CITY OF HOPE NATIONAL MEDICAL CENTER
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DUARTE, CA 91010
DEPARTMENT OF MEDICAL ONCOLOGY AND MOLECULAR THERAPEUTICS

TITLE: Phase II Study of the combination of Pembrolizumab, Letrozole and Palbociclib in Postmenopausal Patients with Newly Diagnosed Metastatic Estrogen Receptor Positive Breast Cancer

CITY OF HOPE PROTOCOL VERSION: IRB # 16058 PROTOCOL DATE:09/27/2024
DATE(S)/ OF AMENDMENT(S)/REVISION(S):

COH Initial Approval	(Protocol, Initial Dated 05/26/2016)	Version: 00
COH Amendment # 1	(Protocol Amendment 1, dated 08/16/2016)	Version: 01
COH Amendment # 2	(Protocol Amendment 2, dated 10/25/2016)	Version: 02
COH Amendment # 3	(Protocol Amendment 3, dated 11/15/2016)	Version: 03
COH Amendment # 4	(Protocol Amendment 3, dated 11/15/2016)	Version: 04
COH Amendment # 5	(Protocol Amendment 3, dated 11/15/2016)	Version: 05
COH Amendment # 6	(Protocol Amendment 3, dated 11/15/2016)	Version: 06
COH amendment # 7	(Protocol, Dated 04/14/2017)	Version: 07
COH amendment # 8	(Protocol, dated 07/25/2017)	Version: 08
COH amendment # 9	(Protocol, dated 09/20/2017)	Version: 09
COH amendment #10	(Protocol, dated 09/20/2017)	Version: 10
COH amendment #11	(Protocol, dated 10/26/2017)	Version: 11
COH Amendment #12	(Protocol, dated 11/16/2017)	Version: 12
COH Amendment #13	(Protocol, dated 11/16/2017)	Version: 13
COH Amendment #14	(Protocol, dated 11/16/2017)	Version: 14
COH Amendment #15	(Protocol, dated 11/16/2017)	Version: 15
COH Amendment #16	(Protocol, dated 03/23/2018)	Version: 16
COH Amendment #17	(Protocol, dated 06/25/2018)	Version: 17
COH Amendment #18	(Protocol, dated 10/17/2018)	Version: 18
COH Amendment #19	(Protocol dated 10/18/2018)	Version: 19
COH Amendment #20	(Protocol dated 10/18/2018)	Version: 20
COH Amendment #21	(Protocol, dated 12/13/2018)	Version: 21
COH Amendment #22	(Protocol, dated 12/13/2018)	Version: 22
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COH Amendment #24	(Protocol dated 01/08/2019)	Version: 24
COH Amendment #25	(Protocol dated 04/30/2019)	Version: 25
COH Amendment #26	(Protocol dated 04/30/2019)	Version: 26
COH Amendment #27	(Protocol dated 07/03/2019)	Version: 27
COH Amendment #28	(Protocol dated 07/03/2019)	Version: 28
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COH Amendment #32	(Protocol, dated 07/10/2020)	Version: 32
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SITE: Breast

STAGE (If applicable): Stage IV

MODALITY: Chemotherapy

TYPE: Phase II

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STUDY SPONSOR AND MONITOR:	City of Hope National Cancer Center
AGENT NSC # AND IND #:	MK-3475 (pembrolizumab); 776864 Letrozole; 719345 Palbociclib; 772256
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Clinical Trial Protocol

Phase II Study of the combination of Pembrolizumab, Endocrine Therapy and Palbociclib in Postmenopausal Patients with Newly Diagnosed Metastatic Estrogen Receptor Positive Breast Cancer

Version Date: 09/27/24

Protocol Packet: 53

City of Hope #: 16058

Agent NSC # and IND #:

Pembrolizumab (MK-3475) 776864

Letrozole 719345

Palbociclib 772256

Sponsor: City of Hope

Funding Support: Merck

Industry Partner: Merck

NCT Number: NCT02778685

Participating Sites: City of Hope (Duarte)

Short Title: Pembrolizumab, letrozole and palbociclib in advanced ER+HER2- breast cancer

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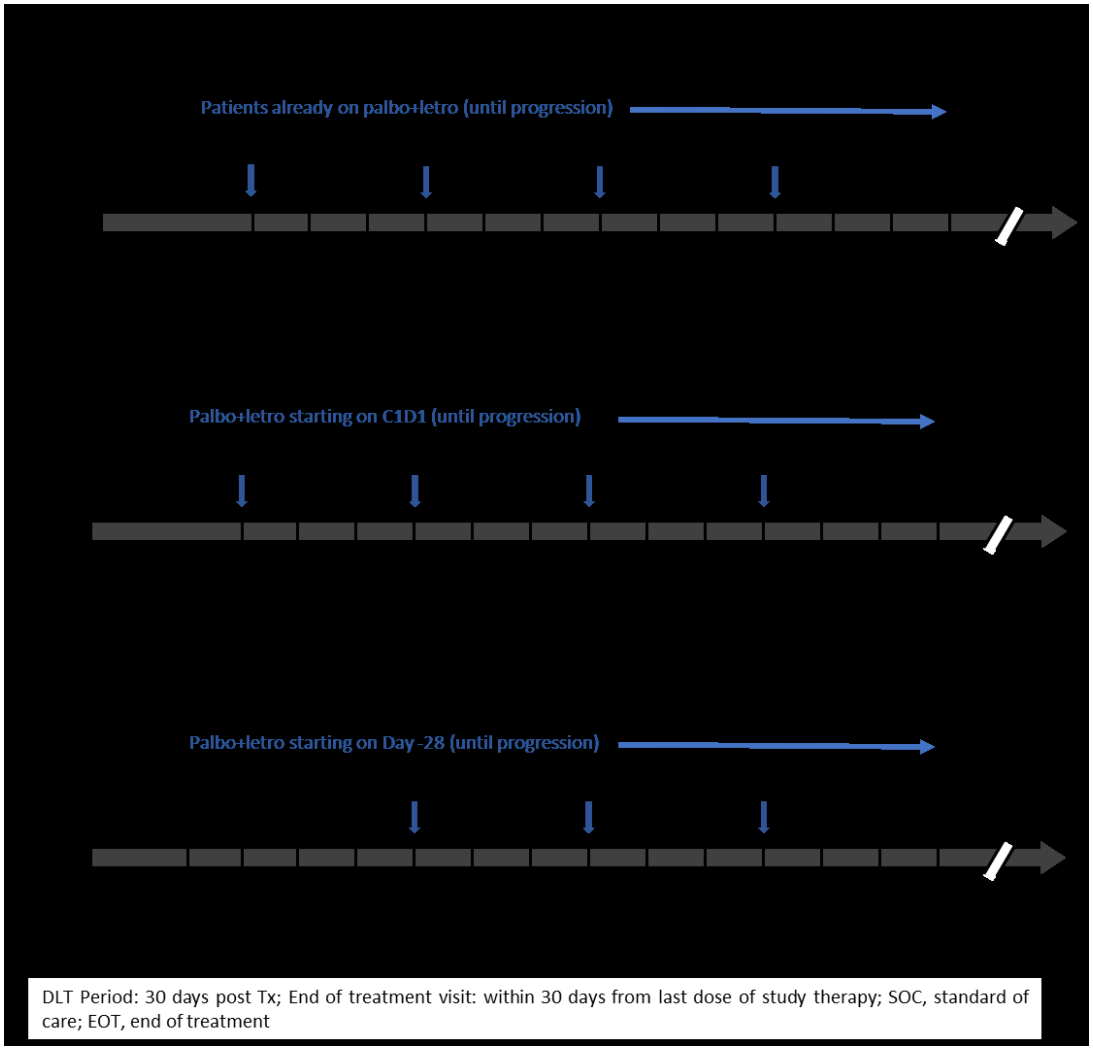
Study Schema



Protocol Synopsis

Protocol Title
Phase II study of the combination of pembrolizumab, letrozole and palbociclib in postmenopausal patients with newly diagnosed metastatic estrogen receptor positive breast cancer
Brief Protocol Title for the Lay Public (if applicable)
Pembrolizumab, letrozole and palbociclib in advanced ER+HER2- breast cancer
Study Phase
Phase II
Participating Sites
City of Hope Comprehensive Cancer Center
Rationale for this Study
The estrogen receptor (ER) pathway plays a pivotal role in ER-positive (ER+) breast cancer development and progression, and endocrine therapy forms the backbone of the therapeutic regimen for patients with ER+ breast cancer. Endocrine therapy, including SERM tamoxifen; aromatase inhibitors letrozole, anastrozole, and exemestane; and ER down-regulator fulvestrant represent the key treatment options targeting the ER pathway. These treatments are highly effective, but their usefulness is limited by common intrinsic and acquired resistance. Multiple mechanisms responsible for endocrine resistance have been proposed and include deregulation of various components of the ER pathway itself, alterations in cell cycle and cell survival signaling molecules, and the activation of escape pathways that can provide tumors with alternative proliferative and survival stimuli. A randomized phase II study comparing palbociclib with letrozole versus letrozole alone as first line treatment of ER+HER2- advanced breast cancer demonstrated a significant progression-free survival (PFS) benefit of 20.2 months for the combination therapy versus 10.2 months for letrozole alone (Hazard Ration [HR] 0.488, 95% CI 0.319-0.748; P = 0.0004). A subsequent Phase III study demonstrated PFS of 24.8 months for the combination, as compared to 14.5 months for single agent letrozole, and a confirmed objective response rate of 55% (95% CI 0.50-0.61) in patients with measurable disease. This combination has become the standard of care for patients with ER+ breast cancer. Despite the progress being made, we seek to continue to improve tumor responsiveness, as 5% of patients treated with letrozole and palbociclib do not have an objective response and only 2% have a complete response. Single agent pembrolizumab has a response rate of 12% in selected patients with PD-L1 positive metastatic ER+HER- breast cancer. CDK4/6 inhibitors may have immune-modulatory effects. Therefore, there is an urgent need to assess the potential efficacy and safety of combination therapy of pembrolizumab, letrozole and palbociclib seeking a deeper and more prevalent response to first-line therapy Although growing evidence suggests that triple negative breast cancer (TNBC) is the most immunogenic breast cancer subtype, PD-L1 expression has also been detected in 33% of luminal B and A tumors and PD-1 expression has been detected in 44% and 25% of luminal B and A tumors (n=58), with concurrent expression seen in 17% and 13%, respectively. There is therefore a strong biological rationale to add pembrolizumab to letrozole and palbociclib to improve clinical response by RECIST and/or immune-related Response Criteria in Solid Tumors (irRECIST) to prolong duration of response (DOR), time to progression, and overall survival (OS). This study will evaluate the activity, feasibility, and tolerability of the triple drug

combination. In addition, a built-in immune correlative study will fill the current knowledge gap in the safety and efficacy data in this extremely important area of research.

Cohort 3 Rationale:

Strong preclinical evidence of the immune-stimulatory effect of the CDK4/6 inhibitor palbociclib, and its complimentary mode of action and minimal overlapping toxicities with anti-PD-1 agents lead to the combination trials of CDK 4/6 inhibitor and immune checkpoint inhibitor. In NCT02779751 study, abemaciclib + pembrolizumab + anastrozole was tested in patients with HR+HER2- MBC, an ORR of 23% (n=26) was observed. 46% had grade >3 transaminase elevation led to discontinued therapy. Interstitial lung disease (ILD)/pneumonitis were found in 4 patients (15%) including 2 patients with fatal outcome (Rugo et. al. AACR 2020). Benefit/risk data do not support further development of abemaciclib + pembrolizumab + anastrozole in this population.

Our current study combining letrozole, palbociclib and pembrolizumab for patients with ER+ metastatic breast cancer (NCT02778685). A total of 23 patients were enrolled in cohort 1&2 and 20 were evaluable for efficacy. The combination is safe with few SAE observed. Response analysis per RECIST 1.1 were as follows: 2/20 complete response (CR, 10%), 11/20 partial response (PR, 55%), 5/20 stable disease (SD, 25%), and 2/20 progressive disease (PD, 10%). Although the response rate of 65% was encouraging, the study was limited by small sample size. Peripheral blood mononuclear cell (PBMC) flow cytometry analysis showed the potential role of PBMCs in predicting response to the combination therapy. We hypothesize that palbociclib may increase CD8+ EMRA T cells and CD4+ EM T cells and enhance immune response to pembrolizumab in HR+HER2- metastatic breast cancer. Here we propose to further investigate the combination of palbociclib, pembrolizumab and endocrine therapy as first-line treatment in patients with metastatic HR^+ $HER2^-$ breast cancer. Patients receiving hormonal therapy, including aromatase inhibitor +/- ovarian suppression or fulvestrant are eligible. A palbociclib + endocrine therapy lead-in design will be used, starting on day -28 followed by combination therapy with pembrolizumab added on C1D1. Peripheral blood and required on-treatment biopsy would allow in-depth analysis of biomarker predicting response to the combination. The current design will also allow a better understanding of the immune potentiating effect of palbociclib prior to the combination with pembrolizumab. The overall goal of cohort 3 is to confirm the response rate of the combination therapy, and to better understand the mechanism of action of the synergy between palbociclib and pembrolizumab.

Objectives

<u>Cohort 1&2 Primary Objective</u>

To evaluate the objective response rate (ORR=PR+CR) based on RECIST version 1.1 when combining pembrolizumab to letrozole and palbociclib in patients with newly diagnosed metastatic ER+HER2- breast.

<u>Cohort 1&2Secondary Objectives</u>

To determine the safety and tolerability of adding pembrolizumab (200 mg every 3 weeks) to letrozole (2.5 mg) and palbociclib (125mg, 3 weeks on, one week off) in patients with metastatic ER+HER2- breast cancer.

<u>Additional Secondary Objectives</u>:

- To evaluate the CR rate.

- To evaluate PFS (progression-free survival).
- To evaluate OS.
- To evaluate DOR using RECIST version 1.1.
- To evaluate clinical benefit rate (CBR) using RECIST version 1.1.
- To evaluate toxicities (using the NCI Common Terminology Criteria for Adverse Events [CTCAE], version 4.0) associated with the triple drug combination (pembrolizumab, letrozole, and palbociclib) in patients with metastatic ER+HER2- breast cancer.
- To evaluate CR, PR, ORR, PFS, DOR, and CBR using irRECIST. Time to treatment failure will also be assessed.
- To evaluate in a separate cohort (cohort 1: patients enrolled prior to amendment to change the study to first-line, n=6) the addition of pembrolizumab to patients who had stable disease after at least 6 months of therapy on palbociclib plus letrozole.

Cohort 1&2 Exploratory Objectives

We will collect serial tumor biopsies (primary/metastatic prior to study treatment, repeat biopsy upon progression) and serial peripheral blood samples. Optional on-treatment biopsy is also allowed for assessment of immune response. Studies include the following

- Cellular/humoral immune response by analyzing immune and stromal cell characteristics before and after treatment that correlate with clinical response. This includes PD-L1 expression levels.
- The peripheral serum thymidine kinase (TK) level and its association with treatment response.
- Circulating tumor DNA (ctDNA) and the effect of combining pembrolizumab, letrozole, and palbociclib on ctDNA profiles.
- To evaluate genomic and phenotypic status of breast tumor

Cohort 3 Primary objectives:

- To evaluate the clinical response rate (CR or PR via RECIST 1.1)
- To evaluate dynamic changes in host peripheral blood in predicting response to treatment: CD8+ EMRA T cells; CD4+ EM T cells; classic monocytes ($CD14^{+}$ $CD16^{-}$) and Non-classic monocytes ($CD14^{Dim-}$ $CD16^{+}$)

Cohort 3 Secondary objectives:

- To further evaluate the safety/tolerability of the combination
- To evaluate the PFS, DOR (time from documentation of tumor response to disease progression or death), Overall survival (OS)
- Cellular/humoral immune response by analyzing immune and stromal cell characteristics before and after treatment that correlate with clinical response.
- Circulating tumor DNA (ctDNA) changes

Study Design:

This is an open-label single institutional trial for evaluating patients with newly diagnosed stage IV, metastatic ER+ breast cancer treated with the combination of pembrolizumab, endocrine therapy and palbociclib. Patients must have confirmed ER+HER2- breast cancer, with measurable disease by RECIST, version 1.1. For cohort 1&2, eligible patients will receive letrozole (2.5 mg)

once a day and palbociclib (125 mg) once a day for 3 weeks on and 1 week off. Pembrolizumab will be given at 200 mg IV every 3 weeks.

For cohort 3, patients will receive palbociclib and endocrine therapy (letrozole or fulvestrant) starting day-28. Palbociclib will be given 3 weeks on 1 week off. Fulvestrant dose 500mg on day -28 and day -14. On cycle 1 day 1, pembrolizumab will be added to the combination of palbociclib and endocrine therapy.

Study treatment will continue until disease progression, unacceptable adverse events (AEs), concurrent illness that prevents further administration of study treatment, investigator's decision to withdraw the subject from study treatment, consent withdrawal, becoming lost-to-follow-up, death, or for administrative reasons requiring cessation of treatment.

After discontinuation of study treatment, each subject will be followed for 30 days for AE monitoring. Serious adverse events (SAEs) and immune-related adverse events (irAEs) will be collected for 90 days after the end of study treatment or until the subject initiates new anticancer therapy for a minimum of 30 days, whichever occurs earlier.

Subjects who discontinue study treatment for reasons other than disease progression will have post-treatment follow-up for disease status approximately every 12 weeks until disease progression (verified by restaging imaging), start of a non-study anticancer treatment, consent withdrawal, becoming lost to follow-up, death, or end of the study. Optional peripheral blood or biopsy sample can be taken if patient was off trial for reasons other than disease progression. In addition, restaging imaging can be assessed for RECIST measurement.

All subjects will be followed by telephone every 4 cycles (±7 days) for OS until consent withdrawal, becoming lost to follow-up, death, or end of the study.

A separate cohort (cohort 1) of patients enrolled were previously on letrozole and palbociclib with stable disease and received the addition of pembrolizumab. This is cohort 1, corresponding to the previous version of the protocol (cohort closed at 6 patients).

Cohort 1 & 2 have completed accrual. Additional cohort 3 is added as described above.

Endpoints

Primary Endpoint

The primary endpoint is the ORR for patient with metastatic ER+HER2- breast cancer when combining pembrolizumab, letrozole and palbociclib.

Secondary Endpoint

A key secondary endpoint is the safety and tolerability of pembrolizumab plus the letrozole/palbociclib combination. Safety analysis will be carried out based on toxicities assessed by CTCAE, version 4.0. Adverse events will be analyzed including but not limited to AEs (grade 2 or higher), SAEs, fatal AEs, and laboratory changes. Immune-related adverse events will also be collected. We will also use CR rate, CBR, DOR, PFS, and OS to test the efficacy of therapy of the novel drug combination assessed by RECIST version 1.1. Any event (toxicity-related study discontinuation or progression) will be counted as "treatment failure" and evaluated in time-to-treatment failure (and failure-free survival). Kaplan-Meier estimates will be generated for PFS, failure-free survival and OS. Patients enrolled on prior protocol version allowing for adding pembrolizumab to at least 6 months of SD on palbociclib and letrozole will be separately evaluated for efficacy and toxicity endpoints (cohort 1).

Exploratory analysis to assess response and clinical benefit will be carried out using irRECIST, an adaptation of RECIST version 1.1 to account for the unique tumor response characteristics to

treatment with new immunotherapeutic agents, including pembrolizumab. RECIST version 1.1 was developed based on treatment with cytotoxic agents.

Sample Size

47 patients: Cohort 1 (6 patients adding pembrolizumab to SD patients on palbociclib plus letrozole- closed to accrual)

Cohort 2 (16 patients first-line therapy, closed to accrual)

Cohort 3 (25 patients first-line therapy)

Estimated Duration of the Study

Cohort 1&2 are closed to accrual. Cohort 3 will require approximately 24 months for accrual, approximately 18 months of minimum follow-up to assess response, and continued follow-up for 36 months to assess PFS, and OS and evaluate for late developing responses.

Summary of Subject Eligibility Criteria

Inclusion Criteria

1. Willing and able to provide written informed consent/assent for the trial.
2. Willing and able to comply with all aspects of the treatment protocol.
3. Men or women ≥18 years of age on day of signing informed consent.
4. Postmenopausal women defined by at least one of the following criteria:
    a. Prior bilateral oophorectomy OR amenorrheic for ≥12 months (if ≤55 years of age and prior to chemotherapy, or on medical ovarian ablative therapy **OR**;
    b. Previous hysterectomy with one or both ovaries left in place (previous hysterectomy in which documentation of bilateral oophorectomy is unavailable AND FSH values consistent with the institutional normal values for the post-menopausal state. FSH levels must be obtained within 28 days prior to registration).
5. Presence of measurable disease meeting the following criteria: At least 1 lesion of >10 mm in long axis diameter for non-lymph nodes or >15 mm in short axis diameter for lymph nodes that is serially measurable according to RECIST, version 1.1 using computerized tomography, magnetic resonance imaging, or panoramic and close-up color photography.
6. *Stage IV metastatic ER+HER2- breast cancer histologically proven per current ASCO/CAP guidelines. Allow up to 30 days prior use of CDK4/6 inhibitors and up to 60 days of prior use of letrozole or other aromatase inhibitors for treatment of metastatic ER+ breast cancer
7. Life expectancy of ≥ 3 months.
8. Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0-2.
9. Willing to provide tissue from a newly obtained core or excisional biopsy of a tumor lesion. Newly obtained is defined as a specimen obtained up-to 6 weeks (42 days) prior to initiation of treatment on Day 1 and day -28 for cohort 3. Subjects for whom newly obtained samples cannot be provided (e.g., inaccessible or subject safety concern) may submit an archived specimen only upon agreement from the study PI.
10. For Cohort 3, baseline, C2 D1(+/- 7 days) on treatment and optional end of treatment biopsy will be acquired.

11. Demonstrate adequate organ function. All screening labs should be performed within 10 days of treatment initiation.
12. Female subjects of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
13. Female subjects of childbearing potential should be willing to use two methods of birth control or be surgically sterile or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication. Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.

* Cohort 1 (accrual to 6 patients) is for patients who had ongoing SD on letrozole + palbociclib, enrolled on prior version were enrolled to receive pembrolizumab after obtaining stable disease on letrozole + palbociclib. These patients must have been on treatment with letrozole and palbociclib for 6 months with SD per RECIST 1.1; received up to 3 lines of previous therapy including endocrine and/or chemotherapy in advanced setting prior to initiation of letrozole and palbociclib; no grade 3 toxicities except alopecia.

Cohort 1&2 are closed to accrual. Only cohort 3 is open to accrual

Exclusion Criteria

1. Patients currently participating and receiving study therapy or who have participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of treatment.
2. Previously received pembrolizumab or other anti-PD-1 or anti-PD-L1 immunotherapy.
3. Does not have measurable disease per RECIST version 1.1.
4. For cohort 2, received > 30 days of prior treatment CDK4/6 inhibitors or received > 60 days of prior use of letrozole before screening.
5. Diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment.
6. Known history of active TB (Bacillus Tuberculosis).
7. Hypersensitivity to pembrolizumab or any of its excipients.
8. Prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to study Day 1 or has not recovered (i.e., ≤ Grade 1 or at baseline) from AEs due to agents administered > 4 weeks earlier.
9. Prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 1 or has not recovered (i.e., ≤ Grade 1 or at baseline) from AEs due to a previously administered agent.

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

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

10. Known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer.
11. Known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Patients with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least 4 weeks prior to the first dose of trial treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis, which is excluded regardless of clinical stability.
12. Active autoimmune disease that has required systemic treatment in the past 2 years (i.e., with use of disease modifying agents, corticosteroids, or immunosuppressive drugs). Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.
13. History of (non-infectious) pneumonitis that required steroids or current pneumonitis..
14. History of interstitial lung disease.
15. Clinically active diverticulitis, intra-abdominal abscess, gastrointestinal (GI) obstruction, or abdominal carcinomatosis (known risks factors for bowel perforation).
16. Active infection requiring systemic therapy.
17. History of significant cardiovascular disease, defined as: congestive heart failure greater than New York Heart Association (NYHA) Class II according to the NYHA Functional Classification; unstable angina or myocardial infarction within 6 months of enrollment; or serious cardiac arrhythmia.
18. Clinically significant electrocardiogram (ECG) abnormality, including a marked baseline prolonged QT/QTc ([QT interval/corrected QT interval], e.g., a repeated demonstration of a QTc interval >480 ms), a family or personal history of long or short QT syndrome, Brugada syndrome or known history of QTc prolongation, or Torsade de Pointes (TdP).
19. Concurrent use of drugs that are known to be moderate or strong CYP3A inhibitors or inducers or drugs that are known to prolong the QT interval (Refer to **Tables 5** and **17**).
20. History or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the patient's participation for the full duration of the trial, or is not in the best interests of the patient to participate, in the opinion of the treating investigator.
21. Pregnant, breastfeeding or expecting to conceive children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment.
22. Known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
23. Known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).
24. Known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).
25. Received a live vaccine within 30 days of planned start of study therapy.

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

Investigational Product Dosage and Administration

Details on preparation and administration of pembrolizumab (MK-3475) are provided in the Pharmacy Manual (200 mg every 3 weeks IV infusion Day 1 of each 3-week cycle; every 4 weeks after completion of pembrolizumab). Letrozole 2.5 mg po daily will be administered as per package insert. For cohort2, palbociclib 125mg po daily will be administered for 3 weeks on and one week off, and otherwise as per approval package insert. For cohort 1, palbociclib dose is established prior to entry of the study (75mg, 100mg or 125mg). Drug compliance will be documented in the EMR at study visits (pill diary collection is not required)

Clinical Observations and Tests to be Performed

Patients who fulfill the eligibility criteria will undergo the full informed consent process. Prior to starting treatment, all subjects will undergo history and physical exam and laboratory studies including CBC, chemistry, and liver function tests.

Baseline disease will be documented with CT scan of chest, abdomen and pelvis and bone scan (within 30 days prior to starting treatment). All subjects will have cardiac function evaluation using an EKG.

CBC with differential and comprehensive serum chemistry panel will be performed on Day 1 of each 3-week cycle (pembrolizumab administration days) through the duration of the study. The study team will monitor the CBCs for dose limiting toxicities (DLTs) evaluation during the first 6 weeks of the study. For Cohort 3, CBC with diff and CMP will be performed on day -28 and day -14.

EKG will be performed at baseline for QTc measurement.

Restaging imaging (CT, bone scan) will be repeated every 12 weeks (+/-7days). At screening, staging imaging within 35 days (5 weeks) of study entry is allowed.

During screening, brain imaging will be performed in subjects with known brain metastases (such subjects also need records of brain imaging performed within the last 3 months prior to screening to establish stability)For cohort 1&2, baseline tumor biopsies will be collected. For cohort 3, tumor biopsies will be collected at baseline (within 6 weeks of study onset), C2D1 (+/-1 week) and at time-of-progression or end-of-treatment.

When a core needle biopsy is used, a minimum of 4 core biopsy samples with a minimum 18-gauge needle is recommended.

Peripheral blood samples will be obtained on Day 1 of cycles 1, 2, 4, and end of study. Three tubes (1 x 10 ml green-top, 2x 10 ml lavender-top) of blood will be collected and delivered to the correlative study PI's laboratory. For cohort 3, day -28 blood will also be collected.

Statistical Considerations

<u>Cohort 1&2 Primary Endpoint</u>

Response (CR or PR by RECIST version 1.1) due to letrozole + palbociclib is 55%, with rare complete responses. This short Phase II study is designed to demonstrate that pembrolizumab enhances the activity of letrozole + palbociclib. As a result, we require, at a minimum, that the RR exceeds 55% or that we see complete responses. As a result, if 8 responses out of 16 patients are observed (50%), the triplet would be considered lacking promise. This would happen with a 7.4% type II error if the true response rate was 70%. If 9-11 (56%-69%) responses are observed, other considerations such as the observation of CRs, and the duration of responses or progression-

free survival will be needed to determine if the combination is worthwhile, and if 12 responses out of 16 patients are observed (75%), the combination would be declared promising. The chance of a 55% response rate resulting in 12 responses is less than 9% (type I error).

Cohort 1&2 Safety Stopping Rules

Cohort 1 is closed for accrual issues and unrelated to toxicity. Cohort 1 previously had the three-at-risk safety lead-in design, with 1 patient with a delay in treatment due to wound healing that was attributed to treatment and called a DLT, 1 patient with only grade 1 AEs and 1 patient who remained on treatment for at least 6 cycles. The study has been amended to accrue patients in cohort 2 (first-line up-front therapy), and the three-at-risk safety lead-in design applied to this cohort, independent from cohort 1. Specifically, for cohort 2, we employ a three-at-risk design (modified rolling design) for this Phase II study to ensure the triplet is well-tolerated.

This design permits only 3 patients to be a risk for DLT at any one time during the "safety lead-in." When the first 6 patients have completed the observation period (1 cycle) and treatment with ≤1 DLT, the safety lead-in for the triplet will be considered successful, and accrual will proceed to a total of 16 patients at dose level 1 in cohort 2.

If two DLTs are observed on the starting dose in the first 6 patients, the dose will be reduced per the dose ladder dose to level -1 (see table 3, section 5). If there are two DLTs in the first 6 patients on dose level -1, the study will hold accrual pending an amendment and discussion between the PI and study drug provider (Merck). Otherwise, additional patients will be accrued to dose level -1 until a total of 16 patients have been treated with the triplet of pembrolizumab, letrozole, and palbociclib in cohort 2.

Cohort 1&2 Additional Secondary Endpoints

With approximately 16 patients (in cohort 2), we expect to have to obtain at least 12 patients with both pre-pembrolizumab and post-pembrolizumab treatment biopsy material adequate for evaluation, and 16 samples with blood collection pre- and post. The correlative studies will be used to potentially refine patient selection for future studies, and to understand the role of immune changes on the activity of the combination of pembrolizumab plus letrozole and palbociclib. These correlative studies are considered exploratory in the context of this limited phase II study; however, 12 samples provide 80% power to detect an effective size of 0.8 (80% of the standard deviation), with a one-sided type I error of 5% for any of these correlative assays in the context of this exploratory study. Samples provided in Cohort 1 (n=6), while limited, will be explored and to determine if there were large reductions in the tumor burden with the addition of pembrolizumab.

Cohort 3 Statistical Design

Clinical statistics: We will treat 25 patients. Patients will go on to receive combination therapy. In the preliminary analysis of 14 patients' flow data (8 responders, 6 non-responders), we noted a statistically significant decrease in classic monocytes (60%, $p<0.001$), and this decrease was more pronounced in responders than non-responders (85% vs. 25%, $p<0.02$). We seek to evaluate, specifically, if palbociclib as a monotherapy is responsible for such changes as the primary endpoint. Other secondary endpoints include other immune cell subsets and changes that follow the combination with pembrolizumab. With 25 patients, assuming a standard deviation of 0.51 in the relative change in classic monocytes in PBMCs, there is 90% power to detect a relative change of log(C1D1/baseline) of 34.5% with a type I error (two-sided) of 0.05. This will confirm that the decrease in classic monocytes is due to palbociclib (secondary endpoints will evaluate the change with the combination therapy). In the preliminary data, we noted a response rate of 65%, and responders had a significantly larger decrease in classic monocytes. With 25 patients, if 16 patients respond, we will have 85% power to detect a difference in the relative change between

responders and non-responders of 0.8 vs. 0.3 (assuming SD of 0.5 and 0.29 from preliminary data) with a type I error of 5% (two-sided). The actual power will depend on the distribution of responders vs. non-responders. Other immune correlatives and the changes after introduction of the combination therapy will also be assessed. Patients will not be pre-selected based on PD-L1 expression; therefore, we will also evaluate response and correlatives both together and by PD-L1 expression. All eligible patients who start treatment will be considered in the calculation of the response rate, and all who complete the pre- and post-biopsy treatment will be considered in the evaluation of the primary objective related to evaluating the impact of palbociclib.

Correlative statistics: With approximately 25 patients with pre-treatment, C2D1 (+/- 1 week), and time of progression biopsy samples, we expect to obtain at least 12 pairs of pre- and post-treatment biopsy material adequate for evaluation of genomic and immune profiling. The correlative studies will be used to potentially refine patient selection for future studies and analyze immune phenotypic and genomic alterations from the combination of pembrolizumab and palbociclib. These correlative studies are considered exploratory in the context of this limited Phase II study; however, 12 samples provide greater than 80% power to detect an effective size of 0.8 (80% of the standard deviation in the change), with a one-sided type I error of 5%. In addition, we will report on changes in PD-L1 expression induced by palbociclib and any relationship to the response rate.

Sponsor/Licensee:

City of Hope National Cancer Center.

Note: Merck provided funding and pembrolizumab. Pfizer is providing palbociclib but is not a study sponsor.

Case Report Forms:

Electronic Data Collection will be used for this protocol. The data will be stored in encrypted, password protected, secure computers that meet all HIPAA requirements.

Table of Contents

1.0 Goals and Objectives (Scientific Aims)

1.1 Primary Objective

To evaluate the objective response rate (ORR), based on Response Evaluation Criteria In Solid Tumors (RECIST, version 1.1), of pembrolizumab in combination with letrozole and palbociclib in patients with newly diagnosed metastatic ER+HER2- breast cancer, and determine if the addition of pembrolizumab to letrozole and palbociclib combination can achieve an improved response rate (ORR = complete response [CR]+ partial response [PR]) measured from the study baseline, based on RECIST version 1.1.

1.2 Secondary Objective

To determine the safety and tolerability of adding pembrolizumab (200 mg every 3 weeks) to letrozole (2.5 mg) and palbociclib (125mg, 3 weeks on, one week off) in patients with metastatic ER+HER2- breast cancer.

Additional Secondary Objectives:

- To evaluate the CR rate.
- To evaluate progression-free survival (PFS).
- To evaluate overall survival (OS).
- To evaluate duration of response (DOR) using RECIST version 1.1.
- To evaluate clinical benefit rate (CBR) using RECIST version 1.1.
- To evaluate toxicities (using the NCI Common Terminology Criteria for Adverse Events [CTCAE], version 4.0) associated with the triple drug combination (pembrolizumab, letrozole, and palbociclib) in patients with metastatic ER+HER2- breast cancer.
- To evaluate CR, PR, ORR, PFS, DOR, and CBR using immune-related Response Criteria in solid tumors (irRECIST). Time to treatment failure will also be assessed.

1.3 Exploratory Objectives

We will collect serial tumor biopsies (primary/metastatic prior to study treatment, repeat biopsy upon progression) and serial peripheral blood samples will be collected. Optional on-treatment biopsies are allowed for study of immune response. Studies include the following

- Cellular/humoral immune response by analyzing immune and stromal cell characteristics before and after treatment that correlate with clinical response. This includes PD-L1 expression levels.
- The peripheral serum thymidine kinase (TK) level and its association with treatment response.
- Circulating tumor DNA (ctDNA) and the effect of combining pembrolizumab, letrozole, and palbociclib on ctDNA profiles.
- To evaluate genomic and phenotypic status of breast tumor

1.4 Cohort 3 Objectives

Primary objectives:

- To evaluate the clinical response rate (CR or PR via RECIST 1.1)
- To evaluate dynamic changes in host peripheral blood in predicting response to treatment: CD8+ EMRA T cells; CD4+ EM T cells; classic monocytes ($CD14^{+}$ $CD16^{-}$) and Non-classic monocytes ($CD14^{Dim-}$ $CD16^{+}$)

Secondary objectives:

- To further evaluate the safety/tolerability of the combination
- To evaluate the PFS, DOR (time from documentation of tumor response to disease progression or death), Overall survival (OS)
- Cellular/humoral immune response by analyzing immune and stromal cell characteristics before and after treatment that correlate with clinical response.
- Circulating tumor DNA (ctDNA) changes

2.0 Background

2.1 Introduction/Rationale for Development

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

2.1.1 Endocrine resistant ER+ breast cancer

The estrogen receptor (ER) pathway plays a pivotal role in ER positive (ER+) breast cancer development and progression, and endocrine therapy forms the backbone of the therapeutic regimen for patients with ER+ breast cancer. Endocrine therapy including SERM tamoxifen; aromatase inhibitors (AIs) letrozole, anastrozole, and exemestane; and ER down-regulator fulvestrant represent the key treatment options targeting the ER pathway. These treatments are highly effective, but their usefulness is limited by common intrinsic and acquired resistance. Multiple mechanisms responsible for endocrine resistance have been proposed and include deregulation of various components of the ER pathway itself, alterations in cell cycle and cell survival signaling molecules, and the activation of escape pathways that can provide tumors with alternative proliferative and survival stimuli [1, 2].

Progress has been made in treating ER+ advanced breast cancer with the following strategies: improved dosing of an ER down-regulator faslodex (500 mg) [3-5]; targeting the activation of the mammalian target of rapamycin (mTOR) pathway by addition of everolimus to exemestane; and addition of CDK4/6 inhibitors to anastrozole. In a Phase II randomized study, faslodex (500 mg) was compared with anastrozole (1 mg) as first-line endocrine therapy for postmenopausal patients with ER+ advanced breast cancer. Median time to progression (TTP) was 23.4 months for fulvestrant versus 13.1 months for anastrozole (Hazard Ration [HR] 0.73, P=0.05) [3]. Objective response rate to faslodex (500 mg) was 33% in the first line setting [6]. Objective response rate to any subsequent systemic therapy was 23.4% in faslodex arm and 21.7% in anastrozole arm. In the landmark phase 3, randomized BOLERO-2 study, everolimus plus exemestane was compared with exemestane alone with PFS of 6.9 months observed in the combination treatment versus 2.8 months for exemestane alone (HR, 0.43; 95% confidence interval [CI], 0.35 to 0.54; P<0.001) [7]. The ORR was 7% and 0.4% in the combination and exemestane-alone groups, respectively (P<0.001). A randomized phase II study comparing palbociclib plus letrozole versus letrozole alone as first line treatment of ER+HER2- (human epidermal growth factor receptor 2-negative) advanced breast cancer demonstrated a significant PFS benefit of 20.2 months for the combination therapy versus 10.2 months for letrozole alone (HR, 0.488, 95% CI 0.319-0.748; P=0.0004)[8]. An ORR of 43% versus 33% was observed comparing the drug combination versus letrozole alone. More recently, the combination of faslodex plus palbociclib has shown an improvement of PFS in the second line setting in patients with ER+ metastatic breast cancer. The median PFS was 9.2 months in the combination therapy arm compared with 3.8 months in the hormonal therapy alone arm (HR, 0.422; P<0.000001).

2.1.2 ER+ metastatic breast cancer and CDK 4/6 inhibitors

Endocrine therapy options for postmenopausal patients with ER+ advanced breast cancer (locally advanced, recurrent, or metastatic breast cancer) include selective ER modulators (SERM; tamoxifen), ER antagonists (fulvestrant), selective nonsteroidal AIs (NSAI, anastrozole, and letrozole) and steroidal AIs (exemestane). For treatment of ER+ metastatic patients, targeted therapy such as everolimus [9, 10] and the novel CDK4/6 (cyclin-dependent kinases 4 and 6) inhibitor palbociclib play a critical role [8].

In the mammalian cell cycle, entry into S phase is achieved by CDK4/6. The cyclin D proteins act through the CDK4 and CDK6 protein kinases (positive regulators) to promote G1 progression. A wide range of human cancers, including breast cancers harbor genetic aberrations that increase the activity of CDK4/6. In the landmark Paloma-1 study, the letrozole plus palbociclib group had a PFS of 20.2 months compared with 10.2 months in letrozole alone arm. The FDA approval to use palbociclib in combination with letrozole for patients with metastatic breast cancer in the first line setting has changed the landscape of ER+ metastatic breast cancer therapy. In the letrozole plus palbociclib arm of the Paloma-1 patient population, 43% of patients achieved PR, 44% had SD, and 4% had progression of disease [8]. However, 48% of patients did not achieve a response by RECIST. The combination of letrozole and palbociclib has become a new standard of care for patients with newly diagnosed metastatic ER+ breast cancer with remarkable PFS benefit [8] but modest response rate. The current study is designed to test the safety and efficacy of adding pembrolizumab to the combination of letrozole and palbociclib.

2.1.3 Rationale for immunotherapy in endocrine resistant breast cancer

Despite the progress being made, 44% of patients treated with letrozole and palbociclib have SD as the best response. Therefore, there is a need to assess the potential benefit of other therapies in addition to letrozole and palbociclib in ER+ patients who have not achieved a tumor response per RECIST in first and possibly second line therapies. Although growing evidence suggests that triple negative breast cancer (TNBC) is the most immunogenic breast cancer subtype, PD-L1 expression has also been detected in 33% of luminal B and A tumors and PD-1 expression has been detected in 44% and 25% of luminal B and A tumors (n=58), with concurrent expression seen in 17% and 13%, respectively [11]. Single agent activity of pembrolizumab inPD-L1 expression ER+ MBC was 12% in the KEYNOTE-28 study[12]. Since CDK 4/6 inhibitor has become the standard care for first time treatment in patient with ER+ metastatic breast cancer, hence there is a strong biological rationale to add pembrolizumab to letrozole and palbociclib to improve clinical response by RECIST and/or irRECIST to prolong DOR, time to progression, and OS. The current study will evaluate the tolerability of the triple drug combination. In addition, the potential efficacy of pembrolizumab in ER+HER2- breast cancer will be evaluated, and a built-in immune correlative study will fill the current knowledge gap in the safety and efficacy data in this extremely important area of research.

2.1.4 Immunotherapy in combination with CDK4/6 inhibitor

The combination of an immune check point inhibitor with CDK 4/6 inhibitors has not been evaluated. ER+ breast cancer is characteristically not enriched with tumor infiltrating lymphocytes (TILs). We hypothesize that ER+ breast cancer may characteristically carry type II immune response [13]. The proliferation of T lymphocytes in adaptive immunity is controlled by the CDK cascade through the phosphorylation of immunologically relevant transcription factors [14]. CDK 4/6 inhibitors may significantly impact the decision between type I and type II immune response. The role of these CDK inhibitors in T lymphocytes is complex in that each regulates the dynamics of T lymphocyte clonal expansion in a unique fashion. Directly targeting the cell cycle in a specific fashion can redirect the immune response, a finding that has significant implications in enhancing immune responses to ER+ breast cancer.

In our study, the addition of pembrolizumab to the letrozole/palbociclib combination may provide a novel way to overcome endocrine resistance. This would provide a novel treatment option for primarily endocrine and CDK4/6 inhibitor resistant ER+ breast cancers.

2.1.5 Rationale for including subjects with PD-L1 negative tumors

The KN 012 TNBC proof-of-concept data was obtained in subjects with PD-L1 positive tumors (PD-L1 staining in ≥1% tumor cells or in stroma) [15]. No data is currently available on the performance of pembrolizumab in breast cancer patients with PD-L1 negative tumors (i.e., PD-L1 staining in <1% tumor cells and no stromal staining). Since there is limited data using PD-L1 expression as a biomarker predicting response to pembrolizumab, and patients with PD-L1 negative tumors may benefit from pembrolizumab, PD-L1 expression will not be used for patient selection. Instead, analysis of PD-L1 expression will be included as a correlative study.

2.1.6 Rationale for dose selection/regimen/modification of pembrolizumab

The dose of pembrolizumab in this trial is 200 mg every 3 weeks (Q3W). The dose recently approved in the United States for treatment of melanoma patients is 2 mg/kg Q3W. Although the dose of pembrolizumab studied in KN 012, which established efficacy and safety in TNBC, was 10 mg/kg Q2W, recent studies in other tumor types have indicated that 10 mg/kg Q2W and 200 mg Q3W are likely to be similar with regard to efficacy and tolerability.

An open-label Phase I trial (Protocol 001) is being conducted to evaluate the safety and clinical activity of single agent MK-3475. The dose escalation portion of Protocol 001 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 [16]. All three dose levels were well tolerated, and no dose-limiting toxicities were observed. This first-in-human study of MK-3475 showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg, and 10 mg/kg Q2W). No maximum tolerated dose (MTD) has been identified to date. The highest dose tested in PN001, 10.0 mg/kg Q2W, will be the dose and schedule utilized in Cohorts A, B, C, and D of this protocol to test for initial tumor activity. Recent data from other clinical studies within the MK-3475 program has shown that a lower dose of MK-3475 and a less frequent schedule may be sufficient for target engagement and clinical activity.

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

A population PK analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance, and volume parameters of MK-3475 were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing and a fixed dose across all body weights.

MK-3475 has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg Q3W body weight-based regimen are anticipated to remain well within the established exposure margins of 0.5-5.0 for MK-3475 in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W versus 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 Non-small-cell lung carcinoma (NSCLC) and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

The rationale for further exploration of 2 mg/kg and comparable doses of pembrolizumab in solid tumors is based on: 1) similar efficacy and safety of pembrolizumab when dosed at either 2 mg/kg or 10 mg/kg Q3W in melanoma patients; 2) the flat exposure-response relationships of pembrolizumab for both efficacy and safety in the dose ranges of 2 mg/kg Q3W to10 mg/kg Q3W; 3) the lack of effect of tumor burden or indication on distribution behavior of pembrolizumab (as assessed by the population PK model); and 4) the assumption that the dynamics of pembrolizumab target engagement will not vary meaningfully with tumor type.

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

The pembrolizumab dose of 200 mg Q3W has formed a foundation for ongoing combination therapeutic protocols.

2.2 Preclinical Studies

2.2.1 Preclinical data for palbociclib/letrozole

Refer to the FDA package insert for palbociclib/letrozole preclinical data.

Embryo-fetal toxicity: Based on findings in animals and mechanism of action, palbociclib can cause fetal harm. Palbociclib caused embryo-fetal toxicities in rats and rabbits at maternal exposures that were greater than or equal to 4 times the human clinical exposure based on area under the curve (AUC). Advise females of reproductive potential to use effective contraception during therapy with palbociclib and for at least two weeks after the last dose (see **Section 3**).

2.2.2 Preclinical data for pembrolizumab

Refer to the IB for pembrolizumab preclinical data.

PD-1 immune checkpoint inhibition: Therapeutic studies in mouse models have shown that administration of antibodies blocking PD-1/PD-L1 interaction enhances infiltration of tumor-specific CD8+ T cells and ultimately leads to tumor rejection, either as a monotherapy or in combination with other treatment modalities [17-19]. Anti-mouse PD-1 or anti-mouse PD-L1 antibodies have demonstrated antitumor responses in models of squamous cell carcinoma, pancreatic carcinoma, melanoma, acute myeloid leukemia, and colorectal carcinoma [19-23]. In such studies, tumor infiltration by CD8+ T cells and increased IFN-γ, granzyme B and perforin expression were observed, indicating that the mechanism underlying the antitumor activity of PD-1 checkpoint inhibition involved local infiltration and activation of effector T cell function *in vivo* [21]. Experiments have confirmed the *in vivo* efficacy of anti-mouse PD-1 antibody as a monotherapy, as well as in combination with chemotherapy, in syngeneic mouse tumor models (see the IB).

2.3 Human Studies

2.3.1 Clinical data for palbociclib/letrozole

Refer to the FDA package insert for palbociclib/letrozole clinical data.

Paloma-1 was a randomized, open-label, multicenter study of palbociclib plus letrozole versus letrozole alone conducted in postmenopausal patients with ER+HER2$^-$ advanced breast cancer who had not received previous systemic treatment for their advanced disease [8]. A total of 165 patients were randomized. Randomization was stratified by disease site (visceral versus bone only versus other) and by disease-free interval (>12 months from the end of adjuvant treatment to disease recurrence versus ≤12 months from the end of adjuvant treatment to disease recurrence or de novo advanced disease). Palbociclib was given orally at a dose of 125 mg daily for 21 consecutive days followed by 7 days off treatment. Patients received study treatment until progressive disease, unmanageable toxicity, or consent withdrawal.

Patients enrolled in this study had a median age of 63 years (range 38 to 89). The majority of patients were Caucasian (90%), and all patients had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1. Forty-three percent of patients had received chemotherapy and 33% had received anti-hormonal therapy in the neoadjuvant or adjuvant setting prior to their diagnosis of advanced breast cancer. Forty-nine percent of patients had no prior systemic therapy in the neoadjuvant or adjuvant setting. The majority of patients (98%) had metastatic disease. Nineteen percent of patients had bone only disease and 48% of patients had visceral disease. The letrozole plus palbociclib arm had medial PFS of 20.2 months compared with 10.2 months in the letrozole alone arm (HR, 0.488, 95% CI, 0.319-0.748). The treatment effect of the combination on PFS was also supported by a retrospective independent review of radiographs with an observed HR of 0.621 (95% CI: 0.378, 1.019). Overall response rate in patients with measurable disease as assessed by the investigator was higher in the palbociclib plus letrozole arm compared to the letrozole alone arm (55.4% versus 39.4%). At the time of the final analysis of PFS, OS data was not mature with 37% of events.

The most common adverse reactions (incidence ≥10%) of palbociclib were neutropenia, leukopenia, fatigue, anemia, upper respiratory infection, nausea, stomatitis, alopecia, diarrhea, thrombocytopenia, decreased appetite, vomiting, asthenia, peripheral neuropathy, and epistaxis. The most frequently reported serious adverse reactions in patients receiving palbociclib plus letrozole were pulmonary embolism (3 of 83; 4%) and diarrhea (2 of 83; 2%). An increased incidence of infection events was observed in the palbociclib plus letrozole arm (55%) compared to the letrozole alone arm (34%). Febrile neutropenia events have been reported in the palbociclib clinical program, although no cases were observed in Study 1. Grade ≥3 neutropenia was managed by dose reductions and/or dose delay or temporary discontinuation consistent with a permanent discontinuation rate of 6% due to neutropenia. Adverse drug reactions (≥10%) reported in patients who received palbociclib plus letrozole or letrozole alone in Study 1 are listed in **Table 1**.

Neutropenia: Decreased neutrophil counts have been observed in clinical trials with palbociclib. Grade 3 (57%) or 4 (5%) decreased neutrophil counts were reported in patients receiving palbociclib plus letrozole in randomized clinical trials [8]. Median time to first episode of any grade neutropenia per laboratory data was 15 days (13-117 days). Median duration of Grade ≥3 neutropenia was 7 days.

Febrile neutropenia events have been reported in the palbociclib clinical program, although no cases of febrile neutropenia have been observed in Study 1. Monitor complete blood count prior to starting palbociclib therapy and at the beginning of each cycle, as well as on Day 14 of the first two cycles, and as clinically indicated. Dose interruption, dose reduction or delay in starting treatment cycles is recommended for patients who develop Grade 3 or 4 neutropenia.

Infections: Infections have been reported at a higher rate in patients treated with palbociclib plus letrozole compared to patients treated with letrozole alone in Study 1. Grade 3 or 4 infections occurred in 5% of patients treated with palbociclib plus letrozole whereas no patients treated with letrozole alone experienced Grade 3 or 4 infections. Monitor patients for signs and symptoms of infection and treat as medically appropriate.

Pulmonary Embolism: Pulmonary embolism has been reported at a higher rate in patients treated with palbociclib plus letrozole (5%) compared with no cases in patients treated with letrozole alone in Study 1. Monitor patients for signs and symptoms of pulmonary embolism and treat as medically appropriate.

Embryo-Fetal Toxicity: Based on findings in animals and mechanism of action, palbociclib can cause fetal harm. Palbociclib caused embryo-fetal toxicities in rats and rabbits at maternal exposures that were greater than or equal to 4 times the human clinical exposure based on area under the curve (AUC). Advise females of reproductive potential to use effective contraception during therapy with palbociclib and for at least two weeks after the last dose (See **Section 3**).

Table 1: Adverse Reactions* (≥10%) in Paloma-1 Study

System Organ Class	Palbociclib plus Letrozole (N=83)			Letrozole Alone (N=77)		
Adverse Reaction (%)	**All Grades**	**Grade 3**	**Grade 4**	**All Grades**	**Grade 3**	**Grade 4**
Infections and infestations						
URI [a]	31	1	0	18	0	0
Blood and lymphatic system disorders						
Neutropenia	75	48	6	5	1	0
Leukopenia	43	19	0	3	0	0
Anemia	35	5	1	7	1	0
Thrombocytopenia	17	2	0	1	0	0
Metabolism and nutrition disorders						
Decreased appetite	16	1	0	7	0	0
Nervous system disorders						
Peripheral neuropathy [b]	13	0	0	5	0	0
Respiratory, thoracic, and mediastinal disorders						
Epistaxis	11	0	0	1	0	0
Gastrointestinal disorders						
Stomatitis [c]	25	0	0	7	1	0
Nausea	25	2	0	13	1	0
Diarrhea	21	4	0	10	0	0
Vomiting	15	0	0	4	1	0
Skin and subcutaneous tissue disorders						
Alopecia	22[d]	N/A	N/A	3[e]	N/A	N/A
General disorders						
Fatigue	41	2	2	23	1	0
Asthenia	13	2	0	4	0	0

*Adverse Reaction rates reported in the table include all reported events regardless of causality; grading according to Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0; N=number of subjects; N/A=not applicable; URI=Upper respiratory infection; [a] URI includes: Influenza, Influenza like illness, Laryngitis, Nasopharyngitis, Pharyngitis, Rhinitis, Sinusitis, Upper respiratory tract infection; [b] Peripheral neuropathy includes: Neuropathy peripheral, Peripheral sensory neuropathy; [c] Stomatitis includes: Aphthous stomatitis, Cheilitis, Glossitis, Glossodynia, Mouth ulceration, Mucosal inflammation, Oral pain, Oropharyngeal discomfort, Oropharyngeal pain, Stomatitis; [d] Grade 1 events -21%; Grade 2 events -1%; [e] Grade 1 events -3%.

2.3.2 Clinical Data for Pembrolizumab

Refer to the Investigator's Brochure for pembrolizumab Clinical data.

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Pembrolizumab was recently approved in the US for the treatment of advanced, unresectable or metastatic malignant melanoma, and for use in melanoma subjects with disease progression after prior treatment with ipilimumab or a BRAF inhibitor, in the case of BRAF V600-mutant disease [24]. It is the first anti-PD-1 therapy to receive regulatory approval in the US and is currently under regulatory review in the EU. Ongoing clinical trials of pembrolizumab are being conducted in advanced melanoma, non-small cell lung cancer, and a number of other advanced solid tumor indications and hematologic malignancies. For study details refer to the IB.

2.3.2.1 PD-1 checkpoint inhibition and cancer treatment

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades [25]. Accumulating evidence shows a correlation between TILs in cancer tissue and favorable prognosis in various malignancies [26-37]. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors [34, 38-43].

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control [44]. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structures of murine PD-1 alone [45] and in complex with its ligands were first resolved [46, 47]and more recently the NMR-based structure of the human PD-1 extracellular region and analyses of its interactions with its ligands were also reported [48]. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail, which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade [49]. The mechanism by which PD-1 down-modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins [50].

PD-1 has been shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells [51]. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells [52] as well as subsets of macrophages [53] and dendritic cells [54]. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types [55], including non-hematopoietic tissues as well as in various tumors. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor.

PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments [55]. 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 [17]. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma [56]. This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention [57].

Preliminary data from a study of pembrolizumab in PD-L1-enriched selected patients with triple negative stage IV breast cancer suggest 18.3% potentially lasting clinical activity [15]. Currently there are no completed studies completed evaluating the efficacy of pembrolizumab in ER+ breast cancer.

2.4 Cohort 3 Rationale and Preliminary data

Strong preclinical evidence of the immune-stimulatory effect of the CDK4/6 inhibitor palbociclib, and its complimentary mode of action and minimal overlapping toxicities with anti-PD-1 agents lead to the combination trials of CDK 4/6 inhibitor and immune checkpoint inhibitor. In NCT02779751 study, abemaciclib + pembrolizumab + anastrozole was tested in patients with HR+HER2- MBC, an ORR of 23% (n=26) was observed. 46% had grade >3 transaminase elevation led to discontinued therapy. Interstitial lung disease (ILD)/pneumonitis were found in 4 patients (15%) including 2 patients with fatal outcome (Rugo et. al. AACR 2020). Benefit/risk data do not support further development of abemaciclib + pembrolizumab + anastrozole in this population.

Our current study combining letrozole, palbociclib and pembrolizumab for patients with ER+ metastatic breast cancer (NCT02778685). A total of 23 patients were enrolled in cohort 1&2 and 20 were evaluable for toxicity (4 in cohort 1, 16 in cohort 2). 19 were eligible for response assessment (one patient was determined to be TNBC upon repeat of biopsy). Median age was 49 years, with 40% Hispanic, and 60% non-Hispanic. There were 2 DLTs (1 biopsy site infection and delay in treatment that was possibly related to treatment in cohort 1, and 1 pneumonitis in cohort 2. The combination is safe with few SAE observed. with the most common cause being neutropenia. Gr 3 and 4 AEs are shown in the **Table**. Immune related AE is consistent with pembrolizumab toxicity profiles. Median follow up was 13.7 (95% CI 6.4-16.9) months and median PFS was not reached. Response analysis per RECIST 1.1 were as follows: 2/20 complete response (CR, 10%), 11/20 partial response (PR, 55%), 5/20 stable disease (SD, 25%), and 2/20 progressive disease (PD, 10%). Although the response rate of 65% was encouraging, the study was limited by small sample size.

Peripheral blood mononuclear cell (PBMC) flow cytometry analysis showed the potential role of PBMCs in predicting response to the combination therapy. PBMCs were collected at day 1 of cycles 1 (baseline), 2, 4 and EOT. At baseline, lower level of naïve CD8+ T cells and higher level of non-naïve effector memory CD8+ T cells (EMRA) were associated with clinical response**.** Responders demonstrated higher baseline frequencies of CD8+ effector memory (CD8+ CCR7- CD45RA-) T cells (p=0.01) and CD4+ effector memory (CD4+ CCR7- CD45RA-) T cells (p=0.01). Further analysis revealed a shift in myeloid cell composition from predominantly classical monocytes (CD14+ CD16-) to non-classical monocytes (CD14- CD16+) in responders between baseline and cycle 2 (p=0.007). These dynamic shifts in circulating monocyte composition are associated with response. Our preliminary results demonstrate that baseline CD8+ EMRA and CD4+ EM are associated with favorable clinical responses, suggesting that T cells expressing markers of effector T cell differentiation may predict response. In addition, dynamic shifts in circulating monocyte composition may also be associated with response. Although our preliminary results are encouraging, the number of evaluable patients was limited to 20 patients. Here we hypothesize that palbociclib may increase CD8+ EMRA T cells and CD4+ EM T cells and enhance immune response to pembrolizumab in HR+HER2- metastatic breast cancer. We propose the addition of cohort 3 to investigate a potential first-line therapy in patients with metastatic HR+HER2- BC. Patients receiving

hormonal therapy, including letrozole +/- ovarian suppression or fulvestrant are eligible for enrollment. Palbociclib +endocrine therapy lead-in design will be implemented in cohort 3 to better understand the immune potentiating effect of palbociclib. Peripheral blood and required on-treatment biopsy would allow in-depth analysis of biomarker predicting response to the combination. The current design will also allow a better understanding of the immune potentiating effect of palbociclib prior to the combination with pembrolizumab. The overall goal of cohort 3 is to confirm the response rate of the combination therapy, and to better understand the mechanism of action of the synergy between palbociclib and pembrolizumab.

	Grade 3 AE	Grade 4 AE
Neutropenia	12	4
Leukopenia	11	3
Lymphocytopenia	4	2
Thrombocytopenia	2	0
Elevated LFT	6	1
Bowel perforation	0	1
Other	7[1]	2[2]

[1]Other: anemia, pain in extremity, influenza pneumonia, respiratory failure, pneumonitis, pruritus, breast infection (n=1 each); [2]Other: abdominal pain, sepsis (n=1 each)

2.5 Overview of Proposed Study

The current study is an open-label single institutional trial for patients with newly diagnosed stage IV, metastatic ER+ breast cancer with measurable disease per RECIST, version 1.1. This study will evaluate the feasibility and tolerability of the triple drug combination of pembrolizumab, letrozole, and palbociclib in these patients. In addition, the potential efficacy of pembrolizumab in ER+HER2- breast cancer will be evaluated, and a built-in immune correlative study will fill the current knowledge gap in the safety and efficacy data in this extremely important area of research.

For cohort 1, patients consented prior to amendment to study the first line (n=6), they become a separate cohort. Pembrolizumab is added to letrozole and palbociclib in these patients who had stable disease per RECIST 1.1 after at least 6 months of therapy on palbociclib plus letrozole (n=6). Patients can have 0-3 lines of previous systemic therapy including endocrine therapy and/or chemotherapy in the advanced setting before starting letrozole and palbociclib. Eligible patients will receive letrozole (2.5 mg) once a day and palbociclib (75, 100 or 125 mg as established prior to study entry) once a day for 3 weeks on and 1 week off. Pembrolizumab will be given at 200 mg IV every 3 weeks. Drug compliance will be documented in the EMR at study visits (pill diary collection is not required).

For cohort 2, patients must have confirmed newly diagnosed ER+HER2- breast cancer, measurable disease by RECIST, version 1.1. Eligible patients will receive letrozole (2.5 mg) once a day and palbociclib 125 mg once a day for 3 weeks on and 1 week off. Pembrolizumab will be given at 200 mg IV every 3 weeks. Drug compliance will be documented in the EMR at study visits (pill diary collection is not required).

For cohort 3, patients will receive palbociclib and endocrine therapy (letrozole or fulvestrant) starting day-28. Palbociclib will be given 3 weeks on 1 week off. Fulvestrant dose 500mg on day -28 and day -14. On cycle 1 day 1, pembrolizumab will be added to the combination of palbociclib and endocrine therapy. Drug compliance will be documented in the EMR at study visits (pill diary collection is not required).

Study treatment will continue until disease progression, unacceptable adverse events (AEs), concurrent illness that prevents further administration of study treatment, investigator's decision to withdraw the subject from study treatment, consent withdrawal, becoming lost-to-follow-up, death, or for administrative reasons requiring cessation of treatment.

After discontinuation of study treatment, each subject will be followed for 30 days for AE (grade 2 or higher) monitoring. Serious adverse events (SAEs) and immune-related adverse events (irAEs) will be collected for 90 days after the end of study treatment or until the subject initiates new anticancer therapy for a minimum of 30 days, whichever occurs earlier.

Subjects who discontinue study treatment for reasons other than disease progression will have post-treatment follow-up for disease status every 12 weeks (±7 days) until disease progression (verified by restaging imaging), start of a non-study anticancer treatment, consent withdrawal, becoming lost to follow-up, death, or end of the study. Optional peripheral blood or biopsy sample can be taken if patient was off trial for reasons other than disease progression. In addition, restaging imaging can be assessed for RECIST measurement.

All subjects will be followed by telephone every 4 cycles (±7 days) for OS until consent withdrawal, becoming lost to follow-up, death, or end of the study.

2.6 Study Endpoints

2.6.1 Primary Endpoint

The primary endpoint is the response rate for patient with newly diagnosed metastatic ER+HER2- breast cancer when combining pembrolizumab, letrozole and palbociclib.

2.6.2 Secondary Endpoints

The secondary endpoint is the safety and tolerability of pembrolizumab plus the letrozole/palbociclib combination. Safety analysis will be carried out based on toxicities assessed by CTCAE version 4.0. Adverse events will be analyzed including but not limited to AEs (grade 2 or higher), SAEs, fatal AEs, and laboratory changes. Immune-related adverse events will also be collected (see **Sections 11** and **13** for further details). We will use CR rate, DOR, PFS, and OS to test the efficacy of therapy of the novel drug combination assessed by RECIST version 1.1. Any event (toxicity-related or progression) will be counted as "treatment failure." Kaplan-Meier estimates will be generated for PFS, failure-free survival and OS.

Exploratory analysis to assess response and clinical benefit will be carried out using irRECIST, an adaptation of RECIST version 1.1 to account for the unique tumor response characteristics to treatment with new immunotherapeutic agents, including pembrolizumab. RECIST version 1.1 was developed based on treatment with cytotoxic agents (see **Sections 11** and **13** for further details).

3.0 Patient Eligibility

Patient MRN:	Patient Initials: (F,M,L):
Institution:	

3.1 Inclusion Criteria

Participants must meet all the following criteria on screening examination to be eligible to participate in the study:

___ 1. Willing and able to provide written informed consent/assent for the trial.

___ 2. Willing and able to comply with all aspects of the treatment protocol.

___ 3. ≥18 years of age on day of signing informed consent.

___ 4. Postmenopausal patients defined by at least one of the following criteria:

- Prior bilateral oophorectomy OR aamenorrheic for ≥12 months (if ≤55 years of age and prior to chemotherapy, or on medical ovarian ablative therapy **OR**;
- Previous hysterectomy with one or both ovaries left in place (previous hysterectomy in which documentation of bilateral oophorectomy is unavailable AND FSH values consistent with the institutional normal values for the post-menopausal state. FSH levels must be obtained within 28 days prior to registration).

___ 5. Presence of measurable disease meeting the following criteria: At least 1 lesion of >10 mm in long axis diameter for non-lymph nodes or >15 mm in short axis diameter for lymph nodes that is serially measurable according to RECIST version 1.1 using computerized tomography, magnetic resonance imaging, or panoramic and close-up color photography.

___ 6. *Stage IV metastatic ER+HER2- breast cancer histologically proven per current ASCO/CAP guidelines. Allow up to 30 days prior use of CDK4/6 inhibitors and up to 60 days of letrozole or other aromatase inhibitors for treatment of metastatic ER+ breast cancer.

___ 7. Life expectancy of ≥ 3 months.

___ 8. Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0-2.

___ 9. Willing to provide tissue from a newly obtained core or excisional biopsy of a tumor lesion. Newly obtained is defined as a specimen obtained up-to 6 weeks (42 days) prior to initiation of treatment on Day 1 and day -28 for cohort 3. Subjects for whom newly obtained samples cannot be provided (e.g., inaccessible, or subject safety concern) may submit an archived specimen only upon agreement from the study PI.

___ 10. For Cohort 3, willing to undergo tumor biopsies at baseline (within 6 weeks of study onset), C2D1 (+/-1 week) and at time-of-progression or end-of-treatment when feasible.

___ 11. Demonstrate adequate organ function (see Table 2). All screening labs should be performed within 10 days of treatment initiation.

Table: 2 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,000 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L without transfusion or EPO dependency (within 7 days of assessment)
Renal	

Serum creatinine **OR** Measured or calculated[a] creatinine clearance. (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR** Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases
Albumin	≥2.5 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants ≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants

[a] Creatinine clearance should be calculated per institutional standard.

___ 12. Female subjects of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

___ 13. Female subjects of childbearing potential should be willing to use two methods of birth control or be surgically sterile or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication. Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.

* Cohort 1 (accrual to 6 patients) is for patients who had ongoing SD on letrozole + palbociclib, enrolled on prior version of eligibility criteria to receive pembrolizumab after obtaining stable disease on letrozole + palbociclib. These patients must have been on treatment with letrozole and palbociclib for 6 months with SD per RECIST 1.1; received up to 3 lies of previous therapy including endocrine and/or chemotherapy in advanced setting prior to initiation of letrozole and palbociclib; no grade 3 toxicities except alopecia.

Cohort 1&2 are closed to accrual. Only cohort 3 is open to accrual

3.2 Exclusion Criteria

___ 1. Patients currently participating and receiving study therapy or who have participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of treatment.

___ 2. Previously received pembrolizumab or other anti-PD-1 or anti-PD-L1 immunotherapy.

___ 3. Does not have measurable disease per RECIST 1.1.

___ 4. For cohort 2, received > 30 days of prior treatment with CDK4/6 inhibitors or > 60 days of letrozole before screening Diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment.

___ 5. Known history of active TB (Bacillus Tuberculosis).

___ 6. Hypersensitivity to pembrolizumab or any of its excipients.

___ 7. Prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to study Day 1 or has not recovered (i.e., ≤ Grade 1 or at baseline) from AEs due to agents administered > 4 weeks earlier.

___ 8. Prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 1 or has not recovered (i.e., ≤ Grade 1 or at baseline) from AEs due to a previously administered agent.

- *Note:* Patients with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study.
- *Note:* If patient received major surgery, she must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

___ 9. Known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer.

___ 10. Known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Patients with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least 4 weeks prior to the first dose of trial treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis, which is excluded regardless of clinical stability.

___ 11. Active autoimmune disease that has required systemic treatment in the past 2 years (i.e., with use of disease modifying agents, corticosteroids, or immunosuppressive drugs). Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

___ 12. History of (non-infectious) pneumonitis that required steroids or current pneumonitis.

___ 13. History of interstitial lung disease.

___ 14. Clinically active diverticulitis, intra-abdominal abscess, gastrointestinal (GI) obstruction, or abdominal carcinomatosis (known risks factors for bowel perforation).

___ 15. Active infection requiring systemic therapy.

___ 16. History of significant cardiovascular disease, defined as: congestive heart failure greater than New York Heart Association (NYHA) Class II according to the NYHA Functional Classification; unstable angina or myocardial infarction within 6 months of enrollment; or serious cardiac arrhythmia.

___ 17. Clinically significant electrocardiogram (ECG) abnormality, including a marked baseline prolonged QT/QTc ([QT interval/corrected QT interval], e.g., a repeated demonstration of a QTc interval >480 ms), a family or personal history of long or short QT syndrome, Brugada syndrome or known history of QTc prolongation, or Torsade de Pointes (TdP).

___ 18. Concurrent use of drugs that are known to be moderate or strong CYP3A inhibitors or inducers or drugs that are known to prolong the QT interval (Refer to **Tables 5** and **17**).

___ 19. History or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the patient's participation for the full duration of the trial, or is not in the best interests of the patient to participate, in the opinion of the treating investigator.

___ 20. Pregnant, breastfeeding, or expecting to conceive children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment.

___ 21. Known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

___ 22. Known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).

___ 23. Known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).

___ 24. Received a live vaccine within 30 days of planned start of study therapy.

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

3.3 Inclusion of Women and Minorities

The study is open to any man or woman regardless of race/ethnicity. Efforts will be made to extend the accrual to a representative population, but in a trial which will accrue approximately 21 subjects, a balance must be struck between subject safety considerations and limitations on the number of individuals exposed to potentially toxic or ineffective treatments on the one hand and the need to explore racial and ethnic aspects of clinical research on the other. If differences in outcome that correlate to racial or ethnic identity are noted, accrual may be expanded, or additional studies may be performed to investigate those differences more fully.

4.0 Screening and Registration Procedures

4.1 Pre-Enrollment Informed Consent and Screening Procedures

Diagnostic or laboratory studies performed exclusively to determine eligibility for this trial will be done only after obtaining written informed consent. Studies or procedures that were for clinical indications (not exclusively to determine study eligibility) may be used for baseline values or to evaluate suitability for treatment, even if the studies were done before informed consent was obtained. The informed consent process is to be fully documented (see Section 14.6), and the prospective participant must receive a copy of the signed informed consent document. Screening procedures are listed in Study Calendar; **Section 10**).

4.2 Registration Requirements/Process

Assignment of Screening Number*:* All consented subjects will be given a unique screening number.

COH DCC Availability and Contact Information

Eligible subjects will be registered on the study centrally by the Data Coordinating Center (DCC) at City of Hope.

DCC staff are available between the hours of 8.00 am and 5.00 pm PST, Monday through Friday (except holidays). DCC contact information is as follows:

- Phone: (626) 256-4673 ext. 83968
- E-mail: DCC@coh.org

Eligible subjects must be registered **prior** to start of protocol therapy. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a subject does not receive protocol therapy following registration, the subject's registration on the study may be canceled after discussion with the PI. The Data Coordinating Center should be notified of cancellations as soon as possible.

Registration Process

To register a participant the subsequent procedure is to be followed:

1. The study team should contact the DCC via telephone or email to provide notification regarding the pending registration and communicate desired timeline of the registration, especially if it must be completed promptly to meet the registration window.
2. The protocol nurse or CRC will email a copy of the following documents to the DCC:
    - Completed eligibility checklist (printed from Section 3.1 of the protocol)
    - Signed Informed Consent
    - Signed subject's bill of Rights
    - Signed HIPAA authorization form and
    - Provide copies of source documentation only if not readily available as a finalized record in the COH Electronic Medical Record (EMR).
3. After having received all transferred documentation, the DCC will complete the review the documents to verify eligibility, working with the study team as needed to resolve any missing required source elements. A participant failing to meet all protocol eligibility requirements will not be registered.
4. Once eligibility has been confirmed, DCC staff will register the participant by: assigning a subject accession number, register the subject on study centrally into a COH clinical trials management system (e.g. MIDAS), and enter the subject into the eCRF system, Medidata RAVE.
5. Once registration has been completed, DCC staff will send a Confirmation of Registration Form within 24 hours, including the participant study number to:
    - The site study team: Principal Investigator, treating physician, protocol statistician, protocol nurse, CRC and COH IDS Pharmacy.
    - the COH sponsor team designees

The DCC is to be notified of all participants who sign consent but do not meet eligibility criteria or do not initiate protocol therapy.

4.3 Randomization and/or Dose Level Assignment

Not applicable.

5.0 Treatment Program

5.1 Treatment Overview

5.1.1 Schedule

For cohort 1, 7 days prior to study initiation, palbociclib needs to be held to synchronize treatment cycle. For cohort 1, dose reduction had likely happened prior to study entry since we require patient to be treated for at least 6 months with letrozole and palbociclib prior to study entry. If dose reduction of palbociclib is required, it will follow the same rule as listed in table 3.

For cohort 2, all three agents will be started on cycle 1 day 1 of therapy. Letrozole can be used continuously. The treatment to be used in cohort 2 of this trial is outlined in **Table 3**. Palbociclib was reported to have over 50% Grade 3 neutropenia event. For cohort 2, most of the dose reduction happens within the first 3 month of initiation of palbociclib. We do not anticipate further dose reduction of palbociclib on study.

If patient developed dose-limiting hematological toxicities (neutropenia, anemia, or thrombocytopenia) at a palbociclib dose of 125mg daily, the next dosing level will be palbociclib at 100mg daily. If this happened at palbociclib dose of 100mg daily, the next dose level will be palbociclib at 75 mg daily. If hematological DLT happened at palbociclib dose of 75mg daily, further dose reduction will not be allowed.

If there is non-hematological dose limiting toxicity (DLT) occurring in the first 6 weeks of the study in the first 6 patients, considered to be definitely, probably, or possibly related to pembrolizumab, the study will be held temporarily. The investigator will consult the co-sponsor Merck to add pembrolizumab reduced dose level. If a non-hematologic DLT is considered unlikely or unrelated to pembrolizumab, a specific likely cause must be specified.

Table 3: Trial Treatment for Cohort 2

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
Letrozole	2.5 mg	Daily	po	daily x 28 days	Standard
Palbociclib dose level 1	125 mg	daily	po	3 weeks on and 1 week off every 28 days	Standard
Palbociclib dose level -1	- 100 mg	Daily	po	3 weeks on and 1 week off every 28 days	Standard
Palbociclib dose level -2	75mg	Daily	po	3 weeks on and 1 week off every 28 days	Standard
Pembrolizumab dose level 1 [a]	200 mg	Q3W	IV infusion	Day 1 of each 21-day cycle	Experimental

[a] If patient developed dose-limiting hematological toxicities at a palbociclib dose of 75 mg daily, there is no available dosage form of 50 mg. As a result, dose level -1 for those patients will be palbociclib 100mg every other day (equivalent to a reduction of 25mg per day).

[a] If there is any non-hematologic dose limiting toxicity (DLT) occurring in the first 6 weeks of the study in the first 6 patients, considered to be definitely, probably, or possibly related to pembrolizumab, the study will be held temporarily. The investigator will consult Merck to add a pembrolizumab reduced dose level.

For cohort 3, patients will receive palbociclib and endocrine therapy (letrozole or fulvestrant) starting day-28. Palbociclib will be given 3 weeks on 1 week off per package insert. Letrozole 2.5mg daily dosing will be given. Fulvestrant dose 500mg on day -28 and day -14, and every 28 days thereafter. The choice of letrozole or fulvestrant will be based of treatment physician's recommendation. On cycle 1 day 1, pembrolizumab 200mg daily will be added to the combination of palbociclib and endocrine therapy.

5.1.2 Trial Procedures

The Study Calendar in **Section 10** summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

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

5.1.3 Clinical procedures/assessments

5.1.3.1 Adverse event (AE) monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the study calendar (see **Section 10**) and more frequently if clinically indicated. Adverse events will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 4. Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment. All AEs (grade 2 or higher) of unknown etiology associated with pembrolizumab exposure should be evaluated to determine if it is possibly an irAE. Refer to **Section 7** for detailed information regarding the assessment and recording of AEs.

5.1.3.2 Full physical exam

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

5.1.3.3 Directed physical exam

The investigator or qualified designee will perform a directed physical exam as clinically indicated and according to the Study Calendar (see **Section 10**).

5.1.3.4 Vital signs

The investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment and at treatment discontinuation as specified in the Study Calendar (**Section 10**). Vital signs should include temperature, pulse, respiratory rate, weight, and blood pressure. Height will be measured at screening only.

5.1.3.5 Eastern Cooperative Oncology Group (ECOG) performance scale

The investigator or qualified designee will assess ECOG performance status at screening, prior to the administration of each dose of trial treatment and discontinuation of trial treatment as specified in the Study Calendar (**Section 10**). Also see **Section 16.1**.

5.1.3.6 Tumor imaging and assessment of disease

To meet screening criteria, tumor imaging must be performed within 3 days prior to the start of the trial. Eligible subjects must have measurable disease based on RECIST version 1.1 as assessed by the investigator and radiology review. Tumor imaging performed as part of routine clinical management is acceptable for use as the screening tumor imaging if it was performed within 35 days prior to study initiation. Tumor imaging for initial staging and restaging may be performed by CT and bone scan. MRI is allowed, but not required. The same technique must be performed throughout the trial. Pre-study staging imaging must occur to ensure patient has achieved only SD per RECIST version 1.1.

Brain imaging at baseline should be performed in patients with known brain metastases (such patients also need records of brain imaging performed at least 4 weeks earlier) and those with worsening and/or new neurological symptoms.

If a patient has a known history of bone metastases or has new bone pain during screening, a bone scan should be obtained prior to study entry. If a patient has no known metastatic disease in the bone at baseline, the routine bone scan repeat is not needed unless clinically indicated during study period. Additionally, plain X-ray evaluation should be obtained for symptomatic skeletal sites with negative bone scan evaluations.

All subjects should have restaging imaging performed every 12 weeks (± 7 days). Imaging should continue to be performed until first radiologic evidence of disease progression (PD) (unless the PI determines the patient would benefit from continued treatment and then follow the patient per irRECIST), the start of new anti-cancer treatment, withdrawal of consent, death, or the end of the study, whichever occurs first.

Bone scans will also be utilized to assess osseous metastases. If a patient has a known history of bone metastases or has new bone pain during screening, a bone scan should be obtained prior to study entry. A bone scan at follow up is required only if they develop new or worsening symptoms or if the site believes they have attained a CR. If a patient has no known metastatic disease in the bone or active symptoms, a bone scan at baseline is not needed. A bone scan can be obtained at follow up if there are new symptoms of bone pain. Additionally, plain X-ray evaluation will be obtained for symptomatic sites with negative bone scan evaluations.

Brain imaging during the trial should be performed in subjects with known brain metastases (Q8W for first year, then Q12W) and those with worsening and/or new neurological symptoms.

Per RECIST version 1.1, PR or CR should be confirmed by a repeat tumor imaging not less than 4 weeks from the date the response was first documented. The tumor image for confirmation of response may be performed at the earliest 4 weeks after the first indication of response, or at the next scheduled tumor imaging (e.g., 8 weeks later) whichever is clinically indicated.

After verification of PD by RECIST version 1.1 in support of the PFS analysis, if the PI determines the patient is clinically stable and will benefit from continued treatment, the patient will then be managed by irRECIST (see **Section 16.4**). Per irRECIST, that initial PD by RECIST version 1.1 must be in clinically stable subjects, disease progression may be confirmed by the investigator/ radiology review at least 4 weeks after verification of first radiologic evidence of PD. Subjects who have unconfirmed disease progression (PD) may continue on treatment until progression PD is confirmed. In subjects who discontinue study therapy without verified disease progression, tumor imaging should be performed at the time of treatment discontinuation (i.e., date of discontinuation ± 4-week window). If previous tumor imaging was obtained within 4 weeks prior to the date of discontinuation, then tumor imaging at treatment discontinuation is not required.

5.1.3.7 Tumor tissue collection and correlative study blood collection

Tumor tissue biopsies will be obtained prior to study initiation and the end of the study. Optional on-treatment biopsies are allowed for assessment of immune response. When a core needle biopsy is used, a minimum of 4 core biopsy samples with a minimum 18-gauge needle is recommended.

For Cohort 3, baseline, C2 D1(+/- 7 days) on treatment and optional end of treatment biopsy will be acquired.

Peripheral blood samples will be obtained on Day 1 of cycles 1, 2, 4, and end of study.

For cohort 3, day -28 peripheral blood will also be collected.

Three tubes (1 green-top, 2 lavender-top) of blood will be collected and delivered to correlative study PI's laboratory.

Optional peripheral blood or biopsy sample can be taken if patient was off trial for reasons other than disease progression.

5.1.3.8 Laboratory procedures/ assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below. Laboratory tests for hematology, chemistry, and others are specified in **Table 4**.

Table 4: Laboratory Tests

Hematology	**Chemistry**	**Other**
Hematocrit	Albumin	Serum β-human chorionic gonadotropin†
Hemoglobin	Alkaline phosphatase	β-hCG†
Platelet count	Alanine aminotransferase (ALT)	
WBC (total and differential)	Aspartate aminotransferase (AST)	
Red Blood Cell Count	Lactate dehydrogenase (LDH)	Total thriiodothyronine (T3)
Absolute Neutrophil Count	Carbon Dioxide ‡	Free tyroxine (T4)
Absolute Lymphocyte Count	*(CO_2 or biocarbonate)*	Thyroid stimulating hormone (TSH)
	Uric Acid	Blood for correlative studies
	Calcium	
	Chloride	
	Glucose	
	Phosphorus	
	Potassium	
	Sodium	
	Magnesium	
	Total Bilirubin	
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*	
	Total protein	

Hematology	Chemistry	Other
	Blood Urea Nitrogen	

Laboratory tests for screening should be performed within 10 days prior to the first dose of treatment. After Cycle 1, pre-dose laboratory procedures can be conducted up to 72 hours prior to dosing. Results must be reviewed by the investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.

CBC with differential and comprehensive serum chemistry panel will be performed on Day 1 of each 3-week cycle (pembrolizumab administration days) through the duration of the study (after completion of pembrolizumab the follow-up can be modified to every 4 weeks). In addition, for cohort 1&2, during the first 12 weeks of therapy, CBC with differential will be checked every 2 weeks for close monitoring of blood count (Day 1 of weeks 3, 5, 7, 9, and 11). The study team will monitor the CBCs and differential for DLT evaluation during the first 6 weeks of the study and continue to monitor CBC and differential through the first 12 weeks.

For cohort 3, CBC diff & CMP will only be collected on day 1 of each cycle.

*EKG***:** All Electrocardiograms (ECGs) will be performed using a 12-lead (with a 10-second rhythm strip) tracing. ECG measurements will include PR interval, QT interval, RR interval, and QRS complex. It is preferable that the machine used has a capacity to calculate the standard intervals automatically. ECG interval readings by the ECG recorder's algorithm will be read and interpreted at the investigational site for eligibility determination and patient safety monitoring and documentation stored in the source documents. Additional ECGs may be performed as clinically indicated at any time.

5.1.3.9 Withdrawal/Discontinuation

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any AEs that are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in **Section 7.8**. Subjects who attain a CR may discontinue treatment. After discontinuing treatment following assessment of CR, these subjects should return for a safety follow-up visit and then proceed to the follow-up period of the study. Also see **Section 5.4.**

5.1.3.10Visit Requirements

Visit requirements are outlined in the Study Calendar (**Section 10**).

5.2 Planned Duration of Therapy

Patients will be treated until criteria for removal from treatment is met. We anticipate the trial will require approximately 24 months from the time the first subject signs the informed consent until the last subject's last study-related follow up or phone call.

For patients who completed 24 months of therapy and have achieved CR/PR or SD, treatment will be continued using commercial supply of letrozole or fulvestrant and pembrolizumab. Palbociclib will be provided through the study or commercial supply.

5.3 Criteria for Subject Removal from Treatment

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 addition, a subject may be withdrawn by the investigator if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons.

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

- The subject withdraws consent.
- Confirmed radiographic disease progression. *Note*: A subject may be granted an exception to continue on treatment with confirmed radiographic progression if clinically stable or clinically improved.
- Unacceptable AEs as described in **Section 7**.
- Concurrent illness that prevents further administration of treatment.
- Investigator's decision to withdraw the subject.
- The subject has a confirmed positive serum pregnancy test.
- Noncompliance with trial treatment or procedure requirements.
- The subject is lost to follow-up.
- Administrative reasons.
- If a subject inadvertently becomes pregnant.

5.4 Discontinuation of Study Therapy after CR

Discontinuation of treatment may be considered for subjects who have attained a confirmed CR, been treated for at least 24 weeks with pembrolizumab, and had at least two treatments with pembrolizumab beyond the date when the initial CR was declared.

5.5 Clinical Criteria for Early Trial Termination

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

- Quality or quantity of data recording is inaccurate or incomplete.
- Poor adherence to protocol and regulatory requirements.
- Life threatening adverse event

5.6 Subject Follow-Up

After the end of study treatment each subject will be followed for 30 days for AE (grade 2 or higher) monitoring. Serious adverse events (SAEs) and immune-related adverse events (irAEs) will be collected for 90 days after the end of study treatment or until the subject initiates new anticancer therapy for a minimum of 30 days, whichever occurs earlier.

Subjects who discontinue study treatment for reasons other than disease progression will have post-treatment follow-up for disease status every 12 weeks (±7 days) until disease progression (verified by restaging imaging), start of a non-study anticancer treatment, consent withdrawal, becoming lost to follow-up, death, or end of the study.

All subjects will be followed by telephone every 4 cycles (±7 days) for OS until consent withdrawal, becoming lost to follow-up, death, or end of the study.

Visit requirements are outlined in **Section 10**.

5.7 Supportive Care, Other Concomitant Therapy, Prohibited Medications

5.7.1 Concomitant use of palbociclib with strong CYP3A inhibitors or moderate/strong CYP3A inducer

5.7.1.1 Palbociclib and CYP3A inhibitors

Coadministration of a strong CYP3A inhibitor (itraconazole) increases the plasma exposure of palbociclib in healthy subjects by 87%. **Avoid concomitant use of strong CYP3A inhibitors** (e.g., clarithromycin, indinavir, itraconazole, ketoconazole, lopinavir/ritonavir, nefazodone, nelfinavir,

posaconazole, ritonavir, saquinavir, telaprevir, telithromycin, verapamil, and voriconazole). **Avoid grapefruit or grapefruit juice during palbociclib treatment.**

5.7.1.2 Palbociclib and CYP3A inducers

Coadministration of a strong CYP3A inducer (rifampin) decreases the plasma exposure of palbociclib in healthy subjects by 85%. **Avoid concomitant use of strong CYP3A inducers** (e.g., phenytoin, rifampin, carbamazepine, and St John's Wort). Coadministration of moderate CYP3A inducers may also decrease the plasma exposure of IBRANCE. **Avoid concomitant use of moderate CYP3A inducers** (e.g., bosentan, efavirenz, etravirine, modafinil, and nafcillin) (**Table 5**).

Table 5: Agents That May Alter Palbociclib Plasma Concentration (Contraindicated).

Class of Agents	Name of Agent
CYP3A inhibitor (increase palbociclib plasma concentration)	amprenavir, atazanavir, boceprevir, clarithromycin, conivaptan, delavirdine, diltiazem, erythromycin, fosamprenavir, indinavir, itraconazole, ketoconazole, lopinavir, mibefradil, miconazole, nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, telaprevir, telithromycin, verapamil, voriconazole, grapefruit , grapefruit juice
Strong CYP3A inducer	phenytoin, rifampin, carbamazepine, St John's Wort
Moderate CYP3A inducer	bosentan, efavirenz, etravirine, modafinil, nafcillin

5.7.1.3 Drugs that may have their plasma concentrations altered by palbociclib

Coadministration of **midazolam** with multiple doses of palbociclib increases the midazolam plasma exposure by 61% in healthy subjects, compared with administration of midazolam alone. The dose of the sensitive CYP3A substrate with a narrow therapeutic index (e.g., alfentanil, cyclosporine, dihydroergotamine, ergotamine, everolimus, fentanyl, pimozide, quinidine, sirolimus and tacrolimus) may need to be reduced as IBRANCE may increase their exposure.

5.8 Trial Blinding/Masking

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

5.9 Randomization or Treatment Allocation

Not applicable.

5.10 Stratification

Not applicable.

5.11 Concomitant Medications/Vaccinations

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial (see **Section 3.2**). If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the Merck clinical team. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician.

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

the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.

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

5.12 Prohibited Concomitant Medications

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

- Antineoplastic systemic chemotherapy or biological therapy.
- Immunotherapy not specified in this protocol.
- Chemotherapy not specified in this protocol.
- Investigational agents other than pembrolizumab, palbociclib, and letrozole.
- Radiation therapy. *Note*: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed at the investigator's discretion.
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Investigator.
- Strong CYP3A inhibitors.
- Strong CYP3A inducers.
- Moderate CYP3A inducers.
- Grapefruit or grapefruit juice
- Drugs that could potentially prolong Q-T interval (see **Table 17**).

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial. Subjects may receive other medications that the investigator deems to be medically necessary (also see **Section 3.2**). There are no prohibited therapies during the post-treatment follow-up phase.

5.13 Rescue Medications & Supportive Care

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of AEs with potential immunologic etiology are outlined below and in Table 7. Where appropriate, these guidelines include the use of oral or IV treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

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

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

5.13.1.1Pneumonitis

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.

Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.

5.13.1.2Diarrhea /colitis

Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood, or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus). All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis. For Grade 2 diarrhea/colitis, administer oral corticosteroids. For Grade 3 or 4 diarrhea/colitis, treat with intravenous steroids followed by high dose oral steroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

5.13.1.3Type 1 diabetes mellitus

If new onset, including diabetic ketoacidosis [DKA]) or ≥ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA):

For **T1DM** or **Grade 3-4** Hyperglycemia:

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

5.13.1.4Hypophysitis

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

5.13.1.5Hyperthyroidism or hypothyroidism:

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

Grade 2 hyperthyroidism events (and **Grade 2-4** hypothyroidism):

- In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
- In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.

Grade 3-4 hyperthyroidism:

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

5.13.1.6Hepatic

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 IV 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.

5.13.1.7Renal failure or nephritis

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

Management of Infusion Reactions: Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. **Table 6** below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab.

Table 6: Infusion Reaction Treatment Guidelines

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

Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.

5.13.2 Diet, activity, and other considerations

5.13.2.1Diet

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

5.13.2.2Contraception

Pembrolizumab may have adverse effects on a fetus in uterus. See **Section 3** for required contraceptive measures. Subjects of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving study drug and for 120 days after the last dose of study drug by complying with one of the following:

(1) Practice abstinence† from heterosexual activity;

OR

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

Acceptable methods of contraception are‡:

Single method (one of the following is acceptable):

- intrauterine device (IUD).
- vasectomy of a female subject's male partner.
- contraceptive rod implanted into the skin.

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

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

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

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

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study subjects of childbearing potential must adhere to the contraception requirement (described above and in **Section 3**) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 120 days after the last dose of trial therapy. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

5.13.2.3Use in Pregnancy

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

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to Merck.

5.13.2.4Use in Nursing Women

It is unknown whether pembrolizumab is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, subjects who are breast-feeding are not eligible for enrollment (also specified in **Section 3**).

6.0 Adverse Events and Unanticipated Problems

The research team is responsible for classifying AEs and UPs as defined in the relevant regulations and reporting to all applicable parties, including but not limited to the COH IRB, DSMC, Food and Drug Administration (FDA), National Institutes of Health (NIH) and other collaborators, e.g., pharmaceutical companies. The research team is responsible for the continued monitoring and tracking of all AEs in order to ensure non-reportable events are reviewed and monitored and do not rise to a reporting level.

6.1 Assessment of Adverse Events

The site Investigator will be responsible for determining the event name, and assessing the severity (i.e. grade), expectedness, and attribution of all adverse events as applicable per the City of Hope Clinical Research Adverse Event and Unanticipated Problem policy. Adverse events will be characterized using the descriptions and grading scales found in version [insert version] of [insert scale]. A copy of the scale can be found at [insert link or appendix].

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

- **Unrelated** – The event is clearly NOT related to study treatment, and is clearly related to other factors such as the participant's clinical state, other therapeutic interventions, or concomitant medications administered to the participant.
- **Unlikely** – The event is unlikely related to the study treatment, and is most likely related to other factors such as the participant's clinical state, other therapeutic interventions, or concomitant drugs.
- **Possible** – The event may be related to study treatment, as it follows a reasonable temporal sequence from the time of drug administration, but could have been produced by other factors such as the participant's clinical state, other therapeutic interventions, or concomitant drugs.
- **Probable** – The event is most likely related to the study treatment, as it follows a reasonable temporal sequence from the time of drug administration and a known response pattern to the study drug, and is unlikely related to the participant's clinical state, other therapeutic interventions, or concomitant drugs.
- **Definite** – The event is clearly related to the study treatment, as it follows a reasonable temporal sequence from the time of drug administration and a known response pattern to the study

drug, and is not reasonably explained by other factors such as the participant's condition, therapeutic interventions, or concomitant drugs.

6.2 Reporting of Adverse Events

6.2.1 Routine Recording of Non-Serious Adverse Events

Routine recording of grade 2 or higher adverse events will occur via data entry into the study eCRF. Collection of adverse events will begin after the patient is given the study treatment or any study related procedures and will continue until end of study. Adverse events will be monitored by the Protocol Management Team (PMT). Adverse events that do not meet the criteria of serious OR are not unanticipated problems do not require expedited reporting. AEs reported through expedited processes (i.e. reported to the IRB, DSMC, FDA, etc.) must also be reported in routine study data submissions.

6.2.2 Expedited Reporting Requirements of SAEs and UPs to the COH Regulatory Committees

Adverse events that meet the criteria of serious OR are unanticipated problems will be reported according to the approved City of Hope Clinical Research Adverse Event and Unanticipated Problem policy. Reporting of SAEs will begin after study treatment or any study related procedures, and must be followed until the event is resolved, stabilized, or determined to be irreversible by the investigator. Follow-up SAE reports must be submitted for all events that require expedited reporting when the status of the event changes and until the resolution or stabilization of the event.

6.2.3 Adverse Events of Special Interest (AESI)

For percentage of subjects with adverse event of special interest by toxicity grade, refer to IB Appendix 7 Tables 27-30. Pembrolizumab has a positive benefit-risk profile and is generally well-tolerated and demonstrates a favorable safety profile in the approved indications. Pembrolizumab immune-mediated adverse reactions (AEOSIs—see discussion in IB (Section 5.4 and Section 5.4.5) are relatively uncommon. The most frequently reported AEOSI is hypothyroidism, with an overall incidence of 8.5%. Furthermore, most AEOSIs are mild to moderate in severity. The immune-mediated adverse reactions are generally readily manageable with appropriate care in the clinical setting (see Section 6.4.1 through Section 6.4.3 for guidance on management). The safety and efficacy data generated to date provide a favorable benefit-risk assessment for the use of pembrolizumab.

The important identified risks for pembrolizumab are of an immune-mediated nature, and include the following: pneumonitis; colitis; hepatitis; nephritis; endocrinopathies that include hypophysitis (including hypopituitarism and secondary adrenal insufficiency), thyroid disorder (hypothyroidism, hyperthyroidism) and Type I diabetes mellitus; uveitis; myositis; Guillain-Barré syndrome; pancreatitis; and severe skin reactions. Information on the nature and frequency of these identified risks is included in the Reference Safety Information in IB Section 7.1. The majority of immune-mediated adverse events AEs were mild to moderate in severity, manageable with appropriate care, and rarely required discontinuation of therapy.

In addition, two important potential risks have been identified, although the data available thus far for these events does not provide sufficient evidence of a causal relationship to pembrolizumab. The 2 important potential risks are: a) myasthenic syndrome, and b) an increased risk of severe complications (such as early severe graft versus host disease and venoocclusive disease) of allogeneic transplant in patients with hematologic malignancies who have previously been treated with PD-1 inhibitors. The Sponsor continues to monitor and collect data on these potential risks in order to further characterize their potential relationship to pembrolizumab.

Table 7:Dose Modification Guidelines for Pembrolizumab-Related Adverse Events (see Section 5.13)

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

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
	3-4	Permanently discontinue	Permanently discontinue
All Other Drug-Related Toxicity[c]	3 or Severe	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue

Note: Permanently discontinue for any severe or Grade 3 drug-related AE that recurs or any life-threatening event.
[a] For patients with liver metastasis who begin treatment with Grade 2 AST or ALT, if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week then patients should be discontinued; [b] If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise, dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose; Refer to **Table 6** for further management details; [c] Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.

6.2.4 Additional AE Reporting Requirements

It is possible that irAEs other than those listed in the guidance document may be observed in subjects receiving pembrolizumab. This is meant to be a general guidance; therefore, recommendations in the current document might not be all inclusive. As such, investigators are encouraged to contact a Sponsor Clinical Monitor as needed to discuss cases that warrant separate discussion outside of the scope of current guidelines.

All AEs are to be graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE),, version 4.0 (http://ctep.cancer.gov). If an irAE does not resolve or improve to ≤Grade 1 within 12 weeks after last administration of pembrolizumab, study therapy discontinuation should be considered after discussion with a Merck Clinical Monitor.

6.2.4.1 Reporting to the FDA

The study PI (or designee) will be responsible for contacting the Office of IND Development and Regulatory Affairs (OIDRA) at COH to ensure prompt reporting of safety reports to the FDA. OIDRA will assist the PI with the preparation of the report and submit the report to the FDA in accordance with the City of Hope Clinical Research Adverse Event and Unanticipated Problem policy.

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

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

- Any unexpected fatal or life threatening adverse experience associated with use of the drug must be reported to the FDA no later than 7 calendar days after initial receipt of the information [21 CFR 312.32(c)(2)]

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

In addition, the study PI will submit annually within 60 days (via COH OIDRA) of the anniversary date of when the IND went into effect, an annual report to the FDA which is to include a narrative summary and analysis of the information of all FDA reports within the reporting interval, a summary report of adverse drug experiences, and history of actions taken since the last report because of adverse drug experiences.

Infusion-related reactions have been reported with pembrolizumab, and are non-immune-mediated events included in the Pembrolizumab Program (MK-3475) Event of Clinical Interest Guidance Document. As noted above in Section 6.4.2, in mid-2015 the guidance document was integrated into new pembrolizumab protocols, and is no longer provided to investigational sites. For those studies that have not integrated the guidance document into the protocol, please refer to the guidance document in conjunction with this IB when assessing and managing potential infusion-related reactions. Infusion-related reactions may present as allergic reaction (hypersensitivity), serum sickness, infusion and infusion-like reactions, cytokine release syndrome, or anaphylaxis. Mild infusion reactions can generally be treated with interruption of the infusion and medical intervention including IV fluids, antihistamines, nonsteroidal anti-inflammatory drugs, acetaminophen, and narcotics as needed. More severe or life-threatening reactions may require vasopressors, corticosteroids, and epinephrine. In the case of severe or life-threatening reactions, subsequent doses of pembrolizumab should not be administered.

6.2.4.2 Reporting to Merck

All serious adverse events and AESIs (initial and follow-up information) will be reported by the study PI to Merck per the following guidelines:

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any SAE, or follow up to a SAE, including death due to any cause other than progression of the cancer under study whether or not related to the Merck product, must be reported within 2 working days to Merck Global Safety.

6.3 Dose Selection

6.3.1 Rationale for dose selection of pembrolizumab

The rationale for selection of doses to be used in this trial is provided in **Section 2.1.6**.

Pembrolizumab treatments should be administered as detailed on the Study Calendar (**Section 10**). Trial treatment may be administered up to 3 days before or after the scheduled Day 1 of each cycle. All trial treatments will be administered on an outpatient basis. Pembrolizumab 200 mg will be administered as a 30-minute IV infusion every 3 weeks (i.e., infusion time is 30 minutes: -5 min/+10 min).

Refer to refer to package insert for specific instructions for the preparation of the pembrolizumab infusion fluid and administration of infusion solution.

6.3.2 Rationale for dose selection of palbociclib

Palbociclib will be administered daily for 3 weeks, with one week off. For cohort 1, the patient will continue palbociclib dose as established prior to study entry (75mg, 100mg or 125mg). For cohort 2, the patient will be started at standard dose level of 125 mg po daily, 3 weeks on and one week off with food on a 28-day cycle.

6.3.3 Rationale for dose selection of letrozole or fulvestrant

Letrozole 2.5 mg will be given daily for 28 days cycle as per package insert. Fulvestrant will be given at 500mg on days -28, -14, day 1 cycle 1 and every 28 days thereafter per package insert.

6.4 Definition of Dose-Limiting Toxicity (DLT)

A dose-limiting toxicity (DLT) is defined as one of the following AEs, occurring in **the first 6 weeks** of the study if considered to be definitely, probably, or possibly related to treatment.

The following define a DLT:

- Grade 3 or 4 non-hematologic toxicity according to the NCI CTCAE Version 4, except for skin toxicity, alopecia, nausea, vomiting, diarrhea, or electrolyte disturbance (see below). For patients with ≤ Grade 2 hepatic transaminase levels at baseline as a result of liver metastases, a transaminase level ≥10x ULN lasting for >7 days will be considered a DLT.
- Grade 4 nausea, vomiting, or diarrhea that persists more than 3 days despite maximal supportive intervention.
- Grade 3 thrombocytopenia with bleeding requiring transfusion, or Grade 4 thrombocytopenia with or without bleeding.
- Grade 4 neutropenia that persists more than 7 days.
- Grade 3 or 4 neutropenia with fever, defined as single temperature of >38.3°C (101°F) or a sustained temperature of ≥38°C (100.4°F) for more than one hour.
- Grade ≥3 skin toxicity despite best supportive care, with exception of Grade 3 rash that resolves to Grade ≤2 within 14 days with appropriate supportive therapy.
- If a total at least 75% of the planned dose cannot be administered in the first two cycles of pembrolizumab due to toxicity.
- Prolonged delay (>2 weeks) in initiating cycle 2 or 3 due to treatment -related toxicity.
- Grade 5 toxicity.

The following toxicities will not be considered DLTs if they are transient (<7 days):

- Hypersensitivity and injection site reactions (if a Grade 3 or 4 hypersensitivity to pembrolizumab occurs, this event will not be considered a DLT; but the patient will not receive any further study therapy and will be replaced in the study by a new patient).
- Grade ≥3 myalgia, fatigue, or constipation, with full supportive therapy.
- Grade ≥3 increase or decrease of electrolytes.
- Grade 3 nausea, vomiting, or diarrhea that resolves to Grade ≤1 within 7 days of appropriate supportive therapy.
- Grade ≥3 elevation of serum creatine kinase level that is asymptomatic that returns to Grade ≤2 within 21 days of treatment interruption.

6.5 Dose Modification

The three agents included in this study (letrozole, palbociclib and pembrolizumab) have non-overlapping toxicity profiles. Side effects such as hot flashes, joint pain, night sweat, and mood swings are most likely to be attributed to letrozole. Bone marrow suppression such as neutropenia, anemia and/or thrombocytopenia are most likely attributed to palbociclib. Immune-related side effects such as diarrhea, colitis, hepatitis, Type I diabetes, hypophysitis, pneumonitis, hypothyroidism, hyperthyroidism and infusional reaction are likely attributed to pembrolizumab as described in Table 7.

6.5.1 Dose modification of pembrolizumab

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These AEs may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per **Table 7** below.

Table 7: Dose Modification Guidelines for Pembrolizumab-Related Adverse Events (see Section 5.13)

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

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
All Other Drug-Related Toxicity[c]	3 or Severe	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue

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

[a] For patients with liver metastasis who begin treatment with Grade 2 AST or ALT, if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week then patients should be discontinued; [b] If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise, dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose; Refer to **Table 6** for further management details; [c] Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.

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

6.5.2 Dose modification of palbociclib

Management of some adverse reactions may require temporary dose interruptions/delays and/or dose reductions, or permanent discontinuation as per dose reduction schedules provided in **Tables 8, 9,** and **10.**

Palbociclib was reported to have over 50% Grade 3 neutropenia event. Dose reduction had likely happened prior to study entry since we require the patient to be treated for at least 6 months with letrozole and palbociclib prior to study entry. Most of the dose reduction happens within the first 3 months of initiation of palbociclib. We do not anticipate further dose reductions of palbociclib on study.

If a patient develops dose-limiting hematological toxicities (neutropenia, anemia, or thrombocytopenia) at a palbociclib dose of 125mg daily, the next dosing level will be palbociclib at 100mg daily. If this happens at palbociclib dose of 100mg daily, the next dose level will be palbociclib at 75 mg daily. If hematological DLT happens at palbociclib dose of 75mg daily, further dose reductions are not feasible due to unavailable dosage form of 50 mg. Patients will then be allowed to have an alternative dosing schedule during the following treatment cycle at the principle investigator's discretion. The option includes palbociclib 75 mg every other day.

Table 8: Recommended Dose Modifications of Palbociclib for Adverse Reactions

Dose Level	Dose
Recommended starting dose	125 mg/day
First dose reduction	100 mg/day
Second dose reduction	75 mg/day
Third dose reduction	75 mg every other day*

*If further dose reduction below 75 mg every other day is required, discontinue the treatment.

Table 9: Dose Modification and Management[a] – Hematologic Toxicities for Palbociclib

CTCAE Grade	Dose Modifications

Grade 1 or 2	No dose adjustment is required.
Grade 3[b]	No dose adjustment is required. Consider repeating complete blood count monitoring one week later. Withhold initiation of next cycle until recovery to Grade ≤2.
Grade 3 ANC (<1000 to 500/mm^3) + Fever ≥38.5ºC and/or infection	Withhold palbociclib and initiation of next cycle until recovery to Grade ≤2 (≥1000/mm3). Resume at next lower dose.
Grade 4[b]	Withhold palbociclib and initiation of next cycle until recovery to Grade ≤2. Resume at next lower dose.

Grading according to CTCAE Version 4.0.; ANC=absolute neutrophil count; CTCAE=Common Terminology Criteria for Adverse Events. [a]**For cohorts 1&2,** Monitor complete blood count prior to the start of palbociclib therapy and at the beginning of each cycle, as well as on Day 14 of the first three cycles, and as clinically indicated; [b] Except lymphopenia (unless associated with clinical events, e.g., opportunistic infections).

Table 10: Dose Modification and Management – Non-Hematologic Toxicities for Palbociclib

CTCAE Grade	**Dose Modifications**
Grade 1 or 2	No dose adjustment is required.
Grade ≥3 non-hematologic toxicity (if persisting despite medical treatment)	Withhold until symptoms resolve to: • Grade ≤1; • Grade ≤2 (if not considered a safety risk for the patient) Resume at the next lower dose.

Grading according to CTCAE Version 4.0; see manufacturer's prescribing information for the coadministered product, letrozole, and dose adjustment guidelines in the event of toxicity and other relevant safety information or contraindications.

6.6 Anticipated Toxicities for Pembrolizumab

Per the IB (Edition 18, 10 March 2020) the expected toxicities for pembrolizumab are as follows (asterisk signifies ≥ 10%; no asterisk signifies 1-10%, † signifies < 1% and * signifies unknown frequency):

Blood and lymphatic system disorders	Anemia, aplasia pure red cell†, neutropenia†, thrombocytopenia†, leukopenia†, lymphopenia†, eosinophilia†, hemolytic anemia†, immune thrombocytopenic purpura†
Cardiac	Myocarditis†, pericardial effusion†, pericaditis†
Endocrine	Addison' disease†, adrenal insufficienty, andrenocortial insufficiency actue†, autoimmune thyroiditis†, Hyperthyroidism, hypothyroidism, hypophysitis †, secondary adrenal insufficiency †, thyroid disorder†, thyroiditis†
Eye	Iridocyclitis†, iritis†, Uveitis †, vogt-koyanagi-harada disease†, eyelid oedema†, periorbital oedema†, dry eye †
Gastrointestinal	Abdominal pain, autoimmune colitis†, autoimmune pacreatitis†, colitis microscopic†, Diarrhea*, entercoloitis haemorrhagic†, enterocolitis†, immune-meiated entercoloitis†, nausea*, abdominal pain, vomiting, colitis, constipation, dry mouth, lip oedema†, pancreatitis†, small intestinal perforation†

General Disorders and Administration Site	Fatigue*, face oedema†,, generalized oedema†, localised oedema, oedema†, oedema periphral, asthenia, edema, pyrexia, influenza-like illness, chills
Hepatobiliary	Drug-induced liver injury†, hepatitis actue†, Hepatitis†immune-mediated hepatitis*, jaundice†
Immune system disorders	Infusion related reactions, severe infusion reactions† sarcoidosis†, liver transplant rejection^, hypersensitivity*, drug hypersensitivity*, cytokine release syndrome*, anaphylactoid reaction*
Infections and infestations	encephalitis†, meningitis aseptic†, pneumonia*
Injury, poisoning, and procedura complications	Infusion related reaction*
Investigations (excluding hematologic)	AST/ALT increased, blood alkaline phosphatase increased, blood creatinine increased, low sodium levels†, low potassium levels†, low calcium levels†, blood bilirubin increased†, amylase increased†, increased calcium†
Metabolism and Nutrition	Decreased appetite, Type 1 diabetes mellitus †, diabetic ketoacidosis*, diabetic ketosis†, fluid overload†, hypercalcaemia, hypocalcaemia†, hypokalaemia, hyponatraemia, ketosis-prone diabetes mellitus†, fluid retention†
Musculoskeletal and Connective Tissue	Arthralgia, back pain, join effusion†, musculoskeletal chest pain†,myositis, musculoskeletal pain, myalgia†, myopathy†, arthritis, pain in extremity, polyarthritis, rhabdomyolysis†, synovitis†, tendonitis†, tenosynovitis†`, joint swelling†, musculoskeletal discomfort†, musculoskeletal stiffness†, polymyalgia rheumatica†
Nervous system	Axonal neuropathy†, demvelinating polyneuropathy†, Headache, dizziness, dysgeusia†, epilepsy†, lethargy†, Guillian-Barré syndrome †, encephalitis†, peripheral neuropathy†, miller fisher syndrome†, myasthenia gravis†, myasthenic syndrome†, neuropathy peripheral†, noninfective encephalitis†,
Psychiatric disorders	Insomnia†
Renal and urinary	Autoimmune nephritis*, Nephritis †, nephrotic syndrome†, renal failure, tubulointerstitial nephritis
Respiratory, Thoracic and Mediastinal	Acute interstitial pneumonitis†, Cough, immune-mediated pneumonitis†, interstitial lung disease, pneumonitis† dyspnea
Skin and Subcutaneous Tissue	Acute febrile neutrophilic dermatosis†, decubitus ulcer, dermatitis†, dermatitis bullous*, dermatitis exfoliative†, drug eruption, epidermal necrosis, erythema multiforme, exfoliative rash, lichen planus, lichenoid keratosis, pemphigoid* pemphigus*, pruritus generalised, Pruritus*, psoriasis†, rash erythematous,* rash generalized*, rash macular*, rash maculo-papular*, rash pruritic*, rash

	pustular*, rash*, rash papular, skin necrosis*, vitiligo, dry skin, erythema, eczema†, alopecia†, urticaria†,severe skin reactions† (includes Steven-Johnson Syndrome† and toxic epidermal necrolysis†, toxic skin eruption*), dermatitis acneiform, dermatitis psoriasiform†, skin hypopigmentation†
Vascular	Inflammation of the blood vessels (vasculitis). Symptoms will depend on the particular blood vessels that are involved in the inflammatory process, for example, if it is your skin, you may get a rash. If your nerves are not getting enough blood, you could have numbness and weakness. You may also experience fever, weight loss, and fatigue.

7.0 Study Oversight, Quality Assurance, and Data & Safety Monitoring

7.1 All Investigator Responsibilities

An investigator is responsible for ensuring that an investigation is conducted according to the signed investigator statement, the investigational plan, and applicable regulations; for protecting the rights, safety, and welfare of subjects under the investigator's care; and for the control of drugs under investigation.

7.2 Study Principal Investigator Responsibilities

The Study Principal Investigator is responsible for the conduct of the clinical trial, including overseeing that sponsor responsibilities are executed in accordance with federal regulations.

7.3 Protocol Management Team (PMT)

The Protocol Management Team (PMT), minimally consisting of the study PI, collaborating investigators, research nurse, clinical research associate/coordinator, and the study biostatistician, is responsible for ongoing monitoring of the data and safety of this study, including implementation of the stopping rules for safety/toxicity.

The PMT is recommended to meet (in person or via teleconference) to review study status. The meeting is a forum to discuss study related issues including accrual, SAE/AE/UPs experienced, study response, deviations/violations, and study management issues. The appropriateness of further subject enrollment and the specific intervention for subsequent subject enrollment are addressed.

7.4 Quality Assurance

Clinical site monitoring is conducted to ensure that the rights of human subjects are protected, that the study is implemented in accordance with the protocol and regulatory requirements, and that the quality and integrity of study data and data collection methods are maintained. Monitoring for this study will be performed by the City of Hope Office of Clinical Trials Monitoring (OCTM), within City of Hope's Office for Safety and Data Quality.

Details of clinical site monitoring are documented in the OCTM SOP and the Risk Based Monitoring (RBM) plan. These documents specifies the frequency of monitoring, monitoring procedures, the amount of subject data to be reviewed, and the distribution of monitoring reports to the study team and the COH DSMC.

7.5 Risk Determination

This is a High Risk Level 4 study as defined in the City of Hope Institutional Data and Safety Monitoring Plan [policy dated 10/8/2015]. This determination was made because the study involves the use of a City of Hope held IND in a Phase II clinical trial.

7.6 City of Hope Data and Safety Monitoring Committee

The COH Data and Safety Monitoring Committee (DSMC) will review and monitor study progress, compliance, toxicity, safety, and accrual data from this trial via the PMT Progress Report (submitted by the Study Principal Investigator according to the frequency outlined in the City of Hope Institutional DSMP). The DSMC is composed of clinical specialists who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Protocol Management Team.

The Protocol Management Team (PMT) is responsible for monitoring the data and safety of this study. The PMT consists of the Principal Investigator (PI), Collaborating Investigators, CRA/protocol nurse, and Biostatistician. The PMT is responsible for monitoring the data and safety of this study, including implementation of the stopping rules for safety and efficacy.

The PMT is required to submit periodic status reports (i.e., the PMT Report) according to the frequency prescribed in the City of Hope Institutional Data and Safety Monitoring Plan [policy dated 10/8/2015]. Important decisions made during PMT meetings only need to be noted in the PMT Report submitted to the Data and Safety Monitoring Committee (DSMC). Protocol specific data collection will include the statistical considerations in **Section 13**.

7.7 Adverse Event Definitions Adverse Events and Serious Adverse Events

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

Adverse Event (AE) - An adverse event is any untoward medical experience or change of an existing condition that occurs during or after treatment, whether or not it is considered to be related to the protocol intervention.

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

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

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

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

Progression of the cancer under study is not considered an AE.

Reporting Non-serious Adverse Events - Adverse events (grade 2 or higher) will be collected after the patient is given the study treatment or any study related procedures. Adverse events will be monitored by the PMT. Adverse events that do not meet the criteria of serious OR are not unanticipated problems will be reported only in the PMT Report. Only clinically significant lab abnormalities will be collected as AEs. Non-Clinically significant labs will not be recorded.

Additionally, all SAEs that occur after the consent form is signed but before treatment begins must be reported by the investigator if they cause the subject to be excluded from the trial or are the result of a protocol-specified intervention.

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

- Death.
- Is life-threatening experience (places the subject at immediate risk of death from the event as it occurred).
- Unplanned hospitalization (equal to or greater than 24 hours) or prolongation of existing hospitalization.
- A persistent or significant disability/incapacity.
- A congenital anomaly/birth defect.
- Secondary malignancy.
- Any other AE (grade 2 or higher) that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias of convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

Note: In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable to Merck in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by Merck for collection purposes.

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

Reporting Serious Adverse Events - begins after study treatment or any study related procedures. All SAEs occurring during this study, whether observed by the physician, nurse, or reported by the patient, will be reported according to the approved City of Hope's Institutional policy [policy effective date: 05/14/14]. Serious Adverse Events that require expedited reporting will be submitted electronically using iRIS.

Additionally, all SAEs that occur after the consent form is signed but before treatment begins must be reported by the investigator if they cause the subject to be excluded from the trial or are the result of a protocol-specified intervention.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any SAE, or follow up to a SAE, including death due to any cause other than progression of the cancer under study whether or not related to the Merck product, must be reported within 2 working days to Merck Global Safety.

Additionally, any SAE, considered by an investigator who is a qualified physician to be related to Merck product that is brought to the attention of the investigator at any time following consent through the end of the specified safety follow-up period specified in the paragraph above, or at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor and to Merck Global Safety.

All subjects with SAEs must be followed up for outcome.

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

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

Adverse Event Name and Severity - The PI will determine the adverse event name and severity (grade) by using the CTCAE version 4.0. In addition, New York Heart Association (NYHA) Class III and IV heart failure will be captured.

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

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

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

- **Definite** - The AE is clearly related to the investigational agent or study procedure and unrelated to any other cause.
- **Probable** - The AE is likely related to the investigational agent or study procedure and unlikely related to other cause(s).
- **Possible** -The AE may be related to the investigational agent or study procedure and may be related to another cause(s).
- **Unlikely** -The AE is doubtfully related to the investigational agent or study procedure and likely related to another cause(s).
- **Unrelated** -The AE is clearly not related to the investigational agent or study procedure and is attributable to another cause(s).

An investigator who is a qualified physician will evaluate all AEs according to the NCI CTCAE, version 4. 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 AEs regardless of CTCAE grade must also be evaluated for seriousness.

7.8 Reporting Related to COH Held IND

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

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

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

The PI or designee will be responsible for contacting the Office of IND Development and Regulatory Affairs (OIDRA) at COH to ensure prompt reporting of safety reports to the FDA. OIDRA will assist the PI with the preparation of the report and submit the report to the FDA in accordance with the approved City of Hope's Institutional policy [policy effective date: 05/14/14].

7.9 Deviations and Unanticipated Problems

Deviation - A deviation is a divergence from a specific element of a protocol that occurred without prior IRB approval. Investigators may deviate from the protocol to eliminate immediate hazard(s) for the protection, safety, and well-being of the study subjects without prior IRB approval. For any such deviation, the PI will notify the COH DSMC and IRB within 5 calendar days of its occurrence via iRIS in accordance with the Clinical Research Protocol Deviation policy [policy effective date: 11/07/11].

Deviations from the protocol should be avoided, except when necessary to eliminate immediate hazard(s) for the protection, safety, and well-being of a research participant. As a result of deviations, corrective actions are to be developed by the study staff and implemented promptly. All protocol deviations and planned protocol deviations will be reported in accordance with the Clinical Research Protocol Deviation policy.

In addition, if contractually obligated, the industry partner/funding source must also approve planned deviations, as necessary.

Single Subject Exception (SSE) - An SSE is a planned deviation, meaning that it involves circumstances in which the specific procedures called for in a protocol are not in the best interests of a specific patient. It is a deviation that is anticipated and receives prior approval by the PI and the IRB. The SSE must be submitted as a "Single Subject Exception Amendment Request" via iRIS in accordance with IRB guidelines and the Clinical Research Protocol Deviation policy [policy effective date: 11/07/11]. An IRB approved SSE does not need to be submitted as a deviation to the DSMC.

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

- Unexpected (in terms of nature, severity, or frequency) given the following: a) the research procedures described in the protocol-related documents such as the IRB approved research protocol, informed consent document or IB; and b) the characteristics of the subject population being studied; **AND**

- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcomes may have been caused by the drugs, devices or procedures involved in the research); **AND**
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm) than previously known or recognized.

Any UP that occurs during study conduct will be reported to the DSMC and IRB in accordance with the City of Hope's Institutional policy [policy effective date: 05/14/14] using iRIS.

7.10 Ethics Review

The final study protocol, including the final version of the Written Informed Consent Form, must be approved in writing by COH IRB. The study PI is responsible for informing the IRB of any amendment to the protocol in accordance with local requirements. In addition, the IRB must approve all advertising used to recruit subjects for the study. The protocol must be re-approved by the IRB annually, as local regulations require. Progress reports and notifications of serious unexpected adverse drug reactions will be provided to the IRB according to local regulations and guidelines. The investigators are also responsible for providing the IRB with reports of any serious adverse drug reactions from any other study conducted with the investigational product. Merck will provide this information to COH. These reports will be reviewed by the investigator and those considered unexpected and possibly related to protocol therapy plus all deaths within 90 days of discontinuing treatment will be submitted to the IRB.

7.11 Ethical Conduct of the Study

The study will be performed in accordance with ethical principles originating from the Declaration of Helsinki, which are consistent with ICH/Good Clinical Practice, and applicable regulatory requirements. Ethical standards for human subjects will be strictly followed. After obtaining permission from the primary treating clinician, patients who meet the eligibility criteria will be approached and recruited for this study by the PI or another trained member of the research team. All investigators and research assistants will have undergone full training in Human Subjects Protection Certification. In addition, all research team members will undergo formal training regarding the research procedures, including the informed consent process. Once initial contact has been established, the PI or their trained research staff will present the study to potential participants, strictly adhering to ethical and regulatory standards for human subject research.

An investigator, who is a qualified physician, will evaluate all AEs as shown in **Table 11**, below.

Table 11: Evaluating Adverse Events

V4.0 CTCAE Grading	**Grade 2**	**Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of Merck product that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization for an elective procedure to treat a pre-existing condition that has not worsened is not a serious adverse event. A pre-existing condition is a clinical condition that is diagnosed prior to the use of a Merck product and is documented in the patient's medical history.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new **cancer** (that is not a condition of the study) **(**although not serious per ICH definition, is reportable to the Sponsor within 24 hours and to Merck within 2 working days to meet certain local requirements); **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event for collection purposes. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported to Merck within 2 working days.	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause Merck product to be discontinued?	
Relationship to Merck Product	Did Merck product cause the adverse event? The determination of the likelihood that Merck product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that support the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between Merck product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely Merck product caused the adverse event (AE):	
Relationship to	**Exposure**	Is there evidence that the subject was actually exposed to Merck product such as: reliable history, acceptable compliance assessment (pill count,

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

7.12 Reporting of Unanticipated Problems and Adverse Events

From the time of treatment allocation through 30 days following cessation of treatment, AEs (grade 2 or higher) must be reported by the investigator. Such events will be recorded at each examination on the AE CRF. The reporting timeframe for AEs meeting any serious criteria is described in **Section 7.3**. The investigator will make every attempt to follow all subjects with non-serious adverse events for outcome. Only clinically significant lab abnormalities will be collected as AEs. Non-Clinically significant labs will not be recorded.

7.13 Definition of an Overdose for and Reporting of Overdose to Merck

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

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

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

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

7.14 Reporting of Pregnancy and Lactation to Merck

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

Pregnancies and lactations that occur after the consent form is signed but before treatment begins must be reported by the investigator if they cause the subject to be excluded from the trial or are the result of a protocol-specified intervention.

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

Such events must be reported within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220).

7.15 Reporting of AEs to Merck

7.15.1 Events of Clinical Interest

Selected non-serious and serious AEs are also known irAEs must be reported within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220).

For the time period beginning when the consent form is signed until treatment begins, any irAE, or follow up to an irAE that occurs to any subject must be reported within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial or is the result of a protocol-specified intervention.

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

Events of clinical interest for this trial include:

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

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

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

7.15.2 Protocol-specific exceptions to serious adverse event reporting

Efficacy endpoints will not be reported to Merck unless there is evidence suggesting a causal relationship between the drug and the event. Any such event will be submitted to Merck Global Safety within 2 working days either by electronic or paper media. Specifically, the suspected/actual events covered in this exception include any event that is disease progression of the cancer under study.

The study team will monitor efficacy endpoint events and safety data to ensure the safety of the subjects in the trial. Any suspected endpoint which upon review is not progression of the cancer under study will be forwarded to Merck Global Safety as a SAE within 2 working days of determination that the event is not progression of the cancer under study. Hospitalization related to convenience (e.g. transportation issues etc.) will not be considered a SAE.

8.0 Agent Information and Risks

8.1 Palbociclib

Refer to package insert for description, toxicology, and pharmacology of palbociclib. For cohort 3, commercial supply of palbociclib is allowed.

8.1.1 Description

Palbociclib is a kinase inhibitor indicated in combination with letrozole for the treatment of postmenopausal patients with ER+HER2- advanced breast cancer as initial endocrine-based therapy for their metastatic disease. This indication is approved under accelerated approval based on PFS. Continued approval for this indication may be contingent upon verification and description of clinical benefit in a confirmatory trial.

8.1.2 Toxicology

Palbociclib was clastogenic in an in vitro micronucleus assay in Chinese Hamster Ovary cells and in vivo in the bone marrow of male rats that received doses ≥100 mg/kg/day for three weeks. Clastogenicity occurred via an aneugenic mechanism. Palbociclib was not mutagenic in an in vitro bacterial reverse mutation (Ames) assay and did not induce structural chromosomal aberrations in the in vitro human lymphocyte chromosome aberration assay.

In a fertility study in female rats, palbociclib did not affect mating or fertility at any dose up to 300 mg/kg/day (approximately 4 times human clinical exposure based on AUC) and no adverse effects were observed in the female reproductive tissues in repeat-dose toxicity studies up to 300 mg/kg/day in the rat and

3 mg/kg/day in the dog (approximately 6 times and similar to human exposure (AUC), at the recommended dose, respectively). Male fertility studies with palbociclib have not been conducted; however, in repeat-dose toxicity studies, testicular degeneration was observed in rats and dogs at 30 and 0.2 mg/kg/day, respectively (approximately 11 and 0.1 times human exposure (AUC), at the recommended dose, respectively), which was partially reversible in the rat and dog following a 12-week non-dosing period.

Altered glucose metabolism (glycosuria, hyperglycemia, decreased insulin) associated with changes in the pancreas (islet cell vacuolation), eye (cataracts, lens degeneration), teeth (degeneration/necrosis of ameloblasts in actively growing teeth), kidney (tubule vacuolation, chronic progressive nephropathy), and adipose tissue (atrophy) were identified in the 27-week repeat-dose toxicology study in rats and were most prevalent in males at doses ≥30 mg/kg/day (approximately 11 times the human exposure (AUC) at the recommended dose). Some of these findings (glycosuria/hyperglycemia, pancreatic islet cell vacuolation, and kidney tubule vacuolation) were present in the 15-week repeat-dose toxicology study in rats, but with lower incidence and severity. The rats used in these studies were approximately 7 weeks old at the beginning of the studies. Altered glucose metabolism or associated changes in pancreas, eye, teeth, kidney, and adipose tissue were not identified in dogs in repeat-dose toxicology studies up to 39 weeks duration.

8.1.3 Pharmacology - handling, storage, dispensing and disposal

Palbociclib is supplied in the following strengths and package**:**

IBRANCE Capsules			
Package Configuration	**Capsule Strength (mg)**	**NDC**	**Capsule Description**
Bottles of 21 capsules	125	NDC 0069-0189-21	opaque, hard gelatin capsules, size 0, with caramel cap and body, printed with white ink “Pfizer” on the cap, “PBC 125” on the body
Bottles of 21 capsules	100	NDC 0069-0188-21	opaque, hard gelatin capsules, size 1, with caramel cap and light orange body, printed with white ink “Pfizer” on the cap, “PBC 100” on the body
Bottles of 21 capsules	75	NDC 0069-0187-21	opaque, hard gelatin capsules, size 2, with light orange cap and body, printed with white ink “Pfizer” on the cap, “PBC 75” on the body

Store at 20°C to 25°C (68°F to 77°F); excursions permitted between 15°C to 30°C (59°F to 86°F).

Disposal: Unused and residual drug should be put into a plastic bag and disposed of in a chemical waste container.

8.2 Letrozole/Fulvestrant

Refer to package insert for description, toxicology, and pharmacology of Letrozole and Fulvestrant.

8.2.1 Description

Letrozole tablets for oral administration contain 2.5 mg of letrozole, a nonsteroidal aromatase inhibitor (inhibitor of estrogen synthesis). It is chemically described as 4,4'-(1H-1,2,4-Triazol-1-ylmethylene) dibenzonitrile. Letrozole is a white to yellowish crystalline powder, practically odorless, freely soluble in dichloromethane, slightly soluble in ethanol, and practically insoluble in water. It has a molecular weight of 285.31, empirical formula $C_{17}H_{11}N_5$, and a melting range of 184°C-185°C. Letrozole tablets are available as 2.5 mg tablets for oral administration.

Inactive Ingredients: Colloidal silicon dioxide, ferric oxide, hydroxypropyl methylcellulose, lactose monohydrate, magnesium stearate, maize starch, microcrystalline cellulose, polyethylene glycol, sodium starch glycolate, talc, and titanium dioxide.

8.2.2 Pharmacology - handling, storage, dispensing and disposal

In postmenopausal patients, estrogens are mainly derived from the action of the aromatase enzyme, which converts adrenal androgens (primarily androstenedione and testosterone) to estrone and estradiol. The suppression of estrogen biosynthesis in peripheral tissues and in the cancer tissue itself can therefore be achieved by specifically inhibiting the aromatase enzyme. Letrozole is a nonsteroidal competitive inhibitor of the aromatase enzyme system; it inhibits the conversion of androgens to estrogens. In adult nontumor- and tumor-bearing female animals, letrozole is as effective as ovariectomy in reducing uterine weight, elevating serum LH, and causing the regression of estrogen-dependent tumors. In contrast to ovariectomy, treatment with letrozole does not lead to an increase in serum FSH.

Letrozole selectively inhibits gonadal steroidogenesis but has no significant effect on adrenal mineralocorticoid or glucocorticoid synthesis. Letrozole inhibits the aromatase enzyme by competitively binding to the heme of the cytochrome P450 subunit of the enzyme, resulting in a reduction of estrogen biosynthesis in all tissues. Treatment of patients with letrozole significantly lowers serum estrone, estradiol and estrone sulfate and has not been shown to significantly affect adrenal corticosteroid synthesis, aldosterone synthesis, or synthesis of thyroid hormones.

Letrozole is supplied as 2.5 mg tablets through regular commercial supply and store at room temperature as instructed per manufacture.

8.3 Pembrolizumab

Refer to IB for description, toxicology, and pharmacology of pembrolizumab.

8.3.1 Description

Pembrolizumab is a human programmed death receptor-1 (PD-1)-blocking antibody indicated for the treatment of the following:

- Unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor.
- Epidermal Growth Factor Receptor (EGFR) mutation-negative, and anaplastic lymphoma kinase (ALK) rearrangement-negative non-small cell lung cancer (NSCLC) which has progressed during or following platinum-based chemotherapy.

These indications are approved under accelerated approval based on tumor response rate and durability of response. Therefore, when treating patients with diseases other than the above, 'off label' use of the agent will be employed

8.3.2 Toxicology

Per the IB (Edition 16, 2018) the expected toxicities for pembrolizumab are as follows (asterisk signifies ≥ 10%; very common; no asterisk signifies 1-10%, common, † signifies 0.1- 1%, uncommon and ^ signifies 0.01-0.1% rare). For percentage of subjects with adverse event of special interest by toxicity grade, refer to table 32 of IB:

Blood and lymphatic system disorders	Anemia, neutropenia†, thrombocytopenia†, leukopenia†, lymphopenia†, eosinophilia†, hemolytic anemia†, †, immune thrombocytopenic purpura†
Cardiac	Myocarditis†, hypertension †
Endocrine	Hyperthyroidism, hypothyroidism, hypophysitis †, secondary adrenal insufficiency †, thyroiditis†

Eye	Uveitis †, dry eye †
Gastrointestinal	Diarrhea*, nausea*, abdominal pain*, vomiting, colitis, constipation, dry mouth, pancreatitis†, †, small intestinal perforation†
General Disorders and Administration Site	Fatigue*, asthenia, edema, pyrexia*, , influenza-like illness, chills
Hepatobiliary	Hepatitis†
Immune system	Infusion related reactions, severe infusion reactions†, sarcoidosis†, solid organ transplant rejection^
Investigations (excluding hematologic)	AST/ALT increased, blood alkaline phosphatase increased, blood creatinine increased, hyponatremia †, low potassium levels†, low calcium levels†, blood bilirubin increased†, amylase increased†, increased calcium†,
Metabolism and Nutrition	Decreased appetite, Type 1 diabetes mellitus †
Musculoskeletal and Connective Tissue	Arthralgia*, back pain*, myositis†, musculoskeletal pain, arthritis, pain in extremity, tenosynovitis^
Nervous system	Headache, dizziness, dysgeusia, epilepsy†, lethargy†, Guillian-Barré syndrome †, encephalitis†, peripheral neuropathy†, myasthenic syndrome†,
Renal and urinary	Nephritis †
Respiratory, Thoracic and Mediastinal	Cough*, pneumonitis, , dyspnea, penumonia †
Skin and Subcutaneous Tissue	Pruritus*, rash*, vitiligo, dry skin, erythema, lichenoid keratosis †, psoriasis†, dermatitis †, dermatitis acneiform †, papule†, hair color changes†, eczema†, alopecia†, severe skin reactions*, erythema nodosum ^, Steven-Johnson Syndrome^ and toxic epidermal necrolysis^)

8.3.3 Pharmacology - handling, storage, dispensing and disposal

Binding of the PD-1 ligands, PD-L1 and PD-L2, to the PD-1 receptor found on T cells, inhibits T cell proliferation and cytokine production. Upregulation of PD-1 ligands occurs in some tumors and signaling through this pathway can contribute to inhibition of active T-cell immune surveillance of tumors. Pembrolizumab is a monoclonal antibody that binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2, releasing PD-1 pathway-mediated inhibition of the immune response, including the anti-tumor immune response. In syngeneic mouse tumor models, blocking PD-1 activity resulted in decreased tumor growth.

Pembrolizumab is provided in 50 mg lyophilized powder for reconstitution, or a 100 mg/ml solution, in single-use vials which should be stored between 2-8°C. Unopened vials of pembrolizumab are stable until the expiration date indicated on the package when stored at the indicated room temperature.

8.3.3.1 Preparation

Add 2.3 ml of Sterile Water for Injection, USP by injecting the water along the walls of the vial (not directly on the lyophilized powder), resulting in a concentration of 25 mg/ml. Slowly swirl the vial. Allow up to 5 minutes for the bubbles to clear. Do not shake the vial. Visually inspect the reconstituted solution for particulate matter and discoloration prior to administration. Reconstituted pembrolizumab is a clear to slightly opalescent, colorless to slightly yellow solution. Discard reconstituted vial if extraneous particulate

matter other than translucent to white proteinaceous particles is observed. Alternatively, Pembrolizumab solution provided at 100 mg/4 ml (25 mg/ml) will be used.

Withdraw the required volume from the vial(s) of pembrolizumab and transfer into an IV bag containing 0.9% Sodium Chloride Injection, USP. Mix diluted solution by gentle inversion. The final concentration of the diluted solution should be 1 mg/ml - 10 mg/ml and administered IV over 30 minutes, through an intravenous line containing a sterile, non-pyrogenic, low-protein binding 0.2 micron to 5 micron in-line or add-on filter.

8.3.3.2 Storage of reconstituted and diluted solutions

Pembrolizumab contains no preservative hence reconstituted diluted solutions should not be stored at room temperature for more than 4 hours after preparation. This includes storage of reconstituted vials, storage of infusion solutions in the IV bag and the duration of infusion. Under refrigeration (2°C-8°C) diluted solutions should not be stored for more than 24 hours from the time of preparation. If refrigerated, allow the diluted solution to come to room temperature prior to administration. Do not freeze. Caution should be exercised in handling pembrolizumab solutions. The use of gloves and gowns is recommended. If the product comes into contact with skin or mucosa, the skin should be washed thoroughly with soap and water and mucosa irrigated with copious amounts of water.

8.3.3.3 Disposal

Unused and residual drug should be put into a plastic bag and disposed of in a chemical waste container.

9.0 Correlative/Special Studies

9.1 Tumor Tissue Immune Correlatives

Previous research data suggested that CD8 T cell infiltration and PD-L1 expression within tumors may predict efficacy for pembrolizumab, but definitive biomarkers are not yet available [58-62]. To identify biomarkers to predict and/or follow efficacy for pembrolizumab in combination with endocrine therapy and CDK 4/6 inhibitors in $ER^{+}HER2^{-}$ metastatic breast cancer, we propose the following correlative studies to analyze the immune and stromal cells within metastatic tumors, as well as immune cells in peripheral blood, before and after therapy. These planned analyses will enhance our understanding of the role of immune check point inhibitors in endocrine resistant ER+ breast cancer; the cross talk of CDK 4/6 pathway with immune check point inhibitor; and the potential future target of patients who failed immunotherapy.

For cohort 1&2, we will obtain biopsies of tumor tissue(s) prior to study initiation and at the end of study (see **Section 10**). Optional on-treatment biopsies are allowed for assessment of immune response.

For Cohort 3, baseline, C2 D1(+/- 7 days) on treatment and optional end of treatment biopsy will be acquired.

When a core needle biopsy is used, a minimum of 4 core biopsy samples with a minimum of 4 cores of minimum 18-gauge needle is recommended.

Key questions we will address include:

1) Immune and stromal cell characteristics before treatment that correlate with clinical response [58]. This includes PD-L1 expression levels.
2) Changes in immune and stromal cells after therapy that correlate with clinical response [59].

Methods

- 12-color FACS analysis to enumerate/phenotype immune cell subsets and functional readouts, including cytokine production and signaling (phosflow).

- TCR repertoire analysis via deep sequencing. Expansion of the T cell repertoire after therapy will be evidence for an immunological response and may indicate epitope spread.
- Immunohistology using a novel quantitative, spatial image analysis system (Vectra, Perkin Elmer) that will enable us to analyze immune, stromal, and cancer cells in metastatic tumors via 8-color histology.
- Gene expression analysis of stromal cells via RNA-Seq or microarrays: Messenger RNA (mRNA) expression profiling in tumor specimens and peripheral blood will be completed to assess expression either by RNAseq or microarrays, to define a gene set critical for clinical response to pembrolizumab. The hypothesis to be tested is that pembrolizumab induces responses in tumors that reflect an inflammatory/immune cell-rich phenotype based on gene expression. Expression of individual genes related to the immune system may also be evaluated, such as immune signatures and critical cytokines.
- Morphological / IHC TIL analysis of tumor biopsies: Tumor infiltrating lymphocytes have been shown to provide prognostic and potentially predictive value, particularly in TNBC and HER2-overexpressing breast cancer. Hematoxylin and eosin (H&E)-stained breast tumor sections can be evaluated for TILs, according to a recently published standardized methodology [63]. We will evaluate: (1) pre-treatment TILS as a predictor of response to combination therapy; (2) specific TIL subsets (e.g. CD4, CD8, Treg distribution) and other immunological correlatives (e.g. TCR repertoire analysis) as possible predictors of response; (3) change in TILs as a result of the combination therapy.

9.2 Tumor Tissue Genomic Correlatives

Sequencing technologies, such as whole genome sequencing or single cell RNA sequencing, allow global assessment of DNA mutations and gene expression profiles, which can provide molecular phenotyping that identifies distinct characteristics of tumors, including the ultimate outcome of disease. We will use single cell sequencing technology to characterize a given tumor for both its mutational profile and phenotypic status.

An overview of collection, processing, and analysis details are shown below:

Tissue Type	Timepoint of collection	Materials per timepoint	Material Submitted	Laboratory Performing the Analysis	Type of Laboratory Analysis
FFPE	• Baseline • *C2D1* • EOT	20 unstained slides	Slides	Dr. Yuan Yuan	Genomic or TME analysis

9.3 Peripheral Blood Immune Biomarkers (Serial Cytokine Measurements)

Blood samples will be obtained as specified in **Section 10** to track the temporal dynamics of the host immune response. 3 tubes (1 x 10 ml green-top and 2 x lavender-top) will be collected. The green-top tube will be used for cytokine and immune analysis in Dr. Synold's lab. The two lavender tops will be used for peripheral blood ctDNA and flow cytometry.

The Human Cytokine Thirty-Plex Antibody Bead Kit (Invitrogen, Camarillo, CA) will be used according to manufacturer's instructions. The 30 cytokine panel includes: epidermal growth factor (EGF), eotaxin, basic fibroblast growth factor (FGF-basic), granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), hepatocyte growth factor (HGF), interferon alpha (IFN-α); IFN-gamma (γ), interleukin-1 beta (IL-1β), interleukin-1 receptor antagonist (IL-1RA), IL-2; interleukin-2 receptor (IL-2R), IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p40/p70, IL-13, IL-15, IL-17, IFN-γ-inducible protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), monokine induced by IFN-γ (MIG),

monocyte inflammatory protein-1 alpha (MIP-1α), monocyte inflammatory protein-1 beta (MIP-1β), regulated upon activation, normal T cell expressed and secreted cytokine (RANTES), tumor necrosis factor (TNF)-α, and VEGF. Cytokine concentrations will be measured using the Bio-plex HTF Luminex instrument and results calculated using Bio-plex Manager 3.0 Software. The inter-assay precision for all cytokines is <10 % and the lower limit of quantitation is between 1 and 15 pg/ml, depending on the target.

9.3.1 Blood Sample Collection

Blood samples will be collected from an indwelling venous catheter or by venipuncture. At each time point indicated in the study calendar, peripheral blood will be collected into one 10 mL green-top tube (sodium or lithium heparin) to prevent blood clotting and two 10 ml lavender-top (EDTA) tubes. Tubes will be inverted several times and then immediately placed on ice for transportation to the CICSL, the processing laboratory. The whole blood should be processed within 4 hours of collection. While awaiting processing, the blood should be kept on a rocker set at low speed to mimic circulation and avoid clot formation.

9.3.2 Sample Processing

9.3.2.1 Plasma

- For plasma preparation, anti-coagulated whole blood (one 10 mL green-top tube) will be processed by centrifugation for 10 minutes at 1000 x g at 4ºC. The resulting upper plasma layer from each tube will be drawn up sequentially into a sterile 5 mL syringe and pushed through a sterile 0.2/0.8 micron disposable filter (PALL Acrodisc PF, Cat. 4658). The filtered plasma will then be transferred in 500 µL aliquots into multiple appropriately-labeled Starstedt microfuge tubes (Starstedt Cat 72.692.005). All the plasma aliquots will be stored frozen at -80˚C until ready for testing.

9.3.2.2 PBMCs

- PBMCs analysis will be performed at the COH Immune-Oncology Core. The two 10 mL lavender-top tubes used to prepare plasma above will be diluted 1:1 with Hank's Balanced Salt Solution ("HBSS", Irvine Scientific, Cat. 9228 or equivalent).Peripheral blood mononuclear cells (PBMC) will then be isolated from the combined whole blood sample by Ficoll-gradient separation as described below;
    - Allow Accuspin-Histopaque tubes ("Accuspin", Sigma Cat. A6929 or A0561, for 12 or 100 tubes, respectively) and HBSS to warm to room temperature. Place a Mr. Frosty container in the refrigerator and prepare the freezing media by adding 10% DMSO to fetal calf serum and chill at 4˚C or on ice.
    - Prepare Accuspin tubes by centrifuging at 1000 x g for 1 minute at room temperature (RT) with brakes on. Each tube can process up to 20 mLs of whole blood; prepare the appropriate amount of tubes necessary. After centrifugation, the Histopaque reagent should be below the barrier of the tube. Add 5 mLs of HBSS to the Accuspin tube. Add up to 20 mls of whole blood to each Accuspin tube until all the blood has been distributed.
    - Centrifuge the blood sample at 800 x g for 15 minutes at RT with brakes on LOW. After centrifugation, three layers should be visible above the barrier of the tube: the plasma layer at the top, a cloudy layer in the middle where the PBMC are, and a clear Histopaque reagent layer right below. Using a pipette, remove the upper plasma layer to within 2 cm of the cloudy interphase. Carefully pipette the cloudy PBMC interphase and transfer to a sterile 50 mL centrifuge tube.
    - Add HBSS up to the 45 mL mark in the centrifuge tube with the PBMC and spin at 400 x g for 10 minutes at RT with brakes on. Decant the supernatant and loosen the cell pellet before adding HBSS to the 45-mL mark again for a second wash. Centrifuge at 300 x g for 10 minutes at RT with brakes on. Decant the supernatant, loosen the cell pellet and then add a known

volume of HBSS to resuspend the cells for counting. Mix the cell suspension up and down with a pipette several times before removing a small aliquot for cell count.

- Centrifuge the cell suspension one final time at 300 x g for 10 minutes at RT with brakes on. PBMC should be frozen down at 0.5 – 1 x 10^7 cells/vial. Determine the volume of freezing media (fetal calf serum with 10% DMSO) needed to give a 1 x 10^7 cell/mL suspension. After the last centrifugation is complete, discard supernatant and loosen the cell pellet before adding freezing media slowly, a small volume at a time with mixing in between (vortex at low speed). Aliquot 0.5 - 1 mL of the final cell suspension into individually labeled cryovials. Transfer the cryovials into Mr. Frosty and store at -80˚C. Twenty four hours later, cryovials will be transferred to liquid nitrogen tanks for long-term storage.

9.4 PD-L1 Biomarker Expression Level

No data is currently available on the performance of pembrolizumab in breast cancer patients with PD-L1 negative tumors (i.e., PD-L1 staining in <1% tumor cells and no stromal staining). Since there is limited data using PD-L1 expression as a biomarker predicting response to pembrolizumab, and patients with PD-L1 negative tumors may benefit from pembrolizumab, PD-L1 expression will not be used for patient selection. Instead, analysis of PD-L1 expression will be included as a correlative study. One unstained slide from FFPE tumor samples will be stained for PD-L1 using commercial testing lab and 22C3 PDL1 antibody.

9.5 Peripheral blood circulating tumor DNA

Blood samples will be obtained as specified in **Section 10** to track the temporal dynamics of the host immune response. One tube (1 x 10 ml purple-top) will be collected and sent on ice to Dr. Tim Synold's lab at COH via COH Biorepository Core for processing and storage.

Procedure for plasma/ buffy coat isolation from EDTA tubes: Collect blood in tube until filled. Mix tube by inverting 3-5 times and transport to Dr. Synold's lab for processing. Samples must be processed within one hour of collection to minimize the possibility of white blood cell lysis. For processing, centrifuge the samples at 820g for 10 min at room temperature and transfer 1 mL aliquots of the plasma to sterile 2 mL microtubes. Freeze plasma aliquots at -80 °C. Remove the Buffy coat layer into a separate pre-labeled tube and store at -80^{0}C. Record corresponding information such as collection time, freezing time, and aliquot number.

Flow Cytometry and single cell RNA sequencing: Peripheral blood mononuclear cells (PBMCs) will be isolated from the blood of patients per manufacturer protocol. For flow cytometry, cells will be stained for immune subtype markers and sorted by fluorescence-activated cell sorting (FACS) or CyTOF. For single cell sequencing, the cells will be loaded in Smarter ICELL8 single cell mRNA chip or the 10x, imaged via microscopy, and single cell libraries will be prepared per manufactures instructions. Next generation sequencing will be performed. Single cell RNA-sequencing of immune cells taken from patients will enable us to determine the phenotypic changes specific to each cell subtype and cell interactions.

Circulating DNA fragments carrying tumor-specific sequence alterations (ctDNA) are found in the cell-free fraction of blood, representing a variable and generally small fraction of the total circulating DNA. Advances in sequencing technologies have enabled the rapid identification of somatic genomic alterations in individual tumors, and these can be used to design personalized assays for the monitoring of ctDNA. Studies have shown the feasibility of using ct DNA to monitor tumor dynamics in a limited number of patients with various solid cancers, but few cases of breast cancer have been analyzed [65].

Biospecimens will be de-identified (coded) prior to submission. The coded identifier will be the COH research patient number (RPN) provided by the COH clinical trial management system, which is devoid of direct participant identifiers. The key to the code is maintained in the COH clinical trial management system which is a secure environment.

9.6 Bacteriomic Profiling

16S Gut Microbiome rRNA Analysis

Gut microbiome has been associated with response to immune checkpoint inhibitors in patients with solid tumors. Fecal samples are relatively easy to collect and non-invasive. They provide an indication of the gut microbiome which may be an indicator of general health, impact drug availability, and indicate the presence of communities associated with inflammation, digestive inefficiencies, and pathogens. Monitoring the gut microbiome may allow us to predict the risk of possible side effects of immune check point inhibitors (colitis and decreased appetite) and therapeutic efficacy.

The exploratory objective was to monitor the gut microbiota at baseline, on treatment and end of treatment using fecal samples. The differences in gut microbiota within and between fecal samples will be compared using alpha and beta diversity metrics based on 16S rRNA sequencing.

Stool Specimen Collection

Fecal material will be collected in a Zymo Research DNA/RNA Shield Fecal Collection Tube by patients as instructed. A standard operating procedure (SOP) has been generated for stool collection, as outlined in Appendix 16.6. A DIET and STOOL FREQUENCY LOG - GENERAL INSTRUCTIONS is listed in Appendix 16.7. Stool collection kit contents are listed in Appendix 16.8. A copy of this SOP will be provided to the patient and their understanding of the SOP will be documented by the PI.

Samples will be collected pre-treatment Day -28 to C1D1 (baseline), C4 Day 1 (+/-7 days), and end of treatment (+/-7 days). Patient will bring samples on the scheduled study visits.

10.0 Study Calendar

Trial Period	**Screening Phase**	**Treatment Cycles [a]**					**To be repeated beyond 8 cycles**				**End of Treatment**	**Post-Treatment**		
Treatment Cycle/Title	**Cohort 1 &2: Screening -28 to 0** **Cohort 3: Screening -56 to -28**	**Cohort3 Day -28, -14**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Discon.**	**Safety Follow-up**	**Follow Up Visits [b]**	**Survival Follow-up**
Scheduling Window (Days)			**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**At time of Discon.**	**30 days post Discon. (±3 days)**	**Every 6 months x 3 years post Discon (±14 days)**	**Every 12-month x 1 year (±14 days)**
Administrative Procedures														
Informed Consent	X													
Inclusion/Exclusion Criteria	X													
Demographics and Medical History	X													
Prior and Concomitant Medication Review	X	X	X	X	X	X	X	X	X	X	X	X		
Trial Treatment Administration [c]														
Pembrolizumab			X	X	X	X	X	X	X	X				
Palbociclib		X	X	X	X	X	X	X	X	X				
Letrozole or Fulvestrant		X	X	X	X	X	X	X	X	X				
Post-study anticancer therapy status													X	X
Survival Status														X
Clinical Procedures/Assessments														
Review Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X		
Full Physical Examination	X	X												
Directed Physical Examination		X	X	X	X	X	X	X	X	X				
Vital Signs and Weight	X	X	X	X	X	X	X	X	X	X				
Menopausal Status	X													
ECOG Performance Status	X	X	X	X	X	X	X	X	X	X	X			
Laboratory Procedures/Assessments: analysis performed by LOCAL laboratory														
Pregnancy Test – Urine or Serum β-HCG	X													
CBC with Differential [d]	X		Every 3 weeks on day 1 of each cycle[l]								X	X		

Trial Period	**Screening Phase**	**Treatment Cycles [a]**									**End of Treatment**	**Post-Treatment**		
Treatment Cycle/Title	**Cohort 1 &2: Screening -28 to 0** **Cohort 3: Screening -56 to -28**	**Cohort3**	**1**	**2**	**3**	**4**	**To be repeated beyond 8 cycles**				**Discon.**	**Safety Follow-up**	**Follow Up Visits [b]**	**Survival Follow-up**
							5	**6**	**7**	**8**				
Scheduling Window (Days)		**Day -28, -14**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**At time of Discon.**	**30 days post Discon. (±3 days)**	**Every 6 months x 3 years post Discon (±14 days)**	**Every 12-month x 1 year (±14 days)**
		X												
Comprehensive Serum Chemistry Panel [d]	X	X	Every 3 weeks on day 1 of each cycle[l]								X	X		
12-Lead EKG [e]	X													
T3, FT4 and TSH	X			X		X		X		X		X		
Laboratory Procedures/Assessments: analysis performed by CENTRAL laboratory														
PD-L1[f]	X													
Efficacy Measurements														
Tumor Imaging [g]	X					X				X	X			
Brain Imaging [h]	X					X				X	X			
Tumor Biopsies/Archival Tissue Collection/Correlative Studies Blood														
New tissue biopsy (or archival tissue) [i]	X			X							X			
Blood collection for correlative studies[j]	X		X	X		X					X			
Stool Microbiome profiling[k]	X					X					X			

Trial Period	**Screening Phase**	**Treatment Cycles [a]**									**End of Treatment**	**Post-Treatment**		
							To be repeated beyond 8 cycles							
Treatment Cycle/Title	**Cohort 1 &2: Screening -28 to 0**		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Discon.**	**Safety Follow-up**	**Follow Up Visits [b]**	**Survival Follow-up**
Scheduling Window (Days)	**Cohort 3: Screening -56 to -28**	**Cohort3 Day -28, -14**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**At time of Discon.**	**30 days post Discon. (±3 days)**	**Every 6 months x 3 years post Discon (±14 days)**	**Every 12-month x 1 year (±14 days)**

a. A cycle is every 21 days (3 weeks) based on pembrolizumab dosing schedule. **For cohort 3, patients will complete screening work up days-56 to day -28. Patients will receive palbociclib and endocrine therapy (letrozole or fulvestrant) starting day-28. Palbociclib will be given 3 weeks on 1 week off. Fulvestrant dose 500mg on day -28 and day -14. On cycle 1 day 1, pembrolizumab will be added to the combination of palbociclib and endocrine therapy**. Once patients complete pembrolizumab, follow-up and testing can be every 4 weeks.

b. Follow up of post-study cancer therapy status after study drug discontinuation will be every 6 months for 3 years, then every 12 months x 1 year.

c. Pembrolizumab will be given on Day 1 every 3 weeks. Palbociclib will be given 3 weeks on, 1 week off every 4 weeks. Letrozole will be given daily every 4 weeks.

d. For cohort 1 & 2, CBC with differential and comprehensive serum chemistry panel will be performed on day 1 of each 3-week cycle (pembrolizumab administration days) through the duration of the study. In addition, during the first 12 weeks of therapy, CBC with differential will be checked every 2 weeks for close monitoring of blood count. Study team will monitor the CBCs for dose limiting toxicities (DLTs) during the first 6 weeks of the study. For cohort 3, CBC diff and CMP will be performed on day -28, -14 and day 1 of all cycles.

e. EKG will be performed at baseline for QTc measurement.

f. PD-L1 will be tested using commercial testing lab and 22C3 PDL1 antibody.

g. Restaging imaging (CT, bone scan) will be repeated every 12 weeks (+/-7 days). At screening, staging imaging within 35 days (5 weeks) of study entry is allowed.

h. During screening, brain imaging should be performed in subjects with known brain metastases (such subjects also need records of brain imaging performed within the last 3 months prior to screening to establish stability).

i. Research tumor tissue (fixed) or archived metastatic specimen will be obtained prior to study initiation and at time of disease progression (end of study). Optional on-treatment biopsies are allowed for assessment of immune response. **When a core needle biopsy is used, a minimum of 4 core biopsy samples with a minimum 18-gauge needle is recommended. For Cohort 3, tumor biopsies will be collected at baseline (within 6 weeks of study onset), C2D1 (+/-1 week) and at time-of-progression or end-of-treatment.**

j. Peripheral blood samples will be obtained on **day -28 for cohort 3, day 1 of cycles 1, 2, 4, and end of study visit**. Three tubes (1 x 10 ml green-top and 2 lavender-top) of blood will be collected and delivered to CICSL (Dr. Tim Synold's lab). Optional peripheral blood or biopsy sample can be taken if patient was off trial for reasons other than disease progression.

k. Stool samples will be collected day -28 to C1D1 (baseline), Cycle 4 Day 1 (+/- 7 days), and end of treatment (+/- 7 days).

l. Once patients complete pembrolizumab, follow-up and testing can be every 4 weeks.

11.0 Endpoint Evaluation Criteria/Measurement of Effect

11.1 Response Criteria

11.1.1 Primary Endpoint

Response rate in ER+HER2- breast cancer when combining a previously untested combination of letrozole, palbociclib and pembrolizumab in patients with newly diagnosed metastatic disease.

11.1.2 Secondary Endpoint

Safety and tolerability of the pembrolizumab plus letrozole-palbociclib combination: Safety analysis will be carried out based on toxicities assessed by CTCAE, version 4. AEs will be analyzed including but not limited to AEs (grade 2 or higher), SAEs, fatal AEs, and laboratory changes. Immune-related adverse events will be collected (also see **Section 13.3**).

11.1.3 Secondary Efficacy Endpoints

Complete response rate, Duration of response, PFS, and OS and time to treatment failure will also be evaluated along with clinical benefit (SD>24 weeks after study initiation). RECIST 1.1 will be used to assess response, DOR, and PFS; Kaplan-Meier estimates will be generated for PFS and OS. Exploratory analysis to assess response and clinical benefit will be carried out using irRECIST. irRECIST is an adaptation of RECIST 1.1 to account for the unique tumor response characteristics to treatment with new immunotherapeutic agents, including pembrolizumab. RECIST 1.1 was developed based on treatment with cytotoxic agents. Immunotherapeutic drugs, such as pembrolizumab, may produce antitumor effects by potentiating endogenous cancer-specific immune responses. 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 clinical responses after initial increases in tumor burden or even the appearance of new lesions. Standard RECIST 1.1 may not provide an accurate assessment of response to immunotherapeutic agents such as pembrolizumab and will therefore be used with the adaptations referred to as irRECIST (see **Sections 13.2** and **16.2**).

12.0 Data Reporting/Protocol Deviations

12.1 Data Reporting

12.1.1 Confidentiality and storage of records

Electronic Data Collection will be used for this protocol. The data will be stored in encrypted, password protected, secure computers that meet all HIPAA requirements. When results of this study are reported in medical journals or at meetings, identification of those taking part will not be disclosed. Medical records of subjects will be securely maintained in the strictest confidence, according to current legal requirements. They will be made available for review, as required by the FDA, HHS, or other authorized users such as the NCI, under the guidelines established by the Federal Privacy Act and rules for the protection of human subjects.

12.1.2 Subject consent form

At the time of registration, the original signed and dated Informed Consent form, HIPAA research authorization form, and the California Experimental Subject's Bill of Rights (for the medical record) and three copies (for the subject, the research record, and the Coordinating Center) must be available. All Institutional, NCI, Federal, and State of California requirements will be fulfilled.

12.1.3 Data collection forms and submission schedule

All data will be collected using electronic data collection, stored as indicated in **Section 12.1.1**, and submitted according to the timelines indicated below (**Table 12)**.

Table 12: Data Submission Schedule

Form	Submission Timeline
Eligibility Checklist	Complete prior to registration
On Study Forms	Within 14 calendar days of registration
Baseline Assessment Forms	Within 14 calendar days of registration
Treatment Forms	Within 10 calendar days of treatment administration
Adverse Event Report Forms	For Cycle 1 only, within 7 calendar days of AE assessment/notification; for all other cycles, within 10 calendar days of AE assessment/notification
Response Assessment Forms	Within 10 calendar days of the response assessment
Other Assessment Forms (as applicable)	Within 10 calendar days of the assessment
Off Treatment/Off Study Forms	Within 10 calendar days of completing treatment or being taken off study for any reason
Follow up/Survival Forms	Within 14 calendar days of the protocol defined follow up visit date or call

12.2 Protocol Deviations

12.2.1 Deviation policy

This protocol will be conducted in accordance with COH's "Clinical Research Protocol Deviation Policy" located at http://www.coh.org/dsmc/Documents/Institutional%20Deviation%20Policy.pdf.

Deviations from the written protocol that could increase patient risk or alter protocol integrity require prior IRB approval of a single subject exception (SSE) request. In addition, if contractually obligated, the sponsor must also approve the deviation. IRB pre-approved SSE protocol modifications are considered an amendment to the protocol and not a deviation. The submission of a deviation report is not required.

Brief interruptions and delays may occasionally be required due to travel delays, airport closure, inclement weather, family responsibilities, security alerts, government holidays, etc. This can also extend to complications of disease or unrelated medical illnesses not related to disease progression. The PI has the discretion to deviate from the protocol when necessary so long as such deviation does not threaten patient safety or protocol scientific integrity. Examples include but are not limited to a) dose adjustments based on excessive patient weight; b) alteration in treatment schedule due to non-availability of the research participant for treatment; c) laboratory test results which are slightly outside the protocol requirements but at levels that do not affect participant safety. These instances are deviations from the protocol. A deviation report will be submitted to the DSMC/IRB within five days.

12.2.2 Reporting of Deviations

All deviations will be reported to the COH DSMC within five days. The DSMC will forward to report to the IRB following review.

12.2.3 Resolving Disputes

The COH Investigational Drug Service (IDS) cannot release a research agent that would cause a protocol deviation without approval by the PI. Whenever the protocol is ambiguous on a key point, the IDS should rely on the PI to clarify the issue.

In situations where there is misperception or dispute regarding a protocol deviation among the persons involved in implementing the protocol, it is the responsibility of the PI to resolve the dispute and the PI may consult with the DSMC chair (or designee) to arrive at resolution.

13.0 Statistical Considerations

13.1 Study Design

This is an open-label single institutional trial evaluating patients with newly diagnosed stage IV (cohort 2), metastatic ER+ breast cancer treated with the combination of pembrolizumab, letrozole and palbociclib. Patients must have confirmed newly diagnosed, ER+HER2- breast cancer, measurable disease documented SD by RECIST 1.1. Eligible patients will receive letrozole (2.5 mg) once a day and palbociclib 125 mg once a day for 3 weeks on and 1 week off. Pembrolizumab will be given at 200 mg IV every 3 weeks.

A separate cohort (cohort 1) of patients enrolled were previously on letrozole and palbociclib with stable disease and received the addition of pembrolizumab. This is cohort 1, corresponding to the previous version of the protocol (cohort closed at 6 patients).

Study treatment will continue until disease progression, unacceptable AEs, concurrent illness that prevents further administration of study treatment, investigator's decision to withdraw the subject from study treatment, consent withdrawal, becoming lost-to-follow-up, death, or for administrative reasons requiring cessation of treatment.

After discontinuation of study treatment, each subject will be followed for 30 days for AE (grade 2 or higher) monitoring. Serious adverse events and irAEs will be collected for 90 days after the end of study treatment or until the subject initiates new anticancer therapy for a minimum of 30 days, whichever occurs earlier.

Subjects who discontinue study treatment for reasons other than disease progression will have post-treatment follow-up for disease status every 12 weeks (±7 days) until disease progression (verified by restaging imaging), start of a non-study anticancer treatment, consent withdrawal, becoming lost to follow-up, death, or end of the study. Optional peripheral blood or biopsy sample can be taken if patient was off trial for reasons other than disease progression. In addition, restaging imaging can be assessed for RECIST measurement.

All subjects will be followed by telephone every 4 cycles (±7 days) for OS until consent withdrawal, becoming lost to follow-up, death, or end of the study.

13.2 Sample Size Accrual Rate

Sample size: 47 patients (6 cohort 1, 16 cohort 2, 25 cohort 3). The estimated accrual rate for cohort 2 (cohort 1 on patients who had previously achieved SD on palbociclib + letrozole is closed) will be approximately 1-2 patients per month for a total of approximately 9 months.

Cohort 1&2 are closed to accrual. Cohort 3 will require approximately 24 months for accrual, approximately 18 months of minimum follow-up to assess response, and continued follow-up for 36 months to assess PFS, and OS and evaluate for late developing responses.

13.3 Statistical Analysis Plan

Primary Endpoint

Response (CR or PR by RECIST version 1.1) due to letrozole+palbociclib is 55%, with rare complete responses. This short Phase II study is designed to demonstrate that pembrolizumab enhances the activity of letrozole+palbociclib. As a result, we require, at a minimum, that the RR exceeds 55% or that we see complete responses. As a result, if 8 responses out of 16 patients are observed (50%), the triplet would be considered lacking promise. This would happen with a 7.4% type II error if the true response rate was 70%. If 9-11 (56%-69%) responses are observed, other considerations such as the observation of CRs, and the duration of responses or progression-free survival will be needed to determine if the combination

is worthwhile, and if 12 responses out of 16 patients are observed (75%), the combination would be declared promising. The chance of a 55% response rate resulting in 12 responses is less than 9% (type I error).

Safety Stopping Rules

Cohort 1 is closed for accrual issues and unrelated to toxicity. Cohort 1 previously had the three-at-risk safety lead-in design, with 1 patient with a delay in treatment due to wound healing that was attributed to treatment and called a DLT, 1 patient with only grade 1 AEs and 1 patient who remained on treatment for at least 6 cycles. The study has been amended to accrue patients in cohort 2 (first-line up-front therapy), and the three-at-risk safety lead-in design applied to this cohort, independent from cohort 1. Specifically, for cohort 2, we employ a three-at-risk design (modified rolling design) for this Phase II study to ensure the triplet is well-tolerated.

This design permits only 3 patients to be a risk for DLT at any one time during the "safety lead-in" When the first 6 patients have completed the observation period (1 cycle) and treatment with ≤1 DLT, the safety lead-in for the triplet will be considered successful, and accrual will proceed to a total of 16 patients at dose level 1 in cohort 2.

If two DLTs are observed on the starting dose in the first 6 patients, the dose will be reduced per the dose ladder dose to level -1 (see table 3, section 5). If there are two DLTs in the first 6 patients on dose level -1, the study will hold accrual pending an amendment and discussion between the PI and study drug provider (Merck). Otherwise, additional patients will be accrued to dose level -1 until a total of 16 patients have been treated with the triplet of pembrolizumab, letrozole, and palbociclib in cohort 2.

Additional Secondary Endpoints

With approximately 16 patients (in cohort 2), we expect to have to obtain at least 12 patients with both pre-pembrolizumab and post-pembrolizumab treatment biopsy material adequate for evaluation, and 16 samples with blood collection pre- and post. The correlative studies will be used to potentially refine patient selection for future studies, and to understand the role of immune changes on the activity of the combination of pembrolizumab plus letrozole and palbociclib. These correlative studies are considered exploratory in the context of this limited phase II study; however, 12 samples provide 80% power to detect an effective size of 0.8 (80% of the standard deviation), with a one-sided type I error of 5% for any of these correlative assays in the context of this exploratory study. Samples provided in Cohort 1 (n=6), while limited, will be explored and to determine if there are large reductions in the tumor burden with the addition of pembrolizumab.

13.4 Statistical Analysis Plan for Cohort 3

Clinical statistics: We will treat 25 patients. Patients will go on to receive combination therapy. In the preliminary analysis of 14 patients' flow data (8 responders, 6 non-responders), we noted a statistically significant decrease in classic monocytes (60%, $p<0.001$), and this decrease was more pronounced in responders than non-responders (85% vs. 25%, $p<0.02$). We seek to evaluate, specifically, if palbociclib as a monotherapy is responsible for such changes as the primary endpoint. Other secondary endpoints include other immune cell subsets and changes that follow the combination with pembrolizumab. With 25 patients, assuming a standard deviation of 0.51 in the relative change in classic monocytes in PBMCs, there is 90% power to detect a relative change of log(C1D1/baseline) of 34.5% with a type I error (two-sided) of 0.05. This will confirm that the decrease in classic monocytes is due to palbociclib (secondary endpoints will evaluate the change with the combination therapy). In the preliminary data, we noted a response rate of 65%, and responders had a significantly larger decrease in classic monocytes. With 25 patients, if 16 patients respond, we will have 85% power to detect a difference in the relative change between responders and non-responders of 0.8 vs. 0.3 (assuming SD of 0.5 and 0.29 from preliminary data) with a type I error of 5% (two-sided). The actual power will depend on the distribution of responders vs. non-responders. Other immune correlatives and the changes after introduction of the

combination therapy will also be assessed. Patients will not be pre-selected based on PD-L1 expression; therefore, we will also evaluate response and correlatives both together and by PD-L1 expression. All eligible patients who start treatment will be considered in the calculation of the response rate, and all who complete the pre- and post-biopsy treatment will be considered in the evaluation of the primary objective related to evaluating the impact of palbociclib.

Correlative statistics: With approximately 25 patients with pre-treatment, C2D1 (+/1 1 week), and time of progression biopsy samples, we expect to obtain at least 12 pairs of pre- and post- treatment biopsy material adequate for evaluation of genomic and immune profiling. The correlative studies will be used to potentially refine patient selection for future studies and analyze immune phenotypic and genomic alterations from the combination of pembrolizumab and palbociclib. These correlative studies are considered exploratory in the context of this limited Phase II study; however, 12 samples provide greater than 80% power to detect an effective size of 0.8 (80% of the standard deviation in the change), with a one-sided type I error of 5%. In addition, we will report on changes in PD-L1 expression induced by palbociclib and any relationship to the response rate.

14.0 Human Subject Issues

14.1 Institutional Review Board

In accordance with City of Hope policies, an Institutional Review Board (IRB) that complies with the federal regulations at 45 CFR 46 and 21 CFR 50, 56 and State of California Health and Safety code, Title 17, must review and approve this protocol and the informed consent form prior to initiation of the study. All institutional, NCI, Federal, and State of California regulations must be fulfilled.

Any documents that the IRB may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB. The IRB's written unconditional approval of the study protocol and the informed consent document will be in the possession of the investigator before the study is initiated.

The IRB will be informed of revisions to other documents originally submitted for review; serious unexpected or unanticipated adverse experiences occurring during the study, and any additional adverse experiences in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

Any amendment to the protocol document and accompanying informed consent document/template, as developed, and provided by the PI, will require review and approval by the COH IRB before the changes are implemented in the study.

14.2 Recruitment of Subjects

Potential research subjects will be identified by a member of the patient's treatment team, the protocol investigator, or research team, from the pool of patients seen by the study center with metastatic breast cancer. Potential subjects will be contacted by their treating physician and will be referred to the investigator/research staff of the study at their institution.

14.3 Study location and Performance Sites

This study will be performed at COH.

14.4 Confidentiality

Participant confidentiality is strictly held in trust by the investigators, study staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples in addition to any study information relating to participants.

This research will be conducted in compliance with federal and state requirements relating to protected health information (PHI), including the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). HIPAA regulations require a signed participant authorization informing the participant of the nature of the PHI to be collected, who will have access to that information and why, who will use or disclose that information, ant the rights of a research participant to revoke their authorization for use of their PHI. In the event that a participant revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of participant authorization. For participants that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the participant is alive) at the end of their scheduled study period.

Release of research results should preserve the privacy of medical information and must be carried out in accordance with Department of Health and Human Services Standards for Privacy of Individually Identifiable Health Information, 45 CFR 164.508. When results of this study are reported in medical journals or at meetings, identification of those taking part will not be disclosed and no identifiers will be used.

Medical records of participants will be securely maintained in the strictest confidence, according to current legal requirements. Data will be entered, analyzed and stored in encrypted, password protected, secure computers that meet all HIPAA requirements. All data capture records, drug accountability records, study reports and communications will identify the patient by initials and the assigned patient number.

The investigator/institution will permit direct access to source data and documents by sponsor representatives, the FDA, and other applicable regulatory authorities. The access may consist of trial-related monitoring/ auditing, IRB reviews, and FDA/regulatory authority inspections. The participant's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

Biospecimens will be de-identified (coded) prior to submission to internal and external research laboratories. The coded identifier will be the COH research patient number (RPN) provided by the COH clinical trial management system, which is devoid of direct participant identifiers. The key to the code is maintained in the COH clinical trial management system which is a secure environment.

14.5 Financial Obligations and Compensation

Neither the research participant nor the insurance carrier will be responsible for the research procedures related to this study. The study drugs pembrolizumab and palbociclib will be provided by the manufacturer free of charge to patients on this study. However, if research participants who have completed 24 months of therapy and will continue to receive treatment, letrozole and pembrolizumab will be provided through commercial source as a standard of care. Pembrolizumab use beyond 24 months is not mandatory. Pharmacokinetic lab draws, processing, and results will also be free of charge as a part of research. Cardiac imaging will be provided free of charge.

The standard of care drugs or procedures provided during the course of study participation will be the responsibility of the research participant and/or the insurance carrier. The research participant will be responsible for all copayments, deductibles, and other costs of treatment and diagnostic procedures as set forth by the insurance carrier. The research participant and/or the insurance carrier will be billed for the

costs of treatment and diagnostic procedures in the same way as if the research participant were not in a research study.

In the event of physical injury to a research participant, resulting from research procedures, appropriate medical treatment will be available at the City of Hope to the injured research participant, however, financial compensation will not be available.

Sponsor will pay for the mandatory study biopsy.

The research participant will not be paid for taking part in this study.

14.6 Informed Consent Processes

The PI or IRB-approved designee will explain the nature, duration, purpose of the study, potential risks, alternatives and potential benefits, and all other information contained in the informed consent document. In addition, they will review the experimental subject's bill of rights and the HIPAA research authorization form. Research subjects will be informed that they may withdraw from the study at any time and for any reason without prejudice, including as applicable, their current or future care or employment at City of Hope or any relationship they have with City of Hope. Research subjects will be afforded sufficient time to consider whether or not to participate in the research.

After the study has been fully explained, written informed consent will be obtained from either the prospective participant or his/her guardian or legal representative before study participation. The method of obtaining and documenting the informed consent and the contents of the consent must comply with all applicable regulatory requirements.

Before implementing any study procedure, informed consent shall be documented by the use of a written consent form approved by the IRB and signed and dated by the prospective participant or his/her legally authorized representative at the time of consent. A copy of the signed informed consent will be given to the participant or his/her legally authorized representative. The original signed consent must be maintained by the investigator and available for inspection sponsor designated representatives, or regulatory authority at any time.

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout study participation.

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16.0 Appendices

16.1 ECOG Performance Status

Table 13: ECOG Performance Status*

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead

* As published in Am. J. Clin. Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.* The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

16.2 CTCAE version 4

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

16.3 RECIST 1.1 for Evaluating Response in Solid Tumors

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

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

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

CATEGORIZING LESIONS AT BASELINE

Measurable Lesions

- Lesions that can be accurately measured in at least one dimension.
- Lesions with longest diameter twice the slice thickness and at least 10 mm or greater when assessed by CT or MRI (slice thickness 5-8 mm).
- Lesions with longest diameter at least 20 mm when assessed by Chest X-ray.
- Superficial lesions with longest diameter 10 mm or greater when assessed by caliper.
- Malignant lymph nodes with the short axis 15 mm or greater when assessed by CT.

NOTE: The shortest axis is used as the diameter for malignant lymph nodes, longest axis for all other measurable lesions.

Non-measurable disease

- Non-measurable disease includes lesions too small to be considered measurable (including nodes with short axis between 10 and 14.9 mm) and truly non-measurable disease such as pleural or pericardial effusions, ascites, inflammatory breast disease, leptomeningeal disease, lymphangitic involvement of skin or lung, clinical lesions that cannot be accurately measured with calipers, abdominal masses identified by physical exam that are not measurable by reproducible imaging techniques.
- Bone disease: Bone disease is non-measurable with the exception of soft tissue components that can be evaluated by CT or MRI and meet the definition of measurability at baseline.
- Previous local treatment: A previously irradiated lesion (or lesion subjected to other local treatment) is non-measurable unless it has progressed since completion of treatment.

Normal sites

- Cystic lesions: Simple cysts should not be considered as malignant lesions and should not be recorded either as target or non-target disease. Cystic lesions thought to represent cystic metastases can be measurable lesions, if they meet the specific definition above. If non-cystic lesions are also present, these are preferred as target lesions.
- Normal nodes: Nodes with short axis <10 mm are considered normal and should not be recorded or followed either as measurable or non-measurable disease.

Recording Tumor Assessments

All sites of disease must be assessed at baseline. Baseline assessments should be done as close as possible prior to study start. For an adequate baseline assessment, all required scans must be done within 28 days prior to treatment and all disease must be documented appropriately. If baseline assessment is inadequate, subsequent statuses generally should be indeterminate.

Target Lesions

All measurable lesions up to a maximum of 2 lesions per organ, 5 lesions in total, representative of all involved organs, should be identified as target lesions at baseline. Target lesions should be selected on the basis of size (longest lesions) and suitability for accurate repeated measurements. Record the longest diameter for each lesion, except in the case of pathological lymph nodes for which the short axis should be recorded. The sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions at baseline will be the basis for comparison to assessments performed on study.

- If two target lesions coalesce the measurement of the coalesced mass is used.
- If a large target lesion splits, the sum of the parts is used.
- Measurements for target lesions that become small should continue to be recorded. If a target lesion becomes too small to measure, 0 mm should be recorded if the lesion is considered to have disappeared; otherwise a default value of 5 mm should be recorded.

NOTE: When nodal lesions decrease to <10 mm (normal), the actual measurement should still be recorded.

Non-target Disease

All non-measurable disease is non-target. All measurable lesions not identified as target lesions are also included as non-target disease. Measurements are not required but rather assessments will be expressed as ABSENT, INDETERMINATE, PRESENT/NOT INCREASED, INCREASED. Multiple non-target lesions in one organ may be recorded as a single item on the case report form (e.g., ‘multiple enlarged pelvic lymph nodes’ or ‘multiple liver metastases’).

OBJECTIVE RESPONSE STATUS AT EACH EVALUATION

Disease sites must be assessed using the same technique as baseline, including consistent administration of contrast and timing of scanning. If a change needs to be made the case must be discussed with the radiologist to determine if substitution is possible. If not, subsequent objective statuses are indeterminate.

Target Disease

- Complete Response (CR): Complete disappearance of all target lesions with the exception of nodal disease. All target nodes must decrease to normal size (short axis <10 mm). All target lesions must be assessed.
- Partial Response (PR): Greater than or equal to 30% decrease under baseline of the sum of diameters of all target measurable lesions. The short diameter is used in the sum for target nodes, while the longest diameter is used in the sum for all other target lesions. All target lesions must be assessed.
- Stable: Does not qualify for CR, PR or Progression. All target lesions must be assessed. Stable can follow PR only in the rare case that the sum increases by less than 20% from the nadir, but enough that a previously documented 30% decrease no longer holds.
- Objective Progression (PD): 20% increase in the sum of diameters of target measurable lesions above the smallest sum observed (over baseline if no decrease in the sum is observed during therapy), with a minimum absolute increase of 5 mm.
- Indeterminate: Progression has not been documented, and
    - One or more target measurable lesions have not been assessed; or
    - Assessment methods used were inconsistent with those used at baseline; or
    - One or more target lesions cannot be measured accurately (e.g., poorly visible unless due to being too small to measure); or
    - One or more target lesions were excised or irradiated and have not reappeared or increased.

Non-target disease

- CR: Disappearance of all non-target lesions and normalization of tumor marker levels. All lymph nodes must be 'normal' in size (<10 mm short axis).
- Non-CR/Non-PD: Persistence of any non-target lesions and/or tumor marker level above the normal limits.
- PD: Unequivocal progression of pre-existing lesions. Generally the overall tumor burden must increase sufficiently to merit discontinuation of therapy. In the presence of SD or PR in target disease, progression due to unequivocal increase in non-target disease should be rare.
- Indeterminate: Progression has not been determined and one or more non-target sites were not assessed or assessment methods were inconsistent with those used at baseline.

New Lesions

The appearance of any new unequivocal malignant lesion indicates PD. If a new lesion is equivocal, for example due to its small size, continued assessment will clarify the etiology.

If repeat assessments confirm the lesion, then progression should be recorded on the date of the initial assessment. A lesion identified in an area not previously scanned will be considered a new lesion.

Supplemental Investigations

- If CR determination depends on a residual lesion that decreased in size but did not biopsy or fine needle aspirate. If no disease is identified, objective status is CR.
- If progression determination depends on a lesion with an increase possibly due to necrosis, the lesion may be investigated with biopsy or fine needle aspirate to clarify status.

Subjective Progression:

Patients requiring discontinuation of treatment without objective evidence of disease progression should not be reported as PD on tumor assessment CRFs. This should be indicated on the end of treatment CRF as off treatment due to Global Deterioration of Health Status. Every effort should be made to document objective progression even after discontinuation of treatment.

Also see **Table 14** below.

Table 14: Objective Response Status at each Evaluation

Target Lesions	Non-target Disease	New Lesions	Objective status
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
CR	Indeterminate or Missing	No	PR
PR	Non-CR/Non-PD, Indeterminate, or Missing	No	PR
SD	Non-CR/Non-PD, Indeterminate, or Missing	No	Stable
Indeterminate or Missing	Non-PD	No	Indeterminate
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Best Overall Response

The best overall response (BOR) is the best response recorded from the randomization until disease progression or death due to any cause. This is derived from the sequence of objective statuses. Objective statuses are not considered after objective progression is documented or after start of the first anticancer treatment post discontinuation of protocol treatment. BOR for each patient will be derived as one of the following categories.

- **Complete response (CR)**: At least one objective status of CR documented before progression.
- **Partial response (PR)**: At least one objective status of PR documented before progression.
- **Stable disease (SD)**: At least one objective status of stable documented at least 8 weeks after randomization date and before progression but not qualifying as CR, PR.
- **Progressive Disease (PD):** Objective status of progression within 16 weeks of randomization, not qualifying as CR, PR or SD.
- **Indeterminate (IND):** Progression not documented within 16 weeks after randomization and no other response category applies.

16.4 Immune Related Response Criteria (irRECIST)

This study will utilize the Immune Related Response Criteria (irRECIST) as one of the secondary objectives. These response criteria were developed to overcome the variable and unusual patterns of response to immunotherapeutic agents, in particular ipilimumab. The development of the guidelines was prompted by observations, mostly in patients with metastatic melanoma, of initial disease progression followed by later response, late responses, and mixed responses with an overall decrease in tumor burden.

Antitumor response based on total measurable tumor burden

For the irRECIST, only index and measurable new lesions are taken into account (in contrast to conventional WHO criteria, which do not require the measurement of new lesions, nor do they include new lesion measurements in the characterization of evolving tumor burden; see **Table 15**, below). At the baseline tumor assessment, the sum of the products of the two largest perpendicular diameters (SPD) of all index lesions (five lesions per organ, up to 10 visceral lesions) is calculated. At each subsequent tumor assessment, the SPD of the index lesions and of new, measurable lesions (up to 5 new lesions per organ; 10 visceral lesions) are added together to provide the total tumor burden: Tumor Burden = SPD index lesions + SPD new, measurable lesions.

Time-point response assessment using irRECIST

Percentage changes in tumor burden per assessment time point describe the size and growth kinetics of both conventional and new, measurable lesions as they appear. At each tumor assessment, the response in index and new, measurable lesions is defined based on the change in tumor burden (after ruling out irPD).

Decreases in tumor burden must be assessed relative to baseline measurements (i.e., the SPD of all index lesions at screening). The irRECIST was derived from WHO criteria and, therefore, the thresholds of response remain the same. However, the irRECIST response categories have been modified from those of WHO criteria as detailed in **Table 15**.

Table 15 Comparison of WHO and irRECIST

	WHO	irRECIST
New, measurable lesions	Always represent PD	Incorporated into tumor burden
New, nonmeasurable lesions	Always represent PD	Do not define progression (but preclude irCR)
Non-index lesions	Changes contribute to defining BOR of CR, PR, SD, and PD	Contribute to defining irCR (complete disappearance required)
CR	Disappearance of all lesions in two consecutive observations not less than 4 wk apart	Disappearance of all lesions in two consecutive observations not less than 4 wk apart
PR	≥50% decrease in SPD of all index lesions compared with baseline in two observations at least 4 wk apart, in absence of new lesions or unequivocal progression of non-index lesions	≥50% decrease in tumor burden compared with baseline in two observations at least 4 wk apart
SD	50% decrease in SPD compared with baseline cannot be established nor 25% increase compared with nadir, in absence of new lesions or unequivocal progression of non-index lesions	50% decrease in tumor burden compared with baseline cannot be established nor 25% increase compared with nadir
PD	At least 25% increase in SPD compared with nadir and/or unequivocal progression of non-index lesions and/or appearance of new lesions (at any single time point)	At least 25% increase in tumor burden compared with nadir (at any single time point) in two consecutive observations at least 4 wk apart

Overall response using the irRECIST

The sum of the products of diameters at tumor assessment using the immune-related response criteria (irRECIST) for progressive disease incorporates the contribution of new measurable lesions. Each net Percentage Change in Tumor Burden per assessment using irRECIST accounts for the size and growth kinetics of both old and new lesions as they appear.

Definition of Index Lesions Response Using irRECIST

- **irComplete Response (irCR):** Complete disappearance of all *index* lesions. This category encompasses exactly the same subjects as "CR" by the mWHO criteria.
- **irPartial Response (irPR):** Decrease, relative to baseline, of 50% or greater in the sum of the products of the two largest perpendicular diameters of all *index* and all new measurable lesions (i.e., Percentage Change in Tumor Burden). Note: the appearance of new measurable lesions is factored into the overall tumor burden, but does not automatically qualify as progressive disease until the SPD increases by ≥25% when compared to SPD at nadir.
- **irStable Disease (irSD):** Does not meet criteria for irCR or irPR, in the absence of progressive disease.
- **irProgressive Disease (irPD):** At least 25% increase Percentage Change in Tumor Burden (i.e., taking sum of the products of all *index* lesions and any new lesions) when compared to SPD at nadir.

Definition of Non-Index Lesions Response Using irRECIST

- **irComplete Response (irCR):** Complete disappearance of all *non-index* lesions. This category encompasses exactly the same subjects as "CR" by the mWHO criteria.
- **irPartial Response (irPR) or irStable Disease (irSD):** *non-index* lesion(s) are not considered in the definition of PR, these terms do not apply.

- **irProgressive Disease (irPD):** Increases in number or size of *non-index* lesion(s) does not constitute progressive disease unless/until the Percentage Change in Tumor Burden increases by 25% (i.e., the SPD at nadir of the index lesions increases by the required amount).

Impact of New Lesions on irRECIST

New lesions in and by themselves do not qualify as progressive disease. However, their contribution to total tumor burden is included in the SPD which in turn feeds into the irRECIST for tumor response. Therefore, new non-measurable lesions will not discontinue any subject from the study.

Definition of Overall Response Using irRECIST

Overall response using irRECIST will be based on these criteria (**Table 16**):

- **Immune-Related Complete Response (irCR):** Complete disappearance of *all* tumor lesions (index and non-index together with no new measurable/unmeasurable lesions) for at least 4 weeks from the date of documentation of complete response.
- **Immune-Related Partial Response (irPR):** The sum of the products of the two largest perpendicular diameters of all index lesions is measured and captured as the SPD baseline. At each subsequent tumor assessment, the sum of the products of the two largest perpendicular diameters of all index lesions and of new measurable lesions are added together to provide the Immune Response Sum of Product Diameters (irSPD). A decrease, relative to baseline of the irSPD compared to the previous SPD baseline, of 50% or greater is considered an immune Partial Response (irPR).
- **Immune-Related Stable Disease (irSD):** irSD is defined as the failure to meet criteria for immune complete response or immune partial response, in the absence of progressive disease.
- **Immune-Related Progressive Disease (irPD):** It is recommended in difficult cases to confirm PD by serial imaging. Any of the following will constitute progressive disease:
- At least 25% increase in the sum of the products of all index lesions over nadir SPD calculated for the index lesions.
- At least a 25% increase in the sum of the products of all index lesions and new measurable lesions (irSPD) over the baseline SPD calculated for the index lesion.

Table 16: Derivation of irRECIST overall responses

Measurable response	**Nonmeasurable response**		**Overall response**
Index and new, measurable lesions (tumor burden),*%	Non-index lesions	New, nonmeasurable lesions	Using irRECIST
↓100	Absent	Absent	irCR†
↓100	Stable	Any	irPR†
↓100	Unequivocal progression	Any	irPR†
↓≥50	Absent/Stable	Any	irPR†
↓≥50	Unequivocal progression	Any	irPR†
↓<50 to <25↑	Absent/Stable	Any	irSD
↓<50 to <25↑	Unequivocal progression	Any	irSD
≥25	Any	Any	irPD†

*Decreases assessed relative to baseline (scan prior to start of any protocol therapy), including measurable lesions only
†Assuming response (irCR) and progression (irPD) are confirmed by a second, consecutive assessment at least 4 wk apart.

Immune-Related Best Overall Response Using irRECIST (irBOR)

irBOR is the best confirmed irRECIST overall response over the study as a whole, recorded between the date of first dose until the last tumor assessment before subsequent therapy (except for local palliative

radiotherapy for painful bone lesions) for the individual subject in the study. For the assessment of irBOR, all available assessments per subject are considered.

irCR or irPR determinations included in the irBOR assessment must be confirmed by a second (confirmatory) evaluation meeting the criteria for response and performed no less than 4 weeks after the criteria for response are first met.

16.5 Drugs Known to Prolong QT interval and Predispose to Torsade De Ponites

Prohibited while on study

Table 17: List of Drugs Known to Prolong QT interval and Predispose to Torsade de Pointes

Generic Name	Brand Name(s)	Generic Name	Brand Name(s)
Amiodarone	Cordarone®, Pacerone®	Haloperidol	Haldol®
Arsenic trioxide	Trisenox®	Ibutilide	Corvert®
Astemizole	Hismanal®	Levomethadyl	Orlaam®
Azithromycin	Zithromax®	Mesoridazine	Serentil®
Bepridil	Vascor®	Methadone	Dolophine®, Methadose®
Chloroquine	Aralen®	Moxifloxacin	Avelox®
Chlorpromazine	Thorazine®	Pentamidine	Pentam®, NebuPent®
Cisapride	Propulsid®	Pimozide	Orap®
Citalopram	Celexa®	Haloperidol	Haldol®
Clarithromycin	Biaxin®	Ibutilide	Corvert®
Disopyramide	Norpace®	Levomethadyl	Orlaam®
Dofetilide	Tikosyn®	Probucol	Lorelco®
Domperidone	Motilium®	Procainamide	Pronestyl®, Procan®
Droperidol	Inapsine®	Quinidine	Cardioquin®, Quinaglute®
Erythromycin	Erythrocin®, E.E.S.®	Sotalol	Betapace®
Flecainide	Tambocor®	Sparfloxacin	Zagam®
Halofantrine	Halfan®	Terfenadine	Seldane®

Adapted from the University of Arizona Cancer Center for Education and Research on Therapeutics: "Torsades List: Drugs with a Risk of Torsades de Pointes," drugs that are generally accepted by the QTdrugs.org Advisory Board to carry a risk of Torsades de Pointes on the University of Arizona CERT website: http://www.azcert.org/medical-pros/drug-lists/bycategory.cfm#. This list is not meant to be considered all inclusive. See website for current list.

16.6 Stool Collection Procedure

As a part of your participation in the current study, we have some specific instructions related to collection of stool. Please abide by these instructions, as they are essential for the proper conduct of the study. You are being asked to collect samples at the following times:

- Day -28 to C1D1 (baseline)
- C4 Day 1 (+/- 7 days)
- End of treatment (+/-7 days)

If you have any questions about sample collection, please call this number: _____

USING THE STOOL COLLECTION KIT

Before you begin, review the following:

a. Make sure you have a *collection hat* and collection tube.
b. Make sure you are able to deliver the sample to City of Hope within 1 week.

STEP ONE: Please place the *collection hat* around the rim of your toilet seat for stool collection.  	
STEP TWO: Unscrew the collection tube cap and use the spoon to scoop one spoonful of feces (about the size of a quarter) from a sample. Place the sample in the collection tube. Tighten the cap and shake to mix the contents thoroughly (invert 10 times) to create a suspension. Note: Some fecal material may be difficult to re-suspend. As long as the material is suspended, the sample is stabilized. Foaming/frothing during shaking is normal.	 Scoop a portion of the stool sample into the DNA/RNA Shield™ Fecal Collection Tube
STEP THREE: Wash hands well and write today's date on the label.	
STEP FOUR: Place the plastic tube in the bag and seal the back using the adhesive tape already present on the bag.	
STEP FIVE: Bring the sample to your City of Hope appointment.	**THANK YOU FOR YOUR PARTICIPATION!**

16.7 Diet and Stool Frequency Log

DIET and STOOL FREQUENCY LOG - GENERAL INSTRUCTIONS

As a part of your participation in the current study, we are requesting that you complete a study log every day.

General pointers:

- When you come to the clinic, bring your logs with you.
- Each page has room for seven days – one row should be completed for each day.
- Please **avoid any intake of yogurt, yogurt-containing foods, or other bacteria-fortified foods**.

Example of how the top part of the log will look:

- A study team member will complete the information in this box before you leave the clinic.

COMPLETED BY STUDY TEAM	Participant Initials: JSM	Participant Research Number: 1001	Group: A

Example of how the information you enter might look:

- You or someone close to can complete the log for you, so long as the information is correct.
- List all prescription and non-prescription medications.
- The person who completes that day's entry should write his or her initials in the last column.

Day and Date	General description of food I ate:	*Did I eat yogurt or take probiotics?*	*How was my stool frequency?*	*Was a stool sample collected?*	*Medications taken*	*Initials of person filling information*
	Eggs, toast, juice Ham sandwich, coke, potato chips Steak, mashed potatoes, wine	○ Yes ☒ No	☒ Seems like baseline ○ 1-3 stools more than baseline ○ 4-6 stools more than normal ○ 7 or more than baseline, or incontinence	○ Yes ☒ No	Vitamin C, Lipitor	JBC
			○ Seems like baseline			

Example of the signature line:

- When you hand over the document to the study team, they will ask to sign and date at the bottom of each log if you agree that the information is complete and correct.

At the time of handing over the document -- Participant Signature: Joseph Black Smith Date 12/18/2002

COMPLETED BY STUDY TEAM	Participant Initials:	Participant Research Number:

Day and Date	General description of food I ate:	*Did I eat yogurt or take probiotics?*	*How was my stool frequency?*	*Was a stool sample collected?*	*Medications taken*	*Initials of person filling information*
	Eggs, toast, juice Ham sandwich, coke, potato chips Steak, mashed potatoes, wine	◯ Yes X	X ike baseline ◯1- re than baseline ◯4-6 stools more than normal ◯7 or more than baseline, or incontinence	◯ Yes X	Vitamin C, Lipitor	JBC
		◯ Yes ◯ No	◯ Seems like baseline ◯1-3 stools more than baseline ◯4-6 stools more than normal ◯7 or more than baseline, or incontinence	◯ Yes ◯ No		
		◯ Yes ◯ No	◯ Seems like baseline ◯1-3 stools more than baseline ◯4-6 stools more than normal ◯7 or more than baseline, or incontinence	◯ Yes ◯ No		
		◯ Yes ◯ No	◯ Seems like baseline ◯1-3 stools more than baseline ◯4-6 stools more than normal ◯7 or more than baseline, or incontinence	◯ Yes ◯ No		
		◯ Yes ◯ No	◯ Seems like baseline ◯1-3 stools more than baseline ◯4-6 stools more than normal ◯7 or more than baseline, or incontinence	◯ Yes ◯ No		
		◯ Yes ◯ No	◯ Seems like baseline ◯1-3 stools more than baseline ◯4-6 stools more than normal ◯7 or more than baseline, or incontinence	◯ Yes ◯ No		
		◯ Yes ◯ No	◯ Seems like baseline ◯1-3 stools more than baseline ◯4-6 stools more than normal ◯7 or more than baseline, or incontinence	◯ Yes ◯ No		

At the time of handing over the document -- Participant Signature: ____ Date _____________

16.8 At Home Sample Collection Kit Contents

Contents of each kit to be provided to participants for at home collection:

[] Copy of Appendix 16.6: Instructions for Stool Specimen Collection

[] Stool collection hat

[] Specimen tube with label attached

Label should have participant identifier added; the participant will be asked to add the date himself/herself.

[] Plastic sealable bag

SAMPLE GUIDELINES

Samples must be de-identified with no PHI. Aim to deliver samples on a **Monday through Thursday**. If this is not feasible, advance arrangements should be made with Dr. Yuan (yuyuan@coh.org).

1. Peripheral Blood Samples:

Dr. Tim Synold
Cc: Lesley Smith-Powell
Analytical Pharmacology Core Facility (APCF)
Shapiro 1042
City of Hope National Medical Center
1500 E. Duarte Road
Duarte, CA 91010
Tel: 626-218-2954
tsynold@coh.org; Lsmith-Powell@coh.org

2. Pathology Slides/Blocks:

Dr. Susan Yost
Department of Medical Oncology & Therapeutic Research
Building 51, City of Hope National Medical Center
1500 East Duarte Rd, Duarte, CA 91010
Direct: 626-218-0499 Internal x 80499
suyost@coh.org

4. **Microbiome specimens:**

Dr. Cui Ke
CC: Biospecimen Coordinator
COH Biorepository Core
City of Hope National Medical Center
1500 E. Duarte Road
Duarte, CA 91010
626-218-1848; 626-218-0462
kcui@coh.org;

Stool specimen will be temporarily stored at -80ºC in the Analytical Pharmacology Core Facility (APCF) in Shapiro 1042 (Dr. Tim Synold/Leslie Smith-Powell) until batch shipping to TGen:

Keehoon Lee, PhD TGen Clinical Microbiome Services Center
Pathogen and Microbiome Division
Translational Genomics Research Institute
3051 W. Shamrell Blvd., Suite 106
Flagstaff, AZ 86005
klee@tgen.org | 928-226-6352

5. DNA and RNA Sequencing

Tumor DNA and RNA sequencing will be performed at Tempus. No identifiable information will be sent to tempus and all unused specimen including DNA/RNA extract, FFPE slides and fresh frozen specimen will returned to COH investigator. Tempus will not store nor conduct any other research with the unused or leftover tissue.

Tempus
600 West Chicago Ave. Ste 510
Chicago, IL 60654
800.739.4137

Tempus xT Panel (595 genes): 15 FFPE +1 H&E for NGS and PD-L1 (20% tumor --- ratio of tumor nuclei to benign nuclei; 5-25mm2), plus normal sample (buffy coat from 1-2 x 8 ml tubes whole blood).