Pembrolizumab Prior to Surgery for Stage 1B, 2 or 3A Non-small Cell Lung Cancer (NSCLC): A Phase II Study
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Statement of Compliance and Signature Page

STUDY TITLE: Pembrolizumab prior to surgery for stage 1B, 2 or 3A Non-Small Cell Lung Cancer (NSCLC): A Phase II Study.

This study will be conducted in compliance with the protocol approved by the Institutional Review Boards of Duke University Health System, Mayo Clinic Medical Center, Dartmouth-Hitchcock Medical Center, and the University of Chicago Medical Center, according to Good Clinical Practice standards. No deviation from the protocol will be implemented without the prior review and approval of the IRB except where it may be necessary to eliminate an immediate hazard to a research subject. In such case, the deviation will be reported to the IRB as soon as possible. The signature below constitutes the approval (by the PI) of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local and state legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

Duke Sponsor Investigator: Neal Ready, MD
Duke University Thoracic Oncology Program

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Neal Ready, MD, Duke Sponsor Investigator **Date**

_____ _____
Site Principal Investigator **Date**

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List of Abbreviations

ADCC	Antibody-Dependent Cellular Cytotoxicity
AE	Adverse Event
ALC	Absolute Lymphocyte Count
ALT	Alanine Transaminase
ANC/AGC	Absolute Neutrophil Count/Absolute Granulocyte Count
APC	Antigen Presenting Cell
AST	Aspartate Transaminase
AUC	Area Under the Curve
BMS	Bristol-Myers Squibb Company
BORR	Best Objective Response Rate
BSA	Body Surface Area
BSC	Best Supportive Care
BW	Body Weight
CDCC	Complement-Dependent Cellular Cytotoxicity
CL	Systemic Clearance
CMax	Peak Concentration
CMin	Trough Concentration
CR	Complete Response
CRF	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.
CRT	Chemotherapy-Radiation Therapy
CRU	Clinical Research Unit
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events; National Cancer Institute, Version 4.0
CTLA4	Cytotoxic T-lymphocyte Antigen
DCI	Duke Cancer Institute
DCR	Disease Control Rate
DOCR	Duke Office of Clinical Research
DUHS/DUMC	Duke University Health System/Duke University Medical Center
ECOG PS	Eastern Cooperative Oncology Group Performance Status
ECL	Electrochemiluminescent
ECRF	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.
Enroll/Randomize	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.
Enter/Consent	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
GI	Gastrointestinal
HepB	Hepatitis B

HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator Brochure
ICD/ICF	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.
irAE	Immune-Related Adverse Event
IRB/ERB	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.
irRC	Immune-Related Response Criteria
ITT	Intention to Treat
IV	Intravenous, usually referring to a medication or substance given into a vein.
LDH	Lactate Dehydrogenase
LFT	Liver Function Test
mAB, mAb	Monoclonal Antibody
MDSC	Myeloid-Derived Suppressor Cells
mg	milligram: 1/100 of a gram
m^2	meters squared
NCA	Non-Compartmental Analysis
NCCN	National Comprehensive Cancer Network
NSCLC	Non-Small Cell Lung Cancer
OS	Overall Survival
Patient	A subject with a defined disease
PBMC	Peripheral Blood Mononuclear Cells
PFS	Progression Free Survival
PD	Progressive Disease
PET	Positron Emission Tomography
PK	Pharmacokinetic
PPK	Population Pharmacokinetics
PR	Partial Response
RECIST	Response Evaluation Criteria in Solid Tumor
RT	Radiation Therapy/Radiotherapy
SAE	Serious Adverse Event
SD	Stable Disease
TAA	Tumor Associated Antigens
TIL	Tumor Infiltrating Lymphocytes
TNM Staging	Tumor, Node and Metastasis Staging
Treg	Regulatory T Cells
Wt	Weight

STUDY SCHEMA

STUDY DESIGN

This is a 30 patient single arm, open label, phase 2 trial adding neoadjuvant and adjuvant pembrolizumab to surgery and standard post-operative therapy in early stage non-small cell lung cancer. Patients with clinical stage IB (> 3cm), II, or IIIA (N0-2) NSCLC and no prior therapy for their current diagnosis of lung cancer will be eligible for the study. Patients will receive two cycles of neoadjuvant pembrolizumab. Patients will undergo standard surgical resection of their lung cancer as deemed appropriate by their surgeon, followed by standard adjuvant chemotherapy (+/- radiation therapy) for their lung cancer as deemed appropriate by their medical oncologist. Patients will receive 4 cycles of adjuvant pembrolizumab after the completion of all standard therapy. Follow-up after completion of therapy will be per institutional standard practice.

PATIENT ELIGIBILITY

- All patients must have histologically documented clinical stage IB ($\geq$ 3cm), IIA/IIB, or IIIA (N0-2) NSCLC.
- **NO** prior chemotherapy, radiation therapy or biologic/targeted therapy for current diagnosis NSCLC.
- ECOG Performance Status of 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.
- Patient has provided informed consent and has signed Informed Consent Form.
- No autoimmune disease that would constitute contraindication to receive pembrolizumab.

REQUIRED LABORATORY DATA

- Hemoglobin $\geq$ 9 g/dL or $\geq$5.6 mmol/L without transfusion
  ANC/AGC $\geq$ 1500/μL
- Platelets $\geq$ 100,000/μL
- Creatinine $\leq$ 1.5 X ULN or creatinine clearance $\geq$ 60 ml/min
- AST/ALT $\leq$2.5x ULN
- Bilirubin $\leq$ 2.0 x institutional ULN
- INR or PT $\leq$1.5 x ULN unless on anticoagulants
  aPTT $\leq$ 1.5 x ULN unless on anticoagulants

Neoadjuvant Treatment:
Pembrolizumab: 200 mg IV over 30 minutes on days 1 & 22 (every 21 days x 2 cycles)
Surgery: Standard surgical evaluation to occur day 29 to day 56 (at least 2 days after second dose of Pembrolizumab)

Adjuvant Treatment:
Chemotherapy: Standard adjuvant chemotherapy as per treating medical oncologist.

Radiation: Standard adjuvant post-operative radiation therapy in selected patients for standard clinical indications such as N2 node metastases and /or close/positive tumor surgical margin.

Pembrolizumab: 200 mg IV over 30 minutes every 21 days times 4 cycles

- Patients who receive both adj CT plus Adj RT should start Pembrolizumab within 8 months of surgery.
- Patients who receive only one modality of adj therapy (e.g. CT alone or RT alone) should start Pembrolizumab within 6 months of surgery
- Patients who do not receive any additional therapy should start Pembrolizumab within 4 months of surgery.

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

1) Tumor samples will be analyzed for tumor infiltrating T cells with specificities against tumor associated antigens. Tumor samples will also be evaluated to monitor populations of infiltrating regulatory T cells, myeloid-derived suppressor cells (MDSC), as well as activated and exhausted T cells.

2) Blood samples will be analyzed for circulating T cells with specificities against tumor associated antigens: prior to treatment, before surgery, after surgery, and after completion of adjuvant pembrolizumab. Blood samples will also be analyzed to monitor circulating populations of regulatory T cells, myeloid-derived suppressor cells (MDSC), as well as activated and exhausted T cells.

1.0 INTRODUCTION

1.1 Lung Cancer Surgery

Surgery can be curative in early stage non-small cell lung cancer (NSCLC); however, there is still a significant chance of relapse. Five year survival in surgical pathologic stage IIA (T1N1M0) and IIB (T2N1M0) disease is 40-63% and 38-45%, respectively. (1) The clinical implications are that pathologically staged patients with early stage tumors (17-32% stage IA, 35-47% stage IB, 37-60% stage IIA, and 55-62% stage IIB) recur because they harbor regional or distant micro-metastasis outside their surgical resection.

1.2 PD1 Checkpoint, Pembrolizumab and Lung Cancer

Programmed death receptor 1 (PD-1) is an important negative immune checkpoint that helps prevent autoimmunity (8, 9). In the setting of inflammation, PD-1 can be activated through interaction with a ligand such as program death receptor ligand 1 or 2 (PDL-1, PDL-2). Activation of PD-1 leads to T cell energy, exhaustion, and/or death. Tumor cells themselves, tumor infiltrating lymphocytes, and/or other cells in the inflamed tumor microenvironment can express PDL, activate PD-1, and inhibit the T cell immune response against the tumor. Masking antibodies to PD-1 or PDL have been developed for cancer therapy that prevents PD1 checkpoint immune inhibition.

Pembrolizumab is a programmed death receptor 1 (PD-1) masking antibody that has been approved for treatment of metastatic malignant melanoma, previously treated advanced stage squamous cell carcinoma of the head and neck, and for previously treated, advanced stage PDL- positive non-small cell lung cancer. Pembrolizumab has shown single agent activity in previously treated and untreated advanced stage non-small cell lung cancer (10, 11,12,13). In a phase 1 trial treating different tumor types, some patients with heavily pretreated lung cancer had deep and durable responses to single agent pembrolizumab therapy (12). Durable responses were seen in both PD-L1-negative and positive tumors (12). Based on the single agent activity of pembrolizumab in previously treated NSCLC, pembrolizumab was given breakthrough therapy designation by the FDA for the treatment of patients with non-small cell lung cancer whose disease has progressed on or following platinum-based chemotherapy. A randomized phase 3 trial comparing docetaxel to pembrolizumab in previously treated non-small cell lung cancer has shown superior survival for pembrolizumab at different levels of tumor PD-L1 positivity (13). A large, randomized, phase 3 trial has been completed comparing pembrolizumab to platinum based chemotherapy in untreated advanced stage, PD-L1-positive non-small cell lung cancer and the results have not yet been presented (14). Additional trials are planned or ongoing to study pembrolizumab in early stage non-small cell lung cancer in the adjuvant or neoadjuvant setting. The presence of program death receptor ligand (PDL) on tumor cells is a candidate predictive biomarker for pembrolizumab that may enrich therapeutic benefit. However, all the data thus far suggest that PDL negative lung cancer tumors can respond to PD-1 checkpoint therapy. Thus, it is not appropriate to exclude PDL negative tumors from clinical trials studying PD-

1 checkpoint therapy. Pembrolizumab (Keytruda™) has recently been approved in the US for the treatment of patients with previously treated, metastatic non-small cell lung cancer in which the cancer is PD-L1 biomarker positive.

1.3 Longitudinal Analyses of T Cell and MDSC Phenotypes

Immunomodulatory cells are present in the peripheral blood. Pembrolizumab has activity in NSCLC through the masking of PD-1 and removal of T cell inhibition through the PD-1 checkpoint. It is our hypothesis that characteristics of the immunomodulatory cells in the blood may be associated with clinical response of NSCLC to pembrolizumab and/or autoimmune adverse events to pembrolizumab.

Phenotypic and functional characterization of T cell populations will be performed on peripheral blood mononuclear cells (PBMC), as well as TILs. It will be of critical importance to better understand the impact of pembrolizumab therapy on: 1) regulatory T cells (Tregs), 2) myeloid-derived suppressor cells (MDSC), 3) T cell activation, and 4) T cell exhaustion.

We will determine if the immunomodulatory effects of the protocol treatment 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 multiple time points. A Wilcoxon rank sum 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.

We will 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 exact confidence interval will be estimated.

1.4 Tumor Infiltrating Lymphocytes (TILs) with Specificities Against Tumor Associated Antigens

The presumed mechanism of action for pembrolizumab is the removal of T lymphocyte inhibition by masking the PD-1 receptor. Our hypothesis is that the masking of the PD-1 receptor by pembrolizumab results in the activation and proliferation of T lymphocytes with specificities against tumor associated antigens. In untreated lung cancer tumors, we would expect few tumors to have TIL cells with specificities against tumor associated antigens. Based on the response rate to pembrolizumab in advanced lung cancer, we hypothesize that at least 20% of lung cancers would have TIL cells with specificities against tumor associated antigens after pembrolizumab therapy.

Fresh tumor samples will be collected at the time of surgical resection for functional TILs and processed per appendices H or I. For each arm, we will determine the percentage of patients with “detectable”

(percentage of ≥ 0.05% with each value also being at least twice that of the background unstimulated control value) tumor infiltrating lymphocytes (TILs) after protocol treatment. The percentage of patients with TILs for patients who have surgical resection and its exact confidence interval will be estimated.

Tumor tissue will be evaluated to determine what antigens commonly expressed in NSCLC are present. The TILs for that tumor will then be analyzed to determine what tumor antigens the TILs are activated against.

We will evaluate whether presence, quantity or quality of detectable TILs is associated with pathologic response to neoadjuvant therapy. 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.

1.5 Biomarkers Based on Blood Profiling

Pembrolizumab has activity in NSCLC through the masking of PD-1 and removal of T cell inhibition through the PD-1 checkpoint. It is our hypothesis that characteristics of blood based biomarkers associated with inflammation and/or immune function may be associated with clinical response of NSCLC to pembrolizumab and/or autoimmune adverse events to pembrolizumab.

The objective of the blood profiling study is to measure circulating levels of cytokines from blood specimens drawn as part of the study before neoadjuvant therapy, after neoadjuvant therapy, after surgery, and after adjuvant pembrolizumab. We will investigate the characteristics of blood analytes over time using exploratory plots and descriptive statistics to assess the prognostic value of the biomarkers on response rate, PFS, OS, and adverse events.

1.6 Rationale for Using Immune-Related Tumor Assessment

Pembrolizumab is an immune-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 (e.g., mWHO) may be inadequate to realize the kinetics and magnitude of clinical benefit achieved with pembrolizumab.

Histopathologic evidence has demonstrated pembrolizumab 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.

2.0 BACKGROUND & RATIONALE

2.1 Background

Refer to the Investigator's Brochure (IB)/approved labeling for detailed background information on pembrolizumab (MK-3475).

2.2 Pharmaceutical and Therapeutic Background

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.
The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to

PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma. This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Keytruda™ (pembrolizumab) has recently been approved in the United Stated for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilumumab and, if BRAF V600 mutation positive, a BRAF inhibitor. Keytruda (pembolizumab) has also been approved in the United States for patients with previously treated, metastatic non-small cell lung cancer in which the cancer is PD-L biomarker positive.

2.3 Preclinical and Clinical Trial Data

Refer to the Investigator's Brochure for Preclinical and Clinical data.

2.4 Rationale for Dose Selection/Regimen/Modification

An open-label Phase I trial (Protocol 001) was conducted to evaluate the safety and clinical activity of single agent pembrolizumab (MK-3475) (pembrolizumab). The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of MK-3475 (pembrolizumab) showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD has been identified to date. Recent data from other clinical studies within the MK-3475 (pembrolizumab) program has shown that a lower dose of MK-3475 (pembrolizumab) and a less frequent schedule may be sufficient for target engagement and clinical activity.

PK data analysis of MK-3475 (pembrolizumab) administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life (refer to IB). Pharmacodynamics data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days). This early PK and pharmacodynamics data provides scientific rationale for testing a Q2W and Q3W dosing schedule.

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of MK-3475 (pembrolizumab) were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. MK-3475 (pembrolizumab) has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg Q3W body weight-based regimen are anticipated to remain well within the established exposure margins of 0.5 – 5.0 for MK-3475 (pembrolizumab) in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. the proposed dose regimen of 2 mg/kg Q3W (i.e. 5-fold higher dose and exposure). The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

The rationale for further exploration of 2 mg/kg and comparable doses of pembrolizumab in solid tumors is based on: 1) similar efficacy and safety of pembrolizumab when dosed at either 2 mg/kg or 10 mg/kg Q3W in melanoma patients, 2) the flat exposure-response relationships of pembrolizumab for both efficacy and safety in the dose ranges of 2 mg/kg Q3W to 10 mg/kg Q3W, 3) the lack of effect of tumor burden or indication on distribution behavior of pembrolizumab (as assessed by the population PK model) and 4) the assumption that the dynamics of pembrolizumab target engagement will not vary meaningfully with tumor type.

The choice of the 200 mg Q3W as an appropriate dose for the switch to fixed dosing is based on simulations performed using the population PK model of pembrolizumab showing that the fixed dose of 200 mg every 3 weeks will provide exposures that 1) are optimally consistent with those obtained with the 2 mg/kg dose every 3 weeks, 2) will maintain individual patient exposures in the exposure range established in melanoma as associated with maximal efficacy response and 3) will maintain individual patients exposure in the exposure range established in melanoma that are well tolerated and safe.

A fixed dose regimen will simplify the dosing regimen to be more convenient for physicians and to reduce potential for dosing errors. A fixed dosing scheme will also reduce complexity in the logistical chain at treatment facilities and reduce wastage.

3.0 STUDY RATIONALE

Studying neoadjuvant pembrolizumab therapy is an attractive strategy for studying the immunologic changes caused by PD-1 checkpoint masking. Most of the immunologic activity associated with pembrolizumab occurs in the tumor and surrounding microenvironment. Evaluation of post-pembrolizumab tumor will be important to understanding factors associated with pembrolizumab activity, immune tolerance, and discovery of other targets for immune therapy. Pembrolizumab has

known benefit in non-small cell lung cancer. The addition of pembrolizumab for two doses prior to surgery and four doses after surgery has the potential to confer clinical benefit. Large randomized phase 3 trials are now testing whether PD-1 checkpoint antibodies improve survival as adjuvant therapy after resection of early stage lung cancer.

At Duke Medical Center, we are conducting an investigator-initiated, neoadjuvant trial of carboplatin, paclitaxel, and ipilimumab in early stage lung cancer. In cases in which there was excess tumor available at surgery, the laboratory of Kent Weinhold PhD has been able to disaggregate ample quantities of functional tumor infiltrating lymphocytes (TILs) to fully characterize the TIL population and ultimately determine what antigens the TILs are activated against. In other studies, in which Duke Cancer Institute has been a coordinating center, tumor specimens have been shipped on wet ice to arrive within 24 hours of surgery and functional TILs have been successfully collected as part of a multi-center trial in glioblastoma mulitforme. One of the most important exploratory, secondary objectives of this trial will be to investigate the methods that would allow assessment of immunologic response to pembrolizumab in the tumor, discover markers of immune therapy resistance, and discover new targets for immune therapy.

3.1 Trial Summary

Abbreviated Title	TOP 1501
Trial Phase	II
Clinical Indication	Stage IB, II or IIIA NSCLC (tumor≥3 cm)
Trial Type	Therapeutic treatment
Type of control	Open Label- no treatment control
Route of administration	Intravenous
Trial Blinding	Unblinded, open-label
Treatment Groups	Single Arm Neoadjuvant: Pembrolizumab 200mg every 21 days' x 2 cycles Surgery Standard adjuvant chemotherapy up to 4 cycles Post-operative radiation as needed as per institutional standard Adjuvant: Pembrolizumab 200 mg every 21 days for 4 cycles
Number of trial subjects	32
Estimated enrollment period	Approximately 3 years; first patient in third quarter 2016
Estimated duration of trial	Estimated at 5 years from the first patient in to last subject's final 2 year follow-up
Duration of Participation	Active treatment for approximately 10-17 months followed by 5 years follow-up

3.2 Trial Design

This a multi-institution phase 2 trial studying 2 doses of pembrolizumab prior to surgery and 4 doses after surgery for stage IB, II or IIIA non-small cell lung cancer. Patients will undergo standard diagnostic workup and pre-surgical staging for early stage lung cancer. Patients with stage IB (> 3 cm), II or IIIA lung cancer will be eligible. We will require that the primary tumor be> 3 cm in order to improve the chances that there will be adequate excess tumor after surgery for planned correlative science studies.

Studying the tumor microenvironment after pembrolizumab is an important secondary endpoint of this trial. Therefore an additional eligibility requirement regardless of the lung cancer stage will be that the primary tumor will need to be > 3 cm by pre-treatment radiographic measurement. For instance, a T1, N1, Mx stage lung cancer would not be eligible for this trial. The requirement that the tumor is > 3 cm will increase the chances that there will be enough excess tumor at surgery to collect for correlative science studies. It will not be possible to know if N1 nodes are pathologic by clinical staging so requiring tumors to be > 3 cm by clinical staging also creates a study population that has been shown to benefit from preoperative or postoperative systemic therapy.

Eligible patients will have ECOG PS of 0-1, good organ function, and no history of active autoimmune disease. Patients will receive 2 doses pembrolizumab on days 1 and 22. Patients will undergo standard surgical resection approximately 29 – 56 days after initiation of pembrolizumab and at least 2 days after the second dose of pembrolizumab. After recovery from surgery, patients will be evaluated to receive standard chemotherapy.

Patients may receive post-operative radiation according to standard clinical indications such as close or positive tumor margin, or presence of N2 metastases. After completion of all standard therapy patients may receive 4 cycles of adjuvant pembrolizumab. Patients will have follow-up as per institutional standard.

3.2.1 Overall Risk/Benefit Assessment

The unique immune-based mechanism of action is reflected in the clinical patterns of anti-cancer activity in some patients. Pembrolizumab 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 pembrolizumab 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 pembrolizumab results in durable disease control, sometimes with novel patterns of response, which contribute to its improvement of 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. 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 in order to initiate steroid therapy promptly 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 pembrolizumab-related GI toxicities, hepatotoxicity, endocrinopathy, and neuropathy are provided in the appendices of the current IB.

In summary, pembrolizumab offers clinically meaningful and statistically significant survival benefit to patients with pre-treated advanced melanoma. Pembrolizumab has shown benefit in NSCLC and has been approved by the FDA for previously treated metastatic non-small cell lung cancer. 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, pembrolizumab has the potential to have additive therapeutic benefit when added to standard surgery and adjuvant 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.

4.0 OBJECTIVES

4.1 Primary objective

The primary objective of the single arm phase II trial is to determine whether the surgery feasibility rate of neoadjuvant pembrolizumab is not significantly worse than the historical surgical feasibility rate of neoadjuvant chemotherapy. Surgery feasibility rate of a neoadjuvant therapy is the percent of

patients who are able to undertake surgery after the neoadjuvant therapy. A total of 30 eligible patients will be registered to the single arm trial. Taking 5% rate of cancellation and ineligibility into account, the trial will enroll a total of 32 patients and eligible subjects (not screen failures) will be followed for 2 years for both disease recurrence and death.

Primary Objective Sample Size Justification

According to Pisters et al (JCO 2010), approximately 80% of subjects undergo surgical exploration within 42 days of day 1 of the last cycle of standard neoadjuvant chemotherapy. We would like to test the following hypotheses:

$$H_0: p >= 0.80 \text{ vs. } Ha: p <= 0.65$$

where p is the true surgery feasibility rates after neoadjuvant pembrolizumab. With 30 evaluable patients receiving protocol treatment, the study has approximately 84.6% power rejecting H_0: p>=0.80 when p=0.65 at one-sided significance level of 15%.

4.2 Secondary Objectives

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. However, if a statistical test on a secondary outcome is significant at the 2-sided significance level of 0.01, the finding will be considered worthy of future investigation.

1) We will estimate the rate of objective response rate for the protocol treatment. 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.

2) We will evaluate disease-free survival and patterns of metastases after protocol treatment. 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 confidence interval. The frequencies of metastases by site will be tabulated.

3) Blood-based biomarkers will be evaluated for changes before and after protocol treatment. The association of the baseline value and the changes of these biomarkers with clinical outcomes, such as objective response, overall survival and disease free survival, will be evaluated using logistics regression and Cox models.

4) We will determine the percentage of patients with "detectable" (percentage of ≥ 0.05% with each value also being at least twice that of the background unstimulated control value) tumor infiltrating lymphocytes (TILs) after protocol treatment. The percentage of patients with TILs for patients who have surgical resection and its exact confidence interval will also be estimated.

5) We will evaluate whether presence, quantity or quality of detectable TILs is associated with pathologic response to neoadjuvant therapy. 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.

6) Treatment-related adverse evaluate will be summarized by type and grade.

7) The proportion of patients with detectable circulating T cells specific against TAA after the protocol treatment will be estimated and its confidence interval will be provided. An exact binominal test will be used to test the increase in the proportion of patients with detectable circulating T cells specific against TAA after the protocol treatment relative to baseline.

8) Estimate the rate of pathologic response rate for neoadjuvant pembrolizumab in early stage NSCLC. The pathologic response rate along with its 95% exact confidence interval will be estimated.

9) Determine if the immunomodulatory effects of neoadjuvant chemotherapy plus pembrolizumab 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 4 time points: baseline, after second dose pembrolizumab, after surgery, and after completion adjuvant pembrolizumab. A Wilcoxon rank sum 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.

10) 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.

11) Perform gene expression analysis on tumor to elucidate genes associated with function and modulation of the PD-1/PD-L1 axis.

5.0 STUDY PLAN /DESIGN

Standard diagnostic and staging work up will be performed prior to or during surgery according to institutional standard practice including: pathologic/histologic diagnosis of non-small cell lung cancer, PET/CT scan, brain imaging, and if clinically indicated, mediastinoscopy. Two doses of neoadjuvant pembrolizumab will be given. Standard surgical evaluation and surgery will be performed following completion of 2 cycles of neoadjuvant pembrolizumab therapy.

After surgery, patients who are appropriate candidates will receive adjuvant chemotherapy, adjuvant radiation, and follow-up as per standard of care and institutional best practice. After all standard therapy, patients will receive 4 additional cycles of pembrolizumab every 21 days for 4 cycles. Patients who are not candidates for adjuvant therapy or who refuse may receive four cycles of adjuvant pembrolizumab.

