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TITLE: A pilot study of changes in PD-L1 expression during preoperative treatment with nab-paclitaxel and pembrolizumab in hormone receptor-positive breast cancer

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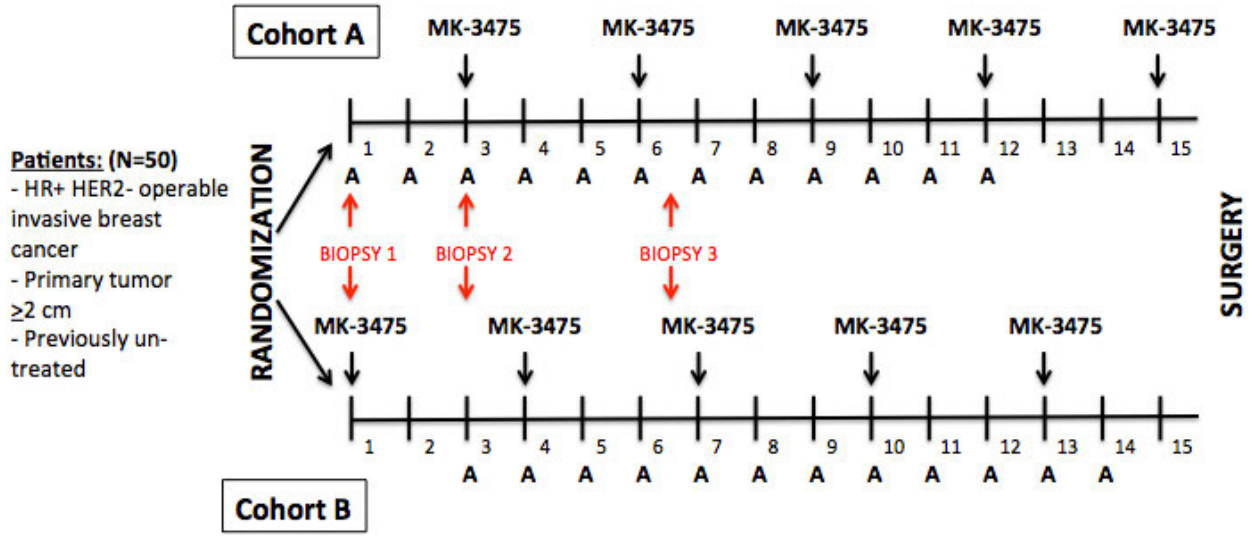
SCHEMA



A: nab-paclitaxel; HR: hormone receptor; MK-3475: pembrolizumab

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1. OBJECTIVES

1.1 Study Design

This is a randomized, open label study assessing changes in immune biomarkers during preoperative treatment with nab-paclitaxel in combination with pembrolizumab. The population to be studied consists of participants with operable hormone receptor-positive, HER2-negative breast cancer who have not yet received treatment for their breast cancer. Participants will receive an upfront randomized window of therapy with either nab-paclitaxel or pembrolizumab, with pre- and post-window biopsies (biopsy 1 and biopsy 2, respectively), followed by nab-paclitaxel in combination with pembrolizumab for an additional 12-13 weeks, with a third biopsy (biopsy 3) on combination treatment. Participants will undergo surgery after completion of combination therapy, and tissue will also be collected at surgery. Post-operatively, administration of further treatment is per the treating investigator's discretion.

1.2 Primary Objectives

Objective: To characterize changes in tumor cell PD-L1 expression after treatment with either nab-paclitaxel or pembrolizumab monotherapy.
Hypothesis: Nab-paclitaxel and pembrolizumab monotherapy will both increase tumor cell PD-L1 expression.

1.3 Secondary Objectives

Secondary biomarker objectives:

- To characterize changes in stromal tumor infiltrating lymphocytes; after treatment with either nab-paclitaxel or pembrolizumab monotherapy.

Safety objective:

- To evaluate the safety and tolerability of combination nab-paclitaxel and pembrolizumab therapy in patients with hormone receptor-positive breast cancer treated in the neoadjuvant setting.

