Protocol #: LCI-BRE-H2N-PEPP-001

TITLE: *A PILOT STUDY OF PACLITAXEL PLUS PEMBROLIZUMAB IN PATIENTS WITH METASTATIC HER2-NEGATIVE BREAST CANCER (THE PePPy TRIAL)*

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Investigational drug/device: Pembrolizumab
Commercial agent: Paclitaxel
Supported by: Merck Sharp & Dohme Corp, a subsidiary of Merck & Co., Inc.

Investigational New Drug (IND)#: 132021

The study will be conducted in compliance with the protocol, ICH-GCP and any applicable regulatory requirements.

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PROTOCOL SUMMARY	
A. Study Title	A Pilot Study of Paclitaxel plus Pembrolizumab in Patients with Metastatic HER2-negative Breast Cancer (The PePPy Trial)
B. Indication	Metastatic HER2-negative breast cancer; no more than three prior lines of cytotoxic chemotherapy for metastatic disease
C. Clinical Phase	Phase II
D. Summary of Rationale	Pembrolizumab is a potent and highly selective humanized monoclonal antibody designed to directly block the interaction between PD-1 (programmed death-1) and its ligands, PD-L1 and PD-L2. A strategy of combining chemotherapy with immunotherapy is a promising approach for treatment of HER2-negative breast cancer. There is little known about the optimal sequence of administration of chemotherapy and immunotherapy in breast cancer. This study will evaluate the safety of a **phased regimen**, paclitaxel only for 2 cycles then pembrolizumab and paclitaxel, and a **concurrent regimen**, pembrolizumab given concurrently with paclitaxel upfront.
E. Study Objectives	The primary objective of this study is to assess the safety and feasibility of the two regimens. Secondary objectives are to assess overall survival (OS), progression-free survival (PFS), objective response rate (ORR), duration of response (DoR), and disease control rate (DCR). Exploratory objectives include assessment of PD-L1 status and genomic profile of tumors.
F. Sample	40 evaluable subjects
G. Inclusion/ Exclusion	**Inclusion:** • HER2-negative metastatic breast cancer

	• Age ≥ 18 years • ECOG ≤ 2 • Measurable or evaluable disease • Adequate organ and bone marrow function **Exclusion:** • More than three prior lines of chemotherapy for metastatic disease • Pregnant and/or breastfeeding • Prior therapy with anti PD-1m, anti PD-L1, anti PD-L2, or other co-inhibitory T-cell receptor agent
H. Dosage/ Dosage Form, Route, And Dose Regimen	Pembrolizumab 200 mg IV over 30 minutes, on day 1 every 21 days Paclitaxel 80 mg/m^2 IV over 60 minutes, on days 1 and 8 every 21 days

SCHEMA

LCI-BRE-H2N-PEPP-001: A Pilot Study of Paclitaxel Plus Pembrolizumab in Patients with Metastatic HER2-Negative Breast Cancer (The PePPY Trial)

Sponsor-Investigator: Dr. Antoinette Tan

Objectives

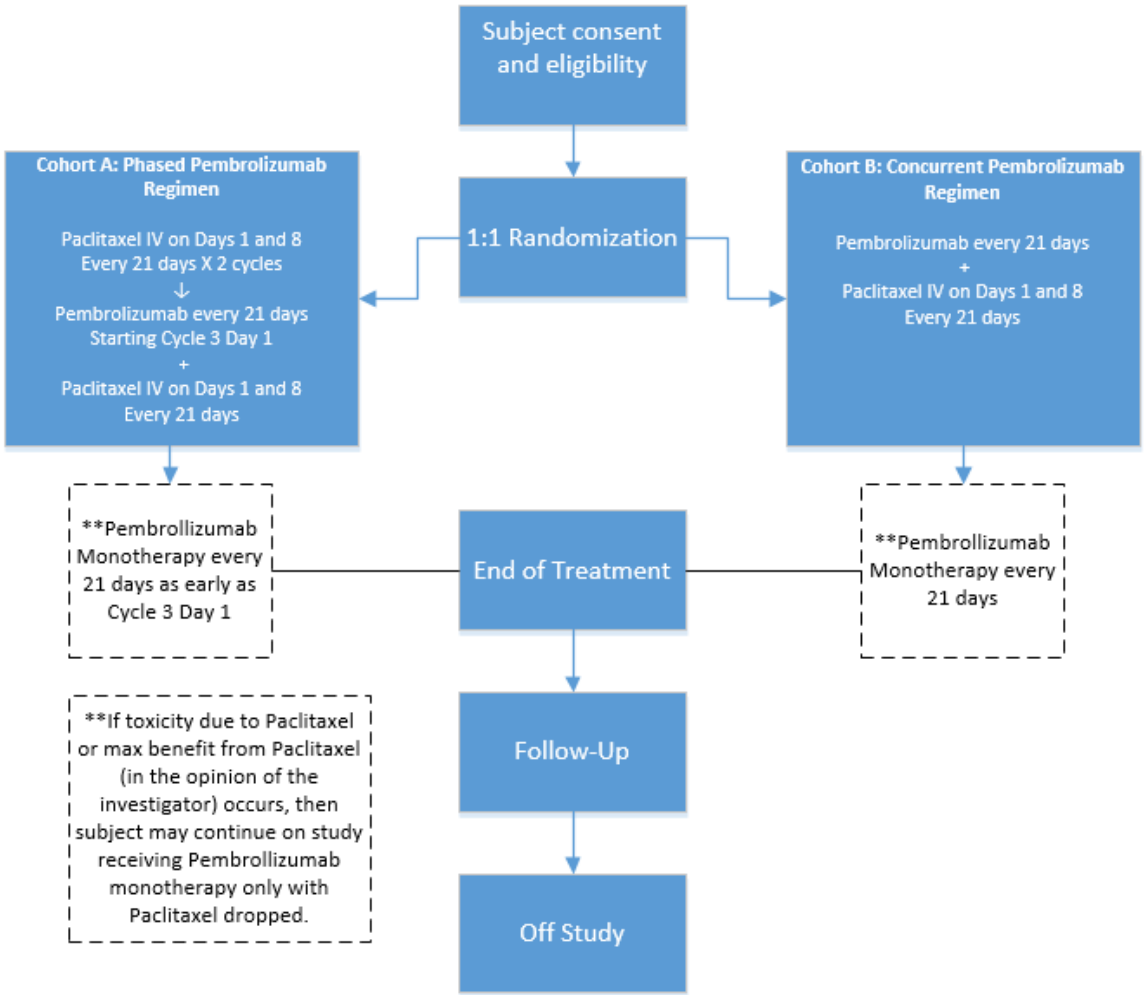
- Primary: to assess the safety and feasibility of the two regimens
- Secondary: to assess overall survival (OS), progression-free survival (PFS) objective response rate (ORR), duration of response (DoR), and disease control rate (DCR).
- Safety: evaluate each cohort in terms of study drug administration, adverse events, serious adverse events including deaths on study, and laboratory parameters
- Exploratory: Assessment of PD-L1 expression, TILs, genomic analyses of the tumor, evaluation of circulating tumor DNA, and collection of biomarkers in blood

Major Inclusion Criteria

- HER2-negative metastatic breast cancer
- Age ≥ 18 years
- ECOG ≤ 2
- Measurable or evaluable disease
- Adequate organ and bone marrow function

Major Exclusion Criteria

- More than three prior lines of chemotherapy for metastatic disease
- Women who are pregnant or breastfeeding
- Prior therapy with anti PD-1m anti PD-L1, anti PD-L2, or other co-inhibitory T-cell receptor agent



LIST OF ABBREVIATIONS

Abbreviation	*Spelled out abbreviation. Listed in alphabetical order.*
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
BRAF	B-Raf proto-oncogene
CTCAE	Common terminology criteria for adverse events
CTLA-4	Cytotoxic T-lymphocyte associated protein 4
CrCL	Creatinine clearance
CNS	Central nervous system
CTMS	Clinical trials management system
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
ECIs	Events of clinical interest
eCRFs	Electronic case report forms
ER	Estrogen receptor
GFR	Glomerular filtration rate
HER2	Human epidermal growth factor receptor type 2
HIV	Human immunodeficiency virus
Ig	Immunoglobulin
ITSM	Immunoreceptor tyrosine-based switch motif
IV	Intravenous
LCI	Levine Cancer Institute
mAb	Monoclonal antibody
MBC	Metastatic breast cancer
MTD	Maximum tolerated dose
NE	Not evaluable

NSCLC	Non-small cell lung cancer
ORR	Objective response rate
OS	Overall survival
PD-1	Programmed death 1
PD-L1	Programmed death ligand 1
PFS	Progression free survival
PK	Pharmacokinetic
PO	By mouth; taken orally
PR	Progesterone receptor
Q2W	Every 2 weeks
Q3W	Every 3 weeks
RECIST	Response evaluation criteria in solid tumors
SCLC	Small cell lung cancer
SHP-1, SHP-2	Src-Homology 2 domain containing phosphatase-1 and -2
TAMs	Tissue associated macrophages
TILs	Tissue infiltrating lymphocyte
TNBC	Triple-negative breast cancer
Tregs	Regulatory T cells
ULN	Upper limit of normal
v-type	Variable-type
WOCBP	Women of childbearing potential

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1. OBJECTIVES

1.1. Primary Objective

The primary objective of this study is to assess the safety and feasibility of the following two regimens: Cohort A) phased regimen of pembrolizumab in which paclitaxel is followed by paclitaxel plus pembrolizumab and Cohort B) concurrent regimen of paclitaxel plus pembrolizumab. The primary safety objective is to evaluate the overall grade 3 or 4 treatment-related adverse event rate for each cohort and compare them to relevant historical controls.

1.2. Secondary Objectives

Secondary objectives are to assess overall survival (OS), progression-free survival (PFS), objective response rate (ORR) duration of response (DoR), and disease control rate (DCR). PFS, ORR, DoR, and DCR will be evaluated using RECIST 1.1 criteria.

1.3. Safety Objectives

The safety objectives are to evaluate each cohort in terms of study drug administration, adverse events assessed by NCI Common Terminology Criteria for Adverse Events version 4.0, serious adverse events including deaths on study, and laboratory parameters.

1.4. Exploratory Objectives

Exploratory objectives include

- Assessment of PFS, ORR, DoR, and DCR using the immune-related RECIST (irRECIST) criteria
- Assessment of tumor-associated PD-L1 (programmed death-ligand 1) expression
- Assessment of tissue infiltrating lymphocytes (TILs) in tumor
- Genomic analyses of the tumor
- Evaluation of circulating tumor DNA
- Collection of biomarkers in blood, including but not limited to cytokines
- Development of a patient derived xenograft model if sufficient tumor is available

2. BACKGROUND AND RATIONALE

2.1. Metastatic HER2-negative Breast Cancer

An estimated 231,840 new cases of breast cancer are expected to be diagnosed in the United States during 2015 and it is estimated that there will be 40,730 deaths from breast cancer.[1] Single agent or combination chemotherapy produces response rates of 40-70% in the treatment of advanced breast cancer. Although numerous regimens are effective in palliating symptoms and inducing tumor regression, the complete response rate is less than 20%. Moreover, the median duration of response is nine months with a median survival of 12-36 months. Most responses are of limited duration and nearly all patients with stage IV disease eventually die of disease progression. Therefore, the treatment of patients with metastatic human epidermal growth factor receptor type 2 (HER2)-negative breast cancer warrants investigation of novel therapeutic strategies.

The treatment algorithm for patients with metastatic breast cancer (MBC) is based on several factors which include clinical, pathologic, and histologic characteristics such as the presence or absence of human epidermal growth factor 2 amplification, hormone receptor status, prior response to endocrine therapy, disease extent and location of metastatic disease, pace of disease progression, and time to relapse since completion of adjuvant chemotherapy. While endocrine therapy is the preferred initial treatment for patients with hormone receptor-positive disease, cytotoxic chemotherapy becomes appropriate for those with hormone refractory disease or rapidly progressive disease to achieve a rapid rate of response. For patients with triple-negative breast cancer, or breast cancer that is estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative, chemotherapy at present is the mainstay of treatment.

2.2. Taxanes in Breast Cancer

Taxanes are among the most active of chemotherapy agents for the treatment of MBC in terms of response rates or survival, based on a metaanalysis of three randomized trials comparing a taxane- with an anthracycline.[2] The role of paclitaxel in the treatment of breast cancer has been well established in both the adjuvant and metastatic settings. The response rates for paclitaxel administered as a single agent to patients with MBC are approximately 25% in firstline- treatment.[3,4]

The most common practice is to administer paclitaxel on a weekly schedule based on the results of a 2010 meta-analysis, which showed that compared with every three-week treatment, weekly administration of paclitaxel resulted in an improvement in overall survival (OS, hazard ratio [HR] 0.78, 95% CI 0.67-0.89).[6] Allergic reactions are a potential side effect of treatment with paclitaxel as a result of the composition of paclitaxel, which is mixed with Cremophor. At most institutions, steroid premedication (IV dexamethasone 8-12 mg 30 minutes prior to the paclitaxel dose) is administered, although it can usually be discontinued if the first two or three doses are tolerated.

2.3. Programmed Death-1 (PD-1) Checkpoint Inhibitor as a Strategy for Treatment of Breast Cancer

Encouraging clinical data emerging in the field of tumor immunotherapy have demonstrated that therapies focused on enhancing Tcell- responses against cancer can result in a significant survival benefit in patients with advanced malignancies.[7-9]

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control.[10] 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 is an immunoglobulin (Ig) superfamily member related to CD28 and Cytotoxic T-Lymphocyte Associated Protein 4 (CTLA-4), which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). 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.

Following T cell stimulation, PD-1 recruits the tyrosine phosphatases Src-Homology 2 domain-containing phosphatase-1 and -2 (SHP-1 and SHP-2) to the ITSM within its cytoplasmic tail, leading to the dephosphorylation of effector molecules, such as CD3ζ, Protein Kinase C theta (PKCθ) and Zeta-chain-Associated Protein kinase 70 (ZAP70), which are involved in the -Homology 2 domain-containing phosphatase-1 and -2 (SHP-1 and SHP-2) to the ITSM within its cytoplasmic tail, leading to the dephosphorylation of effector molecules, such as CD3ζ, Protein Kinase C theta (PKCθ) and Zeta-chain-Associated Protein kinase 70 (ZAP70), which are involved in the CD3 T cell signaling cascade. PD-1 was shown to be expressed on activated lymphocytes, including peripheral CD4+ and CD8+ T cells, B cells, regulatory T cells (Tregs) and Natural Killer (NK) cells.[12] CD3 T cell signaling cascade. PD-1 was shown to be expressed on activated lymphocytes, including peripheral CD4+ and CD8+ T cells, B cells, regulatory T cells (Tregs) and Natural Killer (NK) cells.

The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types. 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.[13]

Binding of either PD-1 ligand to PD-1 inhibits T cell activation triggered through the T cell receptor. 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, which, via its interaction with the PD-1 receptor on

tumor-specific T cells, plays a critical role in immune evasion by tumors.[14] As a consequence, the PD-1/PD-L1 pathway is an attractive target for therapeutic intervention in cancer.[15]

The immune system plays an important and complex role in the biology of breast cancer. There is preclinical and clinical evidence suggesting that immunotherapy has the potential to improve clinical outcomes for patients with breast cancer. Immune modulatory agents have several unique features that make them attractive to combine with standard cytotoxic agents, and includes the observation that cancer immunotherapies cause fewer side effects, enabling them to be given for longer periods of time and in combination with other agents without added toxicity. There also is potentially less likelihood to develop resistance to immunotherapy because of the immune system's ability to simultaneously target multiple cancer antigens and adapt to mutating cancer cells.

2.4. Pembrolizumab

Pembrolizumab (Keytruda®) 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. This blockade enhances functional activity of the target lymphocytes to facilitate tumor regression and ultimately immune rejection.

2.4.1. Nonclinical Studies

The nonclinical strategy of the pembrolizumab program was to demonstrate in vitro and in vivo activity, to determine in vivo pharmacokinetic (PK) behavior, to demonstrate an acceptable safety profile, and to identify a Phase I starting dose. Comprehensive pharmacology, PK, and toxicology evaluations were thus undertaken with pembrolizumab.

The safety, pharmacokinetics, and toxicokinetics of pembrolizumab were investigated in mice and cynomolgus monkeys to support IV administration and to aid in projecting the appropriate starting dose in humans. Given the high amino acid homology of the extracellular regions of PD-1 between cynomolgus monkey and human, the cynomolgus monkey was selected as the primary and relevant nonclinical model for understanding the toxicology of pembrolizumab.

Overall, the nonclinical pharmacokinetics and toxicokinetics observed for pembrolizumab supported entry into clinical studies, including providing adequate safety factors for the proposed Phase I starting doses. The results of the preclinical program were consistent with the anticipated activity of pembrolizumab to enhance the functional activity of tumor-infiltrating lymphocytes to induce tumor regression and ultimately immune rejection by blocking the interaction between PD-1 and its ligands, PD-L1 and

PD-L2 and supported entry into clinical trials in patients. Refer to the pembrolizumab Investigator's Brochure for details on the nonclinical studies.[16]

2.4.2. Clinical Studies – Approval of Pembrolizumab

Pembrolizumab was approved in the United States on September 4, 2014 by the FDA for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor. This indication is approved under accelerated approval based on tumor response rate and durability of response.

Approval was based on the results of a multicenter, open-label, randomized (1:1), dose-comparative, activity-estimating cohort conducted within Trial PN001, which was the first-in human dose cohort escalation study of pembrolizumab that enrolled subjects into 4 parts (Parts A-D, A = any advanced solid tumor, B = melanoma, C = NSCLC, and D = melanoma, 2 doses of pembrolizumab).

In this cohort, 173 patients with unresectable or metastatic melanoma with disease progression within 24 weeks of the last dose of ipilimumab and, if BRAF V600 mutation positive, prior treatment with a BRAF inhibitor, were randomized to receive pembrolizumab 2 mg/kg (n=89) or 10 mg/kg (n=84) intravenously once every 3 weeks until disease progression or unacceptable toxicity.

Key exclusion criteria were an autoimmune disease, a medical condition that required immunosuppression, and/or a history of severe immune-mediated adverse reactions from treatment with ipilimumab. Severe immune-mediated adverse reactions were defined as any CTCAE Grade 4 toxicity requiring treatment with corticosteroids or Grade 3 toxicity requiring corticosteroid treatment (greater than 10 mg/day prednisone or equivalent dose) for greater than 12 weeks.

The major efficacy endpoints were confirmed objective response rate (ORR) according to Response Evaluation Criteria in Solid Tumors (RECIST v1.1) as assessed by a blinded independent review committee and duration of response (DOR). In the lower dose that was ultimately approved, 2 mg/kg, the ORR was 26% (95% CI, 17-37%). A total of 21 patients showed a response, including one patient with a complete response. Three of the patients (14%) had disease progression at 2.8 months, 2.9 months, and 8.2 months after the initial response. The other 18 patients (86%) showed durable responses of 1.4 to 8.5 months. Of note, eight patients (38%) had ongoing responses of ≥ 6 months. Similar ORR results were observed in the 10 mg/kg arm.[17] Treatment was well tolerated, with similar safety profiles in the 2 mg/kg and 10 mg/kg groups and no drug-related deaths. The most common drug-related adverse events of any grade in the 2 mg/kg and 10 mg/kg groups were fatigue (33% vs 37%), pruritus (26% vs 19%), and rash (18% vs 18%). Grade 3 fatigue, reported in five (3%) patients in the 2 mg/kg pembrolizumab group, was the only drug-related grade 3 to 4 adverse event reported in more than one patient.

Additionally, two multicenter, randomized, controlled therapeutic confirmatory trials in patients with unresectable or metastatic melanoma, ipilimumab refractory (KEYNOTE-002/Trial P002) and ipilimumab-naive (KEYNOTE-006/Trial P006), evaluated co-primary endpoints of progression-free survival and overall survival, and have results.

In the KEYNOTE-002 trial, 540 patients with ipilimumab-refractory advanced melanoma were randomly assigned to pembrolizumab at 2 mg/kg (n = 180), 10 mg/kg (n = 181), or chemotherapy (n = 179).[18] Chemotherapy was selected by investigators and consisted primarily of paclitaxel plus carboplatin, paclitaxel, carboplatin, dacarbazine, or temozolomide. Pembrolizumab was administered every 3 weeks. Both pembrolizumab arms were significantly superior to chemotherapy (P <.0001). At the recommended 2-mg/kg dose, pembrolizumab demonstrated a PFS of 34% at 6 months and 24% at 9 months. In the chemotherapy arm, the PFS was 16% and 8%, at the 6- and 9-month analyses, respectively.

At the 2-mg/kg dose, the ORR with pembrolizumab was 21% with a median duration of response that was not yet reached. The ORR in the chemotherapy arm was 4%, the median response duration was 37 weeks. At the analysis, 63% of responses were ongoing. Treatment was relatively well tolerated, with grade 3-5 adverse events reported in 11 and 14 percent of the pembrolizumab treatment arms, and 26 percent of those managed with chemotherapy. The most common pembrolizumab-related adverse events were fatigue, pruritus, and rash. Grade 3 immune related toxicity was reported in two patients treated with pembrolizumab 2 mg/kg (hepatitis, hypophysitis), and in eight patients given pembrolizumab 10 mg/kg (hepatitis, colitis, pneumonitis, and iritis or uveitis).

In the phase III KEYNOTE-006 trial, pembrolizumab demonstrated significantly longer PFS and improved overall survival compared with ipilimumab as immunotherapy in patients with advanced melanoma.[19] This trial included 834 patients with unresectable stage III or IV advanced melanoma who had received no more than one prior systemic therapy. Patients were randomized to receive four cycles of ipilimumab at 3 mg/kg every 3 weeks (n = 278), 10 mg/kg of pembrolizumab every 3 weeks (n = 277), or 10 mg/kg of pembrolizumab every 2 weeks (n = 279). Patient response in KEYNOTE-006 was assessed at week 12 and every 6 weeks thereafter by RECIST 1.1. Median follow-up was 8 months. Both doses of pembrolizumab were found to be superior to ipilimumab. At a 6-month assessment, the PFS with pembrolizumab was 47% and 46% in the 2- and 3-week arms respectively. For ipilimumab, the PFS rate was 27%. At 9 months, PFS rates were 40% and 42% compared with 16%, in the 2-week, 3-week, and ipilimumab arms, respectively. In the 3-week pembrolizumab arm, the 1-year OS rate was 68% compared with 58% for ipilimumab (HR, 0.69). In the 2-week arm, the 1-year OS rate was 74% (HR, 0.63). The objective response rates (ORR) were 33.7% and 32.9%, in the 2- and 3-week arms. In the ipilimumab arm, the ORR was 11.9%. The toxicity profiles of the agents were consistent with other reported studies of the two checkpoint agents. Though pembrolizumab was administered for a longer duration, rates of grade 3-5 adverse events were numerically lower than in the ipilimumab arm (11.7% vs 19.9%).There were no differences in efficacy parameters in any patient subsets, with the exception of a lack of

improvement in overall survival associated with pembrolizumab in those patients whose tumors did not express PD-L1 using the Merck immunohistochemistry assay. The PFS, overall survival, response rates, and side effects profiles were fully consistent with those seen in earlier trials with both pembrolizumab and ipilimumab.

These trials demonstrate the efficacy of pembrolizumab in the treatment of advanced melanoma. Ongoing clinical studies are being conducted in a number of other advanced solid tumor indications, including breast cancer.

2.4.3. Clinical Studies in Breast Cancer with Pembrolizumab

In the first report of clinical activity of an immune checkpoint inhibitor in metastatic breast cancer, a multi-center, non-randomized phase Ib trial (Keynote 012) showed that single-agent pembrolizumab given at 10 mg/kg Q2W is a well-tolerated and effective treatment with significant therapeutic activity in a subset of heavily pre-treated patients with metastatic triple-negative breast cancer (TNBC) [20].

The phase Ib study enrolled 32 patients with TNBC who had recurrent or metastatic disease (47% of which had more than three lines of previous chemotherapy) with PD-L1 expression in their tumor (58% of all patients screened had PD-L1- positive tumors.). Pembrolizumab was administered 10 mg/kg intravenously every 2 weeks, and treatment could continue indefinitely as long as patients were stable and their disease was not clearly progressing as assessed by RECIST v1.1 every 8 weeks. Treatment with PD-1 blockade was tolerable, with 56% of patients reporting an adverse event, but only 16% with grade 3–5 toxicity. There was one treatment-related death caused by disseminated intravascular coagulation. The ORR was 19% (27 patients were evaluable for response) with one (4%) complete response, four (15%) partial responses, and seven patients (26%) with stable disease. Of note, the median time to response was 18 weeks (range, 7 to 32).

There is an international, multi-center phase II clinical trial of pembrolizumab as monotherapy that is currently enrolling patients with metastatic triple-negative breast cancer (Keynote 086; NCT02447003). The results of this trial and others that are evaluating checkpoint inhibitors in the treatment of metastatic breast cancer will help further the clinical development of immunotherapeutic agents in this breast cancer subtype.

2.4.4. Rationale for Flat Dosing of Pembrolizumab

An open-label Phase I trial (Protocol 001) has been conducted to evaluate the safety and clinical activity of single agent pembrolizumab. The dose escalation portion of that 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

pembrolizumab showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD has been identified to date. Recent data from other clinical studies within the pembrolizumab program has shown that a lower dose of pembrolizumab and a less frequent schedule may be sufficient for target engagement and clinical activity. The currently approved dose of pembrolizumab is 2 mg/kg IV over 30 minutes every 3 weeks.

PK data analysis of pembrolizumab administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life[16]. 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 evaluating a 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 pembrolizumab were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. Pembrolizumab has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg Q3W body weight based regimen are anticipated to remain well within the established exposure margins of 0.5 – 5.0 for pembrolizumab in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. the proposed dose regimen of 2 mg/kg Q3W (i.e. 5-fold higher dose and exposure). The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, we do not anticipate changes in exposure between different indication settings.

The choice of the 200 mg Q3W dose of pembrolizumab (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 patient's 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.

2.5. Chemotherapy in Combination with Immunotherapy

Although standard-dose chemotherapy was conventionally thought to suppress the immune system directly by decreasing lymphocyte numbers and limiting the expansion of activated lymphocytes, it has been demonstrated that chemotherapy also has the capacity to be leveraged effectively to potentially augment cancer immunity in several ways. For example, chemotherapy can decrease tumor burden, generate immunogenic cell death and can interrupt mechanisms of immune tolerance.[21]

Paclitaxel is a common first-line regimen for the treatment of metastatic HER2-negative metastatic breast cancer.[22] Paclitaxel therapy has been shown to increase tumor-associated macrophages (TAMs) infiltration in breast tumors.[23] Paclitaxel has also been shown to upregulate PD-L1 expression in breast cancer cells, contributing to immune resistance.[24] Combining pembrolizumab, a human monoclonal immunoglobulin G4 antibody which binds to PD-1, with paclitaxel in HER2-negative breast cancer could be synergistic and potentiate the effect of chemotherapy via an anti-tumor immune response. In designing combination immunotherapy regimens, consideration needs to be given with regards to how chemotherapy impacts the immune system in order to guide the dose and schedule for combining chemotherapy and immunotherapy. The optimal drug dose and timing in relation to immune-based therapy in early-phase clinical studies is necessary to help inform the design of future phase II and III clinical trials.

The cytotoxic effects of paclitaxel are driven primarily by disturbing the microtubule dynamics and inducing apoptosis. In addition to the cytotoxic effect on tumor cells, paclitaxel has been reported to modulate the host immune system in a number of ways.[21] Regulatory T cells, which down-regulate immune effector activity, have been shown to be increased in the peripheral blood of patients with pancreatic, lung and breast cancer, potentially suppressing an anti-tumor response.[25]

Paclitaxel therapy has demonstrated the ability to induce apoptosis and impair these T regulatory cells (potential drivers of peripheral tolerance) preferentially, but not effector T cells (potential drivers or effectors of tumor-cell killing), which could potentially shift the balance to anti-tumor immune activation. In addition, immune activation in lung cancer patients after paclitaxel treatment was reported and the generation of pro-inflammatory cytokines IFN-γ and IL-2, as well as the expression of T-cell activation markers was increased in lung cancer patients after paclitaxel treatment.[24]

Consistent with this observation, paclitaxel has been shown to also induce macrophage activation and release of inflammatory cytokines in mouse models.[26] The perpetuation of an effective anti-tumor response requires a predominantly effector T-cell activation and further expansion, as well as pro-inflammatory cytokine release, which supports cytotoxic T-cell activation and a potential anti-tumor effect. This immune activation process may be subsequently attenuated and reversed by PD-1 expression on activated T cells and the PD-L1 on tumors, conversely supporting T regulation and suppressing and eliminating cytotoxic T effectors. In fact, paclitaxel has also been show to upregulate PD-L1 expression, ultimately reversing its potential tumor immune activation.[24] PD-1 expression is induced when T cells are activated.[27] An important role of PD-1 is to limit T cell effector activation and enhance T regulatory mechanisms.[28]

Pembrolizumab blocks the binding of PD-1 expressed on immune cells to its ligand (PD-L1) expressed in tumors, blocking of which potentially reverses down-regulation of cellular immunity to the tumor. Pembrolizumab has demonstrated promising anti-tumor activity in a variety of tumors, including known immunogenic tumors (i.e. melanoma), as well as tumors previously considered less immunogenic (i.e., NSCLC). Chemotherapy in combination with anti-PD-1 has demonstrated enhanced activity against several mouse tumor models (Pembrolizumab IB) and clinical results support this combination approach.[29]

When combining immunotherapy with chemotherapy, it is important to determine the optimal dose, schedule and sequence. There is some data on the optimal timing and dose of immunotherapy with chemotherapy. Results from two multicenter phase II lung cancer trials showed that a “phased regimen” in which immunotherapy begins after chemotherapy resulted in improved progression-free survival, compared with chemotherapy alone.

Combining ipilimumab with paclitaxel and carboplatin, significantly improved immune-related progression-free survival (irPFS) compared with chemotherapy alone in a phase II study in patients with NSCLC and extensive disease SCLC.[29,30] However, the improvement in irPFS was only evident when the drugs were given on a phased schedule (e.g., two doses of placebo plus paclitaxel/carboplatin followed by four doses of ipilimumab plus paclitaxel/carboplatin), not when they were given concurrently. Phased ipilimumab, concurrent ipilimumab, and control, respectively, were associated with median irPFS of 5.7, 5.5, and 4.6 months in patients with NSCLC, and 6.4, 5.7, and 5.3 months in patients with SCLC. The overall incidence of treatment-related grade 3/4 adverse events was similar across the arms, and ipilimumab did not appear to exacerbate the adverse events associated with chemotherapy.

There is no data with regards to the sequencing of pembrolizumab in relation to chemotherapy in the treatment of breast cancer. This pilot study will primarily assess the safety of dose and dosing schedule of pembrolizumab with the specific chemotherapy agent, paclitaxel. The tolerability will be assessed and the anti-tumor activity of two dosing schedules will be explored. Biomarkers studies to assess immune effects will also be included. Two schedules will be explored. In Cohort A (phased), two cycles of paclitaxel followed by paclitaxel and pembrolizumab with the rationale that giving paclitaxel before pembrolizumab, will allow antigen release to occur, provide an initial tumor response, debulk the tumor, change the tumor's immune microenvironment, and enhance pembrolizumab's effect. For the exploratory comparison of Cohort B (concurrent), paclitaxel in combination with pembrolizumab starting at cycle 1 will be assessed, allowing pembrolizumab to be present at the earliest phase of chemotherapy-induced antigen release, and a schedule that is consistent with several treatment schedules already reported in ongoing trials with other cytotoxic therapy.

2.5.1. Immunotherapy and Steroids

Corticosteroids have nonspecific anti-inflammatory activity and can attenuate immunity.[31] In general, minimal steroid exposure is preferred when being treated with immunotherapy. Corticosteroid premedications are administered before paclitaxel to decrease the hypersensitivity reactions that can occur, possibly a reaction to the vehicle Cremophor EL, a derivative of castor oil. Grade 2 or higher hypersensitivity reactions have been reported to occur at an incidence of less than 3% with weekly paclitaxel at 80 mg/m^2 given as a 1-hour infusion.[32] An 8 to 12 mg dose of IV dexamethasone pre-medication given 30 to 60 minutes prior to paclitaxel is a typical dose given to reduce the incidence and severity of reactions.[33]

Infusion hypersensitivity reactions beyond the second dose are not common. A retrospective study evaluated 449 patients receiving paclitaxel-based chemotherapy for the treatment of breast cancer during January 2011 to June 2013. After receiving the first two doses of paclitaxel-based chemotherapy without experiencing an infusion hypersensitivity reaction, 234 breast cancer patients had their premedications discontinued for all remaining paclitaxel doses. These patients tolerated future paclitaxel doses without severe or life-threatening complications related to infusion hypersensitivity. The majority of patients did not have any symptoms of an infusion reaction, with only two of these patients requiring rescue medication to treat an infusion hypersensitivity reaction with subsequent paclitaxel doses (0.85; 95 % confidence interval (CI), 0.10-3.05 %). Discontinuation of paclitaxel premedications in breast cancer patients who have not experienced an infusion hypersensitivity reaction with the first two doses of paclitaxel is not associated with increased rate of rescue medication use for infusion hypersensitivity.[34]

To reduce prolonged corticosteroid exposure in this trial where immunotherapy is being combined with paclitaxel, it would be reasonable to taper the steroids after the first cycle of chemotherapy. In this trial, dexamethasone 8 mg IV will be given 30 to 60 minutes before each paclitaxel administration during cycle 1. If tolerated, dexamethasone should then be tapered to 4 mg at Cycle 2 and discontinued at Cycle 3 for subsequent infusions.[35] In the event of a future infusion reaction, steroid pre-medication can be added back into the regimen as clinically indicated.

2.6. Study Rationale

The optimal chemotherapy schedule, dose and timing of combination or sequence with pembrolizumab have not been clearly elucidated. Weekly paclitaxel is a mainstay for the adjuvant and neoadjuvant treatment of breast cancer. Combining pembrolizumab with paclitaxel in the treatment of HER2-negative metastatic breast cancer could demonstrate synergy and potentiate the effect of chemotherapy by unleashing an anti-tumor immune response.

This study will assess the feasibility and safety of phased vs concurrent dosing sequences of pembrolizumab with the combination of standard paclitaxel chemotherapy. It is important to determine the dosing, safety, and feasibility of giving weekly paclitaxel and pembrolizumab so that future trials may test the activity of this two-drug regimen in patients with early-stage and/or locally advanced breast cancer.

2.6.1. Safety Endpoints

The primary safety objective of this trial is to characterize the safety and tolerability of pembrolizumab and paclitaxel (phased vs concurrent) in subjects with HER2-negative metastatic breast cancer. The primary safety analysis will be based on subjects who experienced toxicities as defined by CTCAE, Version 4.0 criteria. The attribution to study drug(s), time-of-onset, duration of the event, its resolution, and any concomitant medications administered will be recorded. AEs will be analyzed including but not limited to all AEs, SAEs, fatal AEs, and laboratory changes.

2.6.2. Rationale for Use of irRECIST Criteria

Immunotherapeutic agents may produce antitumor effects by potentiating endogenous cancer-specific immune responses, which may be functionally anergic prior to treatment. The response patterns seen with such an approach may extend beyond the typical time course of responses seen with cytotoxic agents, and can manifest as a clinical response after an initial increase in tumor burden or even the appearance of new lesions (transient tumor flare). Thus, standard RECIST criteria may not provide a complete response

assessment of immunotherapeutic agents, such as pembrolizumab. To address this issue, RECIST 1.1 with the adaptation outlined in Table 10, termed irRECIST, will also be used after the subject's first administration of pembrolizumab.

3. PATIENT SELECTION

3.1. Accrual

A total of 40 evaluable subjects will be enrolled over an enrollment period of up to 36 months. The study is planned to enroll approximately 20 evaluable subjects in each study treatment cohort. Evaluable subjects are those who are randomized and begin study therapy. Patients will be recruited through Levine Cancer Institute locations and through referrals. Women or men of any race or ethnic origin who meet the study criteria may participate in this clinical trial. Children are not included in this clinical trial because the effects of pembrolizumab are not known in the pediatric population, but may be eligible for other pediatric trials.

3.2. Inclusion Criteria

Subjects must meet all of the following criteria:

1. Histologically or cytological confirmed diagnosis of HER2-negative metastatic breast cancer or locally advanced disease not amenable to resection.
    - Available ER and PR status from tumor sample with either hormone receptor positive or negative tumor(s).
    - For subjects with hormone receptor-positive, HER2-negative metastatic breast cancer, they are eligible if they have already received or been intolerant to at least two lines of endocrine therapies (including the adjuvant and/or metastatic setting), or are appropriate candidates for chemotherapy (i.e. large burden of visceral disease).

2. Measurable disease by RECIST 1.1, or evaluable bone disease, i.e., bone lesions that are lytic or mixed (i.e. lytic + sclerotic) in the absence of measurable lesion. Refer to section 11 for the evaluation of measurable disease.
3. Male or female age ≥18 years.
4. ECOG performance status 0, 1 or 2.
5. Must have normal organ and marrow function as defined below:

    - **Hematologic**
        - Absolute neutrophil count ≥1,500/mcL
        - Platelets ≥75,000/mcL
        - Hemoglobin ≥ 9 g/dL

- **Renal**
  - Creatinine ≤ 1.5X ULN or
  - Measured or calculated creatinine clearance (CrCl) ≥ 30 mL/min for subject with creatinine levels > 1.5X ULN [CrCl should be calculated per institutional standard; GFR can also be used in place of creatinine or CrCl]
- **Hepatic**
  - Total bilirubin ≤1.5X ULN *or* for subjects with total bilirubin levels >1.5X ULN, direct bilirubin ≤ULN
  - AST(SGOT)/ALT(SGPT) ≤2.5X ULN
- **Coagulation**
  - PT and PTT ≤ 1.5X ULN; subjects receiving anticoagulant therapy are eligible if PT or PTT is within therapeutic range of intended use of anticoagulants per investigator discretion.
  - INR ≤ 1.5; Patients receiving anticoagulant therapy are eligible if their INR is stable and within the recommended range for the desired level of anticoagulation per investigator discretion.

6. Female subjects of childbearing potential must have a negative serum or urine pregnancy test within 14 days prior to receiving C1D1.
7. Female subjects of childbearing potential must be willing to use an adequate method of birth control as outlined in Section 8.1.10, be surgically sterile, or abstain from heterosexual activity for the course of the study and 120 days after the last dose of study therapy. Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year. Male subjects of reproductive potential should agree to use an adequate method of contraception starting with the first dose of study therapy and 120 days after the last dose of study therapy. Abstinence is acceptable if this is the established and preferred contraception for the subject.
8. Has completed the screening requirement of a core or punch biopsy of a tumor lesion per Section 5 (bone tissue not acceptable). Biopsy of the breast tumor or other regional areas is acceptable (i.e. lymph nodes, skin lesions).
   - Subjects who are unable to meet the screening requirement of a tumor biopsy due to inaccessible tumor, subject safety concern, or bone-only disease may submit an archived tumor specimen from primary tumor or metastatic biopsy collected within 12 months from consent.
   - Subjects who decline tumor biopsy may submit archived tumor specimen as specified above (within 12 months from consent) only after Sponsor-Investigator approval.
9. Ability to understand and the willingness to sign the written informed consent document.

3.3. Exclusion Criteria

Subjects must not meet any of the following criteria:

1. Prior chemotherapy within 3 weeks, prior targeted small molecule therapy or radiation therapy within 2 weeks, or prior anti-cancer monoclonal antibody (mAb) for direct anti-neoplastic treatment within 4 weeks prior to Cycle 1 Day 1.
2. Not recovered (i.e., ≤ Grade 1) from adverse events due to agents previously administered.
    - **Note: Subjects with ≤ Grade 2 neuropathy or alopecia of any grade are an exception and may qualify for the study.**
3. More than three prior lines of chemotherapy for HER2-negative metastatic disease or for locally advanced disease that is not amenable to resection.
    - Note: Non-Chemotherapy regimens do not count as prior lines (ex: hormonals, biologics)
4. Has received prior therapy with an anti-PD-1, anti-PD-L1, anti-PD-L2 agent or with an agent directed to another co-inhibitory T-cell receptor (e.g. CTLA-4, OX-40, CD137) or has participated in Merck MK-3475 trial(s) and received MK-3475 as part of protocol therapy.
5. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to Cycle 1 Day 1
6. Has had major surgery within 3 weeks prior to Cycle 1 Day 1.
7. Has received any other investigational agents within 4 weeks of Cycle 1 Day 1 of study therapy.
8. Known active uncontrolled or symptomatic central nervous system (CNS) metastases and/or carcinomatous meningitis as indicated by clinical symptoms, cerebral edema, and/or progressive growth.
    - Note: Subjects with treated CNS metastases are eligible if they are asymptomatic, have no requirement for steroids, no requirement for anticonvulsants, and stable CNS radiographic study showing no significant vasogenic edema ≥ 4 weeks since completion of radiation and ≥ 2 weeks since discontinuation of steroids.
9. History of known allergic reaction to paclitaxel
10. Has 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.

11. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the screening visit through 120 days after the last dose of study treatment. Pregnant women are excluded from this study because paclitaxel is an agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with paclitaxel, breastfeeding should be discontinued if the mother is treated with paclitaxel. These potential risks also apply to pembrolizumab being used in this study.
12. Has a known history of Human Immunodeficiency Virus (HIV) or known acquired immunodeficiency disorder (AIDS). HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with paclitaxel and pembrolizumab. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy.
13. Has known active infection with hepatitis B or hepatitis C.
14. Has an active autoimmune disease that has required systemic treatment in past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs).
    - Replacement therapy (i.e., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.
15. Has a known additional malignancy that progressed or required active treatment within the last 5 years.
    - Note: Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin that has undergone potentially curative therapy, or in situ cervical cancer.
16. Has a history of (non-infectious) pneumonitis that required steroids or current pneumonitis.
17. Has an active infection requiring systemic therapy.
18. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
19. Has received a live vaccine within 30 days of Cycle 1 Day 1 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.

4. INVESTIGATIONAL PLAN

4.1. Milestone Date Definitions

Eligibility date: the date of the last documented criterion that confirmed subject eligibility.
Enrollment date: the date subject is randomized to one of the study treatment arms.
Treatment discontinuation date: Date investigator decides to discontinue subject from study treatment.

- For subjects who **have** received pembrolizumab study treatment, this date is the date the investigator decides to discontinue subject from pembrolizumab.
- For subjects in cohort A who **have not** yet received pembrolizumab, this date is the date the investigator decides to discontinue subject from paclitaxel.

4.2. Overall Study Design

This is an open-label, randomized pilot study designed to evaluate the safety and feasibility of the following two regimens: Cohort A) phased regimen of pembrolizumab in which paclitaxel is followed by paclitaxel plus pembrolizumab and Cohort B) concurrent regimen of paclitaxel plus pembrolizumab. Following informed consent and eligibility check, subjects will be randomized to either Cohort A or Cohort B.

Data from this study will be collected on electronic case report forms (eCRFs) and stored in the CTMS.

4.3. Randomization/Registration/Enrollment

Following informed consent and eligibility check per standard operating procedures, subjects will be randomized in a 1:1 fashion to either Cohort A (phased) or the Cohort B (concurrent) and assigned a Study ID number. This will be accomplished utilizing the CTMS whereby a list of Study IDs and associated study treatment arm assignments randomly generated prior to study activation will be uploaded by a member of the Levine Cancer Institute Biostatistics Core. The Study ID will be a four digit randomly generated ID number, ranging from 0001 to 9999. A stratified block randomization will be utilized including hormone receptor status (positive versus negative) to reduce confounding of comparisons between the study treatment arms.

4.4. Pre-Treatment

4.4.1. Informed Consent

No protocol-related assessments may be performed prior to obtaining informed consent. Baseline assessments will be conducted according to the Study Calendar. The

investigator or qualified designee must obtain documented consent from each potential subject prior to participating in a clinical trial per standard operating procedures.

4.4.2. Medical History

Demographic information, medical history and baseline conditions 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 subject's MBC will be recorded separately and not listed as medical history.

4.4.3. Prior and Concomitant Medications

All medication that is considered necessary for the subject's welfare, and which is not expected to interfere with the evaluation of the study treatment, may be given at the discretion of the Investigator. All medications taken from the time of informed consent and during study treatment will be recorded in the medical record per standard clinical practice.

4.4.4. Prior Treatment Details for Breast Cancer

The investigator or qualified designee will review and record all prior cancer treatments including systemic treatments, radiation and surgeries. If applicable, documentation of clinical and/or radiographic disease progression on the most recent therapy will be collected.

4.4.5. Baseline Procedures

Physical exam, ECOG performance status, vital signs, clinical laboratory tests, baseline adverse event assessment, and ECG should be performed during screening within the timeframe defined in Section 5.

4.4.5.1. Radiology and Tumor Measurements

Imaging tests, including CT scan of chest, abdomen, and pelvis with contrast or MRI aimed at defining the extent of existing disease must be performed and documented within the timeframe defined in Section 5. A bone scan will be done at Baseline within the timeframe defined in Section 5 to document presence or absence of bone metastasis.

4.4.6. Correlatives

4.4.6.1. Tissue Biopsies

It will be mandatory to obtain a tissue biopsy during screening before study treatment initiation in all subjects and then within 5 days prior to study treatment on Cycle 3,

Day 1. Bone tissue is not acceptable for research biopsies. See Section 3.2 (Inclusion #8) for possible exceptions to tumor biopsy. Optional time points include within 5 days prior to study treatment on Cycle 5, Day 1 and within 21 days following documented disease progression. Subsequent biopsies ideally will be obtained from the same site used at Baseline, but this is not a requirement. Research biopsies may be obtained even if the subject has discontinued study treatment.

An effort should be made to ask subjects in Cohort A if they can undergo the biopsy prior to study treatment on Cycle 5 Day 1 as this would be after 2 cycles of paclitaxel combined with pembrolizumab, and this would provide important information regarding PD-L1 expression and effect on TILs in the tumor after combined chemotherapy and immunotherapy. The overall goal of the trial would be to obtain paired pre-treatment and on-treatment tumor biopsy in at least 10 subjects in each cohort.
Biopsies will be obtained only if they can be performed under local anesthesia. General anesthesia will not be used to obtain biopsies that are to be used only for research purposes. Biopsies may be obtained with the assistance of either a surgical consultant or designee, a dermatological consultant or designee, or a member of the interventional radiology staff.

Biopsies performed for research purposes will only be obtained after the procedure has been explained to the subject, and per institution policy, the clinic specific informed consent has been secured. Subjects will have the opportunity to consent or refuse the use of their tumor samples to be banked at the CHS BioSpecimen Repository for additional unplanned future studies.

4.4.6.2. Archived Tumor Tissue

Tumor tissue samples will be collected (if available) for each subject on-study to evaluate the expression of, but not limited to, PD-L1 and TILs. This includes the following:

1) Sample of tissue from time of original diagnosis (breast cancer primary)
2) Sample of tissue from initial metastatic diagnosis (any site of metastases)

Subjects will have the opportunity to sign the consent form or refuse the use of any archival specimens collected to be banked at the Atrium Health BioSpecimen Repository for any desired unplanned future analyses.

4.4.6.3. PD-L1 Expression in Tumor

Recent data suggests that inducible PD-L1 expression may be important for responses to PD-1 blockade therapy. PD-L1 is expressed in about 20% of TNBCs in tumor samples from constructed tissue microarrays.[36] In hormone receptor-positive breast cancer, PD-L1 expression has been reported at 6%, a smaller incidence compared to TNBC, while still suggestive that checkpoint inhibitors may have a role in luminal breast cancer.[37]

An archived sample or core or punch biopsy (fine needle aspirate not adequate) collected during screening should be submitted to the CHS BioSpecimen Repository for characterization of PD-L1 expression if it can be safely obtained. Tumor tissue collected during screening should be submitted in formalin or as formalin-fixed paraffin embedded tumor tissue blocks (preferred).

Additionally, if available, archived tumor tissue specimens from prior biopsies will be collected for determination of PD-L1 tumor status to compare biomarker expression in archived specimens against the newly obtained tumor tissue that is collected as a requirement for entry into the trial. Tumor tissue collected may also be used to support analysis of additional exploratory biomarkers. Details regarding time points for collection of tumor tissue are outlined in the Study Calendar (Section 5).

4.4.6.4. Tissue Infiltrating Lymphocytes (TILs) in Tumor

TILs have been shown to provide prognostic and predictive value, particularly in TNBC.[38,39] H&E-stained slides will be evaluated for TILs according to standardized methodology described by Salgado et al.[40] Additionally, there is recent data suggestive that the subset of breast cancer tumors with the highest potential to benefit from checkpoint inhibitors may be those breast tumors that have both high TILs and high PD-L1 expression.[41]

4.4.6.5. Genomic Analysis

The application of new technologies such as next generation sequencing has provided the opportunity to define certain tumor types at the genetic level as being "hypermutated." It is possible that the hypermutated states may be correlated with response to pembrolizumab in HER2-negative breast cancer. Recent data in a group of patients with progressive metastatic carcinoma, with or without mismatch repair deficiency and treated with pembrolizumab, showed that mismatch-repair status predicted clinical benefit with pembrolizumab[42].

If tissue not previously sent for genomic analysis, FFPE tumor will be sent for genomic analysis by next-generation sequencing panel (≥400 genes). At least 50

mm^2, or the equivalent of 4 core biopsies using an 18 gauge needle, is required for testing.

4.4.6.6. Patient-Derived Xenografts (PDX) Models

Cancer research and drug development have historically relied upon the use of in vitro cell lines and in vivo models such as genetically-engineered mice and cell line-injected mice (i.e., cell line xenografts). These models lack critical elements such as tumor heterogeneity and its associated microenvironment and therefore do not provide an accurate representation of a patient's tumor.

PDX models have been established by implanting a fragment of a patient's fresh tumor tissue into immune-deficient mice. Tumors are allowed to engraft and grow before they are resected and subsequently implanted into a second generation of mice. Once growth is observed in the second generation of mice, the PDX model is considered successfully established and can be maintained and passaged into subsequent generations of mice or cryopreserved for future use. Extensive characterization of PDX models with regard to histopathology, gene expression and genomic sequence has shown high fidelity of the molecular characteristics of the original patient tumor. Therefore, these models have a distinct advantage over other models and have become an important in vivo model for oncology research and drug development.

The use of PDX models as a robust research platform for optimizing oncology drug development efforts has been well recognized; however, until recently data was lacking to support the accuracy of these models to predict clinical response. Champions Oncology and many academic researchers have data sets that highlight the high degree of accuracy of personalized PDX models in predicting response of individual patients across a variety of tumor types.

For this study, the objective is to collect tumor tissue in metastatic triple-negative breast cancer subjects to create patient-derived xenograft mouse models for translational research purposes. This will give potential insight to mechanisms of response and resistance, pre- and post-treatment, with this combination regimen.

Fresh tumor tissue will be obtained through a biopsy during screening. To maximize the success of tumor engraftment in the immune-deficient mouse, it is requested that approximately 0.5 cm^3 or a minimum of 3 core biopsies using an 18 gauge needle (or equivalent) be obtained.

Freshly acquired specimens will be placed into one to two 50-ml vials containing transport medium that will be provided by Champions and labeled with a unique subject number identifier. Vials containing the tissue specimen will be packaged in an insulated container with coolant blocks. Transportation of the specimen to the designated implantation site will be arranged in advance with Champions Clinical Operations Staff. Ideally, the time from tumor extraction to placement in media should be immediately but can be up to 30 minutes. Exact times will be recorded.

A whole blood sample will also be collected and submitted at the time of tumor biopsy to Champions Oncology. This will enable a comparison of normal DNA profile to tumor DNA for PDX genomic analysis. Lavender-topped, EDTA-containing tubes will be used for collection. Once collected, whole blood samples will be included with tissue specimen collection in an insulated container for transport to the designated implantation site. Exact collection times will be recorded.

Upon receipt of the tumor specimen at the designated implantation site, tumor tissue will be implanted into immune-deficient mice following the standard operating procedures (SOPs). Evaluation for tumor growth will occur as follows: tumor dimensions will measured twice weekly by digital caliper and data, including individual and mean estimated tumor volumes are recorded for each group. Tumor volume will be calculated using the formula: TV= width2 x length x π/2.
Monthly reports of tumor growth status will be provided by Champions to the PI and designated individuals.

Genomic sequencing (whole exome and RNA sequence) will be performed by Champions (or it's designate) on successfully generated PDX models and provided to the Sponsor-Investigator for research purposes.

4.4.6.7. Correlative Studies in the Tissue

A priority ranking of studies on tumor tissue samples are listed below

1) PD-L1
2) TILS
3) Genomic analysis
4) Patient-derived xenografts for metastatic triple-negative breast cancer subjects only

Tissue does not need to be sent for genomic analysis if already performed on previously collected tissue.

Other exploratory biomarkers may also be measured in the tissues.

4.4.6.8. Blood Correlatives

4.4.6.8.1. Circulating Tumor DNA

Circulating tumor DNA (ctDNA) could serve as an early biomarker of therapeutic response to checkpoint inhibitors.[43] Blood (20 ml; 2 BCT or equivalent tubes) will be drawn from the subject and submitted for isolation of ctDNA and analysis by next generation sequencing methods by a panel (≥40 genes). This will be collected pre-treatment and at the timepoints as defined in Section 5.

4.4.6.8.2. BRCA Mutations

A phase II study identified the first genomic marker—mismatch repair deficiency—to predict clinical benefit of immune checkpoint blockade with the anti–PD-1 antibody pembrolizumab. Among 28 patients with colorectal cancer, 40% of the 10 patients with mismatch repair–deficient tumors responded to pembrolizumab, but no responses were seen among the 18 mismatch repair–proficient patients. The difference in disease control rates (response plus stable disease) was even greater: 90% in the mismatch repair–deficient group and only 11% in the mismatch repair–proficient group.[42] Mismatch repair deficiency leads to an accumulation of genetic mutations in a tumor. It is hypothesized that immune checkpoint inhibitors would work particularly well against mismatch repair-deficient tumors. For breast cancer subjects, the determination of BRCA status will be recommended, if they have not had germline testing performed.

4.4.6.8.3. Biomarkers in the Blood

A pre-treatment blood sample will be collected with additional blood collected at subsequent time points according to the study calendar in Section 5, using one serum separator tube (SST) and two cell preparation tubes (CPT) for blood cells separation and storage. Samples will be delivered to the CHS BioSpecimen Repository to be processed and stored for batch analysis at the end of the study. The analysis will include but not be limited to immunotyping and cytokine profiling in Dr. David Foureau's LCI Immune Monitoring Core Laboratory.

4.4.6.8.4. Optional and Future Studies

All study participants will be invited to provide blood samples for future unplanned studies. Participation will be optional for all subjects entering the study. A subject's acceptance of unplanned future analyses will not be a requirement of their participation in the main study. Subjects will have the opportunity to consent or refuse the collection of these samples for additional unplanned studies. If the subject agrees to this collection, two 10 ml tubes of blood will be collected pre-treatment and other timepoints as defined in Section 5,

one red top (for serum) and a heparinized green top (for plasma) and frozen at -70° C to -80° C and stored in the CHS BioSpecimen Repository for future research.

4.5. Treatment

4.5.1. Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for any new or worsening AEs as specified in the Study Calendar and more frequently if clinically indicated. Adverse events will be graded and recorded throughout the study from randomization through 30 days after last administration of study treatment, using CTCAE Version 4.0. Toxicities will be characterized by seriousness, causality, toxicity grading, and action taken with regard to study treatment and recorded in the CTMS.

4.5.2. Vital Signs

The investigator or qualified designee will take vital signs prior to the administration of each dose of study treatment as specified in the Study Calendar. Vital signs include temperature, pulse, respiratory rate, and blood pressure.

4.5.3. ECOG PS

The investigator or qualified designee will assess ECOG status at the timepoints specified in the Study Calendar in Section 5.

4.5.4. Physical Exam

The investigator or qualified designee will perform a complete physical exam including weight at the timepoints specified in the Study Calendar in Section 5. New clinically significant abnormal findings should be recorded as AEs. Height will be measured at screening only.

4.5.5. Laboratory Procedures and Assessments

Laboratory tests for hematology and chemistry at the timepoints specified in the Study Calendar in Section 5.

Results must be reviewed by the investigator or qualified designee and found to be acceptable prior to each dose of study treatment.

4.5.6. On Study Tumor Imaging and Assessment of Disease

Post treatment tumor imaging assessments should be performed at the timepoints specified in the Study Calendar in Section 5. The same modality used at screening should be used at all follow-up imaging assessments.

If no bone lesions were identified at Baseline, bone scans will only be repeated as clinically indicated (i.e. showing signs or symptoms of new or progressing bone metastases). Subjects with bone metastasis present at Baseline will have bone scans at the same time point as study tumor imaging (CT/MRI), based on how long subjects have been on study as defined in Section 5.

Imaging should follow calendar days and not be delayed for any dose interruptions that may occur. Imaging should continue to be performed until disease progression, the start of new anticancer treatment, withdrawal of consent for study participation, death, or notification by the Sponsor-Investigator, whichever occurs first.

Radiographic disease progression must be confirmed at least 4 weeks after the first tumor imaging indicating progressive disease in clinically stable subjects. Subjects in Cohort A will not require confirmatory imaging if radiographic disease progression is identified at the first follow-up imaging time-point (prior to pembrolizumab). Pembrolizumab-treated subjects who have unconfirmed radiographic disease progression may continue on treatment until progression is confirmed (or possibly even later) provided they have met the conditions detailed in irRECIST (Table 10).

For subjects with new symptoms of osseous metastasis [e.g., new bone pain and/or new persistently elevated alkaline phosphatase (AP)] a bone scan should be obtained.

4.6. End of Treatment

An end of treatment visit should be completed after study treatment is discontinued. End of treatment visit is outlined in the Study Calendar.

Subjects will be tracked for at least 30 days following cessation of study treatment to monitor for adverse events and any other unanticipated problems. If the end of treatment visit occurs prior to 31 days from last dose, subjects will be contacted by telephone on day 31 or next business day for monitoring. Subjects will be followed until all treatment-related toxicities have resolved, returned to baseline, stabilized, or are deemed irreversible according to standard of care.

Note: In the event a subject is discontinued from pembrolizumab, but is experiencing benefit (in the investigator's opinion) from the paclitaxel, continuation of paclitaxel will not be considered protocol-directed therapy or start of new or subsequent anticancer therapy.

4.7. Follow-Up

Subjects will be contacted approximately every 6 months from treatment discontinuation date, until death, lost to follow-up, or until the criteria defined for the final analysis (Section 12.4.1) are reached. However, in the event the criteria for the final analysis are met, and there are subjects who have not yet been discontinued from study treatment, subjects in follow up will continue to be followed until all subjects have discontinued study treatment (see Section 6.6) and completed the required 30 day safety monitoring period (see Section 4.5.1). Subjects will be contacted by telephone, in writing, or during clinic visits after treatment discontinuation for collection of follow-up information. Follow-up clinical information may also be obtained through chart reviews. Death information from public sources i.e. death registry, obituary listing, etc. can also be used when available and verifiable.

4.8. Subject Withdrawal and Off Study

Subjects may withdraw consent at any time for any reason or be removed from the trial at the discretion of the investigator should any untoward effect occur.

In this trial, a subject may discontinue from study treatment but continue to participate in the regularly scheduled activities, as long as the subject does not withdraw consent for participation.

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

- The subject withdraws consent for participation on the study
- The subject is lost to follow-up
- Investigator's decision to withdraw the subject from study, which may include noncompliance with study treatment or procedure requirements
- Subject death

In all cases, the reason for withdrawal and/or subject going Off Study must be documented in the subject's medical records and/or research record and recorded in the eCRF.

4.9. Screen Failures/Subject Replacements

A subject who, for any reason (i.e. failure to satisfy the eligibility criteria or withdraws consent), terminates their participation in the study before being randomized is regarded as a "screen failure."

A subject who discontinues from the trial after randomization will not be replaced.

5. STUDY CALENDAR

The following tests and evaluations will be performed according to the schedule below. Baseline (i.e., pre-study) evaluations must be performed within 14 days prior to Cycle 1 Day 1, unless otherwise indicated in one of the footnotes below the table. All imaging must be performed within 28 days prior to Cycle 1 Day 1. A cycle is defined as an interval of 21 (+/- 3) days.

Evaluation	Pre-Study	Cycle 1 Day 1 (C1D1)	C1D8[1]	C2D1	C2D8[1]	C3D1	C3D8[1]	C4 and after; D1	C4 and after; D8[1]	Every 6 weeks (+/- 7 days)	End of Treatment (w/in 31 days of last dose)	Progression	Follow Up[21]
Informed consent	X[5]												
• History[23] • weight/ height[3] • physical exam • ECOG PS	X	X[2]		X[4]		X[4]		X[4]			X		
Vital signs[24]	X	X[2]	X	X	X	X	X	X	X		X		
Serum or urine pregnancy test in WOCBP	X												
CBC with differential, platelets	X	X[2]	X[7]	X[6]	X[7]	X[6]	X[7]	X[6]	X[7]		X		
PT/PTT/ INR	X												
Serum chemistries and LFTs[8]	X	X[2]		X[6]		X[6]		X[6]			X		
Thyroid function tests (TSH and free T4)	X							X[9]					
ECG	X												
Paclitaxel[10]		X	X	X	X	X	X	X	X				

Pembro-lizumab		X[11]		X[11]		X		X					
Tumor biopsy, if accessible	X[19]					X[20]		X[12]				X[12]	
Evaluation ***continued***	Pre-Study	Cycle 1 Day 1 (C1D1)	C1D8[1]	C2D1	C2D8[1]	C3D1	C3D8[1]	C4 and after; D1	C4 and after; D8[1]	Every 6 weeks	End of Treatment (w/in 31 days of last dose)	Progression	Follow Up[21]
Archived tumor tissue	X												
Blood for circulating tumor DNA		X				X		X[13]					
Optional Future research blood draw		X				X		X[14]				X[14]	
Blood for biomarkers		X		X		X		X[15]					
Blood for BRCA mutation[16]	X												
Imaging studies and/or tumor assessments	X[5]									X[17,18]			
Review adverse events[25]	X	X	X	X	X	X	X	X	X		X		
Survival status													X[22]

1. Day 8 visits are not applicable for subjects receiving pembrolizumab monotherapy. Day 8 paclitaxel treatment may have a + 2 day window (it may be given up to 2 days later than Day 8) but paclitaxel doses should not be administered any closer than 7 days apart.
2. Physical exam, ECOG performance status, weight, vital signs, and clinical laboratory tests do not need to be repeated prior to Cycle 1 Day 1 unless the subject's condition is deteriorating.
3. Collect height at Screening only.
4. Physical exam, weight, and ECOG PS within 7 days of Day 1 for Cycles 2 and beyond.
5. Within 28 days prior to study treatment initiation).
6. May be performed within 72 hours
7. May be performed within 1 day on Day 8 for Cycle 1 and beyond.
8. Includes: sodium, potassium, bicarbonate, chloride, glucose, BUN, creatinine, calcium, total protein, and albumin, alkaline phosphatase, total bilirubin, AST and ALT.
9. Blood draws for thyroid function tests should be repeated every 4 cycles starting at Cycle 5 Day 1 (within 72 hours).

10. If paclitaxel needs to be omitted for the subject due to a paclitaxel-related toxicity or the subject has received maximum benefit from paclitaxel as deemed by the treating physician and the Sponsor-Investigator, paclitaxel may be discontinued.
11. ONLY subjects randomized to Cohort B will receive pembrolizumab on Cycle 1 Day 1 and Cycle 2 Day 1 before paclitaxel.
12. Optional within 5 days of C5D1 (strongly encouraged for subjects in Cohort A). If subject discontinues treatment due to disease progression, a biopsy will be performed within 21 days of confirmed progression. If subject has confirmed disease progression but remains on treatment, a biopsy will not be performed.
13. This will be collected at pre-treatment and then within 72 hours prior to Day 1 of every 2 (odd-numbered) cycles starting with Cycle 3.
14. Within 72 hours of C5D1and within 21 days of confirmed progression.
15. Within 72 hours of C4D1only.
16. If applicable as determined by the treating investigator and not previously obtained, can be drawn at any time while on study in conjunction with genetic consultation.
17. The first follow-up imaging timepoint should be 6 weeks (+/- 7 days) from C1D1. Subsequent follow-up imaging frequency as referenced in the calendar and below (based on length of time the subject has been on the study) should be based on the timepoint of the previous scan, not C1D1. If a subject has been on study for ≥ 6 months, the treating physician may decide to lengthen the interval to every 9 weeks (63 days ± 7 days) from previous scan, or more frequently, if clinically indicated. The last scan performed at the 6 week internal must occur on or after the 6 month on study date; subsequent scans can be scheduled at the 9 week interval. The last scan performed at the 9 week interval must occur on or after the 12 month on study date; subsequent scans can be scheduled at the 12 week interval. After 12 months, imaging frequency can be reduced to every 12 weeks (84 ± 7 days) from previous scan, or more frequently if clinically indicated.
18. Imaging should follow calendar days and not be delayed for any dose interruptions that may occur until progression.
19. May be collected anytime during screening prior to study treatment initiation. Subjects for whom tumor biopsies cannot be obtained during screening due to inaccessible tumor or subject safety concern may submit an archived tumor specimen from primary tumor or metastatic biopsy collected within 12 months from consent. Bone tissue is not acceptable for research biopsies.
20. Within 5 days of C3D1
21. Subjects will be contacted approximately every 6 months from treatment discontinuation date, until death, lost to follow-up, or until the criteria defined for the final analysis (Section 12.4.1) are reached.
22. For surviving subjects who receive subsequent anti-cancer therapy prior to documented disease progression, collect start date of first subsequent anti-cancer therapy. In the event a subject is discontinued from pembrolizumab, but is experiencing benefit from the paclitaxel, continuation of paclitaxel will not be considered protocol-directed therapy or start of new or subsequent anticancer therapy.
23. To include baseline conditions present at the time of study treatment initiation
24. VS to include temperature, pulse, respiratory rate, and blood pressure
25. Adverse events will be followed for 30 days after last administration of study treatment.

6. TREATMENT PLAN

6.1. Drug Dosage and Administration

6.1.1. General Considerations

Trial treatment should begin on the day of or within 72 hours of randomization. Study treatment should be administered after all procedures/assessments have been completed as detailed in the Study Calendar. A cycle is defined as an interval of 21 (+/- 3) days (delays due to holidays, weekends and bad weather will be permitted and will **not** be counted as a protocol deviation). **No investigational or commercial**

agents or therapies intended to treat the subject's malignancy may be administered while the subject is receiving study therapy, other than those described below.

6.2. Paclitaxel

Subjects will receive paclitaxel 80 mg/m^2 as an IV infusion over 60 minutes on Days 1 and 8 every 21 days. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted. In the event of a dosing delay, a make-up dose of paclitaxel may be given as outlined in Section 7.2. A window of +/- 3 days is allowed for the Day 1 dose and a + 2 day window is allowed for the Day 8 dose (it may be given up to 2 days later than Day 8) but paclitaxel doses should not be administered any closer than 7 days apart.

Paclitaxel is commercially available. Anaphylaxis precautions should be observed during administration.

Premedications for possible hypersensitivity reactions involving paclitaxel and/or its components will be given approximately 30 to 60 minutes prior to paclitaxel. The following is suggested:

- Dexamethasone 8 mg IV (or equivalent)*
- Diphenhydramine 25 mg IV (or equivalent)
- Famotidine 20 mg IV (or equivalent)

*A suggested regimen to taper dexamethasone is the following: dexamethasone 8 mg IV 30 to 60 minutes prior to each paclitaxel infusion during cycle 1 and then dexamethasone 4 mg IV 30 to 60 minutes prior to each paclitaxel infusion during cycle 2, and then discontinued for subsequent infusions, if tolerated.

The dose of paclitaxel will be calculated each cycle using the subject's most recent weight during screening (baseline weight). If the subject's weight changes ≥10% from baseline weight, recalculate that total dose per standard clinical practice.

6.2.1. Treatment Criteria

On Cycle 1 Day 8 and beyond, paclitaxel can be administered provided that the subject meets the following criteria:

- ANC ≥ 1,000/μl
- Platelets ≥ 75,000/μl

If paclitaxel is held, follow guidelines for dosing pembrolizumab. It is possible for subjects to continue on pembrolizumab even if paclitaxel is held, when appropriate.

6.3. Pembrolizumab

Subjects will receive 200 mg pembrolizumab as an IV infusion over 30 minutes on Day 1 of every 21 days (+/- 3 days).

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.

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

Pembrolizumab 100 mg/ 4mL solution for injection will be supplied by Merck.

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 study treatments in accordance with the protocol and any applicable laws and regulations.

6.4. Treatment Administration

6.4.1. Cohort A, Phased Pembrolizumab

For subjects randomized to Cohort A, paclitaxel will be administered per Section 6.2 during Cycles 1 and 2. No pembrolizumab will be given during Cycles 1 and 2. Starting with cycle 3 and subsequent cycles, pembrolizumab will be administered per Section 6.3 **before** paclitaxel on day 1 every cycle.

6.4.2. Cohort B, Concurrent Pembrolizumab

For subjects randomized to Cohort B, pembrolizumab will be administered per Section 6.3 on day 1 **before** paclitaxel every cycle. Paclitaxel will be administered per Section 6.2, on days 1 and 8 every cycle.

6.4.3. Pembrolizumab Monotherapy

If paclitaxel needs to be omitted for the subject due to a paclitaxel-related toxicity (e.g., subject develops a hypersensitivity reaction or grade 3 or grade 4 neuropathy), the subject may be allowed to continue on protocol with single-agent pembrolizumab.

If the subject has received maximum benefit from paclitaxel as deemed by the treating physician, and in consultation with the Sponsor-Investigator, which could include a situation of complete response, partial response, or stable disease ≥ 6 months, paclitaxel can be discontinued, and treatment with pembrolizumab alone may be continued. Safety assessments, including physical exam, vital signs, laboratory assessments, and monitoring for the occurrence of adverse events will be performed according to Section 5. The dosing of pembrolizumab will be given once every 3 weeks as described above.

6.4.4. Table 1: Trial Treatment

Regimen Description					
Agent	**Premedications; Precautions**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Pembrolizumab	None	200 mg	IV over 30 minutes **before** paclitaxel	Day 1	21 (+/- 3) days (3 weeks)
Paclitaxel	Dexamethasone* Famotidine Diphenhydramine	80 mg/m^2	IV over 60 minutes; give after pembrolizumab	Days 1 and 8	
*Dexamethasone 8 mg IV will be given 30 to 60 minutes before each paclitaxel administration during cycle 1. If tolerated, dexamethasone should then be tapered to 4 mg at Cycle 2 and discontinued at Cycle 3 and beyond for subsequent infusions.					

6.5. Concomitant Medications

6.5.1. 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 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 recorded. All concomitant medications used within 28 days before the first dose of study treatment should be recorded.

6.5.2. Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the screening and treatment phase of this trial:

- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy
- Live vaccines within 30 days prior to the first dose of study 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, intranasal influenza, rabies, BCG, and typhoid vaccine.
- Glucocorticoids for any purpose other than to modulate symptoms from an event of suspected immunologic etiology or as premedication for paclitaxel.
    - Note: The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor-Investigator.
    - Note: Inhaled steroids are allowed for management of asthma.
    - Note: Use of prophylactic corticosteroids to avoid allergic reactions (i.e., to IV contract dye) is permitted.

6.6. Definition of Pembrolizumab Overdose

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

6.7. Duration of Therapy

In the absence of treatment delays due to adverse event(s), study treatment will continue until one of the following criteria applies:

- Disease progression in subjects who have not yet received pembrolizumab (Cohort A)
- Confirmed disease progression in subjects receiving pembrolizumab
- Disease progression if clinically unstable
- Unacceptable adverse event(s)
- Subject decides to withdraw from study treatment or protocol
- General or specific changes in the subject's condition render the subject unacceptable for further study treatment in the judgment of the Investigator.

Note: In the event the criteria for the final analysis are met, and there are subjects who have not yet been discontinued from study treatment, they will continue to receive

therapy beyond the date the criteria for the study final analysis are met and until one of the above criteria applies.

6.8. Drug Accountability

All investigational study drugs will be stored at the investigational site in accordance with Good Clinical Practice (GCP) and Good Manufacturing Practices (GMP) requirements and will be inaccessible to unauthorized personnel.

An adequate record of receipt, distribution, destruction, or return of all investigational study drugs must be kept in the form of a Drug Accountability Form. The Investigator, or responsible party designated by the Investigator, will maintain a careful record of the inventory using the Drug Accountability Form.

6.9. Destruction

The investigator is responsible for keeping accurate records of the clinical supplies received from Merck or designee, including 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 according to LCI IDS pharmacy policies. 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.

7. DOSE MODIFICATIONS

7.1. Pembrolizumab

Pembrolizumab dose reductions are not applicable to this study. However, pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as described in Table 2 below. Held doses will not be replaced. When pembrolizumab is restarted, this dose would be considered Day 1 of the subsequent cycle and should be in alignment with the new schedule. If pembrolizumab is held, paclitaxel may continue, when appropriate.

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 study treatment. See Section 8 for supportive care guidelines, including use of corticosteroids, for treatment-related adverse events.

Table 2: Dose Modification and Toxicity Management Guidelines for Immune-Related Adverse Events Associated with Pembrolizumab Monotherapy and IO Combinations

General instructions:

1. Severe and life-threatening irAEs should be treated with IV corticosteroids followed by oral steroids. Other immunosuppressive treatment should begin if the irAEs are not controlled by corticosteroids.
2. Study intervention must be permanently discontinued if the irAE does not resolve or the corticosteroid dose is not ≤10 mg/day within 12 weeks of the last study intervention treatment.
3. The corticosteroid taper should begin when the irAE is ≤ Grade 1 and continue at least 4 weeks.
4. If study intervention has been withheld, study intervention may resume after the irAE decreased to ≤ Grade 1 after corticosteroid taper.

irAEs	**Toxicity Grade (CTCAE ~~v5.0~~ v4.0)**	**Action With Pembrolizumab**	**Corticosteroid and/or Other Therapies**	**Monitoring and Follow-up**
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper • Add prophylactic antibiotics for opportunistic infections	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment
	Recurrent Grade 2, Grade 3 or 4	Permanently discontinue		
Diarrhea/Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus

	Recurrent Grade 3 or Grade 4	Permanently discontinue		in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus) • Participants with ≥Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis • Participants with 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
AST or ALT Elevation or Increased Bilirubin	Grade 2 [a]	Withhold	• Administer corticosteroids (initial dose of 0.5 to 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable)
	Grade 3 [b] or 4 [c]	Permanently discontinue	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	
~~T1DM or~~ Hyperglycemia	New onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold [d]	• Initiate insulin replacement therapy for participants with T1DM • Administer antihyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes
Endocrine disorders-other (~~H~~hypophysitis)	Grade 2	Withhold		

	Grade 3 or 4	Withhold or permanently discontinue [d]	• Administer corticosteroids and initiate hormonal replacements as clinically indicated	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
Hyperthyroidism	Grade 2	Continue	• Treat with nonselective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hypothyroidism	Grade 2, 3 or 4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyronine) per standard of care	• Monitor for signs and symptoms of thyroid disorders
Nephritis: grading according to increased creatinine or acute kidney injury	Grade 2	Withhold	• Administer corticosteroids (prednisone 1 to 2 mg/kg or equivalent) followed by taper	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Neurological Toxicities	Grade 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 2, 3 or 4	Permanently discontinue		
Exfoliative Dermatologic Conditions	Suspected SJS, TEN, or DRESS	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Confirmed SJS, TEN, or DRESS	Permanently discontinue		

All Other irAEs	Persistent Grade 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Grade 3	Withhold or discontinue based on the event [e]		
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

AE(s)=adverse event(s); ALT= alanine aminotransferase; AST=aspartate aminotransferase; CTCAE=Common Terminology Criteria for Adverse Events; DRESS=Drug Rash with Eosinophilia and Systemic Symptom; GI=gastrointestinal; IO=immuno-oncology; ir=immune related; IV=intravenous; SJS=Stevens-Johnson Syndrome; T1DM=type 1 diabetes mellitus; TEN=Toxic Epidermal Necrolysis; ULN=upper limit of normal.

Note: Non-irAE will be managed as appropriate, following clinical practice recommendations.

[a] AST/ALT: >3.0 to5.0 x ULN if baseline normal; >3.0 to 5.0 x baseline, if baseline abnormal; bilirubin:>1.5 to 3.0 x ULN if baseline normal; >1.5 to 3.0 x baseline if baseline abnormal

[b] AST/ALT: >5.0 to 20.0 x ULN, if baseline normal; >5.0 to 20.0 x baseline, if baseline abnormal; bilirubin:>3.0 to 10.0 x ULN if baseline normal; >3.0 to 10.0 x baseline if baseline abnormal

[c] AST/ALT: >20.0 x ULN, if baseline normal; >20.0 x baseline, if baseline abnormal; bilirubin: >10.0 x ULN if baseline normal; >10.0 x baseline if baseline abnormal

[d] The decision to withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician. If control achieved or ≤ Grade 2, pembrolizumab may be resumed.

[e] Events that require discontinuation include, but are not limited to: encephalitis and other clinically important irAEs (eg, vasculitis and sclerosing cholangitis).

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, subject 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-Investigator. The reason for interruption should be documented in the patient's study record.

See Section 8.1 for supportive care measures to be taken for potential immune-related toxicities associated with pembrolizumab.

7.2. Paclitaxel

Each paclitaxel cycle is defined as two paclitaxel treatments given during a 21-day period. If paclitaxel is held for ≥ 2 weeks, the following instructions apply:

If paclitaxel is held, resume with the missed treatment dose, e.g., if day 1 paclitaxel is held for 7 days, treatment will resume with the "day 1" paclitaxel dose on calendar day 8 and the "day 8" paclitaxel dose will be given on day 15. (In this case, there is no break in therapy during the last week of the cycle. Paclitaxel doses should be given at least 7 days apart.)

If two paclitaxel treatments have not been given by day 21 (+/- 3 days), proceed with treatment day 1 of the next cycle (if toxicities have resolved according to requirements in the dose modification sections/tables).

If paclitaxel is discontinued for neuropathy or musculoskeletal pain or other toxicity, pembrolizumab may continue.

Table 3: Dose levels for paclitaxel

	Dose Level 0 ***Starting Dose***	**Dose Level −1**	**Dose Level −2**	**Dose Level −3**
Paclitaxel	80 mg/m^2	70 mg/m^2	60 mg/m^2	Discontinue

Table 4: Dose Modifications for paclitaxel
Note: dose modifications listed below are only for paclitaxel-related toxicities, do not modify dose if toxicity is not related to paclitaxel.

Toxicity	**Paclitaxel**
No toxicity	Maintain dose level
Febrile Neutropenia Grade 3	Hold until resolved, then ↓ 1 dose level

Grade 4	Hold until resolved, then ↓ 1 dose level
Neutropenia*	
Grade 1	Maintain dose level
Grade 2	Maintain dose level
Grade 3	Hold until resolved to ≥1,000/µl, maintain dose level
Grade 4	Decrease one dose level when resolved to ≤ Grade 2
Thrombocytopenia*	
Grade 1	Maintain dose level
Grade 2	Hold until resolved to ≥75,000/µl, maintain dose level
Grade 3	Hold until resolved to ≥75,000/µl, maintain dose level
Grade 4	Hold until resolved to ≥75,000/µl, then ↓ 1 dose level
Clinically Significant Non-Hematologic Toxicities (excluding neuropathy and musculoskeletal pain)	
Grade 1	Maintain dose level
Grade 2	Hold until resolved to ≤ Grade 1, maintain dose level
Grade 3	Hold and then decrease one dose level when resolved to ≤ Grade 1
Grade 4	Hold and then decrease one dose level when resolved to ≤ Grade 1
*Only if present on the scheduled treatment day	

Table 5: Dose Modifications for paclitaxel-related neuropathy

Paresthesias/Dysesthesias	1–7 Days Duration	Persistent for > 7 Days *or* Caused a Dose Delay
Grade 1 – Paresthesias/dysesthesias that do not interfere with function	Maintain paclitaxel dose	
Grade 2 – Paresthesias/dysesthesias interfering with function, but not activities of daily living	Maintain paclitaxel dose[a]	Decrease paclitaxel one dose level[b]
Grade 3 – Paresthesias/dysesthesias with pain or with function impairment that also interfere with activities of daily living	**First episode:** Decrease paclitaxel one dose level[b] **Second episode:** Discontinue study therapy	**First episode:** Decrease paclitaxel one dose level[b] or discontinue **Second episode:** Discontinue study therapy

Grade 4 – Persistent paresthesias/dysesthesias that are disabling or life-threatening	Discontinue study therapy
a Must be resolved to ≤ grade 1 to receive treatment. **b** Hold paclitaxel for ***persistent*** grade ≥2 neuropathy. When ≤ grade 1, resume treatment with dose modification. If grade ≥2 toxicity persists after 2 weeks of delay, discontinue paclitaxel.	

Table 6: Dose Modifications for paclitaxel-related musculoskeletal pain
Note: Use of narcotics and NSAIDs is encouraged to maintain the paclitaxel dose if possible.

Musculoskeletal Pain	**1–7 Days Duration**	**Persistent for > 7 Days *or* Caused a Dose Delay**
Grade 1 *(despite analgesics)*	Maintain paclitaxel dose	
Grade 2 *(despite analgesics)*	Maintain paclitaxel dose	Maintain paclitaxel dose or Decrease paclitaxel one dose level*
Grade 3 *(despite analgesics)*	**First episode:** Decrease paclitaxel one dose level **Second episode:** Discontinue study therapy	**First episode:** Decrease paclitaxel one dose or Discontinue study therapy **Second episode:** Discontinue study therapy
Grade 4 *(despite analgesics)*	Discontinue study therapy	
* Hold paclitaxel for ***persistent*** grade 2 or 3 musculoskeletal pain. ***(Targeted therapy can be continued while paclitaxel is held.)*** When ≤ grade 1, resume treatment with paclitaxel dose modification. If grade 2 or grade 3 toxicity persists after 2 weeks of delay, discontinue study therapy.		

8. TREATMENT-RELATED ADVERSE EVENTS

8.1. Adverse Events Related to Pembrolizumab

8.1.1. General Considerations

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below. Where appropriate, these guidelines include treatment with oral or

intravenous corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

Note: if after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance as outlined below. Refer to Section 7 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.

8.1.2. 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.

8.1.3. Diarrhea/Colitis

Subjects should be carefully monitored for signs and symptoms of enterocolitis (e.g., diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (e.g., 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 gastrointestinal (GI) consultation and endoscopy to confirm or rule out colitis.
- For Grade 2 diarrhea/colitis administer oral corticosteroids.
- For Grade 3 or 4 diarrhea/colitis, treat with intravenous steroids followed by high dose oral steroids.
- When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

8.1.4. Type 1 Diabetes (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 subjects with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide

8.1.5. Hypophysitis

- 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. 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.

8.1.6. Hyperthyroidism and Hypothyroidism

Thyroid disorders can occur at any time during treatment. Monitor subjects for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.

- Grade 2 hyperthyroidism events (and Grade 2-4 hypothyroidism):
  - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
  - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyronine, 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.

8.1.7. 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.

8.1.8. 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.

8.1.9. 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 7 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab. Subjects who experience an infusion reaction associated with administration of paclitaxel should be treated per institutional policy.

Table 7: Pembrolizumab 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 treating 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	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).

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
	should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing

8.1.10. Reproductive Risks

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. For this trial, male subjects will be considered to be of non-reproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

Female subjects will be considered of non-reproductive potential if they are either:

1) postmenopausal (defined as at least 12 months with no menses without an alternative medical cause; in women < 45 years of age a high follicle stimulating hormone (FSH), luteinizing hormone (LH), and estradiol levels in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy.

OR

2) have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion.

OR

3) has a congenital or acquired condition that prevents childbearing.

Female and male subjects of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving study treatment and for 120 days after the last dose of study treatment by complying with one of the following:

1) practice abstinence from heterosexual activity;

OR

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

Acceptable methods of contraception are:

Single method (one of the following is acceptable):

- Intrauterine device (IUD)
- Vasectomy of a female subject's male partner
- Contraceptive rod implanted into the skin

Combination method (requires use of two of the following):

- Diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- Cervical cap with spermicide (nulliparous women only)
- Contraceptive sponge (nulliparous women only)
- Male condom or female condom (cannot be used together)
- Hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

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

Subjects should be informed that taking the study therapy may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 14 days prior to the initiation of study therapy for oral contraception) throughout the study period up to 120 days after the last dose of study therapy. If

there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

9. DATA AND SAFETY MONITORING PLAN

9.1. Safety Monitoring

This protocol will be monitored according to the processes in effect for all Levine Cancer Institute investigator-initiated studies and the protocol-specific monitoring plan, and will abide by standard operating procedures set forth by both the Atrium Health Office of Clinical and Translational Research and the Levine Cancer Institute Clinical Trials Office. It is the responsibility of the Sponsor-Investigator to monitor the safety data for this study. The Sponsor-Investigator, Statistician, and other team members as needed will meet regularly to monitor subject consents, enrollment and retention, safety data for all subjects [including adverse events (AEs) for all grades and attributions, serious adverse events (SAEs)], study treatment administration, and validity/integrity of the data. Documentation of these meetings will be kept with study records. SAEs will be reported to the Food and Drug Administration (FDA) and the IRB per their requirements. Major protocol deviations that result in a threat to subject safety or the integrity of the study will be reported to the FDA and IRB per their requirements. The Sponsor-Investigator will submit data to the LCI Data and Safety Monitoring Committee according to the overarching LCI Data and Safety Monitoring Plan.

9.2. Data Quality Assurance

This study will be organized, performed, and reported in compliance with the study protocol, standard operating procedures (SOPs) of the Levine Cancer Institute and Atrium Health Office of Clinical and Translational Research, the FDA, and other applicable regulations and guidelines (e.g. GCP).

Subject data will be monitored by Levine Cancer Institute Research Monitors routinely for data quality. This monitoring will be done by comparing source documentation to the eCRFs. Any variation between the two data sets will be discussed with the Sponsor-Investigator and/or appropriate research personnel.

The study database will be reviewed and checked for omissions, apparent errors, and values requiring further clarification using computerized and manual procedures. Data queries requiring clarification will be generated and addressed by the appropriate study team member and/or Sponsor-Investigator. Only authorized personnel will make corrections to the study database and all corrections will be documented in an electronic audit trail.

9.3. Communication Between Sites

Investigational sites will be required to report applicable AEs, SAEs, deviations or any other problem that could affect the validity/integrity of the study data to the Sponsor-Investigator. AEs will be reported within 10 business days of the investigator learning of the event. SAEs will be reported within 1 business day of the investigator learning of the event. Drug administration or any other problem affecting subject safety or the integrity of the data should be communicated to the Sponsor-Investigator in writing as soon as possible but within 2 business days of learning of the event.

10. SAFETY DATA COLLECTION, RECORDING AND REPORTING

Safety assessments will consist of monitoring and recording all adverse events and serious adverse events, regular monitoring of hematology, blood chemistry and liver function test values, regular measurement of vital signs, and performance of history and physical examinations. These assessments should be performed per the study calendar. Adverse events will be evaluated continuously throughout the study. Safety and tolerability, relationship to study treatment and intensity will be assessed according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. All adverse events (Grades 1 – 5) will be documented in subject study charts, and recorded in the eCRF.

10.1. Unanticipated Problem Definition

An UAP is any incidence, experience or outcome that is unexpected, given the information provided in research-related documentation (e.g., Investigator's brochure, informed consent) and the study population characteristics that is related or possibly related to participation in the research study and places the participant at an increased risk.

10.2. Adverse Event

10.2.1. Adverse Event Definition

An adverse event or adverse experience is any untoward medical occurrence in a study subject who is administered a study drug that does not necessarily have a causal relationship with this study treatment. An adverse event can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of the study drug, whether or not considered related to the study drug. Pre-existing conditions that increase in frequency or severity or change in nature during or as a consequence of use of a drug in human clinical trials are also considered adverse events.

Any continuing medical condition or clinically significant laboratory abnormality with an onset date before the first date of study treatment administration should be considered

pre-existing and should be documented in the subject's medical records and/or in the study chart at baseline. If the medical condition or clinically significant laboratory abnormality worsens while the patient is on study, it should be documented as an adverse event.

10.2.2. An Adverse Event does not include

An AE does not include:

- relapse or progression of the underlying malignant disease; however, the associated signs, symptoms, or diagnoses should be recorded as adverse events (e.g., "jaundice" due to new or increasing liver metastases, or "tumor pain" or "bone pain" due to progressive disease);
- medical or surgical procedures (e.g., surgery, endoscopy, tooth extraction, transfusion). The condition that leads to the procedure is the adverse event;
- situations where an untoward medical occurrence has not occurred (e.g., hospitalization for elective surgery, social and/or convenience admissions);
- overdose of paclitaxel or concomitant medication without any signs or symptoms unless the subject is hospitalized for observation

Laboratory abnormalities are usually not recorded as adverse events; however, signs and/or symptoms that are associated with laboratory findings requiring study withdrawal, dose modification, or medical intervention (e.g., anemia requiring transfusions or hyperglycemia requiring treatment) or other abnormal assessments (e.g., ECG, radiographs, vital signs) must be recorded as adverse events if they meet the definition of an adverse event. In addition, laboratory abnormalities marked as clinically significant by the investigator should also be recorded as adverse events in the eCRF. The investigator will record all clinically significant grades for laboratory abnormalities and will evaluate their relationship to the study treatment and subject's clinical condition if/when a clinically significant laboratory abnormality occurs.

10.2.3. Relationship to Study Treatment

The relationship to study treatment therapy should be assessed using the following definitions:

Not Related: Evidence exists that the AE has an etiology other than the study treatment (i.e., pre-existing condition, underlying disease, intercurrent illness, or concomitant medication). This includes events that are considered remotely or unlikely related to study treatment.

Related: A temporal relationship exists between the event onset and administration of the study treatment. It cannot be readily explained by the subject's clinical state, intercurrent illness, or concomitant therapies. In the case of cessation or reduction of the dose, the event abates or resolves and reappears upon rechallenge. It should be emphasized that ineffective study treatment should not be considered as causally related in the context of AE reporting. This includes events that are considered possibly, probably, or definitely related to study treatment.

All adverse events (including event name, grade, start/stop date and attribution) will be documented in the medical record and on the case report form for this protocol.

The Investigator is responsible for verifying and providing source documentation for all adverse events and assigning the attribution for each event for all subjects enrolled on the trial.

10.3. Suspected Adverse Reaction Definition

A SAR is an adverse event in which there is reasonable possibility that the study treatment caused the adverse event as defined by 21 CFR 312.32. The Investigator is responsible for judging whether it is a reasonable possibility that the study treatment caused the adverse event.

10.4. "Unexpected" Definition

An AE or SAR is to be considered unexpected if the event is not listed in the current Investigator Brochure, package insert or label or is not listed in the severity or specificity observed.

10.5. Serious Adverse Event Definition

Adverse events may also be considered serious adverse events. Serious adverse event or SAE shall mean any untoward medical occurrence in a study subject who is administered the study treatment that results in:

- Death;
- Life-threatening situation (subject is at immediate risk of death);
- Inpatient hospitalization or prolongation of an existing hospitalization (excluding those for study treatment administration, protocol-related procedures, palliative or hospice care, or placement of an indwelling catheter, unless associated with other serious events);
- Persistent or significant disability/incapacity;

- Congenital anomaly/birth defect in the offspring of a patient who received study treatment;
- A new cancer if the cancer is the condition of the study; or
- Other: Important medical events that may not result in death, be immediately life-threatening, or require hospitalization, may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed in this definition.
    - **Examples of such events are:**
        - Intensive treatment in an emergency room or at home for allergic bronchospasm;
        - Blood dyscrasias or convulsions that do not result in hospitalization;
        - Development of drug dependency or drug abuse.

The following events do not meet the criteria for seriousness per ICH definition, but must be reported in the same manner as SAEs. Therefore, these events are considered serious for collection purposes:

- Pregnancy or lactation
- Overdose of pembrolizumab, as defined in Section 6.6

10.6. Safety Reporting to the Sponsor-Investigator

SAEs must be reported to the Sponsor-Investigator within 1 business day of awareness.

SAEs will be captured from the time of study treatment initiation through 90 days after the date of the last study treatment administration or 30 days following cessation of treatment if the subject initiates new anti-cancer therapy, whichever is earlier. Events that meet the definition of an SAE that are determined to be related and unexpected to a research procedure or study treatment are reportable starting with informed consent throughout the duration of the subject's participation. In addition, once study treatment is initiated, SAEs that are determined to be related to pembrolizumab or a study procedure (regardless of expectedness) are reportable through the duration of the subject's participation on the trial (treatment and follow-up). SAEs will be followed until clinical recovery is complete and laboratory tests have returned to baseline, until progression has been stabilized, or until there has been acceptable resolution of the event. This may at times cause the follow-up period for SAEs to be greater than 90 days. Similarly, the Sponsor-Investigator is responsible for following the subject during the required follow-up period even if the subject lives elsewhere or has been released from his or her care and is being treated under another service at LCI.

10.7. Safety Reporting to the FDA

For investigator-initiated studies where the Sponsor-Investigator holds an IND, safety reporting requirements for the FDA apply in accordance with the guidance set forth in 21 CFR312.32. The Sponsor-Investigator, or research designee, will be responsible for notifying FDA (using a MedWatch form) and all participating Investigators of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the Sponsor's initial receipt of the information. For all other serious and unexpected safety events, the Sponsor-Investigator, or research designee, will notify the FDA within 15 calendar days.

It is the responsibility of the Sponsor-Investigator, Investigators and the Protocol Team to ensure SAEs are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, Institutional Review Board, and FDA policy.

Planned protocol deviations will be submitted to the FDA for prior approval only if the deviation affects the scientific validity of the study and/or the rights, safety, or welfare of subjects.

10.8. Safety Reporting to the IRB

All UAPs, protocol deviations and SAEs occurring during the conduct of a protocol will be reported to the IRB per IRB reporting requirements.

10.9. Safety Reporting to Merck

All SAEs meeting the definition of an SAE in Section 10.5 will be reportable to Merck.

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 of study treatment initiation through 90 days following cessation of study treatment, or 30 days following cessation of study treatment if the subject initiates new anti-cancer therapy, whichever is earlier, whether or not related to pembrolizumab, will be reported within 2 working days of Sponsor-Investigator awareness to Merck Global Safety by the Sponsor-Investigator.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to pembrolizumab 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-Investigator and to Merck.

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

If the Sponsor-Investigator holds an IND, a copy of all 15 Day Reports and Annual Progress Reports are to be submitted as required by the FDA 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 the appropriate regulatory agency.

10.9.1. Evaluating Adverse Events

The treating investigator or qualified designee 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.

11. MEASUREMENT OF EFFECT

11.1. Anti-tumor Effect – Solid Tumor

Response and progression will be evaluated in this study using the revised response evaluation criteria in solid tumors (RECIST) guideline version 1.1.

11.1.1. Disease Parameters

For the purposes of this study, subjects should be reevaluated for response per section 4.5.6. In addition to the baseline scan(s), confirmatory scans should also be obtained not less than 4 weeks following initial documentation of objective response. Changes in the largest diameter (unidimensional measurement) of the tumor lesions, or shortest axis for lymph nodes, are used in the RECIST criteria.
Note: Lesions are either measurable or non-measurable using the criteria provided below. The term “evaluable” in reference to measurability will not be used because it does not provide additional meaning or accuracy.

Target lesions: When more than one measurable lesion is present at baseline, all lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline. Target lesions should be selected on the

basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. Lymph nodes merit special mention since they are normal anatomical structures which may be visible by imaging even if not involved by tumor. Pathological nodes, which are defined as measurable and may be identified as target lesions, must meet the criterion of a short axis of ≥ 15 mm by CT scan. Only the short axis of these nodes will contribute to the baseline sum. The short axis of the node is the diameter normally used by radiologists to judge if a node is involved by solid tumor. Nodal size is normally reported as two dimensions in the plane in which the image is obtained (for CT scan this is almost always the axial plane). The smaller of these measures is the short axis. All other pathological nodes (those with short axis ≥ 10 mm but <15 mm) should be considered nontarget lesions. Nodes that have a short axis <10 mm are considered nonpathological and should not be recorded or followed. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then as noted above, only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions: All other lesions (or sites of disease) including pathological lymph nodes should be identified as non-target lesions and should also be followed as 'present', 'absent', or in rare cases 'unequivocal progression'.

11.1.2. Methods

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of study treatment and never more than 4 weeks before the beginning of study treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the anti-tumor effect of a treatment. The Sponsor-Investigator or designee will be responsible for performing tumor measurements. The tumor measurements will be recorded on the eCRF.

11.1.3. Response Criteria

Response will be evaluated using RECIST 1.1 Criteria.

Complete Response:

- Target lesion: Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
- Non-target lesion: Disappearance of all non-target lesions. All lymph nodes must be non-pathological in size (<10 mm short axis).

Partial Response:

- Target lesion: At least a 30% decrease in the sum of diameters of target, taking as reference the baseline sum diameters.
- Non-target lesion: Not applicable

Stable Disease:

- Target lesion: Neither sufficient shrinkage to qualify for a partial response, nor sufficient increase to qualify for progressive disease, taking as reference the smallest sum diameters while on study.
- Non-target lesion: Not applicable.

Progressive Disease:

- Target lesion: At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).
- Non-target lesion: Unequivocal progression (as described in RECIST version 1.1) of existing non-target lesions. (Note: the appearance of one or more new lesions is also considered progression).

Non-Complete Response / Non-Progressive Disease:

- Target lesion: Not applicable
- Non-target lesion: Persistence of one or more non-target lesion(s)

Table 8: Summary of RECIST 1.1 (Subjects with measurable disease)

Target Lesions	Non-target Lesions	New Lesions	Overall Response	Best Response for this Category also requires
CR	CR	No	CR	Documented at least once ≥ 4 weeks from baseline
CR	Not-evaluated	No	PR	NA
CR	Non-CR/Non-PD	No	PR	Documented at least once ≥ 4 weeks from baseline
PR	Non-PD or NE	No	PR	
SD	Non-PD or NE	No	SD	Documented at least once ≥ 4 weeks from baseline
Not all evaluated	Non-PD	No	NE	NA
PD	Any	Yes or No	PD	No prior SD, PR or CR
Any	PD*	Yes or No	PD	
Any	Any	Yes	PD	
* In exceptional circumstances, unequivocal progression in nontarget lesions may be accepted as disease progression.				

Table 9: Summary of RECIST 1.1 (Subjects with non-target disease only)

Non-target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/Non-PD	No	Non-CR/ Non-PD
Not all evaluated	No	NE
Unequivocal PD	Yes or No	PD
Any	Yes	PD

RECIST 1.1 will be adapted as follows to account for the unique tumor response seen in this class of therapeutics, **irRECIST**.

If imaging shows PD, tumor assessment should be repeated ≥4 weeks later in order to confirm PD with the option of continuing treatment for clinically stable subjects as discussed below in Table 10.

Clinically stable is defined by the following criteria:

- Absence of signs and symptoms indicating disease progression
- 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 10: irRECIST: Imaging and Treatment after 1st Radiologic Evidence of PD

	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	N/A	Discontinue treatment
Repeat scan confirms PD	No additional imaging required	Discontinue treatment (exception is possible upon consultation with Sponsor).	N/A 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	N/A	N/A

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 study 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 study 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 as outlined in Section 4.5.6 to monitor disease status. If radiologic progression is confirmed by subsequent tumor imaging, then the subject will be discontinued from study treatment.

NOTE: If a subject with confirmed progression by tumor imaging (i.e. two 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 study treatment upon consultation with the Sponsor. Subjects exhibiting intolerable toxicity from trial therapy may NOT continue to receive trial therapy.

NOTE: In subjects who discontinue study therapy without documented disease progression, every effort should be made to continue monitoring their disease status by tumor imaging as

outlined in Section 4.5.6 until (1) the start of new anti-cancer treatment, (2) disease progression (3) death, or (4) the end of the study, whichever occurs first. The same imaging modality (i.e., CT or MRI), acquisition and technical parameters should be used throughout the study for a given subject.

12. STATISTICAL CONSIDERATION

12.1. Sample Size

This is an open-label randomized pilot study designed primarily to evaluate the safety and feasibility of the two regimens described earlier (Cohort A [phased regimen] and Cohort B [concurrent regimen]), in subjects with metastatic HER2-negative breast cancer who have received no more than three prior lines of chemotherapy for metastatic disease. Based on the Bayesian stopping rules described in Section 12.4.4, 20 subjects per arm will enable an assessment for each regimen regarding whether or not evidence suggests the overall grade 3 or 4 study treatment-related adverse event rate convincingly exceeds 0.35 (the reported rate for weekly paclitaxel). Randomization to this study will continue until at least 20 subjects per arm have been randomized and begin study therapy.

12.2. Endpoint Definitions

12.2.1. Grade 3 or 4 Treatment-Related Adverse Event

Grade 3 or 4 study treatment-related adverse events will be determined for each subject as a binary variable indicating whether or not the subject experienced any grade 3 or 4 study treatment-related adverse events according to the NCI Common Terminology for Adverse Events, version 4.0. An adverse event will be considered study treatment related if it is determined that the event is at least possibly related to either paclitaxel, pembrolizumab, or both.

12.2.2. Overall Survival

Overall survival (OS) is defined as the duration of time from randomization to death from any cause. Subjects who are alive or lost to follow-up at the time of the analysis will be censored at the last known date they were alive.

12.2.3. Progression-Free Survival

Progression-Free Survival (PFS) is defined as the duration of time from randomization to first occurrence of either progressive disease or death. Disease progression must be objectively determined as per Section 11, where the date of progression is the date of the radiologic assessment that identified progressive disease. If the subject died without documented disease progression, the date of progression will be the date of death. For surviving subjects who do not have

documented disease progression, PFS will be censored at the date of last radiologic assessment. For subjects who receive subsequent anti-cancer therapy prior to documented disease progression, PFS will be censored at the date of last radiologic assessment prior to the commencement of subsequent therapy. Subjects who have an initial PFS event immediately following 2 or more consecutive missed assessments will be censored at the date of the last assessment prior to those missed assessments. For participants with only one missed assessment, the documented progressive disease status and assessment date will be used. PFS will be determined for each subject using the RECIST 1.1 criteria. For subjects treated with pembrolizumab, PFS will also be calculated using the irRECIST criteria as an exploratory endpoint.

12.2.4. Objective Response

Objective response will be determined for each subject as a binary variable indicating whether or not the subject achieved a best overall response of CR or PR as determined by criteria described in Section 11. Objective response will be determined for each subject using the RECIST 1.1 criteria. For subjects treated with pembrolizumab, objective response will also be calculated using the irRECIST criteria as an exploratory endpoint.

12.2.5. Duration of Response

Duration of Response (DoR) is defined as the duration of time from the first assessment that determined a CR or PR to the date of the first occurrence of progressive disease or death. Progression events and the censoring mechanism for DoR will be the same as described for PFS in Section 12.2.3. DoR will be determined for each subject using the RECIST 1.1 criteria. For subjects treated with pembrolizumab, DoR will also be calculated using the irRECIST criteria as an exploratory endpoint.

