DF/HCC Protocol #: 16-588

TITLE: A phase II study of pembrolizumab in combination with palliative radiotherapy for metastatic hormone receptor positive breast cancer

Coordinating Center: *DF/HCC and Dana-Farber/Partners Cancer Care (DF/PCC)*

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IND #: 132796
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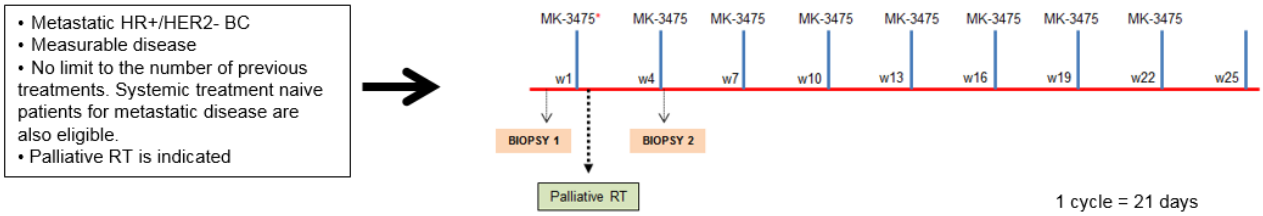
Protocol Type / Version # / Version Date: Amendment / April 9, 2019

SCHEMA



*Cycle 1 day 1 of anti-PD1 therapy will be administered 2-7 days before the initiation of RT.
• Biopsy 1 - Baseline tumor biopsy will be obtained within 7-28 days before starting Pembrolizumab.
• Biopsy 2 – Reassessment biopsy will be obtained within on cycle 2 day 1 (up to 3 days before and 7 days after C2D1).
• Imaging will be performed at screening within 4 weeks before starting Pembrolizumab every 6 weeks thereafter.
• Blood samples will be collected within 7 days before starting Pembrolizumab and every 3 weeks thereafter (just before the dose of Pembrolizumab).

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1. OBJECTIVES

1.1 Study Design

1.1.1 This is a phase II single arm study assessing objective response rate (ORR) according to RECIST 1.1 in patients with metastatic hormone receptor (HR)-positive/HER-2 negative breast cancer who will receive pembrolizumab in combination with palliative radiotherapy (RT). Pembrolizumab will be started 2-7 days before day 1 of RT. Biopsies will be performed at baseline (mandatory if tumor tissue is accessible outside the field of RT) and the same lesion will be repeated within 7-14 days before the day 1 of cycle 3 of pembrolizumab. Pembrolizumab 200mg will be administered intravenously every 21 days until disease progression.

1.2 Primary Objectives

1.2.1 To evaluate the efficacy of pembrolizumab in combination with radiotherapy, as defined by ORR outside the field of radiation according to RECIST 1.1, in patients with metastatic hormone receptor (HR)-positive/HER-2 negative breast cancer.

1.3 Secondary Objectives

Safety objectives

1.3.1 To evaluate the safety and tolerability of pembrolizumab in combination with palliative radiotherapy in patients with metastatic hormone receptor (HR)-positive/HER-2 negative breast cancer.

Efficacy objectives

1.3.2 To evaluate the ORR according to immune-related response criteria (irRC).

1.3.3 To evaluate the abscopal response rate (ARR).

1.3.4 To evaluate the progression-free survival (PFS) and overall survival (OS) of RT combined with pembrolizumab in metastatic hormone-receptor positive, HER2-negative breast cancer.

1.3.5 To evaluate the immune-related progression-free survival (irPFS) of RT combined with pembrolizumab in metastatic hormone-receptor positive, HER2-negative breast cancer.

1.3.6 To evaluate the Clinical Benefit Rate (CR + PR + SD $\geq$ 24 weeks) of RT combined with pembrolizumab in metastatic hormone-receptor positive, HER2-negative breast cancer.

1.3.7 To evaluate the immune-related Clinical Benefit Rate (irCBR) of RT combined with pembrolizumab in metastatic hormone-receptor positive, HER2-negative breast cancer.

1.3.8 To explore if radiation treatment volume or site of palliative radiation correlates with likelihood of achieving clinical benefit.

1.4 Correlative Objectives

1.4.1 To characterize a broad array of immune markers in metastatic HR-positive breast tumors (characterization will be based on histology, protein expression, and mRNA expression).

1.4.2 To explore how different immunosuppressive and/or immune-stimulating immune marker profiles at baseline correlate with disease response to therapy (response assessed by RECIST 1.1 irRC).

1.4.3 To characterize changes in tumor-infiltrating lymphocytes, PD-L1 expression and immune gene signatures in the tissue microenvironment (TME) from baseline to after 2 cycles of

pembrolizumab.

1.4.4 To explore whether induction of changes in the immunosuppressive and/or immune-stimulating immune marker profile in TME correlates with disease response to therapy (response assessed by RECIST 1.1, irRC and ARR).

1.4.5 To characterize serial changes in immune marker profile in peripheral blood mononuclear cells (PBMCs) over the course of the trial treatment.

1.4.6 To explore whether induction of changes in the immunosuppressive and/or immune-stimulating immune marker profile in PBMCs correlates with disease response to therapy (response assessed by RECIST 1.1, irRC and ARR).

1.4.7 To investigate whether there is an immune marker in circulating PBMCs that corresponds to tumor infiltrating lymphocyte (TIL) percentage in baseline tumor.

1.4.8 To collect blood to study cell-free DNA for comparison to tumor specimens before and after immunotherapy.

1.4.9 To characterize the structure and function of the gut microbiome in patients with breast cancer prior to starting this clinical trial.

1.4.10 To determine whether pre-treatment characteristics of the structure and function of the gut microbiome in patients with breast cancer is associated with disease response to therapy (response assessed by RECIST 1.1, irRC and ARR).

1.4.11 To characterize changes in the structure and function of the gut microbiome of patients with breast cancer after two cycles of pembrolizumab compared to baseline.

1.4.12 To determine whether changes in the overall diversity of the gut microbiome, estimated by the Shannon Index, of patients with breast cancer after two cycles of therapy is associated with disease response (response assessed by RECIST 1.1, irRC and ARR).

1.4.13 To determine if the abundance or functional profile of specific gut bacteria are associated with objective response to therapy (response assessed by RECIST 1.1, irRC and ARR).

1.4.14 To explore whether the number and/or type of mutations identified using a next generation sequencing (NGS) panel is correlated with patient outcomes (PFS, ORR, CBR, and OS).

2. BACKGROUND

2.1 Study Disease(s)

Breast cancer (BC) is the most frequently diagnosed cancer and the second cause of cancer death in American women[Jemal *et al.*, 2011, Siegel *et al.*, 2013]. Approximately, 70% of these cancers are HR-positive BC, comprising those expressing estrogen receptor and/or progesterone receptor[Burstein *et al.*, 2010]. Despite many advances in the adjuvant treatment of early-stage disease, 30-40% of women will develop systemic relapse[Greenberg *et al.*, 2015]. Additionally, 6-10% of patients present with de novo metastatic breast cancer[4]. Chemotherapy and endocrine therapy remain the backbone of systemic treatment for HR-positive tumors. More recently, the combination of endocrine therapy plus a molecular-targeted agent (such as CDK 4/6 or mTOR inhibitor), are promising strategies to overcome endocrine resistance[Pritchard *et al.*, 2012, Zhang *et al.*, 2015]. However, despite all these available systemic therapies, in the advanced setting virtually all patients with HR-positive tumors will die of BC and the median survival does not exceed 3 years[Nancy *et al.*, 2015]. Thus, new treatment platforms are needed.

2.2 The PD-1/PD-L1 pathway in cancer

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades[Schreiber *et al.*, Schreiber, 2012]. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies[Mlecnik *et al.*, 2014]. 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[Tosolini *et al.*, 2006, Adams *et al.*, 2014, Denkert *et al.*, 2015].

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene *Pdcd1*) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL). This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention[Intlekofer *et al.*, 2013].

The PD-1/PD-L1 pathway in breast cancer

Unlike melanoma and NSCLC, BC has not been intensively investigated for its susceptibility to immunotherapy in clinical settings. However, there are accumulating preclinical and clinical

evidence suggesting that immune system is critical during natural history of breast cancer and the immune system can be modulated to improve outcomes in this disease[Kroemer *et al.*, 2015]. It has been recognized that BC is capable of stimulating the immune system, as many breast tumors have substantial lymphocyte infiltration [Denkert *et al.*, 2010, Denkert *et al.*, 2015]. Additionally, this pathologic feature has prognostic implications, as lymphocyte predominant breast cancers are associated with improved prognosis [Denkert *et al.*, 2010, Loi *et al.*, 2013]. However, the degree of immune infiltration differs by BC subtype; while a substantial proportion of triple negative BC can be richly infiltrated, hormone-receptor positive BC is poorly T-cell infiltrated[Dushyanthen *et al.*, 2015]. Recently, it has been demonstrated that the expression of PD-1 and PD-L1 differs among breast tumors subtype: HR-positive (30% PD-1; 33% PD-L1), triple-negative (70% PD-1; 59% PD-L1) and HER2-positive (60% PD-1; 20% PD-L1)[Gatalica *et al.*, 2014].

2.3 Pembrolizumab

Pembrolizumab (formerly MK-3475) is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Keytruda™ (pembrolizumab) has recently been approved in the United Stated for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilumumab and, if BRAF V600 mutation positive, a BRAF inhibitor. Pembrolizumab will be used in this protocol at the FDA-approved dose and frequency (200mg every 3 weeks).
Clinical data are derived from an ongoing, first-in-human phase I study (PN001, NCT01295827) to evaluate the safety and clinical activity of Pembrolizumab as a monotherapy, sponsored by Merck Sharp & Dohme. There are five parts to this study (Parts A-D and F) (Investigator's Brochure, 2014).

Part A was a 3+3 dose escalation study in subjects with solid tumors to evaluate safety, tolerability, pharmacokinetics (PK), and pharmacodynamics, and to determine a maximum tolerated dose (MTD) or preliminary recommended phase 2 doses (RP2Ds). Doses were 1, 3, and 10 mg/kg every 2 weeks (Q2W); doses of either 2 mg/kg or 10 mg/kg were also administered every 3 weeks (Q3W). All 3 dose levels were well tolerated and no dose-limiting toxicities (DLTs) were observed; therefore, the MTD was not determined. The RP2D was determined by the sponsor based on safety, PK, and pharmacodynamic measurements, along with the strength of antitumor activity signals observed.

Pharmacokinetics
The half-life ($t_{1/2}$) of pembrolizumab is approximately 4 weeks and there is no indication of dose dependency or half-life in the three dose groups (1,3, and 10 mg/kg) (Investigator's Brochure, 2014). The long $t_{1/2}$ supports a dosing interval of every 2 or 3 weeks.

There was a dose-related increase in exposure from 1 to 10 mg/kg. Serum concentrations of pembrolizumab were lower by a factor of approximately 5 in patients receiving 2 mg/kg Q3W than in those receiving 10 mg/kg Q3W. Steady-state trough concentrations were 20% greater in the patients receiving 10 mg/kg Q2W than in those receiving the same dose Q3W.

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of pembrolizumab were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. Pembrolizumab has been found to have a wide therapeutic range based on the melanoma indication. The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

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

Anti-Drug Antibodies (ADA) Data
The occurrence of ADA has been observed in less than 1% of the patients screened, indicating a low potential of pembrolizumab to elicit the formation of ADA. No impact of ADA on pembrolizumab exposure has been observed.

Efficacy
When treated with pembrolizumab monotherapy, the overall response rate (ORR) for ipilimumab (IPI)-treated patients with melanoma was 25%/27% according to the Response Evaluation Criteria in Solid Tumors (RECIST)/investigator-assessed immune-related response criteria (irRECIST), respectively (Investigator's Brochure, 2014). The ORR for IPI-naïve patients with melanoma was 39%/43% by RECIST/investigator-assessed irRECIST, respectively. The majority of responses were seen in patients with melanoma by 16 weeks of therapy; however, some responses have been reported after 24 weeks or more of therapy with pembrolizumab. Responses can be delayed, and in some patients, a RECIST-defined progression followed by response has been observed.

The preliminary ORR for 38 patients with non-small cell lung cancer was 21%/24% by RECIST/investigator-assessed irRECIST, respectively (Investigator's Brochure, 2014).

Early findings for 27 patients with triple negative breast cancer showed an 18.5% ORR by RECIST (Nanda R, SABCS 2014).

Pharmacodynamics/Biomarkers
Pharmacodynamic data (IL-2 release assay) has suggested that peripheral target engagement is durable (>21 days).

PD-L1 is being investigated as a predictive biomarker for pembrolizumab treatment. At the 15th World Conference on Lung Cancer, Garon et al presented preliminary data on a subset of patients

suggesting that higher levels of tumor PD-L1 expression are associated with increased clinical activity[Leighl *et al.*, 2015]. ORR by RECIST 1.1 occurred in 4 out of 7 patients with higher levels of PD-L1 expression (57%, 95% CI 18-90%) versus 2 out of 22 patients with lower levels of PD-L1 expression (9%, 95% CI 1-29%). These data are extremely preliminarily, and PD-L1 is not being used for patient selection. Mutational load, T-cell infiltration and expression of PD-1/PD-L1 in tumor biopsies, were associated with a high response rate in patients with advanced melanoma and non-small cell lung cancer treated with immune checkpoint inhibitors[Herbst *et al.*, 2014, Snyder *et al.*, 2014, Rizvi *et al.*, 2015].

Safety data
The most frequent treatment-related adverse events (AEs) were fatigue, nausea, cough, pruritis, diarrhea, and rash (Investigator's Brochure, 2014). Most AEs were not considered serious. The most commonly-reported immune-related AEs were rash, pruritis, vitiligo, hypothyroidism, arthralgia, diarrhea, and pneumonitis.

Important identified risks include: pneumonitis, thyroid disorders (hypothyroidism and hyperthyroidism), colitis, diarrhea, hepatitis, nephritis, uveitis, rash/pruritis, and neuropathy.

2.4 Palliative Radiation therapy

Radiation is commonly used throughout the body for patients with metastatic disease for palliative purposes. Patients with breast cancer, specifically, may require palliative radiation for bone pain or metastatic bony, lymph node or other soft tissue sites that could progress and cause fracture or functional compromise. Delivering palliative radiation to a total dose of 20 Gy in 5 fractions is commonly used in clinical practice and achieves high rates of pain control and freedom from further progression at the irradiated site with less need for retreatment of the irradiated site as compared to lower dose regimens[Wu *et al.*, 2003, Hartsell *et al.*, 2005]. Side effects of palliative radiation are largely specific to the site being irradiated, but typically include fatigue, skin erythema, and long term risks of fibrosis and increased propensity for bone fracture.

2.5 Rationale

In the last decade, through the better understanding of tumor immunology, major clinical advances have emerged from T-cell based cancer immunotherapy. One of the most important mechanisms to evade antitumor immunity is the overexpression of checkpoint immune molecules on the T-cell surface, such as CTLA4, LAG3 and PD-1[Mittal *et al.*, 2015]. These molecules, after binding to their ligands, initiate inhibitory pathways that negatively modulate T-cell function. It has been recognized that BC is capable of stimulating the immune system, as many breast tumors have substantial lymphocyte infiltration[Loi *et al.*, 2013]. Additionally, this pathologic feature has prognostic implications, as lymphocyte predominant breast cancers are associated with improved prognosis. However, the degree of immune infiltration differs by BC subtype; while a substantial proportion of triple negative BC can be richly infiltrated, hormone-receptor positive BC is poorly T-cell infiltrated[Loi *et al.*, 2013]. Furthermore, only a small proportion of luminal cancers express PD-1, PD-L1 or both, respectively 30%, 33% and 15%[Gatalica *et al.*, 2014]. These factors make the likelihood of response of hormonal-positive BC to a single anti-PD-1/PD-L1 therapy low. In fact, recently, two phase I clinical trials presented the response rate of immune checkpoint blockade in patients with HR+ BC. In a non-selected population, the use of Avelumab (an anti-PD-L1 inhibitor) presented an objective response rate (ORR) of 2.8% while in patients previously

selected by PD-L1 expression on tumors, the use of Pembrolizumab resulted in an ORR of 12%. Thus, other strategies that elicit an immunogenic tumor microenvironment may be needed in order to enhance the activity of PD1/PD-L1 axis inhibitors in patients with hormone-receptor positive breast cancer.

In this context, there is strong preclinical rational to evaluate the combination of radiotherapy (RT) with anti-PD1 blockade. Previous studies demonstrated that, in addition to its direct cytoreductive effect, RT-induced cell death can be immunogenic, facilitating the recruitment and activation of antigen presenting cells (APCs) and priming of tumor antigen-specific T-cells[Shahabi *et al.*, 2015]. Recently, different groups demonstrated that RT to the tumor bed led to upregulation of PD-L1 on tumor cells, dendritic cells, and on myeloid-derived suppressive cells (MDSCs), which may contribute to impairment of T-cell function in the tumor[Liang *et al.*, 2013, Deng *et al.*, 2014, Sharabi *et al.*, 2014]. Furthermore, these groups also demonstrated that the combination of RT plus blockade of the PD-1/PD-L1 axis improved outcomes in different preclinical models compared with RT or anti-PD1/PD-L1 alone, including breast cancer models (Figure 1).

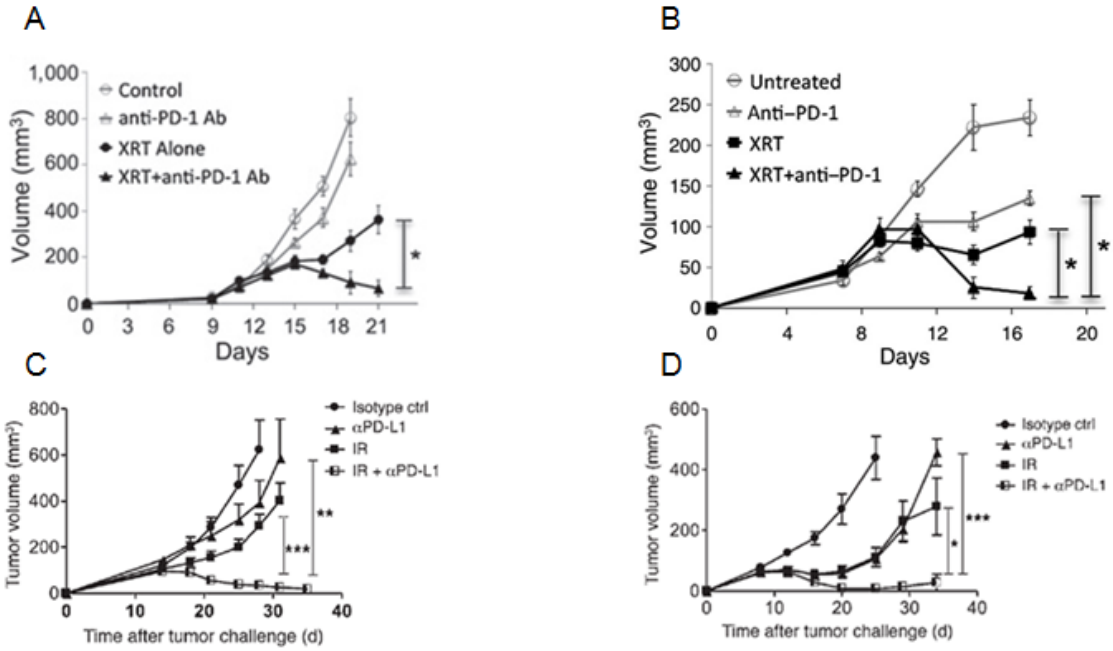


Figure 1. Radiation (XRT or IR) combined with anti– PD-1 immunotherapy significantly improves tumor control in different preclinical models of cancer - A) B16-OVA tumor cells, B) 4T1-HA tumor, C) TUBO tumor and D) MC38.

Modified from J Clin Invest. 2014;124(2):687–695 and Cancer Immunol Res. 2015 Apr;3(4):345-55.

Moreover, the effect of localized radiotherapy mediating systemic responses distant from the field of radiation (the abscopal effect), has been reported in several types of human malignancies, such as melanoma and renal cell carcinoma, after treatment combining RT and immune checkpoint inhibitors[Adamow *et al.*, 2012]. Although there is no definitive data confirming the host immune response caused the response, the tumor responses observed in these cases were accompanied by immunological changes in peripheral blood, including changes in a variety of tumor-associated antigens and a decrease in MDSCs. Additionally, the abscopal effect was recently demonstrated in mice treated with the combination of RT and anti–PD-L1 therapy, but not in groups receiving

either treatment alone, suggesting that this combination may potentiate an abscopal effect on distant tumors (Figure 2)[Deng *et al.*, 2014, Dovedi *et al.*, 2015].

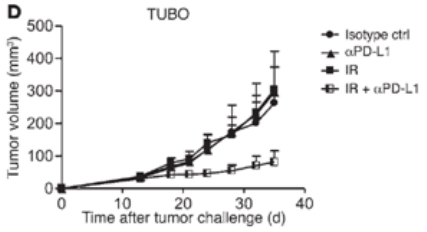


Figure 2. IR (radiation) and PD-L1 blockade synergistically amplify the antitumor effect - systemic effect (abscopal effect) of combination treatment greatly reduced the growth of secondary tumors.

Modified from J Clin Invest. 2014;124(2):687–695. doi:10.1172/JCI67313.

Due to these data that RT may potentiate the efficacy of PD-1 blockade, we propose evaluating the combination of the pembrolizumab with palliative radiotherapy in hormone-positive BC patients. Patients will receive the first dose of pembrolizumab 2-7 days before the beginning of RT. Pembrolizumab will be administered every 3 weeks thereafter until disease progression.

2.6 Correlative Studies Background

2.6.1 Blood and Tissue Analysis

The importance of tumor microenvironment and the immunosurveillance in natural history of cancer and its outcomes was proved to be true in the last years, with clinical approval of immune checkpoint inhibitors[Sharma *et al.*, 2015]. However, less than half of patients with solid tumors will derive benefit with these drugs [Hwu *et al.*, 2012, Smith *et al.*, 2012]. Thus, it is crucial to elucidate the exact mechanisms of antitumor immunity evasion ongoing in tumor microenvironment to successfully develop new cancer immunotherapy and correctly choose the best drug for the right patient. This goal can be pursuit through the discovery and validation of prognostic and predictive biomarkers.

A growing body of evidence suggests that patients with advanced solid tumors shows differences in tumor microenvironment regarding the presence or absence of a gene expression profile indicative of a pre-existing T-cell–inflamed tumor microenvironment[Gajewski, 2015]. Tumors classified as T-cell inflamed present a significant infiltration of CD8+ T cells and a type I IFN signature. In this group, the main mechanisms of immune evasion are the overexpression of immunessupressor molecules acting at the level of the tumor micro- environment, such as immune checkpoint molecules (CTLA-4, PD-1/PD-L1, TIM-3, LAG-3), indoleamine-2,3-dioxygenase (IDO), and FoxP3. Interestingly, such immunosuppressive molecules seem to be upregulated after deflagration of a type I Interferon antitumor response, resulting in T-cell exhaustion, and the so called adaptative immune resistance[Gajewski, 2015, Ribas, 2015]. The other group of patients presents tumors characterized by a low or absence of intratumoral CD8 T cells and a lack evidence of a type I IFN transcriptional signature. This tumor phenotype is called non-T-cell-inflamed[Gajewski, 2015].

The T-cell inflamed phenotype has positive prognostic value for several types of early stage cancer, including breast cancer[Dushyanthen *et al.*, 2015, Perez *et al.*, 2015], suggesting that the attempt by the host to generate an anti-tumor immune response reflects a biologic process

associated with improved patient outcomes[Gajewski, 2015]. In breast oncology, different groups have demonstrated that the amount of tumor-infiltrating lymphocytes (TILs) in a tumor specimen, commonly assessed simply by histological evaluation of a standard hematoxylin and eosin-stained slide by a trained pathologist, is a significant predictor of both response to therapy and overall disease outcomes in the neoadjuvant and adjuvant settings [Denkert *et al.*, 2010, Loi *et al.*, 2013, Adams *et al.*, 2014, Ali *et al.*, 2014, Salgado *et al.*, 2014, Denkert *et al.*, 2015, Denkert *et al.*, 2015]. Recently, more in-depth methods of immunologic profiling are being explored in breast cancer, for example mRNA expression of immune-activating and immunosuppressive factors, and these additional immune profiles also appear to have prognostic significance[Perez *et al.*, 2015]. Furthermore, in metastatic setting, the phenotype T-cell-inflamed appears to be associated with clinical response to several immunotherapies, including checkpoint blockade[Herbst *et al.*, 2014]. Patients with this tumor phenotype seem to be good candidates for immune checkpoint inhibitor therapy, alone or in combination. Thus, the bulk of our correlative science in this trial highlights our especial interest in characterize a broad array of immune markers in metastatic HR-positive breast cancer, investigating whether those markers predict disease response to therapy.

Thus, considering the mechanism of action of drugs like anti-PD-1/anti-PD-L1, is the lack of a significant T-cell infiltrate and low expression of immune checkpoint molecules may explain the reason that certain non inflamed tumor phenotype are associated with de novo resistance to those class of drugs. For this group of patients, therapeutic strategies that promote a boost in innate immunity, such as a course of radiation therapy, will be crucial to successfully overcoming T-cell exclusion and improve the likelihood of benefit of PD-1 blockers.

In breast tumors, particularly the HR-positive subset, the vast majority of patient's tumors do not harbor significant TILs or demonstrate PD-L1 expression and will therefore most likely be classified as non-T-cell-inflamed tumors. This explains why ORR recently reported in this population ranges from 2.8%-12%[Dirix *et al.*, 2015, Hugo *et al.*, 2015]. Clearly, new approaches to boost antitumor immunity are needed in this population. RT can potentially improve the activity of immune checkpoint inhibitors. Because of the preclinical data supporting RT induced immune modulation of the tumor microenvironment, we intend to explore how immune biomarkers change after the beginning of treatment, including the expression of immune checkpoint molecules, TILs and T-cell receptor diversity.

Additionally, as a correlative study to this trial, we will characterize the immune marker profile of peripheral blood mononuclear cells (PBMCs) in enrolled breast cancer patients. Furthermore, given the demonstrated clinical significance of TILs in breast cancer specimens, we will investigate whether there is a peripheral marker whose level corresponds to TIL percentage. Lastly, we will evaluate whether there is a correlation between changes in PBMC immune profiles and disease response. Evidence of a correlation would be of significant interest as it would suggest the potential presence of a predictive biomarker in the peripheral blood.

These correlative projects are made possible by collaboration with Drs. Scott Rodig and Evisa Gjinin, and Mariano Severgnini, all of whom are lab scientists with extensive experience with immune profiling in melanoma. Further details can be found in Section 9.

2.6.2 Microbiome Analysis

Breast Cancer (BC) is the most frequently diagnosed cancer and the second cause of cancer death in American women [DeSantis *et al.,* 2015]. In the advanced setting, despite multiple available systemic therapies, virtually all patients will die from their disease. Thus, the exploration of new treatments, such as immune checkpoint inhibitors (ICI), including pembrolizumab, is imperative.

An increasing body of preclinical and clinical evidence suggests that breast cancer is an immunogenic malignancy [Kroemer *et al.,* 2015]. It is now recognized that a fraction of breast tumors have substantial lymphocyte infiltration, and that this pathologic feature has prognostic implications [Stanton, 2016]. Early clinical trials assessing the efficacy of PD-1/PD-L1 inhibitors given as monotherapy showed that only a small fraction of patients derive benefit from immunotherapy with an approximate 20% objective response rate (ORR) among patients with PDL1+ TNBC [Dirix *et al.,* 2016, Nanda *et al.,* 2016], and a 12% ORR among those with PDL1+ hormone receptor (HR)-positive BC [Rugo *et al.,* 2016]. Therefore, new research approaches combining therapeutic agents aiming to boost antitumor immunity, as well as developing predictive biomarkers of response, are needed to increase the rates of clinical success of immunotherapy in BC.

In this context, the gut microbiota has been recognized as a modulator of immune system development [Tinchieri, 2015]. Healthy individuals have microbial populations in their intestinal tract that vary markedly in composition [Human Microbiome Project, 2012, Qin *et al.*, 2010]. The diversity of intestinal microbiota represents a significant challenge to the host's immune defenses, which must balance immune tolerance of beneficial microbes with inflammatory responses against pathogens. Alterations in the gut microbiota and their resulting interactions with intestinal epithelieum and the host immune system are associated with many disease, including cancer [Roy *et al.* 2017]. Recently, two preclinical studies provided to ICI, raising the possibility that stool microbiota could be used as biomarker predictors of efficacy to immunotherapy [Sivan *et al.*, 2015, Vetizou *et al.,* 2015]. Interestingly, postmenopausal women with breast cancer have altered composition and low diversity of their gut microbiota compared to healthy controls [Goedert *et al.,* 2015].

Identification of biomarkers that predict response to ICI-based therapies can spare *de novo* resistant patients from the unnecessary risks of immune-related adverse events. In addition, the identification of bacterial species associated with response could open new strategies to maximize the clinical benefit of cancer immunotherapy through the modulation of gut microbiota.

This correlative project is made possible by collaboration with the BWH/Harvard Cohorts Biorepository and Dr. Andrew Chan. Further details can be found in Section 9.

2.6.3 Tumor Genomic Profile

In addition to the immune microenvironment, intrinsic tumor factors may be associated with response to immune checkpoint inhibitors. Although some of the mechanisms related to de novo or acquired resistance to ICI have been recently described, including loss of function in beta-2-

microglobulin or defects in the interferon signaling pathway[Gao et al., 2016, Zaretsky et al., 2016], the knowledge of immune resistance remains largely unknown. Several gene/pathways have been described as possible candidates of having an immunosuppressive role in different advanced solid tumor, including MYC amplification[Casey et al., 2016], activation in WNT-□-catenin pathway[Spranger et al., 2015], activation in MAPK pathway, loss of PTEN[Li et al., 2016, Peng et al., 2016, George et al., 2017]. On the other hand, few possible biomarkers of response to ICI have emerged, including mutational load[Snyder et al., 2014, Rizvi et al., 2015], tumor aneuploidy[Davoli et al., 2017], mismatch repair defects[Le et al., 2015], and BRCA2 mutation[Hugo et al., 2016]. Notably, there is no data on genomic mechanisms of de novo resistance to anti-PD-1 therapy in patients with breast cancer.
Therefore, as a correlative study to this trial, we will to explore whether the number and/or type of mutations identified using a next generation sequencing (NGS) panel – OncoPanel - is correlated with patient outcomes (PFS, ORR, CBR, and OS). This tool is a cancer genomic assay to detect somatic mutations, copy number variations and structural variants in tumor DNA extracted from fresh, frozen or formalin-fixed paraffin-embedded samples. The OncoPanel assay surveys exonic DNA sequences of 447 cancer genes and 191 regions across 60 genes for rearrangement detection. DNA is isolated from tissue containing at least 20% tumor nuclei and analyzed by massively parallel sequencing using a solution-phase Agilent SureSelect hybrid capture kit and an Illumina HiSeq 2500 sequencer. The targeted NGS assay (OncoPanel) will be performed at the Center for Advanced Molecular Diagnostics (Department of Pathology, Brigham and Women's Hospital). This assay has been extensively validated and is used as a CLIA-approved clinical molecular test in our institution without any additional sequencing assays to validate the findings [Wagle et al., 2012].

3. PARTICIPANT SELECTION

Eligibility will be assessed as part of the screening procedures for all patients.

3.1 Eligibility Criteria

3.1.1 Participants must have histologically or cytologically confirmed invasive breast cancer, with metastatic disease. Participants without pathologic or cytologic confirmation of metastatic disease should have unequivocal evidence of metastasis from physical examination or radiologic evaluation.

3.1.2 Invasive disease must have been tested for ER, PR and HER2. Participants must have hormone-receptor positive, HER2-negative breast cancer defined as:

- ER>1% or PR>1%
- HER2-negative per ASCO CAP guidelines, 2013 [Wolff *et al.*, 2013]

3.1.3 Participant must be a candidate for palliative radiation treatment to at least one bone, lymph node, or soft tissue lesion. Radiation of visceral lesions (such as lung or hepatic lesions) is not permitted.

3.1.4 Participant must have measurable disease outside the field of radiation as defined by RECIST 1.1.

3.1.5 If tumor is accessible and outside the field of radiation, the participant must be willing to undergo a research biopsy at baseline and around their second cycle of pembrolizumab. Participants for whom newly-obtained samples cannot be provided (e.g. inaccessible or safety concern) must be willing to submit an archival specimen.

3.1.6 Prior systemic therapy:

- Participant must be at least 14 days from the last dose of prior chemotherapy, endocrine therapy, biological agents (including small molecule targeted therapy) or any investigational drug product with adequate recovery of toxicity to baseline, or grade 1(with the exception of alopecia and hot flashes) at the time of registration.

- There is no limit to the number of prior lines of therapy, including endocrine or cytotoxic agents. Systemic treatment naive patients for metastatic disease are also eligible.

- Participants may initiate or continue bisphosphonate therapy on study.
- Continuation of ovarian suppression is allowed.

3.1.7 Prior radiation therapy:

- Patients must be at least 3 months from prior radiation therapy
- Re-irradiation of the same field is not allowed

3.1.8 Concurrent administration of other cancer specific therapy during the course of this study is not allowed.

3.1.9 The subject is ≥18 years old

3.1.10 ECOG performance status ≤1 (See Appendix A for details)

3.1.11 Participants must have normal organ and marrow function as defined below:

– absolute neutrophil count	≥1,500/mcL
– platelets	≥100,000/mcL
– hemoglobin	≥ 8 g/dl
– total bilirubin	≤1.5 × institutional upper limit of normal (ULN) (or 2.0 x ULN in patients with documented Gilbert's Syndrome)
– AST(SGOT)/ALT(SGPT)	≤2.5 × institutional ULN or ≤ 5 × institutional ULN for participants with documented liver metastases
– creatinine	≤1.5 ×within normal institutional ULN OR creatinine clearance ≥60 mL/min/1.73 m^2 for participants with creatinine levels above institutional ULN.

- International normalized ratio (INR) or Prothrombin Time (PT) <1.5 times the upper limit of normal unless subject is receiving anticoagulant therapy, as long as PT or PTT is within therapeutic range of intended use of anticoagulants.
- Activated Partial Thromboplastin Time (aPTT) <1.5 times the upper limit of normal unless subject is receiving anticoagulant therapy, as long as PT or PTT is within therapeutic range of intended use of anticoagulants.

3.1.12 Female subjects of childbearing potential must have a negative pregnancy test at screening

3.1.13 Female and male subjects of childbearing potential must agree to use an adequate method of contraception as outlined in section 5.4.1. Contraception is required starting with the first dose of study medication through 120 days after the last dose of study medication Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

3.1.14 Resolution of all chemotherapy-related or radiation-related toxicities to Grade 1 severity or lower, except for stable sensory neuropathy (≤ Grade 2) and alopecia.

3.1.15 The subject is capable of understanding and complying with the protocol and has signed the informed consent document.

3.2 Exclusion Criteria

3.2.1 Participants who are receiving any other investigational agents.

3.2.2 Previous treatment with any anti-PD-1, PD-L1, or PD-L2 agent.

3.2.3 History of allergic reactions attributed to compounds of similar chemical or biologic composition to pembrolizumab.

3.2.4 Known brain metastases that are untreated, symptomatic, or require therapy to control symptoms. Participants with previously diagnosed brain metastases are eligible if they have:

- completed treatment (whole brain radiotherapy, radiosurgery, or a combination) at least 3 months prior to trial therapy initiation,
- are neurologically stable, and
- have recovered from effects of radiotherapy or surgery.

Any corticosteroid use for brain metastases must have been discontinued without the subsequent appearance of symptoms for ≥2 weeks before prior to registration.

3.2.5 Radiologic or clinical evidence of Spinal Cord Compression.

3.2.6 Spinal Instability Neoplastic Score ≥ 7 unless lesion reviewed by a neurosurgical service and considered stable.

3.2.7 Participants with bone lesions requiring surgical fixation to provide mechanical stability are ineligible. Participants with previously fixed lesions are allowed.

3.2.8 The participant has an uncontrolled intercurrent illness, including, but not limited to uncontrolled hypertension, unstable angina pectoris, uncontrolled cardiac arrhythmia, congestive heart failure-New York Heart Association Class III or IV, active ischemic heart disease, myocardial infarction within the previous six months, uncontrolled diabetes mellitus, gastric or duodenal ulceration diagnosed within the previous 6 months, severe malnutrition or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.9 Clinically significant electrocardiogram (ECG) abnormality, including a marked baseline prolonged QT/QTc ([QT interval/corrected QT interval], eg, a repeated demonstration of a QTc interval >500 ms).

3.2.10 Participant has a medical condition that requires chronic systemic steroid therapy or on any other form of immunosuppressive medication. For example, patients with autoimmune disease that requires systemic steroids or immunosuppression agents should be excluded. Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

3.2.11 Has history of (non-infectious) pneumonitis that required steroids or current pneumonitis.

3.2.12 Has a history of interstitial lung disease.

3.2.13 The participant is known to be positive for the human immunodeficiency virus (HIV), HepBsAg, or HCV RNA. HIV-positive participants on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with Pembrolizumab.

3.2.14 Individuals with a history of different malignancy are ineligible except for the following circumstances. Individuals with a history of other malignancies are eligible if they have been disease-free for at least 3 years or are deemed by the investigator to be at low risk for recurrence of that malignancy.

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

3.2.16 The participant is pregnant or breast-feeding.

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. Registration cancellations must be made in OnCore as soon as possible.

4.2 Registration Process for DF/HCC Institutions

DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) must be followed.

5. TREATMENT PLAN

5.1 Treatment Regimen

This is a phase II single arm study assessing ORR outside the field of radiation according to RECIST 1.1 in patients receiving pembrolizumab in combination with palliative radiotherapy in metastatic hormone-positive, HER2-negative breast cancer. 27 patients will be enrolled to receive therapy. Pembrolizumab therapy will be started 2-7 days before day 1 of RT. Radiation will then be delivered for five treatments of 4Gy (4Gy x 5) over a period of 1 to 2 weeks. The dose of pembrolizumab planned to be studied in this trial is 200 mg on day 1 of each 21 day cycle. Each cycle will have 21 days (3 weeks – q3w). The dose recently approved in the United States for treatment of melanoma subjects is 2 mg/kg Q3W. Although the dose of pembrolizumab studied in KN 012, which established efficacy and safety in mTNBC, 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.

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

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

Treatment will be administered on an outpatient basis. Reported adverse events and

potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. Details of the regimen are described in Table 1. No investigational or commercial agents of therapies other than those described below may be administered with the intent to treat the participant's malignancy

Table 1: Regimen Description

Agent	Premedication ; Precautions	Dose	Route	Schedule	Cycle Length
Pembrolizumab	Not routinely necessary unless prior infusion reaction.	200 mg D1, *q3w, iv*	IV over approximately 30 minutes (range: 25-40 minutes). Please refer to Section 8 for compatible infusion set materials including in-line filter.	Day 1, q3w	21 days (3 week)

5.2 Pre-Treatment Criteria

5.2.1 Cycle 1, Day 1

- absolute neutrophil count ≥1,500/mcL
- platelets ≥100,000/mcL
- hemoglobin ≥ 8 g/dl
- total bilirubin ≤1.5 × institutional upper limit of normal (ULN) (or 2.0 x ULN in patients with documented Gilbert's Syndrome)
- AST(SGOT)/ALT(SGPT) ≤2.5 × institutional ULN or ≤ 5 × institutional ULN for participants with documented liver metastases
- creatinine ≤1.5 ×within normal institutional ULN OR creatinine clearance ≥60 mL/min/1.73 m^2 for participants with creatinine levels above institutional ULN.

5.2.2 Subsequent Cycles, Day 1

- absolute neutrophil count ≥1,000/mcL
- platelets ≥75,000/mcL
- AST(SGOT)/ALT(SGPT) ≤ 2.5 × institutional ULN or ≤ 5 × institutional ULN for participants with documented liver metastases
- total bilirubin ≤1.5 × institutional ULN (or 2.0 x ULN in patients with documented Gilbert's Syndrome)

5.3 Treatment period

5.3.1 Pembrolizumab

Pembrolizumab administration

Sponsor Merck will provide each investigator with adequate supplies of pembrolizumab. Pembrolizumab will be administered by trained medical personnel at the investigational site. Treatment compliance will be monitored through documentation of study treatment administration in the subject's medical record.

Pembrolizumab will be administered in clinic on day 1 (+/- 3 days) of each cycle. It will be administered as a 30 minute IV infusion. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5/+10 minutes is permitted.

