

**Evaluation of Circulating T Cells and Tumor Infiltrating Lymphocytes with Specificities Against Tumor Associated Antigens During and After Neoadjuvant Chemotherapy and Phased Ipilimumab in Non-small Cell Lung Cancer (NSCLC)**

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## **Statement of Compliance and Signature Page**

### **STUDY TITLE: Evaluation of Circulating T Cells and Tumor Infiltrating Lymphocytes with Specificities Against Tumor Associated Antigens During and After Neoadjuvant Chemotherapy and Phased Ipilimumab in Non-small Cell Lung Cancer (NSCLC).**

PI: Neal Ready, MD

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Principal Investigator: Neal Ready, MD 03-10-2016; Duke University Thoracic Oncology Program

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## **STUDY SCHEMA**

### **STUDY DESIGN**

Patients with clinical stage IB (> 4cm), II, or III (N0-2) NSCLC and no prior therapy for the current diagnosis of lung cancer will be eligible for the study. Patients will receive three cycles of neoadjuvant chemotherapy. Ipilimumab will be given during cycles 2 and 3 of chemotherapy and up to 4 cycles alone after surgery. Patients will undergo standard surgical resection of their lung cancer as deemed appropriate by their surgeon.

### **PATIENT ELIGIBILITY**

- All patients must have histologically documented clinical stage IB (> 4 cm), II, or III(N0-2) NSCLC
- **NO** prior chemotherapy, radiation therapy or biologic/targeted therapy within 1 month
- Performance Status ECOG 0-1
- No active invasive malignancy in the past 2 years other than non-melanoma skin cancer. Cancers that are in-situ are not considered invasive.
- Signed Informed Consent
- No autoimmune disease that would constitute contraindication to receive ipilimumab

### **REQUIRED LABORATORY DATA**

- ANC/AGC  $\geq$  1500/ $\mu$ L
- Platelets  $\geq$  100,000/ $\mu$ L
- Creatinine clearance  $\geq$  45 mL/min (creatinine  $\leq$  2.0 mg/dL to receive cisplatin)
- AST/ALT  $\leq$  2.5x ULN
- Bilirubin  $\leq$  2.0 x institutional ULN

Determination of eligibility and histology



#### **Neoadjuvant:**

Cycle 1: Paclitaxel 175 mg/m<sup>2</sup> IV over 3 hours , followed by Cisplatin 75 mg/m<sup>2</sup> IV over 60 minutes or carboplatin AUC 6 IV over 30-60 minutes on day 1(Every 21 days x 1cycle)

Cycles 2 and 3: Ipilimumab 10 mg/kg IV over 90 minutes, Paclitaxel 175 mg/m<sup>2</sup> IV over 3 hours , followed by Cisplatin 75 mg/m<sup>2</sup> IV over 60 minutes or carboplatin AUC 6 IV over 30-60 minutes on day 1(Every 21 days x 2 cycles)



Surgery: Standard surgical evaluation to occur at least 21 days after the last dose (cycle 3) of chemotherapy followed by surgical therapy



**Post-surgical Therapy:** (A total of 4 doses of ipilimumab will be given post-operatively):

**Adjuvant:** Ipilimumab 10 mg/kg IV every 3 weeks times 2 doses beginning 4 weeks postoperative ( up to 10 weeks if needed for recovery)

**Maintenance:** Ipilimumab 10 mg/kg/IV every 12 weeks times 2 doses.

**Correlative Science Measures:** (refer to Appendix B for further details on the correlative science outcomes)

- 1) Blood for circulating T cells with specificities against tumor associated antigens: prior to treatment, after chemotherapy, before surgery, and after adjuvant ipilimumab. Blood also to monitor circulating populations of regulatory T cells, myeloid-derived suppressor cells (MDSC), as well as activated and exhausted T cells.
- 2) Tumor for tumor infiltrating T cells with specificities against tumor associated antigens. Tumor also to monitor populations of infiltrating regulatory T cells, myeloid-derived suppressor cells (MDSC), as well as activated and exhausted T cells.

## List of Abbreviations

<b>ADCC</b>	Antibody-Dependent Cellular Cytotoxicity
<b>AE</b>	Adverse Event
<b>ALC</b>	Absolute Lymphocyte Count
<b>ALT</b>	Alanine Transaminase
<b>ANC/AGC</b>	Absolute Neutrophil Count/Absolute Granulocyte Count
<b>APC</b>	Antigen Presenting Cell
<b>AST</b>	Aspartate Transaminase
<b>AUC</b>	Area Under the Curve
<b>BMS</b>	Bristol-Myers Squibb Company
<b>BORR</b>	Best Objective Response Rate
<b>BSA</b>	Body Surface Area
<b>BSC</b>	Best Supportive Care
<b>BW</b>	Body Weight
<b>CDCC</b>	Complement-Dependent Cellular Cytotoxicity
<b>CL</b>	Systemic Clearance
<b>CMax</b>	Peak Concentration
<b>CMin</b>	Trough Concentration
<b>CR</b>	Complete Response
<b>CRF</b>	Case Report Form (sometimes referred to as Clinical Report Form). A printed or electronic form for recording study participants' data during a clinical study, as required by the protocol.
<b>CRT</b>	Chemotherapy-Radiation Therapy
<b>CRU</b>	Clinical Research Unit
<b>CT</b>	Computed Tomography
<b>CTCAE</b>	Common Terminology Criteria for Adverse Events; National Cancer Institute, Version 4.0
<b>CTLA4</b>	Cytotoxic T-lymphocyte Antigen
<b>DCI</b>	Duke Cancer Institute
<b>DCR</b>	Disease Control Rate
<b>DOCR</b>	Duke Office of Clinical Research
<b>DUHS/DUMC</b>	Duke University Health System/Duke University Medical Center
<b>ECOG PS</b>	Eastern Cooperative Oncology Group Performance Status
<b>ECL</b>	Electrochemiluminescent
<b>ECRF</b>	Electronic Case Report Form (sometimes referred to as an electronic clinical report form). An electronic form for recording study participants' data during a clinical study, as required by the protocol.
<b>Enroll/Randomize</b>	The act of assigning a patient to a treatment. Patients who are enrolled in the trial are those who have been assigned to a treatment or randomized.
<b>Enter/Consent</b>	The act of obtaining informed consent for participation in a clinical trial from patients deemed eligible or potentially eligible to participate in the clinical trial. Patients entered into a trial are those who sign the informed consent document directly or through their legally authorized representative
<b>GI</b>	Gastrointestinal

<b>HepB</b>	Hepatitis B
<b>HIPAA</b>	Health Insurance Portability and Accountability Act
<b>IB</b>	Investigator Brochure
<b>ICD/ICF</b>	A person responsible for the conduct of the clinical trial at a trial site. If a team of individuals at a trial site conducts a trial, the investigator is the responsible leader of the team and may be called the principal investigator.
<b>irAE</b>	Immune-Related Adverse Event
<b>IRB/ERB</b>	Institutional Review Board/Ethical Review Board: a board or committee (institutional, regional, or national) composed of medical, professional and non-medical members whose responsibility it is to verify that the safety, welfare, and human rights of the subjects participating in a clinical trial are protected.
<b>irRC</b>	Immune-Related Response Criteria
<b>ITT</b>	Intention to Treat
<b>IV</b>	Intravenous, usually referring to a medication or substance given into a vein.
<b>LDH</b>	Lactate Dehydrogenase
<b>LFT</b>	Liver Function Test
<b>mAB</b>	Monoclonal Antibody
<b>MDSC</b>	Myeloid-Derived Suppressor Cells
<b>mg</b>	milligram: 1/100 of a gram
<b>m<sup>2</sup></b>	meters squared
<b>NCA</b>	Non-Compartmental Analysis
<b>NCCN</b>	National Comprehensive Cancer Network
<b>NSCLC</b>	Non-Small Cell Lung Cancer
<b>OS</b>	Overall Survival
<b>Patient</b>	A subject with a defined disease
<b>PBMC</b>	Peripheral Blood Mononuclear Cells
<b>PFS</b>	Progression Free Survival
<b>PD</b>	Progressive Disease
<b>PET</b>	Positron Emission Tomography
<b>PK</b>	Pharmacokinetic
<b>PPK</b>	Population Pharmacokinetics
<b>PR</b>	Partial Response
<b>Recist</b>	Response Evaluation Criteria in Solid Tumor
<b>RT</b>	Radiation Therapy/Radiotherapy
<b>SAE</b>	Serious Adverse Event
<b>SD</b>	Stable Disease
<b>TAA</b>	Tumor Associated Antigens
<b>TIL</b>	Tumor Infiltrating Lymphocytes
<b>TNM Staging</b>	Tumor, Node and Metastasis Staging
<b>Treg</b>	Regulatory T Cells
<b>Wt</b>	Weight

## 1.0 INTRODUCTION

### 1.1 Advanced Stage Lung Cancer

In advanced stage IV NSCLC, meta-analysis of 8 trials using cisplatin based chemotherapy compared to Best Supportive Care resulted in chemotherapy having an absolute survival improvement of 10% at 1 year. (1, 2) Median survival, BSC vs. chemo: 4 vs 8+ months, respectively. (3) Median survival now is 12+ months in more recent trials of VEGF targeted therapy plus platinum doublet. (4) Quality of life benefit was also observed with chemotherapy. (5) First-line chemotherapy for unselected stage IV NSCLC is a platinum doublet as the standard platform. Combining taxanes with other chemotherapeutic agents has been reported to have high activity and to be tolerable. Platinum doublets resulted in 2 year Survival of 15-20%. (6) Scagliotti observed a survival advantage selecting doublet chemotherapy based on tumor histology. Using a platinum based doublet, the author observed 2 month longer median overall survival for adenocarcinoma treated with pemetrexed and 1 month longer for squamous cell carcinoma treated with gemcitabine. (7) Molecular targeted agents added to platinum doublet chemotherapy can also improve survival. Anti-VEGF monoclonal antibody, Bevacizumab, added to platinum and paclitaxel improved overall survival in adenocarcinoma subset with stage IV disease. (8) Selection of targeted agents where target is known can replace first-line chemotherapy (EGFR-TKIs in EGFR mutants; ALK fusion mutants treated with MEK TKIs). (9, 10) Identifying better biomarkers will lead to better targeting.

#### 1.1.2 Ipilimumab and Advanced Melanoma:

Ipilimumab enhanced T-cell activation leads to pan immune stimulation against human solid tumors. Recently reported unresectable stage III or IV melanoma that progressed while they were receiving therapy was treated with Ipilimumab dose of 3 mg per kilogram of body weight. The ipilimumab treated cohort was observed to have improved median overall survival 10.0 months compared with 6.4. (30)

#### 1.1.3 Ipilimumab and Advanced Lung Cancer:

In advanced metastatic NSCLC, Ipilimumab 10 mg/kg has been successfully combined with the platin doublet, paclitaxel 175 mg/m<sup>2</sup> and carboplatin AUC 6, as first-line treatment. -Abstracts of a phase II trial reported that the phased sequence (two courses chemotherapy followed by two courses of chemotherapy + Ipilimumab) had superior progression free survival, overall response, and overall

survival; 6.4 months, 57%, and 12.9 months, respectively; compared to 5.7 months, 49%, and 9.1 months, for the concurrent sequence. (31, 32).

Final publication by Lynch observed immune related PFS (irPFS) significantly improved using the phased regimen compared with the control regimen (HR, 0.72;  $P = .05$ ), whereas the concurrent ipilimumab regimen did not significantly improve irPFS (HR, 0.81;  $P = .13$ ). As assessed by WHO criteria, there was also an improvement in PFS, relative to the control, for phased ipilimumab (HR, 0.69;  $P = .02$ ) but not for concurrent ipilimumab (HR, 0.88;  $P = .25$ ). The median mWHO-PFS was 4.2 months for the control group, 5.1 months for the phased ipilimumab group, and 4.1 months for the concurrent ipilimumab group. Similarly, improved median overall survival for the phased ipilimumab group was 12.2 months, and increase of 3.9 months over the median of 8.3 months for the control group (HR, 0.87;  $P = .23$ ). the median overall survival of 9.7 months for the concurrent ipilimumab group was similar to that of the control group (HR, 0.99;  $P = .48$ ). Hematologic and nonhematologic toxicities were similar between control arm [Paclitaxel and Carboplatin] compared to phased arm [Ipilimumab, Paclitaxel, Carboplatin]; and toxicities were typical of those associated with Paclitaxel and Carboplatin. Specifically, toxicities observed as Grade 1-2 occurring  $> 30\%$  in descending order (control, phased arm): anemia 89,92; alopecia 46,45; thrombocytopenia 35, 43; neutropenia 32,34; diarrhea 31,31; AST elevation 32,31; ALT elevation 35,29. Observed Grade 3-4 toxicities were infrequent, interestingly they more frequent in the control than phased arm, less than 9% and 6%, respectively. (59)

## 1.2 Early Stage Lung Cancer

### 1.2.1 Early Stage and Surgery

The natural history of clinically staged (stage I and stage II) non-small cell lung cancer (NSCLC) following surgery is a five year survival of 50% and 30%, respectively.(1) Five-year survival in surgical pathologic stage IA (T1N0M0) and IB (T2N0M0) disease is 68-83% and 53-65%, respectively. Five year survival in surgical pathologic stage IIA (T1N1M0) and IIB (T2N1M0) disease is 40-63% and 38-45%, respectively.(2) Clinical implications are pathologically staged patients (17-32% stage IA, 35-47% stage IB, 37-60% stage IIA, and 55-62% stage IIB) are destined to recur because they harbor regional or distant micro-metastasis outside their surgical resection.

### 1.2.2 Early Stage and Postoperative Chemotherapy

For early stage NSCLC, the addition of chemotherapy to surgery has been shown to improve survival. Postoperative chemotherapy improved 5-year survival from 59% and 40% to 79% and 60% for stage IB  $> 4\text{cm}$  tumors and stage II NSCLC, respectively. (11, 12) Three randomized controlled trials of postoperative chemotherapy each reported improved overall survival for chemotherapy in

early stages IB, II, and IIIA NSCLC. (13, 14, 15) Similar survival benefit has been seen for adjuvant cisplatin based chemotherapy for stage IIIA NSCLC. (16)

### **1.2.3 Early Stage and Preoperative Chemotherapy**

Preoperative chemotherapy trials have included small phase III and phase II trials which initially reported a threefold median survival advantage favoring chemotherapy. (17) (18) However, long-term results were not durable, although a trend of improved survival favoring chemotherapy persisted. (19) (20) Four additional preoperative chemotherapy phase III trials have been conducted. One reported significant OS advantage using a non-taxane platin triplet (21), two reported trends to OS advantage using paclitaxel/carboplatin (22, 23), and one was completely negative administered older technology chemotherapy (non-taxane/platin) to 88% of patients. (24) Most recently, a phase III trial reported preoperative chemotherapy using gemcitabine plus cisplatin improved survival in patients with clinical stage IIB/IIIA NSCLC. The hazard ratios for PFS and overall survival were both in favor of chemotherapy. A statistically significant impact of preoperative chemotherapy on outcomes was observed in the stage IIB/IIIA subgroup (3-year PFS rate: 36.1% *v* 55.4%; *P* *<* .002). (25) Similar survival benefits have been observed for preoperative and postoperative chemotherapy by Meta-analysis. (26, 27, 28, 29)

## **1.3 Proposal: Ipilimumab and Early Stage Lung Cancer**

In early stage NSCLC we propose adding ipilimumab, a T-Cell targeted agent, to enhance T-Cell immune response combined with preoperative platinum doublet chemotherapy. Ipilimumab is a monoclonal (IgG 1) antibody that co-stimulates T-Cells by targeting the CD28 homolog, CTLA-4. This enhances T-cell immunity by co-stimulation of T-cells at T-cell receptor and CD28 by B7 family on the antigen- presenting cell (APC). The combination of enhanced immune response together with cytotoxic chemotherapy may improve clinical outcome. We propose to study circulating T cells and tumor infiltrating T cells with specificities against tumor associated antigens to better understand the effect that ipilimumab has on the immune system in NSCLC.

## **1.4 Clinical Trial Rationale**

We propose studying the cell mediated effects of ipilimumab in combination with chemotherapy in the neoadjuvant setting for NSCLC. The overall immune assessment strategy for the proposed ipilimumab neoadjuvant trial will be based on the hypothesis that 1) T cells with specificities against tumor associated antigens expressed by the patient's progressing NSCLC are present, but functionally impaired, at baseline, and 2) that the immunomodulatory effects of chemotherapy plus ipilimumab will impact the suppressive mechanisms, restoring functional reactivity to important anti-tumor effector cell populations.

An important potential biomarker for anti-tumor immune response is the proliferation and stimulation of circulating T cells with specificities against tumor associated antigens (TAA). At baseline few patients with cancer have populations of circulating T cells with specificities against TAA above the detectable level of 0.05% CD8 lymphocytes. The primary endpoint of this clinical trial will be to determine if the addition of ipilimumab to neoadjuvant chemotherapy for non-small cell lung cancer increases the percentage of patients with circulating T cells with specificities against TAA. We will also measure tumor infiltrating lymphocytes in resected tumors.

#### **1.4.1 T Cells with Specificities Against Tumor Associated Antigens**

Functional TAA-specific T cell reactivities will be monitored at 4 time points [1) baseline prior to treatment, 2) cycle 2 chemotherapy, prior to ipilimumab administration, 3) 21-36 days after last dose (cycle 3) of chemotherapy prior to surgery, and 4) 3-6 weeks after adjuvant ipilimumab dose #2] utilizing a 12-color intracellular cytokine staining (ICS) assay in which functional reactivities within maturational subsets of CD4 and CD8 T cells will be assessed. In this assay, TAA driven production of IL-2, IFN- $\gamma$ , TNF- $\alpha$ , and the degranulation marker CD107a will be measured, thus permitting examination of qualitative changes in relative T cell polyfunctionality within maturational subsets of CD4+ and CD8+ lymphocytes. Pools of overlapping 15-mer TAA peptides, with 11 amino acid overlaps, will be used for the functional analyses. The 3 most common highly conserved TAA expressed by NSCLC are Survivin, PRAME, and MAGE-A3, but peptide pools for CEA, MAGE-A1, MAGE-A4, MART-1, tyrosinase, NY-ESO-1 and gp100 are also available in the Core laboratory. Although the frequencies of TAA-specific T cells may be too low in PBMC to permit direct *ex vivo* functional assessments, tumor specimens obtained at the time of surgical resection, post chemotherapy plus ipilimumab therapy, will be an especially rich source of tumor infiltrating lymphocytes (TIL) for analyses.

#### **1.4.2 Longitudinal Analyses of T Cell and MDSC Phenotypes**

Phenotypic and functional characterization of T cell populations will be performed on peripheral blood mononuclear cells (PBMC), as well as TIL. It will be of critical importance to better understand the impact of ipilimumab therapy on: 1) regulatory T cells (Tregs), 2) myeloid-derived suppressor cells (MDSC), 3) T cell activation, and 4) T cell exhaustion. These parameters will all be assessed using a 14-color polychromatic flow cytometry panel specifically designed and validated for this trial.

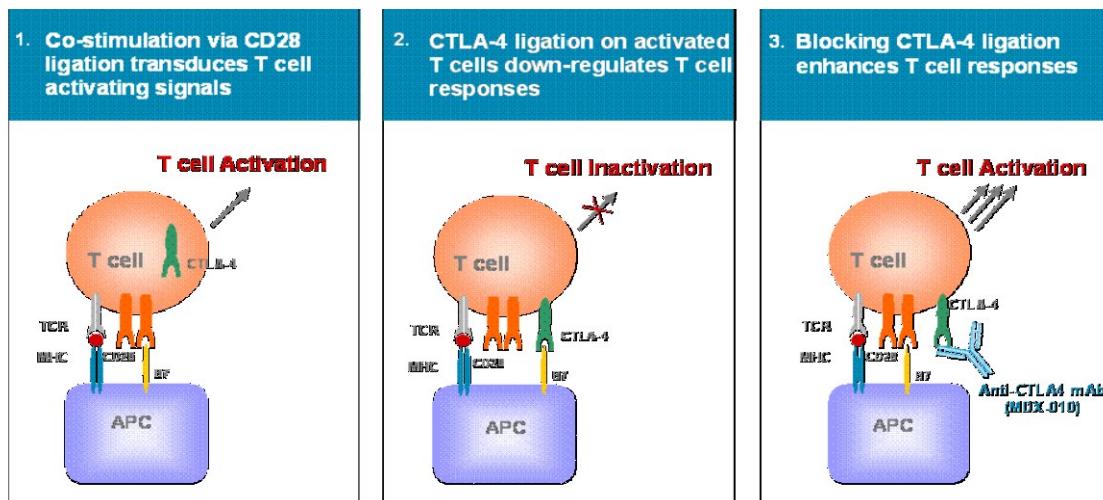
The components of both the 12-color ICS and the 14-color Treg/Activation/Exhaustion/MDSC polychromatic flow cytometry panels are delineated in the Table below.

## BMS IPI Proposed Panels

Panel	Marker	Stain	Purpose	Fluorophore	Clone	Catalog No	Manufacturer
12-color/13-marker ICS/Mat Panel	Viability	Surface	Exclude dead cells	violet	-	L34957	Invitrogen
	CD14	Surface		Pacific Blue	M5E2	558121	Invitrogen
	CD3	Intracellular	T cells	BV605	OKT3	317321	BioLegend
	CD4	Intracellular	Helper T cells	BV785	OKT4	317437	BioLegend
	CD8	Intracellular	Cytotoxic T cells	APC-Cy7	SK1	557834	BD Biosciences
	CD45RA	Surface	Maturation	PE-Cy7	L48	337167	BD Biosciences
	CCR7	Surface	Maturation	PE	150503	560765	BD Biosciences
	CD69	Intracellular	T activation	PerCP-Cy5.5	FN50	560738	BD Biosciences
	CD154	Intracellular	T activation	APC	TRAP1	555702	BD Biosciences
	TNF $\alpha$	Intracellular	T function	Alexa 700	MAb11	557996	BD Biosciences
	IFNg	Intracellular	T function	AF488	4S.B3	502515	BioLegend
	IL-2	Intracellular	T function	BV650	MQ1-17H12	500333	BioLegend
	CD107	Stimulation	T function	PE-Cy5	H4A3	555802	BD Biosciences
14-color Treg/Act/Exh/MDSC Panel	Viability	Surface	Exclude dead cells	aqua	-	423101	BioLegend
	CD3	Surface	T cells	BV605	OKT3	317321	BioLegend
	CD4	Surface	Helper T cells	BV785	OKT4	317437	BioLegend
	Lin	Surface	(CD16,CD19,CD20,CD56)	Exclusion	APC-Cy7		
	CD33	Surface					
	CD11b	Surface	Monocytes/MDSC	PE-Cy7	P67.6	333946	BD Biosciences
	CD28	Surface	Activation	PerCP-Cy5.5	L293	337181	BD Biosciences
	CD14	Surface	Monocytes/MDSC	Alexa 700	M5E2	557923	BD Biosciences
	HLA-DR	Surface	MDSC	BV421	L243	307636	BioLegend
	CD279	Intracellular	Activation (PD-1)	FITC	MIH4	557860	BD Biosciences
	CD152	Intracellular	Activation (CTLA-4)	PE-Cy5	BN13	555854	BD Biosciences
	FoxP3	Intranuclear	T regulatory	PE	PCH101	12-4776-42	eBioscience
	CD25	Surface	T regulatory	BV650	BC96	302633	BioLegend
	CD278	Surface	Activation	APC	ISA-3	17-9948-42	eBioscience

## 1.5 Ipilimumab Development: CTLA-4 and T Cell Activation

Figure 1 Mechanism of Action



Advances in the understanding of the mechanisms that regulate T cell activation have allowed the rational design of new strategies for immunotherapy of tumors, including melanoma. It has been known for some time that engagement of the T cell antigen receptor by itself is not sufficient for full T cell activation; a second co-stimulatory signal is required for induction of IL-2 production, proliferation and differentiation to effector function of naive T cells. Abundant data now indicate that the primary source of this costimulation is mediated by engagement of CD28 on the T cell surface by members of the B7 family on the antigen-presenting cell (APC).<sup>i</sup> (33) (Figure 1.)

Expression of B7 has been shown to be limited to “professional” antigen presenting cells; that is, specialized cells of the hematopoietic lineage, including dendritic cells, activated macrophages, and activated B cells. It has been suggested that this sharply-defined restriction of B7 expression is a fail-safe mechanism for maintenance of peripheral T cell tolerance, insuring that T cell activation can only be stimulated by appropriate APCs.<sup>ii</sup> (34) The fact that tumor cells do not express B7 contributes to their poor capacity to elicit immune responses. (35, 36)

The demonstration that induction of expression of B7 on many tumor cells by transfection, transduction, or other mechanisms can heighten tumor immunogenicity led to great interest in pursuing this as an approach to tumor immunotherapy. As demonstrated in vivo in murine tumor models, the utility of B7 expression as a vaccination approach is limited by the following factors: (1) B7-expressing tumor cell vaccines are only effective when the tumor cells have a high degree of inherent immunogenicity; (2) while B7-expressing vaccines have been shown in many cases to be effective in inducing protective immune responses, they have demonstrated only limited utility in inducing responses to established tumors; and (3) inactivation of tumor cells by radiation has been shown to destroy the immuno-enhancing activity of the B7 gene product. (37, 38)

In the past few years it has become apparent that co-stimulation is even more complex than originally thought. After activation, T cells express CTLA-4, a close homologue to CD28. CTLA-4 binds members of the B7 family with a much higher affinity than CD28. (39) Although there was initially some controversy as to the role of CTLA-4 in regulating T cell activation, it has become clear that CTLA-4 down-regulates T cell responses. (40)

This was initially suggested by the following in vitro observations: (1) blockade of CTLA-4/B7 interactions with antibody enhanced T cell responses; (2) cross-linking of CTLA-4 with CD3 and CD28 inhibited T cell responses; and (3) administration of antibodies to CTLA-4 in vivo enhanced the immune response to peptide antigens or superantigens in mice. (41, 42, 43, 44) Blocking CTLA-4-B7 interaction while preserving signaling via CD28 resulted in enhanced T cell responses in vitro (42).

