SUMMARY OF CHANGES

For Protocol Amendment # 6

NCI Protocol #: 9209
Local Protocol #: PHL-085
Protocol Version / Date: Initial / 13Jul2012
A1 / 07Nov2012
A2 / 29Aug2013
A3 / 30Apr2014
A4 / 01Aug2014
A5 / 30Sep2014
A6 / 31Jan2018

The following revision is in response to CTEP CTCAE v4.0 to CTCAEv5.0 Conversion request letter dated Jan 12/2018.

#	Section	Page(s)	Change
1.	7.2	45	Changes (highlighted text inserted, ~~strikethrough~~ text deleted): **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized ~~for AE reporting~~ until March 31, 2018 for AE reporting. CTCAE version 5.0 will be utilized for AE reporting beginning April 1, 2018. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0~~4.0~~. A copy of the CTCAE version 5.0~~4.0~~ can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. **Rationale:** CTCAE version has been updated as per CTEP request letter dated Jan 12/2018

NCI Protocol #: 9209
Local Protocol #: PHL-085
Protocol Version / Date: Initial / 13Jul2012
A1 / 07Nov2012
A2 / 29Aug2013
A3 / 30Apr2014
A4 / 01Aug2014
A5 / 30Sep2014
A6 / 31Jan2018

TITLE: A Phase 2 Study of Ipilimumab in women with metastatic or recurrent HPV-related cervical carcinoma of either squamous cell or adenocarcinoma histologies

Coordinating Center: The Princess Margaret Hospital Phase II Consortium (PMHC)

***Principal Investigator:** Dr. Amit Oza
610 University Avenue
Princess Margaret Hospital
Toronto Ontario M5G2M9
Phone: 416-946-2818
Fax: 416-946-4467
amit.oza@uhn.ca

Co-Investigators: Dr. Katia Tonkin
11560 University Ave
Cross Cancer Institute
Edmonton Alberta T6G 1Z2
Phone: 780-432-8514
Fax: 780-432-8888
katia.tonkin@albertahealthservices.ca

Dr. Hal Hirte
Juravinski Cancer Centre
699 Concession St.
Hamilton Ontario L8V 5C2
Phone: (905) 387-9495
Fax: (905) 575-6326
hal.hirte@hrcc.on.ca

Dr. Stephen Welch
London Regional Cancer Program
790 Commissioners Road East
London Ontario N6A 4L6
Phone: (519) 685-8640
Fax: (519) 685-8624

Stephen.welch@lhsc.on.ca

Dr. Anna Tinker
BC Cancer Agency
600 West Tenth Avenue
Vancouver British Columbia V5Z 4E6
Phone: (604) 877-6000
Fax: (604) 877-6217atinker@bccancer.bc.ca

Dr. Prafull Ghatage
Tom Baker Cancer Centre
1331-29th Street, NW
Calgary Alberta T2N 4N2
Phone: (403) 521-3721
Fax: (403) 521-3593
Prafull.Ghatage@albertahealthservices.ca

Dr. Johanne Weberpals
Ottawa Regional Cancer Centre
501 Smyth Road
Ottawa Ontario
Phone: (613) 737-8899 x76462
Fax: (613) 737-8828
jweberpals@Ottawahospital.on.ca

Dr. Lainie Martin
Fox Chase Cancer Center
333 Cottman Ave, Room C307
Philadelphia Pensylvannia 19111-2497
Phone: (215)728-3889
Fax: (215)728-3639
lainie.Martin@fccc.edu

Dr. Mihaela Cristea
City of Hope
1500 E. Duarte Road
Duarte, CA 91010
Phone: (626) 256-4673
Fax: (626) 930-5461
mcristea@coh.org

Dr. Stephen Koehler
City of Hope Medical Group, Inc.
209 Fair Oaks Avenue
South Pasadena, CA 91030
Phone: (626) 396-2900
Fax: (626) 396-2770
Skoehler@cohmg.com

Dr. Edwin Alvarez
University of California, Davis Cancer Center
4860 Y Street, Suite 2500
Sacromento, CA 95817
Ph: 916-734-6900
Fax: 916-734-6666
Email: edwin.alvarez@ucdmc.ucdavis.edu

Dr. Gini Fleming
University of Chicago Medical Center
5841 South Maryland Ave., MC 2115
Chicago, IL 60637
Phone: (773) 702-6712
Fax: (773) 702-0963
gfleming@medicine.bsd.uchicago.edu

Statistician:
Lisa Wang
Princess Margaret Hospital
610 University Avenue
Toronto Ontario M5G 2M9
Phone: 416-946-4501 ext. 4883
Fax: 416-946-2048
lisawang@uhnres.utoronto.ca

Study Coordinator:
Smitha Udagani
Princess Margaret Hospital
610 University Avenue
Toronto, Ontario M5G 2M9
Phone: (416) 946-4501 ext. 3408
Fax: (416) 946-2016
smitha.udagani@uhn.ca

Responsible Data Manager:
Chantale Blattler
Princess Margaret Hospital
610 University Avenue
Toronto Ontario M5G 2M9
Phone: 416-946-4501 ext. 3692
Fax: 416-946-2016
chantale.blattler@uhn.ca

NCI Supplied Agent(s): Ipilimumab (NSC 732442)

SCHEMA

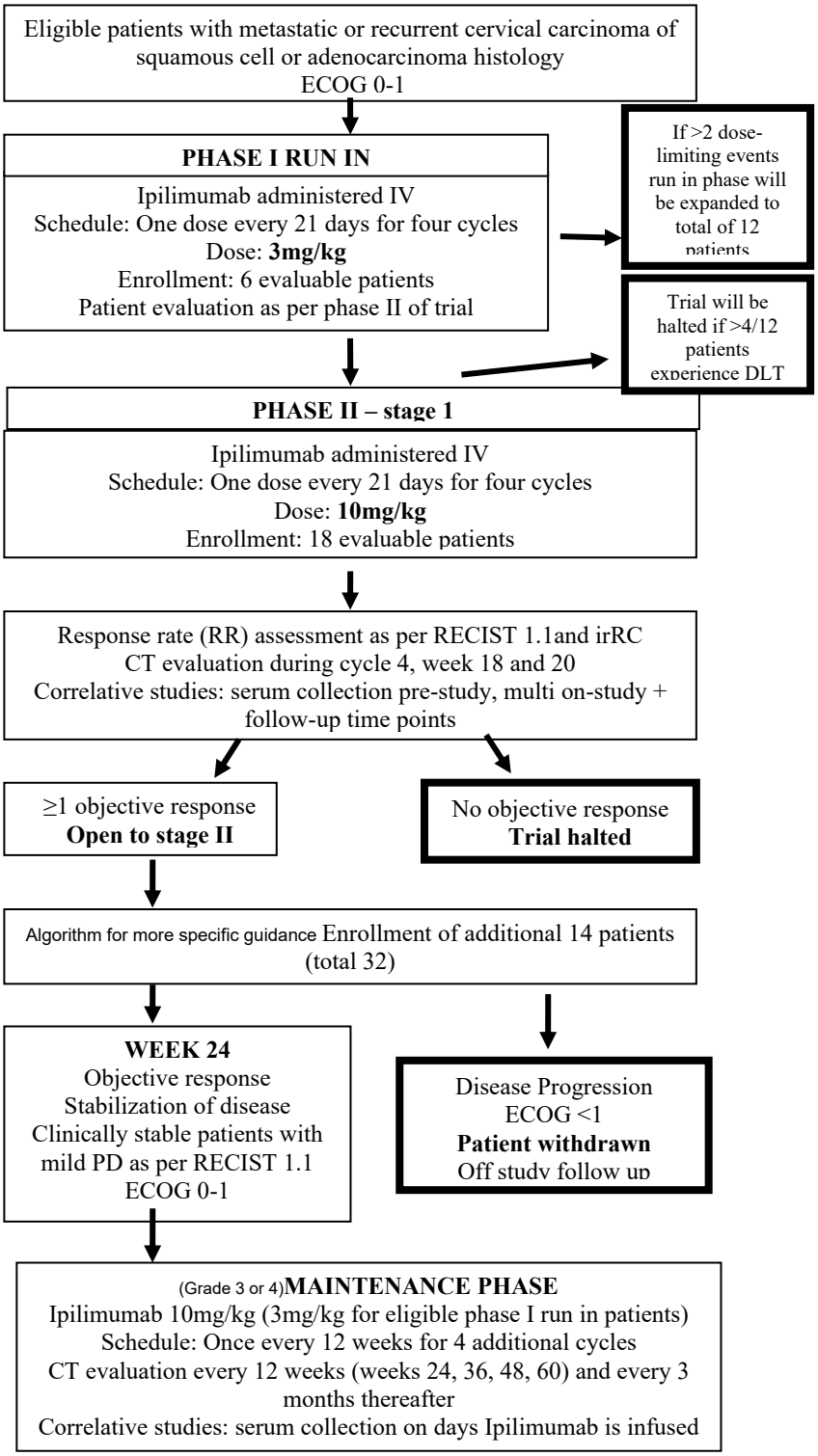

Eligible patients with metastatic or recurrent cervical carcinoma of squamous cell or adenocarcinoma histology
ECOG 0-1
PHASE I RUN IN
Ipilimumab administered IV
Schedule: One dose every 21 days for four cycles
Dose: 3mg/kg
Enrollment: 6 evaluable patients
Patient evaluation as per phase II of trial
If >2 dose-limiting events run in phase will be expanded to total of 12 patients
Trial will be halted if >4/12 patients experience DLT
PHASE II – stage 1
Ipilimumab administered IV
Schedule: One dose every 21 days for four cycles
Dose: 10mg/kg
Enrollment: 18 evaluable patients
Response rate (RR) assessment as per RECIST 1.1and irRC
CT evaluation during cycle 4, week 18 and 20
Correlative studies: serum collection pre-study, multi on-study + follow-up time points
≥1 objective response
Open to stage II
No objective response
Trial halted
Algorithm for more specific guidance Enrollment of additional 14 patients (total 32)
WEEK 24
Objective response
Stabilization of disease
Clinically stable patients with mild PD as per RECIST 1.1
ECOG 0-1
Disease Progression
ECOG <1
Patient withdrawn
Off study follow up
(Grade 3 or 4)MAINTENANCE PHASE
Ipilimumab 10mg/kg (3mg/kg for eligible phase I run in patients)
Schedule: Once every 12 weeks for 4 additional cycles
CT evaluation every 12 weeks (weeks 24, 36, 48, 60) and every 3 months thereafter
Correlative studies: serum collection on days Ipilimumab is infused

TABLE OF CONTENTS

1. OBJECTIVES

1.1 Primary Objectives

- To assess the safety of Ipilimumab in eligible patients with recurrent or metastatic cervical cancer
- To assess the antitumor activity of Ipilimumab in eligible patients with recurrent or metastatic cervical cancer via assessment of objective response rates (ORR).

1.2 Secondary Objectives

- To assess the antitumor activity of Ipilimumab through secondary endpoints including of disease stabilization and PFS.
- Assessment of antitumor activity of Ipilimumab using immune-related response criteria (irRC)
- Assessment of the predictive value of baseline C-reactive protein
- Assess the biologic responses of exposure to Ipilimumab via correlative studies involving analysis of lymphocyte subsets and assessment of cervical cancer-antigen specific T cells anti-tumor response.
- Evaluation of archival tissue with regard to markers of immune population in correlation with clinical stage and response to treatment

2. BACKGROUND

2.1 Study Disease – Cervical Cancer

Cervical cancer is the second leading cause of cancer-related mortality in women world-wide. Over half a million new cases of the disease are diagnosed annually resulting in approximately 260,000 deaths [Frazer 2004]. The incidence of cervical cancer world-wide is not expected to decrease in the near future, despite the existence of the well-established Pap smear screening tool and the recent introduction of effective prophylactic vaccination. Therefore, there is an ongoing need for the development of effective treatments for women suffering from invasive carcinoma of the cervix.

<u>Cervical cancer and Human Papillomavirus (HPV)</u>

Strong epidemiological and molecular evidence have established the importance of human papillomavirus (HPV) in the development of cervical cancer [Munoz 2000]. Persistent infection with a high-risk HPV genotype leads to a substantial increase in the risk of cervical intraepithelial neoplasia (CIN), which if untreated will progress to invasive cancer. HPV infection is most clearly associated with squamous cell carcinoma (SCC) but has also been reported in women diagnosed with adenocarcinoma of the cervix [Frazer 2004].

The HPV virus has a double-stranded DNA genome surrounded by a protein capsid comprising a highly immunogenic L1 protein [Legatt, Frazer 2007]. Following binding to the basement

membrane, viral uptake into basal keratinocytes occurs. These epithelial cells support the replication of the viral episome and cell transformation occurs following the integration of the viral genome into the host cell DNA. The non-structural viral proteins E6 and E7 are considered oncoproteins causing cell transformation by inhibiting cell apoptosis and by promoting degradation of p53 thereby facilitating continued cell proliferation despite cell damage presence [Bhat, Mattarollo, Gosmann et al.].

The oncoproteins E6 and E7 are consistently expressed in cervical cancer cells and as such may be considered to be tumor-associated antigens (TAA). Given that these proteins are absent from normal cells and selectively expressed in tumor cells they can serve as logical targets for the cell-mediated immune response. Equally, they become potential targets for immunotherapy strategies [Valdespino, gorodezky, Vianney et al. 2005].

The rationale for immunotherapy based treatments in cervical cancer

The etiological link between HPV and cervical cancer is undisputed. Epidemiologic data demonstrates a significant increased risk of HPV-related cancer development in immunosuppressed individuals. An increased incidence of cervical dysplasia, genital papillomavirus infection and lower genital neoplasia is well documented in individuals infected by the human immunodeficiency virus (HIV) as well as renal transplant patients receiving long term immunosuppressive medications [Laga, Icenogle, Marsella et al. 1992; Halpert, Fruchter, Sedlis et al. 1986]. These observations suggest that the immune system is central to the prevention of development of these tumors when functioning appropriately.

The natural humoral response to HPV viral capsids can be detected in 50-70% of individuals by 6 months post-infection. Some individuals never seroconvert and whilst a proportion of individuals continue to demonstrate high levels of antibody post infection, this does not appear to prevent future infections [Insinga, Perez, Wheeler et al 2010]. Despite this poor natural humoral response, systemic administration of HPV capsid protein in combination with adjuvant elicits a vigorous host-antibody response in most individuals. This response is effective in preventing persistent HPV infection as well as CIN and malignancy development. It forms the basis for the recently introduced prophylactic vaccinations and further strengthens the belief that immune modulation may serve as an effective treatment strategy in this disease setting [harper, Franco, Wheeler et al 2006; Munoz, Manalastas, Pipisuttithum et al. 2009].

Data suggests that HPV infection does invoke a cellular immune response. Several groups have demonstrated the presence of T cell infiltrates following HPV infection but variability of results is such that the exact role of T-helper cells and cytotoxic T-cells in the immune modulation of this infection remain only partially understood. CD4 T cell infiltrates have been associated with HPV persistence and linked with increasing cervical dysplasia [Molling, deGrijl, Glim et al. 2007; Jaafar, Righi, Lindstrom et al. 2009]. In contrast, CD8 T cells appear abundant in regressing lesions [Woo, van den Hende, Sterling et al. 2010]. These data collectively suggest that cytotoxic T cells may limit disease progression whereas regulatory T cells may be involved in creating a tolerant immunologic environment. This in turn may contribute to viral immune evasion and the persistence of HPV infection resulting in progression of disease [Bhat, Mattarollo, Gosmann et al 2011]. Immune modulating therapies which may be formulated to enhance the innate immune response to cervical cancer cells or alternatively to repress immuno-

protective pathways may be a successful therapeutic strategy to combat this disease.

2.2 CTEP IND Agents

2.2.1 Ipilimumab

Ipilimumab (MDX-010, MDX-CTLA4, and BMS-734016) is being developed by CTEP as an anticancer agent in collaboration with Bristol-Myers-Squibb (BMS). On March 25, 2011, the FDA approved Ipilimumab injection (YERVOY, BMS) for the treatment of unresectable or metastatic melanoma. Ipilimumab is a human $IgG_1\kappa$ monoclonal antibody (mAb); it is specific for human cytotoxic T lymphocyte-associated antigen-4 (CTLA-4, CD152) expressed on activated T cells. Ipilimumab is now produced and formulated from transfected Chinese hamster ovary (CHO) cells.

CTLA-4 is a negative regulator of T-cell responses following T-cell stimulation (Thompson and Allison, 1997; Kuhns *et al*., 2000). CTLA-4 knockout mice suffer from a fatal lymphoproliferative disorder, supporting the idea that CTLA-4 functions as a negative regulator of T-cell responses *in vivo* (Tivol *et al*., 1995; Waterhouse *et al*., 1995; Chambers *et al*., 1997). Disrupting CTLA-4 interaction with its ligands B7-1 (CD80) and B7-2 (CD86), which are expressed on antigen-presenting cells (APCs), with Ipilimumab, augments immune responses (Investigator Brochure, 2011). *In vivo* blockade of CTLA-4, utilizing anti-CTLA-4 mAb, induced regression of established tumors and enhanced antitumor immune responses in several murine tumor models. Blockade of CTLA-4-mediated signals is effective in inducing rejection of immunogenic cancers in mice. Moreover, when anti-CTLA-4 mAb is used in conjunction with granulocyte macrophage-colony stimulating factor (GM-CSF)-secreting tumor vaccines, poorly immunogenic cancers in mice are rejected. These findings suggest that CTLA-4 blockade, alone or in combination with antigenic stimulation and other immune modulating agents can induce a potent antitumor response.

Pharmacology of Ipilimumab

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 or B7.2 negative cell lines, and stains the appropriate cells without non-specific cross-reactivity in normal human tissues. Ipilimumab does cross-react with CTLA-4 in non-human primates including cynomolgus monkeys. Blockade of CTLA-4/B7 interactions enhanced T-cell responses to CD3 / CD28, peptide antigens, or super antigens in mice (Walunas, *et al.,* 1994; Kearney, *et al.,* 1995; Krummel and Allison 1995; Krummel, Sullivan, and Allison, 1996). 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. Genetically engineered mice heterozygous for CTLA-4 (CTLA-4+/-), appeared healthy and gave birth to healthy CTLA-4+/- heterozygous offspring. Mated CTLA-4+/- heterozygous mice also produced offspring deficient in CTLA-4 (homozygous negative, CTLA-4-/-). 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 post- thymic T-cell responses in the periphery following stimulation of naïve, memory, and effector T cells (Krummel, Sullivan, and Allison, 1996).

Pharmacokinetics

The pharmacokinetics (PK) of Ipilimumab was studied in 499 patients with unresectable or metastatic melanoma who received doses of 0.3, 3, or 10 mg/kg administered once every 3 weeks (q3w) for four doses. Peak concentration (Cmax), trough concentration (Cmin), and area under the curve (AUC) of Ipilimumab were found to be dose proportional within the dose range examined. Upon repeated dosing of Ipilimumab administered q3w, Ipilimumab clearance was found to be time-invariant, and minimal systemic accumulation was observed as evident by an accumulation index of 1.5-fold or less. Ipilimumab steady-state concentration was reached by the third dose. The following mean (percent coefficient of variation) parameters were generated through population PK analysis: terminal half-life of 14.7 days (30.1%); systemic clearance (CL) of 15.3 mL/h (38.5%); and volume of distribution at steady-state (Vss) of 7.21 L (10.5%). The mean (±SD) Ipilimumab Cmin achieved at steady-state with the 3-mg/kg regimen was 21.8 mcg/mL (±11.2).

Specific Populations: Cross-study analyses were performed on data from patients with a variety of conditions, including 420 patients with melanoma who received single or multiple infusions of Ipilimumab at doses of 0.3, 3, or 10 mg/kg. The effects of various covariates on Ipilimumab PK were assessed in population PK analyses.
Ipilimumab CL increased with increasing body weight; however, no dose adjustment of Ipilimumab is required for body weight after administration on a mg/kg basis.
The following factors had no clinically meaningful effect on the CL of Ipilimumab: age (range 26 to 86 years), gender, concomitant use of budesonide, performance status, HLA-A2*0201 status, positive anti-Ipilimumab antibody status, prior use of systemic anticancer therapy, or baseline lactate dehydrogenase (LDH) levels. The effect of race was not examined as there were insufficient numbers of patients in non-Caucasian ethnic groups.

Renal Impairment: Creatinine clearance at baseline did not have a clinically important effect on Ipilimumab PK in patients with calculated creatinine clearance values of 29 mL/min or greater.

Hepatic Impairment: Baseline AST, total bilirubin, and ALT levels did not have a clinically important effect on Ipilimumab PK in patients with various degrees of hepatic impairment.

Clinical Pharmacodynamics

In clinical studies, Ipilimumab increased absolute lymphocytes counts (ALC) in peripheral blood (Investigator Brochure, 2011). However, CD4+/CD8+ ratio did not appear to be affected. Across three phase 2 studies in 463 subjects with advanced melanoma, Ipilimumab increased ALC in a dose-dependent manner, with the largest increase observed at 10 mg/kg dose. ALC continued to increase over time during the induction treatment at least until week 12 at the 3

mg/kg and 10 mg/kg dose, but not at the 0.3 mg/kg dose. The slope of ALC increase also suggested the 10 mg/kg dose is more biologically active than the 3.0 mg/kg or 0.3 mg/kg dose.

Mechanism of Action

The proposed mechanism of action for Ipilimumab is T-cell potentiating through interference of the interaction of CTLA-4 with B7 (CD80 or CD86) molecules on APCs, with subsequent blockade of the inhibitory function of CTLA-4 (Investigator Brochure, 2011). Ipilimumab impacts tumor cells indirectly, and measurable clinical effects emerge after the immunological effects. Tumor infiltration with lymphocytes and the associated inflammation is likely the cornerstone of the effect of Ipilimumab and can manifest in various patterns of clinical activity leading to tumor control. These immunologic responses may take time to develop and so tumor responses may be delayed and tumor progression may occur during the initial period followed by responses. In some cases, tumor response based on tumor infiltration with immune cells may be preceded by an apparent increase in initial tumor volume and/or the appearance of new lesions, which may be taken for tumor progression on radiological evaluations. Delayed responses following increasing tumor size or appearance of new lesions have been seen in approximately 10-20% of patients with metastatic melanoma. For patients who are not experiencing rapid clinical deterioration, allowing sufficient time to observe responses including disease stabilization or confirmation of progression is recommended; as discussed in the section "Overall Risk/Benefit Assessment" may allow better assessment of clinical activity and avoid unnecessarily initiating additional therapies in subjects who might be benefitting from treatment. Immune-related (ir) response criteria were developed based on these observations in patients with melanoma to systematically categorize novel patterns of clinical activity and are currently being prospectively evaluated in clinical studies.

Nonclinical Toxicology

Please note relevant toxicity for single agent Ipilimumab has been almost completely derived from clinical studies.

In a study using cynomolgus macaques, anti-melanocyte responses were observed in animals given up to four doses of 10 mg/kg Ipilimumab after receiving a melanoma cell vaccine (Keler *et al*., 2003). Depigmentation has been observed in other nonclinical immunotherapy studies that involve treatment with melanoma peptides (Hara *et al*., 1995; Naftzger *et al*., 1996; Bloom *et al*., 1997; Overwijk *et al*., 1998; Weber *et al*., 1998; Overwijk *et al*., 1999). The symptoms in animals appear to resemble vitiligo observed in clinical immunotherapy trials of melanoma patients and may be an unavoidable consequence of treatment (Rosenberg and White, 1996).

Additional repeat-dose toxicity studies conducted using cynomolgus macaques demonstrated that the IV administration of ≤30 mg/kg every 3 days for three doses, 10 mg/kg weekly for 1 month, 1 mg/kg weekly for 10 weeks, or 10 mg/kg monthly for 6 months was generally well tolerated, without significant clinical, immunotoxicological, or histopathological findings (Investigator Brochure, 2011). However, when Ipilimumab was administered in combination with another immunomodulatory antibody (BMS-663513, a fully human anti-CD137 mAb) and simian immunodeficiency virus (SIV) DNA, two immune-related adverse events (irAEs) were observed:

severe colitis requiring euthanasia in one monkey and reversible dermatitis/rash in the inguinal area and peripheral lymphadenopathy in another monkey.

Complete information on the pre-clinical toxicology studies can be found in the Ipilimumab Investigator Brochure (IB). Non-clinical toxicity assessments included *in vitro* cynomolgus monkeys alone and in the presence of vaccines. Low to moderate ADCC activity was noted at concentrations up to 50 mcg/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. No mortality or signs of toxicity were observed in three independent 14-day intravenous (IV) 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.

Clinical Development of Ipilimumab

Company-Sponsored Studies

BMS and Medarex (acquired by BMS in September 2009) have co-sponsored an extensive clinical development program for Ipilimumab, encompassing more than 4000 subjects in several cancer types in 33 completed and ongoing studies (Investigator Brochure, 2011). The focus of the clinical program is in melanoma, prostate cancer, and lung cancer, with advanced melanoma being the most comprehensively studied indication. Ipilimumab is being investigated both as single agent and in combination with other modalities such as chemotherapy, radiation therapy, and other immunotherapy.

Phase 3 programs are ongoing in melanoma and prostate cancer (Investigator Brochure, 2011). In the phase 3 combination study of Ipilimumab with glycoprotein 100 (gp100) peptide vaccine (melanoma study MDX010-20), Ipilimumab was administered at a dose of 3 mg/kg of body weight, with or without gp100 q3w for up to four treatments (Hodi *et al*., 2010). The median overall survival (OS) in the Ipilimumab plus gp100 group was 10.0 months (95% confidence interval [CI], 8.5 to 11.5 months) compared with 6.4 months (95% CI, 5.5 to 8.7 months) in the gp100-alone group (hazard ratio [HR] for death, 0.68; $p<0.001$). Grade 3 or 4 immune related events (irAEs) occurred in 10 to 15% of patients treated with Ipilimumab and in 3% of patients treated with gp100 alone.

A second phase 3 study in melanoma was reported for Ipilimumab in combination with dacarbazine versus dacarbazine alone in previously untreated advanced melanoma. CA184024 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 predominantly affected skin, GI 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.

There are two ongoing studies of Ipilimumab as adjuvant monotherapy for high-risk Stage III melanoma (CA184029 and ECOG 1609)

Two phase 3 studies are ongoing in subjects with castration-resistant prostate cancer who have received prior chemotherapy (CA184043, Ipilimumab in combination with radiation therapy) and those who are chemotherapy-naïve (CA184095, Ipilimumab monotherapy).

An ongoing large phase 2 study (CA184041) is investigating the addition of Ipilimumab to carboplatin and paclitaxel using two different schedules (concurrent and phased) in subjects with non-small cell lung cancer (NSCLC) or small cell lung cancer (SCLC) (Investigator Brochure, 2011). The results demonstrate an improved immune-related progression-free survival (irPFS) for the subjects who received combination therapy with paclitaxel/carboplatin/Ipilimumab compared with paclitaxel/carboplatin alone. The improvement in irPFS with the combination therapy met the prespecified protocol criteria for significance for both the concurrent and phased schedules (p=0.0935 and 0.0258, respectively). Using modified World Health Organization (mWHO) criteria (a key secondary endpoint), PFS was significantly improved with the phased schedule but not the concurrent schedule (p=0.0240 and 0.2502, respectively).

Other Clinical Studies with Ipilimumab

Renal cell carcinoma (RCC)

Yang and colleagues presented data on a phase 2 study of Ipilimumab conducted in patients with metastatic RCC (Yang *et al*., 2007). Sequential cohorts received either 3 mg/kg followed by 1 mg/kg or all doses at 3 mg/kg q3w. One of 21 patients receiving the lower dose had a PR. Five of 40 patients at the higher dose had PRs (95% CI, cohort response rate 4 to 27%) and responses were seen in patients who had previously not responded to IL-2. Thirty-three percent of patients experienced grade 3 or 4 irAEs. There was a highly significant association between autoimmune events and tumor regression (response rate = 30% with AE, 0% without AE). The authors concluded that CTLA-4 blockade with Ipilimumab induced cancer regression in some patients with metastatic clear cell renal cancer, even if they had not responded to other immunotherapies.

Melanoma (Ipilimumab plus bevacizumab)

At the 2011 ASCO meeting Hodi and colleagues presented results on 21 evaluable patients (22 patients enrolled) with unresectable stage III or stage IV melanoma treated with the combination of 10 mg/kg Ipilimumab and 7.5 mg/kg bevacizumab on a phase 1 study (Hodi *et al*., 2011). AEs included giant cell arteritis (1), hypophysitis (3), thyroiditis (4), grade 3-4 hepatitis (2), bilateral uveitis (2), and grade 2 colitis (2); 5 patients required systemic steroids and stopped treatment. All toxicities were resolved. Eight PRs and 6 SDs were observed. All responses were durable (>6 months). Post-treatment biopsies in 12 patients revealed activated vessel endothelium with extensive T-cell trafficking non-productive central angiogenesis, and peripheral blood monitoring revealed a marked increase in CD4/CCR7/CD45RO central memory cells in the majority of patients, not seen with Ipilimumab alone. The authors concluded that the combination of Ipilimumab with bevacizumab can be safely administered with clinical activity and correlatives suggesting synergistic effects.

Bladder cancer

Carthon and colleaugues reported immunodulatory effects following a brief exposure of anti-CTLA-4 in patients with urothelial carcinoma of the bladder requiring surgery (BMS study CA184027) (Carthon *et al*., 2010). 12 patients were enrolled (6 patients received 3 mg/kg/dose of Ipilimumab and another 6 patients received 10 mg/kg/dose for two doses prior to surgery). The treatment was found to be tolerable in the cohort of patients with 11 of 12 patients receiving both doses of antibody. Grade 1-2 diarrhea and rash were the most common drug-related AEs. The only noted grade 3 irAEs were ischemic papillopathy and diarrhea, which were both responsive to treatment with steroids.

Liakou and colleagues found that CD4 T cells from peripheral blood and tumor tissues of all bladder cancer patients treated with anti-CTLA-4 antibody had markedly increased expression of inducible costimulator (ICOS) (Liakou *et al*., 2008). These $CD4^{+}ICOS^{hi}$ T cells produced interferon-gamma (IFN-γ) and could recognize the tumor antigen NY-ESO-1. Increase in $CD4^{+}ICOS^{hi}$ cells led to an increase in the ratio of effector to regulatory T cells. The authors indicated that these immunologic changes were reported in both tumor tissues and peripheral blood as a result of treatment with anti-CTLA-4 antibody, and they may be used to guide dosing and scheduling of this agent to improve clinical responses. A sustained increased frequency of $CD4^{+}ICOS^{hi}$ T cells may serve as a biomarker of anti-CTLA-4 activity and/or of clinical benefit for patients who are being treated with this novel agent (Carthon *et al*., 2010).

Pancreatic cancer

Royal and colleagues presented the results on 27 patients (metastatic disease: 20 and locally advanced: 7) (Royal *et al*., 2010). Three subjects experienced ≥ grade 3 irAEs (colitis:1, encephalitis:1, hypophysitis:1). One subject experienced a delayed response after initial progressive disease. In this subject, new metastases after 2 doses of Ipilimumab established progressive disease. However, continued administration of the agent per protocol resulted in significant delayed regression of the primary lesion and 20 hepatic metastases with normalization of tumor markers and clinically significant improvement of performance status. The investigators concluded that single agent Ipilimumab at 3.0 mg/kg/dose was ineffective for the treatment of advanced pancreatic cancer. However, a significant delayed response in one subject of this trial suggests that immunotherapeutic approaches to pancreatic cancer deserve further exploration.

CTEP-Sponsored Studies

The DCTD, NCI, has sponsored nine studies with Ipilimumab including one pilot study (5744, lymphoma), three phase 1 studies (5708 [ovarian], 6082 [solid tumors], and 7458 [solid tumors]), one phase 1/2 study (6359 [non-Hodgkin's lymphoma]) with single agent Ipilimumab, two phase 1 combination studies in prostate cancer with GM-CSF (6032) and with prostate-specific antigen (PSA)-TRICOM vaccine (7207), one phase 2 combination study of Ipilimumab with GM-CSF (E1608, melanoma) and one phase 3 study (E1609) of adjuvant Ipilimumab therapy versus high-dose interferon alpha-2b in patients with resected high-risk melanoma.

Results from 11 patients (colon, n=3; non-Hodgkin's lymphoma, n=4; prostate, n=4) who received Ipilimumab on study 5744 included tumor regression in 2 patients with lymphoma; 1 of whom (follicular lymphoma patient) had a partial response (PR) of 14-month duration

(O'Mahony *et al*., 2007). Ipilimumab was well tolerated with predominantly grade 1/2 toxicities. One drug-related grade 3 AE was observed. Tregs, as detected by expression of $CD4^+CD25^+CD62L^+$, declined at early time points but rebounded to levels at or above baseline values at the time of the next infusion. The investigators concluded that Ipilimumab treatment depressed Treg numbers at early time points in the treatment cycle but was not accompanied by an increase in vaccine-specific CD8+ T-cell responses in these patients previously treated with a variety of investigational anticancer vaccines.

Hodi and colleagues reported preliminary results on 20 patients (11 metastatic melanoma patients and 9 metastatic ovarian carcinoma patients) on study 5708 (Hodi *et al*., 2008). None of the 11 patients from the metastatic melanoma cohort manifested grade 3 or 4 inflammatory toxicities; however, all subjects revealed mild inflammatory pathologies associated with low-level constitutional symptoms. The most common toxicity (10/11 subjects) was a grade 1-2 reticular and erythematous rash on the trunk and/or extremities that arose between 3 days and 3 weeks after antibody administration and then gradually resolved without specific intervention. Biopsies of involved skin revealed low-grade interface dermatitis, minor to moderate mononuclear infiltrates surrounding the superficial dermal vasculature, and increased mucin deposition in the papillary and reticular dermis. These pathologic features resembled those observed in mild cutaneous forms of systemic lupus erythematosis. Three PRs (range, 21-34+ months) and five events of stable disease (SD) (range, 4-25 months) were observed. One PR and three SDs (2, 4, and 6+ months) were observed in the ovarian carcinoma group. The investigators concluded that selective targeting of antitumor regulatory T cells (Treg) may constitute a complementary strategy for combination of Ipilimumab and GM-CSF-based antigen tumor cell vaccine therapy.

Results from 29 patients with malignancies that were recurrent or progressive after allogeneic hematopoietic cell transplantation (allo-HCT) demonstrated that drug was well tolerated at single doses up to 3 mg/kg (Bashey *et al*., 2009). Four patients experienced organ-specific irAEs of reversible grade 3 arthritis, grade 2 hyperthyroidism, dyspnea, and grade 4 pneumonitis. Three patients had objective responses: one PR lasting for 2 months, and two durable complete responses (CRs). Two additional patients with Hodgkin's disease who had evidence of rapid disease progression prior to Ipilimumab treatment achieved SD for 3 and 6 months, following infusion at the 3 mg/kg dose level. Median OS was 24.7 months. At a 3.0 mg/kg dose, active serum concentrations of Ipilimumab were maintained for >30 days following a single infusion. Zhou and colleagues reported immunophenotypes of peripheral blood T cells, including T-cell reconstitution, activation, and Treg expression, in 29 patients before and after a single-dose infusion of Ipilimumab (Zhou *et al*., 2011). CTLA-4 blockade by a single infusion of Ipilimumab increased $CD4^+$ and $CD4^+$HLA-DR^+ T lymphocyte counts and intracellular CTLA-4 expression at the highest dose level (3.0 mg/kg). There was no significant change in Treg cell numbers after Ipilimumab infusion. These data demonstrate that significant changes in T-cell populations occur on exposure to a single dose of Ipilimumab.

Harzstark and colleagues reported results on 36 patients with hormone refractory metastatic prostate cancer (Harzstark *et al.*, 2010). Of six patients treated with Ipilimumab at a dose of 3 mg/kg, three patients had confirmed PSA declines of ≥50%, with a time to progression (TTP) of 22, 26, and 103 weeks. One of these patients had a PR in hepatic metastases. Grade 3 IrAEs

consisted of rash in five patients, panhypopituitarism in one patient, temporal arteritis in one patient, and diarrhea in three patients. Non-irAEs included grade 3 and 4 cerebrovascular events (one patient each), grade 3 angina (one patient), grade 3 atrial fibrillation (one patient), grade 3 fatigue (four patients), and grade 5 pulmonary embolism (one patient). One patient treated at 10 mg/kg had a PSA decline of ≥50% with a TTP of 39 weeks. Higher doses of treatment with MDX-010 + GM-CSF induced the expansion of activated circulating $CD25^+$, $CD69^+$, and $CD8^+$ T cells more frequently than was seen in patients who received the same doses of either MDX-010 or GM-CSF alone (Fong *et al*., 2009). The sera screening with protein arrays showed that the treatment can induce antibody responses to the testicular antigen NY-ESO-1.

Patients with metastatic prostate cancer were treated with ProstVac vaccine and Ipilimumab before chemotherapy. The median OS for all patients on study was 31.8 months with a 74% survival probability at 24 months (Madan *et al*., 2010). The median Halabi predicted OS for all patients was 18.5 months. There was no significant difference in OS at different dose levels of antibody (range 1-10 mg). A unique effect of the vaccines on the rate of tumor growth may be a novel method to evaluate the anti-tumor effects of the vaccine (Stein *et al.,* 2011). The authors suggested that the addition of immune checkpoint inhibition may augment the clinical benefit of vaccines.

Ansell and colleagues reported data on 18 treated patients with NHL (Ansell *et al*., 2009). Two clinical responses were observed: one patient with diffuse large B-cell lymphoma (BCL) had an ongoing CR (>31 months), and one with follicular lymphoma had a PR lasting 19 months. In 5 of 16 cases tested, T-cell proliferation to recall antigens was >2 fold increased after Ipilimumab therapy. The investigators have found that blockade of CTLA-4 signaling with the use of Ipilimumab is well tolerated at the doses used. Ipilimumab has antitumor activity in patients with BCL, resulting in durable responses in a minority of patients. Ipilimumab at 3 mg/kg monthly for 4 months can be given safely and is the dose that recommended for future combination studies.

Clinical Safety

Safety Experience

The most common treatment-related AEs (those considered possibly, probably, or definitely related to study drug by the investigator) associated with the use of Ipilimumab were immune related irAEs (Investigator Brochure, 2011). The irAEs primarily involved the gastrointestinal (GI) tract (*e.g.*, diarrhea and colitis) and skin (*e.g.*, pruritus and rash), and less frequently, the liver, endocrine glands (including the thyroid, pituitary, and adrenal glands) and nervous system. IrAEs were generally managed with either symptomatic therapy (grade 1-2 events), systemic corticosteroids (grade 3-4 events), or other immunosuppressants (*e.g.*, infliximab, mycophenolate mofetil) for steroid-unresponsive GI or hepatic irAEs, as appropriate. Management of irAEs was usually paired with omission of dosing for mild or moderate events and permanent discontinuation for severe irAEs. Ipilimumab can result in severe and fatal immune-mediated reactions due to T-cell activation and proliferation. Fatalities due to GI perforation, hepatic failure, toxic epidermal necrolysis, and Guillain-Barré syndrome have been reported in clinical trials of Ipilimumab.

Clinical trials are conducted under widely varying conditions so that extrapolation to novel settings and combinations regarding rates and severity of events may be unreliable. Given the expected rate of toxicity which may require stopping study drug but may also be related to a therapeutic immunologic response, alternative DLT criteria are discussed in section 6.

Min and colleagues reported three patients who received Ipilimumab alone or combined with bevacizumab therapy and developed thyroiditis, and the first report of euthyroid Graves' ophthalmopathy (Min *et al.*, 2011). They recommend that all patients on Ipilimumab alone or combined with bevacizumab therapy have baseline thyroid function tests and careful monitoring for new onset of thyroid disease, particularly during the first 3 months of treatment. See specific events in section 5.

Safety Profile of Ipilimumab at a Dose of 10 mg/kg (Phase 2 data)

The safety profile of Ipilimumab as monotherapy over multiple doses at a dose of 10 mg/kg in 325 subjects was determined from 4 completed melanoma studies. Overall, the incidence of grade 3/4 AEs attributable to study drug was 31%. The target organ system, the incidence, and the severity of the most commonly observed irAEs vary among studies and with drug combinations. Typically, the severity but not necessarily the overall incidence increases with dose. Additional information on specific events is provided in section 7.1 and the IB:

Summary of irAE Safety Data for 10 mg/kg in Melanoma

	Total	Low-grade (Grade 1 - 2) (%)	High-grade (Grade 3 - 4) (%)	Median Time to Resolution of Grade 2 - 4 irAEs (weeks)
All irAEs	72.3	46.2	25.2	-
Skin (*e.g.*, rash, pruritus)	52.0	49.2	2.8	6.14
GI (*e.g.*, colitis, diarrhea)	37.2	24.9	12.3	2.29
Liver (*e.g.*, LFT elevations)	8.0	0.9	6.8	4.0
Endocrine (*e.g.*, hypophysitis, hypothyroid)	6.2	3.7	2.5	20.1

Pregnancy

Preliminary results are available in cynomolgus monkeys. Pregnant monkeys received Ipilimumab every 21 days from the onset of organogenesis in the first trimester through delivery, at dose levels either 2.6 or 7.2 times higher than the clinical dose of 3 mg/kg of Ipilimumab (by AUC). No treatment-related adverse effects on reproduction were detected during the first two trimesters of pregnancy. Beginning in the third trimester, the Ipilimumab groups experienced higher incidences of abortion, stillbirth, premature delivery (with corresponding lower birth weight), and higher incidences of infant mortality in a dose-related manner compared to controls. Based on animal data, Ipilimumab may cause fetal harm. The use of Ipilimumab during human pregnancy has not been formally studied in clinical trials. There have been 7 known pregnancies during Ipilimumab treatment: in 3 female subjects and in the partners of 4 male study subjects. Two (2) of the 3 female pregnancies ended with elected terminations. The third female subject had a history of seizures and delivered the baby at 36 weeks gestation. The baby had respiratory complications that resolved by birth week 16. Three (3) of the 4 partners of male study subjects

had full term, normal babies. The fourth baby had small ureters, which are expected to grow as the baby matures. Although these outcomes do not indicate that stillbirths or other severe abnormalities will occur, pregnancy should be avoided during treatment with Ipilimumab.

Immunogenicity

In clinical studies, 1.1% of 1024 evaluable patients tested positive for binding antibodies against Ipilimumab in an electrochemiluminescent (ECL) based assay. This assay has substantial limitations in detecting anti-Ipilimumab antibodies in the presence of Ipilimumab. Infusion-related or peri-infusional reactions consistent with hypersensitivity or anaphylaxis were not reported in these 11 patients nor were neutralizing antibodies against Ipilimumab detected. Because trough levels of Ipilimumab interfere with the ECL assay results, a subset analysis was performed in the dose cohort with the lowest trough levels. In this analysis, 6.9% of 58 evaluable patients, who were treated with 0.3 mg/kg dose, tested positive for binding antibodies against Ipilimumab. These results are highly dependent on methodology, and comparison of incidence of antibodies to Ipilimumab with the incidences of antibodies to other products may be misleading.

Study Results and Clinical Efficacy

The clinical efficacy of Ipilimumab as a single agent at a dose of 3 mg/kg administered q3w for 4 doses has been established in MDX010-20 (a randomized, controlled study in second line, locally advanced/metastatic melanoma), which led to approval of Ipilimumab by the FDA. In study CA184024, the addition of 10 mg/kg Ipilimumab to dacarbazine led to a prolongation of overall survival in patients with previously untreated melanoma.

In melanoma studies, disease stabilization in subjects receiving Ipilimumab is characteristic of anti-tumor activity. Stable disease, sometimes of long duration, or slow steady decline of tumor lesion size over long periods of time, has been observed. Some subjects demonstrate initial tumor volume increase before response, possibly due to T-cell infiltration as shown by biopsies or to the time required for immunologic activation. Consequently, an initial determination of progressive disease and consequently PFS may not capture all patterns response and may underestimate the clinical activity of Ipilimumab. Please see section “Considerations for Using Immune-Related Tumor Assessment Criteria (irRC).”

10 mg/kg Dosing with Ipilimumab

Several additional trials studied the efficacy and safety of 10 mg/kg dosing, and additional information gained from these trials is listed below:

- A dose of 10 mg/kg may be necessary to ensure blockade of the CTLA-4 pathway; *in vitro,* a concentration of 20 mcg/mL of Ipilimumab was the minimal concentration able to fully abrogate the binding of CTLA-4 to B7.1 and B7.2. With a dose of 3 mg/kg q3w, 30% achieved a trough concentration of Ipilimumab greater than 20 μg/mL, compared to 95% of subjects treated at 10 mg/kg q3w.

- In addition, in all Ipilimumab trials examined to date, mean Absolute Lymphocyte Count (ALC) increased after Ipilimumab treatment throughout the 12-week induction-dosing period, in a dose-dependent manner. In an analysis of Ipilimumab at 0.3, 3, or 10 mg/kg in melanoma studies CA184007, CA184008, and CA184022 combined, the rate of change in

ALC after Ipilimumab treatment was significantly associated with dose (p = 0.0003), with the largest rate at 10 mg/kg Ipilimumab. Moreover, the rate of change in ALC over the first half of the induction-dosing period was significantly associated with clinical activity in these studies (p = 0.009), where clinical activity was defined as CR, PR, or prolonged SD (*i.e.*, SD lasting at least 6 months from first dose). Although these analyses alone could not determine whether the rate of change in ALC was specifically associated with clinical activity in response to Ipilimumab treatment, as opposed to being generally prognostic, these results do suggest a potential benefit to higher rates of ALC increase after Ipilimumab treatment. Among the 3 doses evaluated, 10 mg/kg Ipilimumab led to the greatest such rates.

- In the 3 primary studies conducted in advanced melanoma (CA184007, CA184008, and CA184022), subjects treated with 10 mg/kg single agent Ipilimumab had the highest response, disease control rates, median OS as well as 1-year and 2-year survival rates compared to lower doses. The CA184022 data are summarized in Table below.

Dose, Schedule, and Regimen

While optimal doses and schedules for Ipilimumab have not yet been determined, in proposed proof of principle studies demonstration of efficacy at 10 mg/kg would allow future studies to explore biologic and clinical efficacy at lower doses with reduced toxicity. For most studies in new combinations or settings, a short phase 1 component at 3 mg would be appropriate with a 5 or 6 mg/kg dose added as an additional cohort if needed.

A recommended dose of 10 mg/kg is proposed by the manufacturer for most studies of Ipilimumab. In melanoma, a similar survival benefit was demonstrated in phase 3 trials at the 3 mg/kg and at 10 mg/kg with DTIC. However, the incidence of grade $\geq$3 toxicity was 15 and 25% respectively.

Based on Phase 2 studies, response rates of Ipilimumab appear to be dose dependent up to 10 mg/kg. Exposure-response analyses [C_{min}ss 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 C_{min}ss target threshold of 20 mcg/ml was exceeded in 0%, 30% and 95% subjects respectively. The slope of change in absolute lymphocyte count (ALC) correlated with clinical benefit and T-cell activation markers such as HLA-class II expression may also be dose dependent. Responses have not been compared systematically in randomized phase 2 or phase 3 studies in patients with tumor types other than melanoma.

Regarding schedule, the typical schedule for advanced melanoma at present is once q3w for four doses followed by a maintenance phase of four doses every 12 weeks. Of interest, Ipilimumab was evaluated in NSCLC and SCLC using a dose of 10 mg/kg given concomitantly or following initial paclitaxel/cisplatin. When used in the phased schedule, 10 mg significantly improved irPFS and mWHO defined responses but not PFS determined by Response Evaluation Criteria in Solid Tumors (RECIST). There also was a trend for an improvement in OS in both indications. Doses less than 10 mg/kg have not been evaluated in either NSCLC or SCLC.

Studies comparing doses in non-melanoma and combinations have not been widely done. There

are also no clear data that peak levels, Cmin, AUC, exposure and number of doses given, or the occurrence of autoimmune events, predict responses in individual patients. We note that the incidence of specific events such as hypophysitis may vary from study to study and with different combinations of agents. The severity and possibly time to onset but not necessarily the frequency of events increases with dose. In addition, there are rare but serious events such as toxic epidermal necrolysis (TEN) for which a dose relationship has not been established. Case report forms should include data on the prior treatment, timing, number of doses, duration of event, response to treatment, and complications to allow comparisons among studies.

Considerations in Using Immune-Related Tumor Assessment Criteria (irRC)

New end point definitions for trials of immunologic agents have been proposed based on novel patterns of clinical activity in malignant melanoma (Wolchok, *et al.* 2009; Hoos, *et al.* 2010). These alternative definitions allow time for immunologically mediated effectors to develop that may result in late tumor responses even after initial progression by RECIST. Also, in some patients, tumors necrosis and inflammation may increase tumor size radiographically prior to response. Changing the definitions of OR and PD may alter (increase) the number patients achieving responses and the duration of PFS.

On a protocol by protocol basis, we would consider allowing study treatment to continue during initial progression up to the 12-16 week assessment to allow time for responses to be observed, if the patient is clinically stable, there is no deterioration in PS, and there is no need for immediate additional treatment. While maintaining standard definitions of progression and response, we would allow new lesions and some progression beyond 20% increases in tumor measurements during the initial treatment period to allow time for responses to develop (these delayed tumor responses may be seen in 10-20% of melanoma patients who initially progress during the initial treatment cycles and evaluation). We do not have experience with response patterns with combination therapy nor in diseases other than melanoma. Please use standard response definitions as the primary end point in these studies.

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

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

Overall Risk/Benefit Assessment

The unique immune-based mechanism of action is reflected in the clinical patterns of anti-cancer activity in some patients. Ipilimumab affects tumor cells indirectly, and measurable clinical effects emerge after the immunological effects. Tumor infiltration with lymphocytes and the associated inflammation (documented by biopsy in some subjects) is likely the cornerstone of the effect of Ipilimumab and can manifest in various patterns of clinical activity leading to tumor control. In some cases, response may be preceded by an apparent increase in initial tumor volume and/or the appearance of new lesions, which may be mistaken for tumor progression on

radiological evaluations. Therefore, in subjects who are not experiencing rapid clinical deterioration, confirmation of progression is recommended, at the investigator's discretion, to better understand the prognosis as well as to avoid unnecessarily initiating potentially toxic alternative therapies in subjects who might be benefitting from treatment. Immune-related (ir) response criteria were developed based on these observations to systematically categorize novel patterns of clinical activity and are currently being prospectively evaluated in clinical studies.

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

The immune-based mechanism of action is also reflected in the safety profile. The most common drug-related AEs are immune-mediated, consistent with the mechanism of action of the drug and generally medically manageable with topical and/or systemic immunosuppressants. As previously discussed, the immune-mediated adverse reactions primarily involve the GI tract, skin, liver, endocrine glands, and nervous system.
The early diagnosis of immune-mediated adverse reactions is important to initiate therapy and minimize complications. Immune-mediated adverse reactions are generally manageable using symptomatic or immunosuppressive therapy as recommended through detailed diagnosis and management guidelines, as described fully in the current IB. The management guidelines for general immune-mediated adverse reactions and Ipilimumab-related GI toxicities, hepatotoxicity, endocrinopathy, and neuropathy are provided in the appendices of the current IB.

2.3 Other Agent(s)

N/A

2.4 Rationale

The undisputed etiologic link between HPV and cervical cancer as well as the preclinical data suggestive of a central immune-modulated response to HPV makes cervical cancer a logical target for investigational drugs with immune modulating properties. Ipilimumab as previously described acts via inhibition of CTLA4 which leads to inhibition of self-induced immuno-suppressive activity thereby augmenting the immune response directed against cancer cell presence.

Preclinical data has convincingly demonstrated T-cell induction as a means of inducing local immune suppression of HPV-associated disease. Specifically, Tumor-infiltrating regulatory T cells (Tregs) seem to play a role in local immune suppression in HPV-associated disease. Tregs have been found at higher concentrations in CIN2/3 relative to HPV infection without premalignant changes [Jordanova 2008]. A low ratio of CD8+/regulatory T cells is associated with poor outcome [Tuve, Chen, Liu et al 2007]. A mouse model for cervical cancer (TC-1 cells) was developed and used to investigate the effects of Treg depletion as well as CTLA4 blockade. The group found that Treg depletion alone was not sufficient to control tumor growth. They also

found that systemic CTLA4 blockade did not results in tumor regression but rather induced hepatitis. They subsequently injected CTLA4 antibody locally into tumors and found that this did delay the progression of tumors. In addition this local CTLA4 blockade resulted in a reversal of the intratumoral Treg/CD8 ratio secondary to an increase in CD8 production. A combination of local CTLA4 blockade and Treg inhibition caused permanent tumor regression as a consequence of immune response activation [Tuve, Chen, Liu et al 2007].

In summary, the non-tumor specific nature of the mechanism of action of CTLA4 blockade alongside the accumulating evidence suggesting activity in a wide range of tumors supports its investigation in the context of cervical cancer. This rationale is further strengthened by the significant body of evidence suggesting that HPV related neoplasia would be responsive to immunotherapy treatments. We propose assessing the efficacy of Ipilimumab in recurrent and metastatic cervical cancer patients, for whom the limited treatment options available afford minimal benefit.

2.5 Correlative Studies Background

Biomarkers for Clinical Studies

Retrospective analysis of advanced melanoma patients treated with Ipilimumab showed that increased expression of proliferation and polarization markers in $CD4^+$ and $CD8^+$ cells, decreased CCR7 and IL-7R on $CD4^+$ T cells, and upregulated Granzyme B in $CD8^+$ T cells may serve as pharmacodynamic indicators of the effects of Ipilimumab (Weber *et al*., 2011). Increased activation markers ICOS and eomesodermin (EOMES) were significantly associated with fewer AEs and less favorable outcome, respectively, but baseline elevated expression of EOMES appears to predict favorable relapse-free survival in this analysis.

ICOS potentially could be a useful marker for $CD4^+$ T-cell responses generated after CTLA-4 blockade (Simpson *et al*., 2010). Human leukocyte antigen (HLA-DR) expression could also potentially serve as a T-cell marker (Small *et al*., 2007; Zhou *et al*., 2011).

Retrospective gene expression analysis was performed on 46 primary or metastatic melanoma tumors, collected in a phase 2 study in advanced melanoma patients (CA184004) (Ji *et al*., 2011). Ipilimumab treatment was associated with downregulation of a number of melanoma-associated transcripts in the tumors within 3 weeks after administering the first dose. Expression of immune-related genes such as CD8A, GZMA, LCK, CXCL-9, -10, -11, and CXCR3 in tumors at week 3 post-treatment was associated with the total lymphocyte infiltrate, clinical benefit, and overall survival. The preliminary results suggest that high pre-existing immune activity favors a clinical response to Ipilimumab therapy. These analyses also provide a list of candidate biomarkers with potential predictive value for response to Ipilimumab.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have histologically or cytologically confirmed metastatic or recurrent cervical cancer of squamous, adenocarcinoma or mixed histology type not suited to definitive localized therapy. HPV status will be confirmed for all patients following enrollment.

3.1.2 Patients must have measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded for non-nodal lesions and short axis for nodal lesions) as >10 mm with CT scan, MRI, or calipers by clinical exam. See Section 11 for the evaluation of measurable disease.

3.1.3 Previous Therapy:

3.1.3.1 Patients may have undergone surgery and/or received definitive radiation or chemo-radiation for localized disease in the past.

3.1.3.2 Radiation treatment with curative intent (radical chemoradiotherapy or adjuvant chemoradiotherapy) must have been completed ≥ 3 months prior to enrollment.

Note: *Patients who completed Palliative radiation therapy 2 weeks before start of Ipilimumab are allowed as long as this does not affect measurable disease*

3.1.3.3 Patients must have been exposed to platinum chemotherapy either as part of definitive chemo-radiation OR as first line systemic treatment for metastatic disease.

3.1.3.4 Patients MAY have received up to two prior lines of systemic chemotherapy for metastatic or recurrent disease. Patients with metastatic disease at first presentation MUST have received one platinum based line of chemotherapy.

3.1.3.5 All chemotherapy must have been completed ≥ 4 weeks prior to enrollment with radiologic evidence of radiological disease progression.

3.1.4 Age ≥18 years. Because no dosing or adverse event data are currently available on the use of Ipilimumab in patients <18 years of age, children are excluded from this study.

3.1.5 ECOG performance status 0-1.

3.1.6 Life expectancy of greater than 3 months.

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

– leukocytes	≥3.0 x 10^9/L
– absolute neutrophil count	≥1.5 x 10^9/L
– platelets	≥100 x 10^9/L
– total bilirubin	within normal institutional limits (except in Gilbert's syndrome)
– TSH	≤ ULN
– AST(SGOT)/ALT(SGPT)	≤2.5 X institutional upper limit of normal
– creatinine	<1.25 ULN OR
– creatinine clearance	≥50mL/min/1.73 m^2 as calculated by the Cockroft and Gault formula

3.1.8 All radiology studies must be performed ≤ 3 weeks prior to the start of therapy

3.1.9 Subjects with treated and asymptomatic brain metastases are eligible. Patients that received palliative radiation (for brain metastases) are eligible if they have been asymptomatic for at least 2 weeks with use of maintenance steroid therapy, and last received radiation at least 4 weeks prior to start of therapy.

3.1.10 Ability to understand and willing to sign a written informed consent document.

3.1.11 Ongoing prior toxicities related to previous treatments must be recovered to ≤ grade 1 at the time of registration (with the exception of alopecia or skin depigmentation).

3.1.12 Patients are willing to undergo tumour biopsy pre-treatment (within 14 days prior to registration) and post-treatment (within the first week of cycle 2 onset). Patients who consent but have tumour that is not amenable to safe biopsy will be allowed to enter the trial / continue therapy as per protocol if this has been addressed and permission is granted from the lead consortium PI prior to registration continuation of treatment.

3.2 Exclusion Criteria

3.2.1 Patients who have had chemotherapy < 4 weeks prior to enrollment (<6 weeks for nitrosoureas or mitomycin C) or who had radiation therapy with curative intent < 3months prior to enrollment (< 2 weeks for palliative radiation therapy) or those who have not recovered (≤ grade 1) from adverse events related to previous treatments are excluded.

3.2.2 Patients with a history of prior treatment with Ipilimumab or other CTLA4 agonists or antagonists, anti-PD 1 antibody, CD137 agonist or other immune activating therapy such as anti-CD 40 antibody are excluded.

3.2.3 Patients who are receiving any other investigational agents.

3.2.4 Autoimmune disease: Patients with a history of inflammatory bowel disease, including ulcerative colitis and Crohn's Disease, are excluded from this study, as are patients with a history of symptomatic disease (*e.g.*, rheumatoid arthritis, systemic progressive sclerosis [scleroderma], systemic lupus erythematosus, autoimmune vasculitis [*e.g.*, Wegener's Granulomatosis]); CNS or motor neuropathy considered of autoimmune origin (*e.g.* Guillain-Barre Syndrome and Myasthenia Gravis, multiple sclerosis.).

3.2.5 Patients requiring immunosuppressive agents, unless required for treating potential immune related adverse effects. Steroids at their lowest effective dose in patients with radiated brain metastases is permitted.

3.2.6 Patients with known immune impairment who may be unable to respond to anti-CTLA 4 antibody.

3.2.7 Any other prior malignancy from which the patient has been disease free for less than 3 years, with the exception of adequately treated and cured basal or squamous cell skin cancer, superficial bladder cancer, carcinoma in situ of any site or any other cancer.

3.2.8 History of allergic reactions attributed to compounds of similar chemical or biologic composition to Ipilimumab.

3.2.9 *Excluded Medication*
3.2.9.1 Patients requiring systemic steroids are excluded, as these drugs may interfere with the activity of Ipilimumab if administered at the time of the first Ipilimumab dose.
3.2.9.2 Narcotics should be used with caution as they may mask the signs and symptoms of serious gastrointestinal irAEs including intestinal perforation.

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

Patients with chronic Hepatitis B or hepatitis C infections should be excluded because of potential effects on immune function and/ or drug interactions.

3.2.11 Pregnant women are excluded from this study because Ipilimumab is an agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with Ipilimumab, breastfeeding should be discontinued if the mother is treated with Ipilimumab.

3.2.12 Patients with active or chronic infection with HIV who have raised viral loads or uncontrolled disease are ineligible because of the potential for anticipated and unknown adverse immune related effects secondary to treatment with Ipilimumab. Those patients however who exhibit minimal viral loads with good control whilst on stable anti-viral regimen may be considered if they meet all other eligibility criteria.

3.3 Pregnancy

The effects of Ipilimumab on the developing human fetus are unknown. Due to potential teratogenic effects, women of child-bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

3.4 Inclusion of Women and Minorities

Women of all races and ethnic groups are eligible for this trial. This study is designed to include minorities as appropriate. However, the trial is not designed to measure differences in intervention effects. The population of Southern Ontario is ethnically diverse. The proportion of different ethnic groups in the community is provided in the table below. Universal access to

health care will ensure that there is no discrimination on the basis of race or gender (Guide to Canadian Human Rights Act: www.chrc-ccdp.ca/public/guidechra.pdf). Individual hospital registries and databases do not routinely collect racial data, under the direction of the Canadian Human Rights Code.

The population demographics and distribution of minorities in Southern Ontario is included in the following table:

Table: Visible minority population by Consortium Provinces (2001 Census)

	British Columbia		**Alberta**		**Ontario**		**Nova Scotia**		**Total**	
Total population of province	3,868,870		2,941,150		11,285,550		897,570		**18,993,140**	
Visible Minorities	**Population**	**%**	**Population**	**%**	**Population**	**%**	**Population**	**%**	**Population**	**%**
Black	25,465	1%	31,390	1%	411,095	4%	19,670	2%	487,620	**3%**
Asian	768,435	20%	268,660	9%	1,513,825	13%	12,630	1%	2,563,550	**13%**
Latin American (Hispanic)	23,880	1%	18,745	1%	106,835	1%	520	0%	149,980	**1%**
Visible minority, not included elsewhere	4,195	0%	4,220	0%	78,915	1%	1,170	0%	88,500	**0%**
Multiple visible minority	14,465	0%	6,910	0%	42,375	0%	535	0%	64,285	**0%**
Total Visible minority population	836,440	22%	329,925	11%	2,153,045	19%	34,525	4%	3,353,936	**18%**

Source: Statistics Canada, Census of Population.

Data (2004) from our consortium has been compiled regarding the representation of minorities on previous clinical trials, and the distribution is as follows:

Population Percentage of Minority and Gender of entering PMHC Trials			
	2002	2003	2004
Visible Minorities			
Black	3.6	0	2.8
Asian	7.2	9.0	8.4
Hispanic	2.4	3.0	1.7
Total	**13.2**	**12**	**12.9**
Women	**44.6**	**49.3**	**46.9**

Accrual Targets			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	4	0	4
Not Hispanic or Latino	40	0	40
Ethnic Category: Total of all subjects	44	0	44
Racial Category			
American Indian or Alaskan Native	0	0	0

Asian	5	0	5
Black or African American	4	0	4
Native Hawaiian or other Pacific Islander	1	0	1
White	34	0	34
Racial Category: Total of all subjects	44	0	44

4. REGISTRATION PROCEDURES

4.1 General Guidelines

The Study Coordinator at the Princess Margaret Hospital Consortium Central Office will enter eligible patients on study centrally. All sites should call the Study Coordinator (listed on cover page) to verify dose level availabilities. The required forms (Eligibility Checklist) will be provided upon site activation.

Following registration, patients should begin protocol treatment within 72 hours. Issues that would cause treatment delays should be discussed with the Principal Investigator (cc the central office study coordinator). If a patient does not receive protocol therapy following registration, the patient's registration on the study may be cancelled. The Study Coordinator should be notified of cancellations as soon as possible.

Except in very unusual circumstances, each participating institution will order DCTD-supplied agents directly from CTEP. A participating site may order agents only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO (PIO@ctep.nci.nih.gov) except for Group studies.

4.2 Registration Process

Prior to registering a patient, each institution must have submitted all necessary regulatory documentation to the PMH Phase II Consortium Central Office. The eligibility CRF will only be sent once this has been received.

No patient can receive protocol treatment until registration with the Central Office has taken place. All eligibility criteria must be met at the time of registration. There will be no exceptions. Any questions should be addressed with the Central Office prior to registration.

To register a patient, the following documents are to be completed by the research nurse or data manager and sent / faxed to the Central Office Study Coordinator:
- Signed patient consent form
- Eligibility Checklist CRF signed by the investigator

To complete the registration process, central office will review the checklist and once eligibility has been confirmed:
- Assign a patient study number
- Assign the patient a dose
- Register the patient on the study

• Fax or e-mail the confirmation worksheet with the patient study number and dose to the participating site

To ensure immediate attention is given to the faxed checklist, each site is advised to also call the study coordinator listed on the front sheet. Patient registration will be accepted between the hours of 9am to 5pm Monday to Friday, excluding Canadian statutory holidays when the central office will be closed.

5. TREATMENT PLAN

5.1 Agent Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

- All patients will receive single agent Ipilimumab administered as an intravenous infusion over 90 minutes. One cycle is 21 days for the phase I and II.

- Phase I Run In (safety phase):
    - An initial cohort of 6 patients will receive four doses of Ipilimumab administered at a dose of 3mg/kg once every 21 days.
    - Patients in this cohort will be monitored for 12 weeks from treatment onset. Should 3 or more of the initial 6 patients suffer one of the following SAE considered to be dose-limiting, the phase I cohort will be expanded to include an additional 6 patients, 12 in total:
        - 1. Patients experiencing unexpected non-autoimmune related grade 3 adverse effects.
        - 2. Patients experiencing grade 4 adverse effects.
        - 3. Patients experiencing autoimmune related adverse effects which do not respond to steroid treatment and do not resolve to a grade 1 level within a 4 week period of initiation of steroid treatment. If an expanded phase I is required monitoring of SAE's will continue without modification for the expanded cohort.
    - If more than 4 individuals demonstrate SAE's considered to be dose limiting as per the afore-mentioned criteria, the trial will be halted and not proceed to the second stage.
    - All patients from the first phase of the study who are eligible to proceed to the maintenance phase of treatment will continue to receive Ipilimumab at a dose of 3mg/kg and will not escalate to a dose of 10mg/kg. They will be evaluated and monitored in a similar way to patients in phase II of the study.
- Phase II:
    - An additional 18 evaluable patients will be enrolled and will receive four cycles of Ipilimumab administered at a dose of 10mg/kg once every three weeks.

- A safety assessment will be conducted after 12 patients have been treated. If >4 have DLT by same definition as Phase I run in, the study will be put on hold and discussed with CTEP.
- If one patient has an objective response by week 24, the trial will continue to the second stage and an additional 14 evaluable patients will be enrolled.

- Tumor assessment will be done at week 12 (+/- 1 week), at week 18 and 24. Patients demonstrating clinical or radiological progression of disease or toxicity prohibiting ongoing participation of study will be taken off study. Patients showing evidence of an objective response or stabilization of disease subsequent to treatment will continue on study unless they become ineligible for alternative reasons. Modified RECIST 1.1 criteria may be used at week 12 and beyond to determine on going eligibility. This will allow patients with modest progression of disease (≤ 30% uni-dimensional growth in measurable lesions, development of no more than two new lesions) but stable clinical condition with no alteration in ECOG to continue to participate at the investigators discretion.

- Maintenance Phase:
  - One cycle is 12 weeks.
  - Eligibility for this treatment will be assessed at 24 weeks (-1 week). Should the patient's performance status remain at the required level (ECOG 0-1) and radiologic assessment of disease continue to confirm response or stabilization of disease secondary to treatment, patients may enter the maintenance phase of treatment.
  - Ipilimumab will be administered at 10mg/kg (or 3mg/kg for the phase I cohort) once every 12 weeks for four additional treatments. Response to treatment will be evaluated once every 12 weeks and formal RECIST 1.1 criteria alone will be used to determine on-going participation on study during the maintenance phase of the trial.

5.1.1 Ipilimumab

- Ipilimumab is an intravenous treatment which may be given at any time of day. No particular instruction is required with regard to the ingestion of food prior or during treatment.
- All patients will receive 650mg of oral acetaminophen for the prevention of fever, prior to receiving Ipilimumab. If the patient has an allergy or intolerance to acetaminophen, the patient can take ibuprofen 400mg prior to receiving ipilimumab. In case of allergy to both, acetanminophen and ibuprofen, the case should be discussed with the sponsor.
- Ipilimumab will then be administered intravenously over 90 minutes.
  In the event that a patient develops rigors or chills during or following administration of Ipilimumab, intravenous Meperidine HCl at a dose of 25mg is recommended.

5.2 General Concomitant Medication and Supportive Care Guidelines

5.2.1 Concomitant Medications

- There are no known interactions between Ipilimumab and the cytochrome P450 system

- The chronic use of systemic steroids for other comorbidities should be avoided, or the subject should be on the lowest clinically effective dose, as these drugs may interfere with the activity of Ipilimumab if administered at the time of the first Ipilimumab dose. Patients with a suspected immune adverse event are allowed to receive oral corticosteroids according to the toxicity management guidelines.
- Opioid medications should be used with caution as these may mask the signs and symptoms of serious gastrointestinal immune related adverse effects including intestinal perforation.

5.2.2 Supportive Care Guidelines (refer to appendix C)

- **Rash.** Patients experiencing rash following treatment with Ipilimumab will be advised to administer hydrocortisone cream 1% twice daily in addition to moisturizing lotion as needed until resolution of symptoms. Should symptoms not resolve, oral corticosteroid treatment will be initiated.
- **Diarrhea.** This is a common side effect which if left undetected and untreated may become potentially serious. Patients will be educated as to the importance of monitoring bowel movements and informing trial staff of early signs of diarrhea. At the first sign of diarrhea patients are advised to take 4mg of Loperamide HCl followed by an additional 2mg once every four hours OR after each loose stool, whichever occurs first, to a maximum daily dose of 16mg. This treatment should continue until the patient is free from symptoms of diarrhea for at least 12 hours. Should diarrhea remain unresolved and only after consultation with treating physician, Budesonide SR may be recommended. Patients will receive 9mg daily of budesonide for an initial period of two weeks. Patients will then be advised to reduce the daily dose of budesonide to 6mg for an additional two weeks and subsequently 3mg for a final two weeks before discontinuing this medication.

5.3 Duration of Therapy

Treatment may continue for 4 cycles followed by four maintenance phase treatment cycles or until one of the following criteria applies at which point the patient is to be discontinued from study therapy and withdrawn from study:

- The development of PD by RECIST criteria with modifications to allow continued treatment as outlined:
  - possibility for investigator discretion at week 12 and beyond for patients demonstrating radiologic PD but otherwise stable clinical condition (see section 5.6)
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s) (discretion of the physician) or an adverse event that results in the permanent stopping of study drug defined in section 6.
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition (clinical adverse event, laboratory abnormality or intercurrent illness) which renders the patient unacceptable for further treatment in the judgment of the investigator.
- Clinical progression
- Pregnancy:

- All women of child bearing potential (WOCBP) should be instructed to contact the investigator immediately if they suspect they might be pregnant (*e.g.*, missed or late menstrual period) at any time during study participation. Institutional policy and local regulations should determine the frequency of on study pregnancy tests for WOCBP enrolled in the study.
- The investigator must immediately notify CTEP in the event of a confirmed pregnancy in a patient participating in the study.

- Termination of the study by sponsor.

5.4 Duration of Follow Up

Patients who discontinue treatment for any reason other than PD with evidence of SD, PR or CR will be followed for 1 year on a 3 monthly basis, until progression of disease or death, whichever occurs first. Subsequent therapy to Ipilimumab data will be collected for these patients for the duration of 1 year.

Patients removed from study due to PD will be seen only once at 1 month after withdrawn from treatment.

Any patient experiencing an adverse event will be given medical treatment as appropriate and followed monthly until resolution to a ≤ grade 1 or until the adverse event remains stable at the same grade for at least 2 months and then every 3 months until progression of disease or death, whichever occurs first.

5.5 Criteria for Removal from Study

Patients will be removed from study when any of the criteria listed in Section 5.3 applies. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

Ipilimumab is expected to trigger immune-mediated responses, which require activation of the immune system prior to the observation of clinical responses. Such immune activation may take weeks to months to be evident. Some patients may have objective volume increase of tumor lesions or other disease parameters (based on study indication, *i.e.*, hematologic malignancies) within (specific 12-24) weeks following the start of Ipilimumab dosing. Such patients may not have had sufficient time to develop the required immune activation or, in some patients, tumor volume or other disease parameter increases may represent infiltration of lymphocytes into the original tumor or blood. In conventional studies, such tumor volume or relevant laboratory parameter increases during the first 12 weeks of the study would constitute PD and lead to discontinuation of imaging to detect response, thus disregarding the potential for subsequent immune-mediated clinical response.

Therefore, patients with tumor progression by RECIST imaging (≤ 30% uni-dimensional growth in measurable lesions, development of no more than two new lesions) or laboratory parameters at week12 and beyond without rapid clinical deterioration or change in ECOG who do not require

additional immediate therapy (are in stable condition), may continue to be treated with Ipilimumab.

6. DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and off treatment criteria for Ipilimumab associated immune/inflammatory events are provided below.

No dose modifications are indicated as part of this protocol. Adverse effects as specified will warrant withholding of treatment dose until resolution as specified within the protocol.

6.1 Ipilimumab Administration Rule

Decisions to administer an Ipilimumab dose must be made on specified safety criteria. Treatment with Ipilimumab will be discontinued if the subject experiences at least one adverse event, specified below, considered by the investigator to be "possibly," probably," or "definitely" related to Ipilimumab treatment.

Each site should specify a procedure for reviewing all AEs that require holding drug treatment and or a regularly scheduled conference call with the PI and co-investigators.

The investigator should contact the study PI to discuss any questions.

<u>It is necessary to avoid study drug dosing and initiate appropriate evaluation and/or treatment for the following adverse events:</u>

- Any ≥ grade 3 skin related adverse event regardless of causality.
- Any ≥ grade 2 non-skin related adverse event (including immune-mediated adverse reactions), except for easily correctly laboratory abnormalities that do not reflect underlying organ pathology.
- Any ≥ grade 3 laboratory abnormality.
- Any adverse event, laboratory abnormality or intercurrent illness that, in the judgment of the investigator, presents a substantial clinical risk to the subject with continued dosing.
- It may be necessary to hold study drug to evaluate Grade 1 events that suggest ongoing or incipient autoimmune disease including GI toxicity, diarrhea, pancreatitis, hepatitis, pituitary insufficiency, early evidence of neurologic events, skin toxicity until it is first clinically safe to continue, in discussion with central office.

  - <u>For the induction phase</u>

<u>Ipilimumab may be restarted if/when the adverse event(s) resolve(s) to ≤ grade 1 or returns to baseline within 2 weeks of previous dose administration:</u>

If the adverse event has been determined not to be related to Ipilimumab or is not an autoimmune/inflammatory event:
Plan to administer the total number of planned doses. If > 2-week delay is expected, the dosing schedule modifications must be discussed with the principal investigator prior to implementation.

If the adverse event has been determined to be related to Ipilimumab or is an autoimmune/inflammatory event:

- If the adverse event has resolved to ≤ grade 1, Ipilimumab dosing may be restarted at the next scheduled time point per protocol (±3 days). Please follow guidelines for specific events. Please note that re-initiating treatment may be associated with recurrence or exacerbation of autoimmune or inflammatory events. In some instances, clinical resolution of events such as colitis may be associated with residual pathologic changes and should require evaluation of complete resolution prior to restarting therapy.
- If the adverse event has not resolved ≤ grade 1, the next scheduled dose will be delayed until resolution to grade ≤ 1. Please note that re-initiating treatment may be associated with recurrence or exacerbation of autoimmune or inflammatory events.
- Events which require intervention with immunosuppressant therapy, steroids, surgery, or hormone replacement generally require permanently stopping study treatment. Consult guidelines for exceptions and specific events.
- Autoimmune/inflammatory events are presumably related to the mechanisms of action of Ipilimumab and potentially to a therapeutic effect. The incidence and severity of these events may be dose related, but once initiated, there is no evidence that lowered doses can be administered without continued autoimmune activity and there has so far been no demonstrable benefit to continuing Ipilimumab after an autoimmune event during the initial treatment. The significance and benefit of toxicity or continued treatment in the maintenance phase has not been determined. Typically no dose modification is used for Ipilimumab.

➢ <u>For the maintenance phase</u>

Ipilimumab may be restarted if/when the adverse event(s) resolve(s) ≤ grade 1 or returns to baseline within 4 weeks of previous dose administration:

If the adverse event has been determined not to be related to Ipilimumab or is not an autoimmune/inflammatory event:
Plan to administer the total number of planned doses. If > 4 week delay is expected due to current events, the dosing schedule modifications must be discussed with the principal investigator prior to implementation.

If the adverse event has been determined to be related to Ipilimumab or is an autoimmune/inflammatory event:

- If the adverse event has resolved to ≤ grade 1, Ipilimumab dosing may be restarted at the next scheduled time point per protocol (±3 days). Please follow guidelines for specific events. Please note that re-initiating treatment may be associated with recurrence or exacerbation of autoimmune or inflammatory events. In some instances clinical resolution of events such as colitis may be associated with residual pathologic changes and should require evaluation of

complete resolution prior to restarting therapy.

- If the adverse event has not resolved to ≤ grade 1, the next scheduled dose will be delayed until resolution to grade ≤ 1.
- Events which require intervention with immunosuppressant therapy, steroids, surgery, or hormone replacement generally require permanently stopping study treatment. Consult guidelines for exceptions and specific events.
- Autoimmune/inflammatory events are presumably related to the mechanisms of action of Ipilimumab and potentially to a therapeutic effect. The incidence and severity of these events may be dose related, but once initiated, there is no evidence that lowered doses can be administered without continued autoimmune activity and there has so far been no demonstrable benefit to continuing Ipilimumab after an autoimmune event during the initial treatment. The significance and benefit of toxicity or continued treatment in the maintenance phase has not been determined. Typically no dose modification is used for Ipilimumab.

6.2 Discontinuation of Study Therapy

Subjects MUST be discontinued from study therapy AND withdrawn from the study for the reasons outlined in section 5.3.

6.2.1 Permanent Discontinuation for Related Adverse Events

Permanently discontinue Ipilimumab for any of the following:

- Persistent adverse reactions that require holding more than 2 treatment doses.
- Any grade 3 or higher non-dermatologic autoimmune breakthrough event considered to be secondary to treatment with Ipilimumab
- Any event that requires immunosuppressive treatment or systemic steroids
  **Exception: Short term oral steroids to treat potentially reversible Grade 2 diarrhea and skin toxicities as per algorithm in appendix C*
- Severe or life-threatening adverse reactions, including any of the following:
  - Colitis with abdominal pain, fever, ileus, or peritoneal signs; increase in stool frequency (7 or more over baseline), stool incontinence, need for IV hydration for more than 24 hours, gastrointestinal hemorrhage, and gastrointestinal perforation
  - Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >5 times the upper limit of normal or total bilirubin >3 times the upper limit of normal
  - Stevens-Johnson syndrome, toxic epidermal necrolysis, or rash complicated by full thickness dermal ulceration, or necrotic, bullous, or hemorrhagic manifestations
  - Severe motor or sensory neuropathy, Guillain-Barré syndrome, or myasthenia gravis
  - Severe immune-mediated reactions involving any organ system (*e.g.*, nephritis, pneumonitis, pancreatitis, non-infectious myocarditis)
  - Immune-mediated ocular disease that is unresponsive to topical immunosuppressive therapy
  - Any adverse event, laboratory abnormality or intercurrent illness which, in the judgment of the investigator, presents organ specific injury and/or a substantial clinical risk to the patient with continued dosing.

The following neurological adverse event requires permanent discontinuation of Ipilimumab and defines unacceptable neurotoxicity:

- Any motor neurologic toxicity ≥ grade 3 regardless of causality
- Any ≥ grade 3 treatment related sensory neurologic toxicity

Please refer to Appendix C and the IB for specific treatment algorithms.

6.2.2 Exceptions to Permanent Discontinuation Permanent Discontinuation

- 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.
- Hospitalization for ≤ grade 2 adverse events where the primary reason for hospitalization is to expedite the clinical work-up or to monitor clinical condition felt to be warranted due to minimal experience with Ipilimumab treatment.
- Patients with the following conditions where in the investigator's opinion continuing study drug administration is justified based on the potential for continued clinical benefit:
  - Patients treated with systemic steroids for less than 2 weeks without evidence of autoimmune disease requiring steroids treatment
  - Grade 2 skin rash treated with topical steroids for less than 4 weeks
  - Grade 2 Ocular toxicity that has completely responded to topical therapy .within 4 weeks
  - Endocrinopathies where clinical symptoms are controlled with appropriate hormone replacement therapy. Note: 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.

6.2.3 Immune-Related Adverse Events (irAEs)Reactions and Immune-mediated Adverse Reactions: Definition, Monitoring, and Treatment

For the purposes of this study, an immune-related adverse reaction is defined as an adverse reaction 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 event an irAEs. Serologic, immunologic, and histologic (biopsy) data should be used to support the diagnosis of an immune-related toxicity. Suspected immune-related adverse reactions must be documented on an AE or SAE form. Another term for an irAE is an immune-mediate adverse reaction, as it is termed in the Ipilimumab US Prescribing Information. Both terms may be used in this protocol document.

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

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. Prior experience suggests that colitis manifested as ≥ grade 3 diarrhea requires corticosteroid treatment.

Recommended guidelines for specific immune-mediated adverse reactions are included in section 6.2.4 below, in Appendix C, and the package insert. These recommendations should be utilized as clinically appropriate for the treatment of individual patients. Please contact the PI or drug monitor for any questions.

6.2.4 **Other Guidance**

The following guidance is provided for the management of Ipilimumab treatment related events. These recommendations, treatment algorithms in Appendix C, and further information in the IB, should be considered in the context of appropriate medical treatment for each patient. Patients removed from study due to Ipilimumab related adverse events will receive medical treatment as appropriate to manage Ipilimumab related adverse effects until their resolution.

6.2.4.1 **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 except acetaminophen should 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 additional pre-medication (diphenhydramine) 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).

6.2.4.2 **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 with continue and / or the addition of a 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.

6.2.4.3 **Monitoring and Management of Immune-mediated Adverse Reactions**

<u>Immune-mediated Enterocolitis</u>
The clinical presentation of GI immune-related AEs included diarrhea, increase in the frequency of bowel movements, abdominal pain, or hematochezia, with or without fever. However inflammation may occur in any part of the GI tract including esophagitis and gastritis. Fatalities due to GI perforation have been reported in clinical trials of Ipilimumab. Patients should be carefully monitored for GI symptoms that may be indicative of immune-related colitis, diarrhea, or GI perforation. Diarrhea or colitis occurring after initiation of Ipilimumab therapy should be

evaluated to exclude infectious or alternate etiologies. In clinical trials, immune-related colitis was associated with evidence of mucosal inflammation, with or without ulcerations, and lymphocytic infiltration.

Monitor patients for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, mucus or blood in stool, with or without fever) and bowel perforation (such as peritoneal signs and ileus). In symptomatic patients, rule out infectious etiologies and consider endoscopic evaluation to establish etiology and for persistent or severe symptoms. C.difficile toxin has been detected in several patients with colitis and may be an independent entity or may co-exist with Ipilimumab induced inflammatory colitis.

Withhold Ipilimumab dosing for any patient with enterocolitis pending evaluation; administer anti-diarrheal treatment and, if persistent evaluate with colonoscopy and initiate systemic corticosteroids at a dose of 0.5 mg/kg/day prednisone or equivalent.

Permanently discontinue Ipilimumab in patients with severe enterocolitis and initiate systemic corticosteroids at a dose of 1 to 2 mg/kg/day of prednisone or equivalent. Upon improvement to grade 1 or less, initiate corticosteroid taper and continue to taper over at least one month. In clinical trials, rapid corticosteroid tapering has resulted in recurrence or worsening symptoms of enterocolitis in some patients.

Patients have been treated with anti-TNF agents for persistent colitis not responding to steroids. Please note autoimmune pancreatitis may cause abdominal pain and should be included in all evaluations. Enteritis may occur occasionally with other autoimmune events including hepatitis, pancreatitis, and endocrine insufficiency, which should be evaluated as clinically indicated.

<u>Immune-mediated Hepatitis and Pancreatitis</u>
Hepatic immune-related AEs were mostly clinically silent and manifested as transaminase or bilirubin laboratory abnormalities. Fatal hepatic failure has been reported in clinical trials of Ipilimumab. Serum transaminase and bilirubin and lipase levels must be evaluated before each dose of Ipilimumab as early laboratory changes may be indicative of emerging immune-related hepatitis/ pancreatitis and elevations in liver function tests (LFTs) may develop in the absence of clinical symptoms. Increase in LFT or total bilirubin should be evaluated to exclude other causes of hepatic injury, including infections, disease progression, or other medications, and monitored until resolution. Liver biopsies from patients who had immune-related hepatotoxicity showed evidence of acute inflammation (neutrophils, lymphocytes, and macrophages).

Monitor liver function tests (hepatic transaminase and bilirubin levels, lipase) and assess patients for signs and symptoms of hepatotoxicity/ pancreatitis before each dose of Ipilimumab. In patients with hepatotoxicity, rule out infectious or malignant causes and increase frequency of liver function test monitoring until resolution. Withhold Ipilimumab in patients with grade 2 hepatotoxicity.

Permanently discontinue Ipilimumab in patients with grade 3–5 hepatotoxicity/pancreatitis and administer systemic corticosteroids at a dose of 1 to 2 mg/kg/day of prednisone or equivalent. When liver function tests show sustained improvement or return to baseline, initiate

corticosteroid tapering and continue to taper over 1 month. Across the clinical development program for Ipilimumab, mycophenolate treatment has been administered in patients who have persistent severe hepatitis despite high-dose corticosteroids.

Immune-mediated Dermatitis
Skin immune-related AEs presented mostly frequently as a rash and/or pruritus. Some subjects reported vitiligo associated with Ipilimumab administration. Fatal toxic epidermal necrolysis has been reported in clinical trials of Ipilimumab.

Monitor patients for signs and symptoms of dermatitis such as rash and pruritus. Unless an alternate etiology has been identified, signs or symptoms of dermatitis should be considered immune-mediated.

Permanently discontinue Ipilimumab in patients with Stevens-Johnson syndrome, toxic epidermal necrolysis, or rash complicated by full thickness dermal ulceration, or necrotic, bullous, or hemorrhagic manifestations. Administer systemic corticosteroids at a dose of 1 to 2 mg/kg/day of prednisone or equivalent. When dermatitis is controlled, corticosteroid tapering should occur over a period of at least 1 month. Withhold Ipilimumab dosing in patients with moderate to severe signs and symptoms.

For mild to moderate dermatitis, such as grade 1 or 2 pruritus, treat symptomatically. For grade 1 or 2 rash treat with topical corticosteroids (such as hydrocortisone or betamethasone). For persistent grade 2 (more than 7 days, despite treatment with topical steroids) or grade 3 or greater, oral steroids may be administered.

Immune-related Neurological Events
Fatal Guillain-Barré syndrome has been reported in clinical trials of Ipilimumab. Patients may present with muscle weakness and myasthenia gravis, cranial nerve palsy (n VII Bells palsy), and aseptic meninigits, encephalopathy. Unexplained motor neuropathy, muscle weakness, or sensory neuropathy lasting more than 4 days should be evaluated and non-inflammatory causes such as disease progression, infections, metabolic syndromes, nerve entrapment, and medications should be excluded as causes.

Withhold Ipilimumab dosing in patients with any evidence of neuropathy pending evaluation. Monitor for symptoms of motor or sensory neuropathy such as unilateral or bilateral weakness, sensory alterations, or paresthesia. Permanently discontinue Ipilimumab in patients with severe neuropathy (interfering with daily activities) such as Guillain-Barré-like syndromes. Institute medical intervention as appropriate for management of neuropathy and other neurologic events. Consider initiation of systemic corticosteroids at a dose of 1 to 2 mg/kg/day prednisone or equivalent for severe neuropathies.

Immune-mediated Endocrinopathies
Ipilimumab can cause inflammation of endocrine organs including thyroid (Hashimoto's thryroiditis with positive antibodies) and adrenal glands, hypophysitis, hypopituitarism, and resulting thyroid and adrenal insufficiency, low ADH, prolactin, FSH, LH. Hyperthyroid with Graves' disease and positive antibody has been reported. Patients may present with subtle and

nonspecific symptoms. The most common clinical presentation includes headache and fatigue. Symptoms may also include visual field defects, behavioral changes, and electrolyte disturbances including hyponatremia and hypotension. Adrenal crisis as a cause of the patient's symptoms should be excluded. Based on the available data with known outcome, most of the subjects symptomatically improved with hormone replacement therapy. Long-term hormone replacement therapy with HC and synthroid will typically be required for subjects developing hypophysitis/hypopituitarism after treatment with Ipilimumab. Some patients have regained partial function following steroid treatment.

Monitor patients for clinical signs and symptoms of hypophysitis, adrenal insufficiency (including adrenal crisis), and hyper or hypothyroidism. Headache is often the first symptoms of hypophysitis. Patients may present with fatigue, headache, mental status changes, loss of libido, abdominal pain, unusual bowel habits, and hypotension, or nonspecific symptoms which may resemble other causes such as brain metastasis or underlying disease. Unless an alternate etiology has been identified, signs or symptoms of endocrinopathies should be considered immune-mediated and drug withheld pending evaluation. Patients may demonstrate both central (hypophysitis) and peripheral adrenal and thyroid insufficiency. Evaluation of hypophysitis should include pituitary MRI.

Monitor thyroid function tests and clinical chemistries at the start of treatment and hold blood for possible evaluation should clinical events require determining baseline function and anti-thyroid antibodies. A plan for evaluating endocrine function at each visit either by history or monitoring TSH should be included in the protocol with further evaluation as clinically indicated. In a limited number of patients, hypophysitis was diagnosed by imaging studies through enlargement of the pituitary gland. Withhold Ipilimumab dosing in symptomatic patients. Initiate systemic corticosteroids at a dose of 1 to 2 mg/kg/day of prednisone or equivalent, and initiate appropriate hormone replacement therapy.

<u>Other Immune-mediated Adverse Reactions, Including Ocular Manifestations</u>
Ocular inflammation, manifested as grade 2 or grade 3 episcleritis or uveitis, was associated with concomitant diarrhea in a few subjects (<1%) and occasionally occurred in the absence of clinically apparent GI symptoms. Other presumed immune-related AEs reported include, but were not limited to, arthritis/arthralgias, pneumonitis, pancreatitis, autoimmune (aseptic) meningitis, autoimmune nephritis, pure red cell aplasia, noninfective myocarditis, polymyositis, and myasthenia gravis, of which were individually reported for <1% of subjects.

The following clinically significant immune-mediated adverse reactions were seen in less than 1% of Ipilimumab-treated patients in Study 1: nephritis, pneumonitis, pulmonary granuloma resembling sarcoidosis, meningitis, pericarditis, uveitis, iritis, ITP, neutropenia and hemolytic anemia.

Across the clinical development program for Ipilimumab, the following likely immune-mediated adverse reactions were also reported with less than 1% incidence: myocarditis, angiopathy, temporal arteritis, vasculitis, polymyalgia rheumatica, conjunctivitis, blepharitis, episcleritis, scleritis, leukocytoclastic vasculitis, erythema multiforme, psoriasis, pancreatitis, arthritis, and autoimmune thyroiditis.

Permanently discontinue Ipilimumab for clinically significant or severe immune-mediated adverse reactions. Initiate systemic corticosteroids at a dose of 1 to 2 mg/kg/day prednisone or equivalent for severe immune-mediated adverse reactions.

Administer corticosteroid eye drops to patients who develop uveitis, iritis, or episcleritis. Permanently discontinue Ipilimumab for immune-mediated ocular disease that is unresponsive to local immunosuppressive therapy.

Overall, immune-related AEs commonly started within 3 to 10 weeks from first dose, were successfully managed in most instances by omitting doses, discontinuing dosing, and/or through administering symptomatic or immunosuppressive therapy, including corticosteroids, as mentioned above and detailed in Section 7. Immune-related AEs generally resolved within days to weeks in the majority of subjects.

6.2.4.4 **Prohibited and Restricted Therapies**

Patients may not receive standard non-oncology vaccines for the prevention of infectious diseases for up to 4 weeks before or 4 weeks after any dose of Ipilimumab with the exceptions of amantadine and flumadine.

Concomitant systemic or local anti-cancer medications or treatments are prohibited in this study while receiving Ipilimumab 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 or other immunologic activation agonists
- Immunosuppressive agents
- Chronic systemic corticosteroids

6.2.4.5 **Precautions**

Combination therapy may result in unexpected toxicity especially in novel combinations with other immune modifying agents. A striking example in macaques is presented in Vaccari, et al. 2012.

Please note that while unproven, there is a suggestion that autoimmune events, including hepatitis, may occur more frequently at sites of metastases or prior injury.
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. The management guidelines for general inflammatory AEs and Ipilimumab-related GI toxicities, hepatotoxicity, endocrinopathy, and neuropathy (Investigator Brochure, 2011) are provided in Appendix C.

Patients who have received Ipilimumab may potentially develop autoimmune disease with subsequent therapy including the appearance of colitis, hypophysitis or adrenal insufficiency.

6.2.4.6 Study Procedures by Visit and Treatment Cycle

Note that results of all safety laboratory tests (that is, all chemistry and all hematology results) must be obtained and reviewed before Ipilimumab administration, as applicable before administration of Ipilimumab.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

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

7.1 Comprehensive Adverse Events and Potential Risks List (CAEPR)

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

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

7.1.1 CAEPRs for CTEP IND Agent

7.1.1.1 CAEPR for Ipilimumab

Below is the CAEPR for Ipilimumab (MDX-010). Frequency is provided based on 2678 patients.

Version 2.6, July 8, 2014[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]		
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]		
INFECTIONS AND INFESTATIONS			
	Infections and infestations - Other (aseptic meningitis)[2]		
INVESTIGATIONS			
	Alanine aminotransferase increased		
	Aspartate aminotransferase increased		
	Neutrophil count decreased		
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		
	Dehydration		

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%)**	
	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.

Non-serious adverse events also reported on ipilimumab (MDX-010) trials but with the relationship to ipilimumab (MDX-010) still undetermined due to low frequency (i.e., <3%):

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 - 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.

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting until March 31, 2018 for AE reporting. CTCAE version 5.0 will be utilized for AE reporting beginning April 1, 2018. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the

CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- For expedited reporting purposes only:
  - AEs for the agent that are ***bold and italicized*** in the CAEPR (*i.e.*, those listed in the SPEER column, Section 7.1.1) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.
  - Other AEs for the protocol that do not require expedited reporting are outlined in section 7.3.4.

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

7.3 Expedited Adverse Event Reporting

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

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

7.3.2 CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Study Coordinator of the Lead Organization, Principal Investigator, and the local treating physician. Each site will submit the electronic version of the CTEP-AERS report to the PMH Phase II Consortium Central Office. Once review by the lead group coordinator has taken place the report will be forwarded to NCI. CTEP-AERS provides a copy feature for other e-mail recipients.

7.3.3 Expedited Reporting Guidelines

Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.
Death due to progressive disease should be reported as **grade 5 "Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (Progressive Disease)"** under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Use the NCI protocol number and the protocol-specific patient ID assigned during trial

registration on all reports.

In order to ensure the timely fulfillment of both US and Canadian IND regulatory reporting requirements, all CTEP-AERS reports must be sent to the PMH Phase II Consortium Central Office within 3 working days from the date the event was known to the investigator.

- In the unlikely event that an adverse event occurs that does not meet the reporting requirements for CTEP-AERS, but does meet the definition of a Serious Adverse Event, an CTEP-AERS report must still be completed and sent to the Central Office within 3 working days of the event being known to the investigator. The event must be telephoned or e-mailed to Central Office within 1 working day.

- The PMH Phase II Consortium Central Office will be responsible for reporting to Canadian regulatory authorities all Serious Adverse Events that are both unexpected and related to study drug. The Central Office will notify all Investigators of all Serious Adverse Events that are reportable to regulatory authorities in Canada from this trial or from other clinical trials as reported to the Central Office by the NCI U.S.

Investigators must notify their local Research Ethics Boards (REB/IRBs), according to their guidelines, of all SAE reports from their centre and file the report in their regulatory study binder. In addition, all reports sent out to centres by the PMH Phase II Consortium Central Office must be sent to local REB/IRBs, according to their guidelines. Documentation from the REB/IRB of receipt of these reportable events must be kept on file in each institution's regulatory binder.

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

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)

NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)

An adverse event is considered serious if it results in **ANY** of the following outcomes:

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

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

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

Expedited AE reporting timelines are defined as: ○ "24-Hour; 5 Calendar Days" - The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report. ○ "10 Calendar Days" - A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE.
[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows: **Expedited 24-hour notification followed by complete report within 5 calendar days for:** • All Grade 3, 4, and Grade 5 AEs **Expedited 10 calendar day reports for:** • Grade 2 AEs resulting in hospitalization or prolongation of hospitalization [2] For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period. Effective Date: May 5, 2011

7.4 Routine Adverse Event Reporting

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

7.5 Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.
CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported viaCTEP-AERS. Three options are available to describe the event:

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

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

7.6 Second Malignancy

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

8. PHARMACEUTICAL INFORMATION

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

8.1 CTEP IND Agent(s)

8.1.1 **Ipilimumab (NSC 732442)**

Chemical Name or Amino Acid Sequence: 4 polypeptide chains, 2 identical heavy chains with 447 amino acids and 2 identical light chains consisting of 215 amino acids.

Other Names: Anti-CTLA-4 monoclonal antibody, MDX-010

Classification: Human monoclonal antibody

M.W.: 147,991 Daltons

Mode of Action: Ipilimumab is specific for the CTLA-4 antigen expressed on a subset of activated T-cells. CTLA-4 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 CTLA-4 with the B7 ligand, resulting in a blockade of the inhibitory effect of T-cell activation. The CTLA-4/B7 creates the interaction.

Description: Ipilimumab is a fully human immunoglobulin ($IgG_1\kappa$) with two manufacturing processes – ongoing trials have been using substances manufactured using Process B. New clinical trials will be using 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.

How Supplied: Bristol-Myers-Squibb (BMS) supplies Ipilimumab to the DCTD/NCI. Ipilimumab is available as a 50 mg/10 mL (5 mg/ mL) and a 200 mg /40 mL (5 mg/mL) single-use vial. The vial is a clear, colorless, sterile, isotonic aqueous solution (pH of 7) that may contains particles.

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.

Preparation: Ipilimumab is given undiluted or further diluted in 0.9% NaCl Injection, USP or 5% Dextrose Injection, USP in concentrations between 1 mg/mL and 4 mg/mL. Ipilimumab is stable in a polyvinyl chloride (PVC), non-PVC/non DEHP (di-(2-ethylhexyl) phthalate) IV bag or glass container up to 24 hours refrigerated at (2^0 to 8^0 C) or at room temperature/ room light.

Storage: Store intact vials refrigerated at (2^0 to 8^0 C), protected from light. Do not freeze.

Stability: Shelf-life surveillance of the intact vials is ongoing.
Solution as described above is stable up to 24 hours refrigerated at (2^0 to 8^0 C) or at room temperature/ room light.

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

Route*(s)* of Administration: IV. Do not administer Ipilimumab as an IV push or bolus injection.

Method of Administration: Can use a volumetric pump to infuse Ipilimumab at the protocol-specific dose(s) and rate(s) via a PVC IV infusion set with an in-line, sterile, non-pyrogenic, low-protein-binding filter (0.2 micron to 1.2 micron).

Patient Care 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.

Availability: Ipilimumab is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI. Ipilimumab is provided to the NCI under a Collaborative Agreement between the Bristol-Myers-Squibb and the DCTD, NCI (see Section 12.3).

8.1.2 Agent Ordering and Agent Accountability

8.1.2.1 NCI-supplied agents may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent 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). The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Active CTEP-registered investigators and investigator-designated shipping designees and

ordering designees can submit agent requests through the PMB Online Agent Order Processing (OAOP) application (https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx). Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account (https://eapps-ctep.nci.nih.gov/iam/) and the maintenance of an "active" account status and a "current" password. For questions about drug orders, transfers, returns, or accountability, call (301) 496-5725 Monday through Friday between 8:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov anytime.

8.1.2.2 Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Drug Accountability Record Form (DARF). (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

Immune assessment studies will be performed using peripheral blood obtained from study subjects prior to and following ipilimumab therapy. These studies are exploratory and are designed to identify immune biomarkers that may be associated with ipilimumab treatment and/or clinical response. These studies will be used in the design of future larger studies.

Previous studies have investigated potential biomarkers in patients treated with anti-CTLA-4 monoclonal antibodies (mAbs). Monitoring peripheral blood T cells has shown that T cell activation markers such as HLA-DR and ICOS are increased following treatment. Studies evaluating more specific biomarkers have not yielded consistent results, including those evaluating T cells specific for tumor antigens or negative regulatory T cell populations (Phan et al., 2003; Maker et al., 2005; O'Mahony et al., 2007; Reuben et al., 2006; Yuan et al., 2008; Liakou et al., 2008). Evaluation of tumor tissues suggests that anti-CTLA-4 treatment may increase the number of tumor infiltrating lymphocytes (TIL) and increase the ratio of effector T cells to regulatory T cells (Hodi et al., 2003; Hodi et al., 2008). In bladder cancer patients, ipilimumab treatment resulted in increases in ICOShigh CD4+ TIL, which produce the pro-inflammatory cytokine IFN-□ (Liakou et al., 2008). In a small study that included biopsies from four melanoma patients before and after anti-CTLA-4 therapy with tremelimumab, immunohistochemical (IHC) staining suggested that clinical responders tended to show increased CD8+ TIL following treatment (Ribas et al., 2009). Another study that examined pre- and post-ipilimumab treatment biopsies was reported in abstract form. This study found that clinical activity correlated with higher levels of FOXP3 and IDO staining at baseline and with an increase in TIL post-treatment (Hamid et al., 2009).

9.1 Biomarker Studies

9.1.1 Peripheral Blood Immune Assessment – Laboratory Correlative Study #1

Peripheral blood cells, plasma, and serum will be isolated from phlebotomy specimens. These samples will be used fresh or stored for future analysis and will undergo immunologic assessment using established protocols (Butler et al., 2007; Butler et al., 2011, Crome et al. 2010; Nguyen et al. 2010). We anticipate that the samples will undergo the following analysis,

however, samples may also undergo additional studies in the future as part of exploratory studies. Peripheral blood mononuclear cells (PBMC) will be analyzed phenotypically by flow cytometry using mAbs specific for molecules such as CD4, CD8, CD3, CD16, CD19, CD56, CD11c and CD11b to delineate immune populations present. Furthermore, markers will be used to characterize the function of immune populations.

These include molecules upregulated with activation (ie CD25, CD69, HLA-DR), molecules that indicate stimulatory versus inhibitory immune responses (CD28, CD80, CD86, CTLA-4, PD-1, PDL-1, ICOS), markers of central versus effector memory T cells (CD62L, CCR7) and molecules which are differentially expressed between CD4+ T helper subsets (CD161, CXCR3, CCR6, CCR4, GARP and FOXP3). In parallel, circulating lymphocytes will be analyzed for cytotoxicity and the ability to secret cytokines such as interferon-gamma, tumor necrosis factor alpha, Interleukin (IL)-2, IL-4, IL-6, IL-9, IL-10, IL-17 and IL-22. Antigen-specific responses may be analyzed using overlapping peptides spanning the HPV-derived proteins, E6 and E7, to assess immune reactivity to these tumor associated antigens antigens. Samples may also undergo HLA serotyping and/or genotyping.

9.1.1.1 **Collection of Specimens**

Immune study bloods include nine 6ml tubes for heparinized blood, and one 5 ml tubes for sera (SST tube). Blood will be obtained at the following study visits: Pre-study, Wk 1 (prior to ipilimumab infusion), Wk 2, Wk 4, Wk7, and Wk 10. Immune study bloods will also be obtained during the maintenance phase of the study on days that ipilimumab is infused (ie Wk 24, 36, 48, 60 and every 3 months thereafter.). After the end of the maintenance phase of treatment immune study blood samples will be obtained at the time of follow-up appointments (every 3 months). A final immune study blood sample will be obtained on the follow-up visit one month after withdrawal from study.

9.1.1.2 **Shipping of Specimens**

Heparinized blood must be kept at room temperature after collection. Blood for sera should be kept upright at room temperature and clot for 30-60 minutes. Centrifuge blood at 1500g for 15 minutes. Divide serum evenly into two aliquots. Freeze samples at -70^{o} C and store until shipping.
Refer to the study-specific Laboratory Manual for detailed collection and processing procedures.

9.1.1.3 **Handling of Specimens**

Blood specimens will be transferred by overnight shipment to the Correlative Studies Program (PMH).

Correlative Studies Program
Princess Margaret Hospital
610 University Avenue 9-718
Toronto, Ontario M5G 2M9
Tel: (416) 946-4501 ext 5047
Fax: (416) 946-4431
Email: CCRUcorrelativestudies@uhn.ca

9.1.1.4 Site Performing Correlative Study

PMH will be the site for performing correlative studies for peripheral blood.

9.1.2 Tumor Biopsy Assessment

We will perform immunohistochemistry on archived tumour tissue and fresh tumour tissue (within 14 days prior to registration and within the first week of cycle 2 onset) to assess the density of tumor infiltrating lymphocytes using validated markers for CD3, CD4, and CD8 which are in routine clinical use. Exploratory analysis will be performed to assess for markers associated with immune suppression such as Foxp3 and IDO. When sufficient tumor tissue is available, enzymatic dissociation will be performed and tumor infiltrating lymphocytes will be expanded. Exploratory analysis by flow cytometry will be performed to phenotypically assess expanded cells using monoclonal antibodies such as CD4, CD8, CD3, CD16, CD19, CD56, CD11c, CD11b, CD25, CD69, HLA-DR, CD28, CD80, CD86, CTLA-4, PD-1, PD-L1, CCR7, CD62L, and Foxp3.

9.1.2.1 Collection of Specimens

Archival slides and tumor biopsies will be obtained. Refer to the study-specific Laboratory Manual for detailed collection and processing procedures.

9.1.2.2 Handling of Specimens

Refer to the study-specific Laboratory Manual for detailed collection and processing procedures.

9.1.2.3 Shipping of Specimens

Correlative Studies Program
Princess Margaret Hospital
610 University Avenue 9-718
Toronto, Ontario M5G 2M9
Tel: (416) 946-4501 ext 5047
Fax: (416) 946-4431
Email: CCRUcorrelativestudies@uhn.ca

9.1.2.4 Site Performing Correlative Study

PMH will be the site for performing correlative studies for archival tissue.

Statistical Considerations

Clinical response to treatment as defined by the main drug treatment clinical protocol will be compiled and results of biomarker analysis from “responders” and “non-responders” will be compared. These correlative studies are exploratory in nature and will be used in the design of future larger studies.

9.2 Special Studies

N/A

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 1 week prior to start of protocol therapy unless stated otherwise. Scans and x-rays must be done ≤3 weeks prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy. If pre-study blood work, vitals and weight were done within 7 days of treatment initiation, they do not need to be repeated for cycle 1 day 1. If pre-study physical exam and performance status were done within 3 days prior to treatment initiation, they do not need to be repeated for cycle 1 day 1. For subsequent cycles (2+), day 1 assessments can be completed up to 48hrs prior to day 1; however blood samples can be collected and analyzed up to 72hrs prior to dosing. A cycle is 21 days long for phase I and II.

		C1		C2	C3	C4		Post C4 Treatment		
	Pre-study	D1	W2	D1	D1	D1	W3	Wk 16	Wk 18	Wk 20
Ipilimumab		A		A	A	A				
Informed consent	X									
Demographics	X									
Medical history	X									
Concurrent meds	X	X		X	X	X	X	X		X
Physical exam	X	X		X [d]	X [d]	X [d]	X [d]	X [d]		X [d]
Vital signs	X	X		X	X	X	X	X		X
Height	X									
Weight	X	X		X	X	X		X		X
ECOG assessment	X	X		X	X	X	X	X		X
CBC w/diff, plts [g]	X	X		X	X	X	X	X		X
Serum chemistry [a, g]	X	X		X	X	X	X	X		X
TSH, T3, T4 [g]	X	X		X	X	X	X	X		X
LFT's (AST, ALT, total bilirubin, lipase) [e,g]	X	X		X	X	X	X	X		X
EKG (as indicated) [c]	X									
CRP	X	X					X			
Adverse event evaluation [d]		X----------								----------X
Tumor measurements	X					X			X	
Radiologic evaluation	X						X [f]		X	
B-HCG (serum) [b]	X	X		X	X	X				
Archival tissue (if available)	X									
Pre and Post treatment biopsy	X (pre) [h]			X (post) [i]						
Peripheral blood immune assessment	X	X [1,j]	X [j]	X [2,j]	X [3,j]	X [4,j]				

1. Prior to Ipilimumab infusion
2. To be performed during week 4 of the study, preferably day 1
3. To be performed during week 7 of the study, preferably day 1
4. To be performed during week 10 of the study, preferably day 1

a. Sodium, potassium, chloride, albumin, creatinine, magnesium, phosphate
b. To be performed on women of childbearing potential

c. As clinically indicated beyond screening
d. Review for autoimmune events
e. LFT results must be reviewed by the principal investigator (or designee) to meet dosing criteria. The subject should be evaluated and managed referring to the IB, management section, and algorithm in Appendix C, as clinically appropriate
f. Cycle 4 week 3 (week 12) radiological assessment can be performed +/- 1 week
g. Any change from baseline levels within the first 4 cycles which may be related to known immune events are to be followed on a weekly basis until the event is no longer related or returns to baseline levels.
h. Pre-treatment biopsy must be done within 14 days prior to registration
i. Post treatment biopsy to be completed within the first week of cycle 2 onset
j. Can be done up to 24hrs prior to Ipilimumab infusion

	Maintenance Phase							Off Study [a,g]
	C5		C6		C7		C8	
	W24	W30	W36	W42	W48	W54	W60	
Ipilimumab	A		A		A		A	
Informed Consent								
Demographics								
Medical history								
Concurrent meds	X	X	X	X	X	X	X	X
Physical exam [c]	X	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X	X
Height								
Weight	X	X	X	X	X	X	X	X
ECOG assessment	X	X	X	X	X	X	X	X
CBC w/diff, plts	X	X	X	X	X	X	X	X
Serum chemistry [e]	X	X	X	X	X	X	X	X
TSH, T3, T4	X	X	X	X	X	X	X	X
LFT's (AST, ALT, total bilirubin, lipase) [d]	X	X	X	X	X	X	X	X
EKG (as indicated) [b]								
CRP	X		X		X		X	
Adverse event evaluation [c]	X----------X							
Tumor measurements	X[i]		X[i]		X[i]		X[i]	X[h]
Radiologic evaluation	X[i]		X[i]		X[i]		X[i]	X[h]
B-HCG (serum)	X		X		X		X	
Peripheral blood immune assessment	X		X		X		X	X [f]
Subsequent therapy received post Ipilimumab								X

a. Off study follow-up appointments should occur every 3 months in individuals with PR, CR or SD at the end of treatment for a maximum period of 1 year or until progression of disease or death whichever occurs sooner. Individuals withdrawn from study due to PD will have only one follow-up visit after withdrawal from treatment and will not be followed for a year. Individuals withdrawn from study due to Ipilimumab related adverse effects will be given medical treatment as appropriate and followed up on a monthly basis until resolution of the adverse event and then every 3 months for 1 year or until progression or death whichever occurs sooner.

b. As clinically indicated beyond screening
c. Review for autoimmune events
d. LFT results must be reviewed by the principal investigator (or designee) to meet dosing criteria If, during the course of treatment abnormal LFT values are detected, Ipilimumab should not be administered. The subject should be evaluated and managed referring to the IB, management section, and algorithm in Appendix C, as clinically appropriate
e. Sodium, potassium, chloride, albumin, creatinine, magnesium, phosphate
f. Obtained every 3 months during the follow-up visit.
g. Patients that are not going on to the maintenance cycle will complete their off study on week 24 or when they stop receiving treatment as applicable
h. Radiological assessment only necessary for individuals demonstrating PR, CR or SD at completion of treatment.
i. Radiological evaluations in maintenance phase can be done up to 1 week prior to Ipilimumab dosing for week 24 and onwards (-1 week)

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be evaluated for response as stipulated on the study calendar.

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

11.1.1 **Definitions**

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

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

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

11.1.2 **Disease Parameters**

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as >20 mm by chest x-ray or as >10 mm

with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters). Tumor lesions situated in a previously irradiated field are considered measurable if they have previously been demonstrated to progress as per RECIST 1.1 criteria on radiologic imaging prior to enrollment on study.

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

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

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

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

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

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

11.1.3 **Methods for Evaluation of Measurable Disease**

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and

never more than 4 weeks before the beginning of the treatment.

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

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

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

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

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

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

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

or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

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

Tumor markers Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

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

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

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

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

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

11.1.4 **Response Criteria**

11.1.4.1 **Evaluation of Target Lesions**

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

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

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

Stable Disease (SD) modified:(to be used at week 12 and beyond of study)

Clinically stable patients with no change in ECOG status and not requiring any additional medical interventions who demonstrate an increase of ≤30% in the sum of diameters of target lesions, taking as reference the smallest sum on study. In addition, the sum must also demonstrate an absolute increase of at least 5mm.

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

11.1.4.2 **Evaluation of Non-Target Lesions**

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

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

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

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

11.1.4.3 **Evaluation of Best Overall Response**

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

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

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

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

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

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

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

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

11.1.5 **Duration of Response**

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

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

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

11.1.6 **Progression-Free Survival**

Progression free survival (PFS) is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first. If such event is not observed after 1 year of follow up, patients are censored.

11.1.7 Response Review

All responses will be reviewed by an expert(s) independent of the study at the study's completion by simultaneous review of patient files and radiologic images.

11.2 Other Response Parameters

Tumors will be assessed for response as per suggested immune related response criteria (irRC).

12. DATA REPORTING / REGULATORY REQUIREMENTS

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

12.1 Data Reporting

12.1.1 **Method**

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

Note: If your study has been assigned to CDUS-Complete reporting, **all** adverse events (both routine and expedited) that have occurred on the study and meet the mandatory CDUS reporting guidelines must be reported via the monitoring method identified above. If your study has been assigned to CDUS-Abbreviated reporting, no adverse event reporting (routine or expedited) is required to be reported via CDUS.

12.1.2 **Responsibility for Data Submission**

Study participants are responsible for entering their data into the Medidata Rave system and submitting copies of their source notes to the Central Office / Coordinating Centre within 3 weeks of the end of cycle. Please refer to Appendix G, Data Management Guidelines, for further details regarding data submission requirements.

The Central Office / Coordinating Centre is responsible for compiling and submitting CDUS data to CTEP for all participants and for providing the data to the Principal Investigator for review.

12.2 CTEP Multicenter Guidelines

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

- The Principal Investigator/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports received from CTEP to all participating institutions for submission to their individual IRBs for action as required.

- Except in very unusual circumstances, each participating institution will order DCTD-supplied agents directly from CTEP. Agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO (PIO@ctep.nci.nih.gov) except for Group studies.

12.3 Collaborative Agreements Language

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (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/industryCollaborations2/intellectual_property.htm) 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 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 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 NCI, 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 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 Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm). 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 by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies 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:

Email: 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.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

The run in phase of this study is to assess the safety of Ipilimumab in eligible cervical cancer patients. Six patients will be enrolled in this phase of the study and followed for a period of 12 weeks. If 3 patients experience toxicity considered to be dose-limiting the safety cohort will be expanded to include an additional 6 individuals. In the event that the phase I cohort is expanded, should >4 patients experience a dose limiting toxicity, as defined earlier, the trial will be halted at this point.
Should minimum safety criteria be fulfilled we will proceed to the second phase of the trial which will be a single-arm, non-randomized, open-label multi-center two-stage phase II study to evaluate the antitumor activity of Ipilimumab as measured by the primary endpoint of objective response rate using RECIST.

In phase II of the trial a safety assessment will be conducted after 12 patients have been treated. If >4 have DLT by same definition as Phase I run in, the study will be put on hold and discussed with CTEP.

Sample size calculations are based on the two stage design according to Simon (Simon, 1989). The design will allow the study to terminate after enrollment of 18 evaluable patients if no objective response is observed. If the stopping rule is not met, a further 14 evaluable patients will be enrolled for a total of 32 patients. If the total number of patients responding is 4 or more, the drug is accepted.

The null hypothesis is that the response rate is less than 5% and the alternative is that the response rate is more than 20%. The design has alpha error 10% and power of 90%.

13.2 Sample Size/Accrual Rate

6-44

13.3 Stratification Factors

N/A

13.4 Analysis of Secondary Endpoints

Secondary Endpoints:

- To assess the antitumor activity of Ipilimumab through secondary endpoints including of disease stabilization and PFS.
- Assessment of antitumor activity of Ipilimumab using immune-related response criteria (irRC)
- Assessment of the predictive value of baseline C-reactive protein
- Assess the biologic responses of exposure to Ipilimumab via correlative studies involving analysis of lymphocyte subsets and assessment of cervical cancer-antigen specific T cells anti-tumor response.
- Evaluation of archival tissue with regard to markers of immune population in correlation with clinical stage and response to treatment

Summary statistics, such as mean, median, counts and proportion, will be used to summarize the patients. Survival estimates will be computed using the Kaplan-Meier method. Potential association between variables will be measured using Pearson correlation coefficients, chi-square tests, one- or two-sample t-tests or logistic regression analyses as appropriate. Non-parametric tests such as Spearman correlation coefficients, Fisher's exact tests and Wilcoxon rank sum test may be substituted if necessary. Ninety-five percent confidence intervals will be constructed and selected results will be illustrated using figures and plots.

Frequency and severity of adverse events will be tabulated using counts and proportions detailing frequently occurring, serious and severe events of interest.

13.5 Reporting and Exclusions

13.5.1 Evaluation of Toxicity

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

13.5.2 Evaluation of Response

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 8) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 8 usually designates the "unknown" status of any type of data in a clinical database.]

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) should be included in the main analysis of the response rate. Patients in response categories 4-7 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate

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

REFERENCES

Ansell, S.M., S.A. Hurvitz, P.A. Koenig*, et al.* (2009). Phase I study of Ipilimumab, an anti-CTLA-4 monoclonal antibody, in patients with relapsed and refractory B-cell non-Hodgkin lymphoma. *Clin Cancer Res*. 15:6446-6453.

Bashey, A., B. Medina, S. Corringham*, et al.* (2009). CTLA4 blockade with Ipilimumab to treat relapse of malignancy after allogeneic hematopoietic cell transplantation. *Blood*. 113:1581-1588.

Bhat P, Mattarollo SR, Gosmann C. et al. Regulation of immune responses to HPV infection and during HPV-directed immunotherapy. Immunological Reviews 2011; 239:85-98

Bloom, M.B., D. Perry-Lalley, P.F. Robbins*, et al.* (1997). Identification of tyrosinase-related protein 2 as a tumor rejection antigen for the B16 melanoma. *J Exp Med*. 185:453-459.

BMS Protocol CA184004. (2010). An exploratory study to determine potential predicitve markers of response and/or toxicity in patients with unresectable stage III or IV malignant melanoma randomized and treated with Ipilimumab (MDX-010/BMS-734016) at two dose levels. Bristol-Myers Squibb Research and Development. Document Control No. 930043855.

BMS Protocol CA184007. (2010). Randomized, double-blind, placebo-controlled, Phase 2 study comparing the safety of Ipilimumab administered with or without prophylactic oral budesoride (Entocort™ EC) in patients with unresectable stage III or IV malignant melanoma. Bristol-Myers Squibb Research and Development. Document Control No. 930043852.

BMS Protocol CA184008. (2010). A multicenter, single arm Phase 2 study of MDX-010 (BMS-734016) monotherapy in patients with previously treated unresectable stage III or IV melanoma. Bristol-Myers Squibb Research and Development. Document Control No. 930043858.

BMS Protocol CA184022. (2010). A randomized, double-blind, multicenter, Phase 2, fixed study of multiple dose of Ipilimumab (MDX-010) monotherapy in patients with previously treated unresectable stage III or IV melanoma. Bristol-Myers Squibb Research and Development. Document Control No. 9.0043860.

Carthon, B.C., J.D. Wolchok, J. Yuan*, et al.* (2010). Preoperative CTLA-4 blockade: tolerability and immune monitoring in the setting of a presurgical clinical trial. *Clin Cancer Res*. 16:2861-2871.

Chambers, C.A., T.J. Sullivan, and J.P. Allison. (1997). Lymphoproliferation in CTLA-4-deficient mice is mediated by costimulation-dependent activation of CD4+ T cells. *Immunity*. 7:885-895.

Fong, L., S.S. Kwek, S. O'Brien*, et al.* (2009). Potentiating endogenous antitumor immunity to prostate cancer through combination immunotherapy with CTLA4 blockade and GM-CSF. *Cancer Res*. 69:609-615.

Frazer IH. Prevention of cervical cancer through papillomavirus vaccination. Nat. Rec. Immunol. 2004; 4:46-54

Halpert R, Fruchter RG, Sedlis A. et al. Human papillomavirus and lower genital neoplasia in renal transplant patients. Obstet Gynecol 1986; 68(2):251-8

Hara, I., Y. Takechi, and A.N. Houghton. (1995). Implicating a role for immune recognition of self in tumor rejection: Passive immunization against the brown locus protein. *J Exp Med*. 182:1609-1614.

Harper DM, Franco EL, Wheeler CM et al. HPV vaccine study group. Sustained efficacy of up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: Follow-up from a randomised control trial. Lancet 2006; 26:K17-K28

Harzstark, A.L., L. Fong, V.K. Weinberg*, et al.* (2010). Final results of a phase I study of CTLA-4 blockade in combination with GM-CSF for metastatic castration resistant prostate cancer (mCRPC). *J Clin Oncol*. 28:A6032.

Hodi, F.S., M. Butler, D.A. Oble*, et al.* (2008). Immunologic and clinical effects of antibody blockade of cytotoxic T lymphocyte-associated antigen 4 in previously vaccinated cancer patients. *Proc Natl Acad Sci U S A*. 105:3005-3010.

Hodi, F.S., S.J. O'Day, D.F. McDermott*, et al.* (2010). Improved survival with Ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 363:711-723.

Hodi, F.S., P.A. Friedlander, M.B. Atkins*, et al.* (2011). A phase I trial of Ipilimumab plus bevacizumab in patients with unresectable stage III or stage IV melanoma. *J Clin Oncol*. 29:A8511.

Hoos, A., A.M. Eggermont, S. Janetzki*, et al.* (2010). Improved endpoints for cancer immunotherapy trials. *J Natl Cancer Inst*. 102:1388-1397.

Insinga RP, Perez G, Wheeler CM et al. Incidence, duration and reappearance of type-specific cervical human papillomavirus infections in young women. Cancer Epidemiol Biomarkers Prev 2010; 19:1585-1594

Investigators Brochure. (2011). Ipilimumab; BMS-734016; MDX-010. 14th Edition Bristol-

Myers Squibb. Wallingford, CT.

Jaafar F, Righi E, Lindstrom V. et al. Correlation of CXCL12 expression and FoxP3+ cell infiltration with human papillomavirus infection and clinicopathological progression of cervical cancer. Am J Pathol 2009; 175:1525-1535

Ji, R.-R., O. Hamid, S.D. Chasalow*, et al.* (2011). An immune-active tumor microenvironment favors clinical response to Ipilimumab *AACR Abstracts*. A5513.

Jordanova ES et al. Human leukocyte antigen class I. MHC class I chain-related molecule A, and CD8+/regulatory T-cell ratio: which variable determines survival of cervical cancer patients? Clin Cancer Res 2008; 14: 2028-2035

Kearney, E.R., T.L. Walunas, R.W. Karr*, et al.* (1995). 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*. 155:1032-1036.

Keler, T., E. Halk, L. Vitale*, et al.* (2003). Activity and safety of CTLA-4 blockade combined with vaccines in cynomolgus macaques. *J Immunol*. 171:6251-6259.

Krummel, M.F., and J.P. Allison. (1995). CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. *J Exp Med*. 182:459-465.

Krummel, M.F., T.J. Sullivan, and J.P. Allison. (1996). Superantigen responses and co-stimulation: CD28 and CTLA-4 have opposing effects on T cell expansion in vitro and in vivo. *Int Immunol*. 8:519-523.

Kuhns, M.S., V. Epshteyn, R.A. Sobel, and J.P. Allison. (2000). Cytotoxic T lymphocyte antigen-4 (CTLA-4) regulates the size, reactivity, and function of a primed pool of $CD4^+$ T cells. *Proc Natl Acad Sci USA*. 97:12711-12716.

Laga M, Icenogle Jp, Marsella R. et al. Genital papillomavirus infection and cervical dysplasia opportunistic complications of HIV infection. Int J Cancer 1992; 50(1):45-48

Legatt GR, Frazer IH. HPV vaccines: the beginning of the end for cervical cancer. Curr Opin IMmunol 2007; 19:232-238

Liakou, C.I., A. Kamat, D.N. Tang*, et al.* (2008). CTLA-4 blockade increases IFNgamma-producing CD4+ICOShi cells to shift the ratio of effector to regulatory T cells in cancer patients. *Proc Natl Acad Sci U S A*. 105:14987-14992.

Madan, R.A., M. Mohebtash, P.M. Arlen*, et al.* (2010). Overall survival (OS) analysis of a phase l trial of a vector-based vaccine (PSA-TRICOM) and Ipilimumab (Ipi) in the treatment of metastatic castration-resistant prostate cancer (mCRPC). *2010 Genitourinary Cancers Symposium*. A38.

Min, L., A. Vaidya, and C. Becker. (2011). Thyroid autoimmunity and ophthalmopathy related to melanoma biological therapy. *Eur J Endocrinol*. 164:303-307.

Molling JW, de Gruijl TD, Glim J. et al. CD4+CD25hi regulatory T-cell frequency correlates with persistence of human papillomavirus type 16 and T helper cell responses in patients with cervical intraepithelial neoplasia. Int J cancer 2007; 121:1749-1755

Munoz N. Human papillomavirus and cancer: The epidemiological evidence. J. Clin Virol 2000; 19:1-5

Munoz N, Manalastas R, Pipisuttithum P. et al. Safety, immunogenicity, and efficacy of quadrivalent human papillomavirus (types 6, 11,16,18) recombinant vaccine in women aged 24-45 years: A randomised, double-blind trial. Lancet 2009; 373:1949-1957

Naftzger, C., Y. Takechi, H. Kohda*, et al.* (1996). Immune response to a differentiation antigen induced by altered antigen: A study of tumor rejection and autoimmunity. *Proc Natl Acad Sci USA*. 93:14809-14814.

O'Mahony, D., J.C. Morris, C. Quinn*, et al.* (2007). A pilot study of CTLA-4 blockade after cancer vaccine failure in patients with advanced malignancy. *Clin Cancer Res*. 13:958-964.

Overwijk, W.W., A. Tsung, K.R. Irvine*, et al.* (1998). gp100/pmel 17 is a murine tumor rejection antigen: Induction of "self"-reactive, tumoricidal T cells using high-affinity, altered peptide ligand. *J Exp Med*. 188:277-286.

Overwijk, W.W., D.S. Lee, D.R. Surman*, et al.* (1999). Vaccination with a recombinant vaccinia virus encoding a "self" antigen induces autoimmune vitiligo and tumor cell destruction in mice: Requirement for $CD4^+$ T lymphocytes. *Proc Natl Acad Sci USA*. 96:2982-2987.

Rosenberg, S.A., and D.E. White. (1996). Vitiligo in patients with melanoma: Normal tissue antigens can be targets for cancer immunotherapy. *J Immunother Emphasis Tumor Immunol*. 19:81-84.

Royal, R.E., C. Levy, K. Turner*, et al.* (2010). Phase 2 trial of single agent Ipilimumab (anti-CTLA-4) for locally advanced or metastatic pancreatic adenocarcinoma. *J Immunother*. 33:828-833.

Simpson, T.R., S.A. Quezada, and J.P. Allison. (2010). Regulation of CD4 T cell activation and effector function by inducible costimulator (ICOS). *Curr Opin Immunol*. 22:326-332.

Small, E.J., N.S. Tchekmedyian, B.I. Rini*, et al.* (2007). A pilot trial of CTLA-4 blockade with human anti-CTLA-4 in patients with hormone-refractory prostate cancer. *Clin Cancer Res*. 13:1810-1815.

Stein, W.D., J.L. Gulley, J. Schlom, *et al.* (2011). Tumor regression and growth rates determined in five intramural NCI prostate cancer trials: the growth rate constant as an indicator of therapeutic efficacy. *Clin Cancer Res*. 17:907-917.

Thompson, C.B., and J.P. Allison. (1997). The emerging role of CTLA-4 as an immune attenuator. *Immunity*. 7:445-450.

Tivol, E.A., F. Borriello, A.N. Schweitzer, *et al.* (1995). Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity*. 3:541-547.

Tuve S, Chen BM, Liu Y. et al. Combination of Tumor Site-Located CTL-assocaited Antigen-4 Blockade and Systemic Regulatory T-cell Depletion Induces Tumor-Destructive Immune Responses. Cancer Res 2007; 67(12): 5929-5939

Vaccari, M., A. Boasso, C. Fenizia, *et al.* (2012). Fatal Pancreatitis in Simian Immunodeficiency Virus SIVmac251-Infected Macaques Treated with 2',3'-Dideoxyinosine and Stavudine following Cytotoxic-T-Lymphocyte-Associated Antigen 4 and Indoleamine 2,3-Dioxygenase Blockade. *J Virol*. 86:108-113.

Valdespino V, Gorodezky C, Vianney O. et al. HPV 16-specific cytotoxic T lymphocyte responses are detected in all HPV 16-positive cervical cancer patients. Gynecologic Oncology 2005; 96:92-102

Woo YL, van den Hende M, Sterling JC et al. A prospective study on the natural course of low-grade squamous intra-epithelial lesions and the presence of HPV16 E2-, E6- and E7- specific T-cell responses. Inc J Cancer 2010;126:133-141

Walunas, T.L., D.J. Lenschow, C.Y. Bakker, *et al.* (1994). CTLA-4 can function as a negative regulator of T cell activation. *Immunity*. 1:405-413.

Waterhouse, P., J.M. Penninger, E. Timms, *et al.* (1995). Lymphoproliferative disorders with early lethality in mice deficient in CTLA-4. *Science*. 270:985-988.

Weber, L.W., W.B. Bowne, J.D. Wolchok, *et al.* (1998). Tumor immunity and autoimmunity induced by immunization with homologous DNA. *J Clin Invest*. 102:1258-1264.

Weber, J.S., B. Yu, M. Hall, *et al.* (2011). Pharmacodynamic and predictive markers of Ipilimumab on melanoma patients' T-cells. *J Clin Oncol*. 29:A2503.

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

Yang, J.C., M. Hughes, U. Kammula, *et al.* (2007). Ipilimumab (anti-CTLA4 antibody) causes regression of metastatic renal cell cancer associated with enteritis and hypophysitis. *J*

Immunother. 30:825-830.

Zhou, J., A. Bashey, R. Zhong*, et al.* (2011). CTLA-4 Blockade following relapse of malignancy after allogeneic stem cell transplantation is associated with T cell activation but not with increased levels of T regulatory cells. *Biol Blood Marrow Transplant*. 17:682-692.

Butler, M. O., Lee, J. S., Ansen, S., Neuberg, D., Hodi, F. S., Murray, A. P., Drury, L., Berezovskaya, A., Mulligan, R. C., Nadler, L. M., and Hirano, N. (2007). Long-lived antitumor CD8+ lymphocytes for adoptive therapy generated using an artificial antigen-presenting cell. Clin. Cancer Res. *13*, 1857-1867.

Butler, M. O., Friedlander, P., Milstein, M. I., Mooney, M. M., Metzler, G., Murray, A. P., Tanaka, M., Berezovskaya, A., Imataki, O., Drury, L., Brennan, L., Flavin, M., Neuberg, D., Stevenson, K., Lawrence, D., Hodi, F.S., Velazquez, E.F., Jaklitsch, M.T., Russell, S.E., Mihm, M., Nadler, L.M., Hirano N. (2011). Establishment of antitumor memory in humans using in vitro-educated CD8+ T cells. Sci. Transl. Med. *3*, 80ra34.

Crome S. Q. Clive, B., Wang, A. Y., Kang, C. Y., Chow, V., Yu, J., Lai, A., Ghahary, A., Broady, R., and Levings, M. K. (2010). Inflammatory effects of ex vivo human Th17 cells are suppressed by regulatory T cells. J Immunol.*185*, 3199-208.

Hamid, O., Chasalow, S. D., Tsuchihashi, Z., Alaparthy, S., Galbraith, S., and Berman, D. Association of baseline and on-study tumor biopsy markers with clinical activity in patients (pts) with advanced melanoma treated with Ipilimumab. J Clin Oncol 27, 15s. 2009.

Hodi,F.S., Butler,M., Oble,D.A., Seiden,M.V., Haluska,F.G., Kruse,A., MacRae,S., Nelson,M., Canning,C., Lowy,I., Korman,A., Lautz,D., Russell,S., Jaklitsch,M.T., Ramaiya,N., Chen,T.C., Neuberg,D., Allison,J.P., Mihm,M.C., and Dranoff,G. (2008). Immunologic and clinical effects of antibody blockade of cytotoxic T lymphocyte-associated antigen 4 in previously vaccinated cancer patients. Proc. Natl. Acad. Sci. U. S. A. *105*, 3005-3010.

Hodi,F.S., Mihm,M.C., Soiffer,R.J., Haluska,F.G., Butler,M., Seiden,M.V., Davis,T., Henry-Spires,R., MacRae,S., Willman,A., Padera,R., Jaklitsch,M.T., Shankar,S., Chen,T.C., Korman,A., Allison,J.P., and Dranoff,G. (2003). Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. Proc. Natl. Acad. Sci. U. S. A. *100*, 4712-4717.

Liakou,C.I., Kamat,A., Tang,D.N., Chen,H., Sun,J., Troncoso,P., Logothetis,C., and Sharma,P. (2008). CTLA-4 blockade increases IFNgamma-producing CD4+ICOShi cells to shift the ratio of effector to regulatory T cells in cancer patients. Proc. Natl. Acad. Sci. U. S. A *105*, 14987-14992.

Nguyen,L.T., Yen,P., Nie,J., Liadis,N., Ghazarian,D., Al-Habeeb,A., Easson,A., Leong,W., Lipa,J., McCready,D., Reedijk,M., Hogg,D., Joshua,A.M., Quirt,I., Messner,H., Shaw,P., Crump,M., Sharon,E., Ohashi,P.S. (2010). Expansion and characterization of melanoma tumor-infiltrating lymphocytes (TILs). PLoS ONE, *5*, e13940.

Maker,A.V., Phan,G.Q., Attia,P., Yang,J.C., Sherry,R.M., Topalian,S.L., Kammula,U.S., Royal,R.E., Haworth,L.R., Levy,C., Kleiner,D., Mavroukakis,S.A., Yellin,M., and Rosenberg,S.A. (2005). Tumor regression and autoimmunity in patients treated with cytotoxic T lymphocyte-associated antigen 4 blockade and interleukin 2: a phase I/II study. Ann. Surg. Oncol. *12*, 1005-1016.

O'Mahony,D., Morris,J.C., Quinn,C., Gao,W., Wilson,W.H., Gause,B., Pittaluga,S., Neelapu,S., Brown,M., Fleisher,T.A., Gulley,J.L., Schlom,J., Nussenblatt,R., Albert,P., Davis,T.A., Lowy,I., Petrus,M., Waldmann,T.A., and Janik,J.E. (2007). A pilot study of CTLA-4 blockade after cancer vaccine failure in patients with advanced malignancy. Clin. Cancer Res. *13*, 958-964.

Phan,G.Q., Yang,J.C., Sherry,R.M., Hwu,P., Topalian,S.L., Schwartzentruber,D.J., Restifo,N.P., Haworth,L.R., Seipp,C.A., Freezer,L.J., Morton,K.E., Mavroukakis,S.A., Duray,P.H., Steinberg,S.M., Allison,J.P., Davis,T.A., and Rosenberg,S.A. (2003). Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. Proc. Natl. Acad. Sci. U. S. A. *100*, 8372-8377.

Reuben,J.M., Lee,B.N., Li,C., Gomez-Navarro,J., Bozon,V.A., Parker,C.A., Hernandez,I.M., Gutierrez,C., Lopez-Berestein,G., and Camacho,L.H. (2006). Biologic and immunomodulatory events after CTLA-4 blockade with ticilimumab in patients with advanced malignant melanoma. Cancer. *106*, 2437-2444.

Ribas,A., Comin-Anduix,B., Economou,J.S., Donahue,T.R., de la,R.P., Morris,L.F., Jalil,J., Dissette,V.B., Shintaku,I.P., Glaspy,J.A., Gomez-Navarro,J., and Cochran,A.J. (2009). Intratumoral immune cell infiltrates, FoxP3, and indoleamine 2,3-dioxygenase in patients with melanoma undergoing CTLA4 blockade. Clin. Cancer Res. *15*, 390-399.

Yuan,J., Gnjatic,S., Li,H., Powel,S., Gallardo,H.F., Ritter,E., Ku,G.Y., Jungbluth,A.A., Segal,N.H., Rasalan,T.S., Manukian,G., Xu,Y., Roman,R.A., Terzulli,S.L., Heywood,M., Pogoriler,E., Ritter,G., Old,L.J., Allison,J.P., and Wolchok,J.D. (2008). CTLA-4 blockade enhances polyfunctional NY-ESO-1 specific T cell responses in metastatic melanoma patients with clinical benefit. Proc. Natl. Acad. Sci. U. S. A. *105*, 20410-20415.
.

APPENDIX A PERFORMANCE STATUS CRITERIA

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

APPENDIX B CTEP MULTICENTER GUIDELINES

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

Responsibility of the Protocol Chair

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

Responsibilities of the Coordinating Center

- Each participating institution will have an appropriate assurance on file with the Office for Human Research Protection (OHRP), NIH. The Coordinating Center is responsible for assuring that each participating institution has an OHRP assurance and must maintain copies of IRB approvals from each participating site.
- Prior to the activation of the protocol at each participating institution, an OHRP form 310 (documentation of IRB approval) must be submitted to the CTEP PIO.
- The Coordinating Center is responsible for central patient registration. The Coordinating Center is responsible for assuring that IRB approval has been obtained at each participating site prior to the first patient registration from that site.
- The Coordinating Center is responsible for the preparation of all submitted data for review by the Protocol Chair.
- The Coordinating Center will maintain documentation of AE reports. There are two options for AE reporting: (1) participating institutions may report directly to CTEP with a copy to the Coordinating Center, or (2) participating institutions report to the Coordinating Center who in turn report to CTEP. The Coordinating Center will submit AE reports to the Protocol Chair for timely review.
- Audits may be accomplished in one of two ways: (1) source documents and research records for selected patients are brought from participating sites to the Coordinating Center for audit, or (2) selected patient records may be audited on-site at participating sites. If the NCI chooses to have an audit at the Coordinating Center, then the Coordinating Center is responsible for having all source documents, research records, all IRB approval documents, NCI Drug Accountability Record forms, patient registration lists, response assessments scans, x-rays, etc. available for the audit.

Inclusion of Multicenter Guidelines in the Protocol

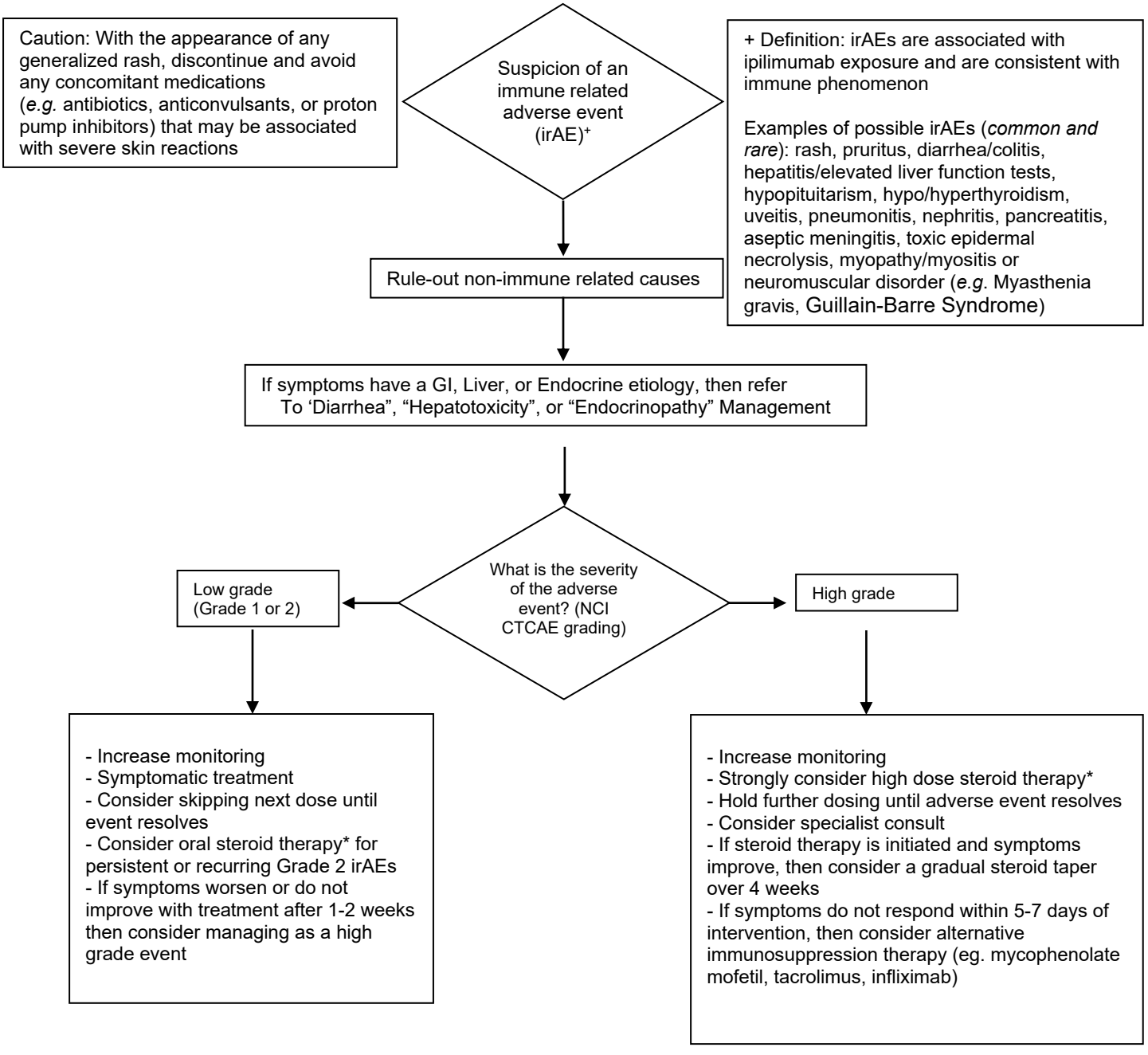
- The protocol must include the following minimum information:
  - The title page must include the name and address of each participating institution and the name, telephone number and e-mail address of the responsible investigator at each participating institution.
  - The Coordinating Center must be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

Agent Ordering

- Except in very unusual circumstances, each participating institution will order DCTD-supplied investigational agents directly from CTEP. Investigational agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO.

APPENDIX C MANAGEMENT OF IMMUNE-RELATED ADVERSE EVENTS, DIARRHEA, HEPATOTOXICITY, ENDOCRINOPATHY, AND NEUROPATHY*

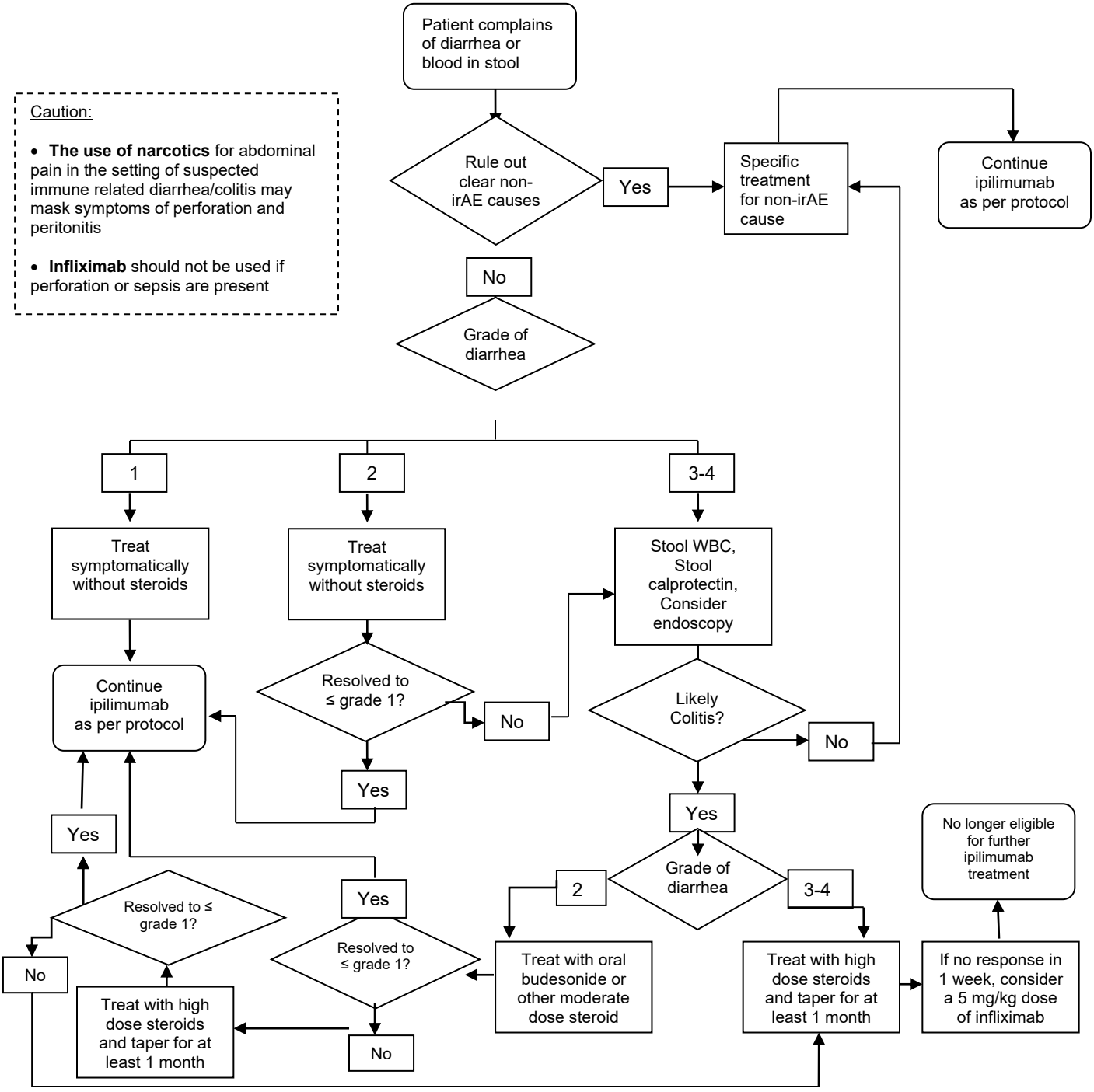
General recommendations for management of suspected inflammatory events



* Based on clinical experience to date, systemic steroids for treatment of irAEs do not appear to impact the development or maintenance of ipilimumab clinical activity in advanced melanoma.

*Investigator Brochure (2011)

Diarrhea Management Algorithm



Diarrhea				
GRADE 1 Increase of <4 stools per day over baseline; mild increase in ostomy output compared with baseline	**GRADE 2** Increase of 4-6 stools per day over baseline; IV fluids indicated < 24 hrs; moderate increase in ostomy output compared to baseline; not interfering with activities of daily living (ADL)	**GRADE 3** Increase of ≥7 stools per day over baseline; incontinence; IV fluids ≥24 hrs; hospitalization; severe increase in ostomy output compared to baseline; interfering with ADL	**GRADE 4** Life-threatening consequences (*e.g.*, hemodynamic collapse)	**GRADE 5** Death

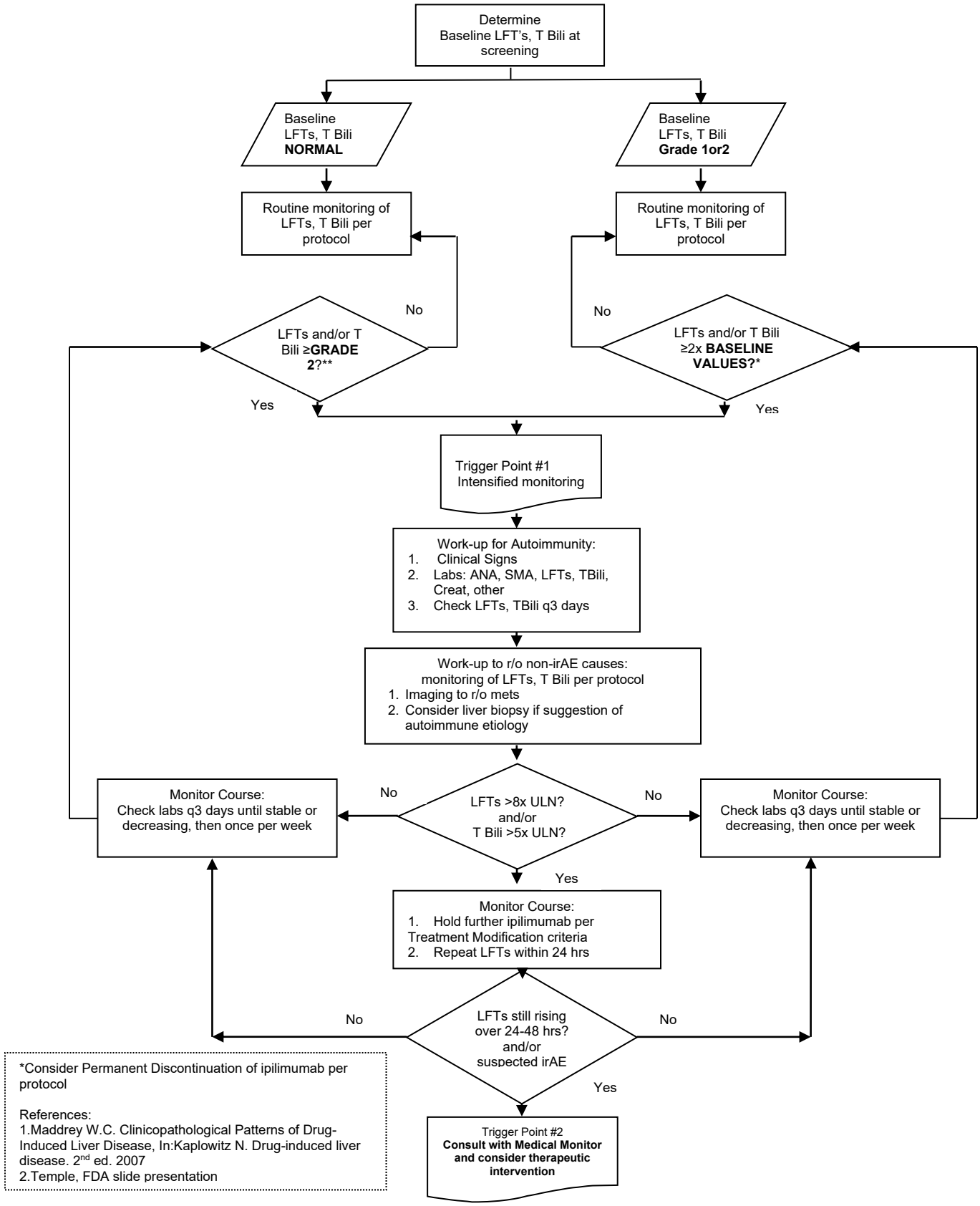
Hepatoxicity Management Algorithm

Situation: rising liver function tests (LFTs) >8X ULN or suspected immune-mediated hepatitis

1) Admit subject to hospital for evaluation and close monitoring
2) Stop further Ipilimumab dosing until hepatotoxicity is resolved. Consider permanent discontinuation of Ipilimumab per protocol
3) Start at least 120 mg methylprednisolone sodium succinate per day, given IV as a single or divided dose
4) Check liver laboratory test values (LFTs, T-bilirubin) daily until stable or showing signs of improvement for at least 3 consecutive days
5) 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)
6) 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)
7) If target trough level is achieved with tacrolimus but no improvement is observed after 5 to 7 days, consider infliximab, 5 mg/kg, once
8) Continue to check LFTs daily for at least 2 weeks to monitor sustained response to treatment

A flow chart of the algorithm is depicted on the following page.

Hepatoxicity Management Algorithm

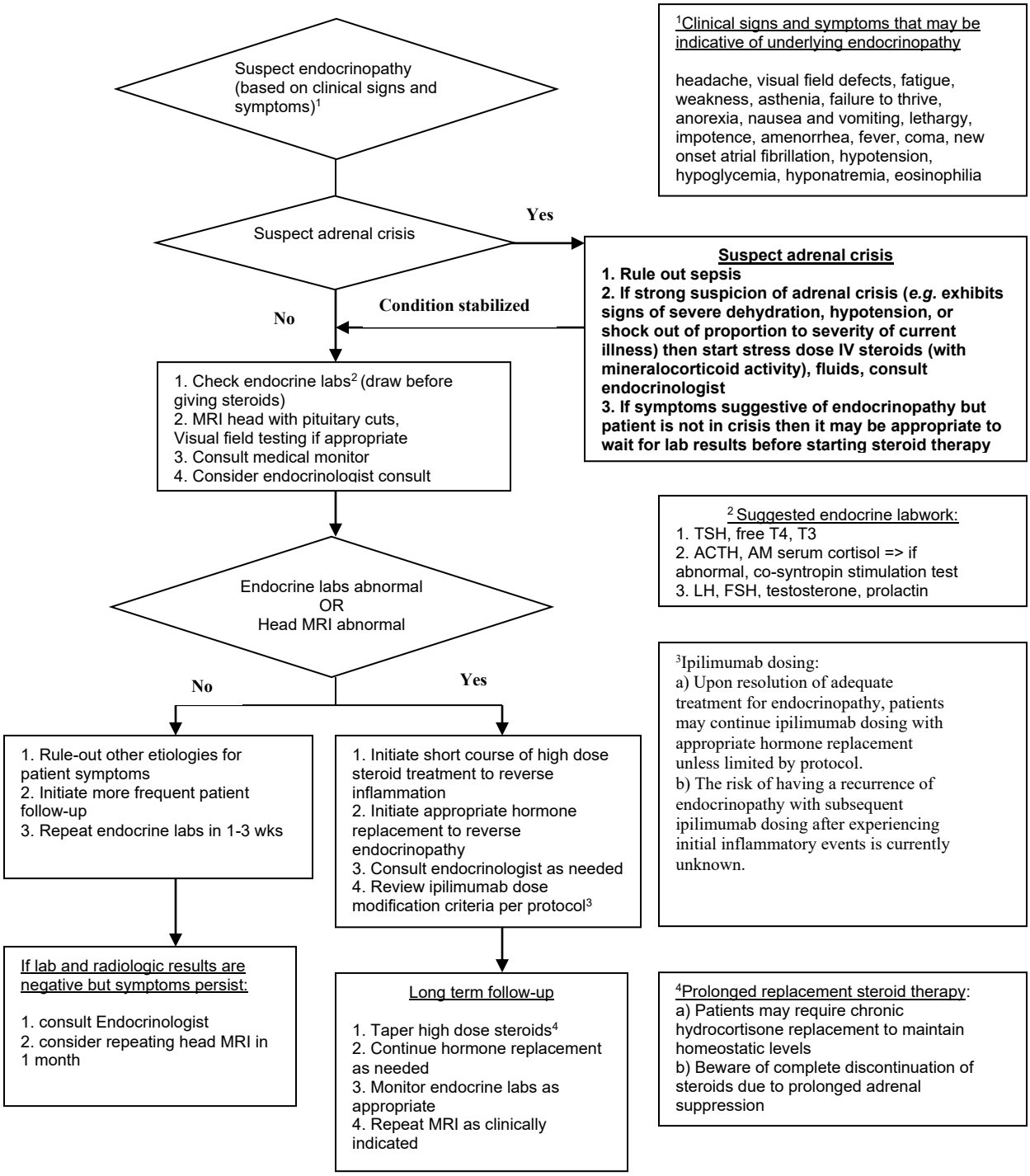


*Consider Permanent Discontinuation of ipilimumab per protocol

References:

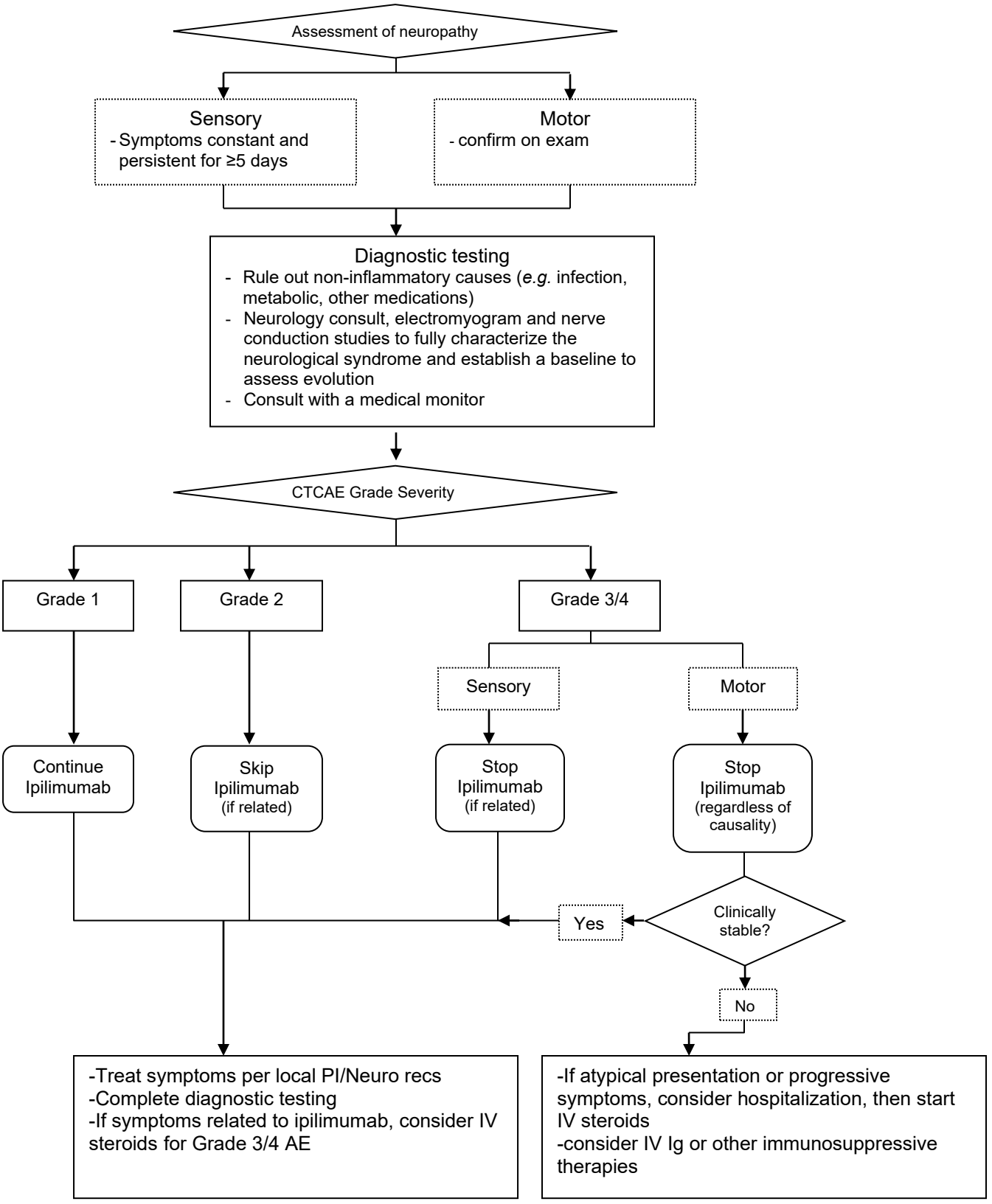
1.Maddrey W.C. Clinicopathological Patterns of Drug-Induced Liver Disease, In:Kaplowitz N. Drug-induced liver disease. 2nd ed. 2007
2.Temple, FDA slide presentation

Endocrinopathy Management Algorithm



Footnote
For numbered footnotes ([1,2,3,4]), please refer to further explanation and text found in the corresponding dotted line boxes to the right side of the algorithm

Neuropathy Management Algorithm



APPENDIX D IMMUNE RELATED RESPONSE CRITERIA (IRRC)*

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

Immune Related Response Criteria

Definition of Tumor Response Using irRC

The sum of the products of diameters (SPD) at tumor assessment using the immune-related response criteria (irRC) for progressive disease incorporates the contribution of new measurable lesions. Each net Percentage Change in Tumor Burden per assessment using irRC criteria accounts for the size and growth kinetics of both old and new lesions as they appear.

Definition of Index Lesions Response Using irRC

- **irComplete Response (irCR):** Complete disappearance of all *index* lesions. This category encompasses exactly the same subjects as "CR" by the mWHO criteria.
- **irPartial Response (irPR):** Decrease, relative to baseline, of 50% or greater in the sum of the products of the two largest perpendicular diameters of all *index* and all new measurable lesions (*i.e.*, Percentage Change in Tumor Burden). Note: the appearance of new measurable lesions is factored into the overall tumor burden, but does not automatically qualify as progressive disease until the SPD increases by ≥25% when compared to SPD at nadir.
- **irStable Disease (irSD):** Does not meet criteria for irCR or irPR, in the absence of progressive disease.
- **irProgressive Disease (irPD):** At least 25% increase Percentage Change in Tumor Burden (*i.e.*, taking sum of the products of all *index* lesions and any new lesions) when compared to SPD at nadir.

Definition of Non-Index Lesions Response Using irRC

- **irComplete Response (irCR):** Complete disappearance of all *non-index* lesions. This category encompasses exactly the same subjects as "CR" by the mWHO criteria.
- **irPartial Response (irPR) or irStable Disease (irSD):** *non-index* lesion(s) are not considered in the definition of PR, these terms do not apply.
- **irProgressive Disease (irPD):** Increases in number or size of *non-index* lesion(s) does not constitute progressive disease unless/until the Percentage Change in Tumor Burden increases by 25% (*i.e.*, the SPD at nadir of the index lesions increases by the required amount).

Impact of New Lesions on irRC

New lesions in and by themselves do not qualify as progressive disease. However their contribution to total tumor burden is included in the SPD which in turn feeds into the irRC criteria for tumor response. Therefore, new non-measurable lesions will not discontinue any subject from the study.

Definition of Overall Response Using irRC

Overall response using irRC will be based on these criteria:

- **Immune-Related Complete Response (irCR):** Complete disappearance of *all* tumor lesions (index and nonindex together with no new measurable/unmeasurable lesions) for at least 4 weeks from the date of documentation of complete response.
- **Immune-Related Partial Response (irPR):** The sum of the products of the two largest perpendicular diameters of all index lesions is measured and captured as the SPD baseline. At each subsequent tumor assessment, the sum of the products of the two largest perpendicular diameters of all index lesions and of new measurable lesions are added together to provide the Immune Response Sum of Product Diameters (irSPD). A decrease, relative to baseline of the

irSPD compared to the previous SPD baseline, of 50% or greater is considered an immune Partial Response (irPR).

- **Immune-Related Stable Disease (irSD):** irSD is defined as the failure to meet criteria for immune complete response or immune partial response, in the absence of progressive disease.
- **Immune-Related Progressive Disease (irPD):** It is recommended in difficult cases to confirm PD by serial imaging. Any of the following will constitute progressive disease:
  - At least 25% increase in the sum of the products of all index lesions over baseline SPD calculated for the index lesions.
  - At least a 25% increase in the sum of the products of all index lesions and new measurable lesions (irSPD) over the baseline SPD calculated for the index lesions.

Table: Immune-Related Response Criteria Definitions

Index Lesion Definition	Non-Index Lesion Definition	New Measurable Lesions	New Unmeasurable Lesions	Percent change in tumor burden (including measurable new lesions when present)	Overall irRC Response
Complete Response	Complete Response	No	No	-100%	irCR
Partial Response	Any	Any	Any	≥ -50%	irPR
				<-50% to <+25%	irSD
				>+25%	irPD
Stable Disease	Any	Any	Any	<-50% to <+25%	irSD
				>+25%	irPD
Progressive Disease	Any	Any	Any	≥+25%	irPD

Immune-Related Best Overall Response Using irRC (irBOR)

irBOR is the best confirmed irRC overall response over the study as a whole, recorded between the date of first dose until the last tumor assessment before subsequent therapy (except for local palliative radiotherapy for painful bone lesions) for the individual subject in the study. For the assessment of irBOR, all available assessments per subject are considered.

irCR or irPR determinations included in the irBOR assessment must be confirmed by a second (confirmatory) evaluation meeting the criteria for response and performed no less than 4 weeks after the criteria for response are first met.

APPENDIX E DATA MANAGEMENT GUIDELINES

Case Report Form Submission Schedule

Data required for the study will be collected in Case Report Forms provided by the PMH Phase 2 Consortium Central Office. The site will be required to complete a paper Eligibility Checklist case report form (CRF) at the time of patient registration. All other data will be collected on electronic case report forms (eCRFs) in the Medidata Rave system. Site staff access to Medidata Rave will be initiated at the time of site activation. The form submission schedule is outlined below.

Case Report Form	Submission Schedule
Eligibility Checklist	At the time of registration
Baseline eCRFs	Within 3 weeks of on study date
On Treatment (Cycle) eCRFs	Within 3 weeks of the end of each cycle of treatment
Off Treatment eCRFs	Within 3 weeks of the patient coming off-study
Short Follow-up eCRFs	Within 3 weeks of the patient coming to clinic.
Final eCRFs	Within 3 weeks from the follow-up period being complete or of the patient's death being known to the investigator unless this constitutes a reportable adverse event when it should be reported according to CTEP-AERS guidelines

Case Report Form Completion

The paper Eligibility Checklist CRF must be completed using black ink. Any errors must be crossed out so that the original entry is still visible, the correction clearly indicated and then initialed and dated by the individual making the correction.

eCRFs will be completed according to the schedule noted above and all relevant supporting documentation such as scans, progress notes, nursing notes, blood work, pathology reports, etc., will be submitted to the PMHC Phase 2 Consortium Central Office for review. All patient names or other identifying information will be removed prior to being sent to the Central Office and the documents labeled with patient initials, study number and the protocol number.

eCRF completion guidelines are available for all sites.

Monitoring

Central data monitoring will take place throughout the trial at the Central Office. On-site monitoring will be performed once a year at participating sites during which a subset of PMHC

studies will be picked for on-site monitoring.

Data in the Medidata Rave eCRFs will be monitored on a regular basis and quality assurance measures will be performed. Electronic data queries as well as paper query letters may be issued to the site prior to the quarterly submission of data to CDUS.

Patient Registration

- Refer to section 4 of the protocol

Data Safety

A Data Safety and Monitoring Board, an independent group of experts, will be reviewing the data from this research throughout the study to see if there are unexpected or more serious side effects than described in the consent.

Regulatory Requirements

- Please submit all required documents to the PMH Phase 2 Consortium Central Office.
- Canadian Principal Investigators must submit a completed Qualified Investigator Undertaking.
- All investigators must have a current NCI investigator number on file with the PMH Phase 2 Consortium Central Office.
- All investigators must have an up-to-date CV (signed within 2 years) on file with the PMH Phase 2 Consortium Central Office.
- Laboratory certification/accreditation and normal ranges are required
- Confirmation of all investigators having undergone training in the Protection of Human Research Subjects is required. It is preferred that other staff involved in the trial also undergoes such training.
- Investigators and site staff are required to complete Medidata eCRF training modules depending on delegated tasks
- OPRR assurance numbers for each institution are required
- Consent forms must be reviewed by the Central Office before submission to the local ethics regulatory board (REB/IRB) and must include a statement that 1) information will be sent to and 2) medical records will be reviewed by the PMH Phase 2 Consortium Central Office.
- A Membership list of the local ethics board is required.
- A copy of the initial approval letter from the ethics board must be submitted to the PMH Phase 2 Consortium Central Office.
- A completed Site Participant List/Training Log is required and must be submitted to PMHC

Continuing approval will be obtained at least yearly until follow-up on patients is completed