS will be compared for the low-dose Ipi vs, high-dose Ipi arms. Similarly, if the low-dose Ipi vs. HDI is significant and high-dose Ipi vs. HDI is significant for RFS then RFS will be compared for the the low-dose Ipi vs, high-dose Ipi arms. It is likely this comparison will be underpowered.

9.8.2 Toxicity data analysis

Adverse events (AE) data will be summarized based on the data collected in ECOG CRFs as well as the data reported via CTEP-AERS. Individual toxicity type AE and categorized AE data (by autoimmune disorders, endocrine, GI, liver, nervous system, pancrease, psychiatric disorders, skin, thromboemblic disorders) will be summarized by grade and treatment arm. The percentages of patients experiencing the worst degree toxicities (highest grade event per AE type per patient) will be evaluated and the distribution of the worst degree toxicities will be compared among the treatment arms. The proportion of patients with worst degree toxicities with 3 or higher will be summarized and compared among the treatment arms. Chi-square test will be used for AE comparisons. For the AE data, any patient who received treatment will be included.

9.8.3 Other secondary analyses

The QOL study is open to patients enrolling from CCOPs. FACT-G, FACIT-D and FACT-BRM forms will be collected every 3 months while on treatment and every 6 months off-treatment for one year.

The main comparison for all forms will be the change in QOL measurements between the baseline and 3 month time point by treatments (high-dose Ipi vs, HDI or low-dose ipi vs. HDI). Descriptive statistics on QOL measurements will be provided. Two-sample t-test

will be used for each comparison and two-sided p-values will be reported. ITT analysis on all cases with QOL data will be conducted.

At the final analysis, subgroup analyses of the treatment effects on RFS and OS will be conducted. Subgroups by age, sex, ECOG PS, AJCC stage (IIIB, IIIC, M1a, M1b), ulceration status of the primary, type of lymph node involvement (microscopic / macroscopic), extent of lymph node involvement (1 vs 2-3 vs ≥ 4 positive LN) will be considered in the ITT patient population. This analysis will be summarized using the forest plots with HRs and 95% CIs.

In the event of missing data, it will be assumed that the data will be missing at random and no imputation will be performed.

9.9 Gender and Ethnicity

Based on previous data from E4697 and E1694, the anticipated accrual in subgroups defined by gender and race is:

Ethnic Category	**Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	2	6	8
Not Hispanic or Latino	567	925	1492
Ethnic Category: Total of all subjects	569	931	1,500

Racial Category			
American Indian or Alaskan Native	0	0	0
Asian	0	2	2
Black or African American	3	3	6
Native Hawaiian or other Pacific Islander	0	0	0
White	566	926	1492
Racial Category: Total of all subjects	569	931	1,500

The accrual targets in individual cells are not large enough for definitive treatment comparisons to be made within these subgroups. Therefore, overall accrual to the study will not be extended to meet individual subgroup accrual targets.

9.10 Study Monitoring

Rev. 11/13
Rev. 11/13

This study will be monitored by the ECOG-ACRIN Data Safety Monitoring Committee (DSMC). The DSMC meets twice each year. For each meeting, all monitored studies are reviewed for safety and progress toward completion. When appropriate, the DSMC will also review interim analyses of outcome data. Copies of the toxicity reports prepared for the DSMC meetings are included in the study reports prepared for the ECOG-ACRIN group meeting (except that for double blind studies, the DSMC may review unblinded toxicity data, while only pooled or blinded data will be made public). These group meeting reports are made available to the local investigators, who may provide them to their IRBs. Only the study statistician and the DSMC members will have access to interim analyses of outcome data. Prior to completion of this study, any use of outcome data will require approval of the DSMC. Any DSMC recommendations for changes to this study will be circulated to the local investigators in the form of addenda to this protocol document. A complete copy of the ECOG-ACRIN DSMC Policy can be obtained from the Coordinating Center.

9.10.1 Arm A Toxicity Monitoring

Rev. 11/13

Grade 5 AE data reported via CTEP-AERS system has been reviewed by ECOG-ACRIN DSMC. Based on the data as of 9/23/13, there had been 8 grade 5 AEs potentially treatment-related in arm A. At that time, there were 405 patients who were treated with 10mg/kg Ipi dose. While AE data was being reviewed by ECOG-ACRIN DSMC, accrual to arm A was suspended and treatment for the patients randomized to arm A was held. Upon reopening the arm A for accrual and grade 5 AE data will be reviewed carefully. When there is a new grade 5 AE event in arm A, a study statistician will be alerted. The rate of grade 5 potentially treatment-related AE will be estimated by dividing the total number of cases with grade 5 potentially treatment-related AEs by (total accrual in arm A – cases never started therapy). If this rate is greater than or equal to 2.5%, ECOG-ACRIN DSMC will be notified and this matter will be discussed. If this rate is reached, it will require suspension of accrual to arm A while the review is conducted.

For a cutoff rate of 2.5%, the rule would be 11 events in 440 or fewer treated patients, 12 events in 480 or fewer patients, or 13 in > 480 (assuming < 520 entered). Given that we already have observed 8 potentially treatment-related grade 5 AE events in 405 patients, the conditional power for the proposed rule can be estimated by: the probability of 3 or more (to reach 11) in 35, plus the probability of 4 or more in 75 given < 3 in the first 35, plus the probability of 5 or more in 95 given < 3 in the first 35 and < 4 in the first 75. Then the conditional power is 3.7% for a true grade 5 AE rate of 1.5%, 8.5% for a true grade 5 AE rate of 2.0%, 15.3% for a true rate of 2.5%, 23.6% for a true rate of 3.0%, 32.9% for a true rate of 3.5%, 42.5% for a true rate of 4.0%, and 60.4% for a true rate of 5.0%. Conditional power is not particularly high given that we have already observed 8 cases with grade 5 AEs out of 405 treated patients. However this toxicity monitoring rule will provide some guidance for a formal review by ECOG-ACRIN DSMC.

Rev. 9/14, 9/14

9.11 Adolescent Patients

As of amendment #10 activation, E1609 age eligibility criteria will be expanded to allow adolescent patients ages 12–17 years old. This is based on preliminary safety and tolerability data reported in study NCI 7458. UP to 45 patients will be randomized into the 3 study arms as follows: up to 15 patients on Arm D (ipilimumab 10 mg/kg), up to 15 patients on Arm E (HDI) and up to 15 patients on Arm F (ipilimumab 3 mg/kg). This patient sample size of 45 will be in addition to the approximately 1500 adult patients planned to be enrolled on this trial. The primary study objective for the adolescent patient population is safety. This includes the frequency of study treatment related adverse events with a focus on the frequency and severity of ipilimumab induced immune mediated adverse reactions in adolescent patients with resected high risk melanoma (Stage IIIB, IIIC, M1a, M1b) as the primary endpoint in this study population. Further, the plans for the adult population study analysis will not be impacted by the accrual in the adolescent population part of the study.

Rev. 3/15

10. Pathology Review

NOTE: ECOG-ACRIN requires that all biological samples submitted be entered and tracked via the online ECOG-ACRIN Sample Tracking System (STS). An STS shipping manifest form must be generated and shipped with the sample submissions. See Section 10.4.

NOTE: An informed consent must be signed prior to the submission of any samples, including mandatory diagnostic reviews and banking.

10.1 Pathology samples must be submitted for review and classification (baseline only) and banking.

The submitting pathologist and clinical research associate should refer to Appendix II (Pathology Submission Guidelines) for guidelines and summary of submission requirements.

10.2 Materials Required For This Protocol

Rev. 9/14

10.2.1 Forms (must be submitted with all tissue submissions)

- A copy of the surgical pathology report
- Immunologic studies, if available

Rev. 2/12, 2/13

- Sample Tracking System Shipping Manifest (see Section 10.4)

In addition to the surgical pathology report, if immunologic studies have been performed at the home institution, it is necessary that these be forwarded as well.

Rev. 9/14

10.2.2 Pathology Submissions (Mandatory for Central Diagnostic Review)

10.2.2.1 Primary Melanoma (for patients with known primary cutaneous melanoma)

One (1) H & E of the primary melanoma and fifteen (15) unstained slides preferably from the thickest portion of the tumor for immunostains (please do not deparaffinise slides) OR, if the primary pathologist is willing, please request the corresponding block, which will be promptly returned upon request. If the patient has more than one primary lesion, please include above slides and/or block for each primary.

10.2.2.2 Lymphadenectomy Specimen

One (1) H & E section of the tumor bearing lymph nodes ONLY, with a copy of pathology report. An alternative is to send two (2) unstained slides or two (2) H & E slides on each positive lymph node. Pathology report should indicate:

Rev. 2/12

a. Total number of lymph nodes
b. Number of lymph nodes positive
c. Size of the largest lymph node
d. Whether there was gross soft tissue tumor involvement

e. Whether there was evidence of extra-capsular spread

f. Whether a lymph node was a sentinel lymph node

(Please include report on immunostains if any).

10.2.2.3 Resected in-transit or satellite metastases, or cutaneous metastases, or lymph node metastases, or lung metastases

One (1) H & E section of the metastatic lesion and fifteen to twenty (15-20) unstained slides from the thickest part of the tumor or, if the primary pathologist is willing, please request the corresponding block, which will be promptly returned upon request.

NOTE: Sections of surgical margins of skin, unless positive for tumor, are not required. A surgical pathology report is sufficient.

NOTE: Submission of pathology samples for diagnostic review is mandatory in order for the patient to be considered evaluable. If insufficient material is available after diagnostic pathology, the pathologist must contact the Study Chair and send a letter stating this to the ECOG-ACRIN Central Biorepository and Pathology Facility.

10.2.3 Progression/Relapse Biopsy (if performed, for banking for future research per patient consent)

- One (1) H & E section of the metastatic lesion and fifteen to twenty (15-20) unstained slides from the thickest part of the tumor or, if the primary pathologist is willing, please request the corresponding block, which will be promptly returned upon request.

NOTE: A copy of the completed submission form will be sent to the ECOG-ACRIN Operations Office - Boston by the Central Biorepository and Pathology Facility.

10.3 Shipping Procedures

Log the samples into the ECOG-ACRIN Sample Tracking System (STS) the day of shipment. If the STS is unavailable, an Generic Specimen Submission Form (#2981) must be submitted with the samples. Once STS is available, retroactively log the shipment into STS, using the actual collection and shipping dates.

Rev. 9/14

Ship using the CBPF's FedEx account via the FedEx on-line ship manager.

Rev. 2/13, 9/14

Access to the FedEx shipping account for specimen shipments to the ECOG-ACRIN CBPF at MD Anderson Cancer Center can only be obtained by logging into fedex.com with an account issued by the ECOG-ACRIN CBPF. For security reasons, the account number will no longer be given out in protocols, over the phone, or via email. If your site needs to have an account created, please contact the ECOG-ACRIN CBPF by email at eacbpf@mdanderson.org .

10.3.1 Submission Schedule

The required diagnostic materials must be submitted within one month of patient randomization.

The optional pathology samples from progression/relapse should be submitted within one month of disease progression.

10.3.2 Shipping Address

ECOG-ACRIN Central Biorepository and Pathology Facility
MD Anderson Cancer Center
Department of Pathology, Unit 085
Tissue Qualification Laboratory for ECOG-ACRIN, Room G1.3586
1515 Holcombe Blvd
Houston, TX 77030
Phone: Toll Free 1-844-744-2420 (713-745-4440 Local or International Sites)
Fax: 713-563-6506
Email: eacbpf@mdanderson.org

An STS shipping manifest form must be generated and shipped with all sample submissions.

10.3.3 Central Biorepository and Pathology Facility: Processing and Routing

Rev. 9/14

Diagnostic materials will be forwarded to Dr. Uma Rao for central review.

10.4 ECOG-ACRIN Sample Tracking System

It is **required** that all samples submitted on this trial be entered and tracked using the ECOG-ACRIN Sample Tracking System (STS). The software will allow the use of either 1) an ECOG-ACRIN user-name and password previously assigned (for those already using STS), or 2) a CTSU username and password.

When you are ready to log the collection and/or shipment of the samples required for this study, please access the Sample Tracking System software by clicking https://webapps.ecog.org/Tst.

Rev. 9/14

Important: Please note that the STS software creates pop-up windows, so you will need to enable pop-ups within your web browser while using the software. A user manual and interactive demo are available by clicking this link: http://www.ecog.org/general/stsinfo.html. Please take a moment to familiarize yourself with the software prior to using the system.

Rev. 2/12

An STS generated shipping manifest form should be generated and shipped with all sample submissions.

Please direct your questions or comments pertaining to the STS to ecog.tst@jimmy.harvard.edu.

10.4.1 Study Specific Notes

Rev. 9/14

An Generic Specimen Submission Form (#2891) will be required only if STS is unavailable at the time of sample submission. Notify the laboratory of the shipment by FAXing a copy of the completed form to

the laboratory. Indicate the appropriate laboratory on the submission form:

- ECOG-ACRIN Central Biorepository and Pathology Facility
- ECOG-ACRIN Immunologic Monitoring and Cellular Products Laboratory
    - The day of shipment, notify the IMCPL ECOG-ACRIN study coordinator by FAX (412-623-6625) using the Specimen Shipment/Requisition Form (Appendix IX). If you are unable to get through to the laboratory by FAX, telephone the ECOG-ACRIN study coordinator at: (412) 624-0078 and provide the FedEx tracking number.

      Retroactively enter all collection and shipping information when STS is available.

Rev. 2/12

10.5 Banking

The residuals and/or derivatives of the blocks/slides submitted will be retained at the ECOG-ACRIN Central Repository for possible use in future ECOG-ACRIN approved studies. Any residual blocks will be available for purposes of individual patient management on specific written request. If future use is denied or withdrawn by the patient, the samples will be removed from consideration for use in any future study.

11. Correlative Studies

NOTE: An informed consent MUST be signed prior to the submission of any sample, including banking for research.

NOTE: Institutions outside of the United States and Canada must confer with the receiving laboratory and the ECOG-ACRIN Operations Office - Boston regarding logistics for submission of fresh samples.

NOTE: ECOG-ACRIN requires that all biological samples submitted be entered and tracked via the online ECOG-ACRIN Sample Tracking System. An STS shipping manifest form must be generated and shipped with the sample submissions. See Section 10.4.

11.1 Blood Sample Collection

Rev. 9/14

Blood samples are being collected for future use in laboratory corollary studies, including the performance of novel biomarker evaluations that are of prognostic and therapeutic predictive value. Due to the potential future therapeutic predictive implications (targeting treatment to those who are likely to benefit) and economic implications (avoiding the toxicities and cost for those who are not likely to benefit) of such studies, patients are encouraged to participate. However, participation in providing these blood samples is optional.

Please refer to Appendix XIX for the full description of the laboratory research studies being performed.

Rev. 9/14

11.1.1 Sample Submission Schedule

Rev. 8/12, 9/14

Blood should be collected as specified below for each tube type at baseline (prior to start of treatment), after three (Ipilimumab Arms A, D and C, F) / four (IFN Arm B, E) weeks of initiating study treatment, after twelve (12) weeks of initiating treatment, at 48 weeks or at disease relapse (if relapse occurs earlier than the specified time point). No further blood samples are to be submitted after the relapse blood is sent.

Blood samples should be shipped the day they are drawn.

Instructions to order kits are outlined below.

Questions are to be directed to the Immunological Monitoring and Cellular Products laboratory (IMCPL) ECOG-ACRIN study coordinator at (412) 624-0078.

Rev. 2/12

11.1.2 Sample Preparation Guidelines

1. At EACH time point please submit the following:
   - Three (3) FULL 10 cc RED top tubes (BD cat #367820 or SST 367988 gel separator/gold top/ tiger tubes if the center can centrifuge them)
   - Ten (10) FULL 10 cc GREEN top heparin tubes (BD cat # 366480).

Rev. 8/12, 9/14

2. At Baseline, after three (Ipilimumab Arms A, D and C, F) / four (IFN Arm B, E) weeks, and after twelve (12) weeks of initiating treatment ONLY, please submit the following:

- One (1) FULL PAXgene RNA tube (Fisher #23 021 01).

3. At Baseline ONLY please submit the following:

- One (1) FULL 10 cc YELLOW top ACD tube (BD cat # 364606)

Please completely fill all blood tubes as full as possible and collect the correct number and tube type as outlined above:

Each tube must be clearly labeled to include:

- ECOG-ACRIN protocol number E1609
- ECOG-ACRIN five-digit patient sequence number
- Patient initials
- Originating institution/investigator name
- Date and time drawn
- Collection time point

Rev. 9/14

11.1.2.1 Shipping Kits

Rev. 2/12

Specimen shipping kits are available to order for the collection of the blood samples and must be requested from the IMCPL. Kits will contain the supplies and instructions for collecting, processing, and shipping the blood samples. Please fax the request using the Shipping Kit Request Facsimile Form (Appendix VIII) to (412) 623-6625 or call the IMCPL at (412) 624-0078. Please allow ten working days for shipment and provide the following information:

- Study Number
- Participating Site Number
- Contact Person and Telephone Number

The kits will be shipped via FedEx Express Saver. Please plan ahead, priority overnight shipment is not possible.

All blood samples should be shipped the day of collection using the shipping kit. Follow the shipping instructions provided in the kit carefully.

The shipping kit consists of the following:

- Insulated shipping container and packing material
- FedEx Priority Overnight return label
- Shipping Instructions
- Shipping Kit Request Form

11.2 Shipping Procedures

Log the samples into the ECOG-ACRIN STS the day of shipment. If the STS is unavailable, an Generic Specimen Submission Form (#2981) must be submitted with the samples. Once STS is available, retroactively log the shipment into STS, using the actual collection and shipping dates.

If the STS is unavailable, notify the IMCPL ECOG-ACRIN study coordinator by fax (412-623-6625) using the Specimen Shipment/Requisition Form (Appendix IX). If you are unable to get through to the laboratory by fax, telephone the ECOG-ACRIN study coordinator at (412) 624-0078 and provide the FedEx tracking number.

Rev. 2/12, 9/14

Blood collected into the appropriate tubes should be sealed, wrapped and placed in the specimen shipper kit and shipped on the same day they are drawn by Federal Express Priority Overnight courier using the return label provided in the kit. The green top tubes should be shipped at ambient temperature (no wet or dry ice). The red, yellow, and PAXgene RNA tubes should be refrigerated immediately and shipped at 2-8°C. Shipments must be timed to arrive during normal working hours and should be shipped in one box.

Rev. 2/12, 9/14

The laboratory will be open Monday through Friday to receive samples. Do NOT ship on Fridays or Saturdays, or the day before a legal holiday. Ship by overnight courier Monday - Thursday only to:

Rev. 2/12

Immunologic Monitoring and Cellular Products Laboratory
University of Pittsburgh Cancer Institute
UPCI-IMCPL, Suite L 1.26
ECOG-ACRIN Study Coordinator
Hillman Cancer Center
5117 Centre Avenue
Pittsburgh, PA 15213
Tel: (412) 624-0078
FAX: (412) 623-6625

An STS shipping manifest form must be generated and shipped with all sample submissions.

Rev. 2/12, 9/14

NOTE: Blood should **NOT** be collected on Fridays.

11.2.1 Federal Guidelines for the Shipment of Blood Products: Sites should follow IATA regulations for Packaging UN3372 shipments. Please refer to FedEx guidelines.

11.3 Banking

Blood samples collected will be retained at the UPCI-IMCPL for use in future ECOG-ACRIN approved studies. If future use is denied or withdrawn by the patient, the samples will be removed from consideration for use in any future study.

11.4 Sample Inventory Submission Guidelines

Rev. 2/12

Inventories of all specimens collected and aliquoted will be submitted electronically by secure web application to the ECOG-ACRIN Operations Office - Boston on a monthly basis or upon request by any laboratory holding and/or using specimens associated with this study.

Rev. 9/14

11.5 Lab Data Transfer Guidelines

The data collected or generated on the above mentioned correlative studies will be submitted electronically via secure data portal to the ECOG-ACRIN Operations Office – Boston by the central laboratory.

12. Records to Be Kept

Please refer to the E1609 Forms Packet for the forms submission schedule and copies of all forms. The E1609 Forms Packet may be downloaded by accessing the ECOG World Wide Web Home Page (http://www.ecog.org). Forms must be submitted to the ECOG-ACRIN Operations Office - Boston, FSTRF, 900 Commonwealth Avenue, Boston, MA 02215 (ATTN: DATA).

This study will be monitored by the CTEP Data Update System (CDUS) version 3.0. Cumulative CDUS data will be submitted quarterly from the ECOG-ACRIN Operations Office - Boston to CTEP by electronic means.

12.1 Records Retention

FDA regulations (21 CFR 312.62) require clinical investigators to retain all trial-related documentation, including source documents, long enough to allow the sponsor to use the data to support marketing applications.

This study will be used in support of a US marketing application (New Drug Application), all records pertaining to the trial (including source documents) must be maintained for:

- two years after the FDA approves the marketing application, or
- two years after the FDA disapproves the application for the indication being studied, or
- two years after the FDA is notified by the sponsor of the discontinuation of trials and that an application will not be submitted.

Please contact the ECOG-ACRIN Operations Office - Boston prior to destroying any source documents.

13. Patient Consent and Peer Judgment

Current FDA, NCI, state, federal and institutional regulations concerning informed consent will be followed.

14. References

1. Balch, C.M., et al., Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. J Clin Oncol, 2001. **19**(16): p. 3635-48.
2. Manola, J., et al., Prognostic factors in metastatic melanoma: a pooled analysis of Eastern Cooperative Oncology Group trials. J Clin Oncol, 2000. **18**(22): p. 3782-93.
3. Kirkwood JM, A.S., Systemic cytotoxic and biologic therapy melanoma., in Cancer Principles and Practice of Oncology. 1993. p. 1-16.
4. Clemente, C.G., et al., Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma. Cancer, 1996. **77**(7): p. 1303-10.
5. Hakansson, A., et al., Tumour-infiltrating lymphocytes in metastatic malignant melanoma and response to interferon alpha treatment. Br J Cancer, 1996. **74**(5): p. 670-6.

6. Mihm, M.C., Jr., C.G. Clemente, and N. Cascinelli, Tumor infiltrating lymphocytes in lymph node melanoma metastases: a histopathologic prognostic indicator and an expression of local immune response. Lab Invest, 1996. **74**(1): p. 43-7.

7. Moschos, S.J., et al., Neoadjuvant treatment of regional stage IIIB melanoma with high-dose interferon alfa-2b induces objective tumor regression in association with modulation of tumor infiltrating host cellular immune responses. J Clin Oncol, 2006. **24**(19): p. 3164-71.

8. Tatsumi, T., et al., Disease-associated bias in T helper type 1 (Th1)/Th2 CD4(+) T cell responses against MAGE-6 in HLA-DRB10401(+) patients with renal cell carcinoma or melanoma. J Exp Med, 2002. **196**(5): p. 619-28.

9. Tatsumi, T., et al., Disease stage variation in CD4+ and CD8+ T-cell reactivity to the receptor tyrosine kinase EphA2 in patients with renal cell carcinoma. Cancer Res, 2003. **63**(15): p. 4481-9.

10. Kirkwood, J.M., et al., Interferon alfa-2b adjuvant therapy of high-risk resected cutaneous melanoma: the Eastern Cooperative Oncology Group Trial EST 1684. J Clin Oncol, 1996. **14**(1): p. 7-17.

11. Kirkwood, J.M., et al., High-dose interferon alfa-2b significantly prolongs relapse-free and overall survival compared with the GM2-KLH/QS-21 vaccine in patients with resected stage IIB-III melanoma: results of intergroup trial E1694/S9512/C509801. J Clin Oncol, 2001. **19**(9): p. 2370-80.

12. Kirkwood, J.M., et al., High- and low-dose interferon alfa-2b in high-risk melanoma: first analysis of intergroup trial E1690/S9111/C9190. J Clin Oncol, 2000. **18**(12): p. 2444-58.

13. Kirkwood, J.M., et al., A pooled analysis of eastern cooperative oncology group and intergroup trials of adjuvant high-dose interferon for melanoma. Clin Cancer Res, 2004. **10**(5): p. 1670-7.

14. Ives, N.J., Chemotherapy compared with biochemotherapy for the treatment of metastatic melanoma: a meta-analysis of 18 trials involving 2,621 patients. J Clin Oncol, 2007. **25**(34): p. 5426-34.

15. Wheatley, K., et al., Interferon-à as adjuvant therapy for melanoma: an individual patient data meta-analysis of randomised trials. Proceedings of the American Society of Clinical Oncology, 2007.

16. Kirkwood, J.M., et al., High-dose interferon alfa-2b does not diminish antibody response to GM2 vaccination in patients with resected melanoma: results of the Multicenter Eastern Cooperative Oncology Group Phase II Trial E2696. J Clin Oncol, 2001. **19**(5): p. 1430-6.

17. Wheatley, K., et al., Does adjuvant interferon-alpha for high-risk melanoma provide a worthwhile benefit? A meta-analysis of the randomised trials. Cancer Treat Rev, 2003. **29**(4): p. 241-52.

18. Wheatley K, I.N., Eggermont A, KirkwoodJM. INTERFERON-ALFA AS ADJUVANT THERAPY FOR MELANOMA: AN INDIVIDUAL PATIENT DATA META-ANALYSIS OF RANDOMISED TRIALS. in ASCO Annual Meeting. 2007. Chicago.

19. Creagan, E.T., et al., Phase II trials of recombinant leukocyte A interferon in disseminated malignant melanoma: results in 96 patients. Cancer Treat Rep, 1986. **70**(5): p. 619-24.

20. Creagan, E.T., et al., Recombinant leukocyte A interferon (rIFN-alpha A) in the treatment of disseminated malignant melanoma. Analysis of complete and long-term responding patients. Cancer, 1986. **58**(12): p. 2576-8.

21. Creagan, E.T., et al., Phase I study of recombinant leukocyte A human interferon combined with BCNU in selected patients with advanced cancer. J Clin Oncol, 1986. **4**(3): p. 408-13.

22. Creagan, E.T., et al., Phase II study: the combination DTIC, BCNU, actinomycin D, and vincristine in disseminated malignant melanoma. Med Pediatr Oncol, 1986. **14**(2): p. 86-7.

23. Lenschow, D.J., T.L. Walunas, and J.A. Bluestone, *CD28/B7 system of T cell costimulation.* Annu Rev Immunol, 1996. **14**: p. 233-58.

24. Schwartz, R.H., Costimulation of T lymphocytes: the role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. Cell, 1992. **71**(7): p. 1065-8.

25. Chen, L., et al., Costimulation of antitumor immunity by the B7 counterreceptor for the T lymphocyte molecules CD28 and CTLA-4. Cell, 1992. **71**(7): p. 1093-102.

26. Townsend, S.E. and J.P. Allison, Tumor rejection after direct costimulation of CD8+ T cells by B7-transfected melanoma cells. Science, 1993. **259**(5093): p. 368-70.

27. Townsend, S.E., et al., Specificity and longevity of antitumor immune responses induced by B7-transfected tumors. Cancer Res, 1994. **54**(24): p. 6477-83.

28. Allison, J.P., A.A. Hurwitz, and D.R. Leach, *Manipulation of costimulatory signals to enhance antitumor T-cell responses.* Curr Opin Immunol, 1995. **7**(5): p. 682-6.

29. Linsley, P.S., et al., CTLA-4 is a second receptor for the B cell activation antigen B7. J Exp Med, 1991. **174**(3): p. 561-9.

30. Thompson, C.B. and J.P. Allison, *The emerging role of CTLA-4 as an immune attenuator.* Immunity, 1997. **7**(4): p. 445-50.

31. Walunas, T.L., et al., CTLA-4 can function as a negative regulator of T cell activation. Immunity, 1994. **1**(5): p. 405-13.

32. Kearney, E.R., et al., Antigen-dependent clonal expansion of a trace population of antigen-specific CD4+ T cells in vivo is dependent on CD28 costimulation and inhibited by CTLA-4. J Immunol, 1995. **155**(3): p. 1032-6.

33. Krummel, M.F. and J.P. Allison, CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. J Exp Med, 1995. **182**(2): p. 459-65.

34. Krummel, M.F., T.J. Sullivan, and J.P. Allison, Superantigen responses and co-stimulation: CD28 and CTLA-4 have opposing effects on T cell expansion in vitro and in vivo. Int Immunol, 1996. **8**(4): p. 519-23.

35. Tivol, E.A., et al., Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunity, 1995. **3**(5): p. 541-7.

36. Waterhouse, P., et al., Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. Science, 1995. **270**(5238): p. 985-8.

37. Chambers, C.A., T.J. Sullivan, and J.P. Allison, Lymphoproliferation in CTLA-4-deficient mice is mediated by costimulation-dependent activation of CD4+ T cells. Immunity, 1997. **7**(6): p. 885-95.

38. Hamid O. Dose effect of ipilimumab in patients with advanced melanoma: Results from a phase II, randomized, dose-ranging study. in J Clin Oncol 26: 2008 (May 20 suppl; abstr 9025). 2008.

39. Weber J. Safety and efficacy of ipilimumab with or without prophylactic budesonide in treatment-naive and previously treated patients with advanced melanoma. in Clin Oncol 26: 2008 (May 20 suppl; abstr 9010). 2008.

40. O'Day, S., J. Weber, and C. Lebbe. Ipilimumab Treatment may be Associated with a Long-term Survival Benefit : 18-month Survival Rate of Patients with Advanced Melnaoma Treated with 10 mg/kg Ipilimumab in Three Phase II Clinical Trials. Abstract ID 9033. in ASCO Annual Meeting. 2009.

41. Kirkwood, J.M., et al., Mechanisms and management of toxicities associated with high-dose interferon alfa-2b therapy. J Clin Oncol, 2002. **20**(17): p. 3703-18.

42. Asim Amin, A., De Pril, V., Hamid, O., et al. Systemic Corticosteroids for the Treatment of Immune-Related Adverse Events (irAEs) Do Not Impact the Development or Maintenance of Ipilimumab Clinical Activity. [Poster presentation: #9037]. Presented at the 2009 American Society of Clinical Oncology Annual Meeting; May 29-June 2, 2009. Orlando, FL, USA.

43. Blansfield, J. A., Beck, K. E., Khoi, T., et al. Cytotoxic Tlymphocyte-associate antigen-4 blockage can induce autoimmune hypophysitis in patients with metastatic melanoma and renal cancer. J Immunother 2005;28(6): 593-598.

44. Jaber S.H., Edward W., et al. Skin reactions in a subset of patients with stage IV melanoma treated with anti-cytotoxic Tlymphocyte antigen 4 monoclonal antibody as a single agent. Arch Dermatol 2006;142:166-172.

45. Robinson, M. R., Chi-Chao C., Yang J. C., et al. Cytotoxic Tlymphocyte-associate antigen-4 blockage in patients withmetastatic melanoma: A new cause of uveitis. J Immunother 2004;27(6):478-479].

46. Hodi. Engl J Med, 2010. 363(8): p. 711-23

47. Compassionate Use Trial for Unresectable Melanoma With Ipilimumab *http://www.clinicaltrials.gov/ct2/results?term=ipilimumab*

48. Yurkovetsky ZR, Kirkwood, JM, et al. Multiplex analysis of serum cytokines in melanoma patients treated with interferon-alpha2b. Clin Cancer Res. 2007 Apr 15;13(8):2422-8.

A Phase III Randomized Study of Adjuvant Ipilimumab Anti-CTLA4 Therapy Versus High Dose Interferon a-2b for Resected High risk Melanoma

Appendix I

Informed Consent Template for Cancer Treatment Trials (English Language) [Deleted in Amendment #6]

INFORMED CONSENT INTENTIONALLY REMOVED FROM PROTOCOL DOCUMENT

Appendix I was removed from the protocol document in amendment #6 and is posted as a separate document on the ECOG website. This was removed from the protocol to comply with NCI formatting guidelines

A Phase III Randomized Study of Adjuvant Ipilimumab Anti-CTLA4 Therapy Versus High Dose Interferon a-2b for Resected High risk Melanoma

Appendix I-A

**Informed Consent Template for Cancer Treatment Trials
(English Language, Non-CCOP Institutions) [Deleted in Amendment #6]**

INFORMED CONSENT INTENTIONALLY REMOVED FROM PROTOCOL DOCUMENT

Appendix I-A was removed from the protocol document in amendment #6 and is posted as a separate document on the ECOG website. This was removed from the protocol to comply with NCI formatting guidelines

A Phase III Randomized Study of Adjuvant Ipilimumab Anti-CTLA4 Therapy Versus High Dose Interferon a-2b for Resected High risk Melanoma

Appendix II
Pathology Submission Guidelines

The following items are included in Appendix II:

1. Guidelines for Submission of Pathology Materials (instructional sheet for Clinical Research Associates [CRAs])
2. Instructional memo to submitting pathologists
3. List of Required Materials for E1609
4. ECOG-ACRIN Generic Specimen Submission Form (#2981)

Rev. 3/15

Rev. 3/15

Guidelines for Submission of Pathology Materials

The following items should always be included when submitting pathology materials to the ECOG-ACRIN Central Biorepository and Pathology Facility:

- Institutional Surgical Pathology Report
- Pathology materials (see attached List of Required Material)
- ECOG-ACRIN Generic Specimen Submission Form (#2981)

Instructions:

1. Complete blank areas of the pathologist's instructional memo and forward it, along with the List of Required Material, to the appropriate pathologist.
2. The pathologist should return the required pathology samples and surgical pathology reports. If any other reports are required, they should be obtained from the appropriate department at this time.
3. Double-check that ALL required forms, reports and pathology samples are included in the package to the Central Biorepository and Pathology Facility. (See appropriate List of Required Material.)
   Pathology specimens submitted WILL NOT be processed by the Central Biorepository and Pathology Facility until all necessary items are received.
4. Mail pathology materials to:

   ECOG-ACRIN Central Biorepository and Pathology Facility
   MD Anderson Cancer Center
   Department of Pathology, Unit 085
   Tissue Qualification Laboratory for ECOG-ACRIN, Room G1.3586
   1515 Holcombe Blvd
   Houston, TX 77030

If you have any questions concerning the above instructions or if you anticipate any problems in meeting the pathology material submission deadline of one month, contact the Pathology Coordinator at the ECOG-ACRIN Central Biorepository and Pathology Facility by telephone 1-844-744-2420or by email at eacbpf@mdanderson.org .

List of Required Material

E1609: A Phase III Randomized Study of Adjuvant Ipilimumab Anti-CTLA4 Therapy Versus High-Dose Interferon α-2b for Resected High-Risk Melanoma

Baseline (submit within one month of patient randomization)

1. STS shipping manifest or ECOG-ACRIN Generic Specimen Submission Form (#2981)
2. Institutional pathology report (**must be included with EVERY pathology submission**).
3. Pathology Materials:
   - Primary Melanoma (for patients with known primary cutaneous melanoma):
     - One (1) H & E of the primary melanoma and fifteen (15) unstained slides preferably from the thickest portion of the tumor for immunostains (please do not deparaffinise slides) OR, if the primary pathologist is willing, please request the corresponding block, which will be promptly returned upon request. If the patient has more than one primary lesion, please include above slides and/or block for each primary.
   - Lymphadenectomy Specimen:
     - One (1) H & E section of the tumor bearing lymph nodes ONLY, with a copy of pathology report. An alternative is to send two (2) unstained slides or two (2) H & E slides on each positive lymph node. Pathology report should indicate:

Rev. 2/12

       a. Total number of lymph nodes
       b. Number of lymph nodes positive
       c. Size of the largest lymph node
       d. Whether there was gross soft tissue tumor involvement
       e. Whether there was evidence of extra-capsular spread
       f. Whether a lymph node was a sentinel lymph node

     (Please include report on immunostains, if any).
   - Resected in-transit or satellite metastases, or cutaneous metastases, or lymph node metastases or lung metastases:
     - One (1) H & E section of the metastatic lesion and fifteen to twenty (15-20) unstained slides from the thickest part of the tumor OR, if the primary pathologist is willing, please request the corresponding block, which will be promptly returned upon request.

NOTE: Sections of surgical margins of skin, unless positive for tumor, are not required. A surgical pathology report is sufficient.

NOTE: A copy of the completed submission form will be sent to the ECOG-ACRIN Operations Office - Boston by the Central Biorepository and Pathology Facility.

NOTE: Submission of pathology samples for diagnostic review is mandatory in order for the patient to be considered evaluable.

Progression/Relapse (submit within one month of disease progression)

1. STS shipping manifest or ECOG-ACRIN Generic Specimen Submission Form (#2981)

2. Institutional pathology report (must be included with EVERY pathology submission)
   If biopsy was performed submit:

   - One (1) H & E section of the metastatic lesion and fifteen to twenty (15-20) unstained slides from the thickest part of the tumor or, if the primary pathologist is willing, please request the corresponding block, which will be promptly returned upon request.

MEMORANDUM

TO: _____
(Submitting Pathologist)

FROM: Stanley Hamilton, M.D., Chair
ECOG-ACRIN Laboratory Science and Pathology Committee

DATE: _____

SUBJECT: *Submission of Pathology Materials for E1609: A Phase III Randomized Study of Adjuvant Ipilimumab ANTI-CTLA4 Therapy Versus High-Dose Interferon α-2b for Resected High-Risk Melanoma*

A patient named on the attached request has been entered onto an ECOG-ACRIN protocol by _____ (ECOG-ACRIN Investigator). This protocol requires the submission of pathology materials for ***pathology review and banking (for research studies in consenting patients and only in accordance with the patient consent).***

Keep a copy for your records and return the completed Submission Form, the surgical pathology report(s), the slides and/or blocks and any other required material (see List of Required Material) to the Clinical Research Associate (CRA). The CRA will forward all required pathology material to the ECOG-ACRIN Central Biorepository and Pathology Facility.

Blocks and/or slides submitted for this study will be retained at the ECOG-ACRIN Central Repository for future studies. Blocks will be returned for purposes of patient management care upon request.

If you have any questions regarding this request, please contact the Central Biorepository and Pathology Facility at 1-844-744-2420 or EMAIL eacbpf@mdanderson.org.

The ECOG-ACRIN CRA at your institution is:

Name:_____

Address: _____

Phone: _____

Thank you.

ECOG-ACRIN Generic Specimen Submission Form

Form No. 2981v3

Institution Instructions: This form is to be completed and submitted with **all specimens** ONLY if the Sample Tracking System (STS) is not available. **Use one form per patient, per time-point.** All specimens shipped to the laboratory must be listed on this form. Enter all dates as MM/DD/YY. Keep a copy for your files. Retroactively log all specimens into STS once the system is available. **Contact the receiving lab to inform them of shipments that will be sent with this form.**

Protocol Number _____ **Patient ID** _____ **Patient Initials** Last _____ First _____

Date Shipped _____ **Courier** _____ **Courier Tracking Number** _____

Shipped To (Laboratory Name) _____ **Date CRA will log into STS** _____

FORMS AND REPORTS: Include all forms and reports as directed per protocol, e.g., pathology, cytogenetics, flow cytometry, patient consult, etc.

Required fields for all samples				**Additional fields for tissue submissions**				**Completed by Receiving Lab**
Protocol Specified Timepoint:								
Sample Type (fluid or fresh tissue, include collection tube type)	**Quantity**	**Collection Date and Time 24 HR**		**Surgical or Sample ID**	**Anatomic Site**	**Disease Status** (e.g., primary, mets, normal)	**Stain or Fixative**	**Lab ID**

Fields to be completed if requested per protocol. Refer to the protocol-specific sample submissions for additional fields that may be required.					
Leukemia/Myeloma Studies:	**Diagnosis**	**Intended Treatment Trial**	**Peripheral WBC Count (x1000)**	**Peripheral Blasts %**	**Lymphocytes %**
Study Drug Information:	**Therapy Drug Name**	**Date Drug Administered**	**Start Time 24 HR**	**Stop Time 24HR**	
Caloric Intake:	**Date of Last Caloric Intake**		**Time of Last Caloric Intake 24HR**		

CRA Name _____ **CRA Phone** _____ **CRA Email** _____

Comments

9/12/14

A Phase III Randomized Study of Adjuvant Ipilimumab Anti-CTLA4 Therapy Versus High Dose Interferon a-2b for Resected High risk Melanoma

Appendix III

Patient Thank You Letter

We ask that the physician use the template contained in this appendix to prepare a letter thanking the patient for enrolling in this trial. The template is intended as a guide and can be downloaded from the ECOG web site at http://www.ecog.org. As this is a personal letter, physicians may elect to further tailor the text to their situation.

This small gesture is a part of a broader program being undertaken by ECOG-ACRIN and the NCI to increase awareness of the importance of clinical trials and improve accrual and follow-through. We appreciate your help in this effort.

[PATIENT NAME] [DATE]

[PATIENT ADDRESS]

Dear [PATIENT SALUTATION],

Rev. 4/14

Thank you for agreeing to take part in this important research study. Many questions remain unanswered in cancer. With the participation of people like you in clinical trials, we will improve treatment and quality of life for those with your type of cancer.

We believe you will receive high quality, complete care. I and my research staff will maintain very close contact with you. This will allow me to provide you with the best care while learning as much as possible to help you and other patients.

On behalf of ***[INSTITUTION]*** and the ECOG-ACRIN Cancer Research Group, we thank you again and look forward to helping you.

Sincerely,

[PHYSICIAN NAME]

A Phase III Randomized Study of Adjuvant Ipilimumab Anti-CTLA4 Therapy Versus High Dose Interferon a-2b for Resected High risk Melanoma

Appendix IV

Patient Interferon Diary (for patients who self-administer Interferon)

Diary Card

Interferon

Dates: ____/____/____ to ____/____/____

Patient Number: _____

Patient Initials:_____

ECOG-ACRIN patient sequence number:

Dose No.	Date			TIME Record Time of Dose (Circle AM or PM)	Use the space below to make notes about things you would like to tell the doctor (include any unusual symptoms you experience, other medicine you have taken and anything else you think may be of interest.
	Month	Day	Year		
1				: AM PM	
2				: AM PM	
3				: AM PM	
4				: AM PM	
5				: AM PM	
6				: AM PM	
7				: AM PM	
8				: AM PM	
9				: AM PM	
10				: AM PM	
11				: AM PM	
12				: AM PM	
13				: AM PM	
14				: AM PM	
15				: AM PM	
16				: AM PM	
17				: AM PM	
18				: AM PM	
19				: AM PM	
20				: AM PM	
21				: AM PM	
22				: AM PM	
23				: AM PM	
24				: AM PM	
25				: AM PM	
26				: AM PM	
27				: AM PM	
28				: AM PM	
29				: AM PM	
30				: AM PM	

A Phase III Randomized Study of Adjuvant Ipilimumab Anti-CTLA4 Therapy Versus High Dose Interferon a-2b for Resected High risk Melanoma

Appendix V

E1609 Cooperative Research and Development Agreement (CRADA)

The ipilimumab supplied by CTEP, DCTD, NCI used in this protocol is provided to the NCI under a Collaborative Agreement (CRADA, CTA) between Bristol-Myers Squibb (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http://ctep.cancer.gov/industry) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.
2. For a clinical protocol where there is an investigational Agent used in combination with (an)other investigational Agent(s), each the subject of different collaborative agreements , the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data."):
    a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.
    b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own investigational Agent.
    c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational Agent.
3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order. Additionally, all Clinical Data and Results and Raw Data will be collected , used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

Rev. 9/14

Jan M. Casadei, Ph.D.,
Cheif, Regulatory Affairs Branch
Cancer Therapy Evaluation Program
Cancer Therapy Evaluation Program
Division of Cancer Treatment and Diagnosis, NCI
9609 Medical Center Drive
Room 5-W532, MSC 9740
Bethesda, MD 20892-9740 [if US Postal Service]
Rockville, MD 20850 [if non-USPS/private carrier]
Tel 240-276-6125
E-mail: casadeij@mail.nih.gov E-mail: ncicteppubs@mail.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

A Phase III Randomized Study of Adjuvant Ipilimumab Anti-CTLA4 Therapy Versus High Dose Interferon a-2b for Resected High risk Melanoma

Appendix VI

ECOG Performance Status

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

A Phase III Randomized Study of Adjuvant Ipilimumab Anti-CTLA4 Therapy Versus High Dose Interferon a-2b for Resected High risk Melanoma

Appendix VII

Suggested Surgical Considerations and Techniques for Lymphadenectomy

Surgical Considerations - Surgery is to be completed prior to randomization.

Stage IIIB, IIIC (except in-transit or satellite metastasis)

Primary Excision: All patients with initial presentation of melanoma T_{1-4} are preferably treated by wide excision of the primary after the initial biopsy. Biopsy for initial documentation may be performed by punch biopsy, or excisional biopsy, at the discretion of the treating dermatologist, surgeon, or physician.

It is important that definitive surgery will include wide excision of the primary and lymphadenectomy according to the guidelines below.

For patients with known primary cutaneous melanoma lesion and no history of wide local excision of that primary lesion, an adequate wide excision of the primary lesion is recommended. Patients with nodal relapse after an inadequate primary excision should undergo wide excision at the time of complete lymphadenectomy. The recommendation for adequate wide excision is the same for patients enrolled at the time of lymph node recurrence as for those enrolled at the time of initial treatment of the primary.

Wide excision with a minimum 1 cm margin surrounding the primary lesion or biopsy scar is important for local surgical control. For lesions whose Breslow's thickness is > 1 mm, a 2 cm minimum margin is preferred when anatomically feasible (i.e., for lesions of the trunk and proximal extremity). For subungual melanoma, a distal interphalangeal amputation with histologically negative margins constitutes an adequate wide excision.

The specimen should be excised to include skin and all subcutaneous tissue down to the muscular fascia. Fascia may be included at the discretion of the operating surgeon. Closure of the defect may be via primary closure, split thickness skin graft, or flap rotation flap at the discretion of the surgeon.

Rev. 8/12

Regional Lymphadenectomy: Note: For patients undergoing sentinel node mapping and lymphoscintigraphic and dye lymphographic identification of regional nodal drainage, the minimum number of nodes may be less than the mandatory minimum numbers of 5/groin, 10/axilla, and 15/cervical node dissection. Patients with clinically positive nodes in the groin, axilla, or neck should have full lymphadenectomy in those sites.

Staging lymphadenectomies:

Head and Neck Lesions

a. Face, ear, and anterior scalp Modified radical neck or radical neck dissection. Parotidectomy to be included if lymphoscintigram indicates flow to the area (to yield a minimum of 15 nodes).
b. Submandibular and anterior neck: Modified radical or radical neck dissection (to yield a minimum of 15 nodes).
c. Posterior scalp, posterior neck and uppermost trunk (areas that drain to posterior cervical triangle): Modified posterior triangle neck dissection with suboccipital nodes (to yield a minimum of 8 nodes).

Upper Extremity (to yield a minimum of 10 nodes)

Axillary node dissection to include at least 10 nodes taken from levels I, and II. Level III nodes should be dissected if they are clinically involved. The pectoralis minor muscle may be divided or sacrificed at the surgeon's discretion.

Lower Extremity (to yield a minimum of 5 nodes)

Superficial inguinal node dissection to be performed. A deep inguinal node dissection will be at the discretion of the surgeon, but if performed, the established guidelines should be followed as closely as possible.

Lymphadenectomy for Nodal Recurrence: Regional node recurrences to be treated using the appropriate lymphadenectomy as described above. Whenever possible, diagnosis of regional node recurrence to be made using fine needle aspiration technique to avoid contaminating the region with tumor.

Sentinel Node (SLN) Dissection and Nodal Staging

Patients with recurrent nodal disease (grossly palpable) or a positive SLN must undergo a complete node dissection to be eligible for the trial.

Patients should have pre-operative lymphoscintigraphy prior to the nodal staging procedure. If drainage is to 2 or fewer basins, all basins need to be dissected or sampled with SLN biopsy techniques.

The SLN to be examined by making at least 5 sections of the node and staining with H&E and S-100 immunohistochemical stain. A 2 mm^2 sample may be submitted to a central laboratory after snap freezing for mRNA isolation and RT-PCR analysis.

At a minimum, the SLN will be examined with step sections and immunohistochemical staining with S-100. In those institutions who do not have mapping capability, 3 lymph nodes from each level of the dissection can be examined with S-100 immunohistochemistry.

Stage IV: M1a, M1b

All patients must have disease that is completely surgically resected in order to be eligible. Patients must have been surgically rendered free of disease with negative margins on resected specimen.

In-transit or satellite metastasis

All patients must have disease that is completely surgically resected in order to be eligible. Patients must have been surgically rendered free of disease with negative margins on resected specimen.

Consideration should be given to performing lymphatic mapping and a SLN biopsy in patients with small volume in transit disease. The identification of regional lymph node involvement should be followed by regional lymphadenectomy. While there is no consensus about the utilization of SLN biopsy in this setting, the technique is similar to that described for patients with primary melanoma. SLN biopsy is best reserved for individuals who have only one to two lesions that are close together and where there is a clearly defined optimal site to inject the radionucleotide or dye for SLN localization. This site should be next to the in transit disease.

Surgical Techniques

1. Axillary Lymphadenectomy: Complete axillary lymph node dissection will be performed including nodes at levels, I, and II. Level III nodes should be dissected if they are clinically involved. The boundaries of the dissection should include the axillary vein superiorly beginning at the thoracic outlet and coursing to the latissimus dorsi tendon. The lateral border of the dissection is the anterior edge of the latissimus dorsi muscle. The posterior boundary is the subscapular muscle. The anterior border of the resection is the pectoralis major group. The inferior boundary of the dissection should be the juncture of the latissimus dorsi and the serratus anterior muscles.

   The contents within these boundaries should be completely removed with the exception of the long thoracic nerve and the thorocodorsal nerve which should be identified during the dissection and preserved throughout. As stated, the pectoralis minor muscle may be divided or sacrificed with the specimen at the discretion of the surgeon. Care should be exercised that in the superior part of the dissection, the medial pectoral nerve is not injured. The preferable approach to the axilla is through a horizontal incision in the line of the skin crease, 3 or 4 cm below the apex of the skin fold of the axilla.
2. Inguinal Lymphadenectomy: A superficial femoral node dissection should be performed by excising all of the nodes inferior to the inguinal ligament and bounded by the medial border of the Sartorius muscle in the lateral border of the adductor magnus muscle. The fatty and lymphatic tissues should be dissected carefully off of the femoral vessels and nerves all the way up to the inguinal canal and for 3 cm superior to the inguinal ligament. Care should be exercised and the obdurator nerve should be spared. Ideally, this area should be entered through a curvilinear incision starting laterally over the inguinal ligament and curving medially and inferiorly ending over the mid-point of the adductor magnus muscle.
3. Deep Inguinal and External Iliac Node Dissection: This can be most easily approached by incising the abdominal wall musculature 3 or 4 cm superior to the inguinal ligament. This incision is taken down through the external oblique, internal oblique and transversus muscles and the surgeon at that point stays extraperitoneally as in the approach to the iliac vessels for renal transplantation. With this approach, the external, internal and common iliac arteries are exposed and the lymphatics coursing among the iliac vessels are excised.
4. Radical Neck Dissection: Classic or modified radical neck dissection may be performed for patients with melanoma of the head and neck. As noted above, patients with melanoma located on the ear and anterior scalp and face will require superficial parotidectomy along with a radical neck procedure. The boundaries of the radical neck dissection are inferiorly the clavicle; the mandible, the mastoid and the tail of the parotid gland superiorly; the anterior border of the trapezius muscle posteriorly; and the strap muscle of the larynx anteriorly. The sternocleidomastoid muscle may be sacrificed or preserved at the surgeon's discretion. For posterior lesions the radical neck incision must be extended posteriorly or a second incision must be make so that the sub-occipital nodal group can be sampled.

A Phase III Randomized Study of Adjuvant Ipilimumab Anti-CTLA4 Therapy Versus High Dose Interferon a-2b for Resected High risk Melanoma

Appendix VIII

Shipping Kit Request Facsimile Form

ECOG-ACRIN – PROTOCOL E1609

Rev. 2/12

Immunologic Monitoring and Cellular Products Laboratory	UPCI Research Pavilion at the Hillman Cancer Center Room L 1.26 5117 Centre Avenue Pittsburgh, PA 15213-1863 Telephone: 412-624-0078 FAX: 412-623-6625

To: ECOG-ACRIN Study Coordinator Fax: 412-623-6625

From: Name:_____

Institution:_____

Telephone: _____

Fax: _____

Number of Kits Requested: _____

Shipping Address:

_____

_____

_____

_____

_____

PLEASE ALLOW 10 WORKING DAYS FOR RECEIPT OF SHIPPING KITS

Rev. 2/12 **NOTE:** To order collection and shipping kits for E1609, patients must be registered to or in the process of being worked up for the E1609 trial. Due to funding

restrictions institutions cannot order multiple collection and shipping kits in advance.

A Phase III Randomized Study of Adjuvant Ipilimumab Anti-CTLA4 Therapy Versus High Dose Interferon a-2b for Resected High risk Melanoma

Appendix IX

Specimen Shipment Requisition Form

ECOG-ACRIN – PROTOCOL E1609

It is required that samples submitted from patients participating in E1609 be entered and tracked via the online ECOG-ACRIN Sample Tracking System (see Section 10.4). This form is used only in the event that the STS is inaccessible and then the shipments are to be logged in retroactively, indicating the actual dates of collection and shipment.

Rev. 2/12, 9/14

Immunologic Monitoring and Cellular Products Laboratory	UPCI Research Pavilion at the Hillman Cancer Center Room L 1.26 5117 Centre Avenue Pittsburgh, PA 15213-1863 Telephone: 412-624-0078 FAX: 412-623-6625

Ship specimens by FedEx Priority Overnight express to arrive the next morning unless otherwise directed by the protocol. Do NOT ship on Friday or Saturday, or the day before a legal holiday.

Rev. 2/12, 9/14 Call the IMCPL ECOG-ACRIN Study Coordinator at 412-624-0078 with questions on collection and shipping.

Please complete the following information and include this form in the shipment.

ECOG Patient Sequence Number: _____ ECOG Patient Initials: _____
Last First

Clinical Site:_____ Site Contact: _____

Telephone Number: _____ Fax Number: _____

Federal Express® Air Bill No.: _____ Date of Shipment: _____

Specimen
Collection Date __ __/__ __/__ __
mm dd yy

Specimen
Collection Time: ____ ____:____ ____
(24 hour clock)

Time Points (check one):

☐	Baseline	Three (3) red top tubes, Ten (10) green top tubes, One (1) PAXgene RNA tube and One (1) yellow top tube
☐	3 weeks (Arms A/D, C/F) 4 weeks (Arm B, E)	Three (3) red top tubes, Ten (10) green top tubes, one (1) PAXgene RNA tube
☐	12 weeks	Three (3) red top tubes, Ten (10) green top tubes, One (1) PAXgene RNA tube
☐	48 weeks	Three (3) red top tubes and Ten (10) green top tubes

Rev. 8/12, 9/14

Rev. 8/12 [Deleted in Addendum #5]

☐	Relapse	Three (3) red top tubes and Ten (10) green top tubes

Shipping checklist: (kits will be shipped / delivered to you from UPCI IMCPL upon request)

____ Label vials with patient initials/sequence number, and date and time of draw.

Rev. 2/12 ____ [Deleted in Addendum #4]

____ Seal, wrap, and place specimen tubes in specimen shipper kit

Rev. 2/12 ___ STS Shipping Manifest Form. Make a copy for your records and place the original form inside the specimen shipper kit.

Rev. 2/12 ___ [Deleted in Addendum #4]

To be completed by IML Staff:	IML Study Number
IML Accession Number:	Specimen Type received (if different from above):
Specimen Acceptability:	Comment:

A Phase III Randomized Study of Adjuvant Ipilimumab Anti-CTLA4 Therapy Versus High Dose Interferon a-2b for Resected High risk Melanoma

Appendix X

Administration of Interferons (Directions for Administration of Subcutaneous Doses)

Patients deemed competent to self administer the Interferon may do so. The hospital or clinic staff will instruct the patient or interested family member in this technique. Patients should be allowed to administer these injections at home when they can independently perform a return demonstration for their instructor. The instructor will note this fact in the patient's record. Adequate contact persons and telephone numbers should be provided so that the patient will always be able to reach someone familiar with this procedure should they need assistance.

For emergency contact: _____

(insert site contact information)

Instructions for Self-Administering Medication

1. **Preparation**
   a. Wash hands well. Take required dose of acetaminophen (Tylenol®).
   b. Assemble necessary supplies. The Interferon should be kept refrigerated until several minutes before each treatment. You also need a syringe with a needle, at least 3 alcohol prep pads, and a container (an unbreakable, leak-proof, reclosable container - milk carton, coffee can) for used materials.
2. **Reconstituting the Interferon Powder**
   a. If this is the first dose that will be coming from a vial, the Interferon powder must be dissolved, using the diluent provided. Snap the plastic cap off both vials, and cleanse both rubber stoppers with an alcohol pad and allow to air dry.
   b. There may be a different syringe/needle provided that you will use to reconstitute the Interferon. Open the package containing one of these syringes and attach (or tighten) the appropriate needle to it. Pull back the plunger of the syringe so that the top of it rests right on the line representing the volume of diluent that you are to add to the Interferon powder.
   c. Insert the needle through the stopper of the diluent vial, and invert the vial/syringe in front of you at eye level, holding the syringe in your dominant hand and the vial in the other.
   d. Inject the air from the syringe into the vial slowly. If you feel like you are forcing it, pull back the plunger to allow some solution into the syringe, then push the remaining air into the vial. Ultimately, your syringe should be filled with diluent solution up to the correct line and no air will be left in the syringe. If you have bubbles, tap the syringe with your finger until they rise to the top, push them up into the vial and recheck the plunger to insure that it is still at the correct volume mark.
   e. Withdraw the needle from the diluent vial and insert it into the vial containing the Interferon powder. This time keep the vial on the surface and push the plunger down to

inject the diluent into the powder vial. If you meet resistance, allow some air to rise into the syringe before pushing down and expelling the remaining solution into the vial. Eventually, all the solution will be in the vial. Pull back the plunger to return it to the line that is the same as the volume of solution that you injected. This will prevent pressure build-up in the Interferon vial. Remove the needle and discard it appropriately.

f. To help dissolve the Interferon powder, you may need to roll the vial between your palms or swirl the solution around. **DO NOT shake the vial.** Be sure that all the powder is dissolved before proceeding to #3.

3. **Withdrawing Your Dose From the Vial**

a. Cleanse the rubber stopper of the vial containing the Interferon solution with an alcohol pad and allow to air dry.

b. Open syringe package and needle package (if separate) and attach or tighten needle by twisting until tight. Pull back the plunger to the mark that represents your dose (i.e., 3 mU/0.5 ml, top of plunger should rest at the 0.5 ml mark). This fills the syringe with air in a volume equal to the volume of your dose.

c. Uncap the needle and push it through the stopper, at least half-way into the vial. Now pick up the vial (with syringe/needle in it) with your left hand and turn it upside down, holding it at eye-level, about 12 inches from your face. You should now have the vial in one hand and your other hand free to manipulate the syringe. (Note: Left-handed persons should have the vial in their right hand, so that they can manipulate the syringe with their left hand.)

d. Inject air from the syringe into the vial slowly, and then withdraw the plunger. The syringe will gradually fill with drug solution. Repeat this procedure until only solution is in the syringe, solidly, to the mark that indicates your dose. Withdraw needle and recap it.

4. **Administration**

a. Thoroughly clean the area to be injected with an alcohol pad. Areas appropriate for this type of injection have been shown to you. A new site should be used for each injection whenever possible.

b. As demonstrated, pinch 1½ to 2 inches of loose skin from the site to be injected.

c. Uncap the needle, and insert the needle approximately ¼ inch into the skin and push the syringe plunger in all the way, thereby giving the dose of Interferon.

d. Remove needle and wipe injection site with a new alcohol pad, but do not massage the area to any great extent.

e. Needles are not to be recapped. Used needles and used syringes should be disposed of in the proper disposal container provided by your pharmacy and returned to the clinic pharmacy for disposal. If the Interferon vial contains more than one dose, write the date on the label. It is usable for 30 days. INTRON A injection vials should be stored in the refrigerator between 2° and 8° C (36° and 46°F), not in the freezer.

f. If a drug administration diary has been provided, remember to complete it after each dose. Enter the date and time of day given, along with any notable side effects that you may have experienced since previous dose was given. Also record vial strength and dose volume at each entry.

A Phase III Randomized Study of Adjuvant Ipilimumab Anti-CTLA4 Therapy Versus High Dose Interferon a-2b for Resected High risk Melanoma

Appendix XI

TNM Staging for Melanoma

Table 1. TNM Staging Categories for Cutaneous Melanoma

Classification	Thickness (mm)	Ulceration Status/Mitoses
T		
Tis	NA	NA
T1	≤ 1.00	a: Without ulceration and mitosis < 1/mm^2 b: With ulceration or mitoses ≥ 1/mm^2
T2	1.01-2.00	a. without ulceration b. with ulceration
T3	2.01-4.00	a: without ulceration b: with ulceration
T4	> 4.00	a. without ulceration b: with ulceration
N		
	No. of Metastatic Nodes	Nodal Metastatic Burden
N0	0	NA
N1	1	a: Micrometatasis* b: Macrometastasis†
N2	2-3	a: Micrometatasis* b: Macrometastasis† c: In transit metastases/satellites without metastatic nodes
N3	4+ metastatic nodes, or matted nodes, or in transit metastases/ Satellites with metastatic nodes	
M	Site	Serum LDH
M0	No distant metastases	NA
M1a	Distant skin, subcutaneous, or nodal metastases	Normal
M1b	Lung metastases	Normal
M1c	All other visceral metastases	Normal
	Any distant metastasis	Elevated

Abbreviations: NA, not applicable; LDH, lactate dehydrogenase,
*Micrometastases are diagnosed after sentinel lymph node biopsy.
†Macrometastases are defined as clinically detectable nodal metastases confirmed pathologically.

Table 2. Anatomic Stage Groupings for Cutaneous Melanoma

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

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

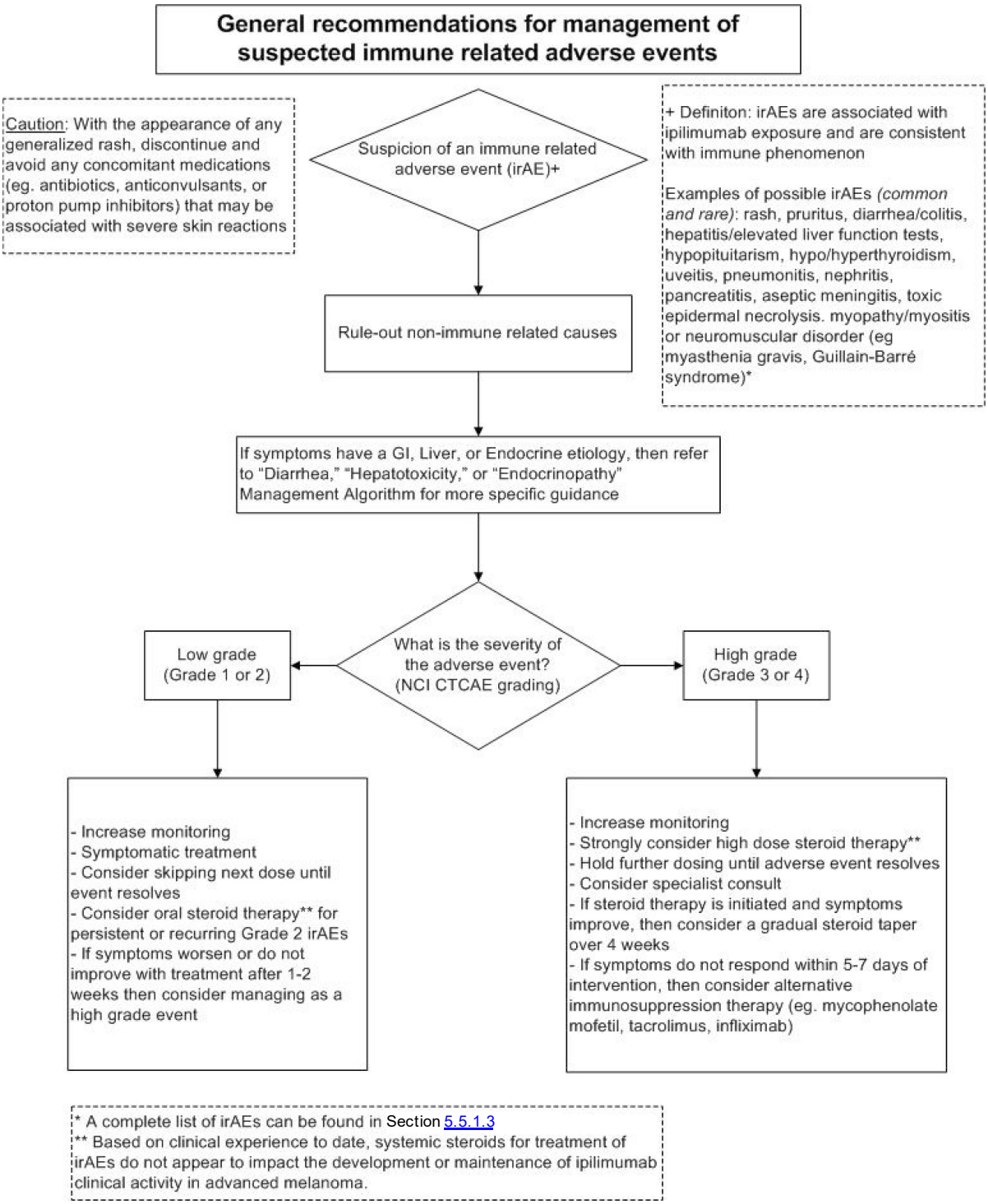
†Pathologic staging includes microstaging of the primary melanoma and pathologic information about the regional lymph nodes after partial (ie, sentinel node biopsy) or complete lymphadenectomy. Pathologic stage 0 or stage IA patients are the exception; they do not require pathologic evaluation of their lymph nodes.

Reference: Balch CM,et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol. 2009 Dec 20;27(36):6199-206.

A Phase III Randomized Study of Adjuvant Ipilimumab Anti-CTLA4 Therapy Versus High Dose Interferon a-2b for Resected High risk Melanoma

Appendix XII

General Recommendations for immune-related Adverse Events (IrAEs)



Rev. 2/14

A Phase III Randomized Study of Adjuvant Ipilimumab Anti-CTLA4 Therapy Versus High Dose Interferon a-2b for Resected High risk Melanoma

Appendix XIII

Suggested Work-up and Treatment for Immune-Related Adverse Events (irAEs)

An IRAE is defined as an adverse event of unknown etiology, associated with drug exposure and is consistent with an immune phenomenon. Efforts should be made to rule out neoplastic, infectious, metabolic, toxin or other etiologic causes prior to labeling an adverse event a non-dermatologic, immune-mediated event. Serological, immunological, and histological (biopsy) data should be used to support the diagnosis of an immune-mediated toxicity. Documentation of test results should be included in the patient's medical record. Effective Amendment #7, the dose delay and discontinuation criteria have been made stricter on Arms A or D (ipilimumab 10 mg/kg) than on Arms C or F (ipilimumab 3 mg/kg) due to a higher incidence of severe toxicities observed on Arms A and D compared to Arms C and F.

Rev. 11/13
9/14

Gastrointestinal (diarrhea) and skin (rash)-related toxicities have been the most common IRAEs noted in prior studies with ipilimumab. Suggested work-up procedures for suspected IRAEs of the gastrointestinal tract, liver, skin, eye, pituitary, and adrenal gland are listed below. When symptomatic therapy is inadequate or inappropriate, an IRAE should be treated with steroids followed by a slow taper.

Gastrointestinal Tract: Diarrhea (defined as either first watery stool, or increase in frequency 50% above baseline with urgency or nocturnal bowel movement, or bloody stool) should be further evaluated and infectious or alternate etiologies ruled out. Patients should be advised to inform the investigator if any diarrhea occurs, even if it is mild. An algorithm for working up patients with diarrhea or suspected colitis is provided in Appendix XIV.

If the event is of significant duration or magnitude or is associated with signs of systemic inflammation or acute phase reactants (e.g., increased CRP or platelet count; or bandemia), it is recommended that sigmoidoscopy (or colonoscopy, if appropriate) with colonic biopsy with 3 to 5 specimens for standard paraffin block be performed. If possible, 1 to 2 biopsy specimens should be snap frozen and stored. All patients with confirmed colitis should also have an opthomological examination, including a slit-lamp exam, to rule out uveitis. Tests should also be performed for WBCs and for stool calprotectin.

Patients with colitis should discontinue any non-steroidal anti-inflammatory medications or any other medications known to exacerbate colitis symptoms. Investigators should use their clinical judgment as to whether corticosteroids are necessary to treat colitis associated with ipilimumab therapy and as to what dose should be used. As guidance prior experience suggests that colitis manifested as ≥ Grade 3 diarrhea requires corticosteroid treatment and possibly other immunosuppressants as noted in the E1609 study protocol (Sections 5.5.1.3, 5.5.1.5) and Appendix XIV.

Rev. 11/13

Liver: Elevation of LFTs from baseline should instigate an investigation into the underlying etiology for suspected IRAEs, in accordance with Sections 5.5.1.3, 5.5.1.5 and Appendix XV. Neoplastic, concurrent medications, viral hepatitis, and toxic etiologies should be considered and addressed, as appropriate. Imaging of the liver, gall bladder, and bile duct should be performed to rule out neoplastic or other causes for the increased LFTs. An ANA, pANCA, and anti-smooth muscle antibody test should be performed if an autoimmune etiology is considered. Consultation with a hepatologist is appropriate for a suspected liver IRAE and a biopsy should be considered.

Rev. 11/13

Patients presenting with right upper-quadrant abdominal pain and/or unexplained nausea or vomiting should have LFTs performed immediately and reviewed before administering the next dose of study drug.

Pancreas: Symptoms of abdominal pain associated with elevations of amylase and lipase, suggestive of pancreatitis, may be associated with anti-CTLA-4 monoclonal antibody administration. The differential diagnosis of acute abdominal pain should include pancreatitis. Appropriate workup should include serum amylase and lipase tests.

Rev. 11/13

Skin: A dermatologist should evaluate persistent or severe rash or pruritus. A biopsy should be performed if appropriate and if possible, photos of the rash should also be obtained. Any non protocol drugs that could contribute to a drug reaction should be stopped if possible pending evaluation. Patients with low-grade ipilimumab-mediated skin toxicity (Grade 1 or 2) may remain on therapy and could be treated with symptomatic therapy (e.g., antihistamines). Low-grade symptoms persisting for 1 to 2 weeks and relapsing should be treated with topical or moderate dose oral corticosteroid therapy (e.g., prednisone 1 mg/kg once daily or equivalent). High-grade (Grade 3 or 4) symptoms require high-dose IV corticosteroid therapy (e.g., methylprednisolone 2 mg/kg once or twice per day or equivalent) to control initial symptoms. A skin biopsy should be performed if appropriate. Once rash or pruritis is controlled, the initiation of corticosteroid taper should be based on clinical judgment; however, the corticosteroid dose should be gradually tapered over a period of at least 1 month.

Patients with any high-grade skin related toxicity (Grade 3 regardless of causality) have to skip ipilimumab and may only continue treatment with ipilimumab if the initial symptoms have improved to ≤ Grade 1, while patients with grade 4 skin toxicities have to permanently discontinue ipilimumab. Please see Sections 5.5.1.3, 5.5.1.5 and Appendix XVIII.

Rev. 11/13

Eye: An ophthalmologist should evaluate visual complaints with examination of the conjunctiva, anterior and posterior chambers and retina; visual field testing and an electroretinogram should also be performed. Patients with ipilimumab related uveitis or episcleritis have been treated with topical corticosteroid eye drops.

Endocrine: Subjects with unexplained symptoms such as fatigue, myalgias, impotence, mental status changes, or constipation should be investigated for the presence of thyroid, pituitary or adrenal endocrinopathies. An endocrinologist should be consulted if an endocrinopathy is suspected. If there are any signs of adrenal crisis such as severe dehydration, hypotension, or shock, intravenous corticosteroids with mineralocorticoid activity (e.g., methylprednisolone) should be initiated immediately. If the patient's symptoms are suggestive of an endocrinopathy but the patient is not in adrenal crisis, endocrine laboratory results should be evaluated before corticosteroid therapy is initiated.

Endocrine work up should include at least Thyroid stimulating hormone and free T4 levels to determine if thyroid abnormalities are present. TSH, prolactin and a morning cortisol level will help to differentiate primary adrenal insufficiency from primary pituitary insufficiency. Radiographic imaging (e.g., MRI) with pituitary cuts should be performed. If the pituitary scan and/or endocrine laboratory tests are abnormal suggestive of pituitary endocrinopathy, a short course of high dose corticosteroids (e.g., dexamethasone 4 mg every 6 hours or equivalent) should be strongly considered in an attempt to treat the presumed pituitary inflammation, but it is currently unknown if this will reverse the pituitary dysfunction. Abrupt discontinuation of corticosteroids should be avoided due to possible prolonged adrenal suppression. Once symptoms or laboratory abnormalities are controlled, and overall patient improvement is evident, the initiation of steroid taper should be based on clinical judgment; however the corticosteroid dose should be gradually tapered over a period of at least 1 month. Appropriate

hormone replacement therapy should be instituted if an endocrinopathy is documented, and it is possible that subjects may require life-long hormone replacement.

Patients diagnosed with hypophysitis should be permanently discontinued from additional ipilimumab therapy. Please see Sections 5.5.1.3, 5.5.1.5 and Appendix XVI.

Rev. 11/13

Suspected irAEs should be documented in the patient's medical record.

A Phase III Randomized Study of Adjuvant Ipilimumab Anti-CTLA4 Therapy Versus High Dose Interferon a-2b for Resected High risk Melanoma

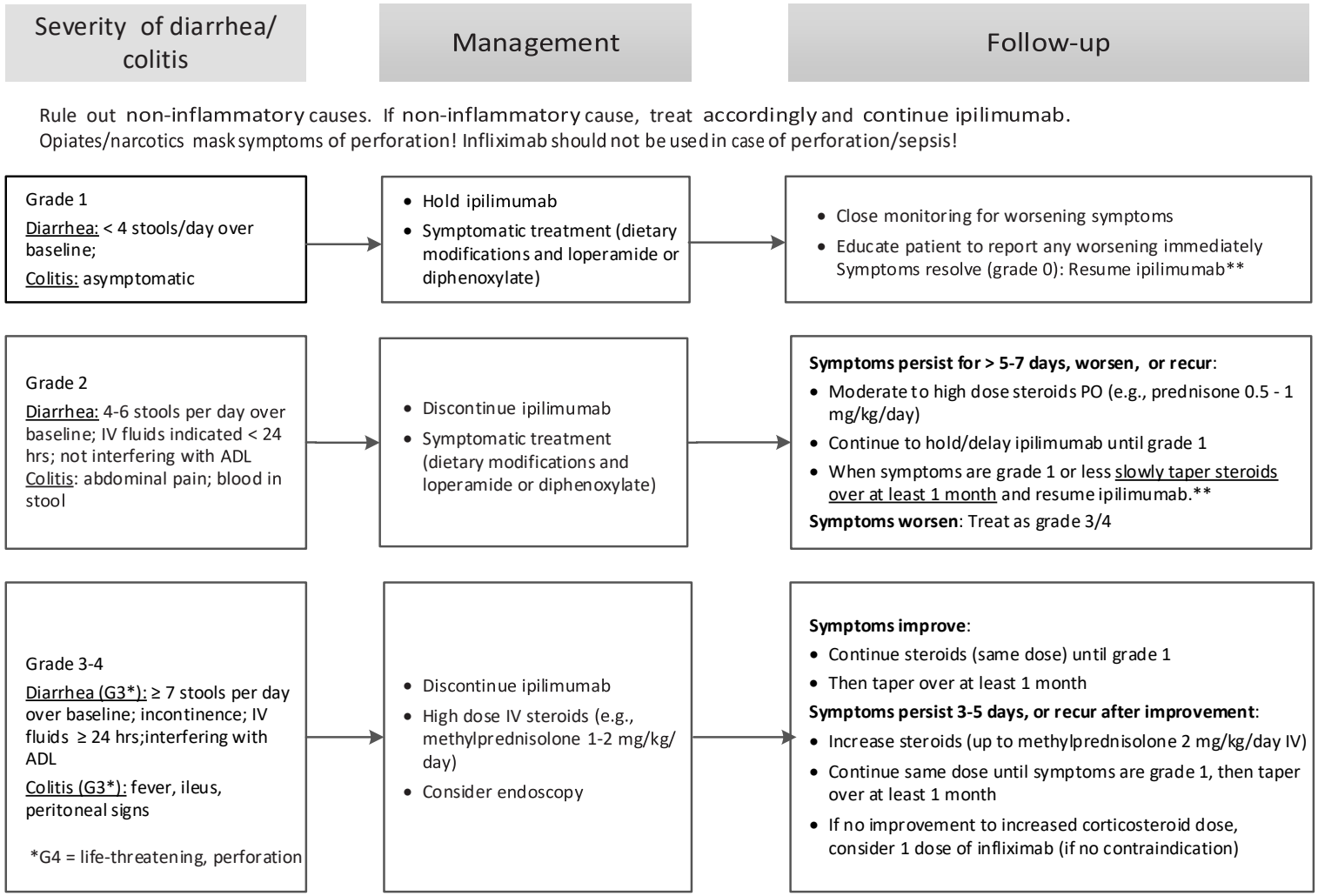
Rev. 9/14

Appendix XIV
GI Management Algorithms

PLEASE NOTE: THERE ARE TWO (2) SEPARATE E1609 GI MANAGEMENT ALGORITHMS. ONE IS TO BE USED FOR ARMS A AND D PATIENTS ONLY, AND THE OTHER FOR ARMS C AND F PATIENTS ONLY. PLEASE REFERENCE THE CORRECT VERSION OF THE ALGORITHM BASED ON THE ARM DESIGNATION OF THE PATIENT. THE ALGORITHMS APPEAR ON THE FOLLOWING 2 PAGES.

Rev. 2/13, 11/13

E1609 (Arms A and D) GI Management Algorithm



** Patients on Arm A and Arm D with Grade 1 diarrhea/colitis must have ipilimumab held until resolution to Grade 0 (baseline) before resuming dosing with ipilimumab. Patients on Arm A and Arm D with Grade 2 diarrhea/colitis must have ipilimumab permanently discontinued.

Patients on IV steroids may be switched to oral corticosteroid (e.g., prednisone) at an equivalent dose at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of PO corticosteroids. Ipilimumab may not be restarted while the patient is being treated with systemic corticosteroids except for patients on stable doses of hormone replacement therapy such as hydrocortisone.

Rev. 11/13

E1609 (Arms C and F) GI Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue ipilimumab.
Opiates/narcotics mask symptoms of perforation! Infliximab should not be used in case of perforation/sepsis!

Severity of diarrhea/ colitis	Management	Follow-up
Grade 1 Diarrhea: < 4 stools/day over baseline; Colitis: asymptomatic	• Continue ipilimumab • Symptomatic treatment (dietary modifications and loperamide or diphenoxylate)	• Close monitoring for worsening symptoms • Educate patient to report any worsening immediately
Grade 2 Diarrhea: 4-6 stools per day over baseline; IV fluids indicated < 24 hrs; not interfering with ADL Colitis: abdominal pain; blood in stool	• Withhold/delay ipilimumab • Symptomatic treatment (dietary modifications and loperamide or diphenoxylate)	**Symptoms improve/resolve** (grade 0/1): Resume ipilimumab** **Symptoms persist for > 5-7 days, worsen, or recur**: • Moderate to high dose steroids PO (e.g., prednisone 0.5 - 1 mg/kg/day) • Continue to hold/delay ipilimumab until grade 1 • When symptoms are grade 1 or less slowly taper steroids over at least 1 month and resume ipilimumab.** **Symptoms worsen**: Treat as grade 3/4
Grade 3-4 Diarrhea (G3*): ≥ 7 stools per day over baseline; incontinence; IV fluids ≥ 24 hrs;interfering with ADL Colitis (G3*): fever, ileus, peritoneal signs *G4 = life-threatening, perforation	• Permanently discontinue ipilimumab • High dose IV steroids (e.g., methylprednisolone 1-2 mg/kg/day) • Consider endoscopy	**Symptoms improve**: • Continue steroids (same dose) until grade 1 • Then taper over at least 1 month **Symptoms persist 3-5 days, or recur after improvement**: • Increase steroids (up to methylprednisolone 2 mg/kg/day IV) • Continue same dose until symptoms are grade 1, then taper over at least 1 month • If no improvement to increased corticosteroid dose, consider 1 dose of infliximab (if no contraindication)

** Patients with Grade 2 colitis who require steroid therapy with resolution to ≤ Grade 1 severity must have a follow-up colonoscopy to document endoscopic (with or without pathologic) resolution of inflammation before resuming dosing with ipilimumab.

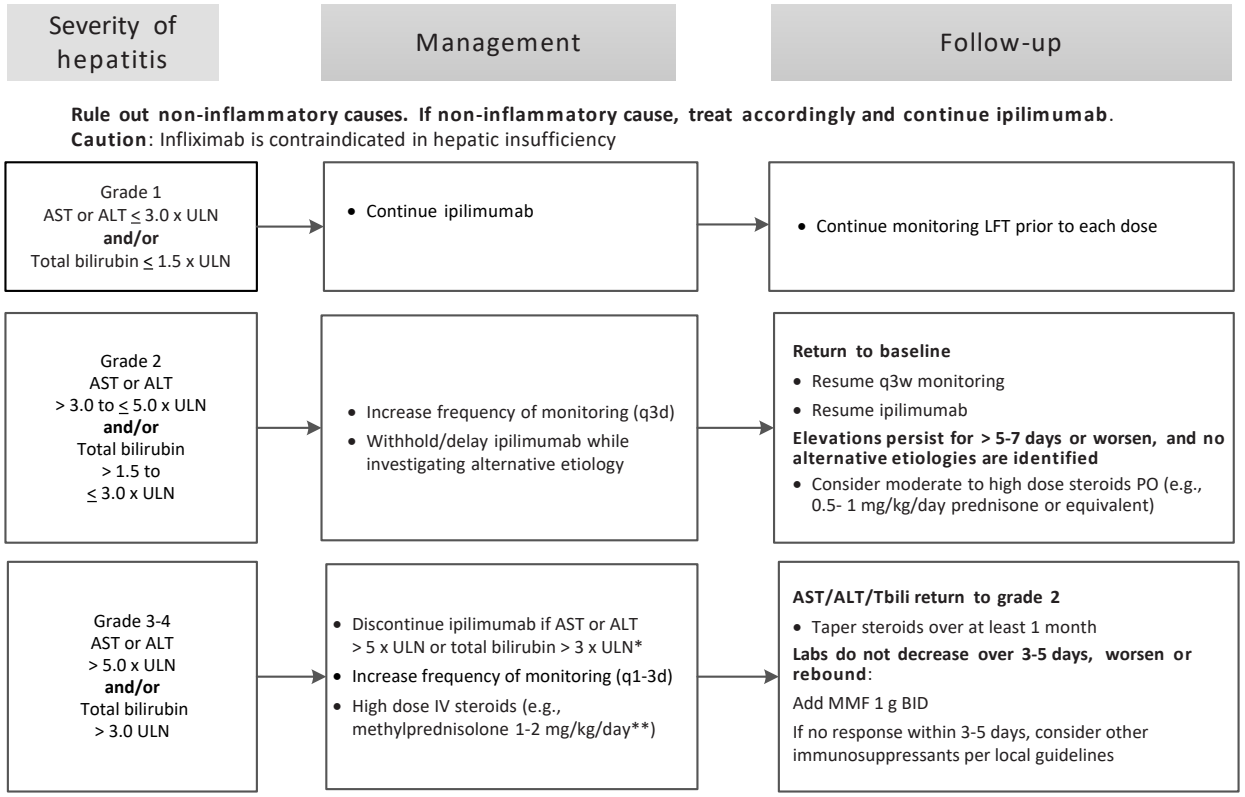
Patients on IV steroids may be switched to oral corticosteroid (e.g., prednisone) at an equivalent dose at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of PO corticosteroids. Ipilimumab may not be restarted while the patient is being treated with systemic corticosteroids except for patients on stable doses of hormone replacement therapy such as hydrocortisone.

A Phase III Randomized Study of Adjuvant Ipilimumab Anti-CTLA4 Therapy Versus High Dose Interferon a-2b for Resected High risk Melanoma

Appendix XV
Hepatotoxicity Management Algorithms

Rev. 2/13, 11/13, 9/14

E1609 (Arms A and D & C and F) Hepatotoxicity Management Algorithm



*Ipilimumab may be held/delayed rather than discontinued if AST/ALT ≤ 5 x ULN and Tbili ≤ 3 x ULN. Resume ipilimumab when AST/ALT/Tbili return to grade 1 and meet protocol specific retreatment criteria.

**The recommended starting dose for grade 4 hepatitis is 2 mg/kg/day methylprednisolone IV.

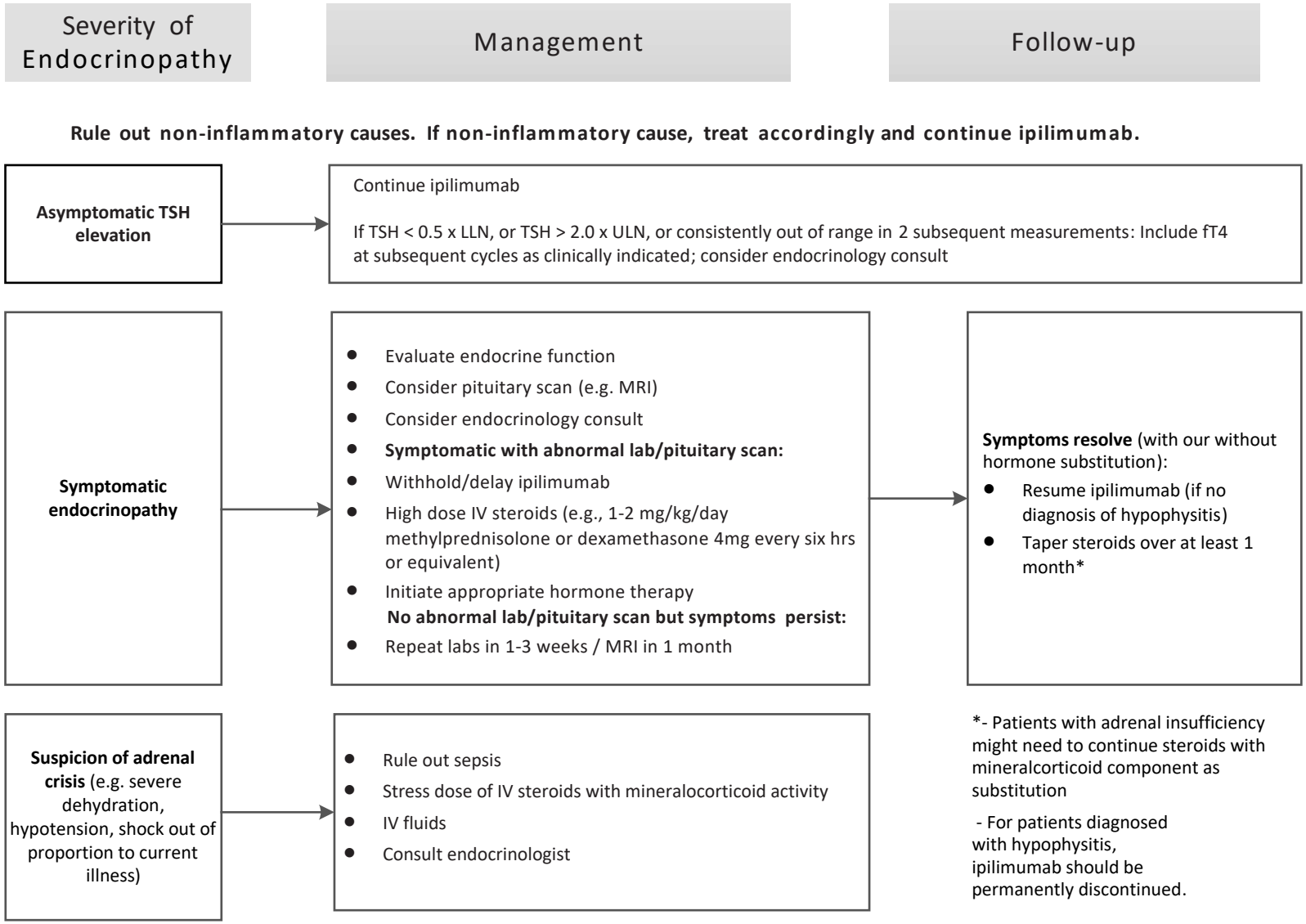
Patients on IV steroids may be switched to oral corticosteroid (e.g., prednisone) at an equivalent dose at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of PO corticosteroids. Ipilimumab may not be restarted while the patient is being treated with systemic corticosteroids except for patients on stable doses of hormone replacement therapy such as hydrocortisone.

A Phase III Randomized Study of Adjuvant Ipilimumab Anti-CTLA4 Therapy Versus High Dose Interferon a-2b for Resected High risk Melanoma

Appendix XVI
Endocrinopathy Management Algorithm

Rev. 2/13, 11/13, 9/14

E1609 (Arms A and D & C and F) Endocrinopathy Management Algorithm



Patients on IV steroids may be switched to oral corticosteroid (e.g., prednisone) at an equivalent dose at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of PO corticosteroids.Ipilimumab may not be restarted while the patient is being treated with systemic corticosteroids except for patients on stabl e doses of hormone replacement therapy such as hydrocortisone.

A Phase III Randomized Study of Adjuvant Ipilimumab Anti-CTLA4 Therapy Versus High Dose Interferon a-2b for Resected High risk Melanoma

Rev. 9/14

Appendix XVII
Neuropathy Management Algorithms

PLEASE NOTE: THERE ARE TWO (2) SEPARATE E1609 NEUROPATHY MANAGEMENT ALGORITHMS. ONE IS TO BE USED FOR ARMS A AND D PATIENTS ONLY, AND THE OTHER FOR ARMS C AND F PATIENTS ONLY. PLEASE REFERENCE THE CORRECT VERSION OF THE ALGORITHM BASED ON THE ARM DESIGNATION OF THE PATIENT. THE ALGORITHMS APPEAR ON THE FOLLOWING 2 PAGES.

Rev. 2/13, 11/13

E1609 (Arms A and D) Neuropathy Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue ipilimumab. Discontinue ipilimumab for any grade 3-4 motor neuropathy, regardless of relationship.

Severity of neurological toxicity	Management	Follow-up
Grade 1 (Asymptomatic, clinical observations only)	• Continue ipilimumab	Continue monitoring the patient. If symptoms worsen, treat as below.
Grade 2 (Moderate symptoms, limiting instrumental ADL)	• Discontinue ipilimumab **regardless of relationship** • Treat symptoms per local guidelines	If symptoms worsen, treat as below.
Grade 3-4 sensory (Severe symptoms, limiting self-case ADL, life-threatening consequences)	• Discontinue ipilmumab **regardless of relationship** • Obtain neuro consult • Treat symptoms per local guidelines • High dose IV steroids (e.g., 1-2 mg/ kg/ day methylprednisolone)	**Symptoms resolve / return to grade 2:** • Taper steroids over at least 1 month
Grade 3-4 motor (Severe symptoms, limiting self-case ADL, life-threatening consequences)	• Discontinue ipilimumab **regardless of relationship** • Obtain neuro consult • Treat symptoms per local guidelines • High dose IV steroids (e.g., 1-2 mg/kg/ day methylprednisolone)	**Symptoms resolve / return to grade 2:** • Taper steroids over at least 1 month **Symptoms do not resolve or progress; atypical presention:** • Consider IVIg or other immunosuppressive therapies per local guidelines

Patients on IV steroids may be switched to oral corticosteroid (e.g., prednisone) at an equivalent dose at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of PO corticosteroids.Ipilimumab may not be restarted while the patient is being treated with systemic corticosteroids except for patients on stabl e doses of hormone replacement therapy such as hydrocortisone.

Rev. 11/13

E1609 (Arms C and F) Neuropathy Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue ipilimumab. Discontinue ipilimumab for any grade 3-4 motor neuropathy, regardless of relationship.

Severity of neurological toxicity	Management	Follow-up
Grade 1 (Asymptomatic, clinical observations only)	• Continue ipilimumab	Continue monitoring the patient. If symptoms worsen, treat as below.
Grade 2 (Moderate symptoms, limiting instrumental ADL)	• Hold/delay ipilimumab • Treat symptoms per local guidelines	Resume ipilimumab when resolved / grade 1 If symptoms worsen, treat as below.
Grade 3-4 sensory (Severe symptoms, limiting self-case ADL, life-threatening consequences)	• Discontinue ipilmumab **regardless of relationship** • Obtain neuro consult • Treat symptoms per local guidelines • High dose IV steroids (e.g., 1-2 mg/ kg/ day methylprednisolone)	**Symptoms resolve / return to grade 2:** • Taper steroids over at least 1 month
Grade 3-4 motor (Severe symptoms, limiting self-case ADL, life-threatening consequences)	• Discontinue ipilimumab **regardless of relationship** • Obtain neuro consult • Treat symptoms per local guidelines • High dose IV steroids (e.g., 1-2 mg/kg/ day methylprednisolone)	**Symptoms resolve / return to grade 2:** • Taper steroids over at least 1 month **Symptoms do not resolve or progress; atypical presention:** • Consider IVIg or other immunosuppressive therapies per local guidelines

Patients on IV steroids may be switched to oral corticosteroid (e.g., prednisone) at an equivalent dose at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of PO corticosteroids.Ipilimumab may not be restarted while the patient is being treated with systemic corticosteroids except for patients on stabl e doses of hormone replacement therapy such as hydrocortisone.

A Phase III Randomized Study of Adjuvant Ipilimumab Anti-CTLA4 Therapy Versus High Dose Interferon a-2b for Resected High risk Melanoma

Rev. 9/14

Appendix XVIII
Skin Toxicity Algorithm

E1609 (Arms A and D & C and F) Skin Toxicity Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue ipilimumab.

Severity of Skin Toxicity	Management	Follow-up
Grade 1-2 (Mild to moderate, localized; papules/pustules covering < 10%–30% of body surface)	• Symptomatic therapy, e.g.topical thick moisturizers and oatmeal baths, antihistamines, topical steroids • Continue ipilimumab	**Symptoms persist > 1-2 weeks with topical corticostroids:** • Moderate to high dose steroids PO (e.g., prednisone 0.5 - 1 mg/kg/day) • Once controlled, taper steroids over at least 1 month
Grade 3-4 (Intense or widespread > 30%; skin sloughing < 10%–30% of body surface; epidermal or mucus membrane detachment)	• Hold/delay ipilimumab (regardless of relationship) • Take photos of rash • Consider skin biopsy • Dermatology consult • High dose IV steroids (e.g., methylprednisolone 1-2 mg/kg/day)	**Symptoms resolve / return to grade 1:** • Taper steroids over at least 1 month • Resume ipilimumab if grade 3 • Discontinue if grade 4 toxicity considered related

Patients on IV steroids may be switched to oral corticosteroid (e.g., prednisone) at an equivalent dose at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of PO corticosteroids.Ipilimumab may not be restarted while the patient is being treated with systemic corticosteroids except for patients on stabl e doses of hormone replacement therapy such as hydrocortisone.

A Phase III Randomized Study of Adjuvant Ipilimumab Anti-CTLA4 Therapy Versus High Dose Interferon a-2b for Resected High risk Melanoma

Appendix XIX
Biomarkers of the Proinflammatory Response and Elements of Immune Suppression

Study PI: Ahmad A. Tarhini, MD, PhD

XIX.1 Hypotheses

1. Based on significant preliminary data generated by our group and others we hypothesize that a baseline and /or early-on treatment prognostic biomarker signature in relation to ipilimumab and IFNα can be generated by assessing specific markers of the pro-inflammatory immune response and of immunosuppression in both tumor tissue and in circulating blood, when evaluated simultaneously due to the common systems biology.
2. The prognostic biomarker signature generated will have therapeutic predictive value in relation to ipilimumab.
3. Based on significant preliminary data we hypothesize that clustered genomic variants specific to patients who develop immune-related colitis after ipilimumab predict this toxicity.

XIX.2 Objectives

1. Primary Objective: Generating prognostic biomarker signature for patients treated with Ipilimumab and high dose IFNα (HDI).
   a. Aim 1: Testing of specific circulating biomarkers of therapeutically predictive value generated by significant preliminary data
      i Cellular populations: (1) absolute lymphocyte count; (2) T regulatory (CD4+CD25hi+Foxp3+, CD4+CD25hi+CD39+) and MDSC (monocyte gate HLA-DR+ low/CD14+, monocyte gate Lin1-/HLA-DR-/CD33+/CD11b+, lymphoid gate Lin1-/HLA-DR-/CD33+/CD11b+); (3) IFNg+CD4+ and IFNg+CD8+ antigen specific T cells (gp-100, MART-1, NY-ESO-1 peptides); (4) CD4+ and CD8+ ICOS-hi T cells; (5) IFNg+CD8+CXCR3+VLA4+ T cells
      ii Serum proteins: CRP, LDH, S100, MIA, YKL40, IL1α, IL1β, IL2, IL2Rα, IL6, IL8, IL10, IL17, IL12p40, TNFα, IFNα, MIP1α, MIP1β, TNF-RII, TGF-α, TIMP-1, CXCL10 (IP10), CXCL11, CXCL9, VEGF
   b. Aim 2: Testing of specific biomarkers within the tumor microenvironment (TME) of therapeutically predictive value generated by significant preliminary data
      i Expression levels of immune related-genes (RNA microarray): CCL2, CCL3, CCL4, CCL5, CD8A, CXCL10, CXCL11, CXCL9, IDO, PRF1, GZMB, HLA-DMA, HLA-DOA, CD79B, IGH, IGKC, IGLC1, HLA-DQA1, IGHM, CD79A, IGHD, CD3G, CD3D, HLA-DPA1, LAT, VAV1, INPP5D, IL2RB, IGHG1, CIITA, IL21R, STAT1

ii Tumor infiltrating lymphocytes and immunosuppressive markers (immunohistochemistry): CD3, CD4, CD8, FOXP3, PD-L1, IDO, CD45RO

Using known clinical covariates and the markers identified in Aims 1 and 2, we will use the entire data set to develop and validate models capable of identifying which patients belong to different prognostic groups.

2. Secondary Objectives:
   a. Testing the therapeutic predictive value in relation to ipilimumab of the prognostic biomarker signature identified in the primary objective.
   b. If a prognostic biomarker signature could not be generated based on the candidate biomarkers selected under the primary objective or if it fails to have a therapeutic predictive value, we will conduct a more comprehensive approach aimed at the generation and validation of a therapeutically predictive biomarker signature in relation to ipilimumab and HDI. This strategy will be feasible since we plan to conduct TME global RNA microarray studies that will generate global gene expression data. This testing approach will allow both global and individual gene expression data analysis.
   c. Testing the "immune-related colitis" predictive value of specific genomic variants generated by significant preliminary data.
   d. Testing for activating mutations (BRAF, NRAS) in patients without known tumor mutational status as tested in a CLIA certified laboratory.

XIX.3 Choice of Trial

These correlatives will be utilizing banked biospecimens (pre-treatment and 3-4 weeks post initiation of treatment) in subjects previously consenting to the future use of their banked biospecimens for research purposes. E1609 trial that is testing adjuvant ipilimumab (at high dose and standard dose) versus IFNα in patients with operable stage IIIB/C and M1a/b melanoma who are at high risk for recurrence and death. The large sample size (N ~ 1600) and diverse banked biorepository for E1609 make it an ideal platform through which to evaluate these interrelated markers for their prognostic and immunotherapeutic predictive value with regard to ipilimumab in reference to IFNα, as assessed individually and in combination based on the common systems biology. E1609 will allow the development of a therapeutic predictive model that links circulating markers of the pro-inflammatory response and immunosuppression with markers in the tumor microenvironment. This trial also allows the evaluation of potentially toxicity-predictive biomarkers, the most devastating of which has been autoimmune colitis.

XIX.4 Preliminary Data

Primary Objective: Generating Prognostic Biomarker Signature

1. Tumor Microenvironment

Immune related pathways/genes identified through tumor gene expression profiling are significantly associated with neoadjuvant ipilimumab clinical benefit (Tarhini et al, AACR 2014)

Patients with regionally advanced melanoma were treated with neoadjuvant ipilimumab in a previously reported study (NCI-ASCO-EORTC Markers in Cancer 2012; PLOS One 2014).[1,2] Gene expression profiles of tumors of treated patients were investigated for their immunotherapeutic predictive value.

Methods: Patients were treated with ipilimumab (10mg/kg IV every 3 weeks x 2 doses) bracketing surgery. Tumor specimens were obtained at baseline and at definitive surgery (week 6-8). Gene expression profiling was performed on the tumor biopsies of 32 patients. The primary endpoint was mRNA expression profiling using U133A 2.0 Affymetrix gene chips. Significance Analysis of Microarrays (SAMR) was performed to test the association of each gene with outcome. Pathway analysis was performed using Ingenuity Pathway Analysis software. The Benjamini and Hochberg method was used to adjust for multiple testing in the pathway analysis.

Results: Pathway analysis identified biologically relevant pathways enriched with genes that are significantly associated with clinical outcome at baseline in relation to progression free survival (PFS) and disease non-progression (NP) as well as early on-treatment outcomes (PFS and overall survival, OS). These pathways and the top associated molecules were notably immune related and highly statistically significant. Associations with clinical outcome overlapped between baseline and on-treatment specimens as well as across clinical endpoints tested. **Table 1** summarizes the top canonical pathways identified at baseline (PRE) and on-treatment (POST) and their association with PFS, NP and OS.

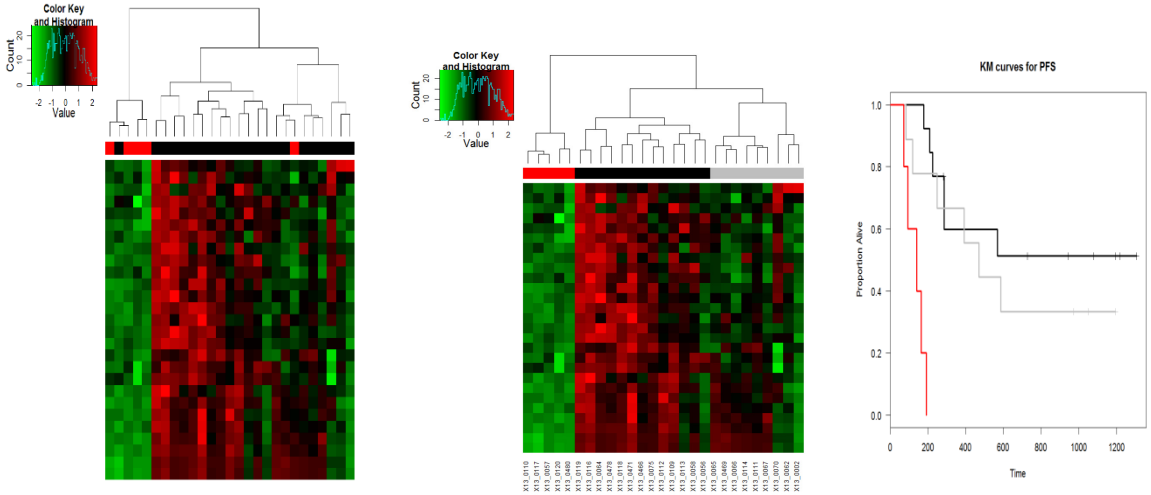
Conclusions: Gene expression profiling identified pathways and genes related to inflammation and autoimmunity that significantly predict clinical benefit from neoadjuvant ipilimumab at baseline and early on-treatment. These findings warrant further investigation in relation to ipilimumab and other immunotherapeutics.

The genes that belong to the top pathways were selected (22 genes, 49 probe sets). We evaluated correlation with clinical outcome (PD, disease progression vs. non-PD). We generated a PD vs. non-PD heatmap using only the probe sets that are significantly differentially expressed between the two groups (27 probe sets from 19 genes). Hierachical clustering was performed and the PD group was found to have a lower expression of these genes. We noticed a sub-group within the non-PD group that has a genomic profile between the PD group and the other non-PD subgroup. As expected, because the genes were selected to correlate with outcome, the clusters produced by these genes also associate with outcome. KM curve shows that this subgroup (in gray) has slightly worse PFS than the others (in black). These results are shown in **Figure 1**.

Table 1. Pathways identified at baseline and on-treatment and their association with PFS, NP and OS				
Pathways	**PRE/PFS** (Adjusted P)	**PRE/NP** (Adj. p)	**POST/PFS** (Adj. p)	**POST/OS** (Adj. p)
Antigen Presentation	0.0002	4.22×10^{-05}	0.004	3.08×10^{-05}
Cytotoxic T Lymphocyte-mediated Apoptosis of Target Cells	0.0004	3.92×10^{-07}	0.004	0.0009
T Helper Cell Differentiation	0.001	0.0005	0.05	0.023
B Cell Development	6.98×10^{-12}	1.14×10^{-13}	2.26×10^{-06}	4.26×10^{-07}
iCOS-iCOSL Signaling in T Helper Cells	0.006	4.23×10^{-07}	0.11	0.11
OX40 Signaling	0.007	1.29×10^{-05}	0.005	0.0002
CD28 Signaling in T Helper Cells	0.04	8.3×10^{-05}	0.04	0.15
IL-4 Signaling	0.02	0.0008	0.06	0.002
PKCÎ¸ Signaling in T Lymphocytes	0.04	7.97×10^{-05}	0.14	0.04
Nur77 Signaling in T Lymphocytes	0.03	0.0001	0.03	0.008
SLE Signaling	1.52×10^{-05}	4.27×10^{-06}		
Allograft Rejection Signaling	0.0003	4.27×10^{-06}	0.004	0.0006
Autoimmune Thyroid Signaling	0.004	4.51×10^{-05}	0.021	0.003
Genes: HLA-DMA, HLA-DOA, CD79B, IGH, IGKC, IGLC1, HLA-DQA1, IGHM, CD79A, IGHD, CD3G, CD3D, HLA-DPA1, GZMB, LAT, VAV1, INPP5D, IL2RB, IGHG1, CIITA, IL21R, STAT1				

Figure 1. Prediction of PD endpoint using baseline expression data. Hierachical clustering was performed and the PD group was found to have a lower expression of these genes. We noticed a sub-group within the non-PD group that has a genomic profile

between the PD group and the other non-PD subgroup. KM curve shows that this subgroup (in gray) has slightly worse PFS than the others (in black).



Time to event analysis was conducted (49 probe sets from the 22 genes). Supervised Principle Components (SPC) were applied to the top genes (probe sets) that are associated with progression free survival (PFS). The plots demonstrated the ability to separate the survival curves by dichotomized value of the first SPC. These signatures will require validation on an independent data set.

Recent data generated by other groups support the importance of the pro-inflammatory gene expression profile as a potential predictor of immunotherapeutic benefit. Studying melanoma activating mutations and methylation status of immune-related genes may complement potential predictive signatures:

- **Chemokine expression in melanoma metastases is important for CD8+ T cell recruitment.** Affymetrix gene expression profiling completed on metastatic melanoma biopsies revealed a major segregation of samples based on the presence or absence of T cell–associated transcripts.[3] The presence of lymphocytes correlated with the expression of defined chemokine genes, and a subset of 6 chemokines (**CCL2, CCL3, CCL4, CCL5, CXCL9, and CXCL10**) was confirmed by protein array and/or quantitative reverse transcription-PCR to be preferentially expressed in tumors that contained T cells. Corresponding chemokine receptors were found to be up-regulated on human CD8+ effector T cells, and transwell migration assays confirmed the ability of each of these chemokines to promote migration of CD8+ effector cells *in vitro*; Chemokine blockade with specific antibodies inhibited migration of CD8+ T cells. These data suggest that lack of critical chemokines in melanoma metastases may limit the migration of activated T cells, which in turn could limit the effectiveness of antitumor immunity.
- **Proinflammatory gene expression signature is associated with survival following GSK MAGE-A3 protein vaccine.**

A phase II trial of immunization with recombinant MAGE-A3 protein using 2 different immune stimulants as an adjuvant (AS15 and AS02B) was conducted by GSK Biologicals in 72 patients with MAGE-A3-positive unresectable stage III or stage IV M1a metastatic melanoma. Gene expression and transcriptional profiling by microarray was performed on baseline biopsies.[4] The predictive gene signature was associated with a significant improvement in median overall survival: 16.2 months in signature (-ve) versus 28 months in signature (+ve) patient population. The high expression of **immune-related cell markers** in patients with clinical activity was confirmed by quantitative reverse transcription PCR,[4] which suggests that a subset of melanoma patients have metastatic tumors that contain a pro-inflammatory infiltrate that includes T cells and chemokines, and that such individuals are more likely to respond clinically to melanoma vaccines and potentially other immunotherapeutic interventions. Further, a phase II study in non-small cell lung cancer evaluated the MAGE-A3 recombinant protein in 182 patients with MAGE-A3-positive, completely resected stage IB or II disease. A signature of immune-related genes, overlapping with the gene set identified for melanoma, was associated with improved outcome from MAGE-A3 treatment,[5] which suggests that a **Type I immune active tumor microenvironment** may have therapeutic predictive value for immunotherapy.

- **Similar pro-inflammatory gene expression profile on pre-treatment biopsies may be associated with clinical response to high-dose interleukin 2 (IL2)** Data presented at the 2009 ASCO Meeting reported a similar **pro-inflammatory tumor microenvironment** to be potentially predictive of IL2 clinical responses.{Sullivan, 2009 #23}
- **Pro-inflammatory/immune-reactive tumor microenvironment favors clinical response to ipilimumab** Affymetrix gene expression profiling was performed on tumor biopsies collected from 45 metastatic melanoma patients before and 3 weeks after the start of treatment in a phase II clinical trial.[6,7] Analysis of pre-treatment tumors indicated that **patients with high baseline expression levels of immune-related genes were more likely to respond favorably**. Associated genes included: T cell surface markers, such as CD8A, CD2, CD247, CD27, CD38, and CD3; members of the TNF receptor family, such as CD40, FAS, and TNFRSF9; cytokines and chemokines, such as CXCL9, CXCL10, CXCL11, CCL4, and CCL5; immune receptors, such as IL10RA, IL12RB2, IL15RA, IL21R, CXCR6, and CCR5; cytotoxic factors, including perforin 1 and various granzymes; and various types of T cell receptors, MHC molecules, and immunoglobulin genes. Pathway analysis of these genes identified the top functional category as “inflammatory response,” with immune-cell trafficking, proliferation, and activation also among the top functional categories. The top 10 canonical pathways that were enriched with the input genes were all associated with the immune system. The most significant pathways were “cytotoxic T lymphocyte-mediated apoptosis of target cells” and “antigen presentation pathway.” Among 22 unique genes with a minimum 2.5-fold difference in pretreatment expression between the clinical activity and no clinical activity groups, most were associated with immune function and had a subcellular localization of plasma membrane or extracellular region. Notably, this list included genes encoding for a surface marker for CD8+ cytotoxic T cells (CD8A and cytolytic components: GZMB and PRF1); Th1 cytokines and chemokines (CCL4, CCL5, CXCL9, CXCL10, and CXCL11); a member of the MHC class II family (HLA- DQA1); and other immune-related genes, such as NKG7, CD38, IGL, and IDO1. Most probe sets also had greater mean post-treatment expression in tumor samples from patients in the clinical activity group. Furthermore, ipilimumab appeared to induce two major changes in tumors from patients who exhibited clinical activity: genes involved in immune response showed increased expression, whereas expression of genes for melanoma-specific antigens and genes involved in cell proliferation decreased. These changes were associated with the total

lymphocyte infiltrate in tumors, and there was a suggestion of association with prolonged overall survival in these patients. Many IFNg-inducible genes and Th1-associated markers showed increased expression after ipilimumab treatment, suggesting an accumulation of this type of T cell at the tumor sites, which might play an important role in mediating the antitumor activity of ipilimumab.

- The aberrant **hypermethylation of CpG islands** located within promoter regions has been associated with the transcriptional silencing of over 50 genes in melanoma.[8] In this context, **Project 1** collaborator Maio et al. recently showed the relevance of aberrant DNA methylation in impairing immunogenicity and immune recognition of melanoma cells, eventually hampering the clinical efficacy of available immunotherapeutic strategies.[8,9] They demonstrated that a global hypomethylation of genomic DNA significantly associates with increased overall survival in stage IIIC CM patients.[10] Furthermore, they defined different methylation-based classifiers able to assign stage III and sub-stage IIIC patients to good or bad prognosis classes, based on the methylation status of specific gene signatures (manuscript in press).

Neoadjuvant ipilimumab induces significant influx of cytotoxic CD8+ tumor infiltrating lymphocytes (TIL) into tumor (Tarhini et al., PLOS One 2014)

Tumor samples (n=24) obtained at baseline and then at definitive surgery and tested by immunohistochemistry showed significant increase in CD8+ TIL after ipilimumab therapy (p=0.02), as shown in **Figure 3**.[1] There were trends observed in examining the change in T reg and clinical benefit ("CR/PR/SD" vs. "PD"); p=0.09. T reg (CD4+CD25hi+ Foxp3+) were higher at week 6 (mean change=1.5; SD=1.46) in the PD group while the opposite was seen in the clinical benefit group ("CR/PR/SD", mean change= -0.64; SD=1.83). The change in Treg is unlike what we observed in the circulation. Ipilimumab was also seen to induce T cell activation evidenced by CD69 without in vitro stimulation. There was an increase in the % CD3+/CD8+/CD45RO+/ TNFα+ TIL (mean change at Week 6 1.38; SD 1.46, p=0.03) and no change in the CD3+/CD8+/CD45RO-/ TNF-α+% TIL (mean change at Week 6 0.20; SD 0.41, p=0.4). This induction/potentiation of T cell memory (CD45RO+) but not naïve (CD45RO-) T cells in tumor requires further testing as a predictive marker given data by Galon et al. in colorectal tumors.[11]

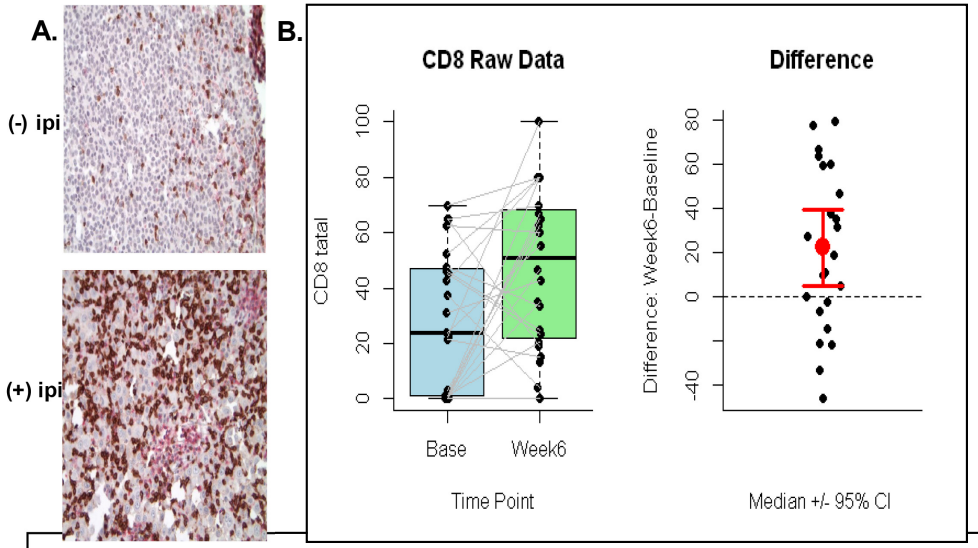


Figure 3. Significant invasion of tumor by CD8+ TIL after ipilimumab (p=0.02).

Recent data generated by other groups support the importance of testing PD-L1, IDO, and Tregs in the melanoma tumor microenvironment in association with CD8+ T Cells

Spranger et al., have reported that the up-regulation of PD-L1, IDO, and Tregs in the melanoma tumor microenvironment is driven by CD8+ T Cells.[12] Although infiltrating CD8+ T cells can be found in melanoma, those tumors are immunologically tolerated and are not rejected. In their report, they showed that the subset of CD8+ T cell infiltrated tumors showed high expression of the three defined immunosuppressive mechanisms (PD-L1, IDO, and Tregs), suggesting that these inhibitory pathways might serve as negative feedback mechanisms that followed, rather than preceded, $CD8^+$ T cell infiltration. They conducted mechanistic studies in mice that revealed that the up-regulated expression of IDO and PD-L1, as well as the recruitment of Tregs in the tumor microenvironment depended on the presence of $CD8^+$ T cells. These results support the hypothesis that these major immunosuppressive pathways are intrinsically driven by the immune system rather than the tumor, and imply that immunotherapy approaches targeting both of these immunosuppressive pathways would best enhance cytotoxic T lymphocytes-mediated cytotoxicity. Therefore, because of the multiple immune inhibitory pathways involved concurrently to facilitate immune escape, these data also support combination therapeutic strategies to target two or more immunosuppressive mechanisms simultaneously. In fact, preclinical data support the idea that combinatorial manipulations of two immunoregulatory pathways can work synergistically to enhance immune-mediated antitumor activity in vivo.[13-15] These data support the notion that the combined inhibition of the immunosuppressive mechanisms mediated through PD1/PDL1 and IDO that are known to inhibit T cell activation leading to tumor escape from immune-mediated destruction is a potentially superior therapeutic approach than the inhibition of either pathway alone.

Immunotype of the tumor-infiltrating lymphocytes correlates with clinical outcome in metastatic melanoma and various other malignancies and may be best evaluated in conjunction with PDL1 and IDO expression

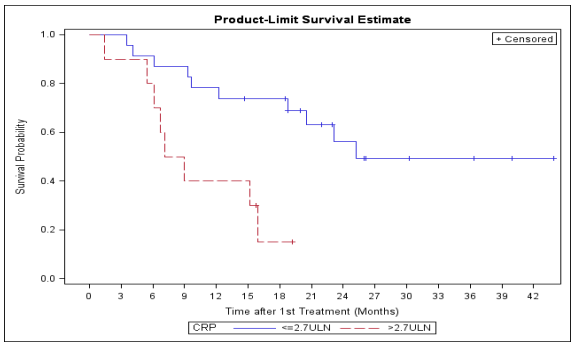
A recent report by Erdag et al showed that an immunotype characterized by a diffuse immune cell infiltrate throughout a metastatic tumor correlated best with survival in patients with metastatic melanoma. Further, higher densities of CD8+ T cells in the tumor microenvironment were the best predictor of improved survival.[16] The presence of CD8+ T cells and the ratio of CD8+ T cells / FoxP3+ regulatory T cells was also reported to correlate with a favorable prognosis and improved survival in metastatic melanoma and other malignancies. [17-19] We and others have also reported that T cell infiltrates within regional nodal metastases correlates with improved survival and is significantly induced by neoadjuvant IFNα2b therapy.[20-22] Spranger et al have also recently reported that the up-regulation of PD-L1, IDO, and regulatory T cells in the melanoma tumor microenvironment is driven by CD8+ T cells.

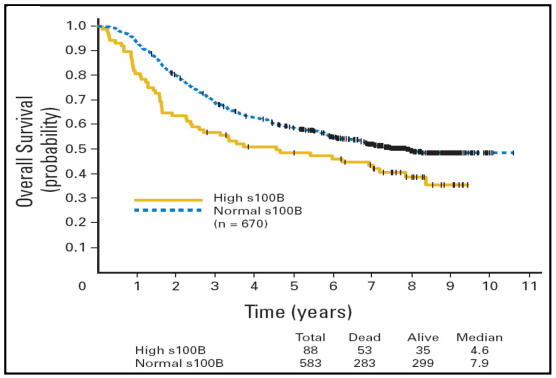
2. Circulating Biomarkers

Potential therapeutic predictive value for baseline ALC in metastatic melanoma patients treated with Anti-CTLA4 antibody and INFα (Tarhini et al, JCO 2012). In a phase II study of the combination of IFNα-2b and tremelimumab, 37 patients with stage IV melanoma were treated.[23] The best response rate by intent to treat (ITT; N=37) was 24% (4 complete response, CR and partial response, PR lasting 6, 6, 12+, 14+, 18+, 20, 28+, 30, 37+ months). Disease control rate (response + stable disease, SD) by ITT was 62% (90% CI=0.53, 0.79). Median PFS was 6.4 months (95% CI = 3.3 – 13.1 months). Median OS was 21 months (95% CI = 9.5 months, -).[23] **Baseline ALC** at ≥1000/uL (N=34 patients) was associated with response (CR/PR versus SD/PD; p=0.02) and clinical benefit (CR/PR/SD versus disease progression, PD; p=0.03 by Wilcoxon two-sample test).[24] No patient ALC<1550/μL had an objective response, and no patient with an ALC<1200/μL had either an objective response or stable disease.

Significant potentiation of Type I CD4 and CD8 antigen specific T cell immunity by neoadjuvant ipilimumab that may play a therapeutic predictive role (Tarhini et al, PLOS One 2014). Patients with clinically palpable stage IIIB-C melanoma were treated with induction ipilimumab (10 mg/kg I.V. every 3 weeks x2 doses) preoperatively and had definitive lymphadenectomy (week ≥ 6), followed by 2 maintenance doses of ipilimumab (q 3 weeks).[1,25] Serum, PBMC, and RNA were collected at baseline, 6 weeks, then at 3, 6, 9, 12 months and/or progression as summarized above. Multicolor flow cytometry utilizing peptide pools (gp-100, MART-1, NY-ESO-1 peptides) showed evidence of spontaneous *in vivo* cross-presentation resulting in type I CD4+ and CD8+ antigen specific T cell immunity. Neoadjuvant ipilimumab induced significant potentiation of type I CD4 and CD8 (fully activated and IFN-γ producing) antigen-specific T cell immunity. Both CD4+ and CD8+ T cells were activated (up-regulated CD69) upon their stimulation with individual antigen peptide pools, and a subset of these cells secreted IFN-γ. The most significant increase "3-10 fold" in CD3+/CD4+/INF-γ+ T cells was seen only in patients who were progression-free at 6 months, suggesting a potential early on treatment therapeutic predictive value. The potential therapeutic predictive value for CD3+/CD4+/INF-γ+ T cells appears to be similar to the observation by Carthon et al. in relation to CD4+ICOShi T cells in bladder cancer patients.[26]

Significant modulation of circulating Treg and MDSC in high-risk melanoma patients treated with neoadjuvant ipilimumab supporting further assessment for a therapeutic predictive value (Tarhini et al, PLOS One 2014) In the same trial testing neoadjuvant ipilimumab, a significant increase in the frequency of circulating Treg (CD4+CD25hi+ Foxp3+; p=0.02 and CD4+CD25hi+CD39+; p=0.001) from baseline to 6 weeks was observed.[25] Significant decreases in circulating MDSC were observed in monocytic HLA-DR+/low/CD14+ MDSC (p<0.0001). Greater increases in Treg were associated with improved PFS (p=0.034; HR=0.57), supporting further evaluation of Treg and MDSC for their therapeutic predictive value. In tumor, Treg (CD4+/CD25hi+, CD4+CD25hi+ Foxp3+) appeared higher at week 6 in the PD group, but the opposite in the clinical benefit (CR/PR/SD) group (p=0.09). **Figure 4** summarizes early-on treatment impact of ipilimumab on MDSC and Treg and the association between increase in Treg and PFS. Interestingly, Walter et al have recently tested 6 predefined populations of MDSC in patients with renal cell cancer treated with the IMA901 vaccine (consisting of multiple tumor-associated peptides) and found 2 to be prognostic for overall survival.[27]





A.

Summary of Most Significant Changes (%) at Week 6			
	Δ at Week 6	SD	p-value
T-Regs			
CD4+	+6.79	9.09	<0.0001
CD4+CD25hi+CD39+	+5.39	8.63	0.0013
CD4+CD25hi+Foxp3+	+4.05	8.13	0.0232
MDSC			
Lymphocytes Lin1-/HLA-DR-/CD33+/ CD11b+	-0.72	3.19	0.3379
Monocytes HLA-DR+low/CD14+	-12.84	12.06	<0.0001
Monocytes Lin1-/HLA-DR-/CD33+ /CD11b+	-2.99	16.18	0.1978

B.

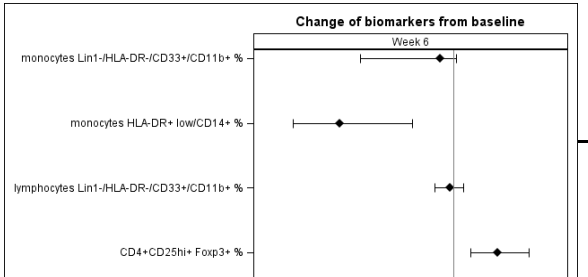


Figure 5 The probability of survival by baseline CRP level (CRP; <=2.7ULN versus >2.7ULN).

Figure 6. Baseline S100B ≥ 0.15 µg/L significantly correlates with OS (*P*=0.010).

Similar pattern of circulating Treg and MDSC in metastatic melanoma patients treated with tremelimumab anti-CTLA4 blocking mAb and INFα supports evaluation of these as potential predictive markers in E1609 (Tarhini et al, JCO 2012, J Immunotherapy 2012). Changes in Treg and MDSC (using the identical markers described above) were compared between baseline, Day 29 (completion of induction IFNα), and Day 85 (completion of one course of the combination of tremelimumab and IFNα). There was a significant increase in the percentage of CD4+CD25hi+CD39+ Treg at Day 85 (p=0.018) but less significant at Day 29 (p=0.09) compared with baseline.[24,25]

In terms of MDSC, there was a significant decrease in the percentage of all MDSC populations at Day 29, most significantly for the monocyte gate MDSC (HLA-DR+ low/CD14+) at Day 29 (p<0.0001) and Day 85 (P=0.001). We noted a less significant decrease in the percentage of the lymphoid gate MDSC phenotype (Lin1-/HLA-DR-/CD33+/CD11b+) at Day 29 (p=0.055) and Day 85 (p=0.07). There was also a decrease in the frequency of the monocyte gate MDSC (Lin1-/HLA-DR-/CD33+/CD11b+) at Day 29 (p=0.04). There was a suggested association between a reduction in lymphoid gate MDSC (Lin1-/HLA-DR-/CD33+/CD11b+) at Day 85 and clinical response (CR/PR vs. SD/PD).[24,25]

Clinical benefit from immunotherapy in melanoma appears to arise from the silencing of MDSC and Treg and the coordinated activation of CXCR3 ligand chemokine production and VCAM1 (Walter et al, IJC 2011). Our preclinical modeling of effective immunotherapies in the setting of solid tumors, including melanoma, suggest that clinical benefit arises from the silencing of MDSC and Treg and the coordinated activation of CXCR3 ligand chemokine (CXCL9, CXCL10 (IP-10) and CXCL11) production and VCAM1 (an adhesion molecule that binds to the integrin VLA4) expression by vascular endothelial cells in the TME.{Rao, 2012 #1059;Bose, 2012 #1060;Bose, 2011 #1061} These alterations allow for the recruitment of peripheral CXCR3+VLA4+Type-1 (i.e., cytotoxic) T effector cells that are capable of mediating direct tumoricidal activity that is requisite for objective clinical response.{Bose, 2011 #1061}

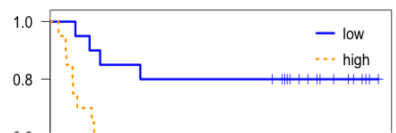
CRP, VEGF, and IL-6 are associated with immune suppression, and their levels at baseline are associated with clinical outcome in the phase II study testing tremelimumab anti-CTLA4 mAb and IFNα (IFN-Treme) (Tarhini et al, JCO 2012, J Immunotherapy 2012). We examined CRP at the cut-off value of 1.5 ULN (based on the report by Marshall et.

al.)[28] but did not detect a significant association with clinical response, clinical benefit, survival, or PFS. Since the distribution of our data suggested that CRP at 2.7ULN might be a cut-off value, we explored baseline CRP at 2.7ULN and found an association with clinical benefit (p=0.05 by Fisher's exact test) and an association with improved probability of survival (p= 0.003 by log-rank test), as illustrated in **Figure 5**. We found an association between baseline VEGF and IL-6 and clinical benefit (CR/PR/SD versus PD) but with lesser significance.

Serum S100B protein is a prognostic biomarker that may improve patient selection for adjuvant therapy (Tarhini et al, JCO 2009). We reported on serum levels of S100B serving as a potential prognostic marker for patients with high-risk melanoma.[29] In this study (see E1694 in **Progress Report**), sera banked at baseline and 3 additional time points were tested for S100B in 691 patients from the E1694 trial by using chemiluminescence. S100B ≥ 0.15 µg/L significantly correlated with OS (*P*=0.010) (**Figure 6**), and a Cox multivariate analysis identified baseline S100B as a significant independent predictor of OS (*P*=0.043) after adjusting for significant prognostic factors and treatment.

Multiplex analysis of serum cytokines in high-risk melanoma patients treated with HDI in E1694 adjuvant trial showed that baseline pro-inflammatory cytokine levels may predict 5-year RFS with HDI but not GMK vaccine (Yurkovetsky et al, CCR 2007). We used Luminex multiplex to simultaneously measure the levels of 29 cytokines, chemokines, and angiogenic and growth factors in the sera of 179 patients from E1694 plus sex-matched controls.[30-33] Serum concentrations of IL1α, IL1ß, IL6, IL8, IL12p40, IL13, G-CSF, MCP1, MIP1α, MIP1ß, IFNα, TNFα, EGF, VEGF, and TNFRII were significantly higher among patients with resected high-risk melanoma compared with controls. Serum levels of immune-suppressive angiogenic/growth stimulatory factors (VEGF, EGF, HGF) were significantly decreased by HDI, while levels of anti-angiogenic IP-10 and IFNα were elevated after treatment. Comparing patients according to relapse outcome, the pretreatment levels of pro-inflammatory cytokines IL1β, IL1α, IL6, TNFα, and chemokines MIP1α, and MIP1β were significantly higher (*P*<.05) in sera of patients with longer relapse-free survival (RFS) of 1-3 years and > 5 years, compared with patients who experienced shorter RFS of < 1 year.

A four-marker signature of TNF-RII, TGF-α, TIMP-1 and CRP is prognostic of worse survival in high-risk surgically resected melanoma (Tarhini et al, JTM 2014). E1694 tested GM2-KLH-QS21 vaccine versus high-dose interferon-α2b (HDI) as adjuvant therapy for operable stage IIB-III melanoma. We tested banked serum specimens from patients in the vaccine arm of E1694 for prognostic biomarkers. Aushon Multiplex Platform was used to quantitate baseline serum levels of 115 analytes from 40 patients. . Leave-one-out cross validation was used to avoid over fitting of the data. In each cross validation step, we hold one sample out, and use the rest of the sample to fit a Least absolute shrinkage and selection operator proportional hazard regression (Lasso PH) model. Markers with coefficients > 0.1 were selected and included in a Cox PH model to predict 1 year RFS and 5 year OS. The linear combination of the CoxPH model was used as the risk score. The hold out sample's risk score is predicted using the CoxPH model fitted by the remaining sample. We continue this until the all the samples were "left out and predicted" once. The ability of the resulting risk scores (from cross validation) to predict 1 year RFS (and 5 year OS) was evaluated by the time-dependent ROC curve. Four markers that include Tumor Necrosis Factor alpha Receptor II (TNF-RII), Transforming Growth Factor alpha (TGF-α), Tissue Inhibitor of Metalloproteinases 1 (TIMP-1), and C-reactive protein (CRP) were found to be most informative for the prediction of OS (high levels correlate with worse prognosis).



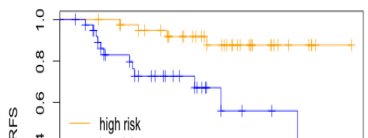


Figure 7. Baseline multi-marker (TGF-α, TNF-RII, TIMP-1, CRP) risk score in GMK arm is prognostic of poor overall survival (OS).

Figure 8. One month pro-inflammatory cytokines (IL2Rα, IL-12p40 and IFN-α) predict 1-Year RFS with IFNα.

As expected, because the markers were selected to correlate with outcome, models built from these selected markers also demonstrated prognostic ability in the whole dataset; **Figure 7**. When using the four-marker PH model to predict 5-year OS, we achieved an area under the curve (AUC) 72% (cross validated). High baseline TNF-RII was also significantly associated with worse RFS. The RFS with high (above median) TNF-RII was significantly lower than low TNF-RII (p=0.01). Therefore, the biomarker signature consisting of **TNFR-II, TGF-α, TIMP-1** and **CRP** could potentially be used as prognostic markers in patients with high-risk melanoma. These preliminary data warrant further investigation.

Early on-treatment (one month) pro-inflammatory cytokines predict 1-Year RFS with IFNα but not observation in E1697 trial (Tarhini et al, SMR 2013). E1697 was a phase III trial that tested observation (Arm A) vs. adjuvant IFN-α2b for one month (Arm B) in patients (pts) with resected intermediate risk stage IIA-B and IIIA melanoma. We evaluated the levels of candidate serum biomarkers for their therapeutic predictive or prognostic value in Arm B versus Arm A in 268 pts with banked biospecimens. Aushon Multiplex Platform was used for serum cytokine tests at baseline and one month. Cox PH modeling and Wald tests were used to test the association of each marker and RFS. Similar leave-one-out cross validation strategy described above was used to avoid over fitting of the data. In the multi-marker modeling analysis in Arm B, one month IL2Rα, IL-12p40 and IFN levels significantly predicted one year RFS with LOOCV AUC=83%. The risk score (linear combination of the 3markers) separated RFS curves of low and high risk groups well (note that the risk score is calculated using the whole data, hence over fitted.); **Figure 8.** This model did not hold for Arm A, indicating a marker-treatment (IFN) interaction in Arm B, that is consistent with our previously published predictive role of the proinflammatory cytokine profile. Therefore, early on-treatment (one month) proinflammatory serum markers (**IL2Rα, IL-12p40, IFN**) significantly predict RFS in pts treated with adjuvant IFN-α2b and warrant further study.

3. Prediction of Autoimmune Colitis

Clustered genomic variants specific to patients who develop immune-related colitis after ipilimumab may predict this toxicity (Tarhini et al, submitted to ASCO 2014)

Data generated in the context of UPCI 08-144 trial of neoadjuvant ipilimumab and funded by NIH award P50CA121973. These data have been submitted for presentation at the 2014 ASCO Annual Meeting (*Tarhini et al., Clustered genomic variants specific to patients who develop immune-related colitis after ipilimumab may predict this toxicity*). We identified a genomic signature to predict colitis predisposition among patients undergoing neoadjuvant ipilimumab therapy for locally/regionally advanced operable melanoma.

Rev. 3/15

XIX.5 Tissue/Specimen Type

1. Tumor tissue: Paraffin Block or Unstained Slides
2. Body fluids: Serum, Lymphocytes
3. Derivatives: DNA (genomic)

The project plans to test specimens from up to 1600 subjects depending on the availability of biospecimens. The tumor tissue blocks/slides are banked at the ECOG-ACRIN Central Biorepository and Pathology Facility. The research blood/blood products are banked at the ECOG-ACRIN Immune Monitoring and Cellular Products Laboratory (IMCPL) at the University of Pittsburgh Cancer Institute (UPCI).

1. Tumor: 15-17 (5 micron scrolls), or 15-17 (5 micron unstained slides); 70% tumor

   (Any extra tissue would be saved and returned to the ECOG-ACRIN Central Biorepository and Pathology Facility.)

   [These will be used for the testing of (1) gene expression profiling , (2) Immunohistochemistry, (3) activating mutations by targeted sequencing in select patients with unknown mutational status, (4) methylation status of DNA sequences]

2. PBMC/Serum: 5 vials PBMC and 3 vials serum

   (An extra vial requested would be saved at the IMCPL and used only for unexpectedly low recoveries and running assays on different days.)

3. DNA (genomic): one yellow top tube

   (Remaining specimen would be saved at the IMCPL.)

   [This will be used for the testing of genomic variants potentially predictive of autoimmune colitis induced by ipilimumab]

XIX.6 Laboratory Methods

1. Tumor Microenvironment

General: These assays will be conducted at the UPCI Cancer Biomarkers Facility (CBF) Genomics Core (Director: William LaFramboise, PhD).

1.a. Baseline expression of immune-related pathways/genes

Microdissection of FFPE tumor specimens will be performed manually using an inverted microscope (Nikon Eclipse TE200) to obtain a minimum of 90% tumor cells for RNA purification. Dissection involves scraping cells from unstained sections of 5 micron thickness on slides aligned in register with serially cut hematoxylin and eosin stained specimens including tumor domains demarcated by a surgical pathologist. RNA purification will be performed using the Qiagen miRNeasy FFPE Kit and protocol (Qiagen, Valencia, CA) with isolated RNA suspended in nuclease-free water. Inclusion in subsequent *in vitro amplification* (IVT) assays is determined both by spectrophotometric absorption ratio [260/280 > 1.8 (NanoDrop, Wilmington, DE)] and RIN values (RNA Integrity Index) determined via microchip electrophoretic analysis (Agilent Bioanalyzer 2100, Agilent Technologies, Santa Clara, CA). We have previously established that RIN values ranging from 5.0 to 8.0 in RNA from FFPE specimens can undergo successful *in vitro transcription* and amplification using a multiple primer approach. Amplification will be performed using the NuGen whole transcription method comprising the Ovation FFPE WTA assay (NuGEN, San Carlos, CA) employing random and 3' primers to eliminate amplification bias beginning with 100 ng total RNA. Confirmation of cDNA diversity will be obtained using the Bioanalyzer 2100 to generate an electrophoretogram for each amplification reaction regarding sample yield, integrity, and size diversity compared to a laboratory human RNA standard and a Universal Human Reference RNA (Stratagene, La Jolla, CA). Five micrograms of purified cDNA will be incubated with fragmentation buffer (NuGEN, San Carlos, CA) at 37°C for 30 minutes, then 95°C for 2 minutes. All cDNA samples will undergo hybridization on Affymetrix GeneChip HG U133A 2.0 arrays which contain overlapping probe sets for transcripts comprehensively representing the functionally characterized human genome. Briefly, fragmented cDNAs are mixed in a hybridization cocktail with water to a final volume of 220µl. 130ul of hybridization cocktail is hybridized on each array at 45°C for 18 hours. The arrays are then washed and stained with streptavidin-phycoerythrin in a GeneChip Fluidics Station 450 (Affymetrix) and scanned using a GeneChip Scanner 3000 (Affymetrix). Quality control (QC) parameters and expression intensity data are derived from the MAS 5.0 (Microarray Suite) and RMA algorithms (Robust Multi-array Average) of the Expression Console software (version 1.2.0.20; Affymetrix). Comparisons of global and individual gene expression data are performed using median normalized and log2 values transferred to Partek Genomics Suite v6.5 (Partek Inc, St Louis, MI). Statistical significance is performed at a false discovery rate (FDR) of less than 5% (q value) to control for Type 1 errors arising from multiple tests.

1.b. Tumor infiltrating lymphocytes and immunosuppressive markers (immunohistochemistry): CD3, CD4, CD8, FOXP3, PD-L1, IDO, CD45RO

Immunohistochemistry will be conducted at UPMC Shadyside Hospital, Department of Pathology, under supervision of Dr Uma Rao (E1609 Pathology Co-chair). Paraffin-embedded tissue sections (5 µm thick) will be scored at × 20 magnifications by a surgical pathologist (Uma Rao, MD) who will be blinded to patient and treatment status. Lymphocytes will be counted in 4 quadrants and added. The total numbers of intratumoral, peritumoral, and perivascular mononuclear cell infiltrates will be enumerated following standard immunohistochemical staining, as previously published,[34] with FOXP3, CD4, CD8, IDO, PDL1 and CD45RO monoclonal antibodies.

2. Circulating Biomarkers

General: These assays will be conducted at UPCI Immune Monitoring and Cellular Products Laboratory (IMCPL; Director: Lisa Butterfield, PhD) which is CAP accredited and CLIA certified. All assays are run according to SOPs by trained and competency tested personnel, and include healthy donor controls. The laboratory participates in external proficiency panels for flow cytometry and Luminex.

2.a. Circulating Cellular Populations

Absolute lymphocyte count will be determined by the hospital clinical lab by standard procedures and is being collected prospectively on study-specific forms. All other cellular assays will be tested by flow cytometry in the IMCPL, according to existing SOPs. The flow cytometer (FC500) is QC'd daily and monitored for any change in laser function. All antibodies are tested before use in patient material testing.

Cellular subpopulations are sequentially gated on, and both MFI and % positive is reported. Dot plots and histograms are reviewed by an experienced supervisor and results inserted in spreadsheets which are sent to the project associated biostatistician. Negative populations are identified by isotype antibodies or negative control cells or unstimulated cells (depending on assay or antibody). Any cell populations with any degree of positive marker expression are listed by the specific MFI or % positive value.

2.b. Circulating Serum Proteins

Most serum proteins will be analyzed by the multiplex Luminex assay, and analyzed on a BioRad reader. Concentrations are automatically determined based on kit standard curves from the software. Additional controls include R&D Systems Multiplex QC controls which are mixed cytokines with expected yield values. Large projects use a single lot of kits whenever possible. Analytes requiring non-standard dilutions (like CRP) are run singly. Analytes not compatible with multiplexing are run singly in ELISA format kits. Results are presented as the pg/ml values reported and results above or below the limits of detection are reported as such. Given the broad range of detection for the Luminex platform, values below the LLD are generally considered negative, and values above the ULD are either considered statistically as equal to the ULD, or, if a reasonable fraction of samples are over the ULD, those samples are diluted further and re-run.

3. Prediction of Autoimmune Colitis

3.a. Targeted Deep Sequencing to identify genomic variants specific to patients who develop immune-related colitis after ipilimumab therapy.

General: These assays will be conducted at the UPCI Cancer Biomarkers Facility (CBF) Genomics Core (Director: William LaFramboise, PhD).

We identified a genomic signature to predict colitis predisposition among patients undergoing neoadjuvant ipilimumab therapy for advanced melanoma (*Tarhini et al., Clustered genomic variants specific to patients who develop immune-related colitis after ipilimumab may predict this toxicity. Submitted to the 2014 ASCO Annual Meeting*). The proposed study will extend these findings in a larger patient cohort. Targeted, deep sequencing will focus on a customized 10 gene/9 SNP panel developed in our laboratory. PBMC (blood) samples (1×10^6 cells) will be extracted using the QIAamp DNA Micro kit (Qiagen, Valencia, CA) and DNA obtained by column purification will undergo QC to identify samples (>100 ng/ul) with OD 260/280 >1.8 (Nanodrop ND-1000 spectrophotometer, NanoDrop, Wilmington, DE) and fragments ≥300 base pairs (Bioanalyzer 2100: 12000 DNA Chip, Agilent Technologies, Santa Clara, CA) for library

construction. Primers for target genes will be used to generate amplicon libraries via PCR selection (Perkin Elmer 9700, Bridgeville, PA) (99°C x 2min, 15 cycles at 99°C x 15 sec, 60°C x 8 min, 10°C x hold) followed by enzymatic digestion and phosphorylation of the multiplexed amplicons. The amplicons are then indexed (Ion Xpress Barcode Adapters, Life Technologies, Grand Island, NY) and adapters ligated for attachment to Ion Spheres (ISP) in micro-reactors for clonal amplification using emulsion PCR (One Touch 2 instrument; 60 cycles, PGM 200bp kit v2). Templated ISPs are recovered and enriched using biotin labeled primers bound to streptavidin coated metallic beads (DynaBeads C1, Life Technologies). The ISPs are then washed, stripped from the streptavidin beads (125mM NaOH; 0.1% Tween 20) and sequencing is performed on the Ion Torrent Personal Genome Machine (PGM) using the 200bp sequencing kit v.2 and a washed, precalibrated semiconductor chip (318v.1 for 200bp libraries, Life Technologies) on the PGM platform. Sequencing comprising 500 flows (125 washes of each of 4 nucleotides) has provided 1000 x base depth of parsed barcoded reads aligned to Human Reference Genome HG19 on 10 samples undergoing simultaneous sequencing. BAM files (binary format), linked BAI files (indexed files) and variant call files (vcf) (Torrent Suite 4.0) are transferred from the Ion Torrent server (Dell Precision T7500, Intel Xeon Quad Core 2.93GHz, Ubuntu 10.04) to the analysis server (MacPro 5.1, Intel Xeon 6 Core 2.4GHz, OSX 10.8.4; GenomOncology Inc., Westlake, OH). A Galaxy Project plug-in (Pennsylvania State University, State College, PA) generates additional vcfs and INDELs using GATK (Genome Analysis Toolkit; UnifiedGenotyper v. 2.3), SAMtools (mpileup v. 0.1.18) and a manual variant caller algorithm (variant: ≥5% base calls differ from HG19 with ≥ 300 calls at Phred Score=20 or $p < 0.01$). All variants and INDELs are validated by manual curation of the BAM file sequence using the Integrated Genomics Viewer (IGV Broad Institute). All patient samples will be compared for commonality of single nucleotide variants and INDELs based on colitis symptoms.

XIX.7 Facilities & Personnel

Principal Investigator:

Name: Ahmad A Tarhini, MD, PhD (UPCI Skin Spore Biomarker Project Co-Leader)
Institution: University of Pittsburgh Cancer Institute
Mailing Address: UPMC Cancer Pavilion, 5150 Centre Avenue, Suite 555, Pittsburgh, PA
Email: tarhiniaa@upmc.edu
Phone: 412 648 6578 Fax: 412 648 6579

Co-Investigators:

Name: Walter Storkus, PhD (UPCI Skin Spore Biomarker Project Co-Leader)
Institution: University of Pittsburgh
Mailing Address: University of Pittsburgh, Biomedical Science Tower, 200 Lothrop Street, W1041, Pittsburgh, PA
Email: storkuswj@upmc.edu

Name: John M Kirkwood, MD
Institution: University of Pittsburgh Cancer Institute
Mailing Address: Hillman Cancer Center Research Pavilion, 5115 Centre Avenue, Suite 1.32, Pittsburgh, PA
Email: kirkwoodjm@upmc.edu

Name: Lisa H Butterfield, PhD
Institution: University of Pittsburgh Cancer Institute
Mailing Address: Hillman Cancer Center, 5117 Centre Avenue, Pittsburgh, PA
Email: butterfieldl@upmc.edu

Name: William LaFramboise, PhD
Institution: University of Pittsburgh Cancer Institute
Mailing Address: UPCI, Shadyside Hospital, 5230 Centre Avenue, Pittsburgh, PA
Email: laframboisewa@upmc.edu

Name: Uma N.M. Rao, MD
Institution: University of Pittsburgh Cancer Institute
Mailing Address: UPCI, Shadyside Hospital, 5230 Centre Avenue, Pittsburgh, PA
Email: raounm@upmc.edu

Biostatistics:

Name: Sandra Lee, PhD (ECOG-ACRIN E1609 Biostatistician)
Institution: Dana Farber Cancer Institute
Mailing Address: 450 Brookline Avenue Boston, MA, 02215
Email: sjlee@jimmy.harvard.edu

Name: Yan Lin, PhD (UPCI Skin Spore Biomarker Project Biostatistician)
Institution: University of Pittsburgh Cancer Institute
Mailing Address: UPCI, Biostatistics Facility, Sterling Plaza Suite 325, 201 North Craig Street, Pittsburgh, PA
Email: yal14@pitt.edu

XIX.8 Statistical Design

Endpoints (outcomes)

The clinical endpoints are relapse free survival (RFS) and overall survival (OS). RFS is defined as the time from study entry to relapse or death, whichever happens first. OS is defined as the time from study entry to death.

Primary Comparison

The primary objective of the study is to build a prognostic model using the candidate biomarkers for each study arm. The secondary objectives are (a) to test for the therapeutic predictive power of prognostic panels discovered; (b) to search for

therapeutic predictive markers to guide the therapeutic choice of either ipilimumab or HDI; (c) to test for association of pre-specified genetic variants and the occurrence of colitis.

Case Selection

We plan to include all subjects accrued in E1609 ipilimumab study arms (A, D and C, F) and HDI study arm. Arms A and D treat patients with high dose ipilimumab (HIP), arms B and E with HDI and arms C and F with low dose ipilimumab (LIP).

Power Justification

The target accrual for E1609, which was activated in May 2011, is 1600 (600 HDI, 500 HIP, 500 LIP), which will be completed by August 2014. Thus, by 2015, we will have at least 1 year of follow-up data for each patient in the trial for analysis and validation of potential biomarkers and the development of prediction models for RFS and OS of the patients in each treatment arm.

We currently estimate that biospecimens are available for testing from approximately 80% of subjects enrolled on E1609. We expect that these numbers will improve as we continue to follow up with participating sites on biospecimen submission.

Power for Primary Objective: Prognostic Modeling

Subjects will be divided into a training set and a validation set (250 each for the LIP and HIP groups, 300 for HDI group). Here we demonstrate our power in the development of a prognosis model in one of the Ipilimumab arms. We base our power calculation on a test that compares the SN and SP simultaneously[35] . Following the E1609 protocol, we expect that the cure rate for the ipilimumab arm will be 40% and the median RFS for the uncured patients is 0.48 years. As described above, we expect to obtain samples from >=80% of the patients, after accounting for 95% assay success rate, we will have about 76 cases (patients who had event within 1 year) and 114 controls (patients who are followed up for 1 year without any event). We define a minimal useful model to be one with a sensitivity of 75% or higher and a specificity of 75% or higher. If the true sensitivity and specificity are 89% and 87% for our model, we will have over 80% power to claim that our model is significantly better than the minimal useful model (α=0.05).

Power for Predictive Modeling

With limited preliminary data, predictive modeling is our secondary objective. Nevertheless, here we estimate our power for validation of a predictive biomarker in the validation set. We base our calculation on the analysis of one of the ipilimumab arms (HIP or LIP) and the HDI arm. However, it is very likely that the RFS profiles are very similar between the two ipilimumab arms. If this turned out to be the case by the time we analyze the data, we will consider combining the data of these two arms. Thus, our power estimate here is a conservative estimate. The goal here is to determine whether there is a significant difference between the clinical benefits of patients in different marker strata. That is, we are testing the interaction of the dichotomized marker value (we discuss how to dichotomize the marker value in details in our analysis plan in the following section) and the treatment group. We calculate our power based on the method by Peterson and George. [36] As shown in Table 1, with 418 samples total, we will be able to detect an interaction term with HR between 1.9-2.1 assuming the marker stratum 1 proportion is between 50-70% with a two-sided test (α=0.01). Details on the power calculation for the predictive modeling is given below.

Table 1 Power calculation for test of interaction ($\alpha = 0.01$, two-sided)

Marker stratum 1 Freq. (g_1)	f_{11}	f_{12}	f_{21}	f_{22}	λ_{11}	λ_{12}	λ_{21}	λ_{22}	Δ_1	Δ_2	Δ	Power
0.5	0.273	0.273	0.227	0.227	1.1	0.734	0.6	0.776	1.833	0.946	1.938	0.69
					1	0.834	0.5	0.876	2	0.952	2.101	0.8
					1.15	0.684	0.6	0.776	1.917	0.881	2.174	0.84
					1.05	0.784	0.5	0.876	2.1	0.895	2.346	0.91
					1.15	0.684	0.55	0.826	2.091	0.828	2.525	0.95
					1.2	0.634	0.5	0.876	2.4	0.724	3.316	1
0.6	0.327	0.218	0.273	0.182	1.05	0.718	0.6	0.82	1.75	0.875	2	0.72
					1	0.793	0.55	0.895	1.818	0.885	2.053	0.76
					0.95	0.868	0.5	0.97	1.9	0.894	2.124	0.8
					1	0.793	0.5	0.97	2	0.817	2.448	0.93
					1.1	0.643	0.5	0.97	2.2	0.662	3.321	1
0.7	0.382	0.164	0.318	0.136	1	0.723	0.6	0.893	1.667	0.81	2.058	0.69
					1.2	0.257	0.8	0.427	1.5	0.602	2.494	0.79
					1	0.723	0.55	1.01	1.818	0.716	2.539	0.92
					1.15	0.373	0.7	0.66	1.643	0.566	2.904	0.95
					1.05	0.607	0.5	1.127	2.1	0.538	3.9	1

XIX.9 Statistical Analysis Plan

- Statistical methods for the main analyses: For the single marker association with PFS and OS, Cox proportional hazards (CoxPH) regression will be used. Because some long-term cures have been observed in patients with stages IIB – III melanoma treated with HDI and ipilimumab, we will also perform exploratory analyses using a cure-rate model for RFS as a supplement to the CoxPH regression analyses proposed above. For multi-marker prognostic model, both the Lasso Cox proportional hazard regression and the Cox proportional hazard regression will be used. And the results will be compared.
- Transformations applied to variables: Distribution of data of different types will be checked and appropriate transformation will be used when necessary.
- Methods for marker cutpoint determination: For the predictive biomarker, we will follow the recommendation of Janes et al 2013 and use the marker-by-treatment predictiveness curves to guide the dichotomization of the marker (or risk score for multi-marker panel) [37]. Specifically, we will dichotomize the marker (or score) around the point where the two predictive curves cross.

- Variable selection procedures: We will use two strategies and compare our results: Cox proportional hazard regression with forward regression and Lasso Cox proportional hazard regression.
- List of standard clinical variables to be incorporated into models or other analyses: Clinical variables that we will consider to be included in the models are age, sex, Breslow thickness, recurrent disease at study entry, ulceration of the primary tumor, lymph-node status, number of positive lymph nodes, completion of lymph node dissection prior to treatment, microscopic extranodal extension, and mitotic rate in the primary tumor.
- Multiple-comparisons adjustment methods: To adjust for the multiple testing, the Benjamini and Hochberg's (BH)[38] method will be used to control the false-discovery rate (FDR) at 5% for all candidate marker analyses. For the whole genome scan, we will use the procedure described in Storey 2002 to control the FDR at 5%.[39]
- For complex studies, methods that will be used to validate the analysis results, or a rationale for not performing a validation study: Given the uniqueness of this study population, we do not a compatible independent validation cohort, but our study population is large enough that we will randomly split the samples into two groups of equal size, using one of them as the **training set** and the other one as the **validation set.** However, to avoid over fitting, the validation set will be held out till the last stage of the study, where it will be used to validate the final models.
- Any other information necessary for the Committee to understand and evaluate the main analyses you are proposing: The detailed analysis plan for our primary objectives is included below.

Secondary Objectives

For the predictive biomarker analysis, again we will randomly split the samples into two groups of equal size, using one of them as the training set and the other one as the validation set. However, to avoid over fitting, the validation set will be held out till the last stage of the study, where it will be used to validate the few (<=5) final biomarkers (or panels).

Searching for predictive biomarkers

Based on our and others studies, we have defined a pool of candidate prognostic biomarkers. We expect that some of these markers are also predictive. Thus, we would consider these markers as our top tier candidate predictive markers. First, we will follow the recommendation by Janes et al, and use the marker-by-treatment predictive curves using 1-year PFS (or 1-year OS) as the "response rates" to evaluate the interaction between the marker and the treatment[37]. Only the markers with a qualitative interaction with the treatment will be further investigated, since they are the only markers that could guide the choice of treatments. We will dichotomize marker around the point where the two predictive curves cross. To test the interaction between the marker and treatment, we will fit the Cox proportional hazard model of the following form: $\lambda(t) = \lambda_0(t) \times \exp\{\beta_1 Md + \beta_2 T + \beta_3 T*Md + \sum \beta_i Covariates\}$, where Md denotes dichotomized marker value, T denotes treatment (HDI/HIP/LIP), the function $\lambda(t)$ represents the event rate at time t, and $\lambda_0(t)$ represents the corresponding baseline event rate. Likelihood ratio test will be used to test the null hypothesis of $\beta_3=0$. To adjust for the multiple testing, the Benjamini and Hochberg's (BH) [38]method will be used to control the false-discovery rate (FDR) at 5%. We may also consider the risk score from the prognostic models developed above as a continuous marker and analyze them in the same way as described above. Markers (and risk scores) that show a significant qualitative interaction with the treatment effect will be further ordered by the estimated 1-year RFS (or OS) rate under the marker-policy. The top few (<5) predictive biomarkers may be validated in the

validation set. Given the lack of preliminary data on predictive biomarkers, if our search among the top tier markers failed, we will consider a genomic scan of gene expression to search for potential predictive biomarkers using the same strategy described above in the training dataset.

We will also consider building a multi-marker prediction model. Given the limited sample size, we will consider only the top 3-5 markers in the model. We will first fit a CoxPH model with main effects of the markers and the clinical factors: $\lambda(t) = \lambda_0(t) \times \exp\{\sum \beta_i M + \sum \beta_i \text{Covariates}\}$, and test the predictive value of the predicted risk score (the linear combination of this model) as described above.

The top few (<=5) predictive markers (and/or panels) will be validated in the validation set. The same cutoff point will be used to dichotomize the marker value (or risk score value). CoxPH models with interaction term between the dichotomized marker and the treatment will be fitted. We will compare the direction and magnitude of the interaction term to that obtained in the training set. Likelihood ratio tests will be used to test the interaction of each marker and the treatment effects. A p-value of 0.01 (0.05/5) will be considered statistically significant.

Testing the association of candidate SNP genotype and "immune-related colitis"

We will test the association of each variant and the risk of immune-related colitis by logistic regression adjusting for potential clinical confounders including age and sex. The Benjamini and Hochberg's (BH)[40] method will be used to control the false-discovery rate (FDR) at 5%.

* * *

Detailed Analysis Plan for the Primary Objective

Association of individual markers with clinical outcome. The association of each marker at each time point with the RFS will be assessed by Cox proportional hazard regression (CoxPH), adjusted for treatment arm and known prognostic factors such as age, sex, Breslow thickness, recurrent disease at study entry, ulceration of the primary tumor, lymph-node status, number of positive lymph nodes, completion of lymph node dissection prior to treatment, microscopic extranodal extension, and mitotic rate. Schematically, we will fit models of the following form: $\lambda(t) = \lambda_0(t) \times \exp\{\beta_1 M + \beta_2 T + \beta_i \text{Covariates}\}$, where M denotes marker value (e.g., the absolute lymphocyte count, ALC), T denotes treatment (HDI/HIP/LIP), the function $\lambda(t)$ represents the relapse rate at time t, and $\lambda_0(t)$ represents the corresponding baseline relapse rate. Because some long-term cures have been observed in patients with stages IIB – III melanoma treated with HDI and ipilimumab, we will also perform exploratory analyses using a cure-rate model for RFS as a supplement to the CoxPH regression analyses proposed above. The cure-rate model assumes that the population being studied is a mixture of two subpopulations: one of which is cured (proportion π of the study population), and the other of which is not (proportion $1 - \pi$ of the study population). We represent the survival function in this latter subpopulation by $S_2(t)$. The survival function S(t) for the entire study population is a simple mixture of the two: $S(t) = \pi + (1 - \pi)S_2(t)$. Similar approaches will be used for the statistical analysis of OS data. To adjust for the multiple testing, the Benjamini and Hochberg's (BH) [38]method will be used to control the false-discovery rate (FDR) at 5%. ***Note that this marker-by-marker analysis is not intended for the feature selection of the multi-marker model building***

described in the next section. This analysis is to test the marginal association of each candidate marker. The feature selection step for the model building is included in the cross-validation loop to avoid over-fitting.

Development and validation of the prediction model. The model development will be carried out at two levels: within each Aim (i.e., using markers within the same category) and across Aims (using markers that are discovered in all Aims). The main goal is to develop a prognosis model of RFS in the HIP and LIP arms. However, we will also take the advantage of the availability of the HDI arm to develop a prognosis model for melanoma patients treated with HDI. Therefore, we will develop a prognosis model for each arm of the study. Given the uniqueness of this study population, we do not a compatible independent validation cohort*, but our study population is large enough that we will randomly split the samples into two groups of equal size, using one of them as the* ***training set*** *and the other one as the* ***validation set.*** *However, to avoid over fitting, the validation set will be held out till the last stage of the study, where it will be used to validate the final models. The small model within each subgroup of the biomarkers will be evaluated through cross-validation within the training set.*

We will first use the Lasso Cox proportional hazard regression (LPHR) to develop a prognosis model for RFS using the training set. The Lasso was developed by Tibshirani 1996[41] and uses an l_1 penalty to achieve a sparse solution, which allows simultaneous variable selection while fitting the model. This method has been applied to generalized linear regression[41] and CoxPH models[42]. Friedman et al. developed a fast algorithm for these methods and implemented it in a user-friendly R package, glmnet [43]Lasso has attracted more and more attention on the analysis of high-dimensional data because of its capability to perform the regression with outstanding accuracy in a large feature space, while simultaneously selecting the features. The tuning parameter will be trained through ten-fold (10x) cross validation within the training set. The linear combination score of the resulting model will be used to classify the patients into low-, intermediate-, and high-risk groups (cutoff at the 33% and 67% percentiles of the score). Hazard ratios (HRs) between the groups will be calculated. Log rank tests will be used to compare the RFS among these groups. The ability of this score to predict 1-year RFS will be evaluated using a time-dependent ROC curve [44].The area under the ROC curve (AUROC) will be calculated. An optimal cutoff point, which produces the best prediction (highest accuracy), will be chosen, and the corresponding sensitivity (SN), specificity (SP), and accuracy will be reported. The moderate number of candidate markers considered in each aim and the relatively large number of samples also enables us to use the regular CoxPH regression as a complement method to the LPHR. Markers will be evaluated for inclusion in the CoxPH model by forward stepwise selection. We will follow the same approach described above to evaluate the predictability of the model. Several popular machine-learning methods have been extended to handle right-censored data and predict subject survival status *at a specific time point*[45,46]. We will use these methods as alternative methods to predict 1-year RFS for these patients and compare the performance of these methods to the LPHR model and Cox regression model. Specifically, univariate Cox regression will be used to evaluate the association of each individual marker and RFS. The BH method will be used to control the FDR at 5%[40]. We will apply the random survival forest [46]and the survival SVM [13] to markers that passed the univariate analysis to generate a prognostic model to predict 1-year RFS. We will also use a bootstrap method in combination with the machine learning algorithm in marker selection. We will cross check the results of both methods for consistencies. The SN, SP, and accuracy will be calculated for each model and compared by McNemar tests between each pair of models. To avoid over fitting of the data, 10x cross validation within the training set will be used. In addition, ***the final models will be validated in the validation set.*** Specifically, the model parameters estimated using the training data will be applied to the validation set. For the survival model, the HR low-, intermediate-, and high-risk

groups (cutoff at the 33% and 67% percentiles of the score) will be calculated. The log rank test will be used to compare the RFS between the groups. The discrimination ability of the risk score on 1-year RFS will be assessed by time-dependent ROC analysis. *The same strategies will be used in the analysis of OS.*

Power Calculation for Predictive Modeling

The goal here is to determine whether there is a significant difference between the clinical benefits of patients in different marker strata. That is, we are testing the interaction of the dichotomized marker value (we discuss how to dichotomize the marker value in details in our analysis plan in the following section) and the treatment group. We calculate our power based on the method by Peterson and George. [36]

We denote the hazard function for each patient subgroup as the following:

	Marker stratum 1	Marker stratum 2
HDI	$\lambda_{11} = \lambda(t)$	$\lambda_{12} = \lambda(t)\lambda_{Marker}(t)$
Ipilimumab	$\lambda_{21} = \lambda(t)\lambda_{Ipi}(t)$	$\lambda_{22} = \lambda_{Ipi}(t)\lambda_{Marker}(t)\lambda_{Interaction}(t)$

where

$\lambda(t)$ is the baseline hazard

$\lambda_{Ipi}(t)$ is the hazard for the Ipilimumab group

$\lambda_{Marker}(t)$ is the hazard for marker stratum2 and

$\lambda_{Interaction}(t)$ is the hazard for the treatment by marker stratum interaction.

Denote N the sample size, f_{ij}, the number of subjects in treatment group i and marker stratum j, and e_{ij}, the event probability in treatment group i and marker stratum j. Define that $\Delta = \frac{\Delta_1}{\Delta_2} = \frac{\lambda_{11}/\lambda_{21}}{\lambda_{12}/\lambda_{22}}$, then the power to test the null hypothesis of $\Delta = 1$ can be calculated using the following formula:

$$Power = \Phi^{-1}\left(\sqrt{N(\log\Delta)^2 / \sum \frac{1}{e_{ij}f_{ij}}} - Z_{1-\alpha/2}\right)$$

- $\alpha = 0.01$: we do not intend to validate more than 5 predictive markers (and/or panels) in the validation set, thus, our type I error is set at 0.05/5=0.01. All tests will be two-sided.

- For the purpose of power calculation, we assume the RFS time follows an exponential distribution.
- N: 250 on the ipilimumab arm + 300 on the HDI arm. We expect that we will be able to obtain samples from approximately 80% of the subjects. We expect that the assay success rate is at least 95%. Thus N is estimated to be 418.
- f_{ij}: since this is a randomized trial, we assumed that the marker distribution in the two arms are balanced. Thus we may estimate $f_{ij} = t_i \times g_j$ where t_i and g_j are the frequencies of treatment *i* and marker stratum *j*. In our data, $t_1 = 300/550$ **and** $t_2 = 250/550$, we calculate the power for g_1 in the range of 50%-70%.
- e_{ij}: with the exponential failure and uniformed accrual assumption, e_{ij} is calculated based the accrual rate, minimum follow up time and λ_{ij}. The accrual rate for E1609 is approximately 40/month, by the time of the analysis we will have at least 1 year of follow up for all the subjects.
- λ_{ij}'s : determine the effect size Δ .Although λ_{ij} (hazard rate for patients in treatment arm *i* marker stratum *j*, which is unknown. However, the hazard rate for treatment arm *i*, λ_i, can be deduced from the E1609 protocol. It was assumed that the RFS function follow a curate model of the following form: $D_A(t) = p + (1-p)\exp(-\beta t)$, where *p*=0.29, and the median RFS for the uncured patients on HDI arm is assumed to be 0.487 year. Thus $\beta = -\log(2)/0.487 = 1.423$. Set $D_A(t_{0.5}) = 0.5$, we estimate that the median survival time for HDI group to be 0.756 year. For the purpose of power calculation, we use exponential distribution to approximate the RFS function in each treatment group. Thus, $\lambda_1 = \log(2)/0.756 = 0.917$. It was assumed that a HR of 0.75 will be observed between the ipilimumab arm and the HDI arm, thus, $\lambda_2 = 0.75 \times \lambda_1 = 0.688$. We further approximate $\lambda_i = \sum_j g_j \times \lambda_{ij}$.

  The following table listed the power for different marker stratum 1 frequencies (g_1), and different effect sizes. We only considered marker with qualitative interaction with the treatment, assuming that patients in marker stratum 1 benefit from the Ipilimumab and those in marker will benefit from HDI. Thus, we consider the situations where $\lambda_{11} > \lambda_{21}$ and $\lambda_{12} < \lambda_{22}$ only. We set λ_{11} **and** λ_{21} within the range of 0.7-1.2. The SWOG webtool (http://stattools.crab.org) was used for the power calculation. Here we assume the accrual time is 40 months (Accrual=40/12=3.3), minimum follow up is 12 months (Follow=12/12=1), Cell Freq Treat1/Strat1= f_{11} , Cell Freq Treat1/Strat2= f_{12}, Cell Freq Treat2/Strat1= f_{21}, Cell Freq Treat2/Strat2= f_{22}, Hazard Rate Treat1/Strat1=λ_{11} , Hazard Rate Treat1/Strat2=λ_{12} , Haz Ratio T1/T2 STrat1=Δ_1, and Haz Ratio T1/T2 Strat2=Δ_2 .

  Table 1 Power calculation for test of interaction ($\alpha = 0.01$, two-sided)

Marker stratum 1 Freq. (g_1)	f_{11}	f_{12}	f_{21}	f_{22}	λ_{c1}	λ_{c2}	λ_{e1}	λ_{e2}	Δ_1	Δ_2	Δ	Power
0.5	0.273	0.273	0.227	0.227	1.1	0.734	0.6	0.776	1.833	0.946	1.938	0.69
					1	0.834	0.5	0.876	2	0.952	2.101	0.8
					1.15	0.684	0.6	0.776	1.917	0.881	2.174	0.84
					1.05	0.784	0.5	0.876	2.1	0.895	2.346	0.91
					1.15	0.684	0.55	0.826	2.091	0.828	2.525	0.95
					1.2	0.634	0.5	0.876	2.4	0.724	3.316	1
0.6	0.327	0.218	0.273	0.182	1.05	0.718	0.6	0.82	1.75	0.875	2	0.72
					1	0.793	0.55	0.895	1.818	0.885	2.053	0.76
					0.95	0.868	0.5	0.97	1.9	0.894	2.124	0.8
					1	0.793	0.5	0.97	2	0.817	2.448	0.93
					1.1	0.643	0.5	0.97	2.2	0.662	3.321	1
0.7	0.382	0.164	0.318	0.136	1	0.723	0.6	0.893	1.667	0.81	2.058	0.69
					1.2	0.257	0.8	0.427	1.5	0.602	2.494	0.79
					1	0.723	0.55	1.01	1.818	0.716	2.539	0.92
					1.15	0.373	0.7	0.66	1.643	0.566	2.904	0.95
					1.05	0.607	0.5	1.127	2.1	0.538	3.9	1

REFERENCES

1. Tarhini AA, Edington H, Butterfield LH, et al. Immune monitoring of the circulation and the tumor microenvironment in patients with regionally advanced melanoma receiving neoadjuvant ipilimumab. PLoS One 2014;9:e87705.
2. Tarhini AA, Edington H, Butterfield LH, et al. Neoadjuvant ipilimumab in locally/regionally advanced melanoma: clinical outcome and immune monitoring. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2012;30:(suppl; abstr 8533).
3. Harlin H, Meng Y, Peterson AC, et al. Chemokine expression in melanoma metastases associated with CD8+ T-cell recruitment. Cancer research 2009;69:3077-85.
4. Louahed J, Gruselle O, Gaulis S, al. e. Expression of defined genes identified by pre-treatment tumor profiling: association with clinical responses to the GSK MAGE-A3 immunotherapeutic in metastatic melanoma patients. J Clin Oncol. 2008;26(suppl):Abstract 9045. 2008.

5. Vansteenkiste JF, Zielinski M, Dahabreh IJ, al. e. Association of gene expression signature and clinical efficacy of MAGE-A3 antigen-specific cancer immunotherapeutic (ASCI) as adjuvant therapy in resected stage IB/II non-small cell lung cancer (NSCLC). J Clin Oncol. 2008;26(suppl): Abstract 7501. . 2008.
6. Ji RR, Chasalow SD, Wang L, et al. An immune-active tumor microenvironment favors clinical response to ipilimumab. Cancer Immunol Immunother 2011.
7. Hamid O, Schmidt H, Nissan A, et al. A prospective phase II trial exploring the association between tumor microenvironment biomarkers and clinical activity of ipilimumab in advanced melanoma. J Transl Med 2011;9:204.
8. Sigalotti L, Covre A, Fratta E, et al. Epigenetics of human cutaneous melanoma: setting the stage for new therapeutic strategies. J Transl Med 2010;8:56.
9. Sigalotti L, Coral S, Fratta E, et al. Epigenetic modulation of solid tumors as a novel approach for cancer immunotherapy. Semin Oncol 2005;32:473-8.
10. Sigalotti L, Fratta E, Bidoli E, et al. Methylation levels of the "long interspersed nucleotide element-1" repetitive sequences predict survival of melanoma patients. J Transl Med 2011;9:78.
11. Galon J, Costes A, Sanchez-Cabo F, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006;313:1960-4.
12. Spranger S, Spaapen RM, Zha Y, et al. Up-regulation of PD-L1, IDO, and T(regs) in the melanoma tumor microenvironment is driven by CD8(+) T cells. Sci Transl Med 2013;5:200ra116.
13. Kline J, Brown IE, Zha YY, et al. Homeostatic proliferation plus regulatory T-cell depletion promotes potent rejection of B16 melanoma. Clinical cancer research : an official journal of the American Association for Cancer Research 2008;14:3156-67.
14. Chen SH, Pham-Nguyen KB, Martinet O, et al. Rejection of disseminated metastases of colon carcinoma by synergism of IL-12 gene therapy and 4-1BB costimulation. Mol Ther 2000;2:39-46.
15. Curran MA, Montalvo W, Yagita H, Allison JP. PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. Proc Natl Acad Sci U S A 2010;107:4275-80.
16. Erdag G, Schaefer JT, Smolkin ME, et al. Immunotype and immunohistologic characteristics of tumor-infiltrating immune cells are associated with clinical outcome in metastatic melanoma. Cancer research 2012;72:1070-80.
17. Hodi FS, Butler M, Oble DA, et al. Immunologic and clinical effects of antibody blockade of cytotoxic T lymphocyte-associated antigen 4 in previously vaccinated cancer patients. Proceedings of the National Academy of Sciences of the United States of America 2008;105:3005-10.
18. Sasaki A, Tanaka F, Mimori K, et al. Prognostic value of tumor-infiltrating FOXP3+ regulatory T cells in patients with hepatocellular carcinoma. European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology 2008;34:173-9.
19. Shen Z, Zhou S, Wang Y, et al. Higher intratumoral infiltrated Foxp3+ Treg numbers and Foxp3+/CD8+ ratio are associated with adverse prognosis in resectable gastric cancer. Journal of cancer research and clinical oncology 2010;136:1585-95.

20. Hakansson A, Gustafsson B, Krysander L, Hakansson L. Tumour-infiltrating lymphocytes in metastatic malignant melanoma and response to interferon alpha treatment. Br J Cancer 1996;74:670-6.
21. Mihm MC, Jr., Clemente CG, Cascinelli N. Tumor infiltrating lymphocytes in lymph node melanoma metastases: a histopathologic prognostic indicator and an expression of local immune response. Lab Invest 1996;74:43-7.
22. Moschos SJ, Edington HD, Land SR, et al. Neoadjuvant treatment of regional stage IIIB melanoma with high-dose interferon alfa-2b induces objective tumor regression in association with modulation of tumor infiltrating host cellular immune responses. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2006;24:3164-71.
23. Tarhini AA, Cherian J, Moschos SJ, et al. Safety and efficacy of combination immunotherapy with interferon alfa-2b and tremelimumab in patients with stage IV melanoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2012;30:322-8.
24. Tarhini AA, Moschos SJ, Tawbi H, et al. Phase II evaluation of tremelimumab (Treme) combined with high-dose interferon alpha-2b (HDI) for metastatic melanoma. J Clin Oncol 28:15s, (suppl; abstr 8524) 2010.
25. Tarhini AA, Butterfield LH, Shuai Y, Gooding WE, Kalinski P, Kirkwood JM. Differing patterns of circulating regulatory T cells and myeloid-derived suppressor cells in metastatic melanoma patients receiving anti-CTLA4 antibody and interferon-alpha or TLR-9 agonist and GM-CSF with peptide vaccination. J Immunother 2012;35:702-10.
26. Carthon BC, Wolchok JD, Yuan J, et al. Preoperative CTLA-4 blockade: tolerability and immune monitoring in the setting of a presurgical clinical trial. Clinical cancer research : an official journal of the American Association for Cancer Research 2010;16:2861-71.
27. Ugel S, Peranzoni E, Desantis G, et al. Immune tolerance to tumor antigens occurs in a specialized environment of the spleen. Cell Rep 2012;2:628-39.
28. Marshall M, Ribas A, Huang B. Evaluation of baseline serum C-reactive protein (CRP) and benefit from tremelimumab compared to chemotherapy in first-line melanoma. J Clin Oncol (2010 ASCO Annual Meeting Proc) 2010; 28: abstract 2609. 2010.
29. Tarhini AA, Stuckert J, Lee S, Sander C, Kirkwood JM. Prognostic significance of serum S100B protein in high-risk surgically resected melanoma patients participating in Intergroup Trial ECOG 1694. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2009;27:38-44.
30. Yurkovetsky ZR, Kirkwood JM, Edington HD, et al. Multiplex analysis of serum cytokines in melanoma patients treated with interferon-alpha2b. Clinical cancer research : an official journal of the American Association for Cancer Research 2007;13:2422-8.
31. Kirkwood JM, Ibrahim JG, Sondak VK, et al. High- and low-dose interferon alfa-2b in high-risk melanoma: first analysis of intergroup trial E1690/S9111/C9190. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2000;18:2444-58.
32. Kirkwood JM, Richards T, Zarour HM, et al. Immunomodulatory effects of high-dose and low-dose interferon alpha2b in patients with high-risk resected melanoma: the E2690 laboratory corollary of intergroup adjuvant trial E1690. Cancer 2002;95:1101-12.

33. Kirkwood JM, Manola J, Ibrahim J, Sondak V, Ernstoff MS, Rao U. A pooled analysis of eastern cooperative oncology group and intergroup trials of adjuvant high-dose interferon for melanoma. Clinical cancer research : an official journal of the American Association for Cancer Research 2004;10:1670-7.
34. Loukas M, Lanteri A, Ferrauiola J, et al. Anatomy in ancient India: a focus on the Susruta Samhita. J Anat 2010;217:646-50.
35. Pepe MS. The Statistical Evaluation of Medical Tests for Classification and Prediction. New York: Oxford University Press; 2003.
36. Peterson B, George SL. Sample size requirements and length of study for testing interaction in a 2 x k factorial design when time-to-failure is the outcome [corrected]. Control Clin Trials 1993;14:511-22.
37. Janes H, Pepe MS, Bossuyt PM, Barlow WE. Measuring the Performance of Markers for Guiding Treatment Decisions. Annals of Internal Medicine 2011;154:253-W80.
38. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J R Statist Soc B 1995;57:289-300.
39. Storey JD. A direct approach to false discovery rates. J R Statist Soc B 2002;64:479-98.
40. Benjamini Y, Hochberg, Yosef. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society B 1995;57:289-300.
41. Tibshirani R. Regression Shrinkage and Selection via the Lasso. Journal of the Royal Statistical Society B 1996;58:267-88.
42. Tibshirani R. The lasso method for variable selection in the Cox model. Stat Med 1997;16:385-95.
43. Friedman J, Hastie T, Tibshirani R. Regularization Paths for Generalized Linear Models via Coordinate Descent. J Stat Softw;33:1-22.
44. Heagerty PJ, Lumley T, Pepe MS. Time-dependent ROC curves for censored survival data and a diagnostic marker. Biometrics 2000;56:337-44.
45. Evers L, Messow CM. Sparse kernel methods for high-dimensional survival data. Bioinformatics 2008;24:1632-8.
46. Ishwaran H, Kogalur, UB, Blackstone, Lauer, MS. Random Survival Forest. The Annals of Applied Statistics 2008;2:841-60.