12.2.6. Disease Control

Disease control will be determined for each subject as a binary variable indicating whether or not the subject achieved a best overall response of CR, PR, or SD (for at least 24 weeks), as determined by the criteria described in Section 11. Disease control will be determined for each subject using the RECIST 1.1 criteria. For subjects treated with pembrolizumab, disease control will also be calculated using the irRECIST criteria as an exploratory endpoint.

12.2.7. Safety Endpoints

Safety endpoints will include study treatment administration (total number of doses taken, and total doses taken as a percent of total number of intended doses),

AEs, SAEs, deaths while on study therapy, and selected laboratory determinations.

12.3. Analysis Populations

The intent-to-treat (ITT) population will consist of all randomized subjects. The ITT population will be used for the purposes of the subject disposition summary. The evaluable population will consist of all randomized subjects who begin study therapy. OS, PFS, and all safety analyses will be conducted on the evaluable population. Analyses of objective response, DoR, and disease control will be conducted on those subjects in the evaluable population who have measureable disease at baseline.

12.4. Analysis Methods

12.4.1. Timing of Analysis

Analyses on this study will be conducted on a continuous basis in order to assess the Bayesian stopping rules for toxicity described in Section 12.4.4. An analysis of all endpoints will occur after the best overall response for each subject has been determined. A final analysis of the study will be conducted after the PFS censoring rate reduces to 20% or when all subjects have been on study for at least two years (whichever occurs first).

12.4.2. Subject Disposition

An accounting of all consenting subjects will be provided at the end of the study. This will include a breakdown of subjects who consented, were randomized, treated, discontinued study treatment, died, and were lost to follow-up or withdrew consent.

12.4.3. Baseline Patient and Disease Characteristics

A summary of subject demographics and disease-related characteristics will be completed and subject medical history will be assessed.

12.4.4. Primary Analysis

This study is planned to enroll approximately 20 subjects in each study treatment arm. The primary objective is to evaluate the overall grade 3 or 4 study treatment related adverse event rate for each study arm. It is not anticipated that the addition of pembrolizumab to standard chemotherapy will increase the grade 3 or 4 toxicity rate. It has been reported that the rate of any grade 3 or 4 toxicity with weekly paclitaxel is 0.35 [Budd, ASCO abstract 2013, CRA1008]. If it becomes evident that the grade 3 or 4 toxicity rate on either arm convincingly exceeds 0.35, the study arm will be halted. The stopping rule will hold enrollment if the posterior probability of grade 3 or 4 toxicity risk exceeding 0.35 is 0.50 or higher. The prior distribution for this monitoring rule is beta

(7,13). This means that our prior assumption regarding the proportion of grade 3 or 4 toxicity is 0.35, and there is 90% probability that this proportion is between 0.188 and 0.530. The operating characteristics of the stopping rule are given in the following table and are based on 5000 simulations.

Table 11: Operating Characteristics of the Stopping Rule

Number of grade 3 or 4Toxicities	Max. Denominator Cutoff for Early Stopping	Posterior Probability Pr(Risk>0.35|Data)
1	2	0.5352
2	5	0.5302
3	8	0.5216
4	11	0.5148
5	14	0.5040
6	16	0.5438
7	19	0.5334

12.4.5. Secondary Analyses

Overall survival and PFS will be analyzed by study arm using Kaplan Meier techniques. Medians, 25th, and 75th percentiles will be estimated. Selected landmarks for OS and PFS rates will be obtained from the Kaplan Meier estimates. Differences between the arms will be assessed qualitatively using a stratified Cox proportional hazards model, including hormone receptor status as the stratification variable. An estimate of the hazard ratio and 95% confidence interval will be provided. Duration of response will be analyzed in a similar fashion as described for OS and PFS. Objective response rate and disease control rate will be estimated for each arm along with corresponding 95% Clopper-Pearson confidence intervals. Differences between the arms will be assessed qualitatively using a stratified logistic regression, including hormone receptor status as the stratification variable. Estimates of the odds ratios and 95% confidence intervals will be provided.

12.4.6. Safety Analyses

Incident rates for treatment-emergent adverse events, SAEs, and deaths while on study therapy will be summarized. Treatment-emergent adverse events are defined as follows:

- An adverse event that occurs after study treatment start that was not present at the time of study treatment start; or

- An adverse event that increases in severity after study treatment start if the event was present at the time of study treatment start.

12.4.7. Exploratory Analyses

Cox proportional hazards models and logistic regression will be used to assess the correlation between additional baseline biomarkers with clinical outcomes. The biomarkers identified in Section 1.4 will be assessed qualitatively as deemed appropriate.

12.5. Interim Analyses

No formal interim analysis for efficacy is planned for this study. However, results will be continuously monitored for safety using the Bayesian stopping rules described in Section 12.4.4.

13. STUDY COMPLETION

13.1. Completion

The study will be considered complete when one or more of the following conditions is met:

- All subjects have completed all study visits.
- All subjects have discontinued from the study.
- The IRB, FDA, LCI DSMC, Sponsor-Investigator discontinues the study because of safety considerations.
- The Sponsor-Investigator defines an administrative or clinical cut-off date.

13.2. Termination

The study will be terminated when one or more of the following conditions occur:

If risk-benefit ratio becomes unacceptable owing to, for example:

- Safety findings from this study (e.g. SAEs)
- Results of any interim analysis
- Results of parallel clinical studies
- Results of parallel animal studies (e.g. toxicity, teratogenicity, carcinogenicity or reproduction toxicity).
- If the study conduct (e.g. recruitment rate; drop-out rate; data quality; protocol compliance) does not suggest a proper completion of the trial within a reasonable time frame.

The Sponsor-Investigator has the right to close the trial at any site and at any time.

For any of the above closures, the following applies:

- Closures should occur only after consultation between involved parties.
- All affected institutions must be informed as applicable according to local law.
- In case of a partial study closure, ongoing subjects, including those in follow-up, must be taken care of in an ethical manner.

14. RETENTION OF RECORDS

Essential documentation (e.g. adverse events, records of study drug receipt and dispensation), including all IRB correspondence, will be retained for at least 2 years after the investigation is completed. Documentation will be readily available upon request.

15. ETHICAL AND LEGAL ISSUES

15.1. Ethical and Legal Conduct of the Study

The procedures set out in this protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the Investigator abide by Good Clinical Practice (GCP) guidelines and under the guiding principles detailed in the Declaration of Helsinki. The study will also be carried out in keeping with applicable local law(s) and regulation(s).

Documented approval from appropriate agencies (e.g. DSMC, IRB, FDA) will be obtained for all participating centers before start of the study, according to GCP, local laws, regulations and organizations. When necessary, an extension, amendment or renewal of IRB approval must be obtained and forwarded to Merck (if applicable).

Strict adherence to all specifications laid down in this protocol is required for all aspects of study conduct; the Investigators may not modify or alter the procedures described in this protocol.

Modifications to the study protocol will not be implemented by the Sponsor-Investigator without discussion and agreement by Merck (if applicable). However, the Investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to the trial subjects without prior approval from applicable agencies. As soon as possible, the implemented deviation or change, the reasons for it and if appropriate the proposed protocol amendment should be submitted to the appropriate agencies. Any deviations from the protocol must be explained and documented by the Investigator.

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

The Sponsor-Investigator will be responsible for assuring that all the required data will be collected and properly documented.

15.2. Confidentiality

All records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available.

16. PUBLICATION POLICY

The Investigator must send a draft manuscript of the publication or abstract to Merck Sharp & Dohme Corp, prior to submission of the final version for publication or congress presentation. All relevant aspects regarding data reporting and publication will be part of the contract between Merck Sharp & Dohme Corp and the Investigator.

The Investigator will ensure that the information regarding the study be publicly available on the internet at www.clinicaltrials.gov.

REFERENCES

1. American Cancer Society. Cancer Facts & Figures 2015. Atlanta: American Cancer Society; 2015.
2. Piccart-Gebhart MJ, Burzykowski T, Buyse M, et al: Taxanes alone or in combination with anthracyclines as first-line therapy of patients with metastatic breast cancer. J Clin Oncol 26:1980-6, 2008
3. Seidman AD, Tiersten A, Hudis C, et al: Phase II trial of paclitaxel by 3-hour infusion as initial and salvage chemotherapy for metastatic breast cancer. J Clin Oncol 13:2575-81, 1995
4. Nabholtz JM, Gelmon K, Bontenbal M, et al: Multicenter, randomized comparative study of two doses of paclitaxel in patients with metastatic breast cancer. J Clin Oncol 14:1858-67, 1996
5. Seidman AD, Berry D, Cirrincione C, et al: Randomized phase III trial of weekly compared with every-3-weeks paclitaxel for metastatic breast cancer, with trastuzumab for all HER-2 overexpressors and random assignment to trastuzumab or not in HER-2 nonoverexpressors: final results of Cancer and Leukemia Group B protocol 9840. J Clin Oncol 26:1642-9, 2008
6. Mauri D, Kamposioras K, Tsali L, et al: Overall survival benefit for weekly vs. three-weekly taxanes regimens in advanced breast cancer: A meta-analysis. Cancer Treat Rev 36:69-74, 2010
7. Hodi FS, O'Day SJ, McDermott DF, et al: Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 363:711-23, 2010
8. Kantoff PW, Higano CS, Shore ND, et al: Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med 363:411-22, 2010
9. Chen DS, Irving BA, Hodi FS: Molecular pathways: next-generation immunotherapy--inhibiting programmed death-ligand 1 and programmed death-1. Clin Cancer Res 18:6580-7, 2012
10. Pedoeem A, Azoulay-Alfaguter I, Strazza M, et al: Programmed death-1 pathway in cancer and autoimmunity. Clin Immunol 153:145-52, 2014
11. Sheppard KA, Fitz LJ, Lee JM, et al: PD-1 inhibits T-cell receptor induced phosphorylation of the ZAP70/CD3zeta signalosome and downstream signaling to PKCtheta. FEBS Lett 574:37-41, 2004
12. Yao S, Chen L: PD-1 as an immune modulatory receptor. Cancer J 20:262-4, 2014
13. Keir ME, Butte MJ, Freeman GJ, et al: PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol 26:677-704, 2008
14. Sanmamed MF, Chen L: Inducible expression of B7-H1 (PD-L1) and its selective role in tumor site immune modulation. Cancer J 20:256-61, 2014
15. Topalian SL, Drake CG, Pardoll DM: Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer Cell 27:450-61, 2015
16. Pembrolizumab Investigator's Brochure. Edition 9.
17. Robert C, Ribas A, Wolchok JD, et al: Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. Lancet 384:1109-17, 2014

18. Ribas A, Puzanov I, Dummer R, et al: Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. Lancet Oncol 16:908-18, 2015

19. Robert C, Schachter J, Long GV, et al: Pembrolizumab versus Ipilimumab in Advanced Melanoma. N Engl J Med 372:2521-32, 2015

20. Nanda R, Chow Q, Dees C, et al. Pembrolizumab in patients with advanced triple-negative breast cancer: Phase Ib KEYNOTE-012 study. J Clin Oncol. 2016:Published online on May 2, 2016.

21. Chen G, Emens LA: Chemoimmunotherapy: reengineering tumor immunity. Cancer Immunol Immunother 62:203-16, 2013

22. National Comprehensive Cancer Network Clincal Practice Guidelines in Oncology. Breast. Version 1.2016.

23. DeNardo DG, Brennan DJ, Rexhepaj E, et al: Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. Cancer Discov 1:54-67, 2011

24. Zhang L, Dermawan K, Jin M, et al: Differential impairment of regulatory T cells rather than effector T cells by paclitaxel-based chemotherapy. Clin Immunol 129:219-29, 2008

25. Nizar S, Copier J, Meyer B, et al: T-regulatory cell modulation: the future of cancer immunotherapy? Br J Cancer 100:1697-703, 2009

26. Chan OT, Yang LX: The immunological effects of taxanes. Cancer Immunol Immunother 49:181-5, 2000

27. Kaufmann DE, Walker BD: Programmed death-1 as a factor in immune exhaustion and activation in HIV infection. Curr Opin HIV AIDS 3:362-7, 2008

28. Pardoll DM: The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 12:252-64, 2012

29. Lynch TJ, Bondarenko I, Luft A, et al: Ipilimumab in combination with paclitaxel and carboplatin as first-line treatment in stage IIIB/IV non-small-cell lung cancer: results from a randomized, double-blind, multicenter phase II study. J Clin Oncol 30:2046-54, 2012

30. Reck M, Bondarenko I, Luft A, et al: Ipilimumab in combination with paclitaxel and carboplatin as first-line therapy in extensive-disease-small-cell lung cancer: results from a randomized, double-blind, multicenter phase 2 trial. Ann Oncol 24:75-83, 2013

31. Rhen T, Cidlowski JA: Antiinflammatory action of glucocorticoids--new mechanisms for old drugs. N Engl J Med 353:1711-23, 2005

32. Sparano JA, Wang M, Martino S, et al: Weekly paclitaxel in the adjuvant treatment of breast cancer. N Engl J Med 358:1663-71, 2008

33. Markman M, Kennedy A, Webster K, et al: An effective and more convenient drug regimen for prophylaxis against paclitaxel-associated hypersensitivity reactions. J Cancer Res Clin Oncol 125:427-9, 1999

34. Berger MJ, Vargo C, Vincent M, et al: Stopping paclitaxel premedication after two doses in patients not experiencing a previous infusion hypersensitivity reaction. Support Care Cancer 23:2019-24, 2015

35. Quock J, Dea G, Tanaka M, et al: Premedication strategy for weekly paclitaxel. Cancer Invest 20:666-72, 2002

36. Mittendorf EA, Philips AV, Meric-Bernstam F, et al: PD-L1 expression in triple-negative breast cancer. Cancer Immunol Res 2:361-70, 2014

37. Basu GD, Ghazalpour A, Vader R, et al. Expression of novel immunotherapeutic targets in luminal breast cancer patients. Presented at San Antonio Breast Cancer Symposium. December 9-13, 2014. San Antonio, TX. Abstract P5-04-08.

38. Loi S, Sirtaine N, Piette F, et al: Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. J Clin Oncol 31:860-7, 2013

39. Adams S, Gray RJ, Demaria S, et al: Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199. J Clin Oncol 32:2959-66, 2014

40. Salgado R, Denkert C, Demaria S, et al: The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol 26:259-71, 2015

41. Schalper KA: PD-L1 expression and tumor-infiltrating lymphocytes: Revisiting the antitumor immune response potential in breast cancer. Oncoimmunology 3:e29288, 2014

42. Le DT, Uram JN, Wang H, et al: PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med 372:2509-20, 2015

43. Lipson EJ, Velculescu VE, Pritchard TS, et al: Circulating tumor DNA analysis as a real-time method for monitoring tumor burden in melanoma patients undergoing treatment with immune checkpoint blockade. J Immunother Cancer 2:42, 2014

APPENDICES

APPENDIX 1: ECOG PERFORMANCE SCALE

Grade	Description
0	Normal activity, fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but fully ambulatory, restricted in physically strenuous but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	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	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead