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DEPARTMENT OF MEDICAL ONCOLOGY

TITLE: MK-3475 (pembrolizumab) in Combination with an Anthracycline or Anti-Estrogen Therapy in Patients with Triple Negative and Hormone Receptor Positive (HR+ HER2-) Metastatic Breast Cancer.

CITY OF HOPE PROTOCOL NUMBER/VERSION: IRB # 15295 **Protocol dated: 06/20/2017**

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COH Amendment 06	Protocol dated 03/03/2017	Version: 06
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COH Amendment 08	Protocol Dated 06/02/2017	Version: 08
COH Amendment 09	Protocol Dated 06/20/2017	Version: 09
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COH Amendment 11	Title Page Dated 02/07/2018	Version: 11
COH Amendment 12	Title Page Dated 10/27/2018	Version: 12
COH Amendment 13	Title Page Dated 10/16/2018	Version: 13
COH Amendment 14	Title Page Dated 11/16/2018	Version: 14
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SITE: Breast

STAGE (If applicable): IV

MODALITY: Immunotherapy: Monoclonal Antibody

TYPE: Phase 2

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SPONSOR: City of Hope Comprehensive Cancer Center

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SPONSOR/IND NUMBER: 129257

IND Supporter: Merck

TABLE OF CONTENTS

1.0 TRIAL SUMMARY

Abbreviated Title	MK-3475 and doxorubicin or an aromatase inhibitor for breast cancer
Trial Phase	Phase II with 2 parallel cohorts; Cohort 1 MK-3475 with doxorubicin and Cohort 2 combines MK-3475 with an aromatase inhibitor; A safety lead-in for the combination of MK-3475 both with doxorubicin or the aromatase inhibitor will be tested.
Clinical Indication	Advanced breast cancer, stage IV
Trial Type	Interventional
Type of control	None
Route of administration	IV MK-3475, IV doxorubicin, oral anti-estrogen (aromatase inhibitor) therapy
Open-label	None
Treatment Groups	Cohort 1 : Triple negative breast cancer Pembrolizumab 200 mg IV every 3 weeks and doxorubicin 50-60 mg/m2 IV every 3 weeks (based on safety lead-in) x 6 cycles followed by a pembrolizumab alone for up to 35 cycles or a maximum of 24 months starting from the first cycle. Cohort 2: HR+ HER2- breast cancer Pembrolizumab 200 mg IV every 3 weeks and anastrozole 1 mg, letrozole 2.5 mg, or exemestane 25 mg (preferred) daily (with a lead-in)
Number of trial subjects	Approximately 60
Estimated enrollment period	24 months
Estimated duration of trial	36 months
Duration of Participation	After signing the informed consent form (ICF), passing the screening phase and initiating therapy, patients will participate until death or discontinuation from trial treatment due to progression. Patients discontinuing treatment for reasons other than progression will be followed until progression and treatment-related toxicities have resolved or another treatment regimen is initiated. Treatment with pembrolizumab for patients of the triple negative cohort (treated with 6 cycles of doxorubicin/pembrolizumab) will continue with pembrolizumab until disease progression, treatment-related unacceptable toxicities, intercurrent illness preventing further administration of study drug, subject or investigator's decision to withdraw, pregnancy, or, if a patient receives a total of 24 months of pembrolizumab (including the cycles given with doxorubicin). Patients in the hormone-receptor positive cohort may also receive up to 24 months of pembrolizumab, as well. They can continue on their selected anti-estrogen therapy (whichever aromatase inhibitor was chosen with pembrolizumab) _until progression. Patients achieving a confirmed complete response may consider stopping pembrolizumab within a year of such response, and could be retreated within 12 months from stoppage. Subjects who stop pembrolizumab with stable disease or better may be eligible for up to one year of additional pembrolizumab therapy if they progress no sooner than 8 weeks after stopping study treatment, for either cohort. After the end of treatment patients will be followed for 30 days for adverse event (AE) monitoring and serious adverse events (SAEs) and events of clinical interest will be collected for 90 days after the end of treatment, or 30 days after the end of treatment if a patient initiates new anti-cancer therapy or until the treatment toxicities clearly stabilize or resolve (whichever is first).

2.0 TRIAL DESIGN

2.1 Trial Design

This is an open-label single institutional Phase II trial for patients with metastatic stage IV breast cancer. The study will include a cohort of patients with stage IV metastatic triple negative (TN) breast cancer who are to receive a combination of pembrolizumab and doxorubicin (a lead-in dose escalation starting at doxorubicin at 50 mg/m^2 and escalating to 60 mg/m^2 will be tested to establish safety using the a 3-at-risk dose escalation design) and a second cohort of patients with stage IV metastatic hormone receptor positive and HER2- (HR+) breast cancer treated at standard dose for both the aromatase inhibitor and pembrolizumab with a lead-in at the standard dosing of both AI and pembrolizumab to help insure safety.

One planned interim analysis to assess safety and futility/efficacy will be carried out in the TN cohort (beyond the initial period required to establish safe dose when combining pembrolizumab and doxorubicin in the triple negative cohort) and the results will reviewed by the PI, sponsor and the City of Hope Data Safety Monitoring Committee.

2.2 Trial Diagram

Stratification

Number of prior treatments in the metastatic setting (none vs. unlimited)

Cohort 1		**Cohort 2**
TN Breast Cancer		**HR+ Breast Cancer**
Pembrolizumab 200 mg IVQ3W Doxorubicin 50-60 mg/m^2 IVQ3W	x 6 cycles	Pembrolizumab 200 mg IVQ3W (24 mos) Anastrozole 1 mg, letrozole 2.5 mg, or
Pembrolizumab 200 mg IV q3 W up to 24 mos		Exemestane 25 mg po (preferred), QD until progression
Progression/off study		Progression/off study
Safety and Survival		Safety and Survival
Follow-up		Follow-up

3.0 OBJECTIVE(S) & HYPOTHESIS(ES)

3.1 Primary Objective(s) & Hypothesis(es)

(1) **Objective:**

1.a To evaluate efficacy (overall response rate) of MK-3475 and doxorubicin in patients with stage IV triple negative breast cancer.

Hypothesis: Interference with breast cancer-associated PD-1 receptor expression will lead to increased cellular (CD8+) immunity. This effect is likely to occur regardless of tumor subtype (i.e. hormone receptor positive, HER2+, or triple negative subtypes), but targeting the PD-1 receptor alone may not provide sufficient therapeutic benefit. Preclinical and clinical data suggest that the prevalence of PD-1 and PD-L1 expression is highest among patients with triple negative primary disease. We postulate that if such is also true in the metastatic setting, a combination of MK-3475 and a DNA-targeting chemotherapeutic agent with the potential to interfere with the interaction of PD-1 and PD-L1/L2 such as doxorubicin may be of additive or even synergistic therapeutic value through facilitating both cellular immune response and chemotherapy effects.

(2) **Objective**:
To evaluate efficacy (overall response rate) of MK-3475 and an oral aromatase inhibitor in patients with stage IV HR+ HER2- breast cancer.

Hypothesis: The combination of MK-3475, and antiestrogen therapy will provide a novel treatment option for primarily endocrine therapy-resistant patients through enhancing cellular immune response while preserving the anti-estrogen effect. Current practice calls for initiating cytotoxic therapy once the first few lines of anti-estrogen treatment options have been exhausted. We postulate that MK-3475, when given together with whichever aromatase inhibitor has not been administered to a patient previously, may provide a less toxic and potentially better therapeutic option.

3.2 Secondary Objective(s) & Hypothesis(es)

(1) **Objectives**:

3.2.1 To assess clinical benefit rate (lack of progression for > 24 weeks), duration of response, time-to-treatment failure, progression-free survival, and overall survival in TN stage IV breast cancer patients based primarily on RECIST 1.1, and irRECIST.

3.2.2 To assess feasibility and toxicity

Hypothesis: A combination of pembrolizumab and doxorubicin in patients with TN breast cancer will provide favorable outcomes vs. the expected single agent response rate with either agent alone, and the combination will have a favorable toxicity profile/risk-benefit ratio.

(3) **Objectives**:

3.2.3 To assess clinical benefit rate (lack of progression for > 24 weeks), duration of response, time-to-treatment failure, progression-free survival, and overall survival in patients with stage IV HR+ breast cancer based primarily on RECIST 1.1, and irRECIST.

(4) **3**.2.4 To assess feasibility and toxicities.

Hypothesis: A combination of pembrolizumab and an aromatase inhibitor in patients with HR+ breast cancer will provide favorable outcomes vs. the expected single agent response rate with an aromatize inhibitor, or with pembrolizumab alone, and the combination will have a favorable toxicity profile/risk-benefit ratio.

3.3 Exploratory Objective

(1) Objective: To procure serial tumor (primary and metastatic) and blood (cellular and serum/plasma) samples and analyze them to better our understanding of cellular and humoral immune response correlates and predictors of clinical benefits, leading to optimized selection of target populations in future phase II and subsequent phase III randomized prospective trials.

4.0 BACKGROUND & RATIONALE

4.1 Background

Refer also to the Investigator's Brochure (IB)/approved labeling for detailed background information on MK-3475 (Appendix 12.4).

4.1.1 Pharmaceutical and Therapeutic Background

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an

immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors . Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL). This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

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

Relevant to this proposal, data from the proof-of concept study KEYNOTE-012 showed that in (primarily) heavily pretreated PD-L1-positive triple negative breast cancer (n=27), pembrolizumab resulted in an overall response rate (ORR) of 18.2 % with durable responses (1).

4.1.2 **Preclinical and Clinical Trial Data**

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

4.1.3. Information on Other Trial-Related Therapy

Anthracyclines are active in stage IV breast cancer, and are prescribed both as single agents and in combination in the metastatic settings (2-3). There are however, no specifics data

available in triple negative stage IV breast cancer patients, whose median survival from first treatment is 9-12 months and their time to progression drops by approximately 50% with each line of therapy (4). There is no "best" or optimal/targeted treatment option recommended currently for such patients except in the rare BRCA-mutated cases with data suggesting a benefit when treatment includes agents interfering with DNA repair.

To complicate matters, there is significant heterogeneity and variable molecular subtypes in the TN phenotypic group of tumors, including the relatively small percentage of immunomodulatory subtype which is characterized by enriched gene expression for immune cell and cytokine signaling and related pathways. Clinical observations point to a prognostic and possibly predictive role of the presence of tumor infiltrating lymphocytes in the tumors. Surface expression of the co-inhibitory programmed cell death receptor 1 (PD-1) on CD8+ cells together with its ligand PD-L1 on tumor cells are considered hallmarks of impaired cellular defense. Consequently, the presence of both PD-1 and PD-L1 are implicated as adverse prognostic factors in patients with a variety of malignancies; PD-1 and PD-L1 expression together with the presence of p53 mutations seems to be of higher prevalence in triple negative breast cancer compared to other breast cancer subtypes (5-11). The idea of combined PD-1 blockade and glucocorticoid-TNFR-related protein (GITR) stimulatory triggering with selected chemotherapeutic agents has also undergone evaluation suggesting that combining PD-1 blockade and DNA-targeting or microtubule-inhibiting agents is worth testing in the clinic (12).Sequential anti-estrogen therapy remains part of standard strategies when treating non-visceral stage IV HR+ breast cancer (13, 14). Indeed, subsequent lines of aromatase inhibitors have been prescribed with variable success, regardless of the sequence, although non-steroidal anastrozole and letrozole) aromatase inhibitor treatment followed by steroidal aromatase inhibition (exemestane) may be preferred (15).

4.1.3 **Rationale for the Trial and Selected Subject Population**

Both in the adjuvant and neoadjuvant setting in patients treated with anthracycline-inclusive regimens immunomodulatory profiles have been implicated as predictors of outcome (16,17). There are preclinical data suggestive of doxorubicin as a downregulator of B7-H1 (PD-L1) expression (18). Since doxorubicin is an active agent in the treatment of metastatic – including triple negative - breast cancers, and it has lost popularity in the adjuvant setting, combining this chemotherapeutic agent with the PD-1 inhibitor pembrolizumab is worth testing in the triple negative stage IV setting, particularly in patients who have not been previously exposed to anthracyclines. PD-L1 and PD-1 expression has recently been described in 59% and 70% of 53 triple negative breast cancer samples with concurrent expression seen in 45% of cases, hence the reason to target this subset of stage IV breast cancer (7). Since the response rate and progression-free and overall survival are suboptimal even in the first line setting when patients are treated with standard agents including platinum and taxanes, new approaches are urgently needed. (19)

As for HR+ disease: PD-L1 has been also observed in 33% of both Luminal B and A tumors and PD-1 expression was seen in 44% and 25% of luminal B and A tumors (n:58), with concurrent expressions seen in 17% and 13%, respectively (7). For patients with stage IV HR+ breast cancer, of Progression-free survival in two small trials describing either the effect

of optimal dose fulvestrant (20), or a combination of letrozole and the moderately toxic agent CDK 4/6 inhibitor palbociclib (20), is approaching 2 years. However, in the second and third line setting, even with novel targeted therapies inclusive of the mTOR inhibitor everolimus and an aromatase inhibitor, response rates are low and progression-free survival is short (22). Response rates in patients with stage IV HR+ breast cancer progressing or relapsing on anti-estrogen therapy are also suboptimal (~ 10%) with a combination of letrozole and palbociclib, although progression-free survival has improved vs. letrozole alone.(23). Since most eligible patients are likely to have been exposed to non-steroidal aromatase inhibitors already either in the adjuvant or metastatic setting, and comparative efficacy data does not reveal a particular advantage using one AI over another in the metastatic first or second line setting, the steroidal aromatase inhibitor exemestane will be the preferred (but not the only) choice for AI in this trial. Clearly, there is an urgent and unmet need to improve response rates and progression-free and overall survival even in the "good-prognosis" HR+ stage IV group of breast cancer patients, and there is good rationale to assess the potential benefit of pembrolizumab and an aromatase inhibitor in HR+ patients who progressed on endocrine therapy, or failed first and beyond line therapies.

Since PDL-1 expression has been observed across all subtypes (7) and stage IV MBC patients need significant improvements in therapeutic options with the goal of extending clinical benefit duration, time to progression, and overall survival, testing the feasibility and potential efficacy of pembrolizumab in both triple negative and HR+ sets of breast cancer may lead to improved therapeutic options in the future. The future directions to take in case of a positive outcome with 6 cycles of combined therapy of pembrolizumab and doxorubicin and maintenance in the triple negative cohort will include testing doxorubicin and pembrolizumab in suitable patients in need of adjuvant therapy. Similarly, adjuvant and neoadjuvant roles could be assessed in HR+ patients with the aromatase inhibitor and pembrolizumab combination.

4.1.4 **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 1 and 2 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.

As for the dose selection of doxorubicin: it is estimated that standard dose of 50-60 mg/m2 for up to 6 cycles with careful cardiac monitoring can be safely administered. Dosing of the chosen aromatase inhibitors will also be standard (anastrozole 1 mg po QD, or letrozole 2.5 mg po QD, or exemestane 25 mg po QD).

4.1.5 **Rationale for Endpoints**

Primary: For Cohort 1 (TN stage IV breast cancer) the primary endpoint is to assess efficacy (overall response rate) in PD-L1 not enriched stage IV breast cancer when combining a previously untested combination of pembrolizumab and doxorubicin.

For Cohort 2, (HR+ HER2- stage IV breast cancer) the primary endpoint is to assess efficacy (overall response rate) in PD-L1 not enriched cancer when combining a previously untested combination of pembrolizumab and an aromatase inhibitor (exemestane preferred).

Safety analysis will be carried out based on toxicities assessed by CTCAE, version 4.0 criteria. AEs will be analyzed including but not limited to all AEs, SAEs, fatal AEs, and laboratory changes. Immune-related adverse events (irAE) will be collected and designated as immune-related events of clinical interest (ECIs)

Secondary: Clinical benefit rate (> 24 weeks of lack of progression, safety, tolerability, duration of response, progression-free survival, and overall survival and time to treatment failure are methods to assess efficacy of therapy of the two novel combinations. These endpoints will help provide information for future trial designs.

4.1.5.1 Efficacy Endpoints

RECIST 1.1 (24) will be used to assess response, duration of response (DOR) , and progression-free survival (PFS) ; Kaplan-Meier estimates will be generated for PFS, time-to-treatment failure, and overall survival (OS).

Exploratory analysis to assess survival endpoints, response and clinical benefit will be carried out also, using irRECIST,

irRECIST
irRECIST is RECIST 1.1 adapted as described below to account for the unique tumor response seen with immuno-therapeutics. irRECIST will be used by site investigators and local radiology review to assess tumor response and progression, and make treatment decisions. This data will be collected in the clinical database.

irRECIST will be used by the central imaging vendor, however, this evaluation will be done retrospectively. irRECIST takes into account the clinical condition/stability of subjects, as described in the Table below, in addition to response or progression via tumor imaging.

Clinically stable is defined by the following criteria:
- Absence of symptoms and signs indicating clinically significant progression of disease, including worsening of laboratory values
- No decline in ECOG performance status
- Absence of rapid progression of disease
- Absence of progressive tumor at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention

Table irRECIST: Tumor Imaging and Treatment after 1st Radiologic Evidence of PD **or** SD, CR or PR

	Clinically Stable		**Clinically Unstable**	
	Imaging	Treatment	Imaging	Treatment
1st radiologic evidence of PD	Repeat imaging at ≥ 4 weeks at site to confirm PD	May continue study treatment at the Investigator's discretion while awaiting confirmatory scan by site	Repeat imaging at ≥ 4 weeks to confirm PD per physician discretion only	Discontinue treatment
Repeat scan confirms PD	No additional imaging required	Discontinue treatment (exception is possible upon consultation with Sponsor).	No additional imaging required	N/A
Repeat scan shows SD, PR or CR	Continue regularly scheduled imaging assessments	Continue study treatment at the Investigator's discretion	Continue regularly scheduled imaging assessments	May restart study treatment if condition has improved and/or clinically stable per Investigator's discretion. Next scan should occur according to the every 9 week (63 ± 7 days) imaging schedule.

In determining whether or not the tumor burden has increased, decreased or stayed stable, site investigators should consider all target lesions as well as non-target lesions.

Any subject deemed **clinically unstable** should be discontinued from trial treatment at first evidence of progressive disease by tumor imaging and is not required to have repeat tumor imaging for confirmation.

For a **clinically stable** subject with first radiologic evidence of progressive disease (i.e., **unconfirmed progression of disease**), it is at the discretion of the site investigator to continue treating the subject with the assigned treatment per protocol until progression of disease is confirmed at least 28 days from the date of the tumor imaging first suggesting PD. If progression is not confirmed on the subsequent tumor imaging, the subject should continue to receive study therapy and have tumor imaging performed every 9 weeks (± 7 days) in the first year or every 12 weeks after the first year, or sooner if clinically indicated, to monitor disease status. If radiologic progression is confirmed by subsequent tumor imaging, then the subject will be discontinued from trial treatment.

NOTE: If a subject with confirmed progression by tumor imaging (i.e. 2 scans at least 28 days apart demonstrating progressive disease) is clinically stable or clinically improved, and there is no further increase in the tumor burden at the confirmatory scan, an exception may be considered to continue treatment upon consultation with the Sponsor.

4.1.5.2 Biomarker Research

Preliminary studies suggest that CD8 T cell infiltration and PD-L1 expression within tumors may predict efficacy for pembrolizumab, but definitive biomarkers are not yet available. To identify biomarkers to predict and/or follow efficacy for pembrolizumab in combination with other agents in metastatic breast cancer, we propose the following correlative studies to analyze the immune and stromal cells within metastatic tumors, as well as immune cells in peripheral blood, before and after therapy:

We will obtain archived biopsies or fresh biopsies as feasible of one or more metastatic lesion(s) before and one month post treatment. In addition, blood samples will be obtained at weeks 0, 2, 4, 6, 8, 10, 20, and 30 to track the temporal dynamics of the host immune response. Key questions we will address include:
1. Immune and stromal cells characteristics before treatment that correlate with clinical response.
2. Changes in immune and stromal cells after therapy that correlate with clinical response.