Perhaps the most convincing demonstration of the down-regulatory role of CTLA-4 came from examination of mice with a null mutation. (45, 46, 47) CTLA-4 knockout mice appear to have spontaneously activated T cells evident at approximately 1 week after birth, followed by rampant lymphoproliferation and lymphadenopathy. These mice die at approximately 3 weeks of age, either as a result of polyclonal T cell expansion and tissue destruction or as a result of toxic shock resulting from lymphokine production by the T cells. Since thymocyte differentiation and selection proceed normally in CTLA-4-deficient mice, the rampant T cell expansion that occurs in the mice indicates that CTLA-4 plays a critical role in down-regulating T cell responses in the periphery. (44)

## **1.6 Summary of Results of Investigational Program**

### **1.6.1 Pharmacology of Ipilimumab**

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

### **1.6.2 Animal Toxicology of Ipilimumab**

The effects of ipilimumab on prenatal and postnatal development in monkeys have not been fully investigated. Preliminary results are available from an ongoing study 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.

Genetically engineered mice heterozygous for CTLA-4 (CTLA-4 $^{+/-}$ ), the target for ipilimumab, 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 $^{-/-}$ ). The CTLA-4 $^{-/-}$  homozygous negative offspring appeared healthy at birth, exhibited

signs of multiorgan lymphoproliferative disease by 2 weeks of age, and all died by 3–4 weeks of age with massive lymphoproliferation and multiorgan tissue destruction.

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

The in vitro studies demonstrated that ipilimumab did not mediate CDCC of PHA- or (CD)3-activated human T cells. However, low to moderate ADCC activity was noted at concentrations up to 50  $\mu$ g/mL. These data are consistent with the requirement of high levels of antigen expression on the surface of target cells for efficient ADCC or CDCC. Since ipilimumab is a human IgG1, an isotype generally capable of mediating CDCC and ADCC, the lack of these activities is likely due to a very low expression of CTLA-4 on activated T cells. Therefore, these data suggest that ipilimumab treatment would not result in depletion of activated T cells in vivo. Indeed, no depletion of T cells or T cell subsets were noted in toxicology studies in cynomolgus monkeys.

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

## **1.7 Clinical Pharmacology**

### **1.7.1 Mechanism of Action**

CTLA-4 is a negative regulator of T-cell activation. Ipilimumab binds to CTLA-4 and blocks the interaction of CTLA-4 with its ligands, CD80/CD86. Blockade of CTLA-4 has been shown to augment T-cell activation and proliferation. The mechanism of action of ipilimumab's effect in patients with melanoma is indirect, possibly through T-cell mediated anti-tumor immune responses.

## 1.7.2 Pharmacokinetics

The pharmacokinetics 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 for four doses. Peak concentration (C<sub>max</sub>), trough concentration (C<sub>min</sub>), 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 every 3 weeks, 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 pharmacokinetic 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 (V<sub>ss</sub>) of 7.21 L (10.5%). The mean (±SD) ipilimumab C<sub>min</sub> 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 pharmacokinetics were assessed in population pharmacokinetic 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 pharmacokinetics 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 pharmacokinetics in patients with various degrees of hepatic impairment.

Pharmacokinetic (PK) profiles for ipilimumab have been analyzed. The primary objective of Protocol MDX010-015 was to determine the safety and PK profile of single and multiple doses of ipilimumab derived from a transfectoma or hybridoma cell line. Mean plasma concentrations of ipilimumab administered at doses of 3 mg/kg (hybridoma-derived drug product); 2.8 mg/kg, 5 mg/kg, 7.5 mg/kg, 10 mg/kg, 15 mg/kg, and 20 mg/kg (transfectoma-derived drug product)

demonstrated approximate dose proportionality. Equimolar doses of hybridoma-derived and transfectoma-derived drug product had comparable PK profiles. The range of mean volume of distribution at steady state ( $V_{ss}$ ) across cohorts 2.8, 3, 5, 7.5, 10, 15, and 20 mg/kg, was 57.3 to 82.6 mL/kg, indicating drug distribution was mostly limited to the intravascular space. The clearance was low (range 0.11 to 0.29 mL/h/kg) and reflective of the half-life (range 297 to 414 h), which is consistent with the long terminal disposition phase of ipilimumab. There was moderate variability in the PK parameters among subjects, with CV of 11% to 48% in AUC (0-21d), 20% to 59% in CL, and 17% to 46% in  $V_{ss}$ .

Ipilimumab was originally produced and purified from a hybridoma clone. Ipilimumab drug substance is currently manufactured using Process B. A new drug substance manufacturing process (Process C) has been developed utilizing a higher producing sub-clone of the current Master Cell Bank, and modified cell culture and purification steps. The new drug substance manufacturing process is intended to replace the current drug substance manufacturing process. The biocomparability of Process C relative to Process B was assessed in Study CA184087.

### **PK in Phase 1 Study CA184087 (Process B and Process C)**

The PK of ipilimumab was assessed when manufactured by a newer process C relative to current process B as an IV infusion (1.5-hr), in subjects with advanced melanoma (CA184087). Upon meeting eligibility criteria, subjects were randomized (1:1) to receive either ipilimumab Process B (Arm A, reference) or ipilimumab Process C (Arm B, test) at a dose of 10 mg/kg IV administered over 90 minutes every 3 weeks on Days 1, 22, 43, and 64 (Weeks 1, 4, 7, and 10) during induction therapy. Randomization was stratified by baseline body weight (BW) and LDH values since both were identified as potential covariates in a population PK assessment.

The primary endpoint of PK data at week 4 demonstrated that the PK of Process B and Process C are biocomparable as the 90% CIs for the ratio of geometric means of AUC(0-21d) and Cmax - both adjusted or not adjusted for covariates - were entirely contained with the pre-specified equivalence interval (80 - 125%).

### **Population Pharmacokinetics**

The population pharmacokinetics (PPK) of ipilimumab was developed with 420 subjects (1767 serum concentrations) with advanced melanoma in phase 2 studies (CA184007, CA184008, and CA184022). (50, 51, 52) subsequently, the final PPK model was evaluated by an external model validation dataset from CA184004 (79 subjects with 328 serum concentration data). The PPK analysis demonstrated that PK of ipilimumab is linear and exposures are dose proportional across

the tested dose range of 0.3 to 10 mg/kg, and the model parameters are time-invariant. The ipilimumab CL of 15.3 mL/h from PPK analysis is consistent with that determined by PK analysis as assessed in MDX010-15 as 12.8 mL/h for a dose of 2.8 mg/kg and 15.7 mL/h for a dose of 10 mg/kg. The terminal half-life and Vss of ipilimumab calculated from the model were 14.7 days, and 7.21 L, which are consistent with that determined by non-compartmental analysis (NCA). Volume of central and peripheral compartment were found to be 4.16 and 3.22 L, respectively, suggesting that ipilimumab first distributes into plasma volume and subsequently into extracellular fluid space. Clearance of ipilimumab was found to increase with increase in body weight, supporting dosing of ipilimumab based on a weight normalized regimen. Other covariates had effects that were either not statistically significant or were of minimal clinical relevance.

## 1.8 Clinical Safety with Ipilimumab

### 1.8.1 Overview of Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, the adverse reaction rates observed cannot be directly compared with rates in other clinical trials or experience with therapeutics in the same class and may not reflect the rates observed in clinical practice.

The clinical development program excluded patients with active autoimmune disease or those receiving systemic immunosuppression for organ transplantation. Exposure to ipilimumab 3 mg/kg for four doses given by intravenous infusion in previously treated patients with unresectable or metastatic melanoma was assessed in a randomized, double-blind clinical study (MDX010-20). One hundred thirty-one patients (median age 57 years, 60% male) received ipilimumab as a single agent, 380 patients (median age 56 years, 61% male) received ipilimumab with an investigational gp100 peptide vaccine (gp100), and 132 patients (median age 57 years, 54% male) received gp100 peptide vaccine alone. Patients in the study received a median of 4 doses (range 1 to 4 doses). Ipilimumab was discontinued for adverse reactions in 10% of patients. (48)

The most common adverse reactions ( $\geq 5\%$ ) in patients who received ipilimumab at 3 mg/kg were fatigue, diarrhea, pruritus, rash, and colitis.

Table 1 presents selected adverse reactions from MDX010-20, which occurred in at least 5% of patients in the ipilimumab-containing arms and with at least 5% increased incidence over the control gp100 arm for all-grade events and at least 1% incidence over the control group for Grade 3–5 events.

**Table 1: Selected Adverse Reactions in MDX010-20**

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

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

Table 2 presents the per-patient incidence of severe, life-threatening, or fatal immune-mediated adverse reactions from MDX010-20.

**Table 2: Severe to Fatal Immune-mediated Adverse Reactions in MDX010-20**

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

<sup>a</sup> Including fatal outcome

<sup>b</sup> Including intestinal perforation

<sup>c</sup> Underlying etiology not established

Source: Yervoy Prescribing Information, Bristol-Myers Squibb, March 2011.

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<sup>2</sup> 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, I tract, liver, and the endocrine system. Events were managed with established guidelines and were generally responsive to dose interruption/discontinuation, corticosteroids and/or other immunosuppressants. Select adverse events associated with the mechanism of action of ipilimumab, regardless of attribution by the investigator are shown in Table 3.

**Table 3: CA184024 Select Adverse Events**

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

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

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

The safety profile of ipilimumab at a dose of 10 mg/kg was characterized in a total of 325 subjects who received multiple doses of 10 mg/kg ipilimumab as monotherapy in the 4 completed melanoma studies (CA184004, -007, -008, and -022). (49, 50, 51, 52) 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 are displayed in Table 4.

**Table 4: Summary of irAE Safety Data for 10 mg/kg in Melanoma**

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

Overall, the 10 mg/kg had an acceptable safety regimen, while being the most active dose. The study drug related deaths across the program are in Section 5 of the investigators brochure.

Across clinical studies that utilized ipilimumab doses ranging from 0.3 to 10 mg/kg, the following adverse reactions were also reported (incidence less than 1% unless otherwise noted): urticaria (2%), large intestinal ulcer, esophagitis, acute respiratory distress syndrome, renal failure, and infusion reaction.

Based on the experience in the entire clinical program for melanoma, the incidence and severity of enterocolitis and hepatitis appear to be dose dependent.

### **1.8.2 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.

Immunogenicity assay results are highly dependent on several factors including assay sensitivity and specificity, assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of incidence of antibodies to ipilimumab with the incidences of antibodies to other products may be misleading.

### **1.8.3 Pregnancy Outcomes**

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.

### **1.8.4 Immune-mediated Adverse Reactions with Ipilimumab**

Ipilimumab can result in severe and fatal immune-mediated reactions due to T-cell activation and proliferation.

## **Immune-related Gastrointestinal Events**

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. 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.

## **Immune-related Hepatotoxicity**

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 levels must be evaluated before each dose of ipilimumab as early laboratory changes may be indicative of emerging immune-related hepatitis. 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 medications, and monitored until resolution. Liver biopsies from patients who had immune-related hepatotoxicity showed evidence of acute inflammation (neutrophils, lymphocytes, and macrophages).

## **Immune-related Skin Toxicity**

Skin immune-related AEs presented mostly 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.

## **Immune-related Endocrinopathy**

Ipilimumab can cause inflammation of the endocrine system organs, specifically hypophysitis, hypopituitarism, and adrenal insufficiency and patients may present with nonspecific symptoms, which may resemble other causes such as brain metastasis or underlying disease. The most common clinical presentation includes headache and fatigue. Symptoms may also include visual field defects, behavioral changes, electrolyte disturbances, 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. It is possible that long-term hormone replacement therapy will be required for subjects developing hypophysitis/hypopituitarism after treatment with ipilimumab.

## **Immune-related Neurological Events**

Neurological manifestations included muscle weakness and sensory neuropathy. Fatal Guillain-Barré syndrome has been reported in clinical trials of ipilimumab. Patients may present with muscle weakness. Sensory neuropathy may also occur. 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, and medications should be excluded.

## **Other Immune-related AEs**

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.

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 11. Immune-related AEs generally resolved within days to weeks in the majority of subjects.

## **1.85 Ipilimumab and Growth Factor Support**

Phase I data observed it was safe to combine ipilimumab with the hematopoietic growth factor, granulocyte macrophage colony-stimulating factor (GM-CSF), sargramostim. Ipilimumab was escalated from 0.5 to 3 mg/kg q 4 weeks with GM-CSF subcutaneous at fixed dose 250mcg/m<sup>2</sup> for 14 days each cycle. Ipilimumab was successfully escalated to 3 mg/kg where 2/6 grade 3 irAEs were observed: 1 pan-hypopituitarism, 1 diarrhea. Both irAEs were easily treated. 1 grade 3 temporal arteritis was also observed unknown if related to ipilimumab? Interestingly, immune induced antitumor T cells were observed at highest dose and tumor response >50% drop in PSA was observed only in patients with grade 3 irAEs.(58)

## **1.9 Clinical Efficacy: Melanoma Program**

The clinical efficacy of ipilimumab as a single agent at a dose of 3 mg/kg administered every 3 weeks 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 for the treatment of unresectable or metastatic melanoma. 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 and was feasible with an acceptable safety profile.

Overall survival and other efficacy endpoints were assessed in ipilimumab studies.

Overall Survival: Prolongs survival in patients with metastatic melanoma who have failed prior treatment.

Best Objective Response Rate (BORR): By the conventional mWHO criteria confirmed objective responses have been observed in subjects receiving ipilimumab. These responses tend to be durable with the majority of subjects who achieve objective responses progression-free at the end of long observation periods.

Disease Control Rate (DCR): Disease stabilization in subjects receiving ipilimumab is a key 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. Consequently, SD as well as objective responses (both captured in DCR) are important for completely characterizing anti-tumor activity of ipilimumab

Progression-Free Survival (PFS): Some subjects demonstrate initial tumor volume increase before response, possibly due to T-cell infiltration as shown by biopsies. Consequently, PFS incompletely captures all patterns of activity and may underestimate the clinical activity of ipilimumab.

### **1.9.1 Rationale for Using Immune-Related Tumor Assessment**

Ipilimumab is an immuno-stimulating antibody with evidence of anti-cancer activity (durable objective response and stable disease) in subjects with advanced melanoma, including subjects with established brain disease. Observations of clinical activity defined by tumor response endpoints defined by conventional criteria (eg, mWHO) may be inadequate to realize the kinetics and magnitude of clinical benefit achieved with ipilimumab.

Histopathologic evidence has demonstrated ipilimumab can produce an influx or expansion of tumor infiltrating lymphocytes. Therefore, early increases in lesion size detected radiologically or upon gross examination could be misinterpreted as progressive tumor growth and precede objective tumor shrinkage. In addition the appearance of new lesions may have categorized a subject to have progressive disease using conventional tumor assessment criteria despite the concurrent observation of objective tumor responses in pre-existing lesions and a net reduction in global tumor burden that includes the new lesions.

Therefore, patients with evidence of possible target lesion growth or new lesion appearance will not be presumed to have progressed and will proceed to surgery as long as all possible sites of disease are deemed resectable by the surgeon.

### **1.9.2 MDX010-20 (Phase 3, 3 mg/kg, previously treated melanoma)**

MDX010-20, a randomized (3:1:1), double-blind, double-dummy study included 676 randomized subjects with unresectable or metastatic melanoma previously treated with one or more of the following: aldesleukin, dacarbazine, temozolomide, fotemustine, or carboplatin. Of these 676 subjects, 403 were randomized to receive ipilimumab at 3 mg/kg in combination with an investigational peptide vaccine with incomplete Freund's adjuvant (gp100), 137 were randomized to receive ipilimumab at 3 mg/kg, and 136 were randomized to receive gp100 alone. The study enrolled only subjects with HLA A2\*0201 genotype; this HLA genotype facilitates the immune presentation of the investigational peptide vaccine. The study excluded subjects with active autoimmune disease or those receiving systemic immunosuppression for organ transplantation. Ipilimumab/placebo was administered at 3 mg/kg as an intravenous infusion every 3 weeks for 4 doses. Gp100/placebo was administered at a dose of 2 mg peptide by deep subcutaneous injection every 3 weeks for four doses. Assessment of tumor response was conducted at Weeks 12 and 24, and every 3 months thereafter. Subjects with evidence of objective tumor response at 12 or 24 weeks had assessment for confirmation of durability of response at 16 or 28 weeks, respectively.(48)

The major efficacy outcome measure was overall survival (OS) in the ipilimumab + gp100 arm compared to that in the gp100 arm. Secondary efficacy outcome measures were OS in the ipilimumab + gp100 arm compared to the ipilimumab arm, OS in the ipilimumab arm compared to the gp100 arm, best overall response rate (BORR) at Week 24 between each of the study arms, and duration of response.

Of the randomized subjects, 61%, 59%, and 54% in the ipilimumab + gp100, ipilimumab, and gp100 arms, respectively, were men. Twenty-nine (29%) percent were  $\geq 65$  years of age, the median age was 57 years, 71% had M1c stage, 12% had a history of previously treated brain metastasis, 98% had ECOG performance status of 0 and 1, 23% had received aldesleukin and 38% had elevated LDH level. Sixty-one (61%) percent of subjects randomized to either

ipilimumab -containing arm received all 4 planned doses. The median duration of follow-up was 8.9 months.

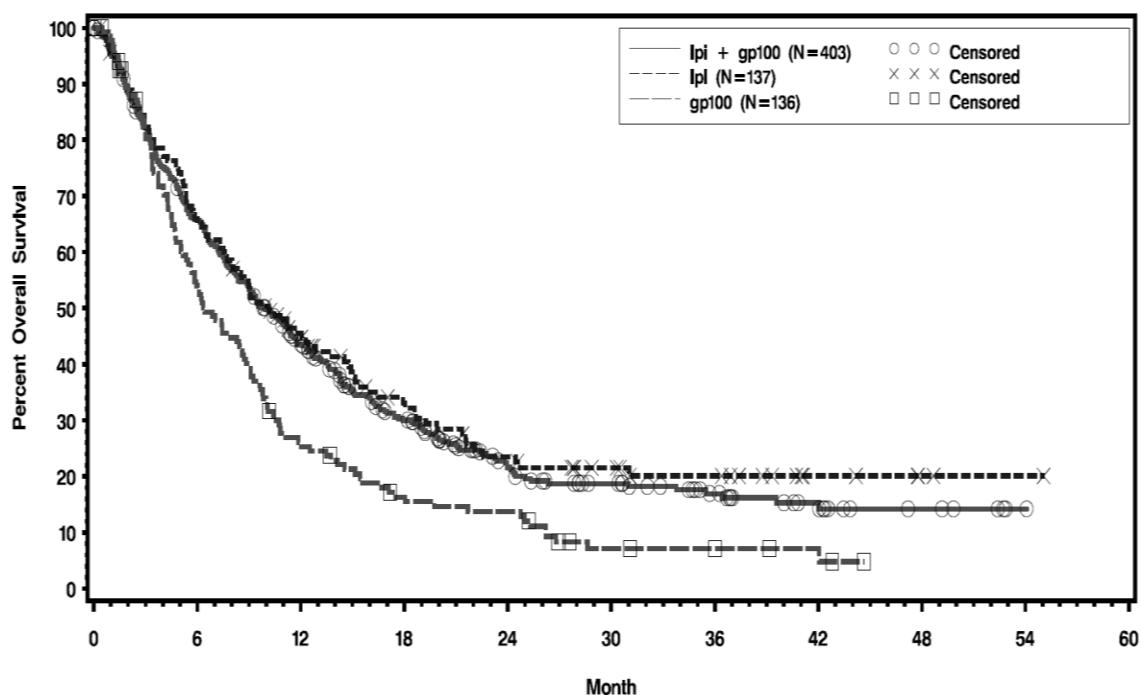
The OS results are shown in Table 5 and Figure 2.

**Table 5: MDX010-20 Overall Survival Results**

	Ipilimumab n = 137	Ipilimumab + gp100 n = 403	gp100 n = 136
Hazard Ratio (vs gp100)	0.66	0.68	
(95% CI)	(0.51, 0.87)	(0.55, 0.85)	
p-value	p = 0.0026 <sup>a</sup>	p = 0.0004	
Hazard Ratio (vs ipilimumab)		1.04	
(95% CI)		(0.83, 1.30)	
Median (months)	10	10	6
(95% CI)	(8.0, 13.8)	(8.5, 11.5)	(5.5, 8.7)

a Not adjusted for multiple comparisons.

**Figure 2: MDX010-20 - Overall Survival by Treatment (ITT Population)**



The best overall response rate (BORR) as assessed by the investigator was 5.7% (95% CI: 3.7%, 8.4%) in the ipilimumab + gp100 arm, 10.9% (95% CI: 6.3%, 17.4%) in the ipilimumab arm, and 1.5% (95% CI: 0.2%, 5.2%) in the gp100 arm. The median duration of response was 11.5 months in the ipilimumab + gp100 arm and has not been reached in the ipilimumab or gp100 arm.

### 1.9.3 CA184024 (Phase 3, previously untreated melanoma, 10 mg/kg)

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<sup>2</sup> q3w, with either ipilimumab 10 mg/kg or placebo cycles 1-4, and as maintenance after completion of chemotherapy.

The two arms were well balanced regarding most baseline characteristics, as shown in Table 6.

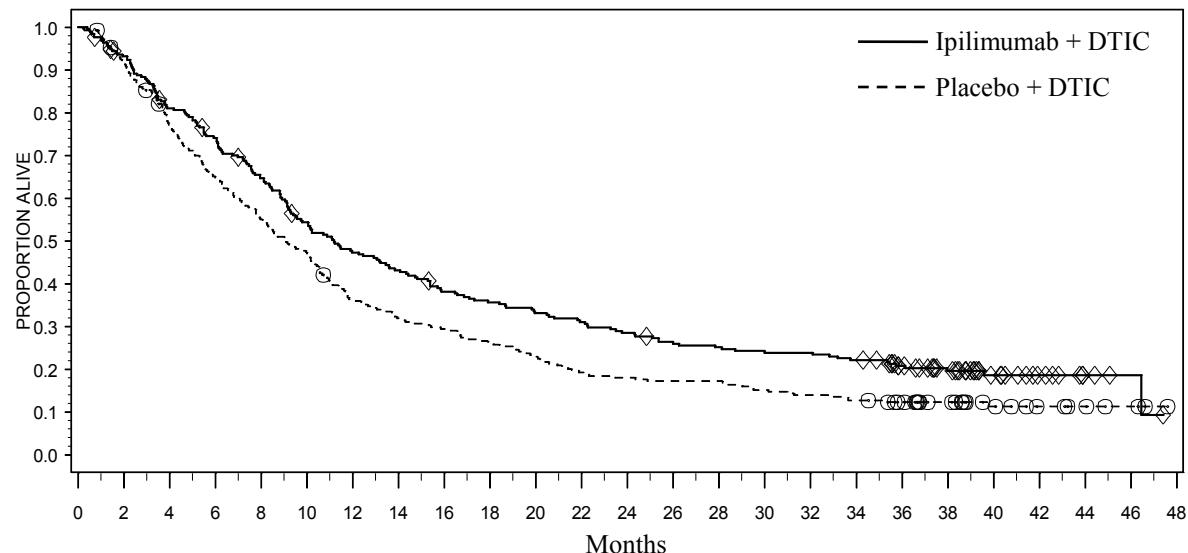
**Table 6: CA184024 Baseline Characteristics**

	<b>Ipilimumab + DTIC n = 250</b>	<b>Placebo + DTIC n = 252</b>
<b>Age (years)</b>		
Mean	57.5	56.4
<b>Gender (%)</b>		
Male	60.8	59.1
Female	39.2	40.9
<b>M Stage (%)</b>		
M0	2.4	3.2
M1a	14.8	17.1
M1b	25.6	24.6
M1c	57.2	55.2
<b>ECOG PS (%)</b>		
0	70.8	71.0
1	29.2	29.0
<b>LDH (%)</b>		
≤ ULN	62.8	55.6
> ULN	37.2	43.7
≤ 2x ULN	86.4	85.3
> 2x ULN	13.6	13.9
<b>Prior adjuvant therapy (%)</b>	26.4	26.6
<b>Prior therapy for advanced disease (%)</b>	0	0

Patients on the ipilimumab arm received a median of 3 ipilimumab induction doses, versus 4 placebo induction doses on the placebo arm. A total of 17.4% and 21.1% of patients continued to receive maintenance ipilimumab or placebo, for a median of 4 and 2 doses, respectively. The number of patients who received all 8 dacarbazine doses was 12.2% in the ipilimumab arm, and 21.5% in the placebo arm.

The study met its primary end-point of prolonging overall survival in patients treated with ipilimumab (HR 0.72 (95% CI, 0.59 – 0.87), median OS 11.2 vs 9.1 months,  $p = 0.0009$ ). The OS Kaplan-Meier curve is presented in Figure 3.

**Figure 3: CA184024 Kaplan-Meier Plot of Overall Survival - All Randomized Subjects**



One, two and three-year survival rates were 47.3%, 28.5% and 20.8% in the ipilimumab arm, and 36.3%, 17.9% and 12.2% in the placebo arm.

PFS, a secondary end-point, was also prolonged by the addition ipilimumab, HR 0.76 (95% CI, 0.63 - 0.93). The median PFS was 2.8 months in the ipilimumab and vs 2.6 months in the placebo arm,  $p = 0.006$ .

BORR was increased from 10.3% in the placebo arm to 15.2% in the ipilimumab arm (Table 7). More importantly, duration of response was more than twice as long in the ipilimumab arm (19.3 months) than in the placebo arm (8.1 months).

**Table 7: CA184024 Tumor Response**

	<b>Ipilimumab + DTIC n = 250</b>	<b>Placebo + DTIC n = 252</b>
<b>Disease Control Rate, n (%)</b>	83 (33.2)	76 (30.2)
<b>BORR (CR + PR), n (%)</b>	38 (15.2)	26 (10.3)
Complete response	4 (1.6)	2 (0.8)
Partial response	34 (13.6)	24 (9.5)
Stable disease	45 (18.0)	50 (19.8)
Progressive disease	111 (44.4)	131 (52.0)
<b>Duration of response, months</b>	19.3	8.1

#### **1.9.4 10 mg/kg Dosing with Ipilimumab**

In melanoma, Phase 3 studies show improved survival at both 3 mg/kg (study MDX010-20) as well as with 10 mg/kg (study CA184024). Several additional conducted 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 is necessary to ensure a blockade of the CTLA-4 pathway: *in vitro* a concentration of 20 µg/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 (ie, 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.

In the three primary studies conducted in advanced melanoma (CA184007, CA184008, and CA184022), subjects treated with 10 mg/kg during the induction period had the highest response, disease control rates, median OS as well as 1-year and 2-year survival rates compared to other

doses. The CA184022 data are summarized in Table 8. (52) Among the 3 doses evaluated, 10 mg/kg ipilimumab led to the greatest such rates.

**Table 8: Summary of Phase 2 Response Data in Melanoma (CA184022)**

	<b>10 mg/kg (n = 72)</b>	<b>3 mg/kg (n = 72)</b>	<b>0.3 mg/kg (n = 73)</b>
<b>BORR (mWHO) - %</b>	11.1	4.2	0
<b>(95% CI)</b>	(4.9 - 20.7)	(0.9 - 11.7)	(0.0 - 4.9)
<b>DCR (mWHO) - %</b>	29.2	26.4	13.7
<b>(95% CI)</b>	(19.0 - 41.1)	(16.7 - 38.1)	(6.8 - 23.8)
<b>Survival rate at 1 year - %</b>	48.64	39.32	39.58
<b>%, 95% CI</b>	(36.84, 60.36)	(27.97, 50.87)	(28.20, 51.19)
<b>Survival rate at 2 year - %</b>	29.81	24.20	18.43
<b>%, 95% CI</b>	(19.13, 41.14)	(14.42, 34.75)	(9.62, 28.22)
<b>Overall median survival</b>	11.43	8.74	8.57
<b>95%CI (months)</b>	(6.90, 16.10)	(6.87, 12.12)	(7.69, 12.71)

Finally, the dose and schedule in study CA184156 is the one that was evaluated in the signal finding study CA184041, with an acceptable safety profile and improvement of irPFS and OS.

Treatment with ipilimumab has demonstrated clinically important and durable tumor responses in several malignancies including melanoma, prostate cancer, and renal cell carcinoma.

### 1.9.5 Advanced Melanoma

Ipilimumab prolonged survival in subjects with pre-treated advanced melanoma are based on results from MDX010-20 (Phase 3) supported by data from Phase 2 studies; the primary efficacy and safety studies are summarized in Table 9. (48, 49, 50, 52, 53, 54, 55, 56) The primary endpoint in MDX010-20 was OS, which was also a key secondary endpoint in Phase 2 studies.

**Table 9: Studies Supporting the Efficacy and Safety of Ipilimumab in Subjects with Advanced Melanoma**

Study No. (Phase)	Populations	Primary Efficacy Endpoint	Doses	# Randomized or Enrolled/Treated		
				Studies	3 mg/kg	10 mg/kg
MDX010-20 (Phase 3)	HLA-A2*0201-positive, previously treated, unresectable Stage III or IV melanoma	OS	3 mg/kg q3 wk x 4 ± gp100 (induction) followed by re-induction	540/512	--/--	676/643 <sup>a</sup>
CA184022 (Phase 2)	Previously treated, unresectable Stage III or IV melanoma	BORR	0.3, 3, or 10 mg/kg q3 wk x 4 (induction) followed by maintenance dosing q12 wk	72/71	72/71	217/214
CA184004 (Phase 2) Biomarker Study	Unresectable Stage III or IV melanoma	BORR	3 or 10 mg/kg q3 wk x 4 (induction) followed by maintenance dosing q12 wk	40/40	42/42	82/82
CA184008 (Phase 2)	Previously treated unresectable Stage III or IV melanoma	BORR	10 mg/kg q3 wk x 4 (induction) followed by maintenance dosing q12 wk	--/--	155/155	155/155
CA184007	Unresectable Stage III or IV melanoma	BORR	10 mg/kg q3 wk x 4 ± budesonide (induction) followed by maintenance dosing q12 wk	--/--	115/115	115/115
<i>Additional Studies</i>						
MDX010-08(Phase 2)	Chemotherapy-naïve advanced melanoma	ORR	3 mg/kg q4 wk x 4 ± DTIC (induction)	78/74	--/--	78/74
CA184042 (Phase 2)	Stage IV melanoma with brain metastases	DCR	10 mg/kg q3 wk x 4 (induction) followed by maintenance	--/--	28/28 <sup>b</sup>	28/28 <sup>b</sup>

**Table 9:****Studies Supporting the Efficacy and Safety of Ipilimumab in Subjects with Advanced Melanoma**

				# Randomized or Enrolled/Treated
		dosing q12 wk		
MDX010-28 (Phase 2) Survival Follow-up Study	Subjects enrolled in earlier Medarex studies, including MDX010-08 and MDX010- 15 <sup>c</sup>	OS	N/A	--/N/A
				--/NA
				--/N/A

BORR = best overall response rate; DCR = disease control rate; DTIC = dacarbazine; N/A = not applicable; ORR = overall response rate; OS = overall survival; PK = pharmacokinetics.

<sup>a</sup> Total includes 136 randomized/131 treated subjects in the gp100 treatment group.

<sup>b</sup> Information is presented only for subjects enrolled in MDX010-20, Arm A.

<sup>c</sup> MDX010-15 was primarily a PK study that evaluated ipilimumab at single and multiple doses.

Source:

## 2.0 Overall Risk/Benefit Assessment

Ipilimumab is the first drug to demonstrate prolonged survival in subjects with pre-treated advanced melanoma, based on a large, multinational, double-blind, pivotal, Phase 3 study supported by a comprehensive Phase 2 program.

The unique immune-based mechanism of action is reflected in the clinical patterns of anti-cancer activity in some patients. Ipilimumab impacts 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, inflammation may not be noted by radiological examination and objective response is observed with the first tumor assessment in a manner seen in patients receiving other types of anti-cancer treatments. In other 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.

In summary, ipilimumab offers clinically meaningful and statistically significant survival benefit to patients with pre-treated advanced melanoma and evidence of clinical activity in randomized studies in other tumor types. These findings, together with evidence of a safety profile that is manageable with careful monitoring and appropriate intervention for treatment of immune-mediated toxicities, suggest an acceptable benefit to risk ratio. In this trial, ipilimumab has the potential to have additive therapeutic benefit with standard NSCLC chemotherapy by enhancing anti-tumor immune activity. The overall risk-benefit ratio for patients entering this protocol is therefore at least comparable to and possibly better than alternative options.

## 3.0 OBJECTIVES

### 3.1 Primary objective:

Evaluate whether the combination of neoadjuvant chemotherapy plus ipilimumab for lung cancer increases the number of subjects with detectable circulating T cells with specificities against tumor associated antigens (TAA) from zero percent of subjects prior to therapy to 20% of subjects after neoadjuvant chemotherapy plus ipilimumab.

### 3.2 Secondary Objectives:

1. Evaluate feasibility/tolerability of neoadjuvant chemotherapy plus ipilimumab and surgery in early stage NSCLC.
2. Evaluate disease-free survival and patterns of metastases after neoadjuvant chemotherapy plus ipilimumab in early stage NSCLC.
3. Evaluate the relationship between pathologic response and the RECIST response after neoadjuvant chemotherapy and ipilimumab.

### 3.3 Exploratory Objectives: (refer to Appendix B for further detail)

1. Determine the percentage of patients with detectable tumor infiltrating lymphocytes (TILs) after neoadjuvant chemotherapy plus ipilimumab.
2. Estimate the rate of pathologic response rate for neoadjuvant chemotherapy plus ipilimumab in early stage NSCLC.
3. Estimate the objective response rate of neoadjuvant chemotherapy and ipilimumab in early stage NSCLC.
4. Determine if the immunomodulatory effects of neoadjuvant chemotherapy plus ipilimumab impact the suppressive mechanisms, restoring functional reactivity to important anti-tumor effector cell populations.
5. Explore an alternative definition for detectability suitable for expression values and determining the percentage of patients with circulating T cells meeting this definition.
6. Evaluate whether presence, quantity or quality of detectable TILs is associated with pathologic response rate to neoadjuvant therapy.

## 4.0 STUDY PLAN / DESIGN

Standard diagnostic and staging work up will be performed including: pathologic/histologic diagnosis of non-small cell lung cancer, PET/CT scan, brain imaging, and mediastinoscopy. Three cycles of neoadjuvant chemotherapy will be given. Ipilimumab will be added to neoadjuvant chemotherapy for cycles 2 and 3. Standard surgical evaluation and therapy will be performed following completion of neoadjuvant therapy. Two cycles of single agent ipilimumab will be given after surgery (adjuvantly), followed by 2 cycles of maintenance therapy.

**Neoadjuvant:**

Cycle 1: Paclitaxel 175 mg/m<sup>2</sup> IV over 3 hours followed by cisplatin 75 mg/m<sup>2</sup> over 60 minutes or carboplatin AUC 6 over 30-60 minutes on day 1 (every 21 days x 1 cycle)

Cycles 2 and 3: Ipilimumab 10mg/kg IV over 90 minutes, Paclitaxel 175 mg/m<sup>2</sup> over 3 hours followed by cisplatin 75 mg/m<sup>2</sup> or carboplatin AUC 6 IV over 30-60 minutes (every 21 days x 2 cycles).

**Surgery:**

Standard surgical evaluation to occur at least 21 days after the last dose (cycle 3) of chemotherapy followed by surgical therapy.

**Post-surgical Therapy:** (Total of 4 doses of ipilimumab will be given post-operatively):

**Adjuvant:** Ipilimumab 10 mg/kg IV every 3 weeks x 2 doses, beginning 4 weeks postoperative (up to 10 weeks if needed for recovery)

**Maintenance:** Ipilimumab 10 mg/kg IV every 12 weeks x 2 doses.

**Correlative Science Measures** (refer to Appendix B for further details on the correlative science outcomes)

1. Peripheral blood mononuclear cells (PBMC) isolated from freshly drawn anti-coagulated blood will be analyzed for circulating T cells with specificities against tumor-associated antigens (TAA) at 4 time points, namely 1) Baseline prior to treatment, 2) cycle 2, prior to ipilimumab therapy, 3) 21-36 days after completion of cycle 3 chemotherapy prior to surgery, and 4) 3-6 weeks after adjuvant ipilimumab dose #2. Additionally, PBMC from each of the time points will be analyzed for the presence of circulating populations of regulatory T cells, myeloid-derived suppressor cells (MDSC), as well as activated and exhausted T cells.
2. Tumor infiltrating lymphocytes (TILs) will be isolated from the patient's resected tumor and analyzed for infiltrating T cells with specificities against tumor-associated antigens. Additionally, TIL will be analyzed for the presence of infiltrating populations of regulatory T cells, myeloid-derived suppressor cells (MDSC), as well as activated and exhausted T cells.

## 5.0 Recruitment

Patients will be recruited for this study as follows: Upon determination that a patient's tumor histology and/or radiographic findings are compatible with the eligibility criteria of this protocol, the clinical study will be briefly explained to the patient by the principal investigator (PI) or colleague. If the patient indicates interest in study participation, patient education sheets (if available) and possibly the approved protocol consent form will be provided to the patient as these provide the most comprehensive explanation of the study in lay terms. If the patient shows continued interest, the PI or designee will thoroughly explain the required elements of informed consent and all aspects of the study to the patient including inclusion/exclusion criteria, risks, benefits and alternatives to study participation.

## **6.0 Study Population/Selection of Patients**

The following guidelines are to assist physicians in selecting patients for whom protocol therapy is safe and appropriate. Physicians should consider the risks and benefits of any therapy, and therefore only enroll patients for whom this treatment is appropriate. For entry into the study, the following criteria MUST be met.

### **6.1. Inclusion Criteria**

Patients are eligible to be included in the study only if they meet **all** of the following criteria:

1. Histologically or cytologically documented NSCLC.
2. Clinical stage IB ( $\geq 4$ cm per CT), Stage IIA/IIB, or Stage III (N0-2) amenable to surgical resection.
3. Patient must be deemed a surgical candidate.
4. ECOG performance status of 0 or 1 (Appendix C).
5. NO prior chemotherapy for current diagnosis of lung cancer.
6. Age  $\geq 18$  years.
7. No active invasive malignancy in the past 2 years other than non-melanoma skin cancer. Cancers that are in-situ are not considered invasive.
8. Signed written informed consent including HIPAA according to institutional guidelines.
9. Adequate Organ Function:
  - ANC or AGC  $\geq 1500$  per  $\mu$ L
  - Platelets  $\geq 100,000$  per  $\mu$ L
  - Total bilirubin  $\leq 1.5$  mg/dL
  - creatinine clearance  $\geq 45$ mL/min (Appendix D); (Creatinine  $<2$  mg/dL to receive cisplatin)
  - SGOT/SGPT  $\leq 2.5$ x institutional ULN.
10. Females of child-bearing potential (not surgically sterilized and between menarche and 1 year post menopause) must test negative for pregnancy within 48 hours prior to any initial study procedure based on a serum pregnancy test. Both sexually active males and females of reproductive potential must agree to use a reliable method of birth control, as determined by the patient and their health care team, during the study and for 3 months following the last dose of study drug. If subject uses appropriate contraceptive methods (the use of two forms at the same time) from the time of the initial serum pregnancy test, then the subsequent pregnancy test can be done within 72 hours of receiving study drug administration. If appropriate contraceptive measures are not begun immediately with the first serum pregnancy test, then subsequent serum pregnancy tests must be done within 48 hours prior to the study drug administration.
11. Patients must agree to research blood sampling to participate in study.

## 6.2. Exclusion Criteria

Patients will be excluded from the study if they meet **any** of the following criteria:

1. Treatment within the last 30 days with a drug that has not received regulatory approval for any indication at the time of study entry.
2. Concurrent administration of any other anti-tumor therapy.
3. Inability to comply with protocol or study procedures.
4. Active infection requiring IV antibiotics, antifungal or antiviral agents, that in the opinion of the investigator would compromise the patient's ability to tolerate therapy.
5. Major surgery (other than definitive lung cancer surgery) within two weeks of study or other serious concomitant systemic disorders that, in the opinion of the investigator, would compromise the safety of the patient or compromise the patient's ability to complete the study.
6. Myocardial infarction having occurred less than 6 months before inclusion, any known uncontrolled arrhythmia, symptomatic angina pectoris, active ischemia, or cardiac failure not controlled by medications.
7. Contraindication to corticosteroids.
8. Unwillingness to stop taking herbal supplements while on study.
9. Female patients who are pregnant or breast-feeding.
10. Autoimmune disease. Patients with a history of inflammatory bowel disease, including ulcerativecolitis 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 [eg, Wegener's Granulomatosis]); motor neuropathy considered of autoimmune origin (e.g. Guillain-Barre Syndrome and Myasthenia Gravis).
11. Any non-oncology vaccine therapy used for prevention of infectious diseases (for up to 1 month before or after any dose of ipilimumab).
12. A history of prior treatment with ipilimumab or prior CD137 agonist or CTLA-4 inhibitor or agonist.
13. Prisoners or subjects who are compulsorily detained (involuntarily incarcerated) for treatment of either psychiatric or physical (e.g., infectious) illness.

## 6.3 Protocol Eligibility Waivers

No waivers of inclusion or exclusion criteria will be granted. All prospective patients must meet all entry criteria prior to enrollment in the study. If there are any questions regarding the interpretation of a criterion for a potential patient, contact the principal investigator to discuss the potential patient to confirm eligibility.

## 7.0 Inclusion of Women and Minorities

There are no exclusions based on gender, race or ethnicity in this trial. There is no evidence to suggest that outcomes will differ.

## 8.0 Registration Procedure

Patient registration for all patients signing informed consent will be completed through the Duke Cancer Institute (DCI) Clinical Research Unit (CRU) into eResearch (DOCR). The investigator and/or designee will enter the patient information into the DOCR Subject Registry within 1 business day of obtaining consent. Patients will be enrolled only after all pre-treatment evaluations are completed and all eligibility criteria are met.

## 9.0 Study Periods

For the purpose of scheduling evaluations and to allow for patient and investigator schedules, holidays and weather or other emergencies requiring clinical facilities to be closed, all patient visits can be performed  $\pm 3$  days of scheduled visits.

## 9.1 Screening

The following procedures and tests are to be performed within **30** days (unless otherwise specified) of study enrollment to confirm eligibility. Baseline and Cycle 1 Day 1 procedures may be completed on the same day. However, screening assessments for *eligibility* **MUST** have already been determined. Refer to appendix A for details.

- Informed Consent

- Medical history including demographics/smoking history
- Height and weight (BSA calculation)
- Vital signs (temp, B/P, pulse)
- Performance status
- Physical exam
- Routine blood work
- Thyroid Functions
- Negative Pregnancy test for WOCBP only
- EKG
- Tumor Histology
- Staging PET/CT of chest/abdomen, Brain MRI or CT (as per standard of care) These may be obtained within 42 days
- Tumor measurements

**Additional Studies:**

- Pretreatment blood will be collected to assess activated CD8+ T cells with specificity against tumor antigens and CD4 and CD8 functional memory. 4 tubes of 8.5-ml each will be collected. The tubes used will be ACD anti-coagulated Vacutainer (yellow tops) and will be processed by Dr. Weinhold's lab.

**9.2 Treatment Study Day 1 Cycle 1 Neoadjuvant Chemohearpy**

(to be performed prior to receiving treatment)

- Weight (BSA calculation)
- Creatinine Clearance
- Vital signs (temp, B/P, pulse)
- Performance status
- Physical exam
- Adverse events assessments
- Routine blood work
- Pregnancy test  $\leq$  3 days (72 hrs) prior to first dose of ipilimumab (for WOCBP only)
- Chemotherapy infusion

**9.3 Treatment Study Day 1 Cycle 2 Neoadjuvant Chemotherapy**

- Weight (BSA calculation)
- Creatinine Clearance
- Vital signs (temp, B/P, pulse)

- Performance status
- Physical Exam
- Adverse events Assessment
- Routine blood work
- Dose # 1 Ipilimumab
- Chemotherapy infusions
- Pregnancy test  $\leq$  3 days (72 hrs) prior to first dose of ipilimumab (for WOCBP only)
- Research blood will be collected to assess activated CD8+ T cells with specificity against tumor antigens and CD4 and CD8 functional memory. 4 tubes of 8.5-ml each will be collected. The tubes used will be ACD anti-coagulated Vacutainer (yellow tops) and will be processed by Dr. Weinhold's lab. **To be collected prior to receiving ipilimumab.**

## 9.4 Treatment Study Day 1 Cycle 3 Neoadjuvant Chemotherapy

- Weight (BSA calculation)
- Creatinine Clearance
- Vital signs (temp, B/P, pulse)
- Performance status
- Physical Exam
- Adverse events assessment
- Routine blood work
- Thyroid Functions
- Pregnancy test  $\leq$  3 days (72 hrs) prior to first dose of ipilimumab (for WOCBP only)
- Dose #2 Ipilimumab
- Chemotherapy infusions

## 9.5 Evaluation at Completion of Neoadjuvant Chemotherapy

Subjects to undergo medical and surgical evaluation at least 21 days after completion of neoadjuvant chemotherapy

- Weight (BSA calculation)
- Vital signs (temp, B/P, pulse)
- Performance Status
- Physical Exam
- Routine Blood work
- Repeat imaging to evaluate tumor (Chest CT)
- Adverse events assessment

Research blood will be collected 21-36 days after completion of cycle 3 chemotherapy to assess activated CD8+ T cells with specificity against tumor antigens and CD4 and CD8 functional memory. 4 tubes of 8.5-ml each will be collected. The tubes used will be ACD anti-coagulated Vacutainer (yellow tops) and will be processed by Dr. Weinhold's lab.

## **9.6 Surgery**

Surgery to occur 4-12 weeks after last dose of chemotherapy or subject will be removed from trial unless approved by PI.

The ability to perform surgery will be assessed by the surgeon and will be based on physical exam and radiological evaluation. Surgical therapy will be individualized based on the surgical judgment and patient preference and is not dictated by study protocol. Tumor tissue samples will also be collected at time of surgical resection

## **9.7 Post-Surgery Therapy**

For subjects with bulky residual adenopathy such that resection was not attempted will be removed from protocol therapy and should receive standard oncology care as deemed appropriate by the treating physician.

Subjects with positive surgical margins or N2 disease will be removed from protocol and considered for standard of care post-operative radiation +/- chemotherapy as deemed appropriate by treating physician.

For subjects that will go on to receive post-operative ipilimumab, please see below.

### **9.71 Adjuvant Ipilimumab**

Ipilimumab to start 4-10 weeks after surgery. Doses will be given every 3 weeks x 2 doses. The following assessments will be done prior to the each dose of adjuvant ipilimumab administration.

- Weight (BSA calculation)
- Creatinine Clearance
- Vital signs (temp, B/P, pulse)
- Performance status
- Physical Exam
- Adverse events Assessment
- Routine blood work
- Thyroid Functions
- Pregnancy test  $\leq$  3 days (72 hrs) prior to first dose of ipilimumab (for WOCBP only)
- Adjuvant Dose Ipilimumab

Research blood will be collected to assess activated CD8+ T cells with specificity against tumor antigens and CD4 and CD8 functional memory. 4 tubes of 8.5-ml each will be collected. The tubes used will be ACD anti-coagulated Vacutainer (yellow tops) and will be processed by Dr. Weinhold's lab. **This blood sample to be collected 3-6 weeks after dose # 2 adjuvant ipilimumab.**

## **9.72 Maintenance Ipilimumab**

Maintenance doses of ipilimumab will be given every 12 weeks x 2 doses starting 12 weeks after dose #2 adjuvant ipilimumab administration. The following assessments will be done prior to each dose of maintenance ipilimumab administration.

- Weight (BSA calculation)
- Creatinine Clearance
- Vital signs (temp, B/P, pulse)
- Performance status
- Physical Exam
- Adverse events Assessment
- Routine blood work
- Thyroid Functions
- Chest CT
- Pregnancy test  $\leq$  3 days (72 hrs) prior to first dose of ipilimumab (for WOCBP only)
- Maintenance Dose Ipilimumab

## **9.8 End of Study (EOS) Final Visit**

For subjects that have completed maintenance ipilimumab, end of study evaluation is to occur approximately 12 weeks after 2<sup>nd</sup> maintenance dose. Final visit for those subjects that come off treatment early for progression of disease, intolerance to protocol therapy, other reasons or consent withdrawal, to occur 30 days (+/- 5 days) after last dose ipilimumab for safety evaluation.

- Vital signs (temp, B/P, pulse)
- Weight
- Performance status
- Physical exam
- Routine blood work
- Adverse events assessment
- Chest CT if applicable (per radiologic evaluation appendix A)

## **9.9 Follow-up**

Surveillance after end of study evaluation will occur every 3-4 months for 2 years then every 6 months until 5 years, then yearly thereafter. These evaluations may be coordinated with visits for radiologic disease evaluation or occur via phone follow-up.

# **10.0 SAMPLE PROCUREMENT AND HANDLING**

## **10.1 Blood Specimens**

Blood will be collected 4 times to assess activated CD8+ T cells with specificity against tumor antigens and CD4 and CD8 functional memory. At each blood collection, 4 tubes of 8.5-ml each will be collected. The tubes used will be ACD anti-coagulated Vacutainer (yellow tops). The following time points will have blood collections (see appendix F):

Collection 1: Baseline prior to treatment

Collection 2: Cycle 2 day 1 chemotherapy prior to ipilimumab administration

Collection 3: 21-36 days after completion of cycle 3 chemotherapy prior to surgery

Collection 4: 3-6 weeks after adjuvant ipilimumab dose #2.

## **10.2 Surgical Specimens**

Patients will be approached to participate in the “DUHS Biospecimen Repository and Processing Core (BRPC)” eIRB 35974 protocol prior to surgery. Tissue will be collected and released as described in this protocol to ensure proper involvement of pathology to minimize the chance that a tissue collection event interferes with appropriate clinical tissue processing and diagnosis.

Tumor specimen samples will be collected at time of definitive surgical resection of tumor. Lung tissue is harvested in the frozen section or gross dissection area of the surgical pathology suite. The tissue is first examined by a certified anatomic pathologist or surrogate (resident, pathology assistant). Relevant margins are inked, removed and examined by frozen section analysis, if necessary. It is imperative that harvesting tissue for use in research trials does not impede accurate initial assessment of critical features of the tumor resection, most notably margin status. After the specimen has been processed for margin status, the frozen section assessment of the specimen is complete, (appendix G), a specimen of excess tumor will be released and acquired by the tumor immunology correlative science staff for isolation of tumor infiltrating lymphocytes for purposes of this protocol.

Patients will be approached to participate in the “DUHS Biospecimen Repository and Processing Core (BRPC)” protocol (eIRB 35974), prior to surgery to help coordinate and facilitate tissue acquisition. Tissue will be collected and released as described in this protocol to ensure proper involvement of pathology to minimize the chance that a tissue collection event interferes with appropriate clinical tissue processing and diagnosis. For those subjects that decline participation in this biorepository tissue will be processed as outlined above.

## **11.0 Treatment**

Bristol-Myers Squibb (BMS) will provide ipilimumab at no cost for this study. The investigational product is defined as a pharmaceutical form of an active ingredient being tested as a reference in the study, whether blinded or unblinded. In this study, the investigational product is ipilimumab. Other medications used in the study as support or escape medication for preventative, diagnostic, or therapeutic reasons, as components of the standard of care for a given diagnosis, are considered non-investigational products. In this protocol, non-investigational product(s) is/are: paclitaxel, carboplatin and cisplatin.

### **11.1. Treatments Administered**

**Patients will receive neoadjuvant combination chemotherapy as follows:**

**Cycle 1:** Paclitaxel 175mg/m<sup>2</sup> intravenously over 3 hours, followed by cisplatin 75 mg/m<sup>2</sup> over 60 minutes or carboplatin AUC 6 (**capped at 900 mg**) intravenously over 30-60 minutes Day 1 (21 day cycle x 1).\*

**Cycles 2 and 3:** Ipilimumab 10 mg/kg IV\*\* intravenously over 90 minutes followed by paclitaxel 175mg/m<sup>2</sup> intravenously over 3 hours, followed by cisplatin 75 mg/m<sup>2</sup> over 60 minutes or carboplatin AUC 6 (**capped at 900 mg**) intravenously over 30-60 minutes Day 1 (every 21 days x 2 cycles)\*

**Surgery:** Standard surgical evaluation to occur at least 21 days after the last dose of chemotherapy followed by surgical therapy.

**Post-surgical Treatment: A total of 4 doses of ipilimumab will be given post-operatively**

**Adjuvant:** Ipilimumab 10 mg/kg/IV\*\* every 3 weeks x2 doses beginning 4 weeks postoperatively (up to 10 weeks if needed for recovery)

**Maintenance:** Ipilimumab 10 mg/kg/IV\*\* every 12 weeks x 2 doses

\*The total administered dose of chemotherapy may be rounded up or down within a range of +/- 5 % of the actual calculated dose.

\*\*The total dose must be calculated using the most recent subject weight (obtained within 3 days of the dosing visit, and prior to the infusion). Dose only to change if there is a weight change >10% from baseline.

## **11.2. Materials and Supplies**

Qualified personnel who are familiar with procedures that minimize undue exposure to themselves and to the environment should undertake the preparation, handling and safe disposal of chemotherapeutic agents in a self-contained, protective environment. Unused portions of injectable chemotherapeutic agents that do not contain a bacteriostatic agent or are prepared with unpreserved diluents (i.e., Sterile Water of Injection USP or 0.9% Sodium Chloride for Injection) should be discarded within eight hours of vial entry to minimize the risk of bacterial contamination.

### **11.2.1. Paclitaxel, Carboplatin, Cisplatin and Ipilimumab**

Paclitaxel, carboplatin, and/or cisplatin and ipilimumab will be intravenously administered only at the investigational site. As a result, patient compliance is ensured.

#### **11.2.2. Paclitaxel**

Paclitaxel is commercially available as a sterile solution concentrate, 6 mg/ml in 5 ml vials (30 mg/vial) in polyoxyethylated castor oil (Cremophor EL) 50% and dehydrated alcohol, USP, 50%. Paclitaxel must be prepared in glass or polyolefin containers due to leaching of diethylexiphthalate (DEHP) plasticizer from polyvinyl chloride (PVC) bags and intravenous tubing by the Cremophor vehicle in which paclitaxel is solubilized. Each bag/bottle should be prepared immediately before administration. Formulation of a small number of fibers in collation (within acceptable limits established by the USP Particulate Matter Test for LVP's) has been observed after preparation of paclitaxel. Therefore, in line filtration is necessary for administration of paclitaxel solution. In line filtration should be accomplished by incorporating a hydrophilic, microporous filter of pore size not greater than 0.22 microns (e.g., IVEX-II or IVEX-HP or equivalent) into the IV fluid pathway distal to the infusion pump. Although particulate formulation does not indicate loss of drug potency, solutions exhibiting excessive particulate matter formation should not be used. The intact vials should be stored under refrigeration (2-8 °C). All solutions of paclitaxel exhibit a slight haziness directly proportional to the concentration of drug and the time elapsed after preparation, although when prepared as described above, solutions of Paclitaxel (0.3-1.2 mg/ml) are physically and chemically stable for 24 hours. Drug will be diluted in order to administer an IV rate of 167 ml per hour for 3 hours. Paclitaxel will be administered via an infusion control device (pump) using non-PVC tubing and connectors, such as the IV administration sets (polyethylene or polyolefin) which are used to infuse parenteral Nitroglycerin. Nothing else is to be infused through the line where paclitaxel is being administered. Patients will be attended by medical

personnel for the first 15 minutes of infusion and then have blood pressure checked every 15 minutes for one hour, then every four hours. Medications for acute management of anaphylaxis should be readily available in the location where the patient is being treated.

### **11.2.3. Cisplatin**

Cisplatin (cis-Diamminedichloroplatinum; cis-DDP) is commercially available. It is available as 1 mg/ml concentration aqueous injection in multidose vials of 50 ml. It should be stored at room temperature (15°C to 25°C) and protected from light. Solutions diluted in 0.9% or 0.45% NaCl to a concentration of 0.05-2 mg/ml are stable for up to 72 hours at room temperature and should be protected from light. Since no antibacterial preservatives are contained in the formulation, it is recommended that any cisplatin solutions admixed for infusion be discarded after 8 hours from the time of dilution. Cisplatin will be administered as an intravenous infusion over 60 minutes. It should be administered in 250-1000 ml NaCl, following intravenous hydration with at least 1000 ml NaCl. Needles, syringes, catheters or IV administration sets containing aluminum parts should not be used as contact with cisplatin yields a black precipitate.

### **11.2.4. Carboplatin**

Carboplatin is supplied commercially as a sterile lyophilized powder available in single-dose vials containing 50 mg, 50 mg and 450 mg of carboplatin. Each vial contains equal parts by weight of carboplatin and mannitol. Preparation: Immediately before use, the content of each vial must be reconstituted with either Sterile Water for injection, USP, 5% dextrose in water, or 0.9% Sodium Chloride injection, USP, to produce a carboplatin concentration of 10 mg/ml. When prepared as directed, Carboplatin solutions are stable for 8 hours at room temperature. Since no antibacterial preservative is contained in the formulation, it is recommended that carboplatin solutions be discarded 8 hours after dilution. Note: aluminum reacts with carboplatin causing precipitate formation and loss of potency; therefore, needles or intravenous sets containing aluminum parts that may come in contact with the drug must not be used for the preparation or administration of carboplatin. Storage and Stability: Unopened vials of carboplatin are stable for the life indicated on the package when stored at controlled room temperature and protected from light. Administration: Administer over 30-60 minutes after completing the paclitaxel infusion. The Crockcroft Gault formula (Dose=AUC (CCC+25) will be used to achieve the desired AUC where CCC=Wt Kg x (140-age)/72xCr (using actual body weight for both female and male and if female use 85%). Capped AUC 6 dose of 900 mg. **Appendix D.**

### **11.2.5 Ipilimumab (see section 15.0 for detailed drug information)**

Ipilimumab is available in concentrations of 5 mg/mL (50 mg/10 mL and 200 mg/40 mL). Ipilimumab injection must not be administered as an IV push or bolus injection. The total dose must be calculated using the most recent subject weight (obtained within three days of the dosing visit prior to the infusion). Dose only to change if there is a weight change >10% from baseline.

For cycles 2-3 neoadjuvant chemotherapy, Ipilimumab will be administered over 90 minutes followed by paclitaxel then cisplatin or carboplatin. Ipilimumab will be administered as a single agent after surgery.

## **11.3 Rationale for Selection of Doses in the Study**

### **11.3.1 Paclitaxel and Cisplatin or Carboplatin and Ipilimumab**

The combination of ipilimumab 10 mg/kg, cisplatin 75 mg/m<sup>2</sup> or carboplatin AUC 6 and paclitaxel 175 mg/m<sup>2</sup> was found to be safe and effective. In advanced metastatic NSCLC, Ipilimumab 10 mg/kg has been successfully combined with the chemotherapy doublet, paclitaxel 175 mg/m<sup>2</sup> and carboplatin AUC 6, as first-line treatment. A recent phase II trial reported that the phased sequence (two courses chemotherapy followed by two courses of chemotherapy + Ipilimumab) had superior progression free survival, overall response, and overall survival; 6.4 months, 57%, and 12.9 months, respectively; compared to 5.7 months, 49%, and 9.1 months, for the concurrent sequence. (31, 32) Overall safety was similar to the control arm with grade 3/4 adverse events being 57%, 54% and 40% in the three study arms, (concurrent ipilimumab+Paclitaxel/carboplatin, phased ipilimumab+Paclitaxel/carboplatin, and paclitaxel/carboplatin alone), respectively. Hematologic abnormalities observed were those generally expected with paclitaxel/carboplatin alone. Most common (> 5% in any arm) treatment related grade 3/4 adverse events (concurrent vs phased vs Paclitaxel/carboplatin alone) were: fatigue (10% vs 7% vs 6%) and diarrhea (10% vs 7% vs 4%) among nonsquamous patients; nausea (0% vs 5% vs 7%), vomiting (0% vs 5% vs 7%) and dyspnea (0% vs 0% vs 7%) among squamous patients. (31)Lynch et al, abstr 701, 14th World Conf on Lung Cancer, July, 2011; (32)Grossi et al, abstr M004, 14th World Conf on Lung Cancer, July, 2011).

## **11.4. Selection and Timing of Doses**

Patients in this study will receive chemotherapy on a 21-day cycle for paclitaxel, plus cisplatin or carboplatin. Chemotherapy alone will be given during cycle 1 of neoadjuvant therapy and ipilimumab will be given with neoadjuvant chemotherapy during cycles 2 and 3. A cycle of ipilimumab, paclitaxel,

and cisplatin and or carboplatin comprises one treatment of each agent on day 1 at least 21 days between day 1 dosing. A delay of cycle as a result of holidays, weekends, bad weather, or other unforeseen circumstances will be permitted and not counted as a protocol violation. The actual dose of ipilimumab will be determined by the patients' weight in kilograms. The actual dose of paclitaxel , or cisplatin, to be administered will be determined by calculating the body surface area at the beginning of each cycle, on day 1. Carboplatin dose will be determined using the Crockcroft Gault Formula (Dose=AUC (CCC+25) will be used to achieve the desired AUC where CCC=Wt (140-age)/72xCr (using actual body weight for both female and male and if female use 85%). A 1.5% variance in the calculated total dose will be allowed for ease of dose administration (Appendix D). Institutional guidelines will be utilized for capping the carboplatin dose. CrCl cap will be 125ml/min using the **(AUC 6 capped dose = 900mg)**. **Ipilimumab will be administered first in sequence**, followed by paclitaxel, followed by cisplatin or carboplatin. Always give ipilimumab 30-60 minutes before premeds for paclitaxel. Cisplatin or carboplatin will be given following administration of paclitaxel on the day of therapy. A total of 3 cycles of chemotherapy will be given, and ipilimumab will be given during cycles 2 and 3.

Ipilimumab will be administered alone beginning 4-10 weeks after surgery to consist of adjuvant therapy every 3 weeks for 2 cycles followed by maintenance therapy every 12 weeks for two cycles. Patients receiving post-operative radiation for standard clinical criteria such as N2 metastatic disease or surgical margin sub-optimal, should receive radiation prior to adjuvant ipilimumab or at least 2 weeks after the most recent dose of ipilimumab.

#### **11.4.1. Special Treatment Considerations: Dose Adjustments or Delays for Chemotherapy Subsequent Cycles. (See section 11.5-Ipilimumab Dose Modifications)**

Ipilimumab dosing will be concurrent with paclitaxel/carboplatin/cisplatin dosing. Therefore, **if paclitaxel/platin is delayed due to AEs, ipilimumab will be delayed.**

If ipilimumab is delayed due to AEs, paclitaxel/platin will be delayed up to two weeks. If ipilimumab is delayed more than two weeks due to ipilimumab related AE, then ipilimumab will be discontinued from cycle 3 of chemotherapy (and will not be made up) and chemotherapy should be given as otherwise appropriate. If ipilimumab is held from cycle three of neoadjuvant chemotherapy, ipilimumab may be given adjuvantly as per protocol, if ipilimumab AE has resolved and all other criteria for giving ipilimumab as per protocol are met.

Any patient who requires a dose reduction of paclitaxel/platin will continue to receive a reduced dose for the remainder of the study. No dose escalations are permitted in this study.

Paclitaxel/platin treatment may be delayed for up to 21 days. Patients on a 21-day cycle regimen may have treatment delayed for up to 42 days from Day 1 of the current cycle to allow sufficient time to recover from study therapy-related toxicity. A patient who cannot be administered neoadjuvant study therapy within 42 days of scheduled day 1 of a cycle will stop neoadjuvant study therapy and proceed to surgery as deemed appropriate by the surgical team so there is no excessive delay in surgery. If there was no grade  $\geq 3$  toxicity from ipilimumab, the patient may still receive adjuvant ipilimumab.

Chemotherapy dose modifications are allowed as per institutional standard practice for treatment-related toxicity. Listed below are guidelines for chemotherapy modifications.

#### 11.4.1.1. Hematologic Toxicity Dose Adjustments

ANC/AGC must be  $\geq 1500/\mu\text{L}$  and platelets  $\geq 100,000/\mu\text{L}$  prior to the 1<sup>st</sup> cycle. Dose adjustments at the start of a subsequent cycle of therapy will be **based on platelet and neutrophil counts on the day of scheduled treatment**. Treatment should be delayed to allow sufficient time for recovery. Upon recovery, if treatment is resumed, it should be according to the guidelines in Table 10 and Table 11.

**Table 10 Chemotherapy dose Adjustments Based on Day 1 Hematologic Values**

ANC/AGC ( $\mu\text{L}$ )		Platelets ( $\mu\text{L}$ )	Percent of Previous Dose
$\geq 1500$	AND	$\geq 100,000$	100%
$< 1500$	OR	$< 100,000$	Repeat counts weekly, when ANC/AGC $\geq 1500$ and platelets $\geq 100,000$ , retreat at 100% previous dose. Remove from the study if delay $> 21$ days.
Febrile Neutropenia as defined by CTC			Delay treatment as appropriate, once fully recovered ( $< 21$ days). Retreat at 75% of previous dose.
ANY	AND	$< 10,000$	Delay until recovered, as above, then retreat at 75% of previous dose. Remove from study if delay $> 21$ days.

For hemoglobin  $< 8\text{ g/dL}$ , transfusion should be considered without interruption of chemotherapy, erythropoietin may be used.

Growth factor support (G-CSF or pegylated G-CSF) may be used at the discretion of the treating physician.

#### **11.4.1.2. Non-Hematologic Toxicity Dose Adjustments**

For non-hematologic toxicities,  $\geq$  Grade 3 (CTCAE), treatment must be delayed until resolution to  $\leq$  CTCAE grade 1 (or patient's original baseline grade) before proceeding. Treatment should resume at 75% of the previous dose level of all chemotherapy agents if deemed appropriate by the treating physician.

#### **11.4.1.3 Renal Toxicity: Cisplatin and Paclitaxel**

##### **Renal Toxicity Cisplatin**

Cisplatin therapy should be delayed if the creatinine is  $>2.0$  mg/dL. If treatment is delayed for renal toxicity, re-evaluate the serum creatinine weekly. If the serum creatinine is  $\leq 2.0$  mg/dL proceed with cisplatin at 75% of previous dose. If serum creatinine is not  $\leq 2.0$  mg/dL after 14 days, start next cycle without cisplatin. Missed cisplatin doses will not be made up. If creatinine is still not  $\leq 2.0$  mg/dL after a total of 42 days, the patient must be discontinued from study therapy unless continuation is approved by the principal investigator. If creatinine is  $\geq 2.0$  mg/dL but creatinine clearance is  $> 45$  mL/min, may switch from cisplatin to carboplatin and continue treatment if all other criteria for therapy are met.

##### **Renal Toxicity Paclitaxel**

No dose adjustments needed for renal toxicity.

#### **11.4.1.4 Neurologic Toxicity: Paclitaxel, Cisplatin or Carboplatin**

Dose of cisplatin, carboplatin and paclitaxel should be reduced for neurologic toxicity (Table 11).

**Table 11**

<b>Grade of Toxicity</b>	<b>Paclitaxel, Cisplatin, or Carboplatin Doses</b>
0	100%
1	100%
2	Hold treatment until patient recovers to grade 1, then resume treatment at 75% dose.*
$\geq 3$	Hold treatment until patient recovers to grade 1, then resume treatment at 50% dose.*

\*Dose reductions for neurotoxicity are permanent.

#### 11.4.1.5 Auditory Toxicity (Cisplatin)

Cisplatin is well known to cause high-frequency hearing loss. Continued use of the drug does not always result in hearing loss, although it may. If grade 2 or worse hearing loss is noted, the patient should be presented with a discussion of the relative risks of hearing loss versus the potential benefit of continuing cisplatin therapy, and a decision made as to continuation of agent. Grade 3 or 4 hearing loss will warrant discontinuation of agent.

#### 11.4.1.6 Hepatic Toxicity (Paclitaxel)

**Table 12:** Treatment day values should be used in determining dose.

SGOT/AST		Alkaline Phosphatase		Bilirubin	Paclitaxel Dose
≤1.5x ULN	and	≤1.5x ULN	and	WNL	100%
>1.5-5x ULN	and	>1.5-5x ULN	and	WNL	50%
>5x ULN	and	>5x ULN	Or	>NL	Hold*

Repeat LFTs weekly. If recovered, reduce dose by 25%. If not recovered within 3 weeks, discontinue protocol therapy. If paclitaxel is withheld due to hepatic toxicity on day 1, cisplatin or carboplatin should also be withheld and administered when paclitaxel is resumed. **There are no dose reductions of cisplatin or carboplatin for hepatic toxicity unless ≥ grade 3.**

#### 11.4.1.7 Hypersensitivity Reactions to Paclitaxel

For grade 3 reactions, in the subsequent cycle, use double dose steroids pretreatment and decrease the paclitaxel infusion rate for the first 1/3 of the infusion. For the last 2/3 of the infusion, double the rate of the infusion. For documented Grade 4 hypersensitivity reactions to paclitaxel, discontinue paclitaxel.

#### 11.4.1.8 Other Toxicities from Paclitaxel

If a patient develops chest pain, hypotension, or arrhythmia other than asymptomatic sinus bradycardia, the paclitaxel infusion should be stopped and patients should not receive further paclitaxel. For asymptomatic sinus bradycardia, the infusion need not be stopped, but the patient should be followed carefully.

## 11.5 Ipilimumab Dose Modifications

### Ipilimumab Dose Skipping Rule

Decisions to skip an ipilimumab dose must be made on specified safety criteria. Treatment with ipilimumab will be skipped or discontinued if the subject experiences at least one adverse event, specified below, considered by the investigator to be “possibly,” “probably,” or “certainly” related to ipilimumab treatment. The investigator should contact BMS for any adverse event that will prompt a skipped dose or discontinuation of ipilimumab. If the adverse event is associated with ipilimumab during preoperative chemotherapy cycle 2 delay up to 2 weeks, then give cycle 3 chemotherapy alone and proceed to surgery as clinically indicated. In the postoperative adjuvant or maintenance ipilimumab therapy, delay ipilimumab up to 4 weeks for ipilimumab related toxicity and if not recovered to ≤ grade 1, discontinue ipilimumab.

The following criteria will be used to determine dose skipping, restarting doses, or discontinuing ipilimumab.

#### **It may be necessary to skip study drug dosing for the following related adverse event(s):**

- Any ≥ Grade 2 non-skin related adverse event (including immune-mediated adverse reactions), except for laboratory abnormalities
- 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 is necessary to skip study drug dosing for the following adverse events:**

- Any ≥ Grade 3 skin related adverse event regardless of causality.

**Restart ipilimumab dosing if/when the adverse event(s) resolve(s) to  $\leq$  Grade 1 severity or returns to baseline:**

- If the adverse event is associated with ipilimumab during preoperative chemotherapy cycle 2, delay up to 2 weeks, then give cycle 3 chemotherapy alone, and proceed to surgery as clinically indicated. If ipilimumab adverse event has resolved to  $\leq$  grade 1 after surgery, then post-operative ipilimumab may be started.
- For postoperative adjuvant or maintenance ipilimumab therapy, delay ipilimumab up to 4 weeks for ipilimumab related toxicity and if not recovered to  $\leq$  grade 1, discontinue ipilimumab.

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

Blocking CTLA-4 function may permit the emergence of auto-reactive T cells and resultant clinical autoimmunity. Rash/vitiligo, diarrhea/colitis, uveitis/episcleritis, hepatitis, and hypopituitarism were drug-related, presumptive autoimmune events, now termed immune-mediated adverse reactions, noted in previous ipilimumab studies.

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 immune-related adverse reaction. 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-mediated 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  $\geq$  Grade 3 diarrhea requires corticosteroid treatment.

**Specific treatment algorithms for immune-mediated adverse reactions adverse events are included see Appendix K.**

### **11.5.2 Treatment of Infusion Reactions Associated with Ipilimumab**

Since ipilimumab contains only human protein sequences, it is less likely that any allergic reaction will be seen in patients. However, it is possible that infusion of ipilimumab will induce a cytokine release syndrome that could be evidenced by fever, chills, rigors, rash, pruritus, hypotension, hypertension, bronchospasm, or other symptoms. No prophylactic pre-medication will be given unless indicated by previous experience in an individual patient. Reactions should be treated based upon the following recommendations.

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

- At the discretion of the treating physician, ipilimumab infusion may be resumed at one half the initial infusion rate, then increased incrementally to the initial infusion rate.
- If symptoms develop after resumption of the infusion, the infusion should be discontinued and no additional ipilimumab should be administered that day.
- The next dose of ipilimumab will be administered at its next scheduled time and may be given with pre-medication (diphenhydramine and acetaminophen) and careful monitoring, following the same treatment guidelines outlined above.
- At the discretion of the treating physician additional oral or IV antihistamine may be administered prior to dosing with ipilimumab.
- For severe symptoms (e.g., any reaction such as bronchospasm, generalized urticaria, systolic blood pressure <80 mm Hg, or angioedema):
  - Immediately discontinue infusion of ipilimumab, and disconnect infusion tubing from the subject.
  - Consider **bronchodilators**, epinephrine 1 mg IV or subcutaneously, and/or diphenhydramine 50 mg IV, with solumedrol 100 mg IV, as needed.
  - Patients should be monitored until the investigator is comfortable that the symptoms will not recur.
  - No further ipilimumab will be administered.
- In case of late-occurring hypersensitivity symptoms (e.g., appearance within one week after treatment of a localized or generalized pruritus), symptomatic treatment may be given (e.g., oral antihistamine, or corticosteroids).

### 11.5.3 Treatment of Ipilimumab-Related Isolated Drug Fever

In the event of isolated drug fever, the investigator must use clinical judgment to determine if the fever is related to the ipilimumab or to an infectious etiology. If a patient experiences isolated drug fever, for the next dose, pre-treatment with acetaminophen or non-steroidal anti-inflammatory agent (investigator discretion) should be instituted and a repeated antipyretic dose at 6 and 12 hours after ipilimumab infusion, should be administered. The infusion rate will remain unchanged for future doses. If a patient experiences recurrent isolated drug fever following premedication and post dosing with an appropriate antipyretic, the infusion rate for subsequent dosing should be decreased to 50% of the previous rate. If fever recurs following infusion rate change, the investigator should assess the patient's level of discomfort with the event and use clinical judgment to determine if the patient should receive further ipilimumab.

## 11.5.4 Monitoring and Management of Immune-Mediated Adverse Reactions

### Suggested Evaluation and Treatment for Immune-related Adverse Events:

Early diagnosis and treatment intervention for high-grade irAEs can help prevent the occurrence of complications such as GI perforation. Gastrointestinal (diarrhea) and skin (rash) related toxicities are the most common irAEs reported in studies with ipilimumab. Suggested evaluation procedures for suspected irAEs of the GI tract, liver, skin, eye, pituitary, and adrenal gland are described below. When symptomatic therapy is inadequate or inappropriate, an irAE should be treated with systemic corticosteroids followed by a gradual taper. Infrequently, subjects with irAEs may require courses of corticosteroid or other immunosuppressive agents that exceed 4 weeks in duration. It is recommended that subjects receiving immunosuppressive agents beyond 4 to 6 weeks should also receive antimicrobial prophylaxis to protect against the emergence of opportunistic infections. Such prophylaxis should include protection against *Pneumocystis jiroveci* (formerly *Pcarinii*) and prevalent fungal strains, as well as considerations for any additional pathogens that may be indicated by the medical history (e.g., herpes simplex virus, cytomegalovirus) or the environment (e.g., occupation, recent travel) of the subject. Consultation with infectious disease specialists may be considered. Immune-related (ir)AE management algorithms for general irAEs, and ipilimumab related diarrhea, hepatotoxicity, endocrinopathy, and neuropathy have been developed and are listed below.

#### 11.5.4.1 Gastrointestinal Tract: Immune-mediated Enterocolitis

Monitor patients for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, mucus or blood in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus). In symptomatic patients, rule out infectious etiologies and consider endoscopic evaluation for persistent or severe symptoms.

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 resulted in recurrence or worsening symptoms of enterocolitis in some patients.

Withhold ipilimumab dosing for moderate enterocolitis; administer anti-diarrheal treatment and, if persistent for more than one week, initiate systemic corticosteroids at a dose of 0.5 mg/kg/day prednisone or equivalent.

The differential diagnosis for subjects presenting with abdominal pain should include colitis, perforation, or pancreatitis. Additionally, a few subjects with abdominal pain also had acute swelling of the cecal wall on CT scan that may have represented localized inflammation.

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

The majority of subjects with ipilimumab-induced diarrhea or colitis responded to symptomatic therapy or corticosteroids. Permanent discontinuation of ipilimumab and starting high dose corticosteroid therapy (e.g., methylprednisolone 2 mg/kg once or twice per day or equivalent) is strongly recommended for ipilimumab related  $\geq$  Grade 3 diarrhea/colitis and steroids should be slowly tapered according to symptomatic response over at least 1 month. Subjects with ipilimumab-related Grade 2 diarrhea/colitis have to skip ipilimumab dosing and may be initially treated conservatively with loperamide, fluid replacement or budesonide, but should be immediately switched to corticosteroids (prednisone 1 mg/kg or equivalent) if symptoms persist for 3-5 days or worsen. Most subjects with diarrhea/colitis will rapidly respond to initiation of corticosteroids. The dose should be gradually tapered over at least a 1-month duration. Lower doses of prednisone may be considered for less severe cases of colitis. It is suggested that prednisone (for oral administration) or methylprednisolone (for IV administration) be the corticosteroids of choice in the treatment of colitis. Caution should be taken in the use of narcotics in patients with abdominal pain or colitis/diarrhea as narcotic use may mask the signs of colonic perforation. Infrequently, subjects will appear refractory to corticosteroids or will flare following taper of corticosteroids. In these subjects, unless contraindicated (i.e., sepsis, perforation and other serious infections), a single dose of infliximab at 5 mg/kg may provide benefit. For patients with concomitant hepatitis, use of mycophenolate mofetil is recommended in place of infliximab. For patients with long term immunosuppressive therapy, administer antimicrobial prophylactics as appropriate per institutional guidelines. Such cases should be discussed with the sponsor study medical

monitor. If the event is prolonged or severe or is associated with signs of systemic inflammation or acute phase reactants (e.g., increased CRP or platelet count; or bandemia), it is recommended that sigmoidoscopy (or colonoscopy, if appropriate) with colonic biopsy of 3 to 5 specimens for standard paraffin block be performed. If possible, 1 to 2 biopsy specimens should be snap-frozen and stored. Tests should also be performed for stool calprotectin and WBCs.

#### **11.5.4.2 Liver: Immune-mediated Hepatitis**

Monitor liver function tests (hepatic transaminase and bilirubin levels) and assess patients for signs and symptoms of hepatotoxicity 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.

Permanently discontinue ipilimumab in patients with Grade 3–5 hepatotoxicity 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. Withhold ipilimumab in patients with Grade 2 hepatotoxicity.

Liver function tests should always be performed and reviewed prior to administration of all ipilimumab doses. Patients treated with ipilimumab may develop elevation in LFT in the absence of clinical symptoms. In addition, subjects presenting with right upper quadrant abdominal pain, unexplained nausea, or vomiting should have LFTs performed immediately and reviewed before administering the next dose of study drug. Any increase in LFT should be evaluated to rule out non-inflammatory causes of hepatotoxicity including infections, disease progression or medications and followed with frequent LFT monitoring at 3-day intervals until resolution.

Any LFTs  $\geq$  Grade 2 (for subjects with normal baseline LFT) or LFT  $\geq$  2 times baseline values (for subjects with baseline LFT of Grade 1 or 2) should prompt treating physicians to: (1) contact the PI; (2) increase frequency of monitoring LFTs to at least every 3 days until LFT have stabilized or improved; (3) investigate to rule out non-irAE etiologies; and (4) initiate an autoimmunity evaluation.

Disease progression, other malignancies, concurrent medications, viral hepatitis, and toxic etiologies should be considered and addressed, as appropriate. Imaging of the liver, gall bladder, and bile ducts

should be considered to rule out neoplastic or other non-irAE-related causes for the increased LFTs. An ANA, perinuclear anti-neutrophil cytoplasmic antibody (pANCA), and anti-smooth muscle antibody test should be performed if an autoimmune etiology is considered. If the LFTs are  $> 5$  times the upper limit of normal (ULN) or total bilirubin is  $> 3$  times the ULN, then ipilimumab dosing should be held according to the dose modification guidelines.

Subjects with LFT elevations  $> 8x$  the ULN that are judged to be due to ipilimumab should initiate high-dose corticosteroid therapy (e.g., methylprednisolone 2 mg/kg once or twice daily or equivalent) and permanently stop administration of ipilimumab. In subjects with  $> 8x$  ULN, LFT should be performed daily until stable or declining for 5 consecutive days. LFT should be monitored for at least 2 consecutive weeks to ensure sustained treatment response. If symptoms or LFT elevations are controlled, the corticosteroid dose should be gradually tapered over a period of at least 1 month. Flare in LFTs during this taper may be treated with an increase in the dose of steroid and a slower taper. In subjects without response to corticosteroid therapy within 3 to 5 days or who have an LFT flare during steroid tapering that is not responsive to an increase in steroids, addition of immunosuppression with mycophenolate mofetil should be considered after a gastroenterology/hepatology consult. Patients receiving immunosuppression for more than 4 weeks should be evaluated for prophylaxis of opportunistic infections per institutional guidelines. (**Appendix H**).

#### **11.5.4.3 Skin: Immune-mediated Dermatitis**

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 localized rash and pruritus, treat symptomatically. Administer topical or systemic corticosteroids if there is no improvement of symptoms within 1 week.

A dermatologist should evaluate persistent or severe rash or pruritus. A biopsy should be performed if appropriate and if possible, photos of the rash should also be obtained.

Patients with low-grade ipilimumab-mediated skin toxicity (Grade 1 or 2) may remain on therapy and could be treated with symptomatic therapy (e.g., antihistamines). Low-grade symptoms persisting for 1 to 2 weeks and relapsing should be treated with topical or oral corticosteroid therapy (e.g., prednisone 1 mg/kg once daily or equivalent). High-grade (Grade 3 or 4) symptoms require high-dose IV corticosteroid therapy (e.g., methylprednisolone 2 mg/kg once or twice per day or equivalent) to control initial symptoms. A skin biopsy should be performed if appropriate. Once rash or pruritis is controlled, the initiation of corticosteroid taper should be based on clinical judgment; however, the corticosteroid dose should be gradually tapered over a period of at least 1 month. Patients with any high-grade skin related toxicity (Grade 3 regardless of causality) have to skip ipilimumab and may only continue treatment with ipilimumab if the initial symptoms have improved to  $\leq$  Grade 1, while patients with grade 4 skin toxicities have to permanently discontinue ipilimumab. (Appendix K)

#### **11.5.4.4 Neuropathy: Immune-mediated Neuropathies**

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 severe neuropathy. Consider initiation of systemic corticosteroids at a dose of 1 to 2 mg/kg/day prednisone or equivalent for severe neuropathies. Withhold ipilimumab dosing in patients with moderate neuropathy (not interfering with daily activities).

Subjects presenting with sensory symptoms lasting more than 5 days or motor symptoms confirmed by physical examinations should be evaluated and non-inflammatory causes such as disease progression, infections (including Lyme disease), metabolic syndromes and medications (such as taxanes and/or platinum salts) should be ruled out. A neurology consult should be obtained and diagnostic

characterization of the neurological syndrome (electromyogram, nerve conduction studies) should be started. The next dose of ipilimumab should be skipped if the event of a Grade 2 neuropathy (sensitive or motor) is related to study drug. The administration of ipilimumab should be permanently discontinued in patients with Grade 3 or 4 sensory neuropathy suspected to be related to study drug. Patients should be treated according to institutional guidelines and the administration of IV steroids (e.g., methylprednisolone 2 mg/kg once or twice per day or equivalent) should be considered.

The administration of ipilimumab should be permanently discontinued in patients with Grade 3 or 4 motor neuropathy regardless of the causality to study drug. Patients with Grade 3 or 4 motor neuropathy who are clinically stable should be treated according to institution guidelines and the administration of IV steroids therapy (e.g., methylprednisolone 2 mg/kg once or twice per day or equivalent) should be considered.

Patients with Grade 3 or 4 motor neuropathy who are not clinically stable or who have atypical symptoms should be hospitalized and the IV administration of steroids should be initiated and IV immunoglobulin (IVIg) or other immunosuppressive therapies (as appropriate) should be considered.

**(Refer to Appendix J)**

#### **11.5.4.5 Endocrine: Immune-mediated Endocrinopathies**

Monitor patient for clinical signs and symptoms of hypophysitis, adrenal insufficiency (including adrenal crisis), and hyper- or hypothyroidism. Patients may present with fatigue, headache, mental status changes, abdominal pain, unusual bowel habits, and hypotension, or nonspecific symptoms that 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.

Monitor thyroid function tests and clinical chemistries at the start of treatment, before each dose, and as clinically indicated based on symptoms. 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.

Most subjects with hypopituitarism presented with nonspecific complaints such as fatigue, visual field defects, confusion, or impotence. Some have had headache as the predominant presentation. The majority of subjects with hypopituitarism demonstrated enlarged pituitary glands based on brain MRI. Low adrenocorticotropic hormone (ACTH) and cortisol were the most common biochemical abnormality; low TSH, free T4, T3, testosterone or prolactin have also been reported in some subjects. Subjects with unexplained symptoms such as fatigue, myalgias, impotence, mental status changes, or constipation should be investigated for the presence of thyroid, pituitary or adrenal endocrinopathies. An endocrinologist should be consulted if an endocrinopathy is suspected. If there are any signs of adrenal crisis such as severe dehydration, hypotension, or shock, IV corticosteroids with mineralocorticoid activity (e.g., methylprednisolone) should be initiated immediately. If the patient's symptoms are suggestive of an endocrinopathy but the patient is not in adrenal crisis, an endocrine laboratory results should be evaluated before corticosteroid therapy is initiated.

Endocrine work-up should include at least thyroid stimulating hormone (TSH) and free T4 levels. These should be obtained to determine if thyroid abnormalities are present. TSH, prolactin and a morning cortisol level will help to differentiate primary adrenal insufficiency from primary pituitary insufficiency.

Radiographic imaging (e.g., MRI) with pituitary cuts should be performed. If the pituitary scan and/or endocrine laboratory tests are abnormal, a short course of high dose corticosteroids (e.g., dexamethasone 4 mg every 6 hours or equivalent) should be considered in an attempt to treat the presumed pituitary inflammation, but it is currently unknown if this will reverse the pituitary dysfunction. Abrupt discontinuation of corticosteroids should be avoided due to possible prolonged adrenal suppression. Once symptoms or laboratory abnormalities are controlled, and overall patient improvement is evident, the initiation of steroid taper should be based on clinical judgment; however the corticosteroid dose should be gradually tapered over a period of at least 1 month. Appropriate hormone replacement therapy should be instituted if an endocrinopathy is documented, and it is possible that subjects may require life-long hormone replacement. (**Refer to Appendix I**)

#### **11.5.4.6 Other: Immune-mediated Adverse Reactions, Including Ocular Manifestations**

The following clinically significant immune-mediated adverse reactions were seen in less than 1% of ipilimumab-treated patients in Study 1: nephritis, pneumonitis, meningitis, pericarditis, uveitis, iritis, 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.

Ocular inflammation (episcleritis or uveitis) was reported in a few subjects. These conditions responded to topical corticosteroid therapy. An ophthalmologist should evaluate visual complaints with examination of the conjunctiva, anterior and posterior chambers, and retina. Visual field testing and an electroretinogram should also be performed. Examination and testing should be documented on the appropriate case report form (CRF). Ipilimumab-related uveitis or episcleritis may be treated with topical corticosteroid eye drops.

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

#### **11.5.4.7 Liver Function Test (LFT) Assessments Required Before Administration of Ipilimumab**

Liver function tests (AST, ALT, T. bilirubin) will be evaluated for every subject prior to administration of ipilimumab. Blood samples must be collected and analyzed at local or central labs within 3 days prior to dosing. LFT results must be reviewed by the principal investigator (or designee) to meet dosing criteria specifications:  $\leq 2.5 \times \text{ULN}$  for AST, ALT and  $\leq 1.5 \times \text{ULN}$  for T. bilirubin unless liver metastases are present in which case LFT  $\leq 5 \times \text{ULN}$  for AST, ALT and T. bilirubin  $\leq 3.0 \times \text{ULN}$  prior to dosing.

If, during the course of treatment abnormal LFT values are detected, the subject must be managed using the hepatotoxicity algorithm **Appendix H**.

### **11.6 Permanent Discontinuation of Ipilimumab**

#### **11.6.1 Permanent Discontinuation for Related Adverse Events**

**Permanently discontinue ipilimumab for any of the following:**

- Persistent moderate adverse reactions or inability to reduce corticosteroid dose to 7.5 mg prednisone or equivalent per day.
- Failure to complete full treatment course within 18 weeks from administration of first dose.
- 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 intravenous 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 (eg, 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 judgement of the investigator presents a substantial clinical risk to the patient with continued dosing.
- Clinical deterioration of subject's condition such that further benefit from ipilimumab dosing is unlikely or requires a change of therapy.

## 11.6.2 Exceptions to 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  $\leq$  Grade 2 adverse events where the primary reason for hospitalization is to expedite the clinical work-up.
- Patients with the following conditions where in the investigator's opinion continuing study drug administration is justified:
  - Ocular toxicity that has responded to topical therapy.
  - 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.

## 11.7 Dose Modifications for Obese Patients

There is no clearly documented adverse impact of treatment of obese patients when dosing is performed according to actual body weight. Therefore, all dosing is to be determined solely by (1) the patient's BSA as calculated from actual weight or (2) actual weight without any modifications unless explicitly described in the protocol. This will eliminate the risk of calculation error and the possible introduction of variability in dose administration. Physicians who are uncomfortable with administering chemotherapy dose(s) based on actual body weight should not enroll obese patients.

## 11.8. Blinding

This is an open-label study; therefore, each patient will be aware of his or her own assigned treatment. All staff involved in treating and caring for study patients will have full knowledge of treatment assignments for those patients under their care.

## 11.9. Concomitant Therapy

Patients are allowed to receive full supportive care therapies concomitantly during the study. All standard pretreatment and supportive care medications for chemotherapy may be used including corticosteroids used for that purpose. Any disease progression requiring other forms of specific anti-

tumor therapy will be cause for early discontinuation of study therapy. The following concomitant therapies warrant special attention:

### **11.9.1 Colony Stimulating Factors (CSFs)**

The American Society of Clinical Oncology guidelines for use of CSF should be followed (<http://www.asco.org/guidelines/wbclf>) [64]. Granulocyte colony-stimulating factor (G-CSF) may be used for patients who have ANC  $<0.5 \times 10^9/L$ , neutropenic fever, or documented infections while neutropenic. Duration of uncomplicated neutropenia before initiation of G-CSF treatment is left to the investigator's discretion. Granulocyte colony-stimulating factor must be discontinued at least 24 hours prior to the start of the next cycle of chemotherapy. If a patient develops hematologic toxicities, chemotherapy dose reduction and acute treatment of neutropenia are recommended, rather than chemotherapy dose maintenance and pre-emptive treatment with G-CSFs. Use of erythropoietin (e.g., Aranesp, Procrit) is allowed. Use of stimulators of thrombopoiesis is not allowed.

### **11.9.2 Antiemetic Agents**

For patients in both study arms, the use of a prophylactic anti-emetic regimen including a 5-HT3 antagonist is suggested. Aprepitant may be used prior to cisplatin. Anti-emetic therapy should be administered according to standard local practice for patients receiving mildly to moderately emetic chemotherapy regimens and is at the discretion of the treating physician.

### **11.9.3 Therapy for Diarrhea**

In the event of CTC Grade 3 or 4 diarrhea, it will be treated like ipilimumab immune related diarrhea (see section 11.5.4 Enterocolitis. **Refer to Appendix L**). The following supportive measures are allowed: corticosteroids, hydration, octreotide, and antidiarrheals.

If diarrhea is severe (requiring intravenous rehydration) **and/or** associated with fever or severe neutropenia (Grade 3 or 4), broad-spectrum antibiotics should be prescribed. Patients with severe

diarrhea or any diarrhea associated with severe nausea or vomiting **should be hospitalized** for intravenous hydration, correction of electrolyte imbalances, and other treatment as necessary.

#### **11.9.4 Therapy for Patients with Febrile Neutropenia**

Patients experiencing chemotherapy related febrile neutropenia should be managed in a hospital setting according to standard procedures, with the urgent initiation of intravenous antibiotic therapy. Refer to section 11.9.1 for use of CSFs.

### **12.0 Prohibited Therapies During the Study**

#### **Prohibited Therapies**

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

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

- Any non-study anti-cancer agent (investigational or non-investigational) this includes chemotherapy, immunotherapy, hormonal cancer therapy, biologics/targeted therapies, radiation therapy, surgery for cancer, or experimental medications patients. Any disease progression requiring other forms of specific anti-tumor therapy will be cause for early discontinuation of study therapy
- Any other investigational agents
- Any other CTLA-4 inhibitors or agonists.
- Any anti-PD1 inhibitor or agonist.
- CD137 agonists
- Immunosuppressive agents [except those used to treat ipilimumab related immune related adverse events (irAEs) and standard antiemetics & premedication for paclitaxel or platin].
- Chronic systemic corticosteroids unless as physiologic dose for adrenal function replacement.
- Any non-oncology vaccine therapies used for the prevention of infectious diseases (for up to 30 days prior to or after any dose of study drug).

## 13.0 Treatment Compliance

Patient compliance with study medication will be assessed at each visit. Deviation(s) from the prescribed dosage regimen should be recorded in the comments section of the CRF.

## 14.0 Discontinuation

### 14.1. Discontinuation of Study Patients

If a patient who does not meet enrollment criteria is inadvertently enrolled, that patient should be discontinued from the study treatment and the principal investigator must be contacted. In addition, patients will be discontinued from study treatment in the following circumstances:

- There is clear evidence of progressive disease.
- In previous studies with ipilimumab in solid tumors there has been transient increase in lesion size due to inflammatory immune response followed by clear evidence of lesion regression. Therefore, CAT scan evidence of progression in the global tumor burden will not be criteria for discontinuation or by itself deemed evidence of progression on therapy. It is possible index tumor may enlarge > 25% or new lesions appear after 2 doses Ipilimumab before repeat CAT scan. Surgical staging at time of resection will verify progression or whether robust inflammatory response was mis-interpreted as progression on repeat CAT scans.
- There is toxicity deemed by the investigator or patient as unacceptable.
- The patient is noncompliant with study procedures.
- The investigator decides that the patient should be withdrawn.
- Pregnancy: All 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 no study pregnancy tests for WOCBP enrolled in the study. **The investigator must immediately notify BMS in the event of a confirmed pregnancy in a patient participating in the study.** The patient or attending physician requests that the patient be withdrawn from the study.
- The investigator, for any reason, stops the study or stops the patient's participation in the study.

- Termination of the study by Bristol-Myers Squibb (BMS)
- The patient, for any reason, requires treatment with another systemic agent potentially effective for treatment of the study indication. In this case, discontinuation from the study occurs immediately upon introduction of the new agent.
- A patient who cannot be administered the study drug after a 21-day delay must be discontinued from the study treatment.
- Bulky residual adenopathy such that resection is not attempted will be removed from protocol therapy and should receive standard oncology care as deemed appropriate by the treating physician.
- Subjects with positive surgical margins or N2 disease post-surgery.
- Withdrawal of informed consent (subject's decision to withdraw for any reason).
- Any clinical adverse event, laboratory abnormality or intercurrent illness which, in the opinion of the investigator, indicates that continued treatment with study therapy is not in the best interest of the subject
- The compulsory detention for the treatment of either a psychiatric or physical (e.g. infections disease) illness.

For patients who discontinue the study treatment early, every effort must be made to complete the post treatment summary visit during which time the end-of-therapy procedures described in the Study Schedule (Appendix A) should be performed. The post treatment summary visit (End of Study=EOS) will commence approximately 30 days from the last dose of study drug.

## 14.2. Discontinuation of Study

This study can be terminated at any time for any reason by the PI-sponsor or the IRB. If this occurs, all subjects on study should be notified as soon as possible. Additional procedures and/or follow up should occur in accordance with Section 9.0 which describes procedures and process for prematurely withdrawn patients.

## **15.0 Ipilimumab Drug Supply**

Bristol-Myers Squibb (BMS) will provide ipilimumab at no cost for this study.

### **15.1. Identification**

Ipilimumab is available in concentrations of 5 mg/mL (50 mg/10 mL and 200 mg/40 mL). The sterile solution in the vial is clear and colorless. Ipilimumab is administered via intravenous infusion only.

### **15.2 Packaging and Labeling**

BMS will provide ipilimumab at no cost for this study. Ipilimumab will be provided in open-label containers. The labels will contain the protocol prefix, batch number, content, storage conditions, and dispensing instructions along with the Investigational New Drug (IND) caution statement.

### **15.3 Storage, Handling, and Dispensing**

#### **15.3.1 Storage**

Ipilimumab must be stored in a secure area according to local regulations. The investigator must ensure that it is stored at a temperature  $\geq 2^{\circ}\text{C}$  and  $\leq 8^{\circ}\text{C}$ .

#### **15.3.2 Handling and Disposal**

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

### 15.3.3 Dispensing

It is the responsibility of the investigator to ensure that ipilimumab is only dispensed to study subjects. The ipilimumab must be dispensed only from official study sites by authorized personnel according to local regulations.

## 15.4 Drug Ordering and Accountability

### 15.4.1 Initial Orders

Following submission and approval of the required regulatory documents, a supply of ipilimumab may be ordered from BMS. Investigators must complete a Drug Request Form and email it to: [distribution.allentown@thermofisher.com](mailto:distribution.allentown@thermofisher.com).

If for any reason the e-mail drug request form is not successfully transmitted, contact Emily Kahora at Fisher Clinical Services:

Phone: +1 484.538.2121

Fax: +1 610.871.9382

Email: [emily.kahora@thermofisher.com](mailto:emily.kahora@thermofisher.com) or [www.fisherclinicalservices.com](http://www.fisherclinicalservices.com).

It is recommended you send a test message to the Fisher Clinical Service e-mail address upon receipt of the Drug Request Form. Please include in the subject line: "BMS IST Drug Order -- Test." This will ensure your site is recognized by Fisher and your future orders will be received without incident.

Ipilimumab vials (40 mL) are shipped in quantities of five. The initial order should be limited to 25 vials (5 cartons of 5 vials each). Allow 5 business days for shipment of drug from BMS receipt of the Drug Request Form. Drug is protocol specific, but not patient specific. All product will be shipped via Federal Express in a temperature-controlled container. Shipments will be made from Fisher Clinical Services on Monday through Thursday for delivery onsite Tuesday through Friday. There will be no weekend or holiday delivery of drugs.

It is possible that sites may have more than one ipilimumab clinical study ongoing at the same time. **It is imperative that only product designated for this protocol number be used for this study.** To help segregate product for this study from other investigational or marketed product, stickers bearing the BMS protocol number will be provided and should be affixed to the front of the outer carton just above the company names so as not to obscure any marking.

#### **15.4.2 Re-Supply**

Reorders should be emailed directly to Fisher Clinical Services (distribution.allentown@thermofisher.com) for shipment within 5 business days. When assessing need for resupply, institutions should keep in mind the number of vials used per treatment dose, and that shipments may take 5 business days from BMS receipt of request. Drug is not patient specific. Be sure to check with your pharmacy regarding existing investigational stock to assure optimal use of drug on hand.

#### **15.4.3 Ipilimumab Accountability**

It is the responsibility of the investigator to ensure that a current record of ipilimumab disposition is maintained at each study site where ipilimumab is inventoried and disposed. Records or logs must comply with applicable regulations and guidelines, and should include:

- Amount received and placed in storage area.
- Amount currently in storage area.
- Label ID number or batch number and use date or expiry date.
- Dates and initials of person responsible for each ipilimumab inventory entry/movement.
- Amount dispensed to and returned by each subject, including unique subject identifiers.
- Amount transferred to another area/site for dispensing or storage.
- Non-study disposition (e.g., lost, wasted, broken).
- Amount destroyed at study site.
- 

#### **15.4.4 Ipilimumab Destruction**

If ipilimumab is to be destroyed on site, it is the investigator's responsibility to ensure that arrangements have been made for disposal and that procedures for proper disposal have been established according to applicable regulations, guidelines, and institutional procedures. Appropriate records of the disposal must be maintained.

### **15.5 Preparation, and Administration**

Ipilimumab injection must not be administered as an IV push or bolus injection. Care must be taken to assure sterility of the prepared solutions, since the drug product does not contain any antimicrobial preservatives or bacteriostatic agents.

- Do not shake product.

- Inspect parenteral drug products visually for particulate matter and discoloration prior to administration. Discard vial if solution is cloudy, there is pronounced discoloration (solution may have pale yellow color), or there is foreign particulate matter other than translucent-to-white, amorphous particles.

*Preparation of Solution*

- Allow the vials to stand at room temperature for approximately 5 minutes prior to preparation of infusion.
- Withdraw the required volume of ipilimumab and transfer into an intravenous bag.
- Store the prepared solution for no more than 24 hours under refrigeration (2°C to 8°C, 36°F to 46°F) or at room temperature (20°C to 25°C, 68°F to 77°F).
- Discard partially used vials or empty vials of ipilimumab.

*Administration Instructions*

- Do not mix ipilimumab with, or administer as an infusion with, other medicinal products.
- Flush the intravenous line with 0.9% Sodium Chloride Injection, USP or 0.5% Dextrose Injection, USP after each dose.
- **Administer diluted solution over 90 minutes through an intravenous line** containing a sterile, non-pyrogenic, low-protein-binding in-line filter 0.2 micron filter.

See the current Investigator Brochure for additional information on allowable filter types. The infusion must be completed in 90 minutes with a 10 ml normal saline flush at the end.

Ipilimumab should be administered under the supervision of a physician experienced in the use of intravenous (IV) agents. Ipilimumab is administered as an IV infusion only.

## 15.6 Dose Calculations

Each patient will receive ipilimumab:

Calculate **Total Dose** as follows:

Patient body weight in kg x (x) mg = total dose in mg

Calculate **Total Infusion Volume** as follows:

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

Calculate **Rate of Infusion** as follows:

Infusion volume in mL ÷ 90 minutes = rate of infusion in mL/min x 60 = ml/hr.

For example, a patient weighing 114 kg (250 lb) would be administered 1140 mg of ipilimumab (114 kg x 10 mg/kg = 1140 mg) with an infusion volume of 228 mL (1140 mg ÷ 5 mg/mL = 228 mL) at a rate of approximately 2.5 mL/min (228 mL ÷ 90 minutes) x 60 minutes = 150 ml/hr.

The total dose must be calculated using the most recent subject weight (obtained within 3 days of the dosing visit, and prior to the infusion). Rounding to a whole number is acceptable. Dose does not change unless weight changes > 10% from baseline.

## **16. OUTCOME MEASUREMENTS**

### **16.1 Criteria for Response, Progression, and Relapse (Solid Tumors)**

Patients should be reevaluated by Response Evaluation Criteria in Solid Tumors (RECIST 1.1) after completion of neoadjuvant ipilimumab and chemotherapy (planned 3 cycles). Unidimensional measurements in the largest diameter will be used.

#### **16.1.1 Measurable Disease**

Measurable lesions are defined as those that can be accurately measured (select largest, most reproducible lesions) in at least one dimension (largest diameter in the plane of measurement is to be recorded) as  $\geq 20$  mm with x-ray or  $\geq 10$  mm with CT scan (CT scan slice thickness  $\leq 5$  mm) or MRI. If scan has slice thickness  $> 5$  mm, the minimum size for a measurable lesion should be twice the slice thickness. Measurable lesions will also include those that can be directly measured on physical exam with calipers and are at least 10 mm. Lesions which cannot be accurately measured with calipers should be recorded as non-measurable. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

#### **16.1.2 Non-Measurable Disease**

All other lesions (or sites of disease), including small lesions (longest diameter  $< 20$  mm with x-ray or  $< 10$  mm using CT or MRI scan), are considered non-measurable disease. Lesions considered non-measurable include: bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitic involvement of skin or lungs, inflammatory breast disease, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

### 16.1.3 Target Lesions

All measurable lesions up to a maximum of two lesions per organ (total 5 maximum target lesions) are considered target lesions. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD.

The baseline sum LD will be used as reference by which to characterize the objective tumor response.

### 16.1.4 Non-Target Lesions

All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Non-target lesions include measurable lesions that exceed the maximum numbers per organ or total of all involved organs as well as non-measurable lesions. Measurements of these lesions are not required but the presence or absence of each should be noted throughout follow-up.

### 16.1.5 Guidelines for Evaluation of Measurable/Evaluable Disease

If subject has measurable disease, all measurements should be taken and recorded in metric notation. For subjects with evaluable disease only, the sites of disease will be noted at baseline and followed for increase or decrease in size and/or new disease. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 30 days before the beginning of the treatment, unless otherwise specified. 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 when both methods have been used to assess the antitumor effect of a treatment.

- Clinical lesions: Clinical lesions will only be considered measurable when they are superficial and  $\geq 10$  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.
- CT and MRI: These techniques should be performed with cuts of 5 mm or less in slice thickness contiguously. If scan has slice thickness  $>5$  mm, the minimum size for a measurable lesion should be twice the slice thickness. CT is currently the best available and reproducible method to measure lesions for response assessment.
- Laryngoscopy, Endoscopy: Such techniques can be useful to evaluate patients for response in SCCHN and may be used in tumor measurement.

- **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).

## 16.2 Response Criteria

### Evaluation of Target Lesions

- Complete Response (CR): Disappearance of all target lesions
- Partial Response (PR): At least a 30% decrease in the sum of the largest diameter (LD) of target lesions, taking as reference the baseline sum LD
- Progressive Disease (PD): At least a 20% increase and a 5 mm absolute increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions. If scan showing new lesion is of anatomical region which was not included in baseline scans, it is PD.
- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started

### Evaluation of Non-Target Lesions

- Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level
- Incomplete Response/Stable Disease (SD): 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 (not attributable to different scanning technique or non-tumor) of existing non-target lesions. ('Unequivocal progression' must be an overall level of substantial worsening in non-target disease that is of a magnitude that, even in the presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy.) If scan showing new lesion is of anatomical region which was not included in baseline scans, it is PD.

**Table 10: Evaluation of Best Overall Response**

<b>Target Lesions</b>	<b>Non-Target Lesions</b>	<b>New Lesions</b>	<b>Overall Response</b>	
CR	CR	No	CR	<b>16.21</b>
CR	Non-CR/non-PD	No	PR	<b>16.1</b>
CR	Not evaluated	No	PR	
PR	Non-PD	No	PR	
SD	Non-PD	No	SD	
PD	Any	Yes or No	PD	
Any	PD	Yes or No	PD	
Any	Any	Yes	PD	

## 16.21 Immune-Related Tumor Assessment

Ipilimumab is an immuno-stimulating antibody with evidence of anti-cancer activity (durable objective response and stable disease) in subjects with advanced melanoma, including subjects with established brain disease. Observations of clinical activity defined by tumor response endpoints defined by conventional criteria (eg, mWHO) may be inadequate to realize the kinetics and magnitude of clinical benefit achieved with ipilimumab.

Histopathologic evidence has demonstrated ipilimumab can produce an influx or expansion of tumor infiltrating lymphocytes. Therefore, early increases in lesion size detected radiologically or upon gross examination could be misinterpreted as progressive tumor growth and precede objective tumor shrinkage. In addition the appearance of new lesions may have categorized a subject to have progressive disease using conventional tumor assessment criteria despite the concurrent observation of objective tumor responses in pre-existing lesions and a net reduction in global tumor burden that includes the new lesions.

Therefore, patients with evidence of possible target lesion growth or new lesion appearance will not be presumed to have progressed and will proceed to surgery as long as all possible site of disease are deemed resectable by the surgeon.

## 16.3 Cytology and Histology

If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology. These techniques can be used to differentiate between PR and CR in rare cases (for example, 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.

### Pathologic Response Criteria

After receiving preoperative chemotherapy + Ipilimumab, criteria for examining resected tumor will be as follows:

- No Response –no evidence of cell death or tumor necrosis.
- Partial Response – $\geq 30\%$  tumor necrosis or cell death.
- Complete Response –no evidence of viable tumor in surgical specimen (includes lung tissue and dissected lymph nodes)

## 16.4 Disease-Free Survival

Disease-free survival (DFS) is defined as the time from surgical resection to disease recurrence (first disease recurrence or death, whichever comes first) after surgery.

## 16.5 Survival

Survival will be measured from the date of enrollment.

## 17.0 Safety

All patients who receive at least one dose of ipilimumab will be considered evaluable for safety parameters. Additionally, any occurrence of non-SAE or SAE from time of consent forward, up to and including follow-up visits will be reported as per Adverse Event Reporting sections below. Safety will be evaluated for all treated patients using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0 (<http://ctep.cancer.gov>). Safety assessments will be based on medical review of adverse event reports and the results of vital sign measurements, physical examinations, and clinical laboratory tests.

### 17.1 Documentation of Adverse Events

Investigators are responsible for monitoring the safety of patients who have entered this study and for alerting BMS or its designee to any event that seems unusual, even if this event may be considered an unanticipated benefit to the patient. The investigator is responsible for appropriate medical care of patients during the study. The investigator remains responsible for following, through an appropriate health care option, adverse events that are serious or that caused the patient to discontinue before completing the study. The patient should be followed until the event is resolved or explained. Frequency of follow-up evaluation is left to the discretion of the investigator. Safety measures that will be used in the study include physical examinations, and clinical laboratory tests (e.g., hematology, blood chemistries, and CrCl). Patients will be rated for toxicity prior to each cycle using the NCI CTCAE scale version 4.0. Refer to the Study Schedule (Appendix A) for timing of safety measures.

An adverse event (AE) is the development of an unfavorable or unintended sign, symptom, disease or the deterioration of a pre-existing condition that occurs while a patient is enrolled on a clinical trial, **whether the event is considered related or unrelated to the study treatment**. An adverse event is any adverse change from the subject's baseline (pretreatment) condition; including any clinical or lab test abnormality that occurs during the course of research after treatment has started. Adverse events will use the descriptions and grading scales found in the NCI Common Toxicity Criteria for Toxicity and Adverse Event Reporting, Version 4.0 (CTCAE; CTEP home page: <http://ctep.info.nih.gov>).

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

### 17.1.1. Adverse Event Reporting

All adverse clinical experiences, whether revealed by observation, physical examination or other diagnostic procedures by the investigator, or reported by the patient, must be recorded regardless of treatment or suspected causal relationship. Information for the adverse event should include a description with details as to the duration and intensity of each event, the causal relationship to the study drug, the action taken with respect to the study drug, and the patient's outcome. Any adverse event that is expected, not serious or not related to the research study should be reported as part of the routine clinical data. All adverse events should be recorded in the patient's medical record and on the case report form.

If known, the diagnosis of the underlying illness or disorder should be recorded, rather than its individual symptoms. The following information should be captured for all AEs: onset, duration, intensity, seriousness, relationship to investigational product, action taken, and treatment required. If treatment for the AE was administered, it should be recorded in the medical record.

Subjects having adverse events will be monitored with relevant clinical assessments and laboratory tests as determined by the investigator. All adverse events are to be followed to satisfactory resolution or stabilization of the event(s).

Any actions taken and follow-up results must be recorded on the appropriate page of the case report form as well as the subject's source documentation (medical record).

For all adverse events that require the subject to be discontinued from the study, relevant clinical assessments and laboratory tests must be repeated at clinically appropriate intervals until satisfactory resolutions or stabilization of the event(s).

Cases of pregnancy that occur during maternal or paternal exposures to study drugs should be reported for tracking purposes. Additional data on fetal outcome and breastfeeding are collected for regulatory reporting and drug safety evaluation. This data also includes cases of pregnancies that occur during paternal exposures to Bristol Myers Squibb study drug. Lack of drug effect is not an adverse event in clinical trials because the purpose of the clinical trial is to establish drug effect.

If a patient **never** receives study drug, but experiences an adverse event after the informed consent document is signed, **ONLY** events the investigator believes may have been caused by a protocol procedure will be reported.

**All** adverse events occurring after enrollment will be documented and recorded on the CRF and any additional required forms. Adverse events are to be reported for 30 days after the patient's last dose of study drug and for any events beyond that time in which the investigator believes the event is related to study drug or study related procedures.

Events leading to the clinical outcome of **death due to NSCLC** will be included as part of the safety and efficacy analyses for this study, and will **not be reported as adverse events via CRF** unless the investigator believes the event may have been caused by the study drug or drug delivery system.

### **17.1.2. Serious Adverse Events**

Study site personnel must alert BMS and the principal investigator immediately of any “serious” (defined below) adverse event experienced by a patient. In addition, adverse events must be reported to regulatory authorities according to the definitions and timelines specified in the local laws and regulations.

An **unexpected adverse event** is any adverse drug experience where the specificity or severity is not consistent with the current investigator brochure; or, if an investigator brochure is not available, the specificity or severity of which is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

Serious events are those that result in:

- death
- initial or prolonged inpatient hospitalization
- a life-threatening experience (that is, immediate risk of dying)
- persistent or significant disability/incapacity
- congenital anomaly/birth defect is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [e.g., medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)

The following hospitalizations are not considered SAEs:

- a visit to the emergency room or other hospital department lasting less than 24 hours that does not result in admission (unless considered an “important medical event” or a life-threatening event)
- admissions as per protocol for a planned medical/surgical procedure
- routine health assessment requiring admission for baseline/trending of health status (eg, routine colonoscopy)

- medical/surgical admission for purpose other than remedying ill health state that was planned before study entry. Appropriate documentation is required in these cases.
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative).

Hospitalization for elective surgery or routine clinical procedure (such as for study drug administration) that are not the result of an adverse experience (e.g. elective surgery for a pre-existing condition) are not considered SAE's and should be recorded on the appropriate case report form. **Hospitalization and/or death that are unequivocally due to progression of disease should not be reported as an SAE.**

Exception: BMS will be alerted to a study-specific clinical outcome of death due to progressive disease as a serious adverse event only if the investigator deems it to be related to use of the study drug.

Serious adverse event collection begins after the patient has signed informed consent and has received a study drug. If a patient experiences an SAE after signing informed consent, but prior to receiving study drug, the event will normally NOT be collected unless the investigator believes the event may have been caused by a protocol procedure.

Serious adverse events will be collected for 30 days after the last dose of study drug, and serious adverse events occurring 30 days after a patient is discontinued from the study will NOT be reported unless the investigator feels that the event may have been caused by the study drug or a protocol procedure.

### **17.1.3. Serious Adverse Event Reporting**

All serious adverse event (SAE) reports should include the patient study number, age, sex, weight, severity or reaction, relationship to the study drug(s), date of administration of test medications and all concomitant medication and medical treatment provided. This information is to be recorded on **FORM 3500**, which can be located at: [www.fda.gov/medwatch/getforms.htm](http://www.fda.gov/medwatch/getforms.htm).

The SAE report should be comprised of full written summary detailing relevant aspects of the adverse event in question. Where applicable, information from relevant hospital case records and autopsy reports should be obtained. If the investigator does not have all information regarding the event, he/she will not wait to receive additional information, but file the information currently available and update the summary when additional information is received. The investigator is to provide an assessment of causality at the time of the initial report.

Any adverse event that is serious, unexpected, and/or study related which occurs in any patient in the course of their treatment on the research study or within 30 days following cessation of treatment must

be reported within 24 hours of the investigator learning of its occurrence. This can be by phone and should be followed promptly by a detailed, written report. In addition, any serious event that is unexpected (i.e. not in the current package inserts or the Investigator Brochures) and associated with use of the study drug(s), must be reported to the FDA within 15 calendar days, or within 7 calendar days if the SAE is life-threatening or fatal.

Copies of completed Medwatch forms (FORM 3500) should be sent to:

- FDA: Fax # 1-800-FDA-0178
- Principal Investigator: Neal Ready, MD: Fax # 919-684-8926
- All SAEs should be faxed or emailed to BMS at:

**Global Pharmacovigilance & Epidemiology  
Bristol-Myers Squibb Company  
Fax Number: 609-818-3804  
Email: [Worldwide.safety@bms.com](mailto:Worldwide.safety@bms.com)**

Serious adverse events that occur on any Duke subject and/or IND safety reports that are received from BMS will be processed by the DCI Safety Desk. SAEs/IND Safety reports will be reported to the DUHS IRB per institutional reporting guidelines.

#### **17.1.4 Assignment of Adverse Event Intensity and Relationship to Investigational Product**

All adverse events, including those that are serious will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) Version 4.0.

The following categories and definitions of causal relationship to investigational product as determined by a physician should be used:

**Related:** There is a reasonable causal relationship to investigational product administration and the adverse event.

**Not Related:** There is not a reasonable causal relationship to investigational product administration and the adverse event.

The expression “reasonable causal relationship” is meant to convey in general that there are facts (e.g., evidence such as de-challenge/re-challenge) or other arguments to suggest a positive causal relationship.

## 17.1.5 Pregnancy

Sexually active WOCBP must use an effective method of birth control during the course of the study, in a manner such that risk of failure is minimized. Before enrolling WOCBP in this clinical study, the investigator must review the guideline about study participation for WOCBP which can be found in the GCP Manual for Investigators. The topics include the following:

- General Information
- Informed Consent Form
- Pregnancy Prevention Information Sheet
- Drug Interactions with Hormonal Contraceptives
- Contraceptives in Current Use
- Guidelines for the Follow-up of a Reported Pregnancy.

Before study enrollment, WOCBP must be advised of the importance of avoiding pregnancy during study participation and the potential risk factors for an unintentional pregnancy. The subject must sign an informed consent form documenting this discussion.

**All WOCBP MUST have a negative pregnancy test within 72 hours before receiving ipilimumab.**

The minimum sensitivity of the pregnancy test must be 25 IU/L or equivalent units of HCG. If the pregnancy test is positive, the subject must not receive ipilimumab and must not be enrolled in the study.

**In addition, all 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.**

If, following initiation of the investigational product, it is subsequently discovered that a study subject is pregnant or may have been pregnant at the time of investigational product exposure, including during at least 6 half-lives after product administration, the investigational product will be permanently discontinued in an appropriate manner (e.g., dose tapering if necessary for subject safety). The investigator must immediately notify BMS of this event and record the pregnancy on the Pregnancy Surveillance Form (not an SAE form). Initial information on a pregnancy must be reported immediately to BMS, and the outcome information provided once the outcome is known. Completed Pregnancy Surveillance Forms must be forwarded to BMS according to SAE reporting procedures.

Any pregnancy that occurs in a female partner of a male study participant should be reported to the sponsor. Information on this pregnancy will be collected on the Pregnancy Surveillance Form.

Protocol-required procedures for study discontinuation and follow-up must be performed on the subject unless contraindicated by pregnancy (e.g., x-ray studies). Other appropriate pregnancy follow-up procedures should be considered if indicated. In addition, the investigator must report to BMS, and follow-up on information regarding the course of the pregnancy, including perinatal and neonatal outcome. Infants should be followed for a minimum of 8 weeks.

### **17.1.6 Other Safety Considerations**

Any significant worsening noted during interim or final physical examinations, electrocardiograms, x-rays, and any other potential safety assessments, whether or not these procedures are required by the protocol, should also be recorded in the medical record.

### **17.1.7 Safety Oversight Committee (SOC)**

The Duke Cancer Institute SOC is responsible for annual data and safety monitoring of DUHS sponsor-investigator phase I and II, therapeutic interventional studies that do not have an independent Data Safety Monitoring Board (DSMB). The primary focus of the SOC is review of safety data, toxicities and new information that may affect subject safety or efficacy. Annual safety reviews includes but may not be limited to review of safety data, enrollment status, stopping rules if applicable, accrual, toxicities, reference literature, and interim analyses as provided by the sponsor-investigator. The SOC in concert with the DCI Monitoring Team (see Section 12.1 for Monitoring Team description) oversees the conduct of DUHS cancer-related, sponsor-investigator therapeutic intervention and prevention intervention studies that do not have an external monitoring plan, ensuring subject safety and that the protocol is conducted, recorded and reported in accordance with the protocol, standing operating procedures (SOPs), Good Clinical Practice (GCP), and applicable regulatory requirements.

## 18.0 Quality Control and Quality Assurance

### 18.1 Monitoring Plan

Review of this protocol begins with an initial **review by the CPC**, Cancer Center Protocol Committee (CPC), which performs a risk assessment of the trial. The PI will abide by their assessment of the level of risk, which will determine the intensity of subsequent external monitoring. Documentation of this assessment will be maintained.

The clinical research study will be monitored both internally by the PI and by the Duke Cancer Institute (DCI) Monitoring Team in accordance with their NCI-approved “Institutional Protocol Monitoring Procedures and Guidelines for NIH-sponsored Research Involving Human Subjects”. The monitoring team will conduct visits to ensure subject safety and to ensure that the protocol is conducted, recorded and reported in accordance with the protocol, standard operating procedures, good clinical practice, and applicable regulatory requirements. As specified in the DCI monitoring plan, they will conduct routine monitoring after the third subject is enrolled, followed by annual monitoring of 1-3 subjects until the study is closed to enrollment and subjects are no longer receiving study interventions that are more than minimal risk.

Additional monitoring may be prompted by findings from monitoring visits, unexpected frequency of serious and/or unexpected toxicities, or other concerns and may be initiated upon request of DUHS and DCI leadership, the DCI Cancer Protocol Committee, the Safety Oversight Committee (SOC), the sponsor, Principal Investigator, or the IRB. All study documents must be made available upon request to the DCI Monitoring Team and other authorized regulatory authorities, including but not limited to the National Institute of Health, National Cancer Institute, and the FDA.

In terms of internal review, the PI (with the help of the statistician) will continuously monitor and tabulate adverse events. Appropriate reporting to the Duke University Medical Center IRB will be made. If an unexpected frequency of grade 3 or 4 events occurs, depending on their nature, action appropriate to the nature and frequency of these adverse events will be taken. This may require a protocol amendment, dose de-escalation, or potentially closure of the study. The PI of this study will also continuously monitor the conduct, data, and safety of this study to ensure that:

- Interim analyses occur as scheduled (if applicable);
- Stopping rules for toxicity and/or response are met;
- Risk/benefit ratio is not altered to the detriment of the subjects;
- Appropriate internal monitoring of adverse events and outcomes is done;
- Over-accrual does not occur;
- Under-accrual is addressed with appropriate amendments or actions;
- Data are being appropriately collected in a reasonably timely manner.

## 18.2 Audits

The Duke School of Medicine Clinical Trials Quality Assurance (CTQA) office may conduct audits to evaluate compliance with the protocol and the principles of GCP. The PI agrees to allow the CTQA auditor(s) direct access to all relevant documents and to allocate his/her time and the time of the study team to the CTQA auditor(s) in order to discuss findings and any relevant issues.

CTQA audits are designed to protect the rights and well-being of human research subjects. CTQA audits may be routine or directed (for cause). Routine audits are selected based upon risk metrics generally geared towards high subject enrollment, studies with limited oversight or monitoring, Investigator initiated Investigational Drugs or Devices, federally-funded studies, high degree of risk (based upon adverse events, type of study, or vulnerable populations), Phase I studies, or studies that involve Medicare populations. Directed audits occur at the directive of the IRB or an authorized Institutional Official.

CTQA audits examine research studies/clinical trials methodology, processes and systems to assess whether the research is conducted according to the protocol approved by the DUHS IRB. The primary purpose of the audit/review is to verify that the standards for safety of human subjects in clinical trials and the quality of data produced by the clinical trial research are met. The audit/review will serve as a quality assurance measure, internal to the institution. Additional goals of such audits are to detect both random and systemic errors occurring during the conduct of clinical research and to emphasize “best practices” in the research/clinical trials environment.

## **19.0. Investigator Responsibilities**

The conduct of the study will comply with all FDA safety reporting requirements.

Serious adverse events (SAE) will be recorded on the Duke IRB Adverse Event Reporting Form and will be reviewed and signed by the principal investigator. Only adverse events that are deemed to be serious, unexpected and related, or possibly related to the research must be reported to the Duke Institutional Review Board (IRB) in accordance with institutional policy.

If the investigator learns of any SAE, including death or congenital abnormality at any time after a subject has been discharged from the study, and he/she considers the event reasonable related to the study drug, the investigator should promptly file a report.

## **20.0. Departure from the Protocol**

There should be no departure from the protocol if at all possible. If an emergency occurs that requires departure from this protocol, the principal investigator or other physician in attendance in such an emergency will, if circumstances and time permit, contact the PI immediately by phone. Such contacts with the PI will be made to permit a decision as to whether or not the subject will be continued on study. Such departures need to be clearly documented on the Duke Notification of Protocol Deviation/Violation form. A protocol deviation or violation is reportable to the IRB if the event is likely to adversely affect: (i) the rights and welfare of the research subject; (ii) the safety of the research subject; (iii) the integrity of the research data; and/or (iv) the subject's willingness to continue study participation. Such events should be reported to the DUHS IRB within 10 business days of the time the PI becomes aware of the event; **however, an unanticipated study-related death must be reported to the DUHS IRB within 24 hours of the occurrence of the event.**

## **21.0. Subject Privacy**

All subject data will be identified by a subject identification number and subject initials only to protect the subject's privacy. The data will be blinded accordingly in all data analysis. Subject names or identifiers will not be used in reports, presentations at scientific meetings, or publications in scientific journals.

However, in compliance with federal guidelines, the investigator will permit representatives from the Duke School of Medicine Compliance Office and/or Duke Cancer Institute Monitoring team to review that portion of the subject's medical record that is directly related to the study. This will include all relevant study documentation including medical histories to verify eligibility, laboratory test results to verify transcription accuracy, X-ray reports, admission/discharge summaries for hospital/outpatient admissions while the subject is on-study, and autopsy reports for deaths occurring during the study. As part of the required content of the informed consent, the subject will be informed that his medical record may be reviewed. Should access to the medical record require a separate waiver or authorization, it is the PI's responsibility to obtain such permission from the patient in writing before the subject is entered into the study.

## **22.0. Institutional Review**

Prior to patient accrual, this protocol and the protocol informed consent must be approved in writing by the Cancer Center Protocol Committee (CPC) of the Duke Comprehensive Cancer Center and the DUHS Institutional Review Board (IRB). A signed and dated statement that the protocol and informed consent have been approved by the CPC/IRB will be kept in the study binder in the Principal Investigator's staff office. The term of study approval will not exceed one year. A progress report will be submitted annually to the IRB and re-approval obtained to continue the study. The IRB will also approve any significant changes to the protocol as well as a change of PI. The PI will report to the IRB any changes in the research protocol and all unanticipated problems involving risks to human subjects and others. No changes will be made in the research activity without IRB approval. Records of all study review and approval documents will be kept on file by the PI and/or his/her staff. Adverse events will be reported to the IRB per institutional reporting guidelines. The IRB will receive notification of the completion of the study and final report within 3 months of study completion or termination. The PI will maintain an accurate and complete record of all submissions made to the IRB, including a list of all reports and documents.

## 23.0 Informed Consent/Informed Consent Process

The PI or designee will fully explain the purpose and potential risks and benefits of the study to the patient prior to enrollment, and address any questions posed by the patient. In accordance with federal guidelines, all patients will sign a statement of informed consent, which has been approved by the IRB. The patient will receive a copy of the executed consent document. The signed consent will be retained at the investigative site for each patient. The informed consent document serves as authorization as deemed under HIPAA and contains the appropriate statements regarding privacy and confidentiality of protected health information (PHI) as well as information on withdrawal from the study. The investigator or designee will explain to each patient (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits, and any discomfort the study may entail. Each patient will be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician. The patient will have as much time as he/she may need to make an informed decision about the study and all treatment related questions will be answered. The consent discussion should occur in an exam room or a private area where it is just the research staff, the patient and his/her family/significant other(s) if desired.

The informed consent will be given by means of a standard written statement, written in non-technical language. For those that cannot read, or are blind, the consenter will read the consent form verbatim in the presence of a witness. The patient will read and consider the statement before signing and dating the document, and should be given a copy of the signed document. If the patient is unable to sign, make another kind of mark (like an X) to indicate consent. The person obtaining consent will document at the bottom of the consent form that the consent was read out loud to the patient by (name of person obtaining consent). If an X or mark is used instead of a signature, the person obtaining consent will note on the consent form that the subject wrote a mark or X instead of a signature. The witness will sign the consent form. Subjects who do not read/understand English may be potentially enrolled following DUHS HRPP policy, including obtaining IRB approval of either a short form or long form consent translation. No patient will enter the study before his/her informed consent document has been obtained. Before, during, and after the consent is signed, the research team and investigators will be available in

person and by phone to answer any questions the participants may have. Any and all other available treatment options are offered to the patient in order to avoid undue influence. Participants are not offered compensation for this study in order to avoid any monetary coercion/influence. The PI is responsible for ensuring that appropriate signatures have been obtained prior to the performance of any protocol procedures and prior to the administration of study drug.

## **24.0 Data Collection and Maintenance**

### **24.1 Study Documentation**

Study documentation includes but is not limited to source documents, case report forms, monitoring logs, appointment schedules, study team correspondence with sponsors or regulatory bodies/committees, and regulatory documents that can be found in the DCI-mandated “Regulatory Binder”, which includes but is not limited to signed protocol and amendments, approved and signed informed consent forms, FDA Form 1572, CAP and CLIA laboratory certifications, and clinical supplies receipts and distribution records.

Source documents are original records that contain source data, which is all information in original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source documents include but are not limited to hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial. When possible, the original record should be retained as the source document. However, a photocopy is acceptable provided that it is a clear, legible, and an exact duplication of the original document.

The research nurse, data manager and PI are responsible for ensuring that data extraction (information required by the protocol) is completed in a timely manner for every patient enrolled on study. The following forms are an integral part of the study data and will be maintained in the patient's clinical

chart. Any errors on the form should be lined through, but not obliterated with the correction inserted, initialed and dated by the person making the correction:

On-study Eligibility Checklist  
DUHS Adverse Event Form (if applicable)  
DUHS Protocol Deviation Form (if applicable)

## 24.2 Case Report Forms

An electronic CRF (eCRF) will be the primary data collection document for the study. The electronic records of subject data will be maintained using a dedicated database (Oracle Clinical or Redcap) which is housed in an encrypted and password-protected DCI file server). Access to electronic databases will be limited to the PI and designated study staff listed on key personnel. Subject data may be stored temporarily on encrypted and password-protected portable memory devices such as flash drives and external hard drives, but only when absolutely necessary. Data stored on portable memory devices will be de-identified. Subject data will be deleted from the portable memory device at the earliest opportunity. The security and viability of the IT infrastructure will be managed by the DCI and/or Duke Medicine.

The CRFs will be updated in a timely manner following acquisition of new source data. Only the PI and designated staff (as listed on key personnel), will be permitted to make entries, changes, or corrections in the eCRF.

An audit trail will be maintained automatically by the eCRF management system. (RedCap or Oracle Clinical database). All users of this system will complete user training as required or appropriate per regulations.

Users of the electronic CRF will have access based on their specific roles in the protocol.

Completeness of entered data will be checked automatically by the eCRF system, and users will be alerted to the presence of data inconsistencies. Additionally, the data manager and/or designess (per key personnel) will cross-reference the data to verify accuracy. Missing or implausible data will be highlighted for the PI requiring appropriate responses (i.e. confirmation of data, correction of data, completion or confirmation that data is not available, etc.).

The database will be reviewed and discussed prior to database closure, and will be closed only after resolution of all remaining queries. An audit trail will be kept of all subsequent changes to the data.

All medical terms will be coded with MedDRA(Medical Dictionary for Regulatory Activities). Medication will coded according to the World Health Organization Drug Dictionary.

## **24.3 Study Closure**

Following completion of the studies, the PI will be responsible for ensuring the following activities have been completed if applicable for the study:

- Date Clarification and/or resolution
- Accounting, reconciliation, and destruction/return of used and unused study drugs
- Review of site study records for completeness
- Shipment of all remaining laboratory samples to designated laboratories.

Upon completion of the study, research records will be archived and handled per DUHS HRPP policy.

## **25.0 Appropriateness of Measurements**

There are no surrogate endpoints used in this study. All efficacy and safety assessments used in this study are standard and appropriate for an oncology study.

## **26.0 Statistical Methods and Analytical Plans**

### **26.1. General Considerations**

#### **STATISTICAL CONSIDERATIONS:**

##### **Sample size justification**

The primary objective of the phase II trial is to determine the proportion of early stage lung cancer patients with detectable circulating T cells specific against TAA after receiving platinum based neoadjuvant chemotherapy plus ipilimumab before surgery. Based on Duke ICS assessments over the past 8 years, “detectable” circulating T cells with specificity against TAA are defined as a CD8, CD4, and DP (CD4+CD8+) lymphocyte percentage of  $\geq 0.05\%$  with each value also being at least twice that of the background unstimulated control value (see Appendix B of the correlative science measures for additional details). It was found that the proportion of patients before or without the treatment with “detectable” circulating T cells specific against TAA is 0-1%. Let  $p$  be the true proportion of patients with “detectable”

circulating T cells at Collection 3 after receiving the protocol treatment ipilimumab of any dose. For the purpose of sample size determination, we assume that the null hypothesis  $H_0: p < 0.01$  is tested against the alternative hypothesis  $H_1: p > 0.20$ . With a sample size of 25 evaluable patients treated with the ipilimumab, we have approximately 90% power to test the above hypothesis using a one-sided binomial test at a significance level of 0.010. The null hypothesis  $H_0$  will be rejected if 3 or more patients are found with detectable circulating T cells specific against TAA after the treatment. Assuming a 5% screening failure rate, the study will enroll a total of 30 patients. A patient is deemed “evaluable” if the patient meets eligibility criteria, receives the protocol treatment ipilimumab of any dose before Collection 3, and the levels of circulating T cells against TAA are measurable at Collection 3. All treated patients will be evaluated for treatment related adverse events and followed for tumor response and disease free survival.

### **Futility Analysis**

Futility analysis is often used for assessing clinical efficacy before the trial reaches target accrual and stops the trial early if the therapy clearly performs worse than control. The primary objective of this one-arm phase II trial is not to evaluate the efficacy of ipilimumab in this population. Since it is unlikely that the therapy will harm the patients, a futility analysis on the percentage of patients with detectable T-cells is unnecessary.

### **Adverse event monitoring**

The first 12 treated patients will be evaluated for AE's attributable to the preoperative treatment. Overall, if 3 or more of the first 12 treated patients (>25%) experience grade 3-5 non-hematologic adverse events (excluding diarrhea and rash) that are probably, possibly, or definitely related to the preoperative treatment, OR if the number of treatment-related deaths from the first treatment until 30 days after surgery is 2 or more among the first 12 patients (>17%), accrual to the study will be suspended to allow for investigation. After consideration by the study team and the IRB, a decision will be made as to whether accrual can be resumed, potentially with modifications to entry criteria and/or study conduct. In addition, all toxicity patterns will be monitored by the DCI Safety Oversight Committee. Postoperative ipilimumab will not be included in toxicity monitoring to determine continued accrual.

### **Evaluation of Investigational Therapy Feasibility**

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Adverse events in the first 10 patients with computerized data were analyzed according to the guidelines in the above section. It became apparent that it was difficult to accurately attribute adverse events to standard chemotherapy, ipilimumab, or other causes. Further it seemed that analysis of treatment toxicities as isolated events did not best represent whether or not the combination of chemotherapy plus ipilimumab follow by surgery is safe and feasible. As of June 28, 2014, a total of 15 patients were registered and treated. Therefore, safety and feasibility for the remainder of the trial will be evaluated whenever additional 5 patients have been treated by assessing: 1) whether the cumulative percentage of deaths among all treated patients related to any of the study treatments from the first ipilimumab treatment until 30 days after surgery exceeds 17%, and 2) if the cumulative percentage of all treated patients deemed unable to go to surgery due to adverse events attributed to neoadjuvant therapy exceeds 20%. The percentage of patients unable to go to surgery after chemotherapy is a conservative estimate based on data reported for a SWOG trial that studied neoadjuvant chemotherapy in early state lung cancer (22). The trial will be stopped for accrual whenever a total of 30 patients are registered or at least one of the thresholds of the safety and feasibility criteria is exceeded. In the event either of these two thresholds is exceeded, the investigational therapy will be considered “infeasible” in this setting.

### **Analytic methods**

The primary outcome will be analyzed using the evaluable patients as defined in the sample size justification section. The exact binomial test will be used to test the increase in the proportion of patients with detectable circulating T cells specific against TAA after the treatment. The proportion will be estimated and its 2-sided 98% confidence interval will be given to be consistent with the nominal 1-sided significance level of 0.01 used in the sample size justification. All secondary objectives are considered exploratory in nature, and type I error will not be controlled for multiplicity. P-values for these statistical tests will be provided for descriptive purposes. All treated patients will be included in the analysis of adverse events.

The following statistical analyses will be conducted on the secondary objectives:

(1) Feasibility/tolerability of neoadjuvant chemotherapy plus ipilimumab and surgery. Based on data provided by Pisters et al (Pisters et al. J Clin Oncol. 2010 April 10; 28 (11): 1843-1849), the following

criteria would be considered evidence of not feasible/tolerable: less than 75% of subjects receive 3 cycles of preoperative therapy (i.e. receive some amount of the expected study medications each cycle), “less than 80% of subjects undergo surgical exploration within 42 days of day 1 of the last cycle of neoadjuvant chemotherapy”, more than 10% of subjects undergoing lobectomy have surgery-related mortality, or more than 20% of subjects experience dose-limiting toxicity during neoadjuvant therapy. Dose-limiting toxicity will be defined as possibly, probably or definitely treatment-related:  $\geq$  Grade 4 hematologic toxicity (excluding Grade 4 neutropenia without fever or infection), or  $\geq$  Grade 3 non-hematologic toxicity (excluding grade 3 fatigue, nausea, vomiting, peripheral neuropathy, chemotherapy infusion reaction to carboplatin or paclitaxel).

- (2) Evaluate disease-free survival and patterns of metastases after neoadjuvant chemotherapy plus ipilimumab in early stage NSCLC. Disease-free survival (DFS) is defined as the time from surgical resection to disease recurrence (first disease recurrence or death, whichever comes first) after surgery. The Kaplan-Meier estimator will be used to estimate median DFS and its 95% confidence interval. The frequencies of metastases by site will be tabulated.
- (3) The relationship between pathologic response and the RECIST response will be explored by looking at a frequency cross-tabulation of pathologic versus RECIST response.

**The following exploratory objectives will be conducted:**

- (1) Determine the percentage of patients with “detectable” (percentage of  $\geq 0.05\%$  with each value also being at least twice that of the background unstimulated control value) tumor infiltrating lymphocytes (TILs) after neoadjuvant chemotherapy plus ipilimumab. The percentage of patients with TILs for patients who have surgical resection and its 95% exact confidence interval will be estimated. See Appendix B of the correlative science measures for additional details on identifying TILs.
- (2) Estimate the pathologic response rate for neoadjuvant chemotherapy plus ipilimumab in early stage NSCLC. The definition of pathologic response rate is given in section 16.3. The pathologic response rate along with its 95% exact confidence interval will be estimated.

(3) Estimate the objective response rate of neoadjuvant chemotherapy plus ipilimumab in early stage NSCLC. Best response rate is of interest and tumor response will be evaluated from treatment initiation to prior to surgery. The definition of objective response will be measured by RECIST 1.1. The objective response rate (ORR=CR+PR) along with its 95% exact confidence interval will be estimated.

(4) Determine if the immunomodulatory effects of neoadjuvant chemotherapy plus ipilimumab impact the suppressive mechanisms, restoring functional reactivity to important anti-tumor effector cell populations. Functional TAA-specific T cell reactivities will be measured in the blood at four time points: Collection 1, 2, 3 and 4. Changes in measures of anti-TAA reactivity (see Appendix B for details about these correlative science measures) across time points will be visualized using boxplots with the mean, median and interquartile range for each time point. For the phenotypic reactivity measures with background unstimulated negative control values (from the ICS panel), the percentage of patients with a positive response (a percentage value greater than the background unstimulated negative control value) will be calculated. A Wilcoxon signed rank test will be used to test for differences from baseline, as well as for any association between each reactivity measure and pathologic response to neoadjuvant therapy.

(5) Explore an alternative definition for detectability suitable for expression values generated using Boolean gating, and determine the percentage of patients with circulating T cells meeting this definition. The percentage of patients with circulating T cells meeting the new definition of detectable and its 95% exact confidence interval will be estimated. See Appendix B for additional details about this outcome measure.

(6) Evaluate whether presence, quantity or quality of detectable TILs is associated with pathologic response to neoadjuvant therapy. Quality of TILs (also called relative polyfunctionality) is defined as  $\geq 2$  functional marker values (out of 4) that meet the definition of detectable described in Objective 5. See Appendix B for additional details about this outcome measure. A Fisher exact test will be used to evaluate the association of both the presence and the quality of TILs with pathologic tumor response to neoadjuvant therapy. A Wilcoxon rank sum test will be used to test the association of the quantity of TILs and pathologic response to neoadjuvant therapy.

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## Appendix A

### Study Schedule

Examination	Baseline (screen) <sup>a</sup> Days -30-0	Cycle 1	Cycle 2	Cycle 3	Eval post chemo <sup>n</sup>	Surgery	Adjuvant q 3 wk x2 and Maintenance q 12 wk x2	End of study EOS <sup>x</sup>	Follow-up <sup>f</sup>	Relapse <sup>l</sup>
<b>Day of Cycle</b>		1	1	1			1			
<b>Informed Consent</b>	X									
<b>Eligibility</b>	X									
<b>Medical History/Demo-Graphics/Smoking Hx</b>	X									
<b>Height</b>	X									
<b>Vital Signs (temp, B/P, pulse)</b>	X	X	X	X	X		X	X		
<b>Weight/BSA<sup>a,b</sup></b>	X	X	X	X	X		X	X		
<b>Physical Exam<sup>a,b,f</sup></b>	X	X	X	X	X		X	X		
<b>Performance Status<sup>a,b</sup></b>	X	X	X	X	X		X	X		
<b>Hematology<sup>a,b,c,g,f</sup></b>	X	X	X	X	X		X	X		
<b>Chemistries<sup>a,b,g,h,f</sup></b>	X	X	X	X	X		X	X		
<b>Calculated Creatinine Clearance<sup>b,d</sup></b>	X	X	X	X						
<b>Serum Beta HCG<sup>e</sup></b>	X		X	X			X			
<b>Radiologic Evaluation<sup>b,f</sup></b>	X				X		X		X	X

<b>EKG</b>	<b>X</b>										
<b>Tumor Histology</b>	<b>X</b>										
<b>Adverse Events<sup>t</sup></b>		<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>			<b>X</b>	<b>X</b>	<b>X</b>	
<b>Chemotherapy</b>		<b>X</b>	<b>X</b>	<b>X</b>							
<b>Ipilimumab<sup>k</sup></b>			<b>X</b>	<b>X</b>				<b>X</b>			
<b>Thyroid Functions<sup>b,L</sup></b>	<b>X</b>			<b>X</b>				<b>X</b>			
<b>Blood Sampling<sup>j</sup></b>	<b>X</b>		<b>X</b>		<b>X</b>			<b>X</b>			
<b>Surgical Specimen<sup>p</sup></b>							<b>X</b>				

**To allow for patient and investigator schedules, holidays, weather or other emergencies requiring facilities to be closed, , visits can be performed ±3 days of scheduled visit**

<sup>b</sup> May be obtained within 3 days of dosing chemo/ipilimumab

<sup>a</sup> Pre-enrollment baseline (screen) assessments are to be performed within -30 to 0 days unless otherwise specified .

<sup>b</sup> Radiologic evaluation: pre-treatment clinical staging PET/CT of chest/abdomen and brain MRI or CT as per standard of care (these may be performed up to 42 days); 3-4 weeks after completing cycle#3 neoadjuvant therapy (prior to surgery) chest CT; post-surgery recommend chest CT prior to each of two doses maintenance Ipilimumab; then every 3-4 months (or per institutional standard of care) for 2 years (this can be timed with follow-up, see footnote f).

<sup>c</sup> Hematology values to include Hgb/Hct, WBC with auto or manual differential, platelets.

<sup>d</sup> Calculated creatinine clearance (see appendix D to be performed at baseline and prior to every dose of cisplatin or carboplatin.

<sup>e</sup> For women of childbearing potential only. All WOCBP MUST test negative for pregnancy within48 hours prior to any initial study procedure based on a serum pregnancy test. If subject uses appropriate contraceptive methods (the use of two form at the same time) from the time of the initial serum pregnancy test, then the subsequent pregnancy test can be done within 72 hours before receiving ipilimumab. If appropriate contraceptive methods are not begun immediately with the first serum pregnancy test, then subsequent serum pregnancy tests must be done within 48 hours prior to the study drug administration. The minimum sensitivity of the pregnancy test must be 25 IU/L or equivalent units of HCG. If the pregnancy test is positive, the subject must not receive ipilimumab and must not be enrolled in the study, or will be removed from treatment.

<sup>f</sup> Surveillance after end of study evaluation: every 3-4 months (or per institutional standard of care) for 2 years and then every 6 months until 5 years, then yearly thereafter. These evaluations may be coordinated with visits for radiologic disease evaluation or occur via phone follow-up. Lab assessments will be at the discretion of treating physician.

<sup>g</sup> Baseline required labs to be performed within 30 days of enrollment Pre-study tests may be used for day 1, cycle 1 tests if obtained within 14 days of day 1 cycle 1 treatment.

<sup>h</sup> Chemistries to include Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, total protein, albumin, calcium, glucose, BUN, creatinine, uric acid, total bilirubin, alkaline phosphatase, SGOT, SGPT, LDH, Mg<sup>+</sup>.

<sup>i</sup> Upon lung cancer relapse, subjects should have chest/abdomen and brain CT or MRI to document sites of failure.

<sup>j</sup> Blood samples to assess activated CD8+ T cells with specificity against tumor antigens and CD4 and CD8 functional memory. will be collected: Collection 1: Baseline: pretreatment. Collection 2: cycle 2 day 1 chemotherapy prior to Ipilimumab infusion.Collection 3: 21-36 days after completion of cycle 3 prior to surgery Collection 4: 3-6 weeks after post-op adjuvant ipilimumab dose #2.

<sup>k</sup> Total dose of ipilimumab to be calculated using weight obtained within 3 days of dosing visit, prior to infusion. Ipilimumab dosing will occur cycles 2 &3(day 1) neoadjuvant therapy and as single therapy post-surgery to consist of adjuvant therapy q3wk x2, followed by maintenance therapy q12 wk x2.

<sup>L</sup> Blood samples for Thyroid Function Tests to include: Thyroid Stimulating Hormone, Free Thyroxine.

<sup>m</sup> Evaluation Post Chemotherapy to occur at least 21 days after last dose of therapy

<sup>n</sup> Medical/Surgical Evaluation to occur at least 21 days after completion of neoadjuvant chemotherapy.

<sup>p</sup> Surgery to occur 4-12 weeks after last dose of chemotherapy. After all tumor specimen necessary for standard pathological testing has been obtained by the surgical pathology staff, a specimen of surplus tumor will be acquired by the tumor immunology correlative science staff for isolation of tumor infiltrating lymphocytes.

<sup>x</sup> EOS=End of Study. Complete once subject has completed maintenance therapy( approximately 12 wks after dose #2) or 30 days after subject comes off treatment for progression of disease, intolerance to protocol therapy, or patient withdraws consent.

## **Appendix B Correlative Science Measures**

### **Primary Objective**

The primary endpoint is the proportion of early stage lung cancer patients with detectable circulating T cells specific against TAA after receiving platinum based chemotherapy plus ipilimumab before surgery. Detectable circulating T cells with specificity against TAA will be identified using the following process:

Whenever individual patient tumor tissue is available for analysis, the expression of 10 highly conserved TAA's (see Table I below) will be assessed using standard Real-Time (RT) PCR technology. Peptide pools representing the three most highly expressed TAA's by each tumor will serve as antigen-specific stimulators for PBMC isolated from the same patient at the 4 specified time points. If no tumor tissue is available for TAA expression analysis, peptide pools representing the three most commonly expressed TAA's on NSCLC, namely Survivin, PRAME, and MAGE-A3, will be chosen as antigen-specific stimulators.

The percentage of CD4, CD8, and double positive (DP=CD4+/CD8+) lymphocytes with specificity against the three selected TAA's, will be calculated for each of 4 individual functional markers (IL-2, TNF- $\alpha$ , IFN- $\gamma$ , and CD107 underlined below). For each peripheral blood collection, the lab will generate a total of 12 lymphocyte values (4 values each for CD4, CD8 and DP) for each of the three TAA's and for CD3/CD28 (used as a positive control), as well as generating 12 background unstimulated negative control values. All values will be

expressed as percentages. A patient will be considered to have detectable circulating T cells if  $\geq 1$  of the 36 percentage values (12 values \* 3 TAA's) satisfies the definition of "detectable" as described in section 26.0 (a lymphocyte percentage of  $\geq 0.05\%$  at the pre-surgical peripheral blood collection (Collection 3) with each value also being at least twice that of the background unstimulated negative control value).

**Table I** **TAA Peptide Pools**  
(15-mers, overlapping by 11 amino acids)

- Survivin (33 peptides)
- PRAME/OIP4 (125 peptides)
- MAGE-A3 (76 peptides)
- Melan A/MART-1 (27 peptides)
- Gp100 (163 peptides)
- Tyrosinase (117 peptides)
- NY-ESO-1 (43 peptides)
- MAGE-A1 (75 peptides)
- MAGE-A4 (77 peptides)
- CEA (173 peptides)

Assessing detectable circulating T cells specific against three TAA's (individually selected from the Table I list of TAA's using the process outlined above) in the following combinations of T cells and functional markers:

\* %**CD4+**/CD69+/IL-2+  
\*%**CD4+**/CD69+/TNF- $\alpha$ +  
\*%**CD4+**/CD69+/IFN- $\gamma$ +  
\*%**CD4+**/CD69+/CD107+

\* %**CD8+**/CD69+/IL-2+  
\*%**CD8+**/CD69+/TNF- $\alpha$ +  
\*%**CD8+**/CD69+/IFN- $\gamma$ +  
\*%**CD8+**/CD69+/CD107+

\* %**DP+**/CD69+/IL-2+  
\*%**DP+**/CD69+/TNF- $\alpha$ +  
\*%**DP+**/CD69+/IFN- $\gamma$ +  
\*%**DP+**/CD69+/CD107+

## Exploratory Objectives

Using the same methods noted above, 12 lymphocyte percentage values (functional markers) for the 3 selected TAA's will also be generated from tumor tissue collected at surgery, and in this context are called tumor infiltrating lymphocytes (TILs). These values will be used to determine the percentage of patients with any detectable TILs (exploratory objective 1). Expression percentage values for each T cell (CD4, CD8, and DP) will also be collected corresponding to each combination of the 4 functional markers (excluding the combination where all four markers are negative) using Boolean gating. These 135 values (15 values \* 3 T cells \* 3 TAA's) will be used to determine a definition of detectability suitable for these measures to avoid missing potential positive values, and to determine the percentage of patients with  $\geq 1$  of the 135 values satisfying the new definition of detectable circulating T cells (exploratory objective 5). In addition, this definition will also be used to explore whether the presence, quantity or quality of TILs is associated with pathologic response (exploratory objective 6).

\* Boolean gating will be performed to calculate percentage values corresponding to the following combinations of T cells and functional markers (excluding the combination where all 4 markers are negative):

- \* %**CD4+**/CD69+/-IL-2+ or -TNF- $\alpha$ + or -IFN- $\gamma$ + or -CD107+ or -
- \* %**CD8+**/CD69+/-IL-2+ or -TNF- $\alpha$ + or -IFN- $\gamma$ + or -CD107+ or -
- \* %**DP+**/CD69+/-IL-2+ or -TNF- $\alpha$ + or -IFN- $\gamma$ + or -CD107+ or -

For exploratory outcome 4, changes in all measures of anti-TAA reactivity listed below across time points will be analyzed, as well as how these values relate to pathologic response. In addition, for the phenotypic reactivity measures with background unstimulated negative control values (those from the ICS panel), the percentage of patients with a positive response (a percentage value greater than the background unstimulated negative control value) will be calculated. The distribution of the changes will be estimated with a boxplot and the median (mean) change will be calculated by pathologic response status (yes/no).

%CD3+CD4+CD8- (CD4 T cells)

%CD3+CD4-CD8+ (CD8 T cells)

%CD3+CD4+CD8+ (CD4/CD8 double positive (DP) T cells)

%CD4+CD152- (CTLA-4 negative CD4 T cells)

%CD4+CD152+ (CTLA-4+ CD4 T cells)

%CD4+/CD69+ (Activated CD4 T cells)

%CD4+CD154+ (Activated CD4 T cells)

%CD4+/-HLA-DR+ (Activated CD4 cells)

%CD4+CD28+

%CD4+CD28-

%CD4+/CD28+/CD152+CD279+ (Activated/Exhausted CD4 T cells)

%CD4+CD28+CD278+ (Activated CD4 T cells/Pharmacodynamic Biomarker)

%CD4+/CD25+FoxP3+/CD152  
%CD4+/CD25+FoxP3+/CD152  
%CD4+/CD25+FoxP3+/CD152  
%CD4+/CD25+FoxP3+/CD152

%CD4+CD45RA+CCR7+ (Naïve CD4 T cells)  
%CD4+CD45RA-CCR7+ (Central memory CD4 T cells)  
%CD4+CD45RA-CCR7- (Effector memory CD4 T cells)  
%CD4+CD45RA+CCR7- (Effector CD4 T cells)

%CD8+CD152- (CTLA-4 negative CD8 T cells)  
% CD8+CD152+ (CTLA-4 positive CD8 T cells)

%CD8+CD69+ (Activated CD8 T cells)  
%CD8+CD152+ (Activated CD8 T cells)

%CD8+/-HLA-DR+ (Activated CD8 T cells)  
%CD8+CD28+  
%CD8+CD28-  
%CD8+/CD28+/CD152+CD279+ (Activated/Exhausted CD8 T cells)

%CD8+CD45RA+CCR7+ (Naïve CD8 T cells)  
%CD8+CD45RA-CCR7+Central memory CD8 T cells)  
%CD8+CD45RA-CCR7- (Effector memory CD8 T cells)  
%CD8+CD45RA+CCR7- (Effector CD8 T cells)

%DP CD69+ (Activated DP T cells)  
%DP CD154+ (Activated DP T cells)

%DP CD45RA+CCR7+ (Naïve DP T cells)  
%DP CD45RA-CCR7+ (Central memory DP T cells)  
%DP CD45RA-CCR7- (Effector memory DP T cells)  
%DP CD45RA+CCR7- (Effector DP T cells)

%CD3-SSC<sup>hi</sup>/HLA-DR<sup>low</sup>CD14+ (Myeloid-derived suppressor cells (MDSC))

FCSxSSC (Monocyte gate) %CD3-CD14- Lin(CD16, CD19, CD20, CD56)- HLA-DR-CD11b+/CD33+ (MDSC)

## **Appendix C     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 D**

### **Standard Cockcroft and Gault Formula for Calculated Creatinine Clearance**

#### **Standard Cockcroft and Gault Formula for Calculated Creatinine Clearance**

##### **For serum creatinine concentration in mg/dL:**

Creatinine clearance (CrCl) will be calculated using the Cockcroft-Gault equation as follows:

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

##### **For serum creatinine concentration in $\mu\text{mol/L}$ :**

$$\text{CrCl} = [(140 - \text{age}) \times (\text{wt in kg})] / [0.81 \times \text{serum creatinine (mol/L)}]$$

Females: Multiply the result  $\times 0.85$

Units: age in years, weight in kilograms.

Source: Cockcroft DW, Gault MH. 1976. Prediction of creatinine clearance from serum creatinine. *Nephron* 16:31-4.

## Appendix E Patient Eligibility Checklist

### TOP 1201: Evaluation of Circulating T Cells and Tumor Infiltrating Lymphocytes with Specificities Against Tumor Associated Antigens During and After Neoadjuvant Chemotherapy and Phased in Ipilimumab in Non-small Cell Lung Cancer (NSCLC).

Initials: \_\_\_\_\_ Sex: M ( ) F ( ) DOB: \_\_\_\_\_ / \_\_\_\_\_ / \_\_\_\_\_ Age: \_\_\_\_\_ Patient ID #: \_\_\_\_\_  
 Race: \_\_\_\_\_ Diagnosis: \_\_\_\_\_ Date of Diagnosis: \_\_\_\_\_ / \_\_\_\_\_ / \_\_\_\_\_  
 Physician: \_\_\_\_\_ Surgeon \_\_\_\_\_

**Inclusion Criteria: Patients are Eligible to be included in the study only if they meet ALL of the following criteria:**

		Yes	No
1.	Histological or pathologic diagnosis NSCLC. Date obtained: _____ / _____ / _____		
2.	Clinical stage IB (>4 cm), II or III (N0-2) NSCLC amenable to surgical resection . Stage: _____ (T _____ N _____ M _____).		
3.	Patient must be deemed a surgical candidate		
4.	ECOG performance status of 0 or 1. Status: _____ Date assessed: _____ / _____ / _____		
5	NO prior chemotherapy for current diagnosis of lung cancer.		
6.	Age $\geq$ 18 years. (DOB/age listed above).		
7.	No active or concomitant invasive malignancy in the past 2 years other than skin or prostate cancer.		
8.	Signed written informed consent including HIPAA according to institutional guidelines,. Date signed: _____ / _____ / _____		
9.	Adequate Organ Function: ABC/diff: Date obtained: _____ / _____ / _____ Platelets _____ ( $\geq$ 100,000 per $\mu$ l) ANC or AGC _____ ( $\geq$ 1500 per $\mu$ l) Chemistries: Date obtained _____ / _____ / _____ Total bilirubin _____ ( $\leq$ 1.5 mg/dL) Creatinine clearance $\geq$ 45 mL/min _____ or creatinine $<$ 2 mg/dL to receive cisplatin) _____ SGOT _____ SGPT _____ ( $\leq$ 2.5 x institutional ULN )		
10.	Females of child-bearing potential (not surgically sterilized and between menarche and 1 year post menopause) must test negative for pregnancy within 48 hours prior to based on a serum pregnancy test. Both sexually active males and females of reproductive potential must agree to use a reliable method of birth control, as determined by the patient and their health care team, during the study and for 3 months following the last dose of study drug. If subject uses appropriate contraceptive methods (the use of two forms at the same time) from the time of initial serum pregnancy test, then the subsequent pregnancy test can be done within 72 hours of receiving study drug administration. If appropriate measures are not begun immediately with the first serum pregnancy test, then subsequent serum pregnancy tests must be done within 48 hours prior to the study drug administration. Beta HCG Result _____ Date obtained: _____ / _____ / _____ If not applicable, please specify why: _____		
11.	Must agree to research blood sampling to participate.		

Patient Eligibility Checklist Continued

**Exclusion Criteria: Patients will be excluded from the study if they meet ANY of the following criteria:**

		YES	NO
1.	Treatment within the last 30 days with a drug that has not received regulatory approval for any indication at the time of study entry.		
2.	Concurrent administration of any other antitumor therapy.		
3.	Inability to comply with protocol or study procedures.		
4.	Active infection requiring IV antibiotics, antifungal, or antiviral agents that in the opinion of the investigator would compromise the patient's ability to tolerate therapy.		
5.	Major surgery (other than definitive lung cancer surgery) within two weeks of study or other serious concomitant systemic disorders, that in the opinion of the investigator, would compromise the safety of the patient or compromise the patient's ability to complete the study.		
6.	Myocardial infarction having occurred less than 6 months before inclusion, uncontrolled arrhythmia, symptomatic angina pectoris, no active ischemia on EKG, or cardiac failure not controlled by medications.		
7.	Contraindication to corticosteroids.		
8.	Unwillingness to stop taking herbal supplements while on study.		
9.	Female patients that are pregnant or breast-feeding.		
10.	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 [eg, Wegener's Granulomatosis]); motor neuropathy considered of autoimmune origin (e.g. Guillain-Barre Syndrome and Myasthenia Gravis).		
11.	Any non-oncology vaccine therapy for prevention of infectious diseases (for up to 1 month before after any dose of ipilimumab).		
12.	A history of prior treatment with ipilimumab or prior CD137 agonist or CTLA-4 inhibitor or agonist.		
13.	Prisoners or subjects who are compulsorily detained (involuntarily incarcerated) for treatment of either psychiatric or physical (e.g., infectious) illness.		

Patient satisfies all criteria: \_\_\_\_\_ (Yes) \_\_\_\_\_ (No)

Comments: \_\_\_\_\_

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Research Nurse/Coordinator's Signature: \_\_\_\_\_

Date

Enrolling Physician's Signature: \_\_\_\_\_

Date

## **Appendix F Blood Specimens for correlative sciences**

Blood will be collected 4 times to assess CD8+ T cells with specificity against tumor antigens. Blood from each of the 4 collections will also be utilized to assess the presence of circulating populations of regulatory T cells, myeloid-derived suppressor cells (MDSC), as well as activated and exhausted T cells.

### **Blood volumes and collection time points:**

At each blood collection, 4 tubes of 8.5-ml each will be collected. The tubes used will be ACD anti-coagulated Vacutainer (yellow tops). The following time points will have blood collections:

Collection 1: Baseline prior to treatment initiation

Collection 2: cycle 2, day 1 chemotherapy prior to ipilimumab infusion

Collection 3: 21-36 days after completion of cycle 3 chemotherapy prior to surgery

Collection 4: 3-6 weeks after post-op ipilimumab dose #2.

Once ACD tubes collected, Dr. Weinhold's lab to be contacted for pick up as PBMC should ideally occur within 4 hours of drawn @ 919-684-3754. Tubes are to remain ambient (refrigeration will limit ability to recover PBMC).

DTRI-IM Sample Transport Record form to be completed to accompany tube to Dr. Weinhold's lab.

## **Appendix G Tumor Tissue Specimen for correlative sciences**

### **SURGICAL SPECIMEN**

Patients will be approached to participate in the “DUHS Biospecimen Repository and Processing Core (BRPC)” eIRB 35974 protocol prior to surgery. Tissue will be collected and released as described in this protocol to ensure proper involvement of pathology to minimize the chance that a tissue collection event interferes with appropriate clinical tissue processing and diagnosis.

#### **PRINCIPLE:**

Lung tissue is harvested in the frozen section or gross dissection area of the surgical pathology suite. The tissue is first examined by a certified anatomic pathologist or surrogate (resident, pathology assistant). Relevant margins are inked, removed and examined by frozen section analysis, if necessary. It is imperative that harvesting tissue for use in research trials does not impede accurate initial assessment of critical features of the tumor resection, most notably margin status. After the specimen has been processed for margin status, the frozen section assessment of the specimen is complete, a specimen of excess tumor will be released and acquired by the tumor immunology correlative science staff for isolation of tumor infiltrating lymphocytes for purposes of this protocol.

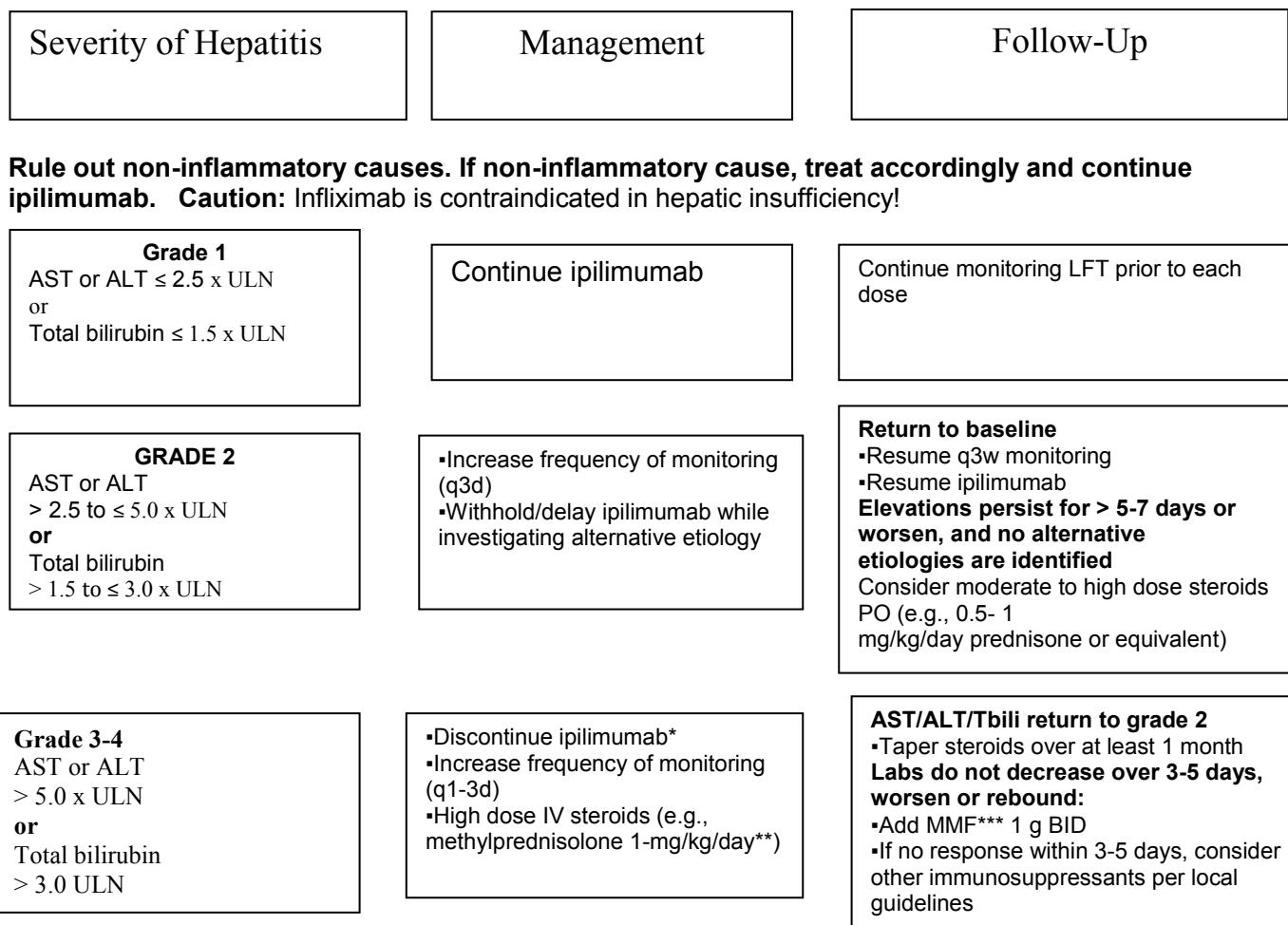
**SPECIMEN:** Lung resection specimen

**LOCATION:** Surgical Pathology Suite

#### **QUALITY CONTROL:**

Lab guidelines for safe handling of all samples must be followed. All tubes used for specimen processing must be labeled with the unique patient identifier or sample number before transfer of the tissue sample.

## Appendix H: Hepatotoxicity Management Algorithm



\*Ipilimumab may be held/delayed rather than discontinued if AST/ALT  $\leq$  8 x ULN and Tbili  $\leq$  5 x ULN. Resume ipilimumab when AST/ALT/

Tbili return to grade 2 and meet protocol specific retreatment criteria.

\*\*The recommended starting dose for grade 4 hepatitis is 2 mg/kg/day methylprednisolone IV.

\*\*\* MMF, mycophenolate mofetil

Patients on IV steroids may be switched to oral corticosteroid (e.g., prednisone) at an equivalent dose at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of PO corticosteroids.

## Appendix I: Endocrinopathy Management Algorithm

Severity of Endocrinopathy	Management	Follow-Up
<b>Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue ipilimumab.</b>		
<b>Asymptomatic TSH elevation</b>	<p>Continue ipilimumab If TSH &lt; 0.5 x LLN, or TSH &gt; 2.0 x ULN, or consistently out of range in 2 subsequent measurements: Include fT4 at subsequent cycles as clinically indicated; consider endocrinology consult</p>	
<b>Symptomatic endocrinopathy</b>	<ul style="list-style-type: none"> <li>Evaluate endocrine function</li> <li>Consider pituitary scan</li> </ul> <p><b>Symptomatic with abnormal lab/pituitary scan:</b></p> <ul style="list-style-type: none"> <li>Withhold/delay ipilimumab</li> <li>High dose IV steroids (e.g., 1-2 mg/kg/day methylprednisolone)</li> <li>Initiate appropriate hormone therapy</li> </ul> <p><b>No abnormal lab/pituitary scan but symptoms persist:</b></p> <p>Repeat labs in 1-3 weeks / MRI in 1 month</p>	<p><b>Symptoms resolve</b> (with or without hormone substitution):</p> <ul style="list-style-type: none"> <li>Resume ipilimumab</li> <li>Taper steroids over at least 1 month*</li> </ul>
<b>Suspicion of adrenal crisis</b> (e.g. severe dehydration, hypotension, shock out of proportion to current illness)	<ul style="list-style-type: none"> <li>Rule out sepsis</li> <li>Stress dose of IV steroids with mineralocorticoid activity</li> <li>IV fluids</li> <li>Consult endocrinologist</li> </ul>	<p>*Patients with adrenal insufficiency might need to continue steroids with mineralocorticoid component</p>

Patients on IV steroids may be switched to oral corticosteroid (e.g., prednisone) at an equivalent dose at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of PO corticosteroids.

## Appendix J: Neuropathy Management Algorithm

Severity of Neurologic Toxicity	Management	Follow-Up
<p><b>Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue ipilimumab.</b></p> <p><b>Discontinue ipilimumab for any grade 3-4 motor neuropathy, regardless of relationship.</b></p>		
<p><b>GRADE 1</b></p>		
	Continue ipilimumab	Continue monitoring the patient. If symptoms worsen, treat as below.
<b>GRADE 2</b>	<ul style="list-style-type: none"><li>Hold/delay ipilimumab</li><li>Treat symptoms per local guidelines</li></ul>	<ul style="list-style-type: none"><li>Resume ipilimumab when resolved / grade 1</li><li>If symptoms worsen, treat as below.</li></ul>
<b>GRADE 3-4 SENSORY</b>	<ul style="list-style-type: none"><li>Discontinue ipilimumab if considered related</li><li>Obtain neuro consult</li><li>Treat symptoms per local guidelines</li><li>High dose IV steroids (e.g., 1-2 mg/kg/day methylprednisolone)</li></ul>	<b>Symptoms resolve / return to grade 2:</b> Taper steroids over at least 1 month
<b>Grade 3-4 MOTOR</b>	<p>Discontinue ipilimumab <b>regardless of relationship</b></p> <ul style="list-style-type: none"><li>Obtain neuro consult</li><li>Treat symptoms per local guidelines</li><li>High dose IV steroids (e.g., 1-2 mg/kg/day methylprednisolone)</li></ul>	<p><b>Symptoms resolve / return to grade 2:</b></p> <ul style="list-style-type: none"><li>Taper steroids over at least 1 month</li></ul> <p><b>Symptoms do not resolve or progress; atypical presentation:</b></p> <ul style="list-style-type: none"><li>Consider IVIg or other immunosuppressive therapies per local guidelines</li></ul>

Patients on IV steroids may be switched to oral corticosteroid (e.g., prednisone) at an equivalent dose at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of PO corticosteroids.

## Appendix K: Skin Toxicity Management

Severity of Skin Toxicity	Management	Follow-up
<b>Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue ipilimumab.</b>		
<b>Grade 1-2</b>	<ul style="list-style-type: none"><li>▪ Symptomatic therapy, e.g. antihistamines, topical steroids</li><li>▪ continue ipilimumab</li></ul>	<b>Symptoms resolve:</b> continue ipilimumab <b>Symptoms persist &gt; 1-2 weeks or recur:</b> <ul style="list-style-type: none"><li>▪ Continue Ipilimumab</li><li>▪ Moderate to high dose steroids PO (e.g., prednisone 0.5-1 mg/kg/day)</li><li>▪ Once controlled, taper steroids over at least 1 month</li></ul>
<b>Grade 3-4</b>	<ul style="list-style-type: none"><li>▪ Hold/delay ipilimumab (regardless of relationship)</li><li>▪ Take photos of rash</li><li>▪ Consider skin biopsy</li><li>▪ Dermatology consult</li><li>▪ High dose IV steroids (e.g., methylprednisolone 1-2mg/kg/day)</li></ul>	<b>Symptoms resolve / return to grade 1:</b> <ul style="list-style-type: none"><li>▪ Taper steroids over at least 1 month</li><li>▪ Resume ipilimumab</li><li>▪ Discontinue if grade 4 toxicity considered related</li></ul>

Patients on IV steroids may be switched to oral corticosteroid (e.g., prednisone) at an equivalent dose at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of PO corticosteroids.

## Appendix L: GI Toxicity Management Algorithm

Severity of diarrhea/ Colitis	Management	Follow-Up
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**Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue ipilimumab.**

**Opiates/narcotics mask symptoms of perforation! Infliximab should not be used in case of perforation/sepsis.**

<b>GRADE 1</b> <u>Diarrhea:</u> < 4 stools/day over baseline <u>Colitis:</u> asymptomatic	<ul style="list-style-type: none"> <li>Continue ipilimumab</li> <li>symptomatic treatment</li> </ul>	<ul style="list-style-type: none"> <li>close monitoring for worsening symptoms</li> <li>Educate patient to report any worsening immediately</li> </ul>
<b>GRADE 2</b> <u>Diarrhea:</u> 4-6 stools per day over baseline; IV fluids indicated < 24 hrs; not interfering with ADL <u>Colitis:</u> abdominal pain; blood in stool	<ul style="list-style-type: none"> <li>Withhold/delay ipilimumab</li> <li>Symptomatic treatment</li> </ul>	<p><b>Symptoms improve/resolve (grade 0/1):</b> Resume ipilimumab</p> <p><b>Symptoms persist for &gt; 5-7 days, worsen, or recur:</b></p> <ul style="list-style-type: none"> <li>Moderate to high dose steroids PO (e.g., prednisone 0.5 - 1 mg/kg/day)</li> <li>Continue to hold/delay ipilimumab until grade 1</li> <li>When symptoms are grade 1 or less slowly taper steroids over at least 1 month and resume ipilimumab.</li> </ul> <p><b>Symptoms worsen:</b> Treat as grade 3/4</p>
<b>Grade 3-4</b> <u>Diarrhea (G3*):</u> ≥ 7 stools per day over baseline; incontinence; IV fluids ≥ 24 hrs; interfering with ADL <u>Colitis (G3*):</u> fever, ileus, peritoneal signs	<ul style="list-style-type: none"> <li>Permanently discontinue ipilimumab</li> <li>High dose IV steroids (e.g., methylprednisolone 1-2 mg/kg/day)</li> <li>Consider endoscopy</li> </ul>	<p><b>Symptoms improve:</b></p> <ul style="list-style-type: none"> <li>Continue steroids (same dose) until grade 1</li> <li>Then taper over at least 1 month</li> </ul> <p><b>Symptoms persist 3-5 days, or recur after improvement:</b></p> <ul style="list-style-type: none"> <li>1 dose* of infliximab 5 mg/kg (if no contraindication)</li> </ul>

Patients on IV steroids may be switched to oral corticosteroid (e.g., prednisone) at an equivalent dose at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of PO corticosteroids.