Efficacy objectives:

- To determine the rate of pCR with combination nab-paclitaxel and pembrolizumab in the neoadjuvant setting
- To determine the overall response rate, based on radiographic assessment, with combination nab-paclitaxel and pembrolizumab in the neoajuvant setting
- To explore disease-free survival with combination nab-paclitaxel and pembrolizumab in the neoadjuvant setting

1.4 Correlative Science Objectives

- To characterize changes in stromal tumor infiltrating lymphocytes after treatment with either nab-paclitaxel or pembrolizumab monotherapy followed by nab-paclitaxel/pembrolizumab combination therapy (at the time of interval biopsy and

definitive breast surgery).
- To explore whether levels of peri-tumoral immune biomarkers ((1) stromal tumor infiltrating lymphocytes; (2) PD-1 expression; (3) PD-L1 expression; (4) PD-L2 expression; (5) CD8 expression) in pre-treatment biopsies correlate with response to treatment as assessed by residual cancer burden (RCB) and pathologic complete response (pCR) at surgery
- To explore whether changes in peri-tumoral immune biomarkers ((1) stromal tumor infiltrating lymphocytes; (2) PD-1 expression; (3) PD-L1 expression; (4) PD-L2 expression; (5) CD8 expression) on serial biopsies and at surgery correlate with response to treatment as assessed by RCB and pCR.
- To characterize an expanded set of immune markers (based on histology, protein expression, and mRNA expression) in hormone receptor-positive breast tumors both pre-treatment and on serial biopsies, and to explore whether any members of this expanded set correlate with response to treatment
- To characterize the immune marker profile in peripheral blood mononuclear cells (PBMCs) both pre-treatment and on serial blood draws
- To explore whether levels of immune markers in the peripheral blood correspond with levels of immune biomarkers in peri-tumoral tissue, both pre-treatment and on therapy
- To explore cell-free DNA (cfDNA) in comparison to tumor specimens before and after immunotherapy
- To characterize the structure and function of the gut microbiome in patients with breast cancer prior to starting this clinical trial.
- To determine whether pre-treatment characteristics of the structure and function of the gut microbiome in patients with breast cancer is associated with response to treatment.
- To characterize changes in the structure and function of the gut microbiome of patients with breast cancer after exposure to Nab-paclitaxel or Pembrolizumab alone (after W1 for both Arms)
- To characterize changes in the structure and function of the gut microbiome of patients with breast cancer after exposure to Nab-paclitaxel and Pembrolizumab together (after W4)
- To determine whether changes in the overall diversity of the gut microbiome, estimated by the Shannon Index, of patients with breast after exposure to Nab-paclitaxel or Pembrolizumab alone (after W1 for both Arms) is associated with response to treatment.
- To determine whether changes in the overall diversity of the gut microbiome, estimated by the Shannon Index, of patients with breast after exposure after exposure to Nab-paclitaxel and Pembrolizumab together (after W4).
- To determine if the abundance and functional profile of specific gut bacteria are associated with response to treatment.
- To evaluate the functional pathways that may play a role as a predictive biomarker of response to treatment.

2. BACKGROUND

2.1 Neoadjuvant therapy for hormone receptor-positive breast cancer

Hormone receptor positive breast cancer (defined as estrogen receptor and/or progesterone receptor expressing tumors) accounts for 60-70% of breast cancer and approximately 140,000

patients are diagnosed with this type of breast cancer in the United States each year, the majority of whom have operable disease.

Preoperative (neoadjuvant) chemotherapy is increasingly being used in the management of operable breast cancer (BC). Randomized trials comparing preoperative and postoperative chemotherapy show similar rates of disease-free and overall survival.[1] However, the preoperative setting offers the advantage of disease downstaging and possibly reducing the extent of surgery. Furthermore, the response to the primary treatment may be used as a prognostic marker as better tumor response at surgery has been shown to be associated with longer disease-free survival.[1] Additionally, the preoperative treatment setting allows access to tissue, providing an optimal setting to identify potential mechanisms of action and biomarkers of response to therapy.

2.2 Pembrolizumab

The PD-1 pathway in cancer

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

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene *Pdcd1*) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors . Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-

hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in participants with melanoma. This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Pembrolizumab

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Keytruda™ (MK-3475; 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. Please refer to the Full Prescribing Information for pembrolizumab for complete safety information: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/.

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 participants 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 participants receiving 2 mg/kg Q3W than in those receiving 10 mg/kg Q3W. Steady-state trough concentrations were 20% greater in the participants 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 participants. 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 participants, 2) the flat exposure-response relationships of pembrolizumab for both efficacy and safety in the dose ranges of 2 mg/kg Q3W to 10 mg/kg Q3W, 3) the lack of effect of tumor burden or indication on distribution behavior of pembrolizumab (as assessed by the population PK model) and 4) the assumption that the dynamics of pembrolizumab target engagement will not vary meaningfully with tumor type.

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

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

Anti-Drug Antibodies (ADA) Data
The occurrence of ADA has been observed in less than 1% of the participants 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 Participants with melanoma was 25%/27% according to the Response Evaluation Criteria in Solid Tumors (RECIST)/investigator-assessed immune-related response criteria (irRC), respectively (Investigator's Brochure, 2014). The ORR for IPI-naïve participants with melanoma was 39%/43% by RECIST/investigator-assessed irRC, respectively. The majority of responses were seen in participants 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 participants, a RECIST-defined progression followed by response has been observed.

The preliminary ORR for 38 participants with non-small cell lung cancer was 21% / 24% by RECIST/investigator-assessed irRC, respectively (Investigator's Brochure, 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 participants suggesting that higher levels of tumor PD-L1 expression are associated with increased clinical activity.[2] ORR by RECIST 1.1 occurred in 4 out of 7 participants with higher levels of PD-L1 expression (57%, 95% CI 18-90%) versus 2 out of 22 participants 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.

Biomarkers to evaluate immune modulation and markers in the tumor microenvironment, such as T-cell infiltration, the baseline expression of markers of T-cell suppression FoxP3 or the immunoregulatory enzyme incoleamine 2,3-dioxygenase (IDO) in tumor biopsies, were associated with a high response rate in participants with advanced melanoma.[3,4]

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.

2.3 Nab-Paclitaxel

Nanoparticle albumin-bound (nab)-paclitaxel is an albumin-bound formulation of paclitaxel that was developed to avoid the toxicities associated with the vehicles that are necessary for parenteral administration of solvent-based (sb)-paclitaxel (polyethylated castor oil and polysorbate 80). Nab-paclitaxel has a shorter infusion time (30 minutes) than sb-paclitaxel and can be administered without steroid or antihistamine premedication. In addition, it has an advantageous PK profile compared with sb-paclitaxel and achieves a 33% higher tumor uptake in preclinical models.[5]

Nab-paclitaxel was approved for the treatment of metastatic breast cancer on the basis of a randomized Phase III study in which participants received either nab-paclitaxel or sb-paclitaxel (control) administered every 3 weeks (q3w) (n = 460).[6] The primary efficacy endpoint of the study was response rate. Participants receiving nab-paclitaxel achieved a higher response rate (21.5% vs. 11.1% for those receiving sb-paclitaxel). The median time to disease progression was longer for participants who received nab-paclitaxel than for those randomized to the sb-paclitaxel arm (23.0 vs. 16.9 weeks, respectively). Although a higher paclitaxel dose was achieved in the nab-paclitaxel arm, the incidence of Grade 4 neutropenia was significantly lower in the nab-paclitaxel arm than in the sb-paclitaxel (control) arm (9% vs. 22%, respectively). Grade 3 sensory neuropathy was more common with nab-paclitaxel than with sb-paclitaxel (10% vs. 2%, respectively) but was reported to be manageable and self-limiting with treatment interruption and dose reduction.

Regimens containing anthracycline and taxanes are standard of care of localized breast cancer. Recent preoperative data from the GeparSepto study suggests that nab-paclitaxel may be more effective than conventional paclitaxel as part of neoadjuvant therapy for breast cancer. In this randomized phase III neoadjuvant study, nab-paclitaxel given as 125 mg/m2 weekly for 12 weeks, followed by 4 cycles of epirubicin/cyclophosphamide, was associated with a pCR rate of 38%, significantly higher than the rate seen with standard paclitaxel (29%, $p<0.01$).[7]

2.4 Rationale for the combination of nab-paclitaxel and pembrolizumab

While chemotherapy has a role in the treatment of hormone receptor positive (HR+) breast cancer, there is compelling evidence that it is less effective in HR+ tumors than in HR- tumors.[8] Accordingly, HR+ participants have low (2-10%) rates of pathologic complete response (pCR) at surgery following neoadjuvant chemotherapy.[9] Therefore, there is a great need to understand how and when to leverage other therapeutic modalities, i.e. immunotherapy, alone or in combination with chemotherapy to improve treatment efficacy in the HR+ breast cancer subset.

Nab-paclitaxel is an attractive chemotherapeutic agent for combining with immunotherapy given the lack of need for steroid premedication. To our knowledge there are no current data documenting the pCR rate from single-agent nab-paclitaxel therapy in HR+ breast cancer. In a study of a different taxane, docetaxel, given as a single agent in the preoperative setting, 8% of estrogen receptor-positive (ER+) participants (N=52) and 13% of progesterone receptor-positive (PR+) participants (N=47) achieved "good" pathologic response, defined as pCR or minimal residual disease based on residual cancer burden (RCB) scores of 0-1.[10,11] This is on par with the low pCR rates seen for HR+ disease in other treatment settings, as detailed above.

There is increasing evidence that in addition to causing tumor cell death, certain conventional chemotherapies may have immunogenic effects.[12] Clinical evidence exists to suggest that T-cell and NK-cell functions are enhanced in participants with breast cancer (stage II/III) treated with taxanes compared with participants who did not receive taxanes.[13] A recent study in the 4 T1 breast cancer mouse model demonstrated remarkable synergy between therapy with PD-1 specific antibody and paclitaxel. As opposed to paclitaxel monotherapy, the combination significantly suppressed tumor growth and 80% of the mice (4 of 5) survived tumor-free until day 90.[14] A small study (N=25) in breast cancer participants undergoing neoadjuvant therapy with paclitaxel showed that in increase in the number of tumor infiltrating lymphocytes (TILs) from baseline to surgery correlated with an increased apoptotic tumor response, and better clinical responses.[15]

In addition, tumor cell killing by cytotoxic chemotherapy can be expected to expose the immune system to high levels of tumor antigens, and this process itself may stimulate antigen presentation and immune activation against tumors.[16] There is therefore strong reason to believe that chemotherapy can work synergistically with immune modulation.

2.5 Dosing of combination nab-paclitaxel and pembrolizumab

A phase I/II study of carboplatin, nab-palictaxel, and pembrolizumab is currently ongoing in

advanced non-small cell lung cancer. The phase I/cohort 1 portion of this study involved enrollment of 12 patients to treatment with carboplatin (AUC 6 q3weeks), nab-paclitaxel (100 mg/m2 weekly), and pembrolizumab (2 mg/kg q3weeks). Cohort 1 completed enrollment (12/12 planned patients) with a total of two dose-limiting toxicities (one grade 4 hyperglycemia; one febrile neutropenia). There was no significant immune-related toxicity observed beyond what would be expected from prior experiences with PD-1 inhibitors given as a single agent; the only instance of high-grade potentially immune-related toxicity was the single episode of grade 4 hyperglycemia, as mentioned. This trial has now moved into phase II enrollment using the phase I/cohort 1 doses, with the exception of changing pembrolizumab to flat dosing of 200 mg q3weeks per industry sponsor preference (Jyoti Patel MD and Ryan Getzler MD, personal communication). Of note, this phase I/II trial differs from our proposed phase II trial in that we plan to treat with nab-paclitaxel/pembrolizumab alone, with omission of carboplatin. As nab-paclitaxel and carboplatin have many overