PROTOCOL AMENDMENT # 6

LCCC 1525: Phase II Study Of Pembrolizumab Therapy Following a Single Priming Dose of Cyclophosphamide in Patients With Metastatic Triple Negative Breast Cancer (TNBC)

AMENDMENT INCORPORATES:

__ Editorial, administrative changes
_X Scientific changes
__ Therapy changes
__ Eligibility Changes

Rationale for amendment: The purpose of this amendment is to decrease the duration of subject follow up after treatment has ended. Currently the protocol states that subjects will be followed for up to 3 years, this time will be changed to reflect that subjects will be followed for up to 2 years. Given that the primary objective has been met, the decrease in subject follow up will allow for subsequent study closure.

Therapy changes:

Section 4.6	Duration of Follow up: Follow up changed from 'up to 3 years' to 'up to 2 years', subsequent follow up changed to 'up to 2 years'
T&E Table	Footnote 3 changed to reflect follow up for up to 2 years.
Section 6.4	Long Term Follow up: Subsequent follow up changed from 'up to 3 years' to 'up to 2 years'

THE ATTACHED VERSION DATED February 22, 2022 INCORPORATES THE ABOVE REVISIONS

PROTOCOL AMENDMENT # 5

LCCC 1525: Phase II Study Of Pembrolizumab Therapy Following a Single Priming Dose of Cyclophosphamide in Patients With Metastatic Triple Negative Breast Cancer (TNBC)

AMENDMENT #5 INCORPORATES (Check all that apply):

X Editorial, Administrative Changes
Scientific Changes
Therapy Changes
Eligibility Changes

AMENDMENT RATIONALE AND SUMMARY
A Principal Investigator change from Dr. Benjamin Vincent to Dr. E. Claire Dees.

Historically, amendments were first submitted to the UNC IRB, and then after approval, submitted to the site IRBs. The change is to submit amendments to the UNC IRB and to site IRBs at the same time. Changes in informed consent documents must be submitted to the Multicenter Regulatory Associate prior to submission to the site IRB.

SUMMARY OF CHANGES

1. The protocol has been revised throughout to reflect the PI change
2. Correction of version date error in page header that was released as part of Amendment 5 version 1.0.
3. Section 9.6 - Amendments to the Protocol now reads:
   For Institutions Relying on Their Own IRB:
   Investigators must submit the amendment to their institution's IRB for approval. For multicenter studies, any multicenter site must submit their informed consent revisions to the Multicenter Regulatory Associate prior to submission to their IRB.

The attached version dated May 24, 2019 incorporates the above revisions

LINEBERGER COMPREHENSIVE CANCER CENTER
CLINICAL ONCOLOGY RESEARCH PROGRAM
UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

PROTOCOL AMENDMENT # 4

LCCC 1525: Phase II Study Of Pembrolizumab Therapy Following a Single Priming Dose of Cyclophosphamide in Patients With Metastatic Triple Negative Breast Cancer (TNBC)

AMENDMENT #4 INCORPORATES (Check all that apply):

X Editorial, Administrative Changes
Scientific Changes
Therapy Changes
Eligibility Changes

AMENDMENT RATIONALE AND SUMMARY
A Principal Investigator change from Dr. Carey Anders to Dr. Benjamin Vincent. Also, the role of Co-Principal Investigator changed from Dr. Jonathan Serody to Dr. Lisa Carey.

The protocol has been revised throughout to reflect these changes.

The attached version dated December 5, 2018 incorporates the above revisions

LINEBERGER COMPREHENSIVE CANCER CENTER
CLINICAL ONCOLOGY RESEARCH PROGRAM
UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

PROTOCOL AMENDMENT # 3

LCCC 1525: Phase II Study Of Pembrolizumab Therapy Following a Single Priming Dose of Cyclophosphamide in Patients With Metastatic Triple Negative Breast Cancer (TNBC)

AMENDMENT #3 INCORPORATES (Check all that apply):

X Editorial, Administrative Changes
X Scientific Changes
Therapy Changes
Eligibility Changes

AMENDMENT RATIONALE AND SUMMARY
This protocol amendment changes the frequency of tumor assessments from every 9 weeks to 12 weeks after the subject has been on treatment for at least 1 year. This change allows for reduced radiation exposure to the subject.

The single subject exception language was updated to align with the Sponsor's updated policy of no single subject exceptions will not be granted for eligibility. This is to incorporate the administrative letter dated December 1, 2017

SUMMARY OF CHANGES

1. Section 6.1, footnote 5: tumor assessment intervals increased to every 12 weeks after one year of study treatment.
2. Section 6.3.7: After the first year, tumor imaging will be performed every 12 weeks, at the end of cycles 22, 26, 30, etc
3. Section 9.5.2: Guidance updated to: Eligibility single subject exceptions are not permitted for Lineberger Comprehensive Cancer Center Investigator Initiated Trials under any circumstances. Other types of single subject exceptions may be allowed if proper regulatory review has been completed in accordance with Lineberger Comprehensive Cancer Center's Single Subject Exception Policy.

The attached version dated February 8, 2018 incorporates the above revisions

LINEBERGER COMPREHENSIVE CANCER CENTER
CLINICAL ONCOLOGY RESEARCH PROGRAM
UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

PROTOCOL AMENDMENT # 2

LCCC 1525: Phase II Study Of Pembrolizumab Therapy Following a Single Priming Dose of Cyclophosphamide in Patients With Metastatic Triple Negative Breast Cancer (TNBC)

AMENDMENT #2 INCORPORATES (Check all that apply):

X Editorial, Administrative Changes
X Scientific Changes
Therapy Changes
Eligibility Changes

AMENDMENT RATIONALE AND SUMMARY
This protocol amendment defines disease progression to correspond with the clinical assessments appropriate for the subject population. Disease progression can be determined by: (1) RECIST 1.1 criteria; (2) Clinical deterioration (3) Clinical progression (i.e. worsening of chest wall lesion). This allows for appropriate documentation of disease progression for subjects who end treatment and continue onto palliative care or hospice.
In addition to other administrative changes, an administrative letter from January 3, 2017, which defines the timeframe for ECHO or MUGO assessments as part of eligibility has been incorporated into this amendment.

SUMMARY OF CHANGES
1. Defined disease progression as: (1) RECIST 1.1 criteria; (2) Clinical deterioration (3) Clinical progression in Section 2.4.1.1
2. Section 4.4 edited to include definition of disease progression
3. Section 4.5 edited to ensure end of treatment time frame (+/- 10 days) is congruent with time and events table in 6.0
4. Time and Events Table 6.0:
    a. [1]Editotial change and clarification of ECHO/MUGO timeline
    b. [5]Language added to allow for CT from PET scan to be used in tumor imaging
    c. [10]Adjusted to correlate with correct assessments in table
5. Language added to allow for CT from PET scan to be used for tumor imaging was added to section 6.2
6. Section 6.4.1 was modified to correspond with definition of disease progression. This indicates subjects do not need imaging to confirm disease progression.

The attached version dated October 17, 2017 incorporates the above revisions

PROTOCOL AMENDMENT # 1

LCCC 1525: Phase II Study Of Pembrolizumab Therapy Following a Single Priming Dose of Cyclophosphamide in Patients With Metastatic Triple Negative Breast Cancer (TNBC)

AMENDMENT #1 INCORPORATES (Check all that apply):

X Editorial, Administrative Changes
X Scientific Changes
Therapy Changes
Eligibility Changes

AMENDMENT RATIONALE AND SUMMARY

1. The primary purpose of this amendment is to update safety language for pembrolizumab that MERCK provides for investigator initiated trial protocols.
2. Minor editorial changes were made to Inclusion criteria 9 and 10
3. The dose modification section 4.2.2.2 was updated to include the most recent dose modification guidelines for pembrolizumab
4. The Time and Events Table in Section 6.0 was updated to ensure that LFTs are collected at the beginning of every cycle of pembrolizumab.
5. Footnote 3 of the Time and Events table was updated to increase the 30-day follow-up period from a 7 day window to a 10 day window.
6. Additional safety data clarifications were incorporated into sections 7.1.1, 7.1.4, 7.3.3.3
7. A new section 7.3.3.2. was added providing protocol-specific exceptions to AE reporting
8. Clarified the use of irRECIST for assessment of immune-related response as described in section 6.7.5.1.

The attached version dated October 27, 2016 incorporates the above revisions

LINEBERGER COMPREHENSIVE CANCER CENTER
CLINICAL ONCOLOGY RESEARCH PROGRAM
UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

LCCC 1525: Phase II Study Of Pembrolizumab Therapy Following a Single Priming Dose of Cyclophosphamide in Patients With Metastatic Triple Negative Breast Cancer (TNBC)

Principal Investigator

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Clinical Protocol Office
Lineberger Comprehensive Cancer Center

[redacted]

Sponsor: Lineberger Comprehensive Cancer Center
Funding Source: Merck & Co., INC
Version Date: February 22, 2022

[redacted]

LINEBERGER COMPREHENSIVE CANCER CENTER
CLINICAL ONCOLOGY RESEARCH PROGRAM
UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

LCCC 1525: **Phase II Study Of Pembrolizumab Therapy Following a Single Priming Dose of Cyclophosphamide in Patients With Metastatic Triple Negative Breast Cancer (TNBC)**

Principal Investigator
E. Claire Dees, MD
[redacted]

Signature Page

The signature below constitutes the approval 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 legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

Principal Investigator (PI) Name: [redacted]

PI Signature: _____

Date:_____

Protocol Version Date: February 22, 2022

Amendment Number 6/ Version #: 1.0

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1.0 BACKGROUND AND RATIONALE

1.1 Study Synopsis

This phase II, single-arm, multicenter study will evaluate pembrolizumab therapy 200 mg IV given every 3 weeks (Q3W), following a single priming dose of cyclophosphamide 300 mg/m^2 IV in patients with advanced triple-negative breast cancer (TNBC) who have received at least one prior line of therapy. The primary objective is to estimate progression-free survival (PFS) for cyclophosphamide + pembrolizumab in advanced TNBC patients compared to pembrolizumab alone (historical PFS = 1.9 months) [1]. A biologically-focused primary objective is to describe the reduction in regulatory T cells (Tregs) in TNBC patients receiving this combination. Secondary objectives include assessments of overall response rate (ORR) per RECIST1.1, duration of response (DOR), disease control rate (DCR) and overall survival (OS) in this population receiving a single dose cyclophosphamide + pembrolizumab. Determination of response to study treatment based on immune-related response criteria (irRECIST) is an exploratory objective of this trial. Patients will continue study therapy until disease progression and toxicity will be assessed during treatment via NCI-CTCAE criteria v4. In addition, tumor samples from archival diagnostic tissue or fresh biopsy material (optional pre and post dose biopsy) and serial blood draws will be obtained for correlative biomarker studies.

Our hypothesis is that a single dose of cyclophosphamide given the day before initiation of pembrolizumab every 3 weeks will improve PFS in advanced TNBC patients by 1 month and that a total of 36 evaluable patients will be needed to detect a clinically-meaningful change in median PFS from 1.9 to 2.9 months. The goal of the proposed collateral biomarker research is to discover immunologic determinants of response to single-dose cyclophosphamide + pembrolizumab in this population. Our group has found that the basal-like and claudin-low molecular subtypes (the two subtypes that comprise virtually all TNBC) are enriched in tumor-infiltrating immune cells with gene-expression patterns consistent with active immunosuppression ([2] and unpublished data]). We anticipate that clinical response to programmed cell death protein 1 and ligand (PD-1/PD-L1) axis inhibition by pembrolizumab will be most pronounced in TNBC patients whose tumors have elicited an immune response that is actively suppressed through PD-1/PD-L1 interaction.

1.2 Disease and Study Background

It is estimated that 200,000 women world-wide are diagnosed annually with TNBC [3]. TNBC lacks expression of the estrogen and progesterone receptors (ER/PR) and the HER2 protein, and is associated with early and visceral recurrences, despite sensitivity to cytotoxics [4, 5]. In particular, TNBC can respond well to anthracyclines in combination with taxanes [6] or to taxanes or platinums as monotherapy [7], but the risks of relapse/recurrence remain quite high. In the metastatic TNBC population and from first presentation of advanced

disease, median PFS with chemotherapy alone is ~5-8 months with an OS of ~18 months [8, 9]. Novel approaches are clearly needed. One exciting area worthy of further exploration is immunotherapy, and in particular agents that inhibit the PD-1 and PD-L1.

1.2.1 Anti-PD1 and PD-L1 Agents in TNBC

Anti-PD1 and PD-L1 agents have demonstrated exciting activity in several human cancers with an ORR of just over 20% [10, 11]. Breast cancer has only recently been included in trials of immunomodulating agents, as this cancer has not been typically viewed as immunosensitive. In fact, intra-tumoral expression of PD1 and PD-L1 has been shown to be associated with decreased survival in basal-like breast cancer [12, 13] and preliminary data in TNBC with immune checkpoint inhibitors have generated enthusiasm for this approach. In the early-phase KEYNOTE-012 trial, treatment with the PD-1 inhibitor pembrolizumab demonstrated an ORR of 18.5%, including one complete response (CR) in 32 heavily pretreated patients with PD-L1-positive recurrent metastatic TNBC [1]. The rate of stable disease (SD) among 27 evaluable patients (median follow-up < 10 months) was 25.9%, and median PFS was 1.9 months. Grade 3/4 adverse events (AEs) occurred in 15.6% of patients, and the most common AEs were arthralgia (18.8%), fatigue (18.8%), myalgia (15.6%), and nausea (15.6%). Clearly this agent is worthy of further study in TNBC.

While promising the overall response rate (ORR) of 18.5% reported in TNBC is modest [1] . This may be due to the presence of intra-tumoral regulatory T cells (Tregs), potent suppressors of the immune response [14]. In vitro, basal-like breast cancer cells induce Treg polarization of undifferentiated CD4+ T cells [15]. Increased ratio of Treg to cytotoxic T lymphocytes (Treg/CTL ratio) in breast tumors has been associated with decreased survival in multiple studies [16].

1.2.2 Modulation of the immune microenvironment by cyclophosphamide

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades. Accumulating evidence shows a correlation between certain subsets of 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 capacity of cyclophosphamide to deplete Tregs is long-established [17]. It is important to note, however, that cyclophosphamide in combination with immunotherapy is not without risk. A recent study showed that repeated doses of cyclophosphamide may worsen outcomes when combined with other immunomodulating agents. Compared to the clinical profile of the CTLA-4 inhibitor ipilimumab when administered as monotherapy, investigators reported reduced efficacy coupled with a doubling of toxicities when cyclophosphamide (300 mg/m^2 IV) was given on D1 followed by ipilimumab on D3 for each of 4

cycles in patients with metastatic melanoma. In addition to depleting Tregs, cyclophosphamide may induce myeloid-derived suppressor cells and thereby promote tumor growth [18]. These effects have not been reported with cyclophosphamide given as a single dose prior to immunotherapy. Further, a single low dose of cyclophosphamide 300 mg/m^2 prior to a novel multi-epitope renal cancer vaccine markedly enhanced the clinical vaccine response in patients who developed an active epitope-specific T cell response to vaccination [19].

While multiple dosing strategies have been shown to diminish circulating Treg numbers, a proper dosing strategy that does not 1) yield depletion of activated cytotoxic T cells and/or 2) cause unacceptable drug-related toxicity is important to define. In one study of an allogeneic HER2-positive GM-CSF–secreting breast tumor vaccine, addition of 200 mg/m^2 cyclophosphamide had no impact on the rate of development of delayed-type hypersensitivity (a measure of effector T cell response), whereas a dose of 450mg/m^2 was associated with diminished effector responses [20]. Anti-HER2 humoral immunity following vaccination was best enhanced with the lower cyclophosphamide dose. In light of these data, we have chosen a one-time immune-priming dose of 300 mg/m^2 to be given prior to the first cycle of pembrolizumab in this study.

We have found in preclinical studies that depletion of Tregs by a drug-dependent genetic method or by a single infusion with low-dose cyclophosphamide results in improved survival and impaired tumor growth in a T11 murine model of TNBC of the claudin-low molecular subtype [Vincent and Serody, unpublished data]. When Treg were depleted using a single administration of low-dose cyclophosphamide or using a genetic depletion method, tumor growth was impaired; however, all mice eventually succumbed to tumor. Treg depletion with low-dose cyclophosphamide was markedly enhanced by concurrent treatment with anti-CTLA4 and anti-PD1 inhibitory antibodies; moreover, tumor impairment was pronounced and a small number of mice failed to develop tumor (3/20 in the combination treatment arm vs. 0/10 mice in the cyclophosphamide-only are and 0/6 mice in the untreated arm). Thus, we hypothesize that Treg depletion with a single priming dose of low-dose cyclophosphamide at 300 mg/m^2 IV given prior to the first cycle of pembrolizumab (200 mg q3W) will provide additional therapeutic benefit in TNBC compared to pembrolizumab alone.

1.3 Pembrolizumab

Pembrolizumab (MK-3475) is a potent and highly selective intravenous humanized mAb of the immunoglobulin (Ig) G4/kappa isotype that directly blocks the interaction between PD-1 and its ligands, PD-L1 and PD-L2. This blockade enhances functional activity of the target lymphocytes to facilitate an antitumor immune response, leading to tumor regression and immune rejection of the tumor. Keytruda^{TM} (Pembrolizumab) has recently been approved at a dose of 2 mg/kg IV every 3 weeks in the United States for the treatment of patients with unresectable or metastatic melanoma with disease progression following ipilumumab and, if BRAF V600 mutation positive, a BRAF inhibitor. Indications

currently under investigation by the manufacturer of pembrolizumab include non-small cell lung cancer and glioblastoma. In addition, preliminary data from the KEYNOTE-012 study of pembrolizumab has generated enthusiasm for further investigation of immune checkpoint inhibition as a treatment strategy for TNBC.

1.3.1 Rationale for Dose Selection/Regimen/Modification

An open-label Phase I trial (Protocol 001) is being conducted to evaluate the safety and clinical activity of single agent MK-3475. 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 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. 10.0 mg/kg Q2W, the highest dose tested in PN001, will be the dose and schedule utilized in Cohorts A, B, C and D of this protocol to test for initial tumor activity. Recent data from other clinical studies within the MK-3475 program has shown that a lower dose of MK-3475 and a less frequent schedule may be sufficient for target engagement and clinical activity.

PK data analysis of MK-3475 administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life (refer to IB). Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days). This early PK and pharmacodynamic 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 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 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 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.

1.4 Correlative Studies

Basal-like and claudin-low molecular subtypes of breast cancer are enriched in tumor-infiltrating immune cells with gene-expression patterns consistent with active immunosuppression ([2] and unpublished data]). A single priming dose of cyclophosphamide + pembrolizumab therapy may counteract the immunosuppressive microenvironment of TNBC and initiate a substantive antitumor response. The efficacy of this combination will likely correlate with the presence of a substantial, but suppressed, tumor immune infiltrate. Correlative biomarker studies in this trial will investigate this hypothesis.

Exploratory objectives based on serial blood collections and tumor samples will investigate whether the phenotype of tumor-infiltrating lymphocytes, including delineation of effector and regulatory T cells, from the diagnostic biopsy will correlate with clinical benefit. An evaluation of the predictive capacity of PD-L1 expression, immune gene signatures, and a characterization of the change in phenotype in tumor-infiltrating lymphocytes (TILs) before and after therapy will be explored. In addition, we hope to define T cell receptor (TCR) and B cell receptor (BCR) repertoire profiles associated with clinical benefit. We hypothesize that each of the following: increased PD-L1 mRNA expression, effector immune infiltrate, clonality in TILs and decreased regulatory cell populations, will be associated with clinical benefit (eg, ORR, PFS, DOR, CBR and OS).

Some or all of these correlative studies may be conducted under the auspices of the Merck sponsored UNC Immunotherapy PAtient Centered Translational

research biorepository (IMPACT), LCCC 1528 (PI Jon Serody, MD, UNC). This will involve co-enrollment in the IMPACT biorepository, which will be a separate consent but linked to IRB-approved Merck immunotherapy protocols.

Additional details regarding correlative studies can be found in the study procedures laboratory manual.

2.0 STUDY OBJECTIVES

2.1 Primary Objectives

2.1.1 Estimate progression-free survival (PFS) in patients with metastatic TNBC receiving single immune-priming dose of cyclophosphamide + pembrolizumab

2.1.2 To measure the reduction in Tregs in patients with metastatic TNBC receiving cyclophosphamide + pembrolizumab

2.2 Secondary Objectives

2.2.1 Estimate overall response rate (ORR) as assessed by RECIST1.1 in patients with metastatic TNBC receiving a single immune-priming dose cyclophosphamide + pembrolizumab

2.2.2 Estimate duration of response (DOR) in patients with metastatic TNBC receiving a single immune-priming dose of cyclophosphamide + pembrolizumab

2.2.3 Estimate disease control rate (DCR) in patients with metastatic TNBC receiving a single immune-priming dose of cyclophosphamide + pembrolizumab

2.2.4 Estimate overall survival (OS) in patients with metastatic TNBC receiving a single immune-priming dose of cyclophosphamide + pembrolizumab

2.2.5 Estimate the rate and intensity of treatment-associated toxicities (as assessed via NCI CTCAE v4), and the rate and intensity of Events of Clinical Interest for pembrolizumab

2.3 Exploratory Objectives

2.3.1 [redacted]

2.3.2 [redacted]

2.3.3 [redacted]

2.3.4 [redacted]

2.4 Endpoints

2.4.1 Primary Endpoint

2.4.1.1 PFS is defined as time from D1 of treatment until disease progression or death. Disease progression is defined as: (1) progression per RECIST1.1 (2) Clinical deterioration (3) Clinical progression (i.e. worsening of chest wall lesions)

2.4.1.2 Quantification of the reduction in T regs prior to and during therapy with cyclophosphamide + pembrolizumab.

2.4.2 Secondary Endpoints

2.4.2.1 ORR will be defined as the percentage of patients with [compete response (CR) + partial response (PR)] per RECIST1.1 (refer to section 6.7 for details)

2.4.2.2 Duration of response (DOR) is defined as the time from documentation of tumor response by RECIST1.1 [(CR) + (PR)] to disease progression (refer to section 6.7 for details)

2.4.2.3 Disease control rate (DCR) **will be defined as the percentage of patients, who achieve** CR, PR and stable disease (SD) per RECIST1.1 (refer to section 6.7 for

details). If best response is SD, then it must last for > 6 months to be included in calculation of DCR

2.4.2.4 OS is defined as the time from D1 of treatment to death from any cause

2.4.2.5 Clinician assessed toxicity will be classified and graded according to NCI-CTCAE criteria v4.0 based on changes in laboratory parameters, vital signs, and other safety assessments per standard of care

2.4.3 Exploratory Endpoints

2.4.3.1 [redacted]

2.4.3.2 [redacted]

2.4.3.3 [redacted]

2.4.3.4 [redacted]

3.0 PATIENT ELIGIBILITY

3.1 Inclusion Criteria

A subject must meet all of the following applicable inclusion criteria to participate in this study:

1. Written informed consent and HIPAA authorization for release of personal health information. **NOTE:** HIPAA authorization may be included in the informed consent or obtained separately.

2. Age ≥ 18 years at the time of consent.

3. Have measurable disease based on RECIST 1.1 (see section 6.7 for details).

4. ECOG Performance Status ≤ 1 as defined in Appendix A.

5. Subject must have histologically confirmed stage IV TNBC (ER-, PR-, HER2-negative) and have received at least 1 prior line of systemic therapy.

- ER- and PR-negative: defined as < 1% staining by immunohistochemistry (IHC)
- HER2-negative disease, defined as IHC 0-1+ or fluorescence in situ hybridization (FISH) ratio < 2.0

6. Patients with stable brain metastases will be allowed provided the following criteria are met:
    - Brain radiation was already provided at least 4 weeks prior to initiating study treatment
    - The subject has no new or progressive neurologic symptoms AND neurological symptom stability for the last 4 weeks prior to the study
    - The subject has been off of corticosteroids for at least 7 days prior to trial treatment
    - The subject does not have carcinomatous meningitis

7. Demonstrate adequate organ function as defined in the table below; all screening labs to be obtained within 72 h of initiating study treatment.

System	Laboratory Value
Hematological[1]	
Hemoglobin (Hgb)	≥ 9.0 g/dL
Absolute Neutrophil Count (ANC)	≥ 1000/mm^3
Platelets	≥ 100,000/mm^3
Renal	
Creatinine **OR** Calculated creatinine clearance	≤1.5 X ULN ≥ 60 mL/min for subject with creatinine levels > 1.5 X ULN (Cockcroft and Gault)
Hepatic	
Bilirubin	≤ 1.5 X upper limit of normal (ULN) or direct bilirubin ≤ ULN for subject with total bilirubin >1.5 X ULN
Aspartate aminotransferase (AST)	≤ 2.5 X ULN **OR** <5 X ULN for liver mets
Alanine aminotransferase (ALT)	≤ 2.5 X ULN **OR** <5 X ULN for liver mets
Albumin	≥2.5 g/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT)	≤ 1.5 × ULN unless subject is receiving anticoagulant therapy as long as PT and PTT is within therapeutic range of intended use of anticoagulants

[1]Note: Transfusions of blood and blood products as well as growth factor support are prohibited within 14 days prior to the first dose of study treatment.

8. Females of childbearing potential must have a negative serum pregnancy test within 72 hrs prior to treatment. **NOTE:** Females are considered of child bearing potential unless they are surgically sterile, have a congenital acquired condition that prevents childbearing (have undergone a hysterectomy, bilateral tubal ligation/occlusion, bilateral salpingectomy or bilateral oophorectomy at least 6 weeks prior to screening) or they are naturally postmenopausal for at least 12 consecutive months without an alternative medical cause. In women < 45 years of age a high follicle stimulating hormone level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

9. Female patients of childbearing potential must be willing to use appropriate birth control as outlined in Section 5.2.8, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication.

10. Male subjects of childbearing potential must agree to use an adequate method of contraception as outlined in Section 5.2.8 or abstain from heterosexual activity, starting with the first dose of study therapy through 120 days after the last dose of study therapy.

11. Consent for the use of any residual material from biopsy (archival tissue) and serial blood draws will be required for enrollment; fresh biopsy (pre and post dose) of tumor tissue will be optional. NOTE: Patients without adequate tissue for bio correlates will not be excluded or required to have a repeat biopsy.

12. As determined by the enrolling physician or protocol designee, the subject should be able to understand and comply with study procedures for the entire length of the study.

13. Has a LVEF within the normal institutional range (or ≥ 50%) based on an ECHO or MUGA, completed within 4 weeks prior to day one of treatment.

3.2 Exclusion Criteria

A subject will be excluded from this study for the following reasons:

1. Active infection requiring systemic therapy

2. Pregnant or breastfeeding (**NOTE:** breast milk cannot be stored for future use while the mother is being treated on study).

3. Has a known history of active TB (Bacillus Tuberculosis)

4. Hypersensitivity to pembrolizumab or any of its excipients.

5. Has a known additional malignancy that is active and/or progressive requiring treatment; exceptions include basal cell or squamous cell skin cancer, in situ cervical or bladder cancer, or other cancer for which the subject has been disease-free for at least five years.

6. Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to receipt of study medication or who has not recovered (i.e., ≤ Grade 1 or at baseline; excludes alopecia and Grade 2 neuropathy) from adverse events due to a previously administered agent.
    - If subject had major surgery, they must have recovered adequately from the toxicity and complications from the intervention prior to starting therapy

7. Has a known additional 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.

8. Has had monoclonal antibody therapy within 4 weeks prior to study Day 1 or who has not recovered (ie, ≤ Grade 1 at baseline; excludes alopecia and Grade 2 neuropathy) from adverse events due to agent(s) administered more than 4 weeks earlier.

9. Treatment with any investigational drug within 4 weeks or 5 half-lives, whichever is shorter, prior to the first dose of study medication.

10. Used an investigational device within 4 weeks of the first dose of treatment.

11. 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.

12. 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 (eg, thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

13. Has known history of (non-infectious) pneumonitis that required steroids, or current pneumonitis.

14. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent.

15. 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.

16. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

17. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

18. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).

19. Has participated in a previous trial and received pembrolizumab therapy

20. Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).

21. Has received a live vaccine within 30 days prior to the first dose of trial treatment.
    - 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

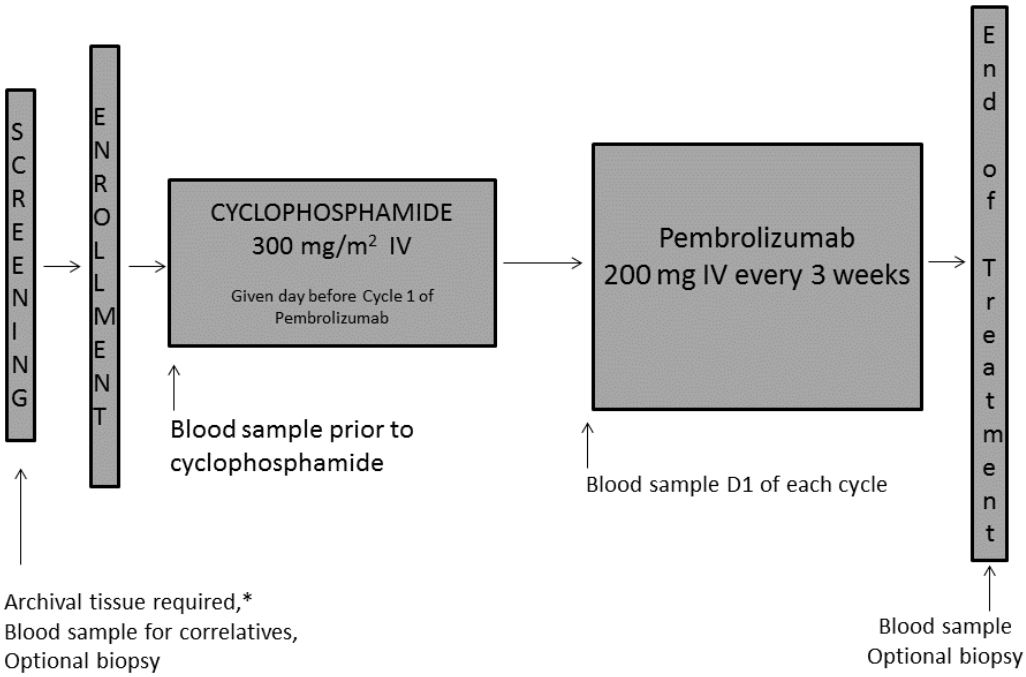
22. Cyclophosphamide is a substrate for cytochromes 2B6, 2C9, 3A4 and 2C19. Patients must not have received any drug that is a moderate or strong inhibitor of 2B6, 2C9, 3A4, and 2C19 within 1 week prior to receiving cyclophosphamide dosing through 72 hours after cyclophosphamide dosing. Patients must not have received any drug that is a moderate or strong inducer of 3A4 within 2 weeks prior to cyclophosphamide dosing.

    Note: A frequently updated P450 drug interaction table can be found at http://medicine.iupui.edu/clinpharm/ddis/. Refer to Appendix C: Prohibited Medications for additional information. A patient handout is provided in Appendix D Patient Handout: Prohibited Medications.

4.0 TREATMENT PLAN

This is a phase II, single-arm, multicenter study of a single dose (300 mg/m^2) of cyclophosphamide given the day before cycle 1 of pembrolizumab (200 mg), which will administered every 3 weeks in patients with metastatic TNBC who have failed at least one prior line of therapy. Toxicity will be assessed during treatment via NCI CTCAEv4. Archival tissue (or tissue from an optional fresh biopsy taken at baseline and at the end of treatment), and serial blood draws will be used for correlative studies.

4.1 Schema



4.2 Treatment Dosage and Administration

After screening and enrollment, treatment will consist of the following:

A single dose of cyclophosphamide 300 mg/m^2 IV (30 min – 60 min infusion) administered the day before cycle 1 of pembrolizumab therapy, followed by pembrolizumab 200 mg IV over 30 minutes on Day 2; Pembrolizumab will be repeated every 3 weeks until progression.

Agent	Dose	Route	Schedule
Cyclophosphamide	300 mg/m^2	IV over 30 - 60 minutes	Single dose on D1 of study
Pembrolizumab	200 mg	IV over 30 minutes	Starting on D2 of study; Every 3 weeks (21 days) until disease progression

4.2.1 Cyclophosphamide Administration

A single 300 mg/m^2 dose of cyclophosphamide IV over 30-60 minutes will be administered on Day 1 (D1) of this study. This is considered a low dose of cyclophosphamide compared to other regimens typically used to treat malignancies.

4.2.2 Pembrolizumab Administration

Pembrolizumab 200 mg will be administered as a 30 minute IV infusion starting on Day 2 (D2) of the study (hematology labs must meet inclusion criteria to initiate therapy with pembrolizumab). Every effort should be made 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. This manual is provided as a document separate from the protocol.

4.2.2.1 Management of Infusion Reactions for Pembrolizumab

Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Refer to the table below for 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	Subject may be premedicated 1.5hr (± 30 minutes) prior to infusion of pembrolizumab with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
	resolve and the subject should be premedicated for the next scheduled dose. Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.	
Grades 3 or 4 Grade 3: 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	Stop Infusion. 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.	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

4.2.2.2 Other Dose Modifications for Pembrolizumab

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 below. See section 4.2.2.3 for supportive care guidelines, including use of corticosteroids.

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)[a]	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
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	Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted	Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted
Infusion Reaction	2[b]	Toxicity resolves to Grade 0-1	Permanently discontinue if toxicity develops despite adequate premedication
	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
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.

[1a] 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.

[2b] 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 Table 2 – 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 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 be placed back on 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.

If a dose of pembrolizumab is delayed, then the subsequent dose should be administered when symptoms have resolved to a level permissive for re-initiation of therapy.

4.2.2.3 Rescue Medications and Supportive Care for Pembrolizumab

Supportive Care Guidelines

Patients 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 l. 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 does not need to follow the treatment guidance (as outlined below). Refer to Section 4.2.2.2 for dose modifications.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event.

- **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** that persists greater than 3 days, administer oral corticosteroids.
  - For **Grade 3 or 4 diarrhea/colitis** that persists > 1 week, 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 should 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 2-4** 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.

- **Infusion Reaction:**

  See section 4.2.2.1

4.3 Concomitant Medications/Treatments

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.

4.3.1 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.

4.3.2 Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase

- Anti-cancer systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol

- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- 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, chicken pox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however intranasal influenza vaccines (e.g. Flu-Mist®) are live attenuated vaccines, and are not allowed
- Glucocorticoids may be used to modulate symptoms from an ECI of suspected immunologic etiology, to prevent allergic reactions, and in supportive care of other adverse events. 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.

Subjects are prohibited from receiving the following therapies prior to and during the Screening Phase

- Cyclophosphamide is a substrate for cytochromes 2B6, 2C9, 2C19 and 3A4. Patients must not have received any drug that is a moderate or strong inhibitor of 3A4, 2B6, 2C9 and 2C19 within 1 week prior to cyclophosphamide dosing through 72 hours post cyclophosphamide dosing. Patients must not have received any drug that is a moderate or strong inducer of 3A4 within 2 weeks prior to cyclophosphamide dosing through 72 hours post cyclophosphamide dosing. A frequently updated P450 drug interaction table can be found at http://medicine.iupui.edu/clinpharm/ddis/. Refer to Appendix C: Prohibited Medications or Those to be used with Caution for additional information. A patient handout is provided in Appendix D Patient Handout: Prohibited Medications.

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.

4.4 Duration of Therapy

Treatment may continue until one of the following occurs:

- Disease progression
  Note: For unconfirmed radiographic disease progression, please see Sections 6.7.3 and 6.7.4
  Note: A subject may be granted an exception to continue on treatment with confirmed radiographic progression if clinically stable or clinically improved, please see Sections 6.7.3 and 6.7.4

Note: Disease progression can be determined by: (1) RECIST 1.1 criteria; (2) Clinical deterioration (3) Clinical progression (i.e. worsening of chest wall lesion).

- Inter-current illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Pregnancy
- Patient decides to withdraw from study treatment, **OR**
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Completed 24 months of uninterrupted treatment with pembrolizumab or 35 administrations of study medication, whichever is later

4.5 End of Treatment

The end of treatment visit should only occur when patients permanently stop study treatment and should be performed 30 days (+/-10 days) after the last dose of study medication. Patients who have an ongoing ≥grade 2 or serious AE (SAE) at this visit will continue to be followed until the event is resolved or deemed irreversible by the investigator.

4.6 Duration of Follow Up

Subjects will be followed for up to 2 years after removal from study treatment or until death, whichever occurs first. Patients removed from study for unacceptable adverse events (AEs) will be followed until resolution or stabilization of the event(s).

The long-term follow-up visit is defined as 90 days (+/- 15 days) after the last dose of treatment (see Section 6.1). All adverse events and concomitant medications should be followed up until this initial long-term follow-up visit (90 days after last pembrolizumab dose). Subsequent follow-up visits, defined as every 60 days thereafter (+/- 15 days) for up to 2 years or until death (whichever is first) may be conducted via telephone. These visits will be limited to history of any subsequent cancer treatments, an assessment of any SAE's considered to be possibly or probably related to study treatment until resolution, and survival status.

4.7 Removal of Patients from Protocol Therapy

Patients will be removed from protocol therapy and the PI notified when any of the criteria listed in section 4.4 apply. The reason for discontinuation of protocol therapy will be documented on the eCRF.

In case a patient decides to prematurely discontinue protocol therapy ("refuses treatment"), the patient should be asked if she or he may still be contacted for further scheduled study assessments. The outcome of that discussion should be documented in both the medical records and in the eCRF.

Excessive patient withdrawals from protocol therapy or from the study can render the study un-interpretable; therefore, unnecessary withdrawal of patients should be avoided.

4.8 Study Withdrawal

If a subject decides to withdraw from the study (and not just from protocol therapy) all efforts should be made to complete and report study assessments as thoroughly as possible. The investigator should contact the subject or a responsible relative by telephone or through a personal visit to establish as completely as possible the reason for the study withdrawal. A complete final evaluation at the time of the subject's study withdrawal should be made with an explanation of why the patient is withdrawing from the study. If the reason for removal of a subject from the study is an adverse event, the principal specific event will be recorded on the eCRF.

5.0 DRUG INFORMATION

5.1 Cyclophosphamide

Indications: Cyclophosphamide is an alkylating agent indicated for treatment of malignant diseases including malignant lymphomas, Hodgkin's disease, lymphocytic lymphoma, mixed-cell type lymphoma, histiocytic lymphoma, Burkitt's lymphoma, multiple myeloma, leukemias, mycosis fungiodes, neuroblastoma, adenocarcinoma of the ovary, retinoblastoma and breast carcinoma.

Mechanism of action: Cyclophosphamide is an alkylating agent that has anticancer activity. It is converted in the liver to active alkylating metabolites such as phosphoramide mustard by cytochrome-P450 enzymes. These alkylating metabolites interfere with the growth of susceptible rapidly proliferating malignant cells. Alkyl radicals intercalate into DNA strands and interfere with DNA replication.

Product description: Cyclophosphamide is supplied as 500 mg, 1 gm and 2 gm vials containing white powder for IV administration. Store vials at or below 25^0C (77^0F). Cyclophosphamide does not contain any antimicrobial preservative and care must be taken to assure the sterility of prepared solutions. USE ASEPTIC TECHNIQUE.

Solution preparation: Cyclophosphamide should be reconstituted in 25 mL (500 mg), 50 mL (1 gm) or 100 mL (2 gm) sterile water for injection, USP, for IV infusion. Shake vigorously to dissolve the drug. To minimize risk of dermal exposure, always wear gloves when handling vials containing cyclophosphamide sterile powder for injection.

Dose and Route of administration: A single IV dose of cyclophosphamide at 300 mg/m^2 over 30-60 min will be administered on D1 of this study.

Possible side effects:

- Nausea, vomiting, diarrhea
- Urinary bladder toxicity
- Bone marrow suppression
- Gonadal suppression
- Myelodysplasia
- Alopecia
- Immunosuppression
- Hyperpigmentation of the skin

Full prescribing information on cyclophosphamide is available at**:**
http://medlibrary.org/lib/rx/meds/cyclophosphamide-1/

Handling and Disposal: Please see policy on hazardous drugs:
http://news.unchealthcare.org/empnews/att/2011/nov/admin0188/.

Local requirements for disposal of hazardous drugs should be followed at each participating clinical site.

Precautions:
See section 4.3.2

5.2 Pembrolizumab

5.2.1 Description

Clinical Supplies will be provided by Merck as summarized in the table below.

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

5.2.2 Supplier/How Supplied

Pembrolizumab will be provided at no cost to the study patient by Merck, the manufacturer of the drug. Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

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.

5.2.3 Handling and Dispensing

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

Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site.

Clinical supplies may not be used for any purpose other than that stated in the protocol.

5.2.4 Storage and Stability

As per the pharmacy manual, provided as a document separate from the protocol.

5.2.5 Return and Retention

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 (eg, UNC IDS drug destruction 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.

5.2.6 Adverse Events Associated with Pembrolizumab

The most common adverse reactions (reported in ≥20% of patients in clinical trials of pembrolizumab) included fatigue, cough, nausea, pruritus, rash, decreased appetite, constipation, arthralgia, and diarrhea. The following warnings are associated with the use of pembrolizumab:

Immune-Mediated Pneumonitis

Pneumonitis occurred in ~3% of melanoma patients treated in clinical trials of pembrolizumab. The median time to development of pneumonitis was 5 months with a median duration of 4.9 months. The one patient with Grade 3 pneumonitis required initial treatment with high-dose systemic corticosteroids (greater than or equal to 40 mg prednisone or equivalent per day) followed by a corticosteroid taper. Pneumonitis completely resolved in seven of the nine patients with Grade 2-3 pneumonitis.

Immune-Mediated Colitis

Colitis (including microscopic colitis) occurred 1% of melanoma patients treated in clinical trials of pembrolizumab. The median time to onset of was 6.5 months with a median duration of 2.6 months. All three patients with Grade 2 or 3 colitis were treated with high-dose corticosteroids (greater than or equal to 40 mg prednisone or equivalent per day).

Immune-Mediated Hepatitis

Hepatitis (including autoimmune hepatitis) occurred in 0.5% of melanoma patients treated in clinical trials of pembrolizumab. The time to onset was 22 days for the case of Grade 4 hepatitis which lasted 1.1 months. The patient with Grade 4 hepatitis permanently discontinued pembrolizumab and was treated with high-dose (greater than or equal to 40 mg prednisone or equivalent per day) systemic corticosteroids followed by a corticosteroid taper. Both patients with hepatitis experienced complete resolution of the event.

Immune-Mediated Hypophysitis

Hypophysitis occurred in 0.5% of melanoma patients treated in clinical trials of pembrolizumab. The time to onset was 1.7 months for the patient with Grade 4 hypophysitis and 1.3 months for the patient with Grade 2 hypophysitis. Both patients were treated with high-dose (greater than or equal to 40 mg prednisone or equivalent per day) corticosteroids followed by a corticosteroid taper and remained on a physiologic replacement dose.

Renal Failure and Immune-Mediated Nephritis
Nephritis occurred in 3 (0.7%) patients of melanoma patients treated in clinical trials of pembrolizumab, consisting of one case of Grade 2 autoimmune nephritis (0.2%) and two cases of interstitial nephritis with renal failure (0.5%), one Grade 3 and one Grade 4. The time to onset of autoimmune nephritis was 11.6 months after the first dose of pembrolizumab (5 months after the last dose) and lasted 3.2 months; this patient did not have a biopsy. Acute interstitial nephritis was confirmed by renal biopsy in two patients with Grades 3-4 renal failure. All three patients fully recovered renal function with treatment with high-dose corticosteroids (greater than or equal to 40 mg prednisone or equivalent per day) followed by a corticosteroid taper.

Immune-Mediated Hyperthyroidism
Hyperthyroidism occurred in 5 (1.2%) of 411 melanoma patients treated in clinical trials of pembrolizumab. The median time to onset was 1.5 months and the median duration was 2.8 months (range 0.9 to 6.1). One of two patients with Grade 2 and the one patient with Grade 3 hyperthyroidism required initial treatment with high-dose corticosteroids (greater than or equal to 40 mg prednisone or equivalent per day) followed by a corticosteroid taper. One patient (0.2%) required permanent discontinuation of pembrolizumab due to hyperthyroidism. All five patients with hyperthyroidism experienced complete resolution of the event.

Immune-Mediated Hypothyroidism
Hypothyroidism occurred in 34 (8.3%) of 411 melanoma patients treated in clinical trials of pembrolizumab. The median time to onset of hypothyroidism was 3.5 months. All but two of the patients with hypothyroidism were treated with long-term thyroid hormone replacement therapy. The other two patients only required short-term thyroid hormone replacement therapy. No patient received corticosteroids or discontinued pembrolizumab for management of hypothyroidism. Thyroid disorders can occur at any time during treatment.

Other Immune-Mediated Adverse Reactions
Other clinically important immune-mediated adverse reactions can occur. The following clinically significant, immune-mediated adverse reactions occurred in less than 1% of patients treated with pembrolizumab, including exfoliative dermatitis, uveitis, arthritis, myositis, pancreatitis, hemolytic anemia, partial seizures arising in a patient with inflammatory foci in brain parenchyma, and adrenal insufficiency.

Across clinical studies with pembrolizumab in approximately 2000 patients, the following additional clinically significant, immune-mediated adverse reactions were reported in less than 1% of patients: myasthenic syndrome, optic neuritis, and rhabdomyolysis.

Embryofetal Toxicity
Based on its mechanism of action, pembrolizumab may cause fetal harm when administered to a pregnant woman. Animal models link the PD-1/PD-L1 signaling pathway with maintenance of pregnancy through induction of maternal immune tolerance to fetal tissue.

5.2.7 Contraindications

There are no reported contraindications associated with the use of pembrolizumab.

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.

5.2.8 Contraception

Female and male subjects of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving study drug and for 120 days after the last dose of study drug by complying with one of the following:
(1) practice abstinence[†] from heterosexual activity;

OR

(2) use (or have their partner use) acceptable contraception during heterosexual activity.

Acceptable methods of contraception are[‡]:

Single method (one of the following is acceptable):
- intrauterine device (IUD)
- vasectomy of a female subject's male partner
- contraceptive rod implanted into the skin

Combination method (requires use of two of the following):
- diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- cervical cap with spermicide (nulliparous women only)
- contraceptive sponge (nulliparous women only)
- male condom or female condom (cannot be used together)
- hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

†Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and ERCs/IRBs. Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

‡If a contraceptive method listed above is restricted by local regulations/guidelines, then it does not qualify as an acceptable method of contraception for subjects participating at sites in this country/region.

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 subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 120 days after the last dose of trial therapy. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

5.2.9 Use in Pregnancy

If a patient inadvertently becomes pregnant while on treatment with pembrolizumab, the patient will immediately be removed from the study. The site will contact the patient at least monthly and document the patient'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 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 patient 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 7.3.3.

5.2.10 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, patients who are breast-feeding are not eligible for enrollment.

5.2.11 Overdose

For purposes of this trial, an overdose will be defined as any dose exceeding the prescribed dose for pembrolizumab by 20% over the prescribed dose. No specific

information is available on the treatment of overdose of pembrolizumab. In the event of overdose, pembrolizumab should be discontinued and the patient should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

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 Sponsor and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220) (see section 7.3.3)

6.0 EVALUATIONS AND ASSESSMENTS

6.1 Time and Events Table

Assessments	Pre-study[1]	Study Day 1	Study Day 2							
		Single Dose cyclo[2]	D1[2] Cycle 1 pembro	D1[2] Cycle 2 pembro	D1[2] Cycle 3 pembro	D1[2] Cycle 4 pembro	D1[2] Cycle 5 pembro	D1[2] Cycle 6-n pembro	End of Treatment[3]	Long-term Follow-up[3]
Informed Consent	X									
History[4]	X	X	X	X	X	X	X	X	X	X
Physical exam[4]	X	X		X	X	X	X	X	X	X
Performance Status	X	X		X	X	X	X	X	X	
ECHO or MUGA	X									
Tumor measurement[5]	X					X		X[5]	X	
Pregnancy test	X[6]									
Hematology[7]	X	X	X	X	X	X	X	X	X	
Serum chemistries[7]	X	X		X	X	X	X	X	X	
Liver function tests[7]	X	X		X	X	X	X	X	X	
Thyroid panel[8]	X				X		X	X	X	
Coagulation[9]	X	If patient is on anticoagulant therapy monitor per investigator's discretion								
Toxicity Assessment	X	X	X	X	X	X	X	X	X	X
Concomitant Meds	X	X	X	X	X	X	X	X	X	
Pembrolizumab IV			X	X	X	X	X	X		
Cyclophosphamide IV		X								
Request archival	X[10]									
Blood sample[11]	X	X	X	X	X	X	X	X	X	
Tumor biopsy Optional	X[10]								X[10]	

Survival analysis										X

Key to Time and Events Table Footnotes

[1]Radiological assessments, ECHO or MUGA, and physical exam may be performed up to 4 weeks prior to day 1 of treatment. Other evaluations except for pregnancy must be performed within 2 weeks prior to Cycle 1 Day 1 of treatment. Serum B-HCG must be performed within 72 hours prior to first dose of study medication for women of child-bearing potential. Screening labs performed within 72 hours prior to Cycle 1 Day 1 do not need to be repeated on C1D1.

[2] On Study D1 a single priming dose of cyclophosphamide will be given the day before initiating treatment with pembrolizumab on Day 2 of the study. Pembrolizumab cycles are to be repeated every 3 weeks (21 days) until progression. A window of +/- 3 days applies to all study visits.

[3]The end of treatment visit should be performed 30 days (+/-10 days) after the last dose of study medication. Patients who have on ongoing ≥grade 2 or serious AE (SAE) at this visit will continue to be followed until the event is resolved or deemed irreversible by the investigator. The first long-term follow-up visit is defined as 90 days (+/- 15 days) after the last dose of treatment. Serious adverse events (SAEs; or follow-up to any SAEs) that occur within 90 days of the end of pembrolizumab (or prior to start of new anti-cancer therapy, whichever is earlier) or any grade of Events of Clinical Interest (see section 7.3 and the ECI Guidance Document that is provided as a document separate from this protocol) that occur within 90 days of the end of pembrolizumab or 30 days after the initiation of a new anti-cancer therapy (whichever is earlier) must be recorded. Subsequent follow-up visits, defined as every 60 days thereafter (+/- 15 days) for up to 2 years or until death (whichever is first) may be conducted via telephone. These visits will be limited to history of any subsequent cancer treatments, an assessment of any SAE's considered to be possibly or probably related to study treatment until resolution, and survival status.

[4]Perform a complete history at baseline only, thereafter focused history on symptoms/toxicity; physical exam to include height (baseline only), examination of the skin, weight, and vital signs.

[5]Tumor imaging should remain consistent throughout study, and should include contrasted computed tomography (CT) of the chest, abdomen and pelvis, bone scan and, if clinically indicated, brain MRI. CT Scan from PET scan can also be used instead of contrast CT. The scans should be performed every 9 weeks at the end of cycles 3, 6, 9, etc., in the first year and every 12 weeks after one year until progression. EOT scan will not be performed if subject cannot withstand scan due to clinical deterioration, or if progression can be determined clinically (i.e. worsening of chest wall lesion).

[6]Serum B-HCG must be performed within 72 hours prior to first dose of study medication for women of child-bearing potential.

[7]Hematology: CBC with differential, Hgb, and platelet count; Liver function tests: total bilirubin, AST (SGOT) and ALT (SGPT). Serum chemistry: potassium, sodium, calcium, creatinine, magnesium, phosphorus, BUN, albumin, and total protein.

[8]Thyroid panel includes TSH, T3 and free T4. If consistent with institutional standard of care, it is acceptable to obtain TSH alone and only obtain T3 and free T4 if clinically useful. This test should be performed at screening and then every 6 weeks during pembrolizumab therapy (D1 of odd numbered cycles of pembrolizumab) and at the end of treatment visit.

[9]Coagulation profile includes prothrombin time or INR, and activated partial thromboplastin time. Patient entering the study while receiving anti-coagulation therapy or those who have the initiation of an anti-coagulation therapy should have their coagulation test performed per the investigator's discretion.

[10]Fixed paraffin-embedded blocks or slides from the original diagnostic specimen or from a recurrence must be requested for patients at baseline. We will collect fresh biopsies (optional) in consenting patients for correlative studies at baseline (predose) and end of treatment (post dose). Additional details are provided in section 6.5 and in the study procedures laboratory manual

[11]Blood samples (taken predose) will be collected for correlative studies. Additional details are provided in section 6.5 and in the study procedures laboratory manual.

6.2 Pre-Study Assessments

Clinical evaluation: complete history; physical exam to include height (baseline only) and weight, examination of skin and vital signs; ECOG performance status (see Appendix A)

Laboratory studies:

- **Pregnancy Test**: A serum pregnancy test (β-HCG) is required for all women of childbearing potential at screening within 72 hours prior to the first dose of study treatment under this protocol.
- **Hematology**: CBC with differential, Hgb, and platelet count
- **Serum Chemistries**: These include the following parameters: potassium, sodium, calcium, creatinine, magnesium, phosphorus, BUN, albumin, and total protein (or calculate creatinine clearance via Cockcroft-Gault (see Appendix B) as noted in inclusion criterion #7)
- **LFTs:** These include total bilirubin, AST (SGOT), ALT (SGPT)
- **Coagulation Studies:** PT or INR and PTT (Note: Patient entering the study while receiving anti-coagulation therapy or those who have the initiation of an anti-coagulation therapy should have their coagulation test performed per investigator's discretion)
- **Thyroid panel:** TSH, T3 and free T4 (see footnote 8 in Time and Events table)

Archival tissue**:** Request access to archival tissue to support correlative studies. (see section 6.5.1); consent for the use of any residual material from biopsy will be required for enrollment. Patients without adequate tissue for bio correlates will not be excluded or required to have a repeat biopsy.

Optional tumor biopsy: Collect a fresh biopsy in consenting subjects for correlative studies.

Blood sample for biomarkers: See section 6.5.2 and refer to the Study Procedures Laboratory Manual for sample collection details.

Concomitant Medications**:** Review (see section 4.3, Appendix C: Prohibited Medications and exclusion criterion #21)

Toxicity Assessment**:** Perform per NCI CTCAEv4

Tumor imaging**:** Baseline tumor imaging should include computed tomography (CT) of the chest, abdomen and pelvis, bone scan and, if clinically indicated, brain MRI. CT Scan from PET scan can also be used instead of contrast CT.

6.3 Treatment Assessments

6.3.1 Study D1 (perform all assessments prior to cyclophosphamide administration)

Clinical evaluation: focused history on symptoms/toxicity; physical exam to include weight, examination of skin, vital signs and ECOG performance status (see Appendix A)
Laboratory studies: (need not be repeated if performed ≤ 72 hours prior to first dose)

- **Hematology:** CBC with differential, Hgb, and platelet count
- **Serum Chemistries:** These include the following parameters: potassium, sodium, calcium, creatinine, magnesium, phosphorus, BUN, albumin, and total protein
- **LFTs:** These include total bilirubin, AST (SGOT), ALT (SGPT)

Concomitant Medications: Review (see section 4.3)
Toxicity: Per NCI CTCAEv4
Cyclophosphamide administration: 300 mg/m^2 IV over 30-60 min
Blood sample for biomarkers: (see section 6.5.2) Taken predose: 3x 8mLs whole blood in ACD tubes required at each scheduled draw (Required)

6.3.2 Study Day 2 (D1 Cycle 1 pembrolizumab)

Clinical evaluation: focused history on symptoms/toxicity
Laboratory studies:

- **Hematology:** CBC with differential, Hgb, and platelet count

Concomitant Medications: Review (see section 4.3)
Toxicity: Per NCI CTCAEv4
Pembrolizumab administration: 200 mg IV over 30 min
Blood sample for biomarkers: (see section 6.5.2); 3x 8mLs whole blood in ACD tubes required at each scheduled draw (Required)

6.3.3 D1 Cycle 2

Clinical evaluation: focused history on symptoms/toxicity. Physical exam to include weight, examination of skin, vital signs and ECOG performance status (see Appendix A)
Laboratory studies:

- **Hematology:** CBC with differential, Hgb, and platelet count
- **Serum Chemistries:** These include the following parameters: potassium, sodium, calcium, creatinine, magnesium, phosphorus, BUN, albumin, and total protein
- **LFTs:** These include total bilirubin, AST (SGOT), ALT (SGPT)

Concomitant Medications: Review (see section 4.3)
Toxicity: Per NCI CTCAEv4

Pembrolizumab administration: 200 mg IV over 30 min
Blood sample for biomarkers: (see section 6.5.2); 3x 8mLs whole blood in ACD tubes required at each scheduled draw (Required)

6.3.4 D1 Cycle 3

Clinical evaluation: focused history on symptoms/toxicity. Physical exam to include weight, examination of skin, vital signs and ECOG performance status (see Appendix A)
Laboratory studies:

- **Hematology:** CBC with differential, Hgb, and platelet count
- **Serum Chemistries:** These include the following parameters: potassium, sodium, calcium, creatinine, magnesium, phosphorus, BUN, albumin, and total protein
- **LFTs:** These include total bilirubin, AST (SGOT), ALT (SGPT)
- **Thyroid panel:** TSH, T3 and free T4

Concomitant Medications: Review (see section 4.3)
Toxicity: Per NCI CTCAEv4
Pembrolizumab administration: 200 mg IV over 30 min
Blood sample for biomarkers: (see section 6.5.2); 3x 8mLs whole blood in ACD tubes required at each scheduled draw (Required)

6.3.5 D1 Cycle 4

Clinical evaluation: focused history on symptoms/toxicity. Physical exam to include weight, examination of skin, vital signs and ECOG performance status (see Appendix A)
Laboratory studies:

- **Hematology:** CBC with differential, Hgb, and platelet count
- **Serum Chemistries:** These include the following parameters: potassium, sodium, calcium, creatinine, magnesium, phosphorus, BUN, albumin, and total protein
- **LFTs:** These include total bilirubin, AST (SGOT), ALT (SGPT)

Concomitant Medications: Review (see section 4.3)
Toxicity: Per NCI CTCAEv4)
Pembrolizumab administration: 200 mg IV over 30 min
Tumor Imaging: repeat assessments performed at baseline
Blood sample for biomarkers: (see section 6.5.2); 3x 8mLs whole blood in ACD tubes required at each scheduled draw (Required)

6.3.6 D1 Cycle 5

Clinical evaluation: focused history on symptoms/toxicity. Physical exam to include weight, examination of skin, vital signs and ECOG performance status (see Appendix A)
Laboratory studies:

- **Hematology:** CBC with differential, Hgb, and platelet count

- **Serum Chemistries:** These include the following parameters: potassium, sodium, calcium, creatinine, magnesium, phosphorus, BUN, albumin, and total protein
- **LFTs:** These include total bilirubin, AST (SGOT), ALT (SGPT)
- **Thyroid panel:** TSH, T3 and free T4

Concomitant Medications: Review (see section 4.3)
Toxicity: Per NCI CTCAEv4)
Pembrolizumab administration: 200 mg IV over 30 min
Blood sample for biomarkers: (see section 6.5.2); 3x 8mLs whole blood in ACD tubes required at each scheduled draw (Required)

6.3.7 D1 Cycle 6-n

Clinical evaluation: focused history on symptoms/toxicity. Physical exam to include weight, examination of skin, vital signs and ECOG performance status (see Appendix A)
Laboratory studies:

- **Hematology:** CBC with differential, Hgb, and platelet count
- **Serum Chemistries:** These include the following parameters: potassium, sodium, calcium, creatinine, magnesium, phosphorus, BUN, albumin, and total protein
- **LFTs:** These include total bilirubin, AST (SGOT), ALT (SGPT)
- **Thyroid panel:** TSH, T3 and free T4 (D1 of cycles 7, 9, 11, etc.)*

Concomitant Medications: Review (see section 4.3)
Toxicity: Per NCI CTCAEv4; Patients who have on ongoing ≥grade 2 or serious AE (SAE) at this visit will continue to be followed until the event is resolved or deemed irreversible by the investigator.
Pembrolizumab administration: 200 mg IV over 30 min
Tumor Imaging: repeat assessments performed at baseline at the end of cycles 6, 9, 12, etc., for the first year. After the first year, tumor imaging will be performed every 12 weeks, at the end of cycles 22, 26, 30, etc until disease progression/end of treatment.
Blood sample for biomarkers: (see section 6.5.2); 3x 8mLs whole blood in ACD tubes required at each scheduled draw (Required)

***Note**: Thyroid panel should be performed every 6 weeks during treatment with pembrolizumab i.e., D1 of odd numbered cycles 7, 9, 11, etc.

6.4 Post-Treatment/Follow-up Assessments

6.4.1 End of Treatment

Clinical evaluation: focused history on symptoms/toxicity. Physical exam to include weight, examination of skin, vital signs and ECOG performance status (see Appendix A)
Laboratory studies:

- **Hematology:** CBC with differential, Hgb, and platelet count

- **Serum Chemistries:** These include the following parameters: potassium, sodium, calcium, creatinine, magnesium, phosphorus, BUN, albumin, and total protein
- **LFTs:** These include total bilirubin, AST (SGOT), ALT (SGPT)
- **Thyroid panel:** TSH, T3, free T4

Concomitant Medications: Review (see section 4.3)
Toxicity: Per NCI CTCAEv4)
Tumor Imaging: repeat assessments performed at baseline. Tumor imaging is not required to assess progression. If subject cannot withstand tumor imaging due to clinical deterioration, imaging will not be required.
Blood sample for biomarkers: (see section 6.5.2); 3x 8mLs whole blood in ACD tubes required at each scheduled draw (Required)
Optional tumor biopsy: Collect a fresh post dose biopsy in consenting subjects for correlative studies.

6.4.2 Long-term follow up (90 days after last dose of pembrolizumab)

Clinical evaluation: focused history on symptoms/toxicity. Physical exam to include weight, examination of skin, and vital signs
Toxicity: Per NCI CTCAEv4; Serious adverse events (SAEs; or follow-up to any SAEs) that occur within 90 days of the end of pembrolizumab (or prior to start of new anti-cancer therapy, whichever is earlier) or any grade of Events of Clinical Interest (see section 7.3 and the ECI Guidance Document that is provided as a document separate from this protocol) that occur within 90 days of the end of pembrolizumab or 30 days after the initiation of a new anti-cancer therapy (whichever is earlier) must be recorded.
Survival analysis:

Note: Subsequent follow-up visits to occur every 60 days (+/- 15 days) for up to 2 years or until death (whichever is first) may be conducted via telephone, and will be limited to history of any subsequent cancer treatments, an assessment of any SAE's considered to be possibly or probably related to study treatment until resolution, and survival status.

6.5 Correlative Studies Procedures

These are described in more detail in the study procedures laboratory manual.

6.5.1 Archival Tissue

Archival tumor tissue (paraffin-embedded blocks/slides) from the patient's original diagnostic biopsy will be requested and collected from all enrolled patients. See accompanying laboratory manual for additional details, including details on shipping and storage.

6.5.2 Fresh Biopsy (optional)

All patients will also be asked if a fresh biopsy is possible prior to initiation of therapy and at the end of treatment but these biopsies are not required (this is optional). See accompanying laboratory manual for additional details, including details on shipping and storage.

6.5.3 Blood samples

Patients will have a three ACD tubes (whole blood, 3 x 8mL) collected at the times as noted in the Time and Event table (sample should be collected predose). Sample collection, storage, and shipment instructions for serum samples will be provided in the Laboratory Manual.

6.6 Assessment of Safety

Any patient who receives treatment on this protocol will be evaluable for toxicity. Each patient will be assessed periodically for the development of any toxicity according to the Time and Events table (section 6.0). Toxicity will be assessed according to the NCI CTCAE v4. 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 adverse events (AEs) of unknown etiology associated with pembrolizumab exposure should be evaluated to determine if it is an Event of Clinical Importance (ECI) with a potential immunologic etiology (termed immune-related adverse events, or irAEs); see the separate ECI guidance document (provided as a document separate from this protocol) regarding the identification, evaluation and management of potential irAEs.

Progression of the cancer under study is not considered an adverse event unless it is considered to be drug related by the investigator.

Please refer to section 7.0 for detailed information regarding the assessment of toxicity and recording of AEs.

6.7 Assessment of Efficacy

Subjects who have received at least 1 dose of pembrolizumab will be evaluable for assessment of response and progression. Progression can be documented: (1) according to RECIST 1.1 criteria per tumor imaging; (2) as Clinical deterioration; or (3) Clinical Progression. Subjects whose cancer growth is documented by physical examination without imaging confirmation will count as progression (i.e. worsening of chest wall lesions). Patients who drop out of the study for any reason (eg. toxicity of treatment, decide to withdraw) will still be followed for PFS and OS.

6.7.1 Assessment of Disease-Tumor Measurement Based on RECIST 1.1

Disease assessment according to RECIST 1.1 criteria will include imaging and physical examination. Refer to the Response Evaluation Criteria in Solid Tumors (RECIST) Committee, version 1.1 (Eur J Cancer 45;2009:228-247) for additional details on RECIST 1.1. To ensure comparability between baseline and subsequent disease assessments, the same method(s) of assessment (eg, CT scan, MRI, etc) will be used throughout the study for determining response. Tumor assessment will be completed as outlined in the Time and Events table in section 6.1.

6.7.2 Baseline Documentation of Target and Non-Target Lesions

All measurable lesions up to a maximum of 5 lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline.

Target lesions should be selected on the basis of their size (lesions with the longer diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements.

A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

All other lesions (or sites of disease) including pathological lymph nodes should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as “present” or “absent”, or in rare cases “unequivocal progression”.

6.7.3 Evaluation of Target Lesions using RECIST 1.1 Criteria

NOTE: In addition to the information below, also see section 4.3.2 in the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee, version 1.1 (Eur J Cancer 45;2009:228-247) for special notes on the assessment of target lesions.

Complete response (CR)−Disappearance of all target lesions. Any pathological lymph node (LN) (whether target or non-target) must have decreased in short axis to <10mm.

Partial response (PR)−At least a 30% decrease in the sum of the longest diameter LD of the target lesions taking as reference the baseline sum LD.

Progressive Disease (PD)−At least a 20% increase in the sum of the LD of the target lesions taking as reference the smallest sum LD recorded since the

treatment started including baseline if that is the smallest on study. In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5mm. The appearance of one or more new lesions also constitutes PD.

Note: Any progressive disease as defined by RECIST1.1 in patients that are asymptomatic without signs or symptoms of impending visceral crisis SHOULD be confirmed by a repeat scan to rule out "tumor flare". In these patients and per MD discretion, pembrolizumab therapy should be continued in these patients until the repeat scan is performed≥ 4 weeks and no more than 6 weeks from the time the initial scan indicated PD. If PD is confirmed, patients will be withdrawn from study. If tumor flare confirmed, patients may continue therapy as per protocol. Subjects that have clear progressive disease as defined by RECIST 1.1 should be withdrawn from treatment and a repeat scan is not necessary.

Stable disease (SD)−Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD since the treatment started.

6.7.4 Evaluation of Non-Target Lesions using RECIST 1.1 Criteria

Complete response (CR)−Disappearance of all non-target lesions and normalization of tumor marker levels. All LN must be non-pathological in size (<10mm short axis).

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

Progressive disease (PD)−Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions.

Note: Any progressive disease as defined by RECIST1.1 in patients that are asymptomatic without signs or symptoms of impending visceral crisis SHOULD be confirmed by repeat scan to rule out "tumor flare". In these patients and per MD discretion, pembrolizumab therapy should be continued in these patients until the repeat scan is performed ≥ 4 weeks but no more than 6 weeks later. If PD is confirmed, patients will be withdrawn from study. If tumor flare confirmed, patients may continue therapy as per protocol. Subjects that have clear progressive disease as defined by RECIST 1.1 should be withdrawn from treatment and a repeat scan is not necessary.

6.7.5 Other Efficacy Parameters

6.7.5.1 Evaluation of Response by irRECIST

An exploratory objective of this study involves assessment of ORR, DCR, PFS and DOR based in immune response criteria as outlined below (see Guidelines for

the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Wolchok JD et al. Clin Cancer Res 2009;15(23):7412-20, Nishino et al. Clin Cancer Res 19(14):3936-43, and Bohnsack O. et al., *Adaptation of the immune related response criteria: irRECIST.* Annals of Oncology (2014) 25 (suppl_4): iv361-iv372. 10.1093/annonc/mdu342, 2014).

irRECIST criteria are based on irRC criteria (Wolchok et al) adapted for unidimensional measurement as outlined by Nishino et al. The adaptation described by Bohnsack et al. further aligns irRECIST to allow for assessment of baseline non-target lesions and new non-measurable lesions, and discusses the impact of those lesions on the overall tumor response assessment. The adaptation by Bohnsack et al. allows for evaluation of patients with non-target disease only and patients in the adjuvant setting (not applicable for this study in subjects with metastatic disease).

Overall response using the irRECIST is based on tumor burden as follows:

irCR	Complete disappearance of all lesions (whether measurable or not, and no new lesions) Lymph nodes must decrease to < 10 mm in short axis. Confirmation of response is not mandatory.
irPR	Decrease in tumor burden ≥30% in TMTB relative to baseline confirmed by a consecutive assessment at least 4 weeks after first documentation, non-target lesions are irNN, and no equivocal progression of new non-measurable lesions.
irSD	Not meeting criteria for irCR, irPR, in absence of irPD
irPD	Increase in tumor burden ≥20% increase and minimum 5 mm absolute increase in TMTB compared to nadir, or irPD for non-target or new non-measurable lesions confirmed by a repeat, consecutive assessment no less than 4 weeks from the date first documented
irNN	No target disease was identified at baseline and at follow-up the patient fails to meet criteria for irCR or irPD.

irNN = irNon-CR/Non-PD
TMTB = Total measureable tumor burden

7.0 ADVERSE EVENTS

7.1 Definitions

7.1.1 Adverse Event (AE)

An adverse event (AE) is any untoward medical occurrence (e.g., an abnormal laboratory finding, symptom, or disease temporally associated with the use of a drug) in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product.

Hospitalization for elective surgery or routine clinical procedures that are not the result of an AE (e.g., surgical insertion of central line) need not be considered AEs and should not be recorded as an AE. Disease progression should not be recorded as an AE, unless it is attributable by the investigator to the study therapy.

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 7.3.3. 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.

7.1.2 Suspected Adverse Reaction (SAR)

A suspected adverse reaction (SAR) is any AE for which there is a *reasonable possibility* that the drug is the cause. *Reasonable possibility* means that there is evidence to suggest a causal relationship between the drug and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Causality assessment to a study drug is a medical judgment made in consideration of the following factors: temporal relationship of the AE to study drug exposure, known mechanism of action or side effect profile of study treatment, other recent or concomitant drug exposures, normal clinical course of the disease under investigation, and any other underlying or concurrent medical conditions. Other factors to consider in considering drug as the cause of the AE:

- Single occurrence of an uncommon event known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome)
- One or more occurrences of an event not commonly associated with drug exposure, but otherwise uncommon in the population (e.g., tendon rupture); often more than once occurrence from one or multiple studies would be needed before the sponsor could determine that there is *reasonable possibility* that the drug caused the event.
- An aggregate analysis of specific events observed in a clinical trial that indicates the events occur more frequently in the drug treatment group than in a concurrent or historical control group

7.1.3 Unexpected AE or SAR

An AE or SAR is considered unexpected if the specificity or severity of it is not consistent with the applicable product information (e.g., Investigator's Brochure (IB) for an unapproved investigational product or package insert/summary of product characteristics for an approved product). Unexpected also refers to AEs or SARs that are mentioned in the IB as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious AE or SAR

An AE or SAR is considered serious if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death;
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- Requires inpatient hospitalization (>24 hours) or prolongation of existing hospitalization;*
- Results in congenital anomaly/birth defect;
- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;

- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definition. For reporting purposes, also consider the occurrences of pregnancy as an event which must be reported as an important medical event.

*Hospitalization for anticipated or protocol specified procedures such as administration of chemotherapy, central line insertion, metastasis interventional therapy, resection of primary tumor, or elective surgery, will not be considered serious adverse events.

Pregnancy that occurs during the study must also be reported as an SAE.

- **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.

7.2 Documentation of non-serious AEs or SARs

For non-serious AEs or SARs, documentation must begin from day 1 of study treatment and continue through the 30 day follow-up period after treatment is discontinued.

Collected information should be recorded in the Case Report Forms (CRF) for that patient. Please include a description of the event, its severity or toxicity grade, onset and resolved dates (if applicable), and the relationship to the study drug. Documentation should occur at least monthly.

7.3 SAEs, Serious SARs or Events of Clinical Interest

7.3.1 Timing

After informed consent but prior to initiation of study medications, only SAEs caused by a protocol-mandated intervention will be collected (e.g. SAEs related to invasive procedures such as biopsies, medication washout).

For any other experience or condition that meets the definition of an SAE or a serious SAR, recording of the event must begin from day 1 of study treatment and

continue through the 90 day follow-up period after treatment is discontinued (or to the initiation of new anti-cancer treatment, whichever is earliest).

7.3.2 Documentation and Notification

These events (SAEs or Serious SARs) must be recorded in the SAE console within Oncore™ for that patient within 24 hours of learning of its occurrence. For Affiliate sites, an email must also be sent to the NCCN Project Manager indicating that an SAE or Serious SAR has been entered into Oncore (email contact will be provided at study start-up).

7.3.3 Reporting

IRB Reporting Requirements:

UNC**:**

- UNC will submit an aggregated list of all SAEs to the UNC IRB annually at the time of study renewal according to the UNC IRB policies and procedures.

The UNC-IRB will be notified of all SAEs that qualify as an Unanticipated Problem as per the UNC IRB Policies using the IRB's web-based reporting system (see section 9.5.3) within 7 days of the Investigator becoming aware of the problem.

Affiliate sites:

- For affiliate sites using a local IRB of record, please submit adverse events per local IRB policy.
- For affiliate sites relying on the UNC-IRB, an aggregated list of all SAEs will be submitted to the UNC IRB annually at the time of study renewal according to the UNC IRB policies and procedures. In addition, any SAEs that qualify as an Unanticipated Problem will be entered into Oncore and reported to the UNC IRB by the UNCCN Project Manager using the IRB's web-based reporting system (see section 9.5.3) within 7 days of the Investigator becoming aware of the problem.

Pregnancy

It is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them), including the pregnancy of a male subject's female partner that occurs during the trial or within 120 days of completing the trial completing the trial, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, as a serious adverse event. The patient is to be discontinued immediately from any protocol directed therapy. All subjects and female partners of male subjects who become pregnant 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 adverse 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 Sponsor and within 2 days to the manufacturer (; see 7.3.3.1 Merck report requirements).

Pregnancies and lactations that occur after the consent form is signed but before treatment allocation 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 through 120 days following cessation of study medication, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, must be reported by the investigator.

For Affiliate sites, the pregnancy, suspected pregnancy, or positive pregnancy test must be reported to the UNCCN Project Manager within 24 hours via facsimile to 919-966-4300. The female subject should be referred to an obstetrician-gynecologist, preferably one experienced in reproductive toxicity for further evaluation and counseling.

FDA Expedited Reporting requirements:
If an investigator deems that an event is both a serious SAR AND unexpected, it must also (in addition to Oncore) be recorded on the MedWatch Form 3500A as per 21 CFR 312.32. For Affiliate Investigators, the MedWatch form should be faxed to the UNCCN Project Manager at 919-966-4300 along with supporting documentation defining the event and causality. The MedWatch 3500a form can be accessed at:
http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm.
(Please be sure and access form 3500a, and not form 3500). UNC, as the Sponsor of the study, will make the final determination regarding FDA submission.

Once the UNC Principal Investigator determines an event is a serious SAR AND unexpected, the MedWatch 3500A form will be submitted to the FDA by the UNCCN Project Manager. If the event is serious, unexpected and considered to be possibly-, probably- or definitely-related to the study treatment, the UCCN Project Manager will inform the Regulatory Associate at UNC who will be responsible for submitting the SAR to the IND. All IND safety reports must be submitted on Form 3500A and be accompanied by Form 1571. The FDA must be notified or any unexpected or life-threatening suspected adverse reactions as soon as possible, but no later than 7 calendar days of learning of the event.

The UNCCN Project Manager will also be responsible for informing each Affiliate site of all serious and unexpected SARs reported to the FDA via fax as soon as possible.

7.3.3.1 Merck Reporting Requirements

Any SAE, or follow up to a SAE, including death due to any cause other than progression of the cancer under study that occurs to any subject from the time the consent is signed through 90 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to Merck product, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. All subjects with serious adverse events must be followed up for outcome.

Non-serious Events of Clinical Interest will be forwarded to Merck Global Safety and will be handled in the same manner as SAEs.

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 outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor and to Merck.

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

All 15-Day Reports and Annual Progress Reports must be submitted as required to FDA. Investigators will cross-reference these reports 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.

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 Sponsor and 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 below under overdose.
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.

Overdose

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 Sponsor and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

Pregnancy and Lactation

See above in this section for additional information. Such events must be reported within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220).

7.3.3.2 Protocol-Specific Exceptions to Serious Adverse Event Reporting

Efficacy endpoints as outlined in this section will not be reported to Merck as AEs 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 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.

7.4 Data and Safety Monitoring Plan

The Principal Investigator will provide continuous monitoring of patient safety in this trial with periodic reporting to the Data and Safety Monitoring Committee (DSMC).

Meetings/teleconferences will be held at a frequency dependent on study accrual, and in consultation with the study Biostatistician. These meetings will include the investigators as well as protocol nurses, clinical research associates, regulatory associates, data managers, biostatisticians, and any other relevant personnel the principal investigators may deem appropriate. At these meetings, the research team will discuss all issues relevant to study progress, including enrollment, safety, regulatory, data collection, etc.

The team will produce summaries or minutes of these meetings. These summaries will be available for inspection when requested by any of the regulatory bodies charged with the safety of human subjects and the integrity of data including, but not limited to, the oversight (Office of Human Research Ethics (OHRE) Biomedical IRB, the Oncology Protocol Review Committee (PRC) or the North Carolina TraCS Institute Data and Safety Monitoring Board (DSMB).

The UNC LCCC Data and Safety Monitoring Committee (DSMC) will review the study on a regular (quarterly to annually) basis, with the frequency of review based on risk and complexity as determined by the UNC Protocol Review Committee. The UNC PI will be responsible for submitting the following information for review: 1) safety and accrual data including the number of patients treated; 2) significant developments reported in the literature that may affect the safety of participants or the ethics of the study; 3) preliminary response data; and 4) summaries of team meetings that have occurred since the last report. Findings of the DSMC review will be disseminated by memo to the UNC PI, PRC, and the UNC IRB and DSMB.

8.0 STATISTICAL CONSIDERATIONS

8.1 Study Design/Study Endpoints

This is a prospective, open-label, single arm, phase 2 trial of single-dose cyclophosphamide +pembrolizumab in patients with metastatic triple negative breast cancer (TNBC). The primary hypothesis being evaluated is that the addition of one dose of cyclophosphamide prior to initiation of pembrolizumab will result in an approximately 50% improvement in progression free survival (PFS) when compared to historical controls that were treated with pembrolizumab alone [1]. A biologically-focused primary objective is to describe the reduction in Tregs in patients with metastatic TNBC receiving cyclophosphamide + pembrolizumab.

Secondary endpoints include the ORR, DCR, DOR, and OS. The tolerability and safety of single-dose cyclophosphamide + pembrolizumab in patients with metastatic TNBC will also be assessed (See section 2.4.2. for end point definitions and Section 8.3).

Correlative studies, based on serial blood collections and tumor samples, will be done under a separate protocol based on availability of archival diagnostic tissue (see section 1.4).

8.2 Sample Size and Accrual

The primary objective of this study is to estimate PFS defined as time from D1 of treatment until disease progression or death from any cause. Based on recent phase 1 data with pembrolizumab alone, we set the null hypothesis for median PFS at 1.9 months. The alternative hypothesis is a median PFS of 2.9 months for patients who receive a single dose cyclophosphamide prior to pembrolizumab. This approximately 50% increase in PFS would be considered clinically significant.

Assuming that accrual occurs within a 12 month period, and all patients are followed for at least 6 months, 36 patients are needed to detect the change in median PFS from 1.9 to 2.9 months. This sample size calculation is based on a one-sided 0.05 significance level, 80% power, and assuming an exponential survival time distribution (calculated using SWOG online One Arm Survival calculator at https://www.swogstat.org/stat/public/one_survival.html). We will also assume a 10% consent and not treat or loss to follow-up rate. Therefore we plan to enroll 40 patients to ensure that 36 are evaluable for the primary endpoint. We expect accrual to take approximately 2 years , with the rate of accrual being 1 to 2 patients per month.

While this study is not specifically powered for the assessment of Treg reduction, with the projected evaluable sample size, we will be able to detect an approximate reduction in Tregs of 67% (i.e.a3-fold reduction) using the Wilcoxon sign-rank test, with 80% power and a one-sided alpha=0.05.

8.3 Toxicity Monitoring

Toxicity will be assessed using NCI CTCAE version 4. Patients will be monitored for excessive toxicity over the duration of the study. A toxicity rate due to the study regimen greater than 25% would be considered unacceptable.

Pocock-type boundaries will be utilized as described in Ivanova, A., et al. Biometrics. 2005;61(2):540-5. If a toxicity boundary is reached, accrual for the study will be suspended and the Data Safety and Monitoring Committee (DSMC) will be alerted. The DSMC (in consultation with the Principal Investigator and MERCK) will evaluate the toxicity events to help determine whether or not to terminate the study.

Accrual to the trial is to be suspended if the number of protocol defined toxic events is equal to or larger than boundary in the table below.

Number of Patients	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Boundary	-	-	-	4	5	5	5	6	6	7	7	7	8	8	9	9	9	10	10	10
Number of Patients	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Boundary	11	11	11	12	12	12	13	13	14	14	14	15	15	15	16	16				

8.4 Data Analysis Plans

The primary efficacy analysis will be based on the primary objective. The primary objective of this study is to estimate PFS defined as time from D1 of treatment until documented disease progression or death from any cause. The Kaplan-Meier method will be used to estimate PFS and median PFS will be reported along with its 95% confidence interval.

Also of primary interest is quantifying the expected reduction in Tregs. While this is listed as a co-primary objective, statistically it will be treated as an exploratory objective. With the projected sample size, we will be able to detect a reduction in Tregs of 67% (ie, 3-fold reduction) using the Wilcoxon sign-rank test. We will report appropriate descriptive statistics to quantitate the number and function of Tregs in these patients.

Secondary objectives include estimating the ORR, DCR, DOR, OS, and characterizing the toxicity profile and safety of this regimen. ORR and DCR will be reported along with their corresponding exact two-sided 95% confidence intervals. The Kaplan-Meier method will be used to estimate DOR and OS. Median DOR and OS will be reported along with their 95% confidence intervals.

The analysis of the toxicity and safety of this regimen will be based on the frequency of adverse events and their severity for patients who received any study medicine. Worst toxicity grades per patient will be tabulated for adverse events and laboratory measurements by using the NCI CTCAEv4 and will be reported in the form of frequency tables.

Demographic and baseline laboratory results will be summarized using descriptive statistics.

Definitions

- Progression Free Survival (PFS): A patient's progression-free survival will be defined as the time from D1 of treatment until the date he or she has documented disease progression or dies. Any patient who has received study treatment but has neither experienced progression nor died will be censored on the date of his or her last tumor assessment
- Overall Survival (OS): A patient's survival time will be defined as the time from start of treatment to the date of his or her death. If the patient has not died, survival will be censored on last date the patient was known to be alive
- Overall Response Rate (ORR): The overall response rate will be defined as the total number of patients whose response are either a CR or PR divided by the number of response evaluable patients
- Disease Control Rate (DCR): Clinical benefit will be defined as the total number of patients whose responses are either a CR, PR, or SD divided by the number of response evaluable patients. Patients will need to be at least a SD for at least 6 months in order to be considered to have received clinical benefit from the treatment regimen
- Duration of Overall Response (DOR): The duration of overall response will be computed for patients whose best response is either PR or CR. It will be measured from when the time measurement criteria are first met for complete response or partial response (whichever status is recorded first) until the first date of progressive disease or death. Patients who neither progress nor die will be censored on the date of their last tumor assessment

Exploratory objectives will be evaluated when sample sizes are such to allow appropriate analyses. To explore possible associations of biological markers with ORR, logistic regression modeling will be used. To explore possible associations of biological markers with PFS and OS, Cox regression modeling will be used. Fisher's exact tests will be used to test for differences in ORR and DCR between subtypes of TNBC. For each T cell phenotype, the change in percentage of T cells will be calculated at the patient level, and descriptive statistics will be reported. TCR and BCR repertoire profiles will be characterized as having high or low sequence 'diversity', and the possible association of these dichotomized variables will be explored by Fisher's exact test with ORR, and Cox regression modeling with PFS and OS. The same statistical methods (outlined above) will used for outcomes defined using the irRECIST criteria.

9.0 STUDY MANAGEMENT

9.1 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

9.2 Required Documentation

Before the study can be initiated at any site, the following documentation must be provided to the Clinical Protocol Office (CPO) at the University of North Carolina.

- A copy of the official IRB approval letter for the protocol and informed consent
- IRB membership list
- CVs and medical licensure for the principal investigator and any sub-investigators who will be involved in the study.
- Form FDA 1572 appropriately filled out and signed with appropriate documentation (NOTE: this is required if UNC holds the IND. Otherwise, the Investigator's signature documenting understanding of the protocol and providing commitment that this trial will be conducted according to all stipulations of the protocol is sufficient to ensure compliance)
- CAP and CLIA Laboratory certification numbers and institution lab normal values
- Executed clinical research contract

9.3 Registration Procedures

All patients must be registered with the LCCC CPO UNCCN at the University of North Carolina before enrollment to study. To register a patient call the UNCCN at [redacted] Monday-Friday 8:30 am – 5:00 pm EST. Fax [redacted] or email (address to be provided at SIM) registration form, signed informed consents and all source documents to confirm eligibility. When sending registration request with eligibility documentation, please allow 24 hours for source to be reviewed.

For Affiliate patients, to register and confirm patient eligibility, please fax registration forms, informed consent, and source documents to [redacted].

9.4 Data Management and Monitoring/Auditing

The CPO UNCCN of the UNC LCCC will serve as the coordinating center for this trial. Data will be collected through a web based clinical research platform, OnCore®. Other study institutions will be given a password to directly enter their own data onto the web site via electronic case report forms (eCRFs). UNCCN personnel will coordinate and manage data for quality control assurance and integrity.

All data will be collected and entered into OnCore® by Clinical Research Associates (CRAs) from UNC LCCC and participating institutions. The investigators at each site will allow monitors to review all source documents supporting data entered into OnCore®. The UNCCN Data Coordinator can be reached at [redacted].

As an investigator initiated study, this trial will also be audited by the Lineberger Cancer Center audit committee every six or twelve months, depending on the participation of affiliate sites.

9.5 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

9.5.1 Emergency Modifications

UNC and Affiliate investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior UNC or their respective institution's IRB/IEC approval/favorable opinion.

<u>For Institutions Relying on UNC's IRB:</u>
For any such emergency modification implemented, a UNC IRB modification form must be completed by UNC Research Personnel within five (5) business days of making the change.

<u>For Institutions Relying on Their Own IRB:</u>

For Affiliate investigators relying on their own institution's IRB, as soon as possible after the modification has been made, the implemented deviation or change and the reasons for it should be submitted to:

- To UNC Principal Investigator for agreement
- The Affiliate institution's IRB for review and approval. (Once IRB's response is received, this should be forwarded to the UNCCN Regulatory Associate).

9.5.2 Single Patient/Subject Exceptions

Eligibility single subject exceptions are not permitted for Lineberger Comprehensive Cancer Center Investigator Initiated Trials under any circumstances. Other types of single subject exceptions may be allowed if proper regulatory review has been completed in accordance with Lineberger Comprehensive Cancer Center's Single Subject Exception Policy.

9.5.3 Other Protocol Deviations/Violations

According to UNC's IRB, a protocol deviation is any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs
- Has no substantive effect on the risks to research participants
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected
- Did not result from willful or knowing misconduct on the part of the investigator(s).

An unplanned protocol variance is considered a violation if the variance meets any of the following criteria:

- Has harmed or increased the risk of harm to one or more research participants.
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

If a deviation or violation occurs please follow the guidelines below:

For Institutions Relying on UNC's IRB:
Protocol Deviations: UNC or Affiliate personnel will record the deviation in OnCore®, and report to any sponsor or data and safety monitoring committee in accordance with their policies. Deviations should be summarized and reported to the IRB at the time of continuing review.

Protocol Violations: Violations should be reported by UNC personnel within one (1) week of the investigator becoming aware of the event using the same IRB online mechanism used to report Unanticipated Problems.

For Institutions Relying on Their Own IRB:
In addition to adhering to the policies regarding protocol compliance set forth by your institution's IRB, the following is also required:

Protocol Deviations: In the event a deviation from protocol procedures is identified, record the deviation in OnCore®.

Protocol Violations: Any protocol violation that occurs must be reported to your IRB per institutional policies and reported to the UNCCN Project Manager within 5 days. UNC-CH will determine if the violation affects the safety of the patient and integrity of the data. Once your institution's IRB response is received, please forward to the UNCCN Regulatory Associate.

Unanticipated Problems:
Affiliate Sites:
Any events that meet the criteria for "Unanticipated Problems (UPs)" as defined by UNC's IRB must also be reported to the UNCCN Project Manager. The UNCCN Project Manager will report the event to the UNC IRB using the IRB's web-based reporting system. Examples of such UPs include a lost or stolen laptop computer that contains sensitive study information.

UNC
Any events that meet the criteria for "Unanticipated Problems" as defined by UNC's IRB must be reported by the Study Coordinator using the IRB's web-based reporting system.

9.6 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator at UNC. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be required.

For Institutions Relying on UNC's IRB:
The written amendment, and if required the amended consent form, must be sent to UNC's IRB for approval prior to implementation.

For Institutions Relying on Their Own IRB:
Investigators must submit the amendment to their institution's IRB for approval. For multi-center studies, any multi-center site must submit their informed consent revisions to the Multi-center Regulatory Associate prior to submission to their IRB.

9.7 Record Retention

Study documentation includes all eCRFs, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

9.8 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered into the eCRFs. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all eCRFs will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

10.0 REFERENCES

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11.0 APPENDICES

11.1 Appendix A ECOG Performance Status

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
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).
2	In bed <50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.
* As published in Am. J. Clin. Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P. Toxicity and Response Criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol* 5:649-655, 1982. The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.	

11.2 Appendix B Cockcroft-Gault Formula

$$\text{Estimated creatinine clearance (mL/min)} = \frac{(140\text{-age in years}) \text{ X (weight in kg)}}{72 \text{ X (serum creatinine in mg/dL)}}$$

For females, use 85% of calculated creatinine clearance value.

11.3 Appendix C: Prohibited Medications

Cyclophosphamide is a substrate for CYP450s (ie, 2B6, 2C9, 3A4, and 2C19). Strong and moderate inhibitors of 2B6, 2C9, 3A4, and 2C19 within 1 week prior to receiving cyclophosphamide dosing through 72 hours after cyclophosphamide dosing are contraindicated. Patients must not have received any drug that is a moderate or strong inducer of 3A4 within 2 weeks prior to cyclophosphamide dosing. ***Note**: this is not an exhaustive list and further details can be found at* http://medicine.iupui.edu/clinpharm/ddis/ and at Expert Opin. Drug Metab Toxicol (2013) 9(6) 737-751. If a drug appears on more than one list follow the conservative rule.

2B6 inhibitor	2C19 inhibitor	2C9 inhibitor
Ticlopidine	Fluvoxamine	Amodiarone
	Ticlopidene	Diosmin
	Efavirenz	Fluconazole
		Miconazole
		Oxandrolone
		Ticynafen

3A4 inhibitor	
Amprenavir	Aprepitant
Atazanvir	Cimetidine
Clarithromycin	Darunavir
Conivaptan	Diltiazem
Fosamprenavir	Erythromycin
Grapefruit juice	Fluconazole
Idinavir	Imatinib
Itraconazole	Nifedipine
Ketoconazole	Posaconazole
Nefazodone	Tofisopam
Nelfinavir	Verapamil
Ritonavir	
Saquinavir	
Telithromycin	
Telaprevir	
Troleandomycin	
Vriconazole	

3A4 Inducer	
Carbamazepine	Efavirenz
Phenytoin	Etravirine
Rifampin	Nafcillin
St John's Wort	Nevirapine
Bosentan	Phenobarbital

11.4 Appendix D Patient Handout: Prohibited Medications

One of the medications you are receiving during this clinical trial is cyclophosphamide. Cyclophosphamide interacts with some drugs that are processed by your liver. Because of this, it is very important to tell your study doctors about all of your medicine before you start this study. It is also very important to tell them if you stop taking any regular medicine, or if you start taking a new medicine while you take part in this study. When you talk about your medicine with your study doctor, include medicine you buy without a prescription at the drug store (over-the counter remedy), or anything that you buy from the health food store or grocery store (herbal supplement). Many health care prescribers can write prescriptions. You must also tell your other prescribers (doctors, physicians' assistants or nurse practitioners) that you are taking part in a clinical trial. **Bring this paper with you**.

- **Cyclophosphamide** is processed by a certain enzyme in the liver called CYP450 enzymes. Drugs that increase the activity of this enzyme are called "inducers", and drugs that decrease the activity of this enzyme are called "inhibitors". Cyclophosphamide must be used very carefully with other medicines that are **inducers** or **inhibitors** of CYP450.
- You and healthcare providers who prescribe drugs for you must be careful about adding or removing any drug in this category
- Before you start the study, your study doctor will work with your regular prescriber to switch the following medications if you are taking them:
    - Ticlopidine, fluvoxamine, efavirenz, amodiarone, diosmin, fluconazole, miconazole, oxandrolone, ticynafen, amprenavir, atazanvir, clarithromycin, conivaptan, fosamprenavir, grapefruit juice, idinavir, itraconazole, ketoconazole, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin, telaprevir, troleansomycin, vriconazole, aprepitant, cimetidine, darunavir, diltiazem, erythromycin, fluconazole, imatinib, nifedipine, posaconazole, tofisopam, verapamil, carbamazepine, phenytoin, rifampin, St John's Wort, bosentan, etravirine, nafcillin, nevirapine, and phenobarbital
- Your regular prescribers should look at this web site: http://medicine.iupui.edu/clinpharm/ddis/table.asp to see if any medicine they want to prescribe is on a list of drugs to avoid. Your study doctor may also have a list of medications for you to show your regular prescribers instead of, or in addition to, this website.
- If you drink grapefruit juice or eat grapefruit, you should avoid these for at least 1 week before receiving cyclophosphamide until 3 days after the dose.
- Other medicines can be a problem with your study drugs.

- You should check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.
- Your regular prescriber should check a medical reference or call your study doctor before prescribing any new medicine for you. Your study doctor's name is _____ and he or she can be contacted at _____.