5.3.2 Other Modality(ies) or Procedures

5.3.2.1 Palliative Radiation Therapy

Palliative radiotherapy will be prescribed to a dose of 20 Gy in 5 fractions fractions to be delivered to a previously unirradiated area for which palliative radiation is indicated with a dose constraint of V20 Gy < 20% for the bilateral lung and V20 Gy < 32% to the bilateral kidney. All patients will undergo CT guided radiation simulation with custom immobilization to be determined based on the area being irradiated. A clinical target volume will be delineated by the treating radiation oncologist based on the CT simulation to treat gross disease and account for any uncertainty, incorporating information from prior diagnostic imaging and physical exam. Irradiation of elective volumes not thought to harbor gross disease is not allowed. A margin of approximately 1 cm and individualized based on the region treated will be added to create the planning tumor volume. Conformal treatment planning is encouraged but not mandatory. Intensity modulated radiation therapy is permitted. Treatment will be administered on an outpatient basis, generally on 5 consecutive business days over a period of one to two weeks. If a treatment is missed because of unavoidable events (e.g. weather, machine maintenance, hospitalization, etc.) it will be made up for so that there are still 5 total delivered treatments.

5.4 General Concomitant Medication and Supportive Care Guidelines

5.4.1 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm.

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

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

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

absence of 12 months of amenorrhea, a single FSH measurement is insufficient.);

OR

- have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion, at least 6 weeks prior to screening;

OR

- has a congenital or acquired condition that prevents childbearing.

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

- practice abstinence† from heterosexual activity;

OR

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

Acceptable methods of contraception are‡:

Single method (one of the following is acceptable):

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

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

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

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

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

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

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

5.4.2 Concomitant Medication Guidelines

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the overall PI.

Acceptable Concomitant Medications

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

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded.

Prohibited Concomitant Medications

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

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy not specified in this protocol
- Any systemically active oral, injected, or implanted hormonal method of contraception except for progesterone coated intrauterine devices (IUDs) that had been previously implanted.
- Estrogen replacement therapy.
- 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 radiation or an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids (10 mg prednisone daily or equivalent) can be used without Sponsor authorization.

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.

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

5.4.3 Supportive Care Guidelines – general medications

The following treatments are permitted throughout the duration of the study treatment phase and during follow-up:

- Standard therapies for pre-existing medical conditions unless listed as prohibited therapy. Any medication intended solely for supportive care (e.g., analgesics, anti-diarrheal, anti-depressants) may be used at the investigator's discretion. Antiemetics and anti-diarrheal medications should not be administered prophylactically before initial treatment with study drugs. At the discretion of the investigator, prophylactic antiemetic and anti-diarrheal medication(s) may be used as per standard clinical practice before subsequent doses of study drugs or before, during or after radiation treatment.
- Bisphosphonate or denosumab therapy to be used in accordance with the approved labeled indication and/or nationally recognized treatment guidelines. Participants already receiving bisphosphonate/denosumab at the time of study entry can continue the treatment.
- Anticoagulants - Anticoagulation with heparin, heparin derivatives, and/or warfarin may be given at the discretion of the treating physician. Coagulation parameters should be checked at least once monthly, or more frequently at discretion of treating physician.
- Pain medications administered per standard clinical practice are acceptable while the patient is enrolled in the study.

Patients who experience toxicities should be treated symptomatically as clinically indicated. Medications that are considered necessary for the subject's welfare and that are not expected to interfere with the evaluation of study treatment or be restricted may be given at the discretion of the investigator. Ancillary treatments will be given as medically indicated.

5.5 Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse event(s), treatment may continue for an indefinite number of cycles, or until one of the following criteria applies:

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Participant demonstrates an inability or unwillingness to comply with the medication regimen and/or documentation requirements
- Participant decides to withdraw from the protocol therapy
- Completed 35 treatments with pembrolizumab

- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF) and in the CTMS system (OnCore). Alternative care options will be discussed with the participant.

For sites using Centralized Subject Registrations with the office of ODQ, the research team submits a completed Off Treatment/Off Study form to ODQ when a participant comes off study. This form can be found on the ODQ website or obtained from the ODQ registration staff.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, Sara Tolaney, MD at 617-632-5743 or stolaney@partners.org.

Participants may elect to stop pembrolizumab with CR after at least 24 weeks of treatment and having had at least two treatments with pembrolizumab after documentation of the CR, or if they have been on pembrolizumab after 35 cycles..

These patients may be eligible for up to 17 additional study treatments if they progress after stopping study treatment provided they meet the requirements detailed in Sections 3.1 and 3.2. This retreatment is termed the Second Course Phase of this study and is only available if the study remains open and the subject meets all the eligibility criteria included in section 3 and the following conditions:

- Stopped initial treatment with pembrolizumab after attaining an investigator-determined a confirmed CR according to RECIST 1.1, was treated for at least 24 weeks with pembrolizumab before discontinuing therapy, and received at least two treatments with pembrolizumab beyond the date when the initial CR was declared

OR

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

OR

- Received 35 cycles of study therapy without disease progression

Subjects who restart treatment will be retreated at the same dose and dose interval as when they last received pembrolizumab. Visit requirements are as outlined for subjects on the initial treatment phase of the trial. Patients must meet cycle 1 day 1 pre-treatment criteria to initiate therapy.

5.6 Duration of Follow Up

Participants removed from protocol therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

For participants who are alive and free of disease progression at the time of removal from protocol therapy, tumor assessments should continue to be performed every 6-12 weeks until progression or death, whichever occurs first.

All participants will be followed annually by medical record or phone until death.

5.7 Criteria for Taking a Participant Off Study

Participants will be removed from study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent for data submission
- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF) and CTMS (OnCore).

For Centralized Subject Registrations, the research team submits a completed Off Treatment/Off Study form to ODQ when a participant comes off study. This form can be found on the ODQ website or obtained from the ODQ registration staff.

6. DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made as indicated in the following table(s). The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

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). Participants held for these reasons require prior approval from the PI and should resume therapy within 3 weeks of the scheduled interruption. The reason for interruption should be documented in the participant's study record.

No dose reductions are allowed for pembrolizumab.

If there are dosing delays for any reason, all study assessments are to be delayed in the same fashion, such that scans and other assessments occur in conjunction with cycles of treatment.

6.1 Management of toxicities attributable to pembrolizumab

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may

represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table 2 below.

Table 2: Dose modification guidelines for Pembrolizumab for drug-related adverse events

General instructions:

1. Corticosteroid taper should be initiated upon AE improving to less than or equal to Grade 1 or baseline and continue to taper over at least 4 weeks.
2. For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to less than or equal to Grade 1 or baseline and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤10 mg prednisone or equivalent per day within 12 weeks.
3. For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.

Immune-related AEs	**Toxicity grade or conditions (CTCAEv4.0)**	**Action taken to pembrolizumab**	**irAE management with corticosteroid and/or other therapies**	**Monitor and follow-up**
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment • Add prophylactic antibiotics for opportunistic infections
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus). • Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.
	Grade 4	Permanently discontinue		
AST / ALT elevation or Increased bilirubin	Grade 2	Continue	• Consider administering corticosteroids (initial dose of 0.5- 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable

	Grade 3 or 4	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold	• Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated.	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hyperthyroidism	Grade 2	Continue	• Treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders.
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyroinine) per standard of care	• Monitor for signs and symptoms of thyroid disorders.
Nephritis and Renal dysfunction	Grade 2	Withhold	• Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1 or 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other cause
	Grade 3 or 4	Permanently discontinue		
Infusion Reaction[2]	Grade 3 or 4	Permanently discontinue	• See Table 5	• See Table 5
All other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	• Based on type and severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Gullain-Barre Syndrome,		

		encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		

1. Withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician.
2. See Table 5 for further guidance on all grades of pembrolizumab infusion reactions.

NOTE:
For participants with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required, pembrolizumab may be resumed when AE resolves to ≤ Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM).

Supportive care for pembrolizumab toxicity, particularly suspected immune-mediated toxicity
Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined in Table 2 and Table 3.

Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

Note: if after the evaluation the event is determined not to be related, the investigator is instructed to follow the events of clinical interest (ECI) reporting guidance in Section 7 but does not need to follow the treatment guidance provided.

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

- **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 3 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab.

Table 3: Infusion Reaction Treatment Guidelines for pembrolizumab

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	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
	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.**	(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.		

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

7.1 Adverse Events Lists

7.1.1 Adverse Event List(s) for pembrolizumab

In the pembrolizumab monotherapy trials (P001/P002, P012, P013, and P028, plus the P011 monotherapy arm), the overall incidence of AEs ranged from 83.0% (73 of 88 subjects in P012) to 100% (10 of 10 subjects in P011). The most commonly reported AEs included fatigue, diarrhea, decreased appetite, nausea, and anemia. The incidence of drug –related AEs (DRAEs) ranged from 39.8% (35 of 88 subjects in P013) to 80.0% (8 of 10 subjects in P011). The most commonly reported DRAEs across all studies were nausea, fatigue, and diarrhea. The incidence of Grade 3-5 DRAEs across studies ranged from 6.8% (6 of 88 in P013) to 12.0% (187 of 1562 subjects) in P001/P002. The most commonly reported Grade 3-5 DRAEs were anemia, alanine aminotransferase increased, and aspartate aminotransferase increased. Most subjects who

experienced an AE continued in the study, with the incidence of AEs leading to discontinuation ranging from 1.9% (8 of 430 subjects in P028) to 12.3% (192 of 1562 subjects in P001/P002). The majority of AEs leading to discontinuation were not considered drug related. Discontinuations due to a DRAE were infrequent and ranged from 0% (no subjects in P011) to 4.5% (4 of 88 subjects in P013). The most commonly reported DRAEs leading to discontinuation were pneumonitis, alanine aminotransferase increased, and aspartate aminotransferase increased.

List of AEs considered expected:

- Endorcine disorders: Adrenal insufficiency, Hyperthyroidism, Hypophysitis, Hypopituitarism, Hypothyroidism, Secondary adrenal insufficiency, Thyroid disorder
- Eye disorders: Uveitis
- Gastrointestinal disorders: Abdominal pain, Colitis, Diarrhoea,Pancreatitis
- General disorders and administration site conditions: Asthenia, Pyrexia
- Hepatobiliary disorders: Autoimmune hepatitis, Hepatitis
- Infusion related reaction
- Metabolism and nutrition disorders: Diabetic ketoacidosis, Hyponatremia, Type 1 diabetes mellitus
- Musculoskeletal and connective tissue disorders: Arthralgia, Back pain, Myositis
- Nervous system disorders: Guillain-Barré syndrome
- Renal and urinary disorders: Nephritis
- Respiratory, thoracic and mediastinal disorders: Cough, Pneumonitis
- Skin and subcutaneous tissue disorders: Pruritis, Rash, Severe skin reaction, Vitiligo

7.1.2 Adverse Event List(s) for Radiation Therapy

The risks of radiation depend on the area of the body that will be treated with radiation. In general, the risks of radiation to an area of the soft tissue, lymph or bone are listed below:

- Tiredness
- Skin reddening & irritation
- thickening of skin in the treatment field
- Blistering, ulceration or tissue death
- Delayed wound healing
- Alopecia
- Swelling
- Pain
- Destruction of bone or cartilage and increased likelihood of fracture
- Damage to muscles

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded

from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent(s) that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Expedited Adverse Event Reporting

7.3.1 Investigators **must** report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

7.3.2 DF/HCC Expedited Reporting Guidelines

Investigative sites within DF/HCC will report AEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy

7.4 Expedited Reporting to the Food and Drug Administration (FDA)

The Overall PI, as study sponsor, will be responsible for all communications with the FDA. The Overall PI will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

7.5 Expedited Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports or sentinel events that require reporting according to institutional policy.

7.6 Expedited Reporting of Adverse Events and Events of Clinical Interest to Merck

7.6.1 Serious Adverse Events

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

- Results in death;
- Is life threatening;

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

Any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject from the time the consent is signed through 30 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to Merck product, must be reported to the Overall PI within 24hrs of being notified of the event and 2 working days working days to Merck Global Safety.

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

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to Merck product that is brought to the attention of the investigator at any time outside of the time period specified in the previous paragraph also must be reported immediately to Merck.

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

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

7.6.2 Events of Clinical Interest (ECIs)

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

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

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

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

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

   *Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology. The trial site guidance for assessment and follow up of these criteria can be found in the Investigator Trial File Binder (or equivalent).

Additional adverse events:

ECIs (both non-serious and serious adverse events) from the date of first dose through 30 days following cessation of treatment or the initiation of a new anticancer therapy, whichever is earlier, need to be reported to the overall PI within 24hrs of being notified of the event and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220), regardless of attribution to study treatment, consistent with standard SAE reporting guidelines.

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

7.6.3 Reporting of Pregnancy and Lactation to Merck

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them), including the pregnancy of a male subject's female partner that occurs during the trial or within 120 days of completing the trial, or 30 days following cessation of treatment if

the subject initiates new anticancer therapy, whichever is earlier. All subjects and female partners of male subjects who become pregnant must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported to the Overall PI within 24hrs of being notified of the event and 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

7.7 Routine Adverse Event Reporting

All Grade 2 or higher Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must also be reported in routine study data submissions.**

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational and other agents administered in this study can be found in Section 7.1.

8.1 PEMBROLIZUMAB

Please refer to the Investigator's Brochure for detailed agent information, and to the FDA label for additional information.

8.1.1 Description

Pembrolizumab is a humanized monoclonal antibody of the IgG4/kappa isotype. Other name: MK-3475, Keytruda. Pembrolizumab blocks negative immune regulatory signaling by binding to the PD-1 receptor, inhibiting the interaction between PD-1 and its ligands.

The molecular weight of Pembrolizumab is 148.9-149.5 KDa.

8.1.2 Form

Clinical supplies will be manufactured and provided by Merck as summarized in Table 4.

Table 4: Product Description

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

8.1.3 Storage and Stability

Store intact vials between 2°C-8°C (36°F-46°F). Do not freeze. Protect from light by storing in the original box.

Stability testing of the intact vials is ongoing.

Administer prepared solutions immediately after preparation. If not administered immediately, prepared solutions may be stored refrigerated for a total cumulative storage time at room temperature and refrigeration of 24 hours. PEMBROLIZUMAB solutions may be stored at room temperature for a cumulative time of up to 6 hours. This includes room temperature storage of liquid drug product solution in vials, room temperature storage of infusion solution in the IV bag, and the duration of infusion.

8.1.4 **Compatibility**

Compatible IV bag materials: PVC plasticized with DEHP, non-PVC (polyolefin), EVA, or PE lined polyolefin.

8.1.5 **Handling**

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

8.1.6 **Availability**

Pembrolizumab is an investigational agent and will be supplied free of charge from Merck.

8.1.7 **Preparation**

Pembrolizumab solution for infusion must be diluted prior to administration. Allow the required number of vials to equilibrate to room temperature. Do not shake the vials. Do not use if opaque or extraneous particulate matter other than translucent to white proteinaceous particles is observed. Do not use if discolored. To prepare the infusion solution add the dose volume of Pembrolizumab to an infusion bag containing 0.9& Sodium Chloride Injection, USP of 5% Dextrose Injection, USP. Gently invert the bag 10-15 times to mix the solution. The final concentration must be between **1 mg/mL to 10 mg/mL.**

8.1.8 **Administration**

Route of administration: IV infusion only. Do not administer as an IV push or bolus injection.

Method of administration: Infuse over approximately 30 minutes (range: 25-40 minutes) using an infusion set containing a low-protein binding 0.2 to 5 m in-line filter make of polyethersulfone or polysulfone. Infusion rate should not exceed 6.7 mL/min. A central

line is not required however if a subject has a central venous catheter in place, it is recommended that it be used for the infusion. Do not co-administer drugs through the same infusion line. Following the infusion, flush the IV line with normal saline.

8.1.9 Ordering

Pembrolizumab will be obtained directly from Merck, the study sponsor.

8.1.10 Accountability

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

8.1.11 Destruction and Return

At the end of the study, unused supplies of Pembrolizumab should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

In all patients in whom a tumor outside the field of radiation is accessible, a baseline tumor biopsy is required. We plan to use baseline biopsy tissue to perform a number of immune profiling assays, detailed below. On baseline tumor biopsies, we will perform characterization based on histology (TILs), protein expression, and mRNA expression. Additionally, we will bank specimens for possible future DNA analysis, and other further testing.

Serial blood draws for correlative science are required on this trial; blood draws will be obtained every 3 weeks prior to the infusion of study drugs, at the end-of-treatment visit in patients who go off study for progressive disease, and all efforts will be made to obtain an additional blood draw at the time of progressive disease, in patients who went off study for anything other than progressive disease. On each blood draw, we will perform flow cytometry to characterize protein expression of immune mediators, detailed below, and additional blood will be banked for future testing.

All patients will additionally be asked to provide a stool sample at three separate timepoints: prior to treatment, during treatment, and at the time of disease progression. A fourth collection may be requested from patients who experience grade ≥ 2 diarrhea after discussion with the PI. This collection is not required, but is strongly encouraged. These samples will be analyzed for microbiota content.

Please refer to the separate laboratory manual for additional correlative details including collection, processing, and shipping instructions.

9.1 Archival Tissue

1 block or 10-20 5 micron unstained, charged slides will be collected for future research.

9.2 Fresh Tissue Biopsy

9.2.1 Objectives:

- Characterizing immune markers in metastatic HR-positive breast tumors

9.2.2 Collection

Biopsies are required at screening and C2D1 (up to 3 days before and 7 days after C2D1).

Biopsies should not be performed on Friday afternoons, as there may not be time for processing of the fresh tissue. If a biopsy must be performed on Friday morning, the lab of Mariano Severgnini must be notified ahead of time to ensure that there will be adequate time for processing fresh tissue, since fresh tissue cannot be stored over the weekend. The specimens in RNALater and formalin may be stored over the weekend and shipped on Monday. Specimens in RNA Later and formalin should be stored at room temperature until shipment.

Ideally five core biopsies will be obtained:
- Two cores should be placed in 10% neutral buffered formalin tube supplied by the study.
- One core should be placed in RNAlater
- Two cores should be placed in sterile DMEM

The order of specimen collection should be:
- First core: 10% neutral buffered formalin
- Second core: Sterile DMEM
- Third core: RNAlater
- Fourth core: Sterile DMEM
- Fifth core: 10% neutral buffered formalin

If additional cores are obtained, they should be processed as follows:
- Sixth core: RNAlater
- Seventh core: 10% neutral buffered formalin

If a skin punch is performed, the goal of tissue collection is 2, 5mm punches. The collection order should be as follows:

- First punch: 10% neutral buffered formalin
- Second punch: RNAlater

Guidelines for biopsy from various metastatic sites can be found in Appendix B.

9.2.3 Handling and Shipping

After being obtained, processing of the cores is as follows:

- All samples should be de-identified and labeled with the Participant initials, Participant Study ID number and date of procedure.
- One core in sterile DMEM should be brought as fresh tissue immediately to the lab of Mariano Severgnini at:

  Center for Immuno-Oncology
  Dana-Farber Cancer Institute
  1 Jimmy Fund Way, JF0406
  Boston, MA 02215
  Phone: (617) 632-2421
  Pager: 42093

  This core must arrive to the lab to be processed for TILs (as described below) within 90 minutes of collection, though an additional 2-hour window is permitted.. In addition, a small piece of this core will be immediately frozen in liquid nitrogen upon arrival to Mariano Severgnini, for later use for RNA sequencing.

- Two cores in formalin should be brought to the Brigham and Women's SHL lab (with appropriate work order submitted and printed) on the 6th floor of the Thorn building, where a block will be made. 5 positively charged, 75mm x 25mm x 1mm slides should be cut from the block to be sent to QualTek for PD-L1 IHC assay. Shipping details will be provided in a lab manual. The block should be brought to Dr. Scott Rodig on the 6th floor of the Thorn building at Brigham and Women's.

  Specialized Histopathology Services – Longwood Core
  Brigham and Women's Hospital, Thorn 604/603B
  75 Francis St.
  Boston MA 02115

- One core in RNAlater should be brought to the DF/HCC Clinical Trial Core Laboratory (Deborah Dillon, MD) on Smith 9. Please email the DF/HCC Clinical Trials Core Laboratory (dfcibreastbank@partners.org) with patient name, study ID, date of collection, approximate time of collection, and study time point the day prior to collection.

Tissue remaining after specific protocol testing described below will be banked in the Clinical Trial Core Laboratory (Deborah Dillon, MD) and may be used for additional or future analyses as

needed.

9.2.4 Potential Testing

- Assay 1: Tumor infiltrating lymphocyte (TIL) percentage and determination of lymphocyte predominant breast cancer (LPBC)

  Paraffinized, hematoxylin and eosin-stained slides taken from two tissue planes will be derived from each biopsy and will be reviewed by certified pathologists. In the research setting, all cases are reviewed by two pathologists and any discordant results resolved by consensus review. The extent of lymphocytic infiltrate in tumor tissue will be assessed, and stromal TIL percentage will be determined. More detailed guidelines for the quantification of stromal TILs in breast cancer can be found in the recommendations from the International TILs Working Group 2014.[Salgado *et al.*, 2015]

  After assessment of the TIL percentage, the pathologists will categorize the specimen as lymphocyte predominant breast cancer (LPBC), defined as a tumor that contains >60% stromal lymphocytes, or non-LPBC.

- Assay 2: Immunohistochemistry

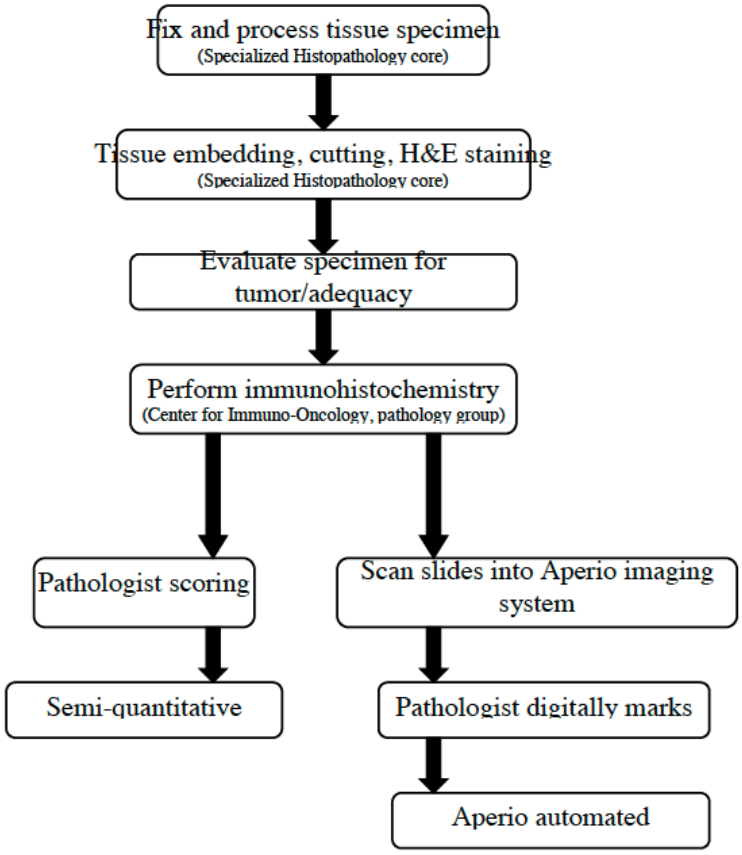
  Tissue will be collected and fixed by 10% neutral buffered formalin overnight, dehydrated, and paraffin embedded. Four micrometer-thick sections will be cut. The paraffin blocks and unstained slides will be stored at room temperature. All immunohistochemical staining will be performed in the Center for Immuno-Oncology Pathology Core at Dana-Farber/Harvard Cancer Center (DF/HCC) Specialized Histopathology Core.

  Formalin fixed-paraffin embedded (FFPE) tumor slides will be prepared and H&E stained for assessment of TIL in pre- and post-treatment tumor samples. To identify subsets of different immune populations (effector/memory CD8 cells, T regulatory cells, dendritic cells, tumor associated macrophages, and Tie-2 expressing monocytes (TEM)), immunohistochemical (IHC) staining will be performed on FFPE tumor slices using some or all of the following antibodies:
  Core set: CD8, PD-1, PD-L1, PD-L2
  Others: CD3, CD4, CD25, FoxP3, Indoleamine 2,3 deoxygenase-1 (IDO1), CD11c, CD83, CD86, CD56, CD14, CD16, Tie2.

  Investigators at our institution have developed IHC staining on paraffin embedded tissues for PD-L1, PD-L2, TIM-3, and LAG-3 through our center for Immuno-Oncology Pathology Core (Scott Rodig MD, PhD Core Director, is a co-investigator on this protocol). PD-L1 IHC has recently been established in a CLIA approved laboratory and the remaining assays for CLIA laboratory conduct are being finalized.

These investigators have published the methods, protocols, and data establishing the sensitivity and specificity of IHC staining assays using the monoclonal antibodies recognizing PD-L1 (CD274, B7-H1, antibody clone 7G11, generated in the lab of Gordon Freeman, DFCI) and PD-L2 (CD273, B7-DC, clone 9E5, generated in the laboratory of Gordon Freeman, DFCI in two recent manuscripts.[Chen *et al.*, 2013, Shi *et al.*, 2014]

Below is a schematic of the workflow for the tissue-based biomarker analysis.



Tumor will be considered positive if >5% (PD-L1)[Topalian *et al.*, 2012] or >10% (PD-L2) of the tumor cell population demonstrates unequivocal staining. PD-1 positivity will be defined as >3% positive cells/high power field.[Bachireddy *et al.*, 2014] All IHC stained slides will be evaluated and scored by a pathologist. A subset of slides will be reviewed by a second pathologist to ensure concordance of interpretation.

The semi-quantitative scoring for this study is in accordance with those published previously and, as described above, will include scores for both the neoplastic and non-neoplastic cells within the tumor microenvironment. Data derived from pathologist visual scoring (semi-quantitative, but with increased specificity for

delineating neoplastic and non-neoplastic cells) and pathologist-assisted, automated scoring (quantitative, but without accurately delineating neoplastic and non-neoplastic cells) for each marker of interest will be assessed for its clinical value. As necessary, the data from combinations of markers and multiplex immunofluorescent assays will also be considered (i.e. combined scores from PD-L1 and PD-L2 expression). All data will be analyzed in conjunction with the biostatistics group.

Further details of the immunohistochemical assay and assessment are described in the lab manual.

- Assay 3: Flow cytometry

  TILs will be isolated from the biopsy specimen as described in the lab manual

  Surface staining followed by flow cytometry on the resultant TILs will then be performed as described in the lab manual. The following antibodies may be used on all specimens: (core set)
  CD8
  PD-1
  PD-L1
  PD-L2

  A selection of the following antibodies may also be used, and additional antibodies may be used as well, as deemed appropriate and informative based on the state of the immune profiling literature at the time of correlative science performance:
  CD4
  FOXP3
  CD127

- Assay 4: RNA analysis

  RNA analysis may be performed, and tissue for RNA analysis will be stored, in the Clinical Trials Core Laboratory (Deborah Dillon, MD).