Approaches:
1. 12-color FACS analysis to enumerate/phenotype immune cell subsets and functional readouts, including cytokine production and signaling (phosflow)
2. TCR repertoire analysis via deep sequencing. Expansion of the T cell repertoire after therapy will be evidence for an immunological response and may indicate epitope spread.
3. Immunohistology using a novel quantitative, spatial image analysis system (Vectra, Perkin Elmer) that will enable us to analyze immune, stromal, and cancer cells in metastatic tumors via 8-color histology.
4. Gene expression analysis of stromal cells via RNA-Seq or microarrays.
5. Morphological / IHC TIL analysis of tumor biopsies.

Serial Cytokine Measurements

We will procure and analyze blood samples prior to initiating cycle one, and prior to cycle 2. The Human Cytokine Thirty-Plex Antibody Bead Kit (Invitrogen, Camarillo, CA) will be used according to manufacturer's instructions. The 30 cytokine panel includes: epidermal growth factor (EGF), eotaxin, basic fibroblast growth factor (FGF-basic), granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), hepatocyte growth factor (HGF), interferon alpha (IFN-α); IFN-gamma (γ), interleukin-1 beta (IL-1β), interleukin-1 receptor antagonist (IL-1RA), IL-2; interleukin-2 receptor (IL-2R), IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p40/p70, IL-13, IL-15, IL-17, IFN-γ-inducible protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), monokine induced by IFN-γ (MIG), monocyte inflammatory protein-1 alpha (MIP-1α), monocyte inflammatory protein-1 beta (MIP-1β), regulated upon activation, normal T cell expressed

and secreted cytokine (RANTES), tumor necrosis factor (TNF)-α, and VEGF. Cytokine concentrations will be measured using the Bio-plex HTF Luminex instrument and results calculated using Bio-plex Manager 3.0 Software. The inter-assay precision for all cytokines is <10 % and the lower limit of quantitation is between 1 and 15 pg/ml, depending on the target. (25-29)

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Diagnosis/Condition for Entry into the Trial

Patients who are 18 years or older with 1) stage IV metastatic triple negative breast cancer (triple negative is defined as ER and PgR status is <1% of tumor cell nuclei are immunoreactive for ER or PgR, and HER2 status is FISH negative or IHC 0 or 1+) or 2) Stage IV HR+ HER2- (HR+) breast cancer (defined as ER or PgR ≥1% of tumor cell nuclei are immunoreactive for ER or PgR and HER2 status is FISH negative or ICH 0 or 1+).

Subject Inclusion Criteria

In order to be eligible for participation in this trial, the subject must:

1. Be willing and able to provide written informed consent/assent for the trial.

2. Be ≥ 18 years of age on day of signing informed consent.

3. Have measurable disease based on RECIST 1.1.

4. Be willing to provide tissue from a newly obtained core or excisional biopsy of a tumor lesion. *Newly-obtained is defined as a specimen obtained up to 6 weeks (42 days) prior to initiation of treatment on Day 1. Subjects for whom newly-obtained samples cannot be provided (e.g. inaccessible or subject safety concern) may submit an archived specimen only upon agreement from the Sponsor.*

5. Have a performance status of 0 or 1 on the ECOG Performance Scale.

6. Demonstrate adequate organ function as defined in Table 1, all screening labs should be performed within 7 days of treatment initiation.

Table 1 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L without transfusion or EPO dependency (within 7 days of assessment)
Renal	
Serum creatinine **OR**	≤1.5 X upper limit of normal (ULN) **OR**

Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Cardiac	
Left ventricular ejection fraction by MUGA or Echocardiogram[#]	[#] ≥55%; [^]≥ upper limit of institutional normal
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases
Albumin	≥2.5 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants ≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
Reproductive status for cohort 2: HR+ stage IV post-menopausal breast cancer	Post-menopausal is defined by at least one of the following criteria: Prior bilateral oophorectomy OR Amenorrheic for ≥ 12 months (if ≤ 55 years of age and prior chemotherapy or on medical ovarian ablative therapy or received ovarian radiation for ablation in the past 5 years and/or tamoxifen or an AI within the past year, then FSH and estradiol must be in the post-menopausal range and obtained within 28 days prior to registration) OR Previous hysterectomy with one or both ovaries left in place (or previous hysterectomy in which documentation of bilateral oophorectomy is unavailable AND FSH values consistent with the institutional normal values for the post-menopausal state. FSH levels must be obtained within 28 days prior to registration.
[a]Creatinine clearance should be calculated per institutional standard. # For patients with triple negative breast cancer ^ For patient with HR+ breast cancer	

7. Female subject of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

8. Female subjects of childbearing potential should be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication (Reference Section 5.7.2). Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.

9. Male subjects should agree to use an adequate method of contraception starting with the first dose of study therapy through 120 days after the last dose of study therapy.

10. Subjects currently on a bisphosphonate or denosumab are eligible for study therapy.

5.1.2 **Subject Exclusion Criteria**

The subject must be excluded from participating in the trial if the subject:

1. Cohort 1: Has triple negative breast cancer, is considered for cohort 1 participation, and received prior anthracycline therapy.

   Cohort 2: Has received prior aromatase inhibitor therapy and is deemed to be resistant to all three (anastrozole, letrozole, exemestane) approved AIs. Resistance is defined as progression within 12 months or while on an AI.

2. Patient is premenopausal (see Table 1, medical ovarian suppression is allowed)Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of treatment.

3. 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.

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

5. Hypersensitivity to pembrolizumab or any of its excipients.

6. Suboptimal cardiac function as defined by decreased left ventricular ejection fraction < 55% for cohort 1, and < 50% for cohort 2.

7. Prior pembrolizumab.

8. Has had a prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.

9. Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.

   - Note: Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study.

- Note: If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

10. Has a known additional malignancy that has progressed or required active treatment in the past 5 years. 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.

11. Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to the first dose of trial treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability.

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

13. Has a history of, active, pneumonitis requiring treatment with steroids or history of/active interstitial lung disease.

14. Has an active infection requiring systemic therapy.

15. 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.

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

17. 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.

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

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

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

21. Has a history of (non-infectious) pneumonitis that required steroids or currently has pneumonitis.

22. Has received a live vaccine within 30 days of planned start of study therapy.

    Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.

5.2 Participant Enrollment

5.2.1 Pre-Enrollment Informed Consent and Screening Procedures

Diagnostic or laboratory studies performed exclusively to determine eligibility will be done only after obtaining written informed consent. Studies or procedures that are performed for clinical indications (not exclusively to determine study eligibility) may be used for baseline values and/or to determine pre-eligibility, even if the studies were done before informed consent was obtained. The informed consent process is to be fully documented, and the prospective participant must receive a copy of the signed informed consent document.

5.2.2 COH DCC Availability and Contact Information

Eligible subjects will be registered on the study centrally by the Data Coordinating Center (DCC) at City of Hope. DCC staff are available **between the hours of 8:00 a.m. and 5:00p.m. PST, Monday through Friday (except holidays).** DCC contact information is as follows:

- phone: (626) 256-4673 ext. 83968
- e-mail: DCC@coh.org

5.2.3 Slot verification and reservation

The study team personnel (including physicians, protocol nurses and/or CRCs) may wish to contact the DCC to verify slot availability and to reserve an open slot or be placed in queue for slot opening. Slots may only be held for a limited time which will be determined by the PMT. The Data Coordinating Center should be notified of cancellations of prospective participants holding slots as soon as possible.

5.2.4 Registration procedure

To register a participant, the subsequent procedure is to be followed.

1. The coordinator/research nurse should contact the DCC via telephone or email to provide notification regarding the pending registration and communicate desired timeline of the registration, especially if it must be completed promptly.

2. The coordinator/research nurse should then e-mail copies to DCC@coh.org of the following documents to the DCC:

- Completed Eligibility Criteria List (printed from Section 5.1.1 and 5.1.2 of the protocol)
- Source documentation to support eligibility criteria**
- Signed informed consent document
- Signed HIPAA authorization form (if separate from the informed consent document)
- Signed subject's Bill of Rights (COH only)

**For COH participants, provide copies of source documentation only if not readily available as a finalized record in the COH EMR.

3. After having received all documentation, the DCC will complete the review the documents to verify eligibility, working with the staff as needed to resolve any missing required source elements. A subject failing to meet all protocol eligibility requirements will not be registered.

4. Once eligibility has been confirmed, DCC staff will register the participant by assigning a participant study number and will enter the subject into the eCRF system, Medidata RAVE (once dosing is confirmed). The COH CRC will directly accession into MIDAS as per current policy and procedure.

5. Once registration has been completed, DCC staff will send a Confirmation of Registration Form, including the participant study number and cohort assignment to:

- the site study team: site PI, treating physician, biostatistician, protocol nurse, CRC and IDS pharmacy.

5.3 Trial Treatments

The treatment to be used in this trial is outlined below in Table 2~~Table 2~~

Table 2 Trial Treatment

Cohort 1 Triple negative breast cancer

Drug	Dose	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
Pembrolizumab	200 mg	Q3W	IV infusion	Day 1 of each 3 week cycle	Experimental
Doxorubicin*	50-60 mg/m^2	Q3W X 6 cycles	IV infusion	Day 1 of each 3 week cycle, standard	

*starting dose 50 mg/m^2 for cohort 1, escalating to 60mg/m^2 based on acceptable toxicity during safety-lead in. see Statistical Section. No intrapatient dose escalation

is allowed. After 6 cycles of doxorubicin and pembrolizumab, patients will continue on pembrolizumab alone up to a total of 24 cycles.

Subjects who stop pembrolizumab with stable disease or better may be eligible for up to one year of additional pembrolizumab therapy if they progress after stopping study treatment.

Cohort 2 HR+ breast cancer

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
Pembrolizumab	200 mg	Q3W	IV infusion	Day 1 of each 3 week cycle	Experimental
Aromatase inhibitor (AI)*	Exemestane 25 mg daily po preferred, Anastrozole 1 mg daily po, or letrozole 2.5 mg daily po are options	QD	Po	QD cycle, standard	

*pending prior exposure: patients could not have failed all three commercially available AIs.

Trial treatment should begin as close as possible to the date on which treatment is allocated/assigned.

Subjects who stop pembrolizumab with stable disease or better may be eligible for up to one year of additional pembrolizumab therapy if they progress after stopping study treatment.

5.3.1 **Dose Selection/Modification**

5.3.1.1 Dose Selection

The rationale for selection of doses to be used in this trial is provided in Section 4.0 – Background and Rationale.

Details on preparation and administration of pembrolizumab (MK-3475) are provided in the Pharmacy Manual (Appendix 12.4.)

5.3.1.2 Dose Modification

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 3 below. See Section 5.6.1 and Events of Clinical Interest Guidance Document for supportive care guidelines, including use of corticosteroids.Table 3

Dose Modification Guidelines for Drug-Related Adverse Events

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Discontinue Subject
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)[1]	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	2-3	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
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue
Hypothyroidism	2-4	Therapy with pembrolizumab can be continued while treatment for the thyroid disorder is instituted	Therapy with pembrolizumab can be continued while treatment for the thyroid disorder is instituted.
Infusion Reaction	3-4	Permanently discontinue	Permanently discontinue
Pneumonitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	3-4	Permanently discontinue	Permanently discontinue
Renal Failure or Nephritis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	3-4	Permanently discontinue	Permanently discontinue
All Other Drug-Related Toxicity[2]	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 (Grade 2 for [pneumonitis) drug-related AE that recurs or any life-threatening event.

[1] 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.

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Discontinue Subject
[2] 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.

5.3.2 **Timing of Dose Administration**

Trial treatment should be administered on Day 1 of each cycle after all procedures/assessments have been completed as detailed on the Trial Flow Chart (Section 6.0). Trial treatment may be administered up to 3 days before or after the scheduled Day 1 of each cycle due to administrative reasons.

All trial treatments will be administered on an outpatient basis.

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

Follow the package insert for the preparation of the pembrolizumab infusion fluid and administration of infusion solution.

5.3.3 **Doxorubicin**

For patients accrued on cohort 1: For dose adjustment see package insert for subjects receiving this agent, once the trial dose is selected. We will have a safety lead-in employing a 3-at-risk rolling design. For each treatment, we will permit only 3 patients to be a risk for first cycle toxicities at any one time during the safety-lead in. Dose escalation from 50mg/m2 to 60mg/m2 will be permitted according the 3-at-risk dose escalation rules (below). Once the safety lead-in is complete, accrual will proceed. Any dose de-escalation from 50mg/m2 will require discussion with the sponsor and an amendment. DLTs are defined in section 8.1. below.

.**5.3.4 Aromatase inhibitors**

Exemestane 25 mg daily po, anastrozole 1 mg po daily, or letrozole2.5 mg po daily will be administered as per package insert. There will be no dose-adjustment for these agents.

5.3.5Trial Blinding/Masking

This is an open-label trial; therefore, the Sponsor, investigator and subject will know the treatment administered.

5.4 Randomization or Treatment Allocation

Patients with triple negative stage 4 breast cancer will receive pembrolizumab and doxorubicin in cohort 1; patients with HR+ stage IV breast cancer will receive pembrolizumab and an aromatase inhibitor.

5.5 Stratification

Accrual will not depend on stratification, but for Cohort 1 we will consider stratified analysis based on first-line (for metastatic or newly diagnosed), versus lines 2 or beyond for metastatic disease.

5.6 Concomitant Medications/Vaccinations (allowed & prohibited)

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.

5.6.1 Acceptable Concomitant Medications

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

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs as defined in Section 7.2.

5.6.2 **Prohibited Concomitant Medications**

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase (including retreatment for post-complete response relapse) of this trial:

Antineoplastic systemic chemotherapy or biological therapy

Immunotherapy not specified in this protocol

Chemotherapy not specified in this protocol

Investigational agents other than pembrolizumab

Radiation therapy

Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed at the investigator's discretion.

- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.

Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiological doses of corticosteroids (≤ 10 mg prednisone or equivalent po daily) is allowed.

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

The Exclusion Criteria describes other medications which are prohibited in this trial.

There are no prohibited therapies during the Post-Treatment Follow-up Phase.

5.7 Rescue Medications & Supportive Care

5.7.1 **Supportive Care Guidelines**

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below and in greater detail in the ECI guidance document. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is

decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

Note: if after the evaluation the event is determined not to be related, the investigator is instructed to follow the ECI reporting guidance but does not need to follow the treatment guidance (as outlined in the ECI guidance document). Refer to Section 5.2.1 for dose modification.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event. Suggested conditional procedures, as appropriate, can be found in the ECI guidance document.

- **Pneumonitis**:
    - For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
    - For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
    - Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.
- **Diarrhea/Colitis**:
  Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).

    - All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
    - For **Grade 2 diarrhea/colitis** 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. Monitor patients for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.

  - **Grade 2** hyperthyroidism events(and **Grade 3-4** hypothyroidism):
    - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
    - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.
  - **Grade 3-4** hyperthyroidism
    - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

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

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

- **Management of Infusion Reactions**: Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

Table

~~Table~~ 4 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab (MK-3475).

Table 4 Infusion Reaction Treatment Guidelines

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

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
(e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

5.8 Diet/Activity/Other Considerations

5.8.1 Diet

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

5.8.2 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm. Non-pregnant, non-breast-feeding women may be enrolled if they are willing to use 2 methods of birth control or are considered highly unlikely to conceive. Highly unlikely to conceive is defined as 1) surgically sterilized, or 2) postmenopausal (a woman who is ≥45 years of age and has not had menses for greater than 1 year will be considered postmenopausal), or 3) not heterosexually active for the duration of the study. The two birth control methods can be either two barrier methods or a barrier method plus a hormonal method to prevent pregnancy. Subjects should start using birth control from study Visit 1 throughout the study period up to 120 days after the last dose of study therapy.

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

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study they must adhere to the contraception requirement (described above) for the duration of the study and during the follow-up period defined in section 7.2.2-Reporting of Pregnancy and Lactation to the Sponsor and to Merck. If there is any question that a subject will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

5.8.3 **Use in Pregnancy**

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

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the Sponsor and to Merck and followed as described above and in Section 7.2.2.

5.8.4 **Use in Nursing Women**

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

5.9 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator or the Sponsor if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding discontinuation or withdrawal are provided in Section 7.1.3 – Other Procedures.

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

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

  Note: For unconfirmed radiographic disease progression, please see Section 4.1.5.1

  Note: A subject may be granted an exception to continue on treatment with confirmed radiographic progression if clinically stable or clinically improved, please see Section 4.1.5.1
- Unacceptable adverse experiences as described in Section 5.2.1.2
- Intercurrent illness that prevents further administration of treatment

- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Completed 24 months of uninterrupted treatment with pembrolizumab or 35 administrations of study medication, whichever is later.

  Note: 24 months of study medication is calculated from the date of first dose. Subjects who stop pembrolizumab after 24 months may be eligible for up to one year of additional study treatment if they progress after stopping study treatment provided they meet the requirements detailed in Section 7.1.4.5

- Administrative reasons

The End of Treatment and Follow-up visit procedures are listed in Section 6 (Protocol Flow Chart) and Section 7.1.4 (Visit Requirements). After the end of treatment, each subject will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment as described in Section 7.1.5.3.1). Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent or becoming lost to follow-up. After documented disease progression each subject will be followed by telephone for overall survival until death, withdrawal of consent, or the end of the study, whichever occurs first.

5.9.1 **Discontinuation of Study Therapy after CR**

Discontinuation of treatment may be considered for subjects who have attained a confirmed CR that have been treated for at least 24 weeks with pembrolizumab and had at least two treatments with pembrolizumab beyond the date when the initial CR was declared. Subjects who then experience radiographic disease progression no sooner than 8 weeks after completion of pembrolizumab may be eligible for up to one year of additional treatment with pembrolizumab via the Second Course Phase at the discretion of the investigator if no cancer treatment was administered since the last dose of pembrolizumab, the subject meets the safety parameters listed in the Inclusion/Exclusion criteria, and the trial is open. Subjects will resume therapy at the same dose and schedule at the time of initial discontinuation. Additional details are provided in Section 7.1.4.5.

5.9.2. Discontinuation of doxorubicin for patients treated in cohort 1 should be considered after either a cumulative dose of 360 mg/m^2, a drop in left ventricular ejection fraction below 50%, or 2 cycles after complete remission is confirmed, whichever occurs sooner.

5.10 Subject Replacement Strategy

A subject discontinuing therapy will not be replaced. Patients who are screen-failed but were never treated will be replaced.

5.11 Clinical Criteria for Early Trial Termination

Early trial termination will be the result of the criteria specified below:

1. Quality or quantity of data recording is inaccurate or incomplete
2. Poor adherence to protocol and regulatory requirements
3. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects
4. Plans to modify or discontinue the development of the study drug

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

6.0 TRIAL FLOW CHART

6.1 Study Flow Chart

Trial Period:	**Screening Phase**		**Treatment Cycles**								**End of Treatment**	**Post-Treatment**		
Treatment Cycle/Title:	Pre-screening (Visit 1)	Main Study Screening (Visit 2)	1	2	3	4	To be repeated beyond 8 cycles 5	6	7	8	Discont.	Safety Follow-up	Follow Up Visits	Survival Follow-Up
Scheduling Window (Days):		-28 to -1		± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discont.	30 days post Discont.	Every 8 weeks post Discont.	Every 12 weeks
Administrative Procedures														
Pre-screening Consent	X													
Informed Consent		X												
Inclusion/Exclusion Criteria		X												
Demographics and Medical History		X												
Prior and Concomitant Medication Review		X	X	X	X	X	X	X	X	X		X		
Trial Treatment Administration			X	X	X	X	X	X	X	X				
Post-study anticancer therapy status													X	X
Survival Status														X
Clinical Procedures/Assessments														
Review Adverse Events		X	X	X	X	X	X	X	X	X	X	X		
Full Physical Examination		X												
Directed Physical Examination			X	X	X	X	X	X	X	X				
Vital Signs and Weight		X	X	X	X	X	X	X	X	X	X			
ECOG Performance Status		X	X	X	X	X	X	X	X	X				
Laboratory Procedures/Assessments: analysis performed by LOCAL laboratory														
Pregnancy Test – Urine or Serum β-HCG		X												
PT/INR and aPTT		X												
CBC with Differential		X	X	X	X	X	X	X	X	X	X	X		

Trial Period:	**Screening Phase**		**Treatment Cycles**								**End of Treatment**	**Post-Treatment**		
							To be repeated beyond 8 cycles							
Treatment Cycle/Title:	Pre-screening (Visit 1)	Main Study Screening (Visit 2)	1	2	3	4	5	6	7	8	Discont.	Safety Follow-up	Follow Up Visits	Survival Follow-Up
Scheduling Window (Days):		-28 to -1		± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discont.	30 days post Discont.	Every 8 weeks post Discont.	Every 12 weeks
Comprehensive Serum Chemistry Panel		X	X	X	X	X	X	X	X	X	X	X		
Urinalysis		X		X		X		X		X		X		
T3, FT4 and TSH		X		X		X		X		X		X		
Efficacy Measurements														
Tumor Imaging with CT (chest, abdomen, pelvis, bone scan, other modalities as deemed appropriate clinically)		X			X			X			X			
Cardiac evaluation:Echo,or MUGA and ECG for triple negative and MUGA or ECHO for the HR+ cohort		X							X@					
Tumor Biopsies/Archival Tissue Collection/Correlative Studies Blood														
Archival or Newly Obtained Tissue Collection		X		X#							X			
Correlative Studies Blood Collection##		X		X							X			

#optional ## if pts agree ask for weeks 4,6,8,10, 20,30

@For triple negative patients, Echo or MUGA evaluations will be repeated after cycle 6, at least a week prior to cycle 7. Subsequent cardiac testing will be carried out as clinically indicated. For hormone receptor positive patients, only a baseline Echo or MUGA scan is required, any subsequent cardiac testing will be as clinically indicated

7.0 TRIAL PROCEDURES

7.1 Trial Procedures

The Trial Flow Chart - Section 6.0 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

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

7.1.1 **Administrative Procedures**

7.1.1.1 Informed Consent

The Investigator must obtain documented consent from each potential subject prior to participating in a clinical trial.

7.1.1.1.1 General Informed Consent

Consent must be documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion.

A copy of the signed and dated consent form should be given to the subject before participation in the trial.

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

Specifics about a trial and the trial population will be added to the consent form template at the protocol level.

The informed consent will adhere to IRB/ERC requirements, applicable laws and regulations and Sponsor requirements.

7.1.1.2 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial.

7.1.1.3 Medical History

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

7.1.1.4 Prior and Concomitant Medications Review

7.1.1.4.1 Prior Medications

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

7.1.1.4.2 Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the subject during the trial. All medications related to reportable SAEs and ECIs should be recorded as defined in Section 7.2.

7.1.1.5 Disease Details and Treatments

7.1.1.5.1 Disease Details

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

7.1.1.5.2 Prior Treatment Details

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

7.1.1.5.3 Subsequent Anti-Cancer Therapy Status

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

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

7.1.1.6 Assignment of Screening Number

All consented subjects will be given a unique screening number according to their designated cohorts (Cohort 1: triple negative; Cohort 2 HR+).

7.1.1.7 Assignment of Cohort Number will be based as above.

7.1.1.8 Trial Compliance (Medication/Diet/Activity/Other)

Interruptions from protocol-specified treatment for greater than 12 weeks require consultations between the investigator/PI and sponsors.

Instructions on how to prepare pembrolizumab will be per Pharmacy manual. Doxorubicin and AIs will be prepared or prescribed as per approved product label and standard practice guidelines.

7.1.2 Clinical Procedures/Assessments

7.1.2.1 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 4.0 (see Section 11.2). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

For subjects receiving treatment with pembrolizumab all AEs of unknown etiology associated with pembrolizumab exposure should be evaluated to determine if it is possibly an event of clinical interest (ECI) of a potentially immunologic etiology (termed immune-related adverse events, or irAEs); see the separate ECI guidance document in Appendix 4 regarding the identification, evaluation and management of potential irAEs.

Please refer to section 7.2 for detailed information regarding the assessment and recording of AEs.

7.1.2.2 Full Physical Exam

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

7.1.2.3 Directed Physical Exam

For cycles that do not require a full physical exam per the Trial Flow Chart, the investigator or qualified designee will perform a directed physical exam as clinically indicated prior to trial treatment administration.

7.1.2.4 Vital Signs

The investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment and at treatment discontinuation as specified in the Trial Flow Chart (Section 6.0). Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only.

7.1.2.5 Eastern Cooperative Oncology Group (ECOG) Performance Scale

The investigator or qualified designee will assess ECOG status (see Section 11.1) at screening, prior to the administration of each dose of trial treatment and discontinuation of trial treatment as specified in the Trial Flow Chart.

7.1.2.6 Tumor Imaging and Assessment of Disease

Tumor imaging may be performed by CT, MRI, FDG/PET/CT but the same technique needs to be performed throughout the trial. Cardiac assessment (Cohort 1 patients) can be performed by MUGA or Echocardiogram.

7.1.2.7 Tumor Tissue Collection and Correlative Studies Blood Sampling

Laboratory Procedures/Assessments

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

Laboratory tests for hematology, chemistry, urinalysis, and others are specified in Table 5.

Table 5 Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hematocrit	Albumin	Blood	Serum β-human chorionic gonadotropin†
Hemoglobin	Alkaline phosphatase	Glucose	(β-hCG)†
Platelet count	Alanine aminotransferase (ALT)	Protein	PT (INR)
WBC (total and differential)	Aspartate aminotransferase (AST)	Specific gravity	aPTT
Red Blood Cell Count	Lactate dehydrogenase (LDH)	Microscopic exam *(If abnormal)*	Total triiodothyronine (T3)
Absolute Neutrophil Count	Carbon Dioxide ‡	results are noted	Free tyroxine (T4)
Absolute Lymphocyte Count	*(CO_2 or bicarbonate)*	Urine pregnancy test †	Thyroid stimulating hormone (TSH)
	Uric Acid		PK
	Calcium		
	Chloride		Blood for correlative studies
	Glucose		
	Phosphorus		
	Potassium		
	Sodium		
	Magnesium		
	Total Bilirubin		
	Direct Bilirubin		
	Total protein		
	Blood Urea Nitrogen		

† Perform on women of childbearing potential only. If urine pregnancy results cannot be confirmed as negative, a serum pregnancy test will be required.
‡ If considered standard of care in your region.

Laboratory tests for screening or entry into the Second Course Phase should be performed within 7 days prior to the first dose of treatment. After Cycle 1, pre-dose laboratory procedures can be conducted up to 72 hours prior to dosing. Results must be reviewed by the investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.

7.1.2.8 Pharmacokinetic/Pharmacodynamic Evaluations

Not applicable.

7.1.2.8.1 Blood Collection for Serum Pembrolizumab

N/A

7.1.2.8.2 Blood Collection for Anti-Pembrolizumab Antibodies NA

7.1.3 Other Procedures

7.1.3.1 Withdrawal/Discontinuation

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 7.2 - Assessing and Recording Adverse Events. Subjects who a) attain a CR or b) complete 24 months of treatment with pembrolizumab may discontinue treatment with the option of restarting treatment if they meet the criteria specified in Section 7.1.4.5. After discontinuing treatment following assessment of CR, these subjects should return to the site for a Safety Follow-up Visit (described in Section 7.1.5.3.1) and then proceed to the Follow-Up Period of the study (described in Section 7.1.5.4).

7.1.3.2 Blinding/Unblinding

Not applicable.

7.1.4 Visit Requirements

Visit requirements are outlined in Section 6.0 - Trial Flow Chart. Specific procedure-related details are provided above in Section 7.1 - Trial Procedures.

7.1.4.1 Screening

Patients will be identified by the breast cancer team at their regular weekly treatment planning conference, or as they are being seen by team members.

7.1.4.1.1 Screening Period

Laboratory information should be within 7 days of initiating treatment, radiographic and other images should be within 28 days prior to starting treatment.

7.1.4.2 Treatment Period

See 6.1.

7.1.4.3 Post-Treatment Visits

See section 6.1

7.1.5.3.1 Safety Follow-Up Visit

The mandatory Safety Follow-Up Visit should be conducted approximately 30 days after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes first. All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Subjects with an AE of Grade > 1 will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-neoplastic therapy, whichever occurs first. SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded. Subjects who are eligible for retreatment with pembrolizumab (as described in Section 7.1.4.5) may have up to two safety follow-up visits, one after the Treatment Period and one after the Second Course Phase.

7.1.4.4 Follow-up Visits

Subjects who discontinue trial treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed every 6 weeks (42 ± 7 days) by radiologic imaging to monitor disease status. After 1 year, the imaging time point will occur every 9 weeks (± 7 days). Every effort should be made to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression, death, end of the study or if the subject begins retreatment with pembrolizumab as detailed in Section 7.1.4.5. Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated.

Subjects who are eligible to receive retreatment with pembrolizumab according to the criteria in Section 7.1.4.5 will move from the follow-up phase to the Second Course Phase when they experience disease progression. Details are provided in Section 6.2 – Trial Flow Chart for Retreatment.

7.1.4.4.1 Survival Follow-up

Once a subject experiences confirmed disease progression or starts a new anti-cancer therapy, the subject moves into the survival follow-up phase and should be contacted by telephone every 12 weeks to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.

7.1.4.5 Second Course Phase (Retreatment Period)

Subjects who stop pembrolizumab with SD or better may be eligible for up to one year of additional pembrolizumab therapy if they progress after stopping study treatment. This retreatment is termed the Second Course Phase of this study and is only available if the study remains open and the subject meets the following conditions:

- **Either**
    - Stopped initial treatment with pembrolizumab after attaining an investigator-determined confirmed CR according to RECIST 1.1, and
        - Was treated for at least 24 weeks with pembrolizumab before discontinuing therapy and has not progressed within 8 weeks of completion of such therapy, but not beyond 12 months of being off therapy.
        - Received at least two treatments with pembrolizumab beyond the date when the initial CR was declared

OR

    - Had SD, PR or CR and stopped pembrolizumab treatment after 24 months of study therapy for reasons other than disease progression or intolerability

AND

- Experienced an investigator-determined confirmed radiographic disease progression after stopping their initial treatment with pembrolizumab
- Did not receive any anti-cancer treatment since the last dose of pembrolizumab
- Has a performance status of 0 or 1 on the ECOG Performance Scale
- Demonstrates adequate organ function as detailed in Section 5.1.2
- Female subject of childbearing potential should have a negative serum or urine pregnancy test within 72 hours prior to receiving retreatment with study medication.
- Female subject of childbearing potential should be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication (Reference Section 5.7.2). Subjects of child bearing potential are those who have not been surgically sterilized or have been free from menses for > 1 year.
- Male subject should agree to use an adequate method of contraception starting with the first dose of study therapy through 120 days after the last dose of study therapy.

- Does not have a history or current evidence of any condition, therapy, or laboratory abnormality that might 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.

Subjects who restart treatment will be retreated at the same dose and dose interval as when they last received pembrolizumab. Treatment will be administered for up to one additional year.

Visit requirements are outlined in Section 6.0 – Trial Flow Chart.

7.2 Assessing and Recording Adverse Events

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

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

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

Adverse events may occur during the course of the use of Merck product in clinical trials or within the follow-up period specified by the protocol, or prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

Adverse events may also occur in screened subjects during any pre-allocation baseline period as a result of a protocol-specified intervention, including washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

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

All adverse events will be recorded from the time the consent form is signed through 30 days following cessation of treatment and 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 7.2.3.1.

7.2.1 Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor and to Merck

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

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

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

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

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

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them), 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. 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 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 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

7.2.3 Data and Safety Monitoring Plan

7.2.3.1 Definition of Risk Level

This is a Risk Level 4 study as defined in the City of Hope Institutional Data and Safety Monitoring Plan. This determination was made because the study involves a COH IND.

7.2.4 Monitoring and Personnel Responsible for Monitoring

The Protocol Management Team (PMT) is responsible for monitoring the data and safety of this study. The PMT consists of the Principal Investigator (PI), Biostatistician, Research Protocol Nurse, and Clinical Research Coordinator.

The PMT is required to submit periodic status reports (i.e., the PMT Report) according to the frequency prescribed in the City of Hope Institutional Data and Safety Monitoring Plan. Important decisions made during PMT meetings (i.e., dose escalation, de-escalation, etc.) only need to be noted in the PMT Report submitted to the Data and Safety Monitoring Committee (DSMC).
Adverse Event (AE) - An adverse event is any untoward medical experience or change of an existing condition that occurs during or after treatment, whether or not it is considered to be related to the protocol intervention.

Reporting Non-serious Adverse Events – Adverse events will be collected after the patient is given the study treatment or any study related procedures. Adverse events will be monitored by the PMT. Adverse events that do not meet the criteria of serious OR are not unanticipated problems will be reported only in the PMT Report.

Serious Adverse Event (SAE) [Modified from the definition of unexpected adverse drug experience in 21 CFR 312.32] - defined as *any expected* or *unexpected adverse event*s that result in any of the following outcomes:

- Death
- Is life-threatening experience (places the subject at immediate risk of death from the event as it occurred)
- Unplanned hospitalization (equal to or greater than 24 hours) or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- Secondary malignancy
- Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias of convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

Reporting Serious Adverse Events - begins after study treatment or any study related procedures. All SAEs occurring during this study, whether observed by the physician, nurse, or reported by the patient, will be reported according to the approved COH policy. Serious Adverse Events that require expedited reporting will be submitted electronically using iRIS.

7.2.5 Adverse Event Name and Severity

The PI will determine the adverse event name and severity (grade) by using the CTCAE version 4.

Expected Adverse Event - Any event that does not meet the criteria for an unexpected event, OR is an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

Unexpected Adverse Event [21 CFR 312.32 (a)] – An adverse event is unexpected if it is not listed in the investigator's brochure and/or package insert; is not listed at the specificity or severity that has been observed; is not consistent with the risk information described in the protocol and/or consent; is not an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

7.2.6 Adverse Event Attribution

The following definitions will be used to determine the causality (attribution) of the event to the study agent or study procedure.

Definite - The AE is clearly related to the investigational agent or study procedure and unrelated to any other cause.

Probable - The AE is likely related to the investigational agent or study procedure and unlikely related to other cause(s).

Possible -The AE may be related to the investigational agent or study procedure and may be related to another cause(s).

Unlikely -The AE is doubtfully related to the investigational agent or study procedure and likely related to another cause(s).

Unrelated -The AE is clearly not related to the investigational agent or study procedure and is attributable to another cause(s).

7.2.7 COH Held IND

Serious Adverse Events meeting the requirements for expedited reporting to the Food and Drug Administration (FDA), as defined in 21 CFR 312.32, will be reported as an IND safety report using the MedWatch Form FDA 3500A for Mandatory Reporting.

The criteria that require reporting using the Medwatch 3500A are:

- Any unexpected fatal or life threatening adverse experience associated with use of the drug must be reported to the FDA no later than 7 calendar days after initial receipt of the information [21 CFR 312.32(c)(2)]
- Any adverse experience associated with use of the drug that is both serious and unexpected must be submitted no later than 15 calendar days after initial receipt of the information [21 CFR 312.32(c)(1)]
- Any follow-up information to a study report shall be reported as soon as the relevant information becomes available. [21 CFR 312.32(d)(3)]

The PI or designee will be responsible for contacting the Office of IND Development and Regulatory Affairs (OIDRA) at COH to ensure prompt reporting of safety reports to the FDA. OIDRA will assist the PI with the preparation of the report and submit the report to the FDA in accordance with the approved COH policy.

7.2.8 Deviations and Unanticipated Problems

Deviation - A deviation is a divergence from a specific element of a protocol that occurred without prior IRB approval. Investigators may deviate from the protocol to eliminate immediate hazard(s) for the protection, safety, and well-being of the study subjects without prior IRB approval. For any such deviation, the PI will notify the COH DSMC and IRB within 5 calendar days of its occurrence via iRIS in accordance with the Clinical Research Protocol Deviation policy.

7.2.9 Single Subject Exception (SSE)

An SSE is a planned deviation, meaning that it involves circumstances in which the specific procedures called for in a protocol are not in the best interests of a specific patient. It is a deviation that is anticipated and receives prior approval by the PI and the IRB. The SSE must be submitted as a "Single Subject Exception Amendment Request" via iRIS in accordance with IRB guidelines and the Clinical Research Protocol Deviation policy. An IRB approved SSE does not need to be submitted as a deviation to the DSMC.

7.2.10 Unanticipated Problem (UP) – Any incident, experience, or outcome that meets all three of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given the following: a) the research procedures described in the protocol-related documents such as the IRB approved research protocol, informed consent document or Investigator Brochure (IB); and b) the characteristics of the subject population being studied; **AND**
2. Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcomes may have been caused by the drugs, devices or procedures involved in the research); **AND**
3. Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm) than previously known or recognized.

Any UP that occurs during study conduct will be reported to the DSMC and IRB in accordance with the City of Hope's Institutional policy using iRIS.

7.2.11 COH Held IND

The Office of IND Development and Regulatory Affairs (OIDRA) will assist the PI in reporting the event to the Food and Drug Administration (FDA).

7.2.12 Adverse Events and Serious Adverse Events for Merck

The PI will be responsible for determining the event name, assessing the severity (i.e., grade), expectedness, and attribution of all adverse events.

7.2.13 Immediate Reporting of Adverse Events to Merck

7.2.13.1 Serious Adverse Events

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

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose;
- Is an other important medical event

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

Progression of the cancer under study is not considered an adverse event unless it results in hospitalization or death.

Any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject 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.

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

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

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

7.2.13.2 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be recorded as such on the Adverse Event case report forms/worksheets and reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)Events of clinical interest for this trial include:

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

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

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

an additional evaluation for an underlying etiology. The trial site guidance for assessment and follow up of these criteria can be found in the Investigator Trial File Binder (or equivalent).

1. Additional adverse events:

   A separate guidance document has been provided entitled "Event of Clinical Interest Guidance Document" (previously entitled, "Event of Clinical Interest and Immune-Related Adverse Event Guidance Document"). This document can be found in Appendix 4 and provides guidance regarding identification, evaluation and management of ECIs and irAEs.

   ECIs (both non-serious and serious adverse events) identified in this guidance document from the date of first dose through 90 days following cessation of treatment, or 30 days after the initiation of a new anticancer therapy, whichever is earlier, need to 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), regardless of attribution to study treatment, consistent with standard SAE reporting guidelines.

   Subjects should be assessed for possible ECIs prior to each dose. Lab results should be evaluated and subjects should be asked for signs and symptoms suggestive of an immune-related event. Subjects who develop an ECI thought to be immune-related should have additional testing to rule out other etiologic causes. If lab results or symptoms indicate a possible immune-related ECI, then additional testing should be performed to rule out other etiologic causes. If no other cause is found, then it is assumed to be immune-related.

7.2.14 **Evaluating Adverse Events**

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

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

Table 6 Evaluating Adverse Events

An investigator who is a qualified physician, will evaluate all adverse events as to:

V4.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of Merck product that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization [including hospitalization for an elective procedure] for a preexisting condition which has not worsened does not constitute a serious adverse event.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new **cancer;** (that is not a condition of the study) **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours.	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause the Merck product to be discontinued?	
Relationship to test drug	Did the Merck product cause the adverse event? The determination of the likelihood that the Merck product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between the Merck product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely the Merck product caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to the Merck product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of the Merck product? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

Relationship to Merck product (continued)	**The following components are to be used to assess the relationship between the test drug and the AE: (continued)**	
	Dechallenge	Was the Merck product discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Merck product; or (3) the trial is a single-dose drug trial); or (4) Merck product(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to the Merck product in this study? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) Merck product(s) is/are used only one time). NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY THE MERCK PRODUCT, OR IF REEXPOSURE TO THE MERCK PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE U.S. CLINICAL MONITOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding the Merck product or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following		**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of a Merck product relationship).**
Yes, there is a reasonable possibility of Merck product relationship.		There is evidence of exposure to the Merck product. The temporal sequence of the AE onset relative to the administration of the Merck product is reasonable. The AE is more likely explained by the Merck product than by another cause.
No, there is not a reasonable possibility Merck product relationship		Subject did not receive the Merck product OR temporal sequence of the AE onset relative to administration of the Merck product is not reasonable OR there is another obvious cause of the AE. (Also entered for a subject with overdose without an associated AE.)

7.2.15 Sponsor Responsibility for Reporting Adverse Events

All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations.

8.0 STATISTICAL ANALYSIS PLAN

8.1 Statistical Analytical Plan Summary

8.1.1 Statistical Analysis Plan Phase I

Phase I lead-in considerations:

Cohort 1: doxorubicin + pembrolizumab

Drug	Doxorubicin	Pembrolizumab
Level 1	50 mg/m^2	200 mg
Level 2	60 mg/m^2	200 mg

Cohort 2: AI + pembrolizumab (AIs are combined for the safety/activity primary endpoints)

Drug	Anti-estrogen	Pembrolizumab
Level 1	Standard*	200 mg

* Exemestane 25 mg daily po preferred, Anastrozole 1 mg daily po, or letrozole 2.5 mg daily po are options.

8.1.1.1 Safety Lead-in and Dose Escalation and De-escalation

For both cohorts we will have a safety lead-in employing a 3-at-risk rolling design. For each treatment, we will permit only 3 patients to be a risk for first cycle toxicities at any one time during the safety-lead in.

8.1.1.1.1 Cohort 1 (Pembrolizumab and Doxorubicin)

For cohort 1, a dose escalation from 50mg/m2 to 60mg/m2 will be permitted according the 3-at-risk dose escalation rules (below). There will not be intra-patient dose-escalation. Once the safety lead-in is complete, accrual will proceed based on the phase II considerations for cohort 1 below. Any dose de-escalation from 50mg/m2 will require discussion with the sponsor and an amendment.

8.1.1.1.2. Cohort 2 (pembrolizumab and Aromatase Inhibitor)

For cohort 2, when the first 6 patients have been completed cycle 1 with at most 1 dose limiting toxicities (DLT), the safety-lead in for that doublet will be considered successful, and accrual will proceed based on the Phase II considerations below. For cohort 2, if 2 DLTs are observed on dose level 1 in the first 6 patients the study will hold accrual pending discussion with sponsor, PI, and COH DSMB for the selection of a dose level -1 or terminate consideration of that treatment doublet.

8.1.2 **Definitions of DLTs**

DLTs for both cohorts are defined as:

Hematologic Toxicities:

• Any Grade 4 thrombocytopenia or neutropenia lasting >7 days

Nonhematologic Toxicities:

• Episcleritis, uveitis, or iritis of Grade 2 or higher

• Any Grade 4 toxicity

• Any Grade 3 toxicity EXCLUDING:

- Nausea, vomiting, or diarrhea controlled by medical intervention within 72 hours
- Grade 3 rash in the absence of desquamation, no mucosal involvement, does not require steroids, and resolves to Grade 1 by the next scheduled dose of pembrolizumab.
- Rapidly reversible electrolyte abnormalities (within 72 hrs.).
- Transient Grade 3 AST or ALT elevation, defined as no more than 3 days with or without steroid use

• Discontinuation or delay of more than 2 weeks of any study drug due to treatment-related AE will be considered as a DLT

Toxicity will be graded according to the NCI CTCAE version 4.0. To be evaluable for toxicity, a patient must receive at least one complete course of treatment and be observed for at least 21 days after the start of the first course or have experienced a DLT. All patients who receive any amount of drug will be considered evaluable for toxicity.

8.1.3 **3-at-risk—Rules of Level Assignment**

3-at-risk -- Rules for Dose Level Assignment

# Patients on Current Level			Action
DLT	EVAL	EVAL+At Risk	
0	0	1-2	Accrue next patient at this level*
0	0	3	Hold accrual
0	1	1-3	Accrue next patient at this level
0	1	4	Hold accrual
0	2	2-4	Accrue next patient at this level
0	2	5	Hold accrual
0	3	3-5	Accrue next patient at the next higher level*
1	1	1-2	Accrue next patient at this level
1	1	3	Hold Accrual
1	2	2	Accrue next patient at this level
1	2	3-4	Hold accrual
1	3-5	3-5	Accrue next patient at this level
1	3-5	6	Hold accrual
1	6	6	Accrue next patient at the next higher level*
2**	any	Any	Accrue next patient at the next lower level to a maximum of 6

*If in dose-escalation portion. If higher dose level already closed, the next lower dose will accrue to a total of 6 patients, with 2 or higher DLTs requiring further dose de-escalation. If there is no next higher dose, the next patient will be accrued to the current level.

**Patients treated on a higher dose during cycle 1 will have their treatment modified to the dose below the dose level with 2 DLTs.

Note: "DLT" – a patient with a documented first-cycle DLT; "PASS" – a patient without a first-cycle DLT fully evaluable for toxicity for the purpose of dose escalations; "EVAL" – a patient who is either DLT or PASS; "Inevaluable" – a patient who is off treatment without being DLT or PASS; "At Risk" – a patient who is on study in the first cycle and not yet DLT, PASS, or Inevaluable

8.2 Statistical Plan Phase II

Phase II considerations:

8.2.1 Cohort 1

Cohort 1 (doxorubicin plus pembrolizumab):

Cohort 1: All patients treated in cohort 1 (regardless of dose level) will be included in the efficacy assessments as it is unclear, based on historical data, if there is a significant clinical advantage to higher dose intensity once beyond 50mg/m2. Patients with triple negative metastatic breast cancer are eligible. All patients must be doxorubicin and pembrolizumab naïve. Patients will be treated with doxorubicin and pembrolizumab. For each treatment, following the safety lead-in, we will accrue to a total of 36 patients over 18-24months.

Recent (19) data on single agent platinum treatment in first and second line metastatic patients are 25.6% (95% CI 16.8-36.1%), with second line cisplatin at 22.2% and first-line carboplatin at 22.9%. First-line patients who will be likely candidates for this study will either have peripheral neuropathy preventing taxanes and platinum, or will have recurred rapidly after taxane-platinum containing adjuvant therapy, or progressed, suggesting low efficacy of standard approach. Patients would need to be doxorubicin naïve. As a result of this patient selection, we have set a discouraging response rate at 15% and an encouraging rate at 34%. The null hypothesis H0: is ORR<=15%, and the alternative H1: ORR>=34%. Specifically, for we follow Simon's MinMax two-stage design with a type I error of 10% and a power of 90%: If 3 or fewer responses are noted in the first 22 patients in this cohort, the study will hold accrual for futility. In the case of early stopping, evaluation of patient subsets (e.g. immune phenotype), in consultation with the PI, statistician, sponsor and DSMB, may permit the study to continue for specific subsets following an amendment if it is deemed to be inadequately evaluated and there appears to be sufficient promise for that subset. If early stopping does not occur, accrual will continue to a total of 36 patients are treated in this cohort. With 36 patients, 9 patients with an ORR (25%) are required to deem this combination worthy of further evaluation. This maintains the type I error at 10% to reject the null hypothesis and the power at 90% to declare a positive finding if the alternative hypothesis holds. Secondary analysis endpoints, including progression-free survival, time-to-treatment failure, and toxicity may influence the overall recommendation for future evaluation. With 36 patients on this cohort, we expect at least 10 patients on this cohort to have pre- and post-treatment biopsy material for correlative analysis and approximately 36 patients with pre-treatment biopsy.

8.2.2 **Cohort 2**

Cohort 2 (AI+pembrolizumab): Patients with ER+ HR2- metastatic breast cancer who have recurred on or after, or progressed on prior endocrine therapy. Objective response in this setting is rare for AI (~10%), which has suggested the following design:

We will accrue 20 patients over 12-24 months. With 20 patients, if 3 or more patients experience a response, this would be considered worthy of further evaluation. This rule has a power of over 80% to detect a true response rate of 20% (encouraging, alternative hypothesis) and a type I error of less than 8% for falsely declaring a 5% response rate (discouraging, null hypothesis) as promising. This rule establishes the minimum response rate for declaring the combination promising, however, it does not preclude the combination could be promising based on secondary endpoints such as progression-free survival. Analysis by prior treatment may also influence the overall recommendation.

In addition, with 20 patients in this cohort, we expect to obtain at least 10 patients with pre and post-treatment biopsy for correlative analysis, although all patients will have tissue for baseline evaluation.

8.3 Correlative Science

The correlative studies will be used to potentially refine patient selection for future studies, and understand the role of immune changes and baseline status on the activity of the combination of pembrolizumab with AIs, or doxorubicin. These correlative studies are considered exploratory in the context of this limited Phase II study.

9.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

9.1 Investigational Product

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

Clinical Supplies will be provided by Merck as summarized in Table 7.

Table 7 Product Descriptions

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

9.2 Packaging and Labeling Information

Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

9.3 Clinical Supplies Disclosure

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

9.4 Storage and Handling Requirements

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.

9.5 Returns and Reconciliation

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

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

10.0 OTHER STUDY DRUGS:

10.1 Doxorubicin

10.1.1 Availability

Doxorubicin is commercially available as lyophilized powder for reconstitution in 10, 20, 50, 100 and 150 mg vials. Also available as 2 mg/ml solution for injection in 10, 20, 50, 75, and 200 mg vials. Please refer to the FDA-approved package insert for complete product information

10.1.2 **Storage and Stability**

Intact vials of doxorubicin solution should be stored in the refrigerator. Intact vials of powder for reconstitution should be stored at room temperature. Reconstituted solutions are stable for 7 days at room temperature and 15 days under refrigeration when protected from light. Commercially available solutions labeled as such are intended to be multidose vials.

10.1.3 **Preparation**

Reconstitute the vials of doxorubicin powder with 5, 10, 25, 50 or 75 ml, respectively, of Sodium Chloride for Injection, USP, resulting in a concentration of 2 mg/ml.

10.1.4 **Toxicities**

Common: Hair loss, vomiting, red colored urine, saliva and/or sweat

Less Common: Nausea, damage to the heart, heart failure, heart attack, swelling of the heart, swelling and redness of the area of radiation (if radiation is part of treatment) or at the site of the medication injection, belly pain, sores in the mouth, throat, or stomach, infection, especially when white blood cell count is low, bruising, bleeding, hepatitis or damage to the liver, tiredness, allergic reaction which may cause rash, low blood pressure, wheezing, shortness of breath, swelling of the face or throat, swelling of the lungs which may cause shortness of breath, cancer of bone marrow (leukemia) caused by chemotherapy, damage to the bone marrow (irreversible) which may cause infection, bleeding, may require transfusions, darkening of the nail beds or skin or hands and feet, loss of nails

Rare: Severe blood infection

10.2 Exemestane

10.2.1 **Availability:**

Exemestane is commercially available as a 25 mg tablet

10.2.2 **Storage and Stability:**

Exemestane should be stored at room temperature

10.2.3 **Toxicity:**

Common: Hot flashes, osteopenia, vaginal discharge and/or dryness, joint/muscle pain, fatigue, headache, insomnia, nausea, hair thinning

Less Likely: Bone fractures from osteoporosis, shortness of breath, diarrhea, constipation, loss of appetite, dry skin/skin rash, diarrhea, stomach ache, indigestion, vision changes, dizziness, nervousness, reduced libido.

Rare: drowsiness, muscle weakness, inflammation of the liver

10.3 Anastrozole

10.3.1 **Availability:**

Anastrozole is commercially available as a 1 mg film coated tablet.

10.3.2 **Storage and Stability**

Anastrozole should be stored at room temperature.

10.3.3 **Toxicity:**

Common: headaches, hot flushes and sweats, nausea, rashes, painful or stiff joints, arthritis, feeling weak, loss of bone density caused by a lack of estrogen over a long period of time, mood changes, tiredness or fatigue, reduced libido

Less Common: Bone pain, carpal tunnel syndrome, hair thinning, loss of appetite, raised cholesterol levels, feeling sleepy, vomiting, liver changes, diarrhea, dryness of the vagina, vaginal bleeding

Rare: Inflammation of the liver (hepatitis), trigger finger

10.4 Letrozole:

10.4.1 **Availability:**

Letrozole (Femara) 2.5 mg tablets are dark yellow, film-coated, round, slightly biconvex, with beveled edges imprinted with the letters FV on one side and CG on the other side. Letrozole is for oral administration. Letrozole is commercially available. Refer to the Product Information Sheet for information regarding the physical and chemical properties of letrozole.

10.4.2 **Storage and Stability**

Letrozole should be stored at room temperature and away from excess heat and moisture.

10.4.3 **Toxicities:**

Common: Hot flushes and sweats, pain in joints or bones, tiredness and weakness (fatigue), increased levels of cholesterol in the blood

Less Common: Skin rashes, headaches , dizziness, malaise, nausea and vomiting, fluid retention, loss of appetite or indigestion, hair thinning, diarrhea, constipation, vaginal dryness, reduced libido, sadness or depression, cough and breathlessness, osteoporosis, vaginal bleeding, muscle pain, weight gain, hypertension, pain in the abdomen

Rare: Nervous disorders such as anxiety, nervousness, feeling irritable, drowsiness, memory problems, difficulty sleeping, changes in sensation, especially touch, eyesight changes such as blurred vision, red, sore eyes, heart palpitations, joint stiffness (arthritis), trigger finger, carpal tunnel syndrome, breast pain, fever, taste changes, dry mouth and feeling thirsty, weight loss, urine infections

11.0 ADMINISTRATIVE AND REGULATORY DETAILS

11.1 Confidentiality

As per City of Hope Standard Operating Procedures.

11.2 Compliance with Financial Disclosure Requirements

As per City of Hope Standard Operating Procedures

11.3 Compliance with Law, Audit and Debarment

11.4 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the Sponsor of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

11.5 Quality Management System

As per City of Hope Data Safety Monitoring and Clinical Trials Office Standard Operating Procedures.

11.6 Data Management

As per City of Hope Standard Bioinformatics Standard Operating Procedures.

12.0 APPENDICES

12.1 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. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about 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.	

12.2 Common Terminology Criteria for Adverse Events V4.0 (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for adverse event reporting. (http://ctep.cancer.gov/reporting/ctc.html)

12.3 Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 Criteria for Evaluating Response in Solid Tumors

RECIST version 1.1* will be used in this study for assessment of tumor response. While either CT or MRI may be utilized, as per RECIST 1.1, CT is the preferred imaging technique in this study.

* As published in the European Journal of Cancer:

E.A. Eisenhauer, P. Therasse, J. Bogaerts, L.H. Schwartz, D. Sargent, R. Ford, J. Dancey, S. Arbuck, S. Gwyther, M. Mooney, L. Rubinstein, L. Shankar, L. Dodd, R. Kaplan, D. Lacombe, J. Verweij. New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1). Eur J Cancer. 2009 Jan;45(2):228-47.

In addition, volumetric analysis will be explored by central review for response assessment.

12.4 Events of Clinical Interest Guidance Documents

12.4.1 **IB**

12.5 Blood Sample Collection

Blood samples will be collected from an indwelling venous catheter or by venipuncture. At each time point indicated in the study calendar, peripheral blood will be collected into three 7 mL green-top tubes (sodium or lithium heparin) to prevent blood clotting. Tubes will be inverted several times and then immediately placed on ice for transportation to the COH Analytical Pharmacology Core or local research specimen processing laboratory. The whole blood should be processed within 4 hours of collection. While awaiting processing, the blood should be kept on a rocker set at low speed to mimic circulation and avoid clot formation.

12.6 Blood Sample Processing

Plasma

- For plasma preparation, anti-coagulated whole blood (two 7 mL green-top tubes) will be processed by centrifugation for 10 minutes at 1000 x g at 4^0 C. The resulting upper plasma layer from each tube will be drawn up sequentially into a sterile 5 mL syringe and pushed through a sterile 0.2/0.8 micron disposable filter (PALL Acrodisc PF, Cat. 4658). The filtered plasma will then be transferred in 500 µL aliquots into multiple appropriately-labeled Starstedt microfuge tubes (Starstedt Cat 72.692.005). To one aliquot, add 0.5 mL glycerol/0.02% sodium azide solution to dilute the plasma 50/50 v/v. **Keep the diluted plasma sample at -20˚C and do not freeze**. All the remaining plasma aliquots will be stored frozen at -80˚C until ready for testing.

PBMCs

- Any blood remaining in the two 7 mL green-top tubes used to prepare plasma above will be diluted 1:1 with Hank's Balanced Salt Solution ("HBSS", Irvine Scientific, Cat. 9228 or equivalent) and combined with the whole blood from the unused green-top tube in a sterile 50 ml conical centrifuge tube. Peripheral blood mononuclear cells (PBMC) will then be isolated from the combined whole blood sample by Ficoll-gradient separation as described below;
    - Allow Accuspin-Histopaque tubes ("Accuspin", Sigma Cat. A6929 or A0561, for 12 or 100 tubes, respectively) and HBSS to warm to room temperature. Place a Mr. Frosty container in the refrigerator and prepare the freezing media by adding 10% DMSO to fetal calf serum and chill at 4˚C or on ice.
    - Prepare Accuspin tubes by centrifuging at 1000 x g for 1 minute at room temperature (RT) with brakes on. Each tube can process up to 20 mLs of whole blood; prepare the appropriate amount of tubes necessary. After centrifugation, the Histopaque reagent should be below the barrier of the tube. Add 5 mLs of HBSS to the Accuspin tube. Add up to 20 mls of whole blood to each Accuspin tube until all the blood has been distributed.
    - Centrifuge the blood sample at 800 x g for 15 minutes at RT with brakes on LOW. After centrifugation, three layers should be visible above the barrier of the tube: the plasma layer at the top, a cloudy layer in the middle where the

PBMC are, and a clear Histopaque reagent layer right below. Using a pipette, remove the upper plasma layer to within 2 cm of the cloudy interphase. Carefully pipette the cloudy PBMC interphase and transfer to a sterile 50 mL centrifuge tube.

- Add HBSS up to the 45 mL mark in the centrifuge tube with the PBMC and spin at 400 x g for 10 minutes at RT with brakes on. Decant the supernatant and loosen the cell pellet before adding HBSS to the 45-mL mark again for a second wash. Centrifuge at 300 x g for 10 minutes at RT with brakes on. Decant the supernatant, loosen the cell pellet and then add a known volume of HBSS to resuspend the cells for counting. Mix the cell suspension up and down with a pipette several times before removing a small aliquot for cell count.
- Centrifuge the cell suspension one final time at 300 x g for 10 minutes at RT with brakes on. PBMC should be frozen down at 0.5 – 1 x 10^7 cells/vial. Determine the volume of freezing media (fetal calf serum with 10% DMSO) needed to give a 1 x 10^7 cell/mL suspension. After the last centrifugation is complete, discard supernatant and loosen the cell pellet before adding freezing media slowly, a small volume at a time with mixing in between (vortex at low speed). Aliquot 0.5 - 1 mL of the final cell suspension into individually labeled cryovials. Transfer the cryovials into Mr. Frosty and store at -80˚C. Twenty four hours later, cryovials will be transferred to liquid nitrogen tanks for long-term storage.

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