All post-surgical therapy is as per institutional standard of care and is not study specific. Post-surgery chemotherapy, radiation may be given through the patient's primary oncology team as per the wishes of the patient. This allows a patient to have neoadjuvant pembrolizumab and surgery at an institution participating in this study, receive standard chemotherapy and radiation therapy near home, and return to the institution participating in the study to receive adjuvant pembrolizumab. Patients will be seen for a visit approximately 30 days after the last dose of pembrolizumab and follow-up will continue until pembrolizumab related side effects are grade 1, or Investigator deems stable. Patients will be followed for outcome for up to 7 years. Long term outcome data may be collected by phone.

5.1 Correlative Science Measures

(refer to Appendix B for further details on the correlative science outcomes)

5.1.1 Longitudinal Analyses of T Cell and MDSC Phenotypes:

Immunomodulatory cells are present in the peripheral blood. Pembrolizumab has activity in NSCLC through the masking of PD-1 and removal of T cell inhibition through the PD1 checkpoint. It is our hypothesis that characteristics of the immunomodulatory cells in the blood may be associated with clinical response of NSCLC to pembrolizumab and/or immune-related adverse events to pembrolizumab.

Phenotypic and functional characterization of T cell populations will be performed on peripheral blood mononuclear cells (PBMC), as well as TILs. It will be of critical importance to better understand the impact of pembrolizumab therapy on: 1) regulatory T cells (Tregs), 2) myeloid-derived suppressor cells (MDSC), 3) T cell activation, and 4) T cell exhaustion.

We will determine if the immunomodulatory effects of the protocol treatment 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 multiple time points. A Wilcoxon rank sum 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.

We will 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 exact confidence interval will be estimated.

5.1.2 TILS

Tumor infiltrating lymphocytes (TILs) with specificities against tumor associated antigens:

The presumed mechanism of action for pembrolizumab is the removal of T lymphocyte inhibition by masking the PD-1 receptor. Our hypothesis is that the masking of the PD-1 receptor by pembrolizumab results in the activation and proliferation of T lymphocytes with specificities against tumor associated antigens. In untreated lung cancer tumors, we would expect few tumors to have TIL cells with specificities against tumor associated antigens. Based on the response rate to pembrolizumab in advanced lung cancer, we hypothesize that at least 20% of lung cancers would have TIL cells with specificities against tumor associated antigens after pembrolizumab therapy.

Fresh tumor samples will be shipped on wet ice for collection of functional TILs within 24 hours of surgery. We will 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 protocol treatment. The percentage of patients with TILs for patients who have surgical resection and its exact confidence interval will be estimated.

Tumor tissue will be evaluated to determine what antigens commonly expressed in NSCLC are present. The TILs for that tumor will then be analyzed to determine what tumor antigens the TILs are activated against.

We will evaluate whether presence, quantity or quality of detectable TILs is associated with pathologic response to neoadjuvant therapy. 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.

5.1.3 Biomarkers based on blood profiling

Pembrolizumab has activity in NSCLC through the masking of PD-1 and removal of T cell inhibition through the PD1 checkpoint. It is our hypothesis that characteristics of blood based biomarkers associated with inflammation and/or immune function may be associated with clinical response of NSCLC to pembrolizumab and/or autoimmune adverse events to pembrolizumab.
The objective of the blood profiling study is to measure circulating levels of cytokines from blood specimens drawn as part of study design before neoadjuvant therapy, after neoadjuvant therapy, after surgery, and following adjuvant pembrolizumab. We will investigate the characteristics of blood analytes over time using exploratory plots and descriptive statistics to assess the prognostic value of the biomarkers on response rate, PFS, OS, and adverse events.

6.0 PATIENT 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 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, possible benefits and alternatives to study participation.

6.1 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.

6.2 Inclusion Criteria

Patients are eligible to be included in the study only if they meet **all** of the following criteria. For time limits on imaging and blood tests see study calendar:

1. Histologically cytologically confirmed NSCLC.
2. Clinical stage IB (≥3cm per CT), Stage IIA/IIB, or Stage IIIA (N0-2) amenable to surgical resection.
3. Primary tumor ≥ 3 cm (for all stages entered) to increase the likelihood that excess tumor will be available after resection.
4. Patient must be deemed a surgical candidate as documented by surgeon within their respective institutional standards.
5. ECOG performance status of 0 or 1 (Appendix C).
6. NO prior chemotherapy, radiation therapy or biologic/targeted therapy for current diagnosis of lung cancer.
7. Age ≥18 years.
8. No active invasive malignancy in the past 2 years other than non-melanoma skin cancer. Cancers that are in-situ are not considered invasive.
9. Signed written informed consent including HIPAA according to institutional guidelines.
10. Adequate Organ Function:

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC) or AGC	≥1500 per uL
Platelets	≥100,000 per uL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L without transfusion or EPO dependency (within 7 days of assessment)
Renal	

Serum creatinine **OR** Measured or calculated creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN
Albumin	≥2.5 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Creatinine clearance should be calculated per institutional standard. (Appendix D)	

11. Females of child-bearing potential (not surgically sterilized or postmenopausal [a woman who is ≥ 45 years of age and has not had menses for greater than 1 year]) 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 120 days 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.
12. Patients must agree to research blood sampling to participate in study.
13. Have measurable disease based on RECIST 1.1.
14. FEV1 and DLCO ≥ 40% predicted (or per institutional standard).

6.3 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 or used an investigational device within 4 weeks of the first dose of treatment.
2. Has a known history of active TB (Bacillus Tuberculosis).
3. Hypersensitivity to pembrolizumab or any of its excipients.
4. Concurrent administration of any other anti-tumor therapy.
5. Has received prior therapy with an anti-PD-1, anti-PD-L-1, or anti-PD-L2 agent.
6. Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative].
7. Inability to comply with protocol or study procedures.
8. Active infection requiring antibiotics, antifungal or antiviral agents, that in the opinion of the investigator would compromise the patient's ability to tolerate therapy.
9. Has known history of, or any evidence of active, non-infectious pneumonitis that required steroids (steroid treatment of COPD or asthma allowed).
10. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).
11. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (e.g. thyroxine, insulin or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency etc.) is not considered a form of system treatment. Patients with a history of inflammatory bowel disease, including ulcerative colitis and Crohn's Disease, are excluded from this study, as are patients with a history of symptomatic disease (e.g., rheumatoid arthritis, systemic progressive sclerosis [scleroderma], systemic lupus erythematosus, autoimmune vasculitis [e.g., Wegener's Granulomatosis]); motor neuropathy considered of autoimmune origin (e.g. Guillain-Barre Syndrome and Myasthenia Gravis).
12. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment.
13. Has a known additional invasive malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer.
14. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment.
15. Has had major surgery (other than definitive lung cancer surgery) within two weeks of study or other serious concomitant 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.
16. Has received any non-oncology vaccine therapy used for prevention of infectious diseases (for up to 30 days before or after any dose of pembrolizumab). *Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however, intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines and are not allowed.*

17. Has history of 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. Patients with CAD recently treated with surgery and/or stent, if stable without symptomatic angina pectoris, active ischemia are eligible.
18. Has evidence or a history of interstitial lung disease.
19. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
20. Prisoners or subjects who are compulsorily detained involuntarily incarcerated) for treatment of either psychiatric or physical (e.g., infectious) illness.

6.4 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. For patients enrolled at Duke Cancer Institute, patient information will also be entered into MaestroCare at the time of enrollment, linking the patient to the research subject record (RSH), to the study encounter and the timeline (if applicable) prior to the close of the study encounter.

8.1 Registration Procedure for Outside Sites

All patients signing informed consent at outside sites must be registered with Duke (including screen failures – indicate screen failure status on last page of eligibility checklist). Redacted enrollment packet will be completed by the investigator and/or designee and faxed to the Thoracic Oncology Protocol Office at 919-684-8926 at the time of registration. This includes:

- TOP 1501 Eligibility Checklist
- Signed informed consent

Patients will be enrolled only after all pre-treatment evaluations are completed and all eligibility criteria are met and confirmed by Duke.

9.0 STUDY PERIODS (Visit Requirements)

The Study Schedule (Appendix A) summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

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 ±3 days of scheduled visits.

Furthermore, additional evaluations/testing may be deemed necessary by the Sponsor and/or Merck for reasons related to subject safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g., HIV, Hepatitis C, etc.), and local regulations may require that additional informed consent be obtained from the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations.

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. Refer to Table 5 for specific laboratory test information.

- Informed Consent
- Eligibility
- Medical history including demographics/smoking history
- Height and weight (BSA calculation)
- Vital signs (temp, B/P, pulse)
- ECOG Performance status
- Physical exam
- Routine blood work (CBC, Chemistries)
- Calculated creatinine clearance
- Thyroid Functions (T3, FT4 & TSH)
- Coagulation (aPTT, PT(INR)
- Routine Urinalysis
- Negative Pregnancy test for WOCBP only
- EKG

- Tumor Histology
- Medical and Surgical Evaluation
- Staging PET/CT of chest/abdomen, Brain MRI or CT (as per standard of care) These may be obtained within 42 days
- Tumor measurement to ensure primary lesion $\geq$ 3 cm
- PFTs (FEV1 and DLCO)
- Research blood (1st Collection): blood will be collected to assess longitudinal analyses of T cell and MDSC phenotypes and blood based biomarkers. 4 tubes of 8.5-ml each will be collected. The tubes used will be ACD anti-coagulated Vacutainer (yellow tops). Samples will be collected prior to pembrolizumab initiation (may be collected Day 1 cycle 1 pre-infusion), and transported/shipped to the Substrate Services Core lab for processing. (Refer to Appendices F and G)

9.2 Neoadjuvant Pembrolizumab Treatment (Cycle 1, Day 1) (To be performed prior to receiving treatment-refer to Appendix A)

- Weight (BSA calculation)
- Vital signs (temp, B/P, pulse)
- ECOG Performance status
- Physical exam
- Adverse events assessment
- Routine blood work
- Pregnancy test $\leq$ 3 days (72 hrs.) prior to first dose of pembrolizumab (for WOCBP only)
- Dose # 1 Pembrolizumab 200 mg IV

9.3 Neoadjuvant Pembrolizumab Treatment (Cycle 2, Day 22)

- Weight (BSA calculation)
- Vital signs (temp, B/P, pulse)
- ECOG Performance Status
- Physical exam
- Adverse events assessment
- Routine blood work
- Dose #2 Pembrolizumab

9.4 Evaluation at Completion of Neoadjuvant Pembrolizumab and Prior to Surgery.

Final visit (if $\leq$1 grade 1 toxicity) for those subjects that come off treatment early for progression of disease, intolerance to protocol therapy, other reasons, or patient withdraws consent. Safety

Evaluation should occur 30 days (+/- 5 days) after last dose of pembrolizumab or before initiation of a new anti-neoplastic cancer treatment (whichever comes first).

- Medical and Surgical evaluation after completion of pembrolizumab
- Weight
- Vital signs (temp, B/P, pulse)
- ECOG Performance status
- Physical Exam
- Routine Blood work
- Thyroid Functions (T3, FT4, & TSH)
- Repeat imaging to evaluate tumor (Chest CT)
- Adverse events assessment
- Research blood (2nd Collection): blood will be collected to assess longitudinal analyses of T cell and MDSC phenotypes and blood based biomarkers. 4 tubes of 8.5-ml each will be collected. The tubes used will be ACD anti-coagulated Vacutainer (yellow tops). Samples will be collected, and transported/shipped to the Substrate Services Core lab for processing. (Refer to Appendices F & G)

9.5 Surgery

Surgery to occur at 4-8 weeks (days 29-56) or subject will be removed from trial unless approved by PI. If pembrolizumab dose 2 is delayed, then there should be at least 2 days between pembrolizumab infusion and surgery.

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. Excess tumor tissue samples will also be collected at time of surgical resection after determination that adequate tumor has been taken by surgical pathology for all standard clinical studies. Specific instructions for processing and shipping are provided in Appendices G & H.

9.6 Post-surgery Follow-Up Visit (_2-6 weeks following surgery)

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 +/_ standard chemotherapy as deemed appropriate by their physician. Subjects who are completely resected or who refuse or cannot receive adjuvant chemotherapy will be given the option of receiving adjuvant Pembrozulimab.

- Vital signs (temp, B/P, pulse)
- Weight
- ECOG performance status
- Physical exam
- Routine blood work
- Adverse events assessment*
- **Research blood sample (3rd collection).** Research blood: blood will be collected to assess longitudinal analyses of T cell and MDSC phenotypes and blood based biomarkers. 4 tubes of 8.5-ml each will be collected. The tubes used will be ACD anti-coagulated Vacutainer (yellow tops). Samples will be collected and transported/shipped to the Substrate Services Core lab for processing. This blood sample to be collected 2-6 weeks after surgery and prior to adjuvant therapy (Refer to Appendices F and G).

*All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Subjects with an AE of grade > 1 will be followed until the resolution of the AE to grade 0-1 or until the beginning of a new anti-neoplastic therapy, whichever occurs first. SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded.

9.7 Post-Surgery Therapy (Adjuvant Standard Chemotherapy +/- Radiation Therapy)

After surgery, patients should be offered all standard therapies including adjuvant chemotherapy and radiation based on the final pathologic stage of the cancer, tumor margins, and other standard clinical criteria. Patients should receive all standard oncology care as deemed appropriate by the treating physician. Since none of the post-surgical therapy is study specific, post-surgical therapy can be given through the primary oncology team.

9.8 Adjuvant Pembrolizumab

Following completion of all standard adjuvant therapy, including chemotherapy and/or radiation therapy, patients will receive 4 cycles of pembrolizumab 200 mg every 21 days. Patients who receive both adjuvant CT plus adjuvant RT should start Pembrolizumab within 8 months of surgery. Patients who receive only one modality of adjuvant therapy (e.g. CT alone or RT alone) should start Pembrolizumab within 6 months of surgery. Patients who do not receive any adjuvant therapy should start Pembrolizumab within 4 months of surgery

9.8.1 Cycle 1 (Day 1)

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

- ECOG Performance status
- Physical exam
- Adverse events assessment
- Routine blood work
- Thyroid functions (T3, FT4, & TSH)
- Pregnancy test ≤ 3 days (72 hrs.) prior to first dose of pembrolizumab for WOCBP only (per institutional guidelines)
- Pembrolizumab 200 mg IV

9.8.2 Cycle 2 (Day 22)

- Weight (BSA calculation)
- Vital signs (temp, B/P, pulse)
- ECOG Performance status
- Physical exam
- Adverse events assessment
- Routine blood work
- Pregnancy test ≤ 3 days (72 hrs.) prior to first dose of pembrolizumab for WOCBP only (per institutional guidelines)
- Pembrolizumab 200 mg IV

9.8.3 Cycle 3 (Day 43)

- Weight (BSA calculation)
- Vital signs (temp, B/P, pulse)
- ECOG Performance status
- Physical exam
- Adverse events assessment
- Routine blood work
- Pregnancy test ≤ 3 days (72 hrs.) prior to first dose of pembrolizumab for WOCBP only (per institutional guidelines)
- Thyroid functions (T3, FT4, & TSH)
- Pembrolizumab 200 mg IV

9.8.4 Cycle 4 (Day 64)

- Weight (BSA calculation)
- Vital signs (temp, B/P, pulse)
- ECOG Performance status
- Physical exam
- Adverse events assessment
- Routine blood work

- Pregnancy test ≤ 3 days (72 hrs.) prior to first dose of pembrolizumab for WOCBP only (per institutional guidelines)
- Pembrolizumab 200 mg IV
-

9.8.5 End of Study Visit (*Safety Follow-up)

End of study evaluation is to occur approximately 30 days or more after last adjuvant Pembrolizumab treatment.

- Vital signs (temp, B/P, pulse)
- Weight
- Performance status
- Physical exam
- Routine blood work
- Thyroid functions (T3, FT4, & TSH)
- Adverse events assessment
- Chest CT if applicable (per radiologic evaluation appendix A)
- Research blood (4th Collection): blood will be collected to assess longitudinal analyses of T cell and MDSC phenotypes and blood based biomarkers. 4 tubes of 8.5-ml each will be collected. The tubes used will be ACD anti-coagulated Vacutainer (yellow tops). Samples will be collected, and transported/shipped to the Substrate Services Core lab for processing (Refer to Appendices F & G).

*All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Subjects with an AE of Grade > 1 related to Pembrolizumab will be followed until the resolutionof the AE to Grade 0-1, or investigator deems stable, or until the beginning of a new anti-neoplastic therapy, whichever occurs first. SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded.

9.8.6 Follow-up

Patients should have follow-up as per standard care of the treating institution. Outcome data (recurrence and survival) will be collected every 3-4 months for 2 years after surgery. Following that, collect follow-up every 6 months for up to five years. Follow-up can occur by primary oncology provider and data can be collected by phone.

Every effort should be made to collect information regarding disease status during this follow-up which may include the start of new anti-neoplastic therapy, disease progression, death, end of the study.

10.0 Sample Procurement and Handling

10.1 Blood Specimens

Blood will be collected 4 times to assess longitudinal analyses of T cell and MDSC phenotypes and other blood based biomarkers.

Samples will be collected to detect 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

(Outside Sites see appendix G for processing/shipping):

Collection 1: Baseline prior to treatment initiation
Collection 2: After dose # 2 pembrolizumab and prior to surgery
Collection 3: Two to six weeks after surgery prior to initiation of adjuvant chemotherapy.
Collection 4: Three to six weeks after last dose of adjuvant pembrolizumab
**** Note: it is imperative tube is filled to the fill line for proper additive to blood ratio. Specimen is not evaluable if not filled to the fill line. If unable to obtain the 4 required tubes, fill as many tubes as possible to the fill line.

******If EOS (End of Study) occurs and no adjuvant treatment with Pembrolizumab has occurred, draw Collection #4 at End of Study visit.

10.2 Surgical Specimens

Patients at Duke will be approached to participate in the "DUHS Biospecimen Repository and Processing Core (BRPC)" eIRB 35974 protocol 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 protocol, tissue will be processed as outlined below.

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 H), 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.

OUTSIDE SITES: Please refer to Appendix I for processing/shipping surgical specimen to Duke to the Tumor Immunology Laboratory for TILs analysis. Note that the ability to obtain high quality TILs from **fresh tissue** is highly dependent on the tissue procurement. Delays in tissue processing usually lead to cell death and RNA degradation. Please carefully observe the precautions/instructions pertaining to shipping.

10.2.1 Genomic Analysis (Slides/Blocks)

Response of tumors to immune therapy is in part related to the expression of novel antigens (neoantigens), abnormal patterns of wild type proteins, and/or viral antigens on the tumors cell surface. Genomic studies of the tumor (optional for the subject) will be of interest for comparison to tumor response, immune related side effects, and functional immune biomarkers. Future studies will utilize FFPE from the tumor resected as part of standard of care to perform relevant genomic analysis on the tumor cells. Future scientific studies would be submitted for scientific and IRB review.

10.2.2 Other Biomarkers (blocks)

To ascertain the impact of PD-L1 status on response and endpoints, PD-L1 analysis will be performed by QUALTEK Molecular Laboratories. Duke and sites will ship formalin-fixed paraffin embedded blocks directly to QUALTEK Molecular Laboratories (Newtown, PA) for analysis of PD-L1. Details about shipment are contained in the QualtekMISP Sample Handling Manual.

11.0 TREATMENT

Merck will provide pembrolizumab 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 un-blinded. In this study, the investigational product is pembrolizumab. 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.

11.1 Treatments Administered

Patients will receive pembrolizumab as follows:**

Neoadjuvant Pembrolizumab: Cycle 1 day 1: pembrolizumab 200 mg IV

Cycle 2 day 1 (21 days after cycle 1 day 1): pembrolizumab 200 mg IV

Pembrolizumab will be administered as a 30-minute IV infusion (treatment cycle may be delayed up to 3 weeks due to toxicity). Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5minutes and +10 minutes is permitted (i.e., allowed infusion time is between 25 and 40 minutes).

Surgery: Standard surgical evaluation to occur after the last dose of pembrolizumab followed by surgical therapy.

Adjuvant chemotherapy: Is a standard consideration as per NCCN guidelines. Patients will be evaluated and offered adjuvant chemotherapy based on standard criteria such as T > 3 cm and/or metastases in N1 and/or N2 lymph nodes. Adjuvant chemotherapy is not study specific and should be given by commercial supply and standard of care.

Adjuvant radiation: Is sometimes a standard consideration as per NCCN guidelines. Patients will be evaluated and offered radiation based on standard criteria such as positive post-operative tumor margin and/or presence of metastases in N2 lymph nodes. Adjuvant radiation is not specific to this study and should be given as per standard of care.

Adjuvant Pembrolizumab: Three to 6 weeks after completion of all standard therapy including adjuvant chemotherapy and/or radiation therapy, patients will receive pembrolizumab 200 mg IV every 21 days for 4 doses.

11.2 Materials and Supplies

Qualified personnel who are familiar with procedures that minimize undue exposure to themselves and to the environment will 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 Pembrolizumab

Pembrolizumab will be intravenously administered only at the investigational site. As a result, patient compliance is ensured.

11.3 Dose Selection/Modification

11.3.1 Dose Selection

The rationale for selection of doses to be used in this trial is provided in Section 2 – Background and Rationale.

Details on preparation and administration of pembrolizumab (MK-3475) are provided in the Pharmacy Manual.

11.3.2 Selection and Timing of Doses

Patients in this study will receive neoadjuvant pembrolizumab on Days 1 and 22, and adjuvant pembrolizumab every 21 days for 4 cycles after completion of all standard therapy.

Trial treatment should be administered on Day 1 of each cycle after all procedures/assessments have been completed as detailed on the Trial Flow Chart (Appendix A). Trial treatment may be administered up to 3 days before or after the scheduled Day 1 of each cycle due to administrative reasons.

All trial treatments will be administered on an outpatient basis. Pembrolizumab 200 mg will be administered as a 30-minute IV infusion every 3 weeks. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

The Pharmacy Manual contains specific instructions for the preparation of the pembrolizumab infusion fluid and administration of infusion solution.

11.4 Pembrolizumab Dose Modifications

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table 1 below.

If pembrolizumab is delayed more than two weeks due to a related AE, then pembrolizumab will be discontinued and patients should proceed to surgical resection when clinically appropriate.

Table 1
Dose Modification Guidelines for Drug-Related Adverse Events

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
Diarrhea/Colitis	2-3	Toxicity resolves to Grade 0-1.	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue
AST, ALT, or Increased Bilirubin	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose.
	3-4	Permanently discontinue (see exception below)	Permanently discontinue
Type 1 diabetes mellitus (if new onset) or Hyperglycemia	T1DM or 3-4	Hold pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure.	Resume pembrolizumab when patients are clinically and metabolically stable.
Hypophysitis	2-4	Toxicity resolves to Grade 0-1 Therapy with Pembrolizumab can be continued while endocrine replacement therapy is instituted	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4		
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue
Hypothyroidism	2-4	Therapy with pembrolizumab can be continued while thyroid	Therapy with pembrolizumab can be continued while thyroid

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
		replacement therapy is instituted	replacement therapy is instituted.
Infusion Reaction	3-4	Permanently discontinue	Permanently discontinue
Pneumonitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	3-4	Permanently discontinue	Permanently discontinue
Renal Failure or Nephritis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	3-4	Permanently discontinue	Permanently discontinue
Myocarditis	1-2	Withhold	Give corticosteroids based on AE severity (pred 1-2mg/kg then taper)
	3-4	Permanently discontinue	
All Other Drug-Related Toxicity[c]	3 or Severe	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue

Note: Permanently discontinue for any severe or Grade 3 drug-related AE that recurs or any life-threatening event.

[a]For patients with liver metastasis who begin treatment with Grade 2 AST or ALT, if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week then patients should be discontinued.

[b] If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose; Refer to – Infusion Treatment Guidelines for further management details.

[c]Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.			

Dosing interruptions are permitted in the case of medical/surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Subjects should resume study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the patient's study record.

11.5 Rescue Medications & Supportive Care

11.5.1 Supportive Care Guidelines

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

Note: if after the evaluation the event is determined not to be related, the investigator is instructed to follow the ECI reporting guidance (Section 20.7).
It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event. Suggested conditional procedures, as appropriate, can be found in the ECI guidance document.

- **Pneumonitis**:
  - For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
  - For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
  - Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.

- **Diarrhea/Colitis**:

Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).

- All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
- For **Grade 2 diarrhea/colitis**, administer oral corticosteroids.
- For **Grade 3 or 4 diarrhea/colitis** treat with intravenous steroids followed by high dose oral steroids.
- When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or ≥ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)**

  - For **T1DM** or **Grade 3-4** Hyperglycemia
    - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.
    - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

- **Hypophysitis**:

  - For **Grade 2** events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
  - For **Grade 3-4** events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hyperthyroidism or Hypothyroidism**:
  Thyroid disorders can occur at any time during treatment. Patients will be monitored for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.

  - **Grade 2** hyperthyroidism events (and **Grade 3-4** hypothyroidism):
    - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.

        - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.

    - **Grade 3-4** hyperthyroidism
        - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hepatic**:
    - For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).
        - Treat with IV or oral corticosteroids
    - For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours.
    - When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

- **Renal Failure or Nephritis**:
    - For **Grade 2** events, treat with corticosteroids.
    - For **Grade 3-4** events, treat with systemic corticosteroids.
    - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Management of Infusion Reactions**: Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.
  Table 2 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab (MK-3475).

Table 2 Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs.	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr. to 50 mL/hr.). Otherwise dosing will be held until symptoms resolve and the subject should be pre-medicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be pre-medicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3:	**Stop Infusion.**	No subsequent dosing

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

11.6 Dose Modifications for Obese Patients

There is no clearly documented adverse impact of treatment of obese patients. Pembrolizumab is being given at a set dose of 200 mg so body weight and/or obesity is not relevant to this study.

12.0 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.

13.0 CONCOMITANT THERAPY/VACCINATIONS

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the Merck Clinical team. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician**.** Any disease progression requiring other forms of specific anti-tumor therapy will be cause for early discontinuation of study therapy. Acceptable and prohibited concomitant therapies are reviewed below.

13.1 Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.

All concomitant medications received within 30 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs as defined in Section 20.5.

13.2 Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase of this trial:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy other than post-operative radiation for lung cancer
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor.

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial. Subjects may receive other medications that the investigator deems to be medically necessary.

The Exclusion Criteria describes other medications which are prohibited in this trial.
There are no prohibited therapies during the Post-Treatment Follow-up Phase.

14.0 DIET/ACTIVITY/OTHER CONSIDERATIONS

14.1 Diet

Subjects should maintain a normal diet unless modifications are required to manage an AE such as diarrhea, nausea or vomiting.

14.2 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm. Non-pregnant, non-breast-feeding women may be enrolled if they are willing to use 2 methods of birth control or are considered highly unlikely to conceive. Highly unlikely to conceive is defined as 1) surgically sterilized, or 2) postmenopausal (a woman who is ≥45 years of age and has not had menses for greater than 1 year will be considered postmenopausal). The two birth control methods can be either two barrier methods or a barrier method plus a hormonal method to prevent pregnancy. Subjects should start using birth control from study Visit 1 throughout the study period up to 120 days after the last dose of study therapy.

The following are considered adequate barrier methods of contraception: diaphragm, condom (by the partner), copper intrauterine device, sponge, or spermicide. Appropriate hormonal contraceptives will include any registered and marketed contraceptive agent that contains an estrogen and/or a progestational agent (including oral, subcutaneous, intrauterine, or intramuscular agents).

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study, they must adhere to the contraception requirement (described above) for the duration of the study and during the follow-up period defined in section 20.4 -Reporting of Pregnancy and Lactation to the Sponsor and to Merck. If there is any question that a subject will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

14.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor and to Merck without delay and within 24 hours to the Sponsor and within 2 working days to Merck if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn).

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the Sponsor and to Merck and followed as described above and in Section 20.4.

14.4 Use in Nursing Women

It is unknown whether pembrolizumab is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, subjects who are breast-feeding are not eligible for enrollment.

15.0 TREATMENT COMPLIANCE

Deviation(s) from the prescribed dosage regimen are to be recorded in the comments section of the CRF.

15.1 Subject Withdrawal/Discontinuation Criteria

The End of Treatment and Follow-up visit procedures are listed in Appendix A (Study Schedule) and Section 9.0 (Study Periods-Visit Requirements). After the end of treatment, each subject will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment (Section 20.5.1). Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent or becoming lost to follow-up. For all other subject's outcome data will be collected for 2 years, then every 6 months for up to five years. Every effort should be made to collect information regarding disease during this follow-up which may include the start of new anti-neoplastic therapy, disease progression, death, or end of study.
When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 9.8.5& 20. .

A subject must be discontinued from the trial for any of the following reasons:

- The subject or legal representative (such as a parent or legal guardian) withdraws consent.
- Confirmed radiographic disease progression

Note: For unconfirmed radiographic disease progression, please see Section 17.3.1. A subject 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(s) of disease are deemed resectable by the surgeon.

- There is toxicity deemed by the investigator or subject to be unacceptable
- Concurrent illness that prevents further administration of treatment
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Administrative reasons
- The investigator, for any reason, stops the study
- Termination of the study by Merck
- 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.
- A patient with 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.
- The compulsory detention for the treatment of either a psychiatric or physical (e.g. infections disease) illness.
- There is clear evidence of progressive disease.

In previous studies with pembrolizumab 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 pembrolizumab before repeat CAT scan. Surgical staging at time of resection will verify progression or whether robust inflammatory response was misinterpreted as progression on repeat CAT scans.

15.2 Clinical Criteria for Early Trial Termination

- Quality or quantity of data recording is inaccurate or incomplete
- Poor adherence to protocol and regulatory requirements

- Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects
- Plans to modify or discontinue the development of the study drug

In the event of Merck decision to no longer supply study drug, ample notification will be provided so that appropriate adjustments to subject treatment can be made.

15.3 Subject Replacement Strategy

Subjects may be replaced during this study if they did not receive pembrolizumab for any reason. Otherwise replacement decisions will be made between the sponsor and investigator on a case-by-case basis.

15.4 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 15.1 which describes procedures and process for prematurely withdrawn patients.

16.0 LABELING, PACKAGING, STORAGE, AND RETURN OF CLINICAL SUPPLIES

Merck Pharmaceuticals will provide pembrolizumab at no cost for this study.

16.1 Investigational Product

Pembrolizumab is a potent and highly selective humanized me of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PDL1and PD-L2.

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.
Clinical Supplies will be provided by Merck as summarized in Table 3.

Table 3 Product Descriptions

Product Name & Potency	Dosage Form
Pembrolizumab 50 mg	Lyophilized Powder for Injection
Pembrolizumab 100 mg/ 4mL	Solution for Injection

16.2 Packaging and Labeling Information

Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements. Pharmacist will receive open label packages of the drug.

16.3 Clinical Supplies Disclosure

This trial is open-label; therefore, the subject, the trial site personnel, the Sponsor and/or designee are not blinded to treatment. Drug identity (name, strength) is included in the label text; random code/disclosure envelopes or lists are not provided.

16.4 Storage, Handling and Disposal

Clinical supplies must be stored in a secure, limited access location under the storage conditions specified on the label.

As with all injectable drugs, care should be taken when handling and preparing pembrolizumab. Whenever possible, pembrolizumab 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 pembrolizumab concentrate or solution comes in contact with skin or mucosa, immediately and thoroughly wash with soap and water. After final drug reconciliation, unused pembrolizumab solution should be disposed at the site following procedures for the disposal of anticancer drugs.

16.5 Dispensing

It is the responsibility of the investigator to ensure that pembrolizumab is only dispensed to study subjects. The pembrolizumab must be dispensed only from official study sites by authorized personnel according to local regulations. Clinical supplies may not be used for any purpose other than that stated in the protocol.

16.6 Drug Ordering and Accountability

16.6.1 Initial and Re-Supply Orders

Following submission and approval of the required regulatory documents, a supply of pembrolizumab may be ordered from Merck. Refer to the pharmacy manual for product ordering.

16.6.2 Pembrolizumab Accountability

It is the responsibility of the investigator to ensure that a current record of pembrolizumab disposition is maintained at each study site where pembrolizumab is inventoried and disposed. This inventory must be available for monitoring. 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 pembrolizumab 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.

All supplies, including unused, partially used or empty containers will be destroyed according to sites drug destruction policy.

16.6.3 Pembrolizumab Returns and Reconciliation

The investigator is responsible for keeping accurate records of the clinical supplies received from Merck or designee, the amount dispensed to and returned by the subjects and the amount remaining at the conclusion of the trial.

Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

16.6.4 Preparation and Administration

Refer to the pharmacy manual for product preparation instructions.

Pembrolizumab will be administered as a 30-minute IV infusion using an infusion pump (treatment cycles may be delayed for 3 weeks due to toxicity as described in Section 11). Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min). Attach the infusion line to the pump and prime the line, either with normal saline (at least 25 mL) or with infusion solution as per local SOP, before

starting the infusion. Maximum infusion rate should not exceed 6.7 ml/min through a peripheral or indwelling catheter. Use 30 mL normal saline to flush the infusion line at the end of the infusion if institutional guidelines allow.

Unused infusion solution should not be used for another infusion of the same participant or a different participant.

DO NOT administer the product as an intravenous push or bolus.

DO NOT combine, dilute or administer it as an infusion with other medicinal products.
A central line is not required for Pembrolizumab administration, but may be used if available.
The following infusion set materials are compatible with Pembrolizumab

- PVC infusion set that is plasticized using Di-2-ethylhexyl Terephthalate DEHP
- PVC and tri-(2-ethylhexyl) trimellitate (TOTM) infusion set
- Polyehtylene lined PVC infusion set
- Polyrethane
- Plybutadiene

A sterile, non-pyrogenic, low-protein binding 0.2 to 5 µm in-line filter made of polyethersulfone (PES) or polysulfone must be used during administration to remove any adventitious particles. If the infusion set does not contain a 0.2 to 5 µm in-line filter, it is recommended to use a 0.2 to 5m add-on filter which may contain an extension line (the materials of the extension line and filter should be as mentioned above).

17.0 OUTCOME MEASUREMENTS

17.1 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 pembrolizumab. Unidimensional measurements in the largest diameter will be used.

17.1.1 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 ≥20 mm with x-ray or ≥10 mm with CT scan (CT scan slice thickness ≤5 mm) or MRI. If scan has slice thickness >5mm, 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).

17.1.2 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.

17.1.3 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.

17.1.4 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.

17.2 Guidelines for Evaluation of Measureable/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 ≥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.
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).

17.3 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 4: Evaluation of Best Overall Response

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
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

17.3.1 Immune-Related Tumor Assessment

Pembrolizumab 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 (e.g., mWHO) may be inadequate to realize the kinetics and magnitude of clinical benefit achieved with pembrolizumab.

Histopathologic evidence has demonstrated pembrolizumab 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.

17.4 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.

17.5 Pathologic Response Criteria

After receiving preoperative pembrolizumab, criteria for examining resected tumor will be as follows:

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

17.6 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.

17.7 Survival

Survival will be measured from the date of enrollment.

18.0 COSTS TO THE SUBJECT

There will be no additional costs to subjects as a result of being in this study. Merck will provide the study drug pembrolizumab free of charge. The research specific blood and tissue testing will also be done at no charge to the subject.

Routine medical care given for the disease under study (which is care a subject would receive whether or not they were in this study), such as the standard chemotherapy drugs and surgery, will be charged to subject's insurance company.

19.0 STUDY ASSESSMENTS

19.1 Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the Investigator. Details regarding the disease for which the subject has enrolled in this study will be recorded separately and not listed as medical history.

19.2 Prior and Concomitant Medications Review

19.2.1 Prior Medications

The investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the subject within 28 days before starting the trial. Treatment for the disease for which the subject has enrolled in this study will be recorded separately and not listed as a prior medication.

19.2.2 Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the subject during the trial. All medications related to reportable SAEs and ECIs should be recorded as defined in Section 20.51.

19.3 Disease Details and Treatments

19.3.1 Disease Details

The investigator or qualified designee will obtain prior and current details regarding disease status.

19.3.2 Prior Treatment Details

The investigator or qualified designee will review all prior cancer treatments including systemic treatments, radiation and surgeries.

19.3.3 Subsequent Anti-Cancer Therapy Status

The investigator or qualified designee will review all new anti-neoplastic therapy initiated after the last dose of trial treatment. If a subject initiates a new anti-cancer therapy within 30 days after the last dose of trial treatment, the 30-day Safety Follow-up visit must occur before the first dose of the

new therapy. Once new anti-cancer therapy has been initiated the subject will move into survival follow-up.

19.4 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Study Schedule (Appendix A) and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 4.0 (see Section 20.0). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

For subjects receiving treatment with pembrolizumab all AEs of unknown etiology associated with pembrolizumab exposure should be evaluated to determine if it is possibly an event of clinical interest (ECI) of a potentially immunologic etiology (termed immune-related adverse events, or irAEs). Please refer to section 20 for detailed information regarding the assessment and recording of AEs.

19.5 Full Physical Exam

The investigator or qualified designee will perform a complete physical exam during the screening period. Clinically significant abnormal findings should be recorded as medical history. A full physical exam should be performed during screening.

19.5.1 Directed Physical Exam

For cycles that do not require a full physical exam per Study Schedule- Appendix A, the investigator or qualified designee will perform a directed physical exam as clinically indicated prior to trial treatment administration.

19.6 Vital Signs

The investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment and at treatment discontinuation as specified in the Study Calendar (Appendix A). Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only.

19.7 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below. Laboratory Safety Evaluations (Hematology, Chemistry and Urinalysis and others) are specified in Table 5 (below).

Table 5- Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hematocrit	Albumin	Blood	Serum β-human chorionic gonadotropin†
Hemoglobin	Alkaline phosphatase	Glucose	(β-hCG)†
Platelet count	Alanine aminotransferase (ALT)	Protein	PT (INR)
WBC (total and differential)	Aspartate aminotransferase (AST)	Specific gravity	aPTT
Red Blood Cell Count	Lactate dehydrogenase (LDH)	Microscopic exam *(If abnormal)*	Total triiodothyronine (T3)
Absolute Neutrophil Count	Carbon Dioxide ‡	results are noted	Free thyroxine (T4)
Absolute Lymphocyte Count	*(CO_2 or bicarbonate)*		Thyroid stimulating hormone (TSH)
	Uric Acid		
	Calcium		
	Chloride		Blood for correlative studies
	Glucose		
	Phosphorus		
	Potassium		
	Sodium		
	Magnesium		
	Total Bilirubin		
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*		
	Total protein		
	Blood Urea Nitrogen		
† Perform on women of childbearing potential only. ‡ If considered standard of care in your region.			

20.0 SAFETY

All patients who receive at least one dose of pembrolizumab will be considered evaluable for safety parameters. Additionally, any occurrence of a 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.

20.1 Safety Monitoring and Reporting

The PI at each institution is responsible for the identification and documentation of adverse events and serious adverse events, as defined below. At each study visit, the PI or designee must assess, through non-suggestive inquiries of the subject or evaluation of study assessments, whether an AE or SAE has occurred. Adverse events that are judged to be Serious Adverse Events by the institutional PI should be reported to the Duke Cancer Institute Safety Desk as outlined in Appendix K- Reporting Procedures for Adverse Events and Serious Adverse Events by Participating Sites.

20.2 Assessing and Recording Adverse Events

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the Merck's product, is also an adverse event.

Changes resulting from normal growth and development that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events. Examples of this may include, but are not limited to, teething, typical crying in infants and children and onset of menses or menopause occurring at a physiologically appropriate time.

Merck product includes any pharmaceutical product, biological product, device, diagnostic agent or protocol-specified procedure, whether investigational (including placebo or active comparator medication) or marketed, manufactured by, licensed by, provided by or distributed by Merck for human use.

Adverse events may occur during the course of the use of Merck product in clinical trials, or as prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

Progression of the cancer under study is not considered an adverse event.

All adverse events that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.
From the time of treatment allocation/randomization through 30 days following cessation of treatment, all adverse events must be reported by the investigator. Such events will be recorded at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in section 20.5.1 & 20.6. The investigator will make every attempt to follow all subjects with non-serious adverse events for outcome.

Adverse events will not be collected for subjects during the pre-screening period (for determination of archival tissue status) as long as that subject has not undergone any protocol-specified procedure or intervention. If the subject requires a blood draw, fresh tumor biopsy etc., the subject is first required to provide consent to the main study and AEs will be captured according to guidelines for standard AE reporting.

20.3 Definition of an Overdose for This Protocol & Reporting to Sponsor and to Merck

For purposes of this trial, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater (≥5 times the indicated dose). No specific information is available on the treatment of overdose of pembrolizumab. Appropriate supportive treatment should be provided if clinically indicated. In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event(s) is associated with ("results from") the overdose of a Merck product, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of Merck's product meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."

All reports of overdose with and without an adverse event must be reported within 24 hours to the Duke Sponsor (DCI Safety Desk) who will report within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

20.4 Reporting of Pregnancy and Lactation to the Sponsor and to Merck

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them) that occurs during the trial.

Pregnancies and lactations that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Pregnancies and lactations that occur from the time of treatment allocation/randomization through 120 days following cessation of Sponsor's product, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, must be reported by the investigator. All reported pregnancies must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the Duke Sponsor (DCI Safety Desk) who will report within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

20.5 Immediate Reporting of Serious Adverse Events

20.5.1 Serious Adverse Events

A serious adverse event is any adverse event occurring at any dose or during any use of Merck's product that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is another important medical event
- **Note:** In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable to the Merck in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by Merck for collection purposes.

- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose.

Refer to Table 6 for additional details regarding each of the above criteria.

For the time period beginning when the consent form is signed until treatment allocation/randomization, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject must be reported within 24 hours to the Duke Sponsor (DCI Safety Desk) who will report within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including, but not limited to, washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study whether or not related to the Merck product, must be reported within 24 hours to the Duke Sponsor (DCI Safety Desk) who will report within 2 working days to Merck Global Safety.
Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to Merck product that is brought to the attention of the investigator at any time following consent through the end of the specified safety follow-up period specified in the paragraph above, or at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Duke Sponsor and to Merck Global Safety.

All subjects with serious adverse events must be followed up for outcome.

SAE reports and any other relevant safety information are to be forwarded to the DCI Safety Desk for forwarding to Merck Global Safety facsimile number: +1-215-993-1220

A copy of all 15 Day Reports and Annual Progress Reports are submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators. Investigators will cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally, investigators will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215 993-1220) at the time of submission to FDA.

20.6 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 24 hours to the Duke Sponsor (DCI Safety Desk) who will report within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220).

For the time period beginning when the consent form is signed until treatment allocation/randomization, any ECI, or follow up to an ECI, that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to Merck product, must be reported within 24 hours to the Sponsor and within 24 hours to Merck Global Safety.

Events of clinical interest for this trial include:

1. An overdose of Merck product, as defined in Section 7.2.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.

2. An elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing. *

*Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology.

20.7 Protocol-Specific Exceptions to Serious Adverse Event Reporting

Efficacy endpoints as outlined in this section will not be reported to Merck as described in Section 20.5- Immediate Reporting of Adverse Events to the Sponsor and to Merck, unless there is evidence suggesting a causal relationship between the drug and the event. Any such event will be submitted to the Sponsor within 24 hours and to Merck Global Safety within 2 working days either by electronic or paper media.

Specifically, the suspected/actual events covered in this exception include any event that is disease progression of the cancer under study.

The Sponsor will monitor un-blinded aggregated efficacy endpoint events and safety data to ensure the safety of the subjects in the trial. Any suspected endpoint which upon review is not progression

of the cancer under study will be forwarded to Merck Global Safety as a SAE within 2 working days of determination that the event is not progression of the cancer under study.

Hospitalization related to convenience (e.g. transportation issues etc.) will not be considered a SAE.

20.8 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 4.0. Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade recorded on the adverse event case report forms/worksheets.

All adverse events regardless of CTCAE grade must also be evaluated for seriousness.

Table 6 EVALUATING ADVERSE EVENTS: An investigator who is a qualified physician will evaluate all adverse events as to:

V4.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic obersvations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of Merck product that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization [for an elective procedure] to treat a pre-existing condition that has not worsened is not a serious adverse event. A pre-existing condition is a clinical condition that is diagnosed prior to the use of a Merck product and is documented in the patient's medical history); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new **cancer** (that is not a condition of the study) **(**although not serious per ICH definition, is reportable to the Sponsor within 24 hours and to Merck within 2 working days to meet certain local requirements); **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event for collection purposes. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours to the Sponsor and to Merck within 2 working days.	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	

Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause Merck product to be discontinued?	
Relationship to Merck Product	Did Merck product cause the adverse event? The determination of the likelihood that Merck product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between Merck product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely Merck product caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to Merck product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of Merck product? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

Relationship to Merck Product (continued)	**The following components are to be used to assess the relationship between the test drug and the AE: (continued)**	
	Dechallenge	Was Merck product discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Sponser's product; or (3) the trial is a single-dose drug trial); or (4) Sponser's product(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to Merck product in this study? If yes, did the AE recur or worsen?

		If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) Sponser's product(s) is/are used only one time). NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY MERCK PRODUCT, OR IF REEXPOSURE TO MERCK PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE SPONSER AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding Merck product or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following		**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of Merck product relationship).**
Yes, there is a reasonable possibility of Merck product relationship.		There is evidence of exposure to Merck product. The temporal sequence of the AE onset relative to the administration of Merck product is reasonable. The AE is more likely explained by Merck product than by another cause.
No, there is not a reasonable possibility of Merck product relationship		Subject did not receive the Merck product OR temporal sequence of the AE onset relative to administration of Merck product is not reasonable OR the AEis more likely explained by another cause than the Merck product. (Also entered for a subject with overdose without an associated AE.)

21.0 QUALITY CONTROL AND QUALITY ASSURANCE

Please refer to the External Site Monitoring Plan developed for the protocol.

21.1 Audits

The Duke School of Medicine Clinical Trials Quality OARC (Office of Audit and Research Compliance) office may conduct audits to evaluate compliance with the protocol and the principles of GCP. The PI agrees to allow the OARC auditor(s) direct access to all relevant documents and to allocate his/her

time and the time of the study team to the OARC auditor(s) in order to discuss findings and any relevant issues.

OARC audits are designed to protect the rights and well-being of human research subjects. OARC 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.

OARC 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.

22.0 ADMINISTRATIVE AND ETHICAL CONSIDERATIONS

This protocol was designed and will be conducted and reported in accordance with the International Conference on Harmonization (ICH) Harmonized Tripartite Guidelines for Good Clinical Practice, the Declaration of Helsinki, and applicable federal, state, and local regulations.

22.1 Investigator Responsibilities

Each participating institution is responsible for appropriately recording adverse events and for reporting serious adverse events to the DCI Safety Desk (per Appendix K) and to their local Institutional IRBs in accordance with the reporting requirements.

22.2 Institutional Review

The protocol, informed consent form, advertising material, and additional protocol-related documents will be submitted to the DUHS Institutional Review Board (IRB) and DCI Cancer Protocol Committee (CPC) for review. The study may be initiated only after the Duke Principal Investigator has received written and dated approval from the CPC and IRB. PIs at participating sites must obtain appropriate institutional approvals, including, but not limited to, local IRB approval.

Principal Investigators must submit and obtain approval from their IRBs for all subsequent protocol amendments and changes to the informed consent form. The CPC at Duke will be informed about any

protocol amendments that potentially affect research design or data analysis (i.e. amendments affecting subject population, inclusion/exclusion criteria, agent administration, statistical analysis, etc.).

Principal Investigators must obtain protocol re-approval from their IRBs within 1 year of the most recent IRB approval. The Duke Principal Investigator will also obtain protocol re-approval from the CPC within 1 year of the most recent IRB approval, for as long as the protocol remains open to subject enrollment.

22.3 Protocol Amendments

All protocol amendments must be initiated by the Duke Principal Investigator and approved by the Duke IRB prior to implementation. Protocol amendments will be disseminated to participating sites for local IRB approval. IRB approval is not required for protocol changes that occur to protect the safety of a subject from an immediate hazard. However, the Principal Investigators must report these to Duke and inform their IRBs and all other applicable regulatory agencies of such action immediately.

Though not yet required, the CPC will be informed about any protocol amendments that potentially affect research design or data analysis (i.e. amendments affecting subject population, inclusion/exclusion criteria, agent administration, etc.).

22.4 Departures from the Protocol and Reporting of Protocol Deviations

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 Duke 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.

Departures from the protocol and protocol deviations or violations need to be clearly documented on a Notification of Protocol Deviation/Violation form. These are events that are 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. Participating sites must report protocol deviations or violations to Duke within 7 business days. These events will be reviewed by Duke and reported to the Duke PI as deemed necessary. H**owever,** any unanticipated study-related death must be reported to Duke within 24 hours of the occurrence of the event.

22.5 Compliance with Trial Registration and Posting Requirements

This clinical trial will be posted to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

22.6 Conflict of Interest

The Principal Investigators and Sub-Investigators must comply with applicable federal, state, and local regulations regarding reporting and disclosure of conflict of interest. Conflicts of interest may arise from situations in which financial or other personal considerations have the potential to compromise or bias professional judgment and objectivity. Conflicts of interest include but are not limited to royalty or consulting fees, speaking honoraria, advisory board appointments, publicly-traded or privately-held equities, stock options, intellectual property, and gifts.

The Duke University School of Medicine's Research Integrity Office (RIO) reviews and manages research-related conflicts of interest. The Duke Principal Investigator and Sub-Investigators must report conflicts of interest annually and within 10 days of a change in status, and when applicable, must have a documented management plan that is developed in conjunction with the Duke RIO and approved by the IRB/IEC. PIs at participating sites must comply with local institutional requirements regarding conflict to interest reporting.

22.7 Informed Consent/Informed Consent Process

Principal Investigators or their 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. Consent must be documented by the subject's (or legal representative) dated/timed signature along with the dated/timed signature of the person conducting the consent discussion. The Principal Investigator or designee is responsible for asking the subject whether the subject wishes to notify his/her primary care physician about participation in the study. If the subject agrees to such notification, the Principal Investigator will inform the subject's primary care physician about the subject's participation in the clinical study.

The initial informed consent form, any subsequent revised written informed consent form and any written information provided to the subject must receive the IRB/ERC's approval/favorable opinion in advance of use. The subject or his/her legally authorized representative should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the subject's dated signature or by the subject's legally authorized representative's dated signature.

23.0 PRIVACY, DATA STORAGE, AND CONFIDENTIALITY

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

However, in compliance with federal guidelines, investigators will permit representatives from the Duke School of Medicine Office of Audit and Research Compliance 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 or the PI's designee's responsibility to obtain such permission from the patient in writing before the subject is entered into the study.

Principal Investigators will ensure that subject privacy and confidentiality of the subject's data will be maintained.

To protect privacy, every reasonable effort will be made to prevent undue access to subjects during the course of the study. Prospective participants will be consented in an exam room where it is just the research staff, the patient and his family, if desired. For all future visits, interactions with research staff (study doctor and study coordinators) regarding research activities will take place in a private exam room. All research related interactions with the participant will be conducted by qualified research staff who are directly involved in the conduct of the research study.

To protect confidentiality, subject files in paper format will be stored in secure cabinets under lock and key accessible only by the research staff. Subjects will be identified only by a unique study number and subject initials. Electronic records of subject data will be maintained using a dedicated database (MediData RAVE) which is housed in an encrypted and password-protected, DCI file server. Access to electronic databases will be limited to authorized key personnel only. 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.

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

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, research coordinator, 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 or research chart in accord with the institution's practice. 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:

Eligibility Checklist
Serious and Non-Serious Adverse Event Forms (if applicable)
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 CRF will be updated within two weeks of acquisition of new source data. The electronic records of subject data will be maintained using a dedicated database (Medidata Rave 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. (Medidata Rave or RedCap). 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 designees (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 be coded according to the World Health Organization Drug Dictionary.

24.3 Data Management Procedures and Data Verification

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 research nurse coordinator 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.

25.0 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 (if applicable).

Upon completion of the study, research records will be archived and handled per DUHS (or participating Institution's) HRPP policy.

25.1 Records Retention

The Principal Investigator will maintain study-related records for the longer of a period of:

-at least two years after the date on which a New Drug Application is approved by the FDA (if an IND is involved)

-at least two years after formal withdrawal of the IND associated with this protocol (if an IND is involved)

-at least six years after study completion (Duke policy)

26.0 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.

27.0 STATISTICAL METHODS AND ANALYTICAL PLANS

27.1 Statistical Considerations

Sample size justification

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 pembrolizumab in this population. Since it is unlikely that the therapy will harm the patients, a futility analysis on the percentage of patients with adequate TIL collection is unnecessary.

Evaluation of Investigational Therapy Safety and Feasibility

Analytic methods

Overview

The primary objective of the single arm phase II trial is to determine whether the surgery feasibility rate of neoadjuvant pembrolizumab is not significantly worse than the historical surgical feasibility rate of neoadjuvant chemotherapy. Surgery feasibility rate of a neoadjuvant therapy is the percent of patients who are able to undertake surgery after the neoadjuvant therapy. A total of 30 evaluable patients will be entered to the single arm trial. Taking 15% rate of cancellation and ineligibility into account, the trial will enroll a total of 35 patients and eligible subjects (not screen failures) will be followed for 2 years for both disease recurrence and death. Following that, patients will be followed every 6 months for up to five years.

Primary Objective Sample Size Justification

According to Pisters et al (JCO 2010), approximately 80% of subjects undergo surgical exploration within 42 days of day 1 of the last cycle of standard neoadjuvant chemotherapy. We would like to test the following hypotheses:

$$H_0: p >= 0.80 \text{ vs. } Ha: p <= 0.65$$

where p is the true surgery feasibility rates after combined neoadjuvant chemotherapy and pembrolizumab. With 30 evaluable patients receiving protocol treatment, the study has approximately 84.6% power rejecting H_0: p>=0.80 when p=0.65 at one-sided significance level of 15%.

Adverse event monitoring: In this trial design it is difficult to accurately attribute adverse events to pembrolizumab, surgery, or other causes. Further, the analysis of treatment toxicities as isolated events will not best represent whether or not the combination of pembrolizumab followed by surgery is safe and feasible. Therefore, this safety and feasibility trial will be evaluated by the following criteria:

1) Whether the cumulative percentage of deaths among all treated patients related to any of the study treatments from the first pembrolizumab treatment until 30 days after surgery exceeds 17%.
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 pembrolizumab is a conservative estimate based on data reported for a SWOG trial that studied neoadjuvant chemotherapy in early stage lung cancer (22).

The first 10 treated patients will be evaluated for safety and feasibility. If in the first ten patients the criteria for overall therapy being not safe or feasible is met, 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.

The trial will be stopped for accrual whenever a total of 35 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.

Accrual and follow-up

With an expected rate of 2 patients being enrolled each month, the trial will take about 16 months to reach the target accrual. All registered patients will be followed for tumor response, disease free survival and overall survival.

Statistical Analysis Plan

Surgery feasibility rate, the primary endpoint of the trial, will be analyzed using the data from all evaluable patients, who are defined as the patients who meet eligibility criteria and has received at least 1 dose of pembrolizumab. The surgery feasibility rate of neoadjuvant pembrolizumab as well as its confidence interval will be estimated.

Secondary Objectives

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. However, if a statistical test on a secondary outcome is significant at the 2-sided significance level of 0.01, the finding will be considered worthy of future investigation.
1) We will estimate the rate of objective response rate for the protocol treatment. 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.

2) We will evaluate disease-free survival and patterns of metastases after protocol treatment. 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 confidence interval. The frequencies of metastases by site will be tabulated.
3) Blood based biomarkers will be evaluated for the changes before and after the protocol treatment. The association of the baseline value and the changes of these biomarkers with clinical outcomes, such as objective response, overall survival and disease free survival, will be evaluated using logistics regression and Cox models.
4) We will 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 protocol treatment. The percentage of patients with TILs for patients who have surgical resection and its exact confidence interval will also be estimated.
5) We will evaluate whether presence, quantity or quality of detectable TILs is associated with pathologic response to neoadjuvant therapy. 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.
6) Treatment-related adverse events will be summarized by type and grade.
7) The proportion of patients with detectable circulating T cells specific against TAA after the protocol treatment will be estimated and its confidence interval will be provided. An exact binominal test will be used to test the increase in the proportion of patients with detectable circulating T cells specific against TAA after the protocol treatment relative to baseline.
8) Estimate the rate of pathologic response for neoadjuvant pembrolizumab in early stage NSCLC. The pathologic response rate along with its 95% exact confidence interval will be estimated.
9) Determine if the immunomodulatory effects of neoadjuvant chemotherapy plus pembrolizumab impact the suppressive mechanisms, restoring functional reactivity to important anti-tumor effector cell populations. Functional TAA-specific T cell reactivity's will be measured in the blood at 4 time points: Collection 1, 2, 3 and 4. A Wilcoxon rank sum 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.
10) 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.
11) Perform gene expression analysis on tumor to elucidate genes associated with function and modulation of the PD-1/PD-L1 axis.

28.0 References

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29.0 LIST OF APPENDICES

Appendix A Study Schedule
Appendix B Correlative Science Measures
Appendix C Performance Status Criteria
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29.1 Appendix A: STUDY SCHEDULE

Examination	Baseline (screen)[a]	Neoadjuvant RX		Evaluation post pembro[k]	Surgery[P]	Post Surgery FU [R]
		Cycle1	Cycle 2			
Day	30 to 0	1 (+/- 3 days)	22 (+/- 3 days)	pre-surgery	29 – 56 (from C1D1)	2-6 wks. post Surgery
Informed Consent	X					
Eligibility	X					
Medical Hx/Demo/ Smoking Hx	X					
Height	X					
Vital Signs (temp, RR, B/P, pulse)	X	X	X	X		X
Weight/BSA	X	X	X	X		X
Physical Exam	X	X	X	X		X
Performance Status	X	X	X	X		X
Hematology [b,c,e]	X	X	X	X		X
Chemistries[b,d,e]	X	X	X	X		X
Serum Beta HCG[h]	X	X[h]				
Calculated Creatinine[ef]Clearance	X					
Thyroid Functions (T3, FT4,TSH) [e]	X			X		
PT(INR), aPTT[e]	X					
Urinalysis	X					
PFTs (FEV1/DLCO) [e]	X					
Radiologic Eval[g]	X			X[g]		X[g]
Medical & Surgical Evaluation	X			X		
EKG	X					
Tumor Histology	X					
Adverse Events		X	X	X		X
Concomitant Meds	X	X	X	X		X

Pembrolizumab		X	X			
Blood Sampling [j]	X[j]			X[j]		X[j]
Surgical Specimen[p]					X	
Tumor Biopsy Slides[n]					X	
Examination	**STD ADJ CT/RT [L]**	**Adjuvant Pembrolizumab[m]**				**EOS[S] Follow-Up**
		Cycle 1[m]	**Cycle 2**	**Cycle 3**	**Cycle 4**	
Day		1	22	43	64	≥30 d post Pembro[j]
Informed Consent						
Eligibility						
Medical History/Demo-Graphics/Smoking Hx						
Height						
Vital Signs (temp, RR, B/P, pulse)		X	X	X	X	X
Weight/BSA		X	X	X	X	X
Physical Exam		X	X	X	X	X
Performance Status		X	X	X	X	X
Hematology [b,c,e]		X	X	X	X	X
Chemistries [b,d,e]		X	X	X	X	X
Serum Beta HCG[h]		X	X	X	X	
Thyroid Functions (T3, FT4,TSH) [e]		X		X		X
PT(INR), aPTT[e]						
Urinalysis						
PFTs (FEV1/DLCO) [e]						
Radiologic Evaluation[g]						
Med/surg Evaluation						
EKG						

Tumor Histology						
Adverse Events		X	X	X	X	X
Concomitant Meds		X	X	X	X	X
Pembrolizumab		X	X	X	X	
Blood Sampling [j]						X[j]
Surgical Specimen[p]						
Tumor Biopsy Slides[n]						

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

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

b May be obtained within 3 days of pembrolizumab dosing

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

d Chemistries to include Na^+, K^+, Cl^-, total protein, albumin, calcium, glucose, BUN, creatinine, total bilirubin (direct bilirubin if total is elevated above upper limit of normal), alkaline phosphatase, SGOT, SGPT, LDH, uric acid, magnesium, phosphorus. **e** 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. PFT's per institutional standards.

f Calculated creatinine clearance (see appendix D)

g Radiologic evaluation: pre-treatment clinical staging PET/CT of chest/abdomen, brain MRI or CT as per standard of care (these may be performed up to 42 days); after completing cycle#2 Pembrolizumab therapy (prior to surgery) chest CT; post- surgery recommend chest CT every 3-4 months (or per institutional standard of care) for 2 years

h All WOCBP MUST test negative for pregnancy within 48 hours prior to any initial study procedure based on a serum pregnancy test. If subject uses appropriate contraceptive methods (section 6.2) from the time of the initial serum pregnancy test, then the subsequent pregnancy test can be done within 72 hours before receiving pembrolizumab. 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. If the pregnancy test is positive, the subject must not receive pembrolizumab and must not be enrolled in the study, or will be removed from treatment.

j Blood samples to assess activated CD8+ T cells with specificity against tumor antigens and CD4 and CD8 functional memory. **Collection 1**: Baseline: pre-dose #1 pembrolizumab**. Collection 2 : After second dose of Pembrolizumab** (cycle 2) and prior to surgery. **Collection 3**: 2-6 weeks after surgery. **Collection #4**: Three to 6 weeks after last dose of adjuvant pembrolizumab. 4 tubes of 8.5 ml each (ACD anti-coagulated Vacutainer yellow tops).

k Evaluation Post pembrolizumab prior to surgery. Final visit (if ≤ grade 1 toxicity) for those subjects that come off treatment early for progression of disease, intolerance to protocol therapy, for other reasons, or patient withdraws consent; safety evaluation should occur 30 days (+/- 5days) after last dose pembrolizumab or before initiation of a new anti-neoplastic cancer treatment (whichever comes first).

L Post-op standard therapy, including adjuvant CT and RT, based on final pathologic stage of cancer, tumor margins, and other standard clinical criteria. Post-op therapy is non-study specific and can be given through the primary oncology team.

m Adjuvant Pembrolizumab: Patients who receive both adj CT plus adj RT should start Pembrolizumab within 8 months of surgery. Patients who receive only one modality of adj therapy (e.g. CT alone or RT alone) should start Pembrolizumab within 6 months of surgery Patients who do not receive any adjuvant therapy should start Pembrolizumab within 4 months of surgery.

n Tumor Biopsy Slides (appendix I)

p Surgery to occur at 4-8 weeks (29-56 days). If dose # 2 pembrolizumab delayed, there is to be at least 2 days between this infusion and surgery. Tumor Specimen collection (appendices G & H)

R Post-Surgery F/U visit: Two to 6 weeks following surgery. 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 +/- standard chemotherapy as deemed appropriate by their physician. Subjects who are completely resected or who refuse or cannot receive adjuvant chemotherapy will be given the option of receiving adjuvant Pembrozulimab. Collection #3 research blood sample 2-6 weeks after surgery prior to initiation of adjuvant CT.

S End of Study Visit: End of study evaluation is to occur approximately 30 days or more after adjuvant Pembrolizumab treatment. SAEs will be collected for 90 days after the end of treatment. Final research blood sample will be collected (collection #4). Chest CT if applicable (per radiologic evaluation, Appendix A)

T Follow-Up: Patients should have follow-up every 3-4 months as per standard care(of the treating institution (recurrence and survival) for 2 years unless patients has experienced > grade 1 toxicity related to Pembrolizumab (in which case follow until resolution of the AE to Grade 0-1, patient deemed stable by investigator, or new anti-neoplastic therapy has begun.). After 2 years follow every 6 months for up to five years. 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.

* Upon lung cancer relapse, radiologic imaging of sites of failure is at the discretion of the treating physician.

29.2 Appendix B- Correlative Science Measures

Correlative Science Primary Objective

Circulating T Lymphocytes with Specificity Against Tumor Associated Antigens:
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-α, IFN-γ, 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 ≥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 ≥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-α+
*%**CD4**+/CD69+/ IFN-γ+
*%**CD4**+/CD69+/ CD107+

* %**CD8**+/CD69+/IL-2+
*%**CD8**+/CD69+/TNF-α+
*%**CD8**+/CD69+/IFN-γ+
*%**CD8**+/CD69+/CD107+

* %**DP**+/CD69+/IL-2+
*%**DP**+/CD69+/TNF-α+
*%**DP**+/CD69+/IFN-γ+
*%**DP**+/CD69+/CD107+

Exploratory Objectives
Tumor Infiltrating Lymphocytes with Specificity Against Tumor Associated Antigens:

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 ≥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-α+ or -IFN-γ+ or -CD107+ or -
* %**CD8**+/CD69+-IL-2+ or -TNF-α+ or -IFN-γ+ or -CD107+ or -

* %**DP**+/CD69+/-IL-2+ or -TNα+ or -IFN-γ+ 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^{hi}/HLA-DR^{low}CD14+ (Myeloid-derived suppressor cells (MDSC)) FCSxSSC (Monocyte gate) %CD3-CD14- Lin(CD16, CD19, CD20, CD56)- HLA-DR- CD11b+/CD33+ (MDSC)

29.3 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.

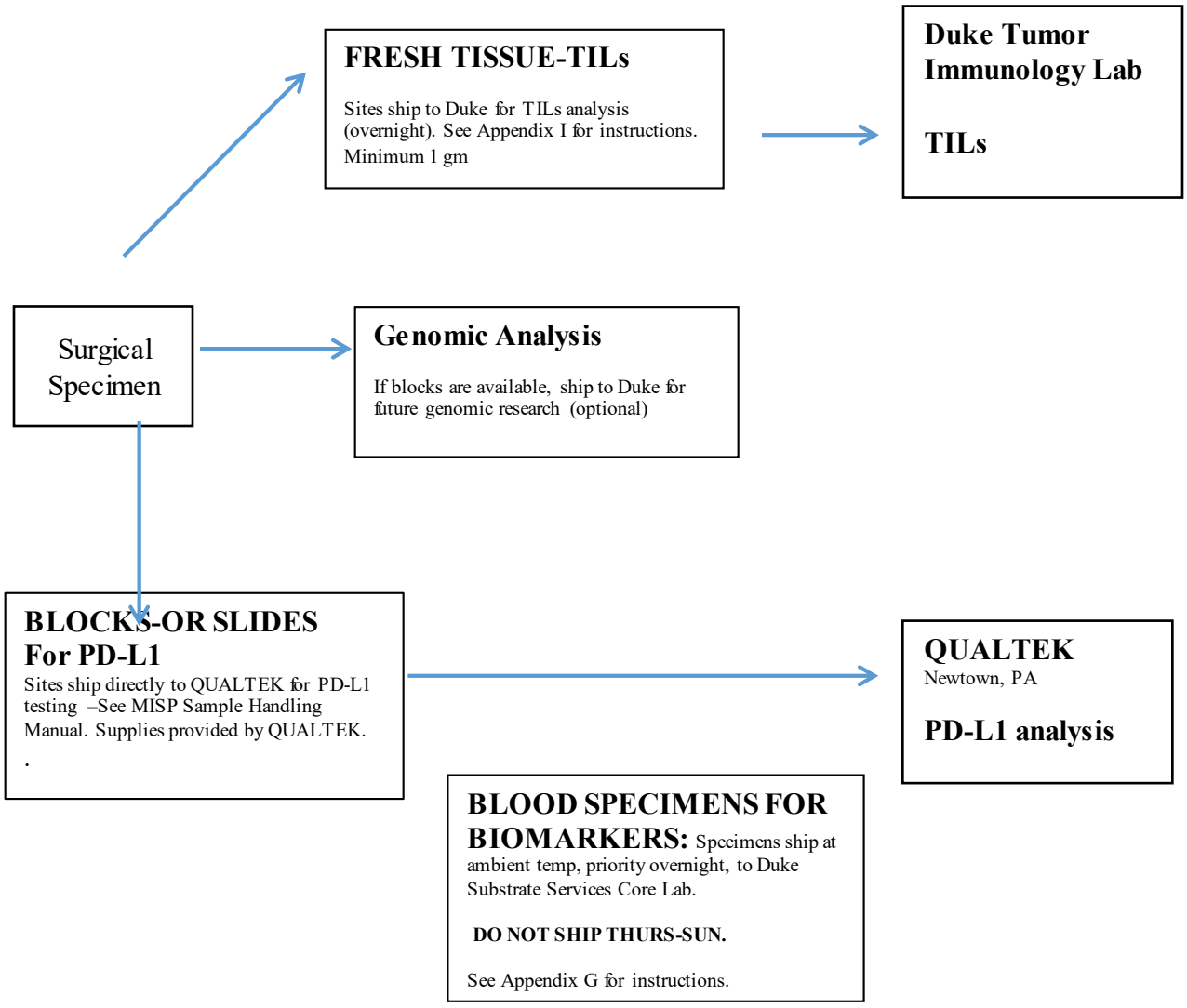
29.4 Appendix D Standard Cockcroft and Gault Formula for Calculated Creatinine Clearance

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

For serum creatinine concentration in Dmol/L:
CrCl = [(140 - age) x (wt. in kg)] / [0.81 x serum creatinine (mol/L)] Females: Multiply the result x 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.

29.5 Appendix E -SITE BIOSPECIMEN PROCESSING FLOW CHART - TOP 1501



29.6 Appendix F-Blood Specimens-Duke

Biomarker Blood Specimens for Correlative Sciences

Duke Processing

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: After dose #2 pembrolizumab prior to surgery
Collection 3: Two to six weeks after surgery prior to initiation of adjuvant therapy
Collection 4: Three to six weeks after the last dose of adjuvant pembrolizumab.
******If EOS (End of Study) occurs and no adjuvant treatment with Pembrolizumab has occurred, draw Collection #4 at End of Study visit

Once ACD tubes collected, Substrate Services Core lab to be contacted for pick up as PBMC should ideally occur within 4 hours of draw @ 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 the Substrate Services Core lab.

** Note: it is imperative tube is filled to the fill line for proper additive to blood ratio. Specimen is not evaluable if not filled to the fill line. If unable to obtain the 4 required tubes, fill as many tubes as possible to the fill line.

29.7 Appendix G-Blood Specimens-Outside Sites

Biomarker Blood Specimens for Correlative Sciences

Blood samples for biomarker analysis will be collected at four time points and labeled with the Participant Study ID, collection date and time. The blood samples will be collected in four (4) ACD (yellow top) tubes.

Collection 1: Baseline prior to treatment initiation
Collection 2: After dose #2 pembrolizumab prior to surgery
Collection 3: Two to six weeks after surgery prior to initiation of adjuvant therapy
Collection 4: Three-six weeks after last dose adjuvant pembrolizumab

Vacutainer ACD (yellow top) tube: Immediately after drawing the blood, gently invert the tube 5 times. Do not store samples on ice, but hold the tubes at room temperature.

** Note: it is imperative tube is filled to the fill line for proper additive to blood ratio. Specimen is not evaluable if not filled to the fill line. If unable to obtain the 4 required tubes, fill as many tubes as possible to the fill line.

******If EOS (End of Study) occurs and no adjuvant treatment with Pembrolizumab has occurred, draw Collection #4 at End of Study visit

Sample Storage Prior to Shipment:

The samples should be held at room temperature, and must be shipped the same day the samples are collected.

Sample Shipping:

- It is imperative that samples be shipped according to IATA regulations and in appropriate packaging.
- Samples should be shipped ambient for next morning delivery (priority overnight).
- Samples are to be shipped Monday-Wednesday to be received Tuesday-Thursday (to avoid a late Friday afternoon or weekend delivery). Do not ship samples Thursday through Sunday!
- Email the designated contact prior to shipping to ensure your samples will not be arriving on a university holiday and to confirm that someone will be available to receive the shipment (contact Robyn Osborne: robyn.osborne@duke.edu).
- Do not ship on a national holiday or a day which is followed by a national holiday.

Shipping address:

Lab Technical Manager
Substrate Services Core Lab

Duke University Medical Center
203 Research Drive.
MSRB I, Room 459
Durham, NC 27710
Phone - (919) 684-3754
Fax - (919) 684-4288
robyn.osborne@duke.edu

29.8 Appendix H-Tissue Specimens-Duke

Surgical Tissue Specimen for Correlative Sciences
Duke Processing

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 surgery 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 impeded accurate initial assessment of critical features of the tumor resection, most notable 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 (TILs) for purposes of this protocol (as much tissue as possible, but a minimum 1 gm).

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.

29.9 Appendix I-Tissue Specimens-Outside Sites

BACKGROUND:

The ability to obtain high quality Tumor infiltrating lymphocytes (TIL) from fresh tissue is highly dependent on the tissue procurement (requires as much tissue as possible, minimum 1 gm). Delays in tissue processing usually lead to cell death and RNA degradation. Inadequate shipping conditions can also be a major source of pre-analytic variance in downstream applications. The following procedure addresses these issues.

PRINCIPLE:

Tumor tissue is harvested after surgical resection, weighed, kept cold at 4°C in antibiotic-antimycotic containing Holding Medium, and shipped overnight.

EQUIPMENT AND SUPPLIES:

Equipment:
Scale

Supplies:
50 ml sterile conical tube
Parafilm
Recloseable Zip Lock Plastic Bag
IATA Certified Cold Shipper

Reagents:

***Holding Media (50ml):** RPMI 1640 plus 10% Human serum, with antibiotic-antimycotic and 2-Mercaptoethanol

RPMI 1640, Hepes	Gibco 22400-089	44.5 ml
Antibiotic-Antimycotic (100X)	Gibco 15240-096	0.5 ml
Human Serum Type AB (Male)	Sigma H4522	5 ml
2-mercaptoethanol	Sigma M6250	0.05 ml

Storage Requirements:
Tissue samples should be kept cold on ice or at 4°C until shipped.

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.

PROCEDURE - STEPWISE:

1. After procurement, weigh the tissue, note the weight on the Sample Processing Request Form, and place it in a sterile 50 ml tube. Fill the tube with Tumor Holding Media and close tightly. Label the tube with the Study Number and Patient ID. Wrap the top with parafilm to prevent spillage.

2. Be sure to fill out the Sample Processing Request Form and include it in the package with the tissue

3. It is imperative that samples be shipped according to IATA regulations and in appropriate packaging that allows shipping with wet ice..
4. Samples are to be shipped Monday-Wednesday to be received Tuesday-Thursday (to avoid a late Friday afternoon or weekend delivery). Do not ship samples Thursday through Sunday! ** Please ship priority overnight.
5. Do not ship on a national holiday or a day which is followed by a national holiday.

Email the designated contact prior to shipping to ensure your samples will not be arriving on a university holiday and to confirm that someone will be available to receive the shipment (contact Kristy Long: kristy.long@duke.edu).**DTRI shipping address**:
Kristy Long
Duke University Medical Center
915 S. LaSalle St.
SORF Bldg. Room 121
Durham, NC 27710
Phone - (919) 684-6834
Fax - (919) 684-4288
kristy.long@duke.edu