  Messenger RNA (mRNA) expression within tumor biopsy specimens may be assessed using the most current and informative methodologies at the time that correlative science is performed on all specimens. NanoString signatures and comprehensive RNA sequencing may be used. Potential genes of interest, based on prior immune profiling of breast tumors,[Denkert *et al.*, 2015] include CXCL9, CCL5, CD8ACD80, CXCL13, IGKC, CD21, IDO1, PD-1, PD-L1, PD-L2, CTLA4, and FOXP3.

9.2.5 Sites Performing Correlatives

BWH
DFCI CIO

9.3 Blood Collection

Research blood collection is mandatory for all patients for flow cytometry and potential DNA isolation. The samples will be banked in the DFCI breast tissue repository for these and future research purposes. These specimens will become the property of the DF/HCC.

Blood draws should not be performed on Friday afternoons, as there may not be time for processing of the blood. If a blood draw must be performed on Friday morning, the lab of Mariano Severgnini must be notified ahead of time to ensure that there will be adequate time for processing the blood, since it cannot be stored over the weekend.

The following research blood samples are required:

Cycle 1 Day 1:

- 1-9mL Streck Tube for whole blood (cfDNA)
- 5- 10mL green top tubes for whole blood (Immune Markers)

Every Cycle Day 1:

- 5- 10mL green top tubes for whole blood (Immune Markers)

Restaging Visits:

- 1-9 mL Streck Tube for whole blood (cfDNA)

Off Treatment (if for progressive disease):

- 1-9 mL Streck Tube for whole blood (cfDNA)
- 5- 10mL green top tubes for whole blood (Immune Markers)

The following Time of Progression research blood samples are optional for patients who came off treatment for a reason other than progressive disease:

- 1-9 mL Streck Tube for whole blood (cfDNA)
- 5- 10mL green top tubes for whole blood (Immune Markers)

If green top tubes for whole blood (Immune Markers) are unavailable, purple or CPT tubes are can be substituted.

9.3.1 Immune Markers

9.3.1.1 Handling and Shipping

All samples should be de-identified and labeled with the Participant initials, Participant Study ID

number and date of collection and time point (e.g., "Baseline" or "Cycle 1" or "Progressive Disease").

- Green Top tubes:

  Must be processed within 3-4hrs of its being drawn at ambient temperature immediately after being drawn to Mariano Severgnini at:

  Center for Immuno-Oncology
  Dana-Farber Cancer Institute
  1 Jimmy Fund Way, JF0406
  Boston, MA 02215
  Phone: (617) 632-2421
  Pager: 42093

9.3.1.2 Potential Testing

- Assay 1: Flow cytometry

PBMCs will be generated as described in the lab manual, and used to assess immune cell populations.

Surface staining with a panel of antibodies and flow cytometry on PBMCs will then be performed as described in Appendices. The following antibodies will be used on all specimens: (core set) CD8, PD-1, PD-L1, PD-L2,
A selection of the following antibodies may also be used, and additional antibodies may be used as well, as deemed appropriate and informative based on the state of the immune profiling literature at the time of correlative science performance: CD4, FOXP3, CD127

9.3.1.3 Sites Performing Correlatives

DFCI CIO

9.3.2 **Cell-free DNA (cfDNA) analysis**

Blood will be collected at baseline, restaging visits and at time of progression for evaluation of cell-free DNA (cfDNA). The cfDNA will be banked in the DF/HCC Clinical Trials Core laboratory for future research purposes. The banked samples will be used to analyze DNA, RNA and protein in future studies.

9.3.2.1 Collection of cfDNA specimen(s)

One 10 ml of whole blood will be collected in Streck Tubes. The blood sample will be collected and processed at baseline, restaging visits and time of progression for evaluation of cfDNA. Fill the Streck tube completely and immediately mix by gentle inversion 8 to 10 times. Inadequate or delayed mixing may result in accurate results. The banked samples will be used to analyze DNA, RNA and protein in future studies.

9.3.2.2 Handling and shipping of cfDNA specimens

One 10 ml Streck tube will be collected and processed at baseline, restaging visits and at time of progression for evaluation of cfDNA. Fill the Streck tube completely and immediately mix by gentle inversion 8 to 10 times. Inadequate or delayed mixing may result in accurate results. Deliver to Clinical Trials Core Lab.

Dana-Farber Cancer Institute
Attn: Lynda Chichester
Smith 9th Floor, Rm 948
450 Brookline Avenue
Boston, MA 02215
dfcibreastbank@partners.org

Email the blood bank (dfcibreastbank@partners.org) and the current Dana-Farber CRC with the sample information and tracking information the day before shipping specimens.

Tube precautions:

- If samples cannot be shipped within 24 hours of collection, contact DFCI. DO NOT FREEZE OR REFRIDGERATE TUBES as this could result in cfDNA breakage. Blood collected in the Streck tube can be stored for 14 days between 6-37 degrees Celsius.
- Do not use tubes after expiration date.
- Fill the tube completely; overfilling or underfilling of tubes will result in an incorrect blood-to-additive ratio and may lead to incorrect analytical results.

Shipping Note: Streck tube samples are sent ambient. Frozen and ambient specimens obtained and shipped on the same day to the DFCI blood bank (e.g., Progression or Off Study Biopsy Specimens, Streck Tubes, and Circulating Tumor Cells) may be placed in a combination shipping box which contains separate compartments for frozen and ambient samples. If a combination shipping box is not available, two shipping boxes should be used.

9.3.2.3 Sites Performing Correlatives

DFCI Clinical Trials Core Lab

9.4 Stool Collection

9.4.1 Handling and shipping

All stool samples will be collected by each patient at home using a home-based kit with a pre-paid mailer that provides nearly equivalent metagenomic and metatranscriptomic data to state-of-the-art fresh-frozen sample-collection protocol. Patients will be asked to provide samples at the following timepoints:

- Baseline
- After two cycles of therapy
- At the time of disease progression
- Optional collection at the time of grade ≥ 2 diarrhea

Most kits will be provided to the patients at their clinic visits. If the study team is unable to provide the kits to the patients in clinic, they may be mailed to patients by members of the study team. All kits will contain a questionnaire for patients to complete and return with their samples regarding timing and conditions surrounding their stool sample.

Please refer to the separate lab manual for collection and processing instructions.

Samples will be stored at the BWH/Harvard Cohorts Repository and will be shipped in batches to an external lab vendor, Microbiome Dx, who will perform the analysis of the samples.

9.4.2 Analysis of DNA extraction from stool samples

Microbial DNA is extracted using the Mag-Bind Universal Pathogen DNA Kit (Omega Bio-Tek). Briefly, 250 mg of the specimen is transferred to a deep-well plate for bead beating followed by DNA precipitation and purification following the manufacturer's instructions. Finally, DNA is eluted in 100 uls of Elution Buffer and stored at -80°C until further use. 16S sequencing libraries are generated by amplifying the v3-v4 hypervariable regions of the 16S gene in a polymerase chain reaction using primers F341and R785. Resulting amplicons are tagged with unique molecular barcodes that are later used to demultiplex sequencing reads into individual sample buckets. Libraries are loaded on a MiSeq flowcell and sequenced following Illumina's loading instructions. Sequence data are retrieved from the instrument by converting base call format files into fastq files for data processing purposes.

MicrobiomeDX uses BacPro™, a proprietary algorithm, to inspect and validate sequencing files by employing demultiplexing, trimming, merging, and quality filtering steps. Paired sequencing reads are merged using an overlap of 25 bp allowing for 10 base mismatches. Merged sequences are dereplicated and clustered in a de-novo fashion using VSEARCH, while filtering out sequence chimeras and singletons. Representative sequences from each cluster are mapped against the SILVA database at 99% sequence identity to obtain accurate taxonomic classifications and relative abundances. In parallel, feature tables are constructed to derive alpha diversity indices, and distance matrices are built to derive beta diversity indices. The BacPro™ pipeline generates a comprehensive report that includes alpha diversity scores describing community richness and evenness, taxonomic composition with relative abundances, and beta diversity metrics to determine the in-between sample differences based on the bacterial communities identified.

9.4.3 Shotgun sequencing and metabolic pathway reconstruction of stool samples

Stool samples from patients included in the trial 2 will be subjected to whole genome shotgun sequencing. Libraries will be constructed with Illumina barcodes from the TruSeq DNA Sample Prep kit (Illumina) and reagents from KAPA Library Preparation kit (Kapa Biosystems), and then sequenced on an Illumina MiSeq platform using 2_250 nucleotide paired-end sequencing, according to the manufacturer's instructions. Sequencing reads will be converted into relative abundances of microbial metabolic modules using HUMAnN35, the Human Microbiome Project metabolic reconstruction pipeline and mapped to the KEGG36. Relative species abundances will be calculated by the MetaPhlAn pipeline37.

9.4.4 Sites performing correlative analysis

- BWH/Harvard Cohorts Biorepository
- Microbiome Dx

9.5 Additional analysis

The above-mentioned analyses may be altered based on novel developments in the field of cancer immune profiling at the time of correlative science. Additional markers or alternative technologies (based on scientific developments and/or novel technologies) may also be used, to explore potential prognostic or predictive candidate markers/panels or markers related to treatment benefit and/or safety, to improve diagnostic tests, or to understand breast cancer biology.

10. STUDY CALENDAR

Screening evaluations are to be conducted within 28 days prior to start of protocol therapy unless otherwise specified. Screening assessments occurring within 3 days prior to initiating study treatment do not need to be repeated on Cycle 1 Day 1.

As detailed in the Study Calendar, laboratory assessments must be documented within 7 days before the first dose of study medication. For women of childbearing potential, as defined in section 5.4.1, a pregnancy test must be completed within 7 days of receiving the first dose of study medication. If a urine pregnancy test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

In the event that the participant's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

Assessments must be performed prior to administration of any study agent. Study assessments and agents should be administered within $\pm$ 3 days of the protocol-specified date, unless otherwise noted.

	Screening[a]	C1 D1	C2 D1	Cycle 3 + D1	Off-Treatment[l]	Follow-Up[p]
Informed consent	X					
Radiotherapy Planning Assessments	X					
Demographics	X					
Medical history	X					
Concurrent medications	X	X	X	X	X	X
Physical exam	X	X	X	X	X	
Radiation Oncology[b]		X				
Performance status	X	X	X	X	X	
Adverse event evaluation		X	X	X	X	
Vital signs[c]	X	X	X	X	X	
Hematology panel[d]	X	X	X	X	X	
Chemistry panel[d]	X	X	X	X	X	
TSH[e]	X	X	X	X		
Coagulation panel (PT/PTT)	X					
Cortisol[m]	X		X	X		
Pregnancy test[f]	X					
Single 12- lead EKG	X					
Tumor Measurements[g]	X			X		X[j]
Archival Tumor[h]	X					
Research Biopsy[i]	X		X			
Research Blood[k]		X	X	X	X	X
Research Stool Collection[n]	X			X	X	
Stool Questionnaire[o]	X			X	X	
Survival						X[p]

Pembrolizumab: 200mg given IV over 30 minutes (-5minutes/+10minutes q3weeks on Day 1 of each cycle

Radiotherapy: Patients will start radiotherapy 2-7 days after their first infusion of pembrolizumab and be delivered over 5 treatments of 4Gy over a period of 1-2 weeks. It should be delivered on 5 consecutive business days but unavoidable delays due to machine maintanence, holidays, etc it is permitted to extend to 10 days.

a. Screening assessments are to be conducted within 28 days prior to start of protocol therapy unless otherwise specified. If these screening assessments occur within 3 days before start of study treatment, then they may serve as the baseline Cycle 1 Day 1 values.
b. Patients will be seen at their final treatment (+/- 4 days) and 10 days after completion of RT (+/- 4 days) by a radiation oncologist in addition to their regularly scheduled medical oncology visits.
c. Vitals to include: diastolic and systolic blood pressure, heart rate, temperature, and weight
d. Labs to include: CBCA + Differential, Chloride, potassium, sodium, BUN, serum creatinine, phosphorus, calcium, albumin, total protein, alkaline phosphatase, ALT, AST, total bilirubin (NOTE: the frequency of checking magnesium levels is left up to the treating provider)
e. TSH will be performed on C1D1, C2D1, C3D1, C4D1 and then every other cycle on Day 1.
f. In female subjects of child-bearing potential as defined in section 5.4, a pregnancy test must be performed within **7 days** of the first dose of study medication. If a urine test is positive or cannot be confirmed as negative, then a serum test is required.
g. Tumor measurements are repeated every 6 weeks for the first 24 weeks and then every 9 weeks thereafter. Documentation (radiologic) must be provided for participants removed from study for progressive disease. Confirmatory scans 4 weeks after documented response should be obtained.
h. Archival tumor sample should be collected (block or if not possible, 10-20 5micron, unstained, positively charged slides).
i. A baseline tumor biopsy obtained within 28 days before starting protocol therapy is required for those with accessible tumor tissue outside the field of radiotherapy. The C tumor biopsy should be performed as close to C2D1 as possible. It would be most preferable to perform the biopsy after the second treatment of pembrolizumab, but it may be performed up to 3 days before and 7 days after C2D1.
j. For those taken off treatment for reasons other than progressive disease, tumor measurements should continue to be repeated every 6-9 weeks and after 1 year of assessments every 12 weeks.
k. cfDNA will be collected at screening, restaging visits and at time of progression

l. Off-Treatment visit is to occur within 30 days of final administration of study treatment. End of treatment assessments do not have to be repeated if the same assessments were performed within 7 days prior to the visit. SAEs and ConMeds need to be recorded for 30 days after the final treatment.
m. Cortisol may be drawn at any time of day, though is most informative in the morning.
n. Baseline stool collection should be obtained within 28 days before starting protocol therapy. The C3D1 stool collection should be performed as close to C3D1 as possible, but may be collected up to 14 days prior. A sample will additionally be collection at the time of disease progression. An optional stool sample may be collected at the time of grade ≥ 2 diarrhea after discussion with the PI. As these collections are for exploratory correlative purposes, failure to provide a sample at these timepoints will not constitute a protocol violation. See section 9 and/or lab manual for stool collection and processing instructions.
o. Each stool collection kit will contain a questionnaire for the patients to complete regarding the conditions surrounding their collection. These will be a part of the kit and are not to be administered in clinic. Failure to complete these questionnaires at the required or optional timepoints will not constitute a protocol violation.
p. Survival status should be collected annually, either by phone or medical record.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [Eisenhauer *et al.*, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

Pembrolizumab, like other immunotherapeutic agents, 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 image responses seen with cytotoxic agents, and can manifest as a clinical response after an initial increase in tumor burden or even the appearance of new lesions.

For any subject who showed first radiologic evidence of progressive disease (PD) by RECIST 1.1 (see Section 11.1) and is deemed clinically stable, it is at the discretion of the investigator to continue treating the subject until progression is confirmed at the next scheduled restaging (or with a confirmatory completed at least 4 weeks from the initial date of PD). If progression is not confirmed on the subsequent scan, the subject should continue to receive treatment and have radiographic scans performed according to the study calendar (every 9 weeks for the first 12 months and then every 12 weeks). If radiologic progression is confirmed, then the subject should be discontinued from all study treatment. If the treating investigator feels that the participant is clinically stable, demonstrates improved condition, or is clearly continuing to benefit from the treatment; the PI may approve the participant to continue to receive study treatment. In all participants, the date of progression will be documented as the first date progression was observed.

11.1.1 Definitions

Evaluable for Target Disease response. Only those participants who have measurable disease outside the field of radiation present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for target disease response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Participants who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

11.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm by chest x-ray or ≥10 mm with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area are not considered measurable.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all considered non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same participant, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should

also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow up.

11.1.3 Methods for Evaluation of Disease

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

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm in diameter as assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung; however, CT is preferable.

Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT thickness is 5mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size of a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

FDG-PET. While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

(a) Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
(b) No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
(c) FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

PET-CT. At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

MIBG (meta-iodobenzylguanidine).The following is recommended, to assure high quality images are obtained.

Patient preparation: Iodides, usually SSKI (saturated solution of potassium iodide), are administered to reduce thyroidal accumulation of free radioiodine, preferably beginning the day prior to injection and continuing for 3 additional days (4 days total). For infants and children, one drop t.i.d. is sufficient, for adolescents 2 drops t.i.d., and for adults 3 drops t.i.d. Participants and/or parents are asked about exposure to potential interfering agents. If none is noted, an indwelling intravenous line is established. The dose of MIBG is administered by slow intravenous injection over 90 seconds.

Images from the head to the distal lower extremities should be obtained.

I-123MIBG scintigraphy is performed to obtain both planar and tomographic images.

Planar: Anterior and posterior views from the top of the head to the proximal lower extremities are obtained for 10 minutes at 24 hours and occasionally at 48 hours following injection of 10 mCi/1.7 square meters of body surface area (~150 μCi/kg, maximum 10 mCi). Anterior views of the distal lower extremities are adequate. A large field of view dual head gamma camera with low energy collimators is preferred.

SPECT: Most participants receiving I-123 MIBG also undergo SPECT at 24 hours, using a single or multi-headed camera with a low energy collimator. The camera is rotated through 360 degrees, 120 projections at 25 seconds per stop. Data are reconstructed using filtered back projections with a Butterworth filter and a cut off frequency of 0.2-0.5. SPECT/CT may be performed at institutions with this capacity.

Ultrasound. Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later data and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure from CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy. The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a participant to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (*e.g.*, residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

11.1.3.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph

nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

11.1.3.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

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

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

11.1.3.3 Evaluation of New Lesions

The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

11.1.3.4 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Participants with Measurable Disease (*i.e.*, Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	4 wks Confirmation**
CR	Non-CR/Non-PD	No	PR	4 wks Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Participants with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration.*" Every effort should be made to document the objective progression even after discontinuation of treatment.

11.1.4 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started, or death due to any cause. Participants without events reported are censored at the last disease evaluation).

Duration of overall complete response: The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented, or death due to any cause. Participants without events reported are censored at the last disease evaluation.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

11.1.5 Clinical Benefit rate

Clinical benefit rate (CBR): defined as CR, PR and stable disease (SD) ≥ 24 weeks.

11.2 Antitumor Effect – Hematologic Tumors

N/A

11.3 Other Response Parameters

11.3.1 Definition of Tumor Response Using Immune-Related Response Criteria (irRC)

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

11.3.1.1 Impact of New Lesions on irRC

New lesions in and of 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 irRC criteria for tumor response. Therefore, new non-measurable lesions will not discontinue any subject from the study.

11.3.1.2 Definition of Target Lesions Response Using irRC

- **irComplete Response (irCR):** Complete disappearance of all target lesions. This category encompasses exactly the same subjects as "CR" by the mWHO criteria.
- **irPartial Response (irPR):** Decrease, relative to baseline, or 50% or greater in the sum of the products of the two largest perpendicular diameters of all target and all new measurable target 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 SBD 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 SPD of all target lesions and any new lesions) when compared to SPD at nadir.

11.3.1.3 Definition of Non-Target Lesions Response Using irRC

- **irComplete Response (irCR):** Complete disappearance of all non-target lesions. This category encompasses exactly the same subjects as "CR" by the mWHO criteria.
- **irPartial Response (irPR) or irStable Disease (irSD):** Non-target 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-target 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 target lesions increases by the required amount).

11.3.1.4 Definition of Overall Response Using irRC

Overall response using irRC will be based on these criteria:

- **Immune-Related Complete Response (irCR):** Complete disappearance of all tumor lesions (target an non-target) 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 target lesions is measured and captured as the SPD baseline. At each subsequent tumor assessment, the SPD of the two largest perpendicular diameters of all target 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 previously SPD baseline of 50% or greater is considered an 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 PD:
  - At least 25% increase in the SPD of all target lesions over baseline SPD calculated for the target lesions.
  - At least 25% increase in the SPD of all target lesions and new measurable lesions (irSPD) over the baseline SPD calculated for the target lesions.

Criteria for determining overall response by irRC are summarized as follows:

Immune-Related Response Criteria Definitions

Target Lesion Definition	Non-Target Lesion Definition	New Measurable Lesions	New Unmeasurable Lesions	Percent change in tumor burden (including measurable new lesions when present)	Overall irRC Response
Complete Response	Complete Response	No	No	–100%	irCR
Partial Response	Any	Any	Any	≥ –50%	irPR
				<–50% to <+25%	irSD
				>+25%	irPD
Stable Disease	Any	Any	Any	<–50% to <+25%	irSD
				>+25%	irPD
Progressive Disease	Any	Any	Any	≥+25%	irPD

11.3.1.5 Immune-Related Best Overall Response Using irRC (irBOR)

irBOR is the best confirmed 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.

11.3.2 Definition of Abscopal response

As described previously, abscopal response is defined as a decrease in the longest diameter of at least 30% in any measurable (≥1 cm) non-irradiated lesion from baseline[Golden *et al.*, 2015]. In patients with more than three lesions, the non-irradiated lesions will be measured individually for response to treatment. The best abscopal responding lesion will be reported.

11.3.2.1 Definition of overall response according abscopal response definition

- Complete abscopal response is defined as the complete disappearance of a measurable non-irradiated lesion.
- Partial abscopal response is defined as at least a 30% decrease in the longest diameter.

- Progressive disease **is** defined as at least a 20% increase in the longest diameter of the best measurable non-irradiated lesion.
- Stable disease is defined as insufficient shrinkage or growth to qualify for a partial abscopal or complete abscopal response or progressive disease.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Method

The ODQ will collect, manage, and perform quality checks on the data for this study.

12.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the ODQ according to the schedule set by the ODQ.

12.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

The DSMC will review each protocol up to four times a year or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring with 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Multicenter Guidelines

N/A

12.4 Collaborative Agreements Language

N/A

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

This is a single-arm phase 2 study to evaluate efficacy of pembrolizumab in combination with radiation therapy for patients with metastatic HR positive breast cancer.

This study uses a Simon "optimal" two-stage design. 8 patients will be enrolled in the first stage, and if the study continues to the second stage, another 19 patients would be enrolled. A total of 27 patients will be enrolled in this study.

Primary Endpoint
The primary endpoint of this trial is ORR outside the field of radiation, according to RECIST 1.1 as defined in Section 11.1.

Secondary endpoints include:
Secondary endpoints include ORR according to immune-related response criteria (irRC), ARR according abscopal response definition (as defined in Section 11.3.2), PFS according to RECIST 1.1 and irRC (as defined in Section 11.3), CBR, irCBR, safety and tolerability.

Blood and Tissue correlative science objectives include:
- To characterize a broad array of immune markers in metastatic HR-positive breast tumors (characterization will be based on histology, protein expression, and mRNA expression).
- To explore how different immunosuppressive and/or immune-stimulating immune marker profiles at baseline correlate with disease response to therapy (response assessed by RECIST 1.1 and immune-related response criteria).
- To characterize changes in tumor-infiltrating lymphocytes and PD-L1 expression immune marker profile in tissue microenvironment (TME) from baseline to after 2 cycles of Pembrolizumab.
- To explore whether induction of changes in the immunosuppressive and/or immune-stimulating immune marker profile in TME correlates with disease response to therapy (response assessed by RECIST 1.1, irRC and ARR).
- To characterize serial changes in immune marker profile in peripheral blood mononuclear cells (PBMCs) over the course of the trial treatment.
- To explore whether induction of changes in the immunosuppressive and/or immune-stimulating immune marker profile in PBMCs correlates with disease response to therapy (response assessed by RECIST 1.1, irRC and ARR).
- To investigate whether there is an immune marker in circulating PBMCs that corresponds to tumor infiltrating lymphocyte (TIL) percentage in baseline tumor
- To collect blood to study cell-free DNA for comparison to tumor specimens before and after immunotherapy

Stool and microbiome correlative science endpoints:

Overall, we plan to describe the landscape of gut microbiota in patients with BC who will receive the combination of radiation therapy plus pembrolizumab, and the changes in their gut microbiota after two cycles of pembrolizumab. Statistical analyses of intestinal microbiota samples will be performed using R Statistical Language (v3.1.1) and GraphPad Prism (version 6.0e) software packages. Unpaired Mann–Whitney rank sum test (two-tailed) will be used for comparisons of continuous variables between two groups. Bar plots will be used to represent the data's mean at the center values, with error bars to indicate standard deviation. In order to explore the association of response (objective response according RECIST 1.1 and progression-free survival) to baseline microbiota diversity, and changes from baseline in microbiota, inference will be based on Wilcoxon rank sum tests and estimates of predictive value along the continuous scales will be visualized using receiver operating characteristic (ROC) curves and reported with c-index and confidence intervals derived from variance estimates of Somers rank correlation. Unadjusted P-values will be considered significant for the Mann–Whitney rank sum test.

We will quantify microbiome features from amplicon, metagenome, metatranscriptome using established pipelines to identify strain-level taxonomic, functional gene, transcriptional, and microbially-mediated metabolite profiles associated with BC patients with and without immunotherapy70[-76]. We will use modified multivariate linear modeling to identify statistically significant features associated with outcomes. Statistical tests for association with these outcomes and covariates will be performed using the sparse generalized linear model MaAsLin, which provides random effects models for both log-Gaussian and zero-inflated negative binomial link functions. Computational workflows for these steps are implemented as AnADAMA2 (http://huttenhower.sph.harvard.edu/anadama) workflows, a reproducible data handling environment that captures all provenance during the analysis process.

Tumor Genomic Profile correlative science endpoints:

All analyses of Oncopanel in correlation with patient outcomes are exploratory and hypothesis-generating. Any promising findings will be explored in future studies.

13.2 Sample Size, Accrual Rate and Study Duration

Based on data of recently presented Javelin (cohort of metastatic breast cancer) and Keynote-028 trials[Dirix *et al.*, 2015, Hugo *et al.*, 2015], and considering that our population will not be previously selected by PD-L1 expression status, a true ORR outside the field of radiation of 3% or less would not be of clinical interest, and is the null hypothesis to the Simon optimal two-stage design. A true rate of 20% would be considered a clinically meaningful level of response, so the sample size was chosen to have higher power (80%) to declare the combination effective at this rate, while controlling the one-sided Type I error at no more than 5% under the null.

Using the Simons "optimal" method, in the first stage, 8 patients will be enrolled. If there is at least 1 response, accrual will continue to the second stage where up to 19 additional patients will be enrolled. If at least 3 of these 27 patients have an objective response (≥10%), the regimen will be considered worthy of further study. With this design, the probability of stopping the trial early

is 78% if the true response rate is 3%. If the true response rate is 20% the chance that the regimen is declared worthy of further study is 80%.

The expected accrual rate is 0-1 patients per month, and the accrual is expected to complete within approximately 54 months.

13.3 Stratification Factors

NA

13.4 Interim Monitoring Plan

An interim analysis will happen after 8 patients enrolled in the first stage. If there is at least 1 response, accrual will continue to the second stage where up to 19 additional patients will be enrolled.

13.5 Analysis of Primary Endpoint

The primary endpoint is objective response outside the field of radiation, which will be assessed among all patients who initiated protocol therapy. Radiographic response will be assessed using RECIST 1.1 criteria as defined in section 11.1. Objective response will require confirmatory scans as indicated. The ORR (CR + PR) will be reported with 90% exact confidence intervals.

13.6 Analysis of Secondary Endpoints

Efficacy Endpoints

All patients who initiated protocol therapy will also be evaluated for ORR outside the field of radiation according irRC, and for CBR and PFS, according RECIST and irRC. In addition, these patients will be evaluated for ARR according abscopal response definition (Section 11.3.2). Clinical benefit is defined as CR, PR or SD $\geq$ 24 weeks according RECIST 1.1. Immune-related clinical benefit is defined as CR, PR or SD $\geq$ 24 weeks according irRC. ORR according to irRC and ARR will be reported with 90% exact confidence intervals. CBR will be reported respectively with 95% exact confidence intervals. The radiation plan will be reviewed and the treated volume (clinical target volume, planning target volume, area receiving 100% and 95% of the planned dose), and the site of treatment will be also evaluated; we will explore if there is any correlation between these parameters and the likelihood of clinical benefit. PFS, irPFS, and OS will be analyzed using Kaplan–Meier product-limit estimates and will be plotted using Kaplan-Meier plots. PFS is defined as the time from study randomization to disease progression according RECIST 1.1, medical judgment or death due to any cause, whichever occurred first. Immune-related PFS is defined as the time from study randomization to disease progression according irRC, medical judgment or death due to any cause, whichever occurred first. Patients alive without disease progression are censored at the date of last disease evaluation. The hazard ratio for each time-to-event endpoint will be estimated with 95% confidence intervals derived from the Cox proportional hazard model, but no hypothesis testing will be conducted.

Safety and tolerability

All patients will be evaluable for toxicity from the time of their first treatment with any study agent. Toxicity will be graded according to NCI CTCAE, Version 4.0. Toxicities will be summarized by maximum grade and by treatment arm. Incidence rate of each toxicity will be reported with 95% exact CI. The incidence rates of any grade 3+ toxicity will be compared between two arms using Fisher's exact test.

Correlative endpoints

- We will describe the presence and abundance of multiple immune markers in metastatic HR-positive breast tumors (characterization will be based on histology, protein expression, and mRNA expression) using frequency tables and descriptive statistics (mean, standard deviation, median , and inter-quartile range).
- We will explore the correlation of immunosuppressive and/or immune-stimulating immune marker profiles at baseline to disease response to therapy (response assessed by RECIST 1.1 and immune-related response criteria) using odds ratios and 95% confidence intervals.
- PD-L1 positivity seen at baseline and C3D1 samples will be summarized using contingency tables. An exploratory analysis is planned to evaluate PD-L1 change in continuous scale.
- We will explore the correlation of immunosuppressive and/or immune-stimulating immune marker profiles in TME to disease response to therapy (response assessed by RECIST 1.1, irRC and ARR) using odds ratios and 95% confidence intervals. Descriptive statistics will be used to characterize serial changes in immune marker profile in peripheral blood mononuclear cells (PBMCs) over the course of the trial treatment
- We will explore the correlation of immunosuppressive and/or immune-stimulating immune marker profiles in PBMCs to disease response to therapy (response assessed by RECIST 1.1, irRC and ARR) using odds ratios and 95% confidence intervals.
- We will explore whether there is an immune marker in circulating PBMCs that corresponds to tumor infiltrating lymphocyte (TIL) percentage in baseline tumor. T-test, ANOVA, or regression will be used, depending on whether immune markers are characterized as categorical or continuous variables.
- Descriptive statistics will be used to characterize cell-free DNA before and after immunotherapy.
- Descriptive statistics will be used to characterize tumor genomic profile by disease response to therapy (response assessed by RECIST 1.1, irRC, and ARR). PFS and OS will be estimated using Kaplan-Meier methods among patients with and without certain gene mutations, respectively.

Previous studies demonstrated that, in addition to its direct cytoreductive effect, RT-induced cell death can be immunogenic, facilitating the recruitment and activation of antigen presenting cells (APCs) and priming of tumor antigen-specific T-cells[Shahabi *et al.*, 2015]. Recently, different groups demonstrated that RT to the tumor bed led to upregulation of PD-L1 on tumor cells, dendritic cells, and on myeloid-derived suppressive cells (MDSCs), which may contribute to impairment of T-cell function in the tumor[Liang *et al.*, 2013, Deng *et al.*, 2014, Sharabi *et al.*, 2014]. Furthermore, these groups also demonstrated that the combination of RT plus blockade of the PD-1/PD-L1 axis improved outcomes in different preclinical models compared with RT or anti-PD1/PD-L1 alone, including breast cancer models.

Recently, Herbst et al have demonstrated that patients who presented an increase of at least 5% in expression of PD-L1 in tumor microenvironment experienced a bigger likelihood to respond to treatment with the anti-PD-L1 Atezolizumab[Herbst *et al.*, 2014]. Also, modifications in molecular signature of tumor microenvironment also correlated with response rate to this drug. Because of this rationale, we plan to perform two research biopsies in tumor lesion outside the field of radiation: one at baseline and the other one just before the begging of cycle 3 of pembrolizumab.

We anticipate that 25 (93%) pairs of tumor specimens will be evaluable for PD-L1 expression. PD-L1 positivity is defined as ≥5%. Assuming 2% of patients unexpectedly show PD-L1 positivity only in the baseline assessment, we have 80% power to detect 30% increases of PD-L1 positivity rate (e.g. 20% at baseline vs. 50% at C3D1). The power calculation is based on McNemar's test with 1-sided alpha of 0.05.

13.7 Reporting and Exclusions

13.7.1 Evaluation of Efficacy

For this Phase II trial, the efficacy evaluable population is a modified intent-to-treat (ITT) population. The modified ITT population consists of all patients who initiate protocol therapy, even if there are major protocol therapy deviations.

Subanalyses may then be performed on the basis of a subset of participants, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding participants from the analysis should be clearly reported. If applicable to the endpoint, the 95% confidence intervals should also be provided.

13.7.2 Evaluation of Safety

The safety population will be used in the safety data summaries. The safety population consists of all patients who took at least one dose of any randomized treatment and who have at least one post-baseline safety assessment. Note that a patient who had no adverse events constitutes a safety assessment. Patients who have received at least one dose of study drug but have no post-treatment safety data of any kind would be excluded.

14. PUBLICATION PLAN

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

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APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B GUIDELINES FOR COLLECTING RESEARCH BIOPSY TISSUE

Tissue specimens will be collected from metastatic lesions using standard institutional procedures. The amount of tissue collected may follow the guidelines listed below:

Skin/chest wall: A goal of 2 4-mm punch biopsies will be obtained.

Lymph node: A goal of 5-7 core biopsy specimens will be obtained using an 18-gauge needle.

Liver: A goal of 5-7 core biopsy specimens will be obtained using an 18-gauge needle.

Lung: Because of the risk of pneumothorax associated with core needle biopsies of lung nodules, no core biopsies of lung nodules are mandated on this protocol, unless they are clinically indicated.

Bone: Because the yield of malignant tissue from bone biopsies tends to be relatively low, if a patient has another accessible site of disease (i.e. skin, lymph node, liver), that site should be biopsied preferentially. If bone is the only biopsy-accessible site, then a goal of 5-7 core biopsy specimens will be obtained using an 11-13 gauge needle.

Please note that the above are guidelines for the amount of tissue to be obtained, and are not meant to replace clinical judgment at the time the procedure is performed. Less than the goal quantity of tissue is accepted for each type of biopsy, and will be left to the clinical judgment of the physician performing the procedure.

If a patient is undergoing resection of a lesion for clinical reasons (i.e. wedge resection of a new lung lesion for confirmation of diagnosis or re-testing of hormone receptor or HER2 status; or, resection of a chest wall lesion; or, resection of a lymph node), then the patient may opt to have a portion of that tissue (roughly equivalent to the goal amount of tissue listed in the guidelines above, i.e. the equivalent of two 5-mm punch biopsies of the skin, or 3-6 18-gauge core biopsies) stored for research at the time of the procedure (provided that the tissue is processed as specified), in which case, the patient would not be required to undergo a separate research biopsy at baseline on this protocol.

Coded laboratory specimens will be stored in the Tumor Bank of the DFCI. These specimens will become the property of DFCI. Patients will be informed that their specimens may be used for research by investigators at DF/HCC and other approved collaborators. Shared specimens will be identified with a sample ID number; all patient identifying material will be removed.

Risks of Research Biopsy and Procedures for Minimizing Risk

Potential risks according to site are:

Skin/chest wall (punch biopsy):

- Likely: local discomfort and minor bleeding
- Less likely: moderate or major bleeding, or infection

Lymph node, liver, or bone (core needle biopsy):

- Likely: local discomfort and minor bleeding
- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due

to bleeding or other complications, infection, damage to adjacent organs. Additional risks may be present if intravenous conscious sedation is required

Breast (core biopsy):

- Likely: local discomfort and minor bleeding.
- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due

to bleeding or other complications, infection, pneumothorax, damage to adjacent organs.

Pleural fluid (thoracentesis):

- Likely: local discomfort and minor bleeding
- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due to bleeding or other complications, infection, pneumothorax, damage to adjacent organs

Ascites fluid (paracentesis):

- Likely: local discomfort and minor bleeding
- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due to bleeding or other complications, infection, bowel perforation or damage to adjacent organs. In order to minimize the risk of a biopsy, only qualified personnel will perform these procedures.

Prior to the procedure, the physician performing the procedure will discuss the risks with each study participant, answer any questions, and obtain separate procedure consent. Patients will be evaluated for comorbidities or concomitant medications that may increase the risk of potential complications. For biopsies of lesions that are not superficial and clearly palpable, imaging studies such as CT or ultrasound will be used to guide the biopsy in order to minimize the risk of damage to adjacent structures. After lymph node biopsies, patients will be observed a minimum of 2 hours (range 2-4 hours) after the procedure, or according to standard institutional guidelines. After liver biopsies, patients will be observed a minimum of 4 hours (range 4-6 hours) after the procedure, or according to standard institutional guidelines. Less than the goal quantity of tissue is accepted for each type of biopsy, and will be left to the clinical judgment of the physician performing the procedure.

Risks of Anesthesia

Local Anesthesia

All biopsy procedures require local anesthesia using lidocaine, xylocaine, or related compounds. There is a small risk of an allergic reaction associated with these drugs.
In order to minimize the risk of local anesthesia, only qualified personnel will perform

the biopsy procedure. Patients will be queried if they have had previous allergic reactions to local anesthetics.

Intravenous Conscious Sedation

Certain biopsy procedures, such as lymph node, liver, or bone biopsies, may require intravenous conscious sedation (IVCS). IVCS is a minimally depressed level of consciousness that retains the patient's ability to maintain a patent airway independently and continuously and respond appropriately to physical stimulation and verbal commands.

The risks of intravenous conscious sedation include: inhibition of the gag reflex and concomitant risk of aspiration, cardiopulmonary complications (myocardial infarction, cardiac arrhythmias, hypoxemia), and allergic reactions to the sedative or analgesic medications. These risks are small but real; for example, in a prospective study of 14,149 patients undergoing IVCS during upper gastrointestinal endoscopies, the rate of immediate cardiopulmonary events was 2 in 1000.[Quine *et al.*, 1995] The 30-day mortality was 1 per 2,000 cases. In this study, there was a strong association between lack of monitoring and use of high-dose benzodiazepines with adverse outcomes. There was also an association between the use of local anesthetic sprays to the oropharynx and the development of pneumonia. In order to minimize the risk of intravenous conscious sedation, only qualified personnel will be responsible for conscious sedation. A minimum of two individuals will be involved in the care of patients undergoing conscious sedation—the physician performing the biopsy procedure, and the individual (M.D. or R.N.) who monitors the patients and his/her response to both the sedation and the procedure, and who is capable of assisting with any supportive or resuscitative measures. The room where the procedure utilizing IVCS takes place will have adequate equipment to provide supplemental oxygen, monitor vital signs, and maintain an airway should this be necessary. An emergency cart will also be immediately accessible to the room where the procedure is to take place, and emergency support services will be available on page. Patients will be screened and evaluated for their fitness to undergo conscious sedation by a trained physician. Patients with active cardiac disease are excluded from this study. No local anesthetic spray to the oropharynx will be necessary, given that endoscopy is not a planned procedure. Following the procedure, patients will be observed closely in the recovery room for a minimum of 2 hours.

General Anesthesia

Because of the higher risk of general anesthesia compared with local anesthesia or intravenous conscious sedation, biopsies that would require general anesthesia in order to be performed *are not permitted* on this protocol, unless they are being done for clinical reasons, and excess tissue that otherwise would have been discarded is then banked for the purpose of this protocol.

For Biopsies of Soft Tissue, Liver, Bone, Breast, Etc:

1. After biopsy is performed, the tissue mass is placed on a sterile gauze
2. Using forceps, separate the tumor tissue
3. Place 2 pieces (cores) of tumor tissue in each cassette (typically end up with 3 cassettes per biopsy); the last cassette will contain many small pieces of tumor tissue
4. Fill cassettes with OCT
    a. Completely cover tissue
    b. Limit the amount of bubbles
5. Place cassettes on dry ice and prepare for transport by limiting OCT leakage
6. Return samples to the lab and complete freezing of samples in OCT with dry ice (about 10 minutes freezing time)
7. Once samples are frozen, place in plastic bag; label bag with date, protocol number, patient number, and number of initials included
8. Store in –80C freezer

For Effusions and Ascites

1. Fluid sample should be split into two equal aliquots
2. One aliquot should be spun down into a pellet and snap frozen in an ETOH/dry ice bath or in liquid N2
3. One aliquot should be fixed and processed as a standard cell block.

Note: if the sample preparation is done by a clinical cytopathology laboratory, it is important to explain that the sample is for research purposes only and that no thin prep should be performed as this uses up a significant portion of the sample.

For Fine Needle Aspiration Samples

A goal of 3 passes:

1. One pass should be evacuated and rinsed directly into 2mL of room temperature Trizol for RNA analysis.
2. One pass should be evacuated and rinsed directly into 2mL of room temperature Trizol for DNA analysis.
3. One pass should be evacuated and rinsed directly into 10-20mL of RPMI to prepare a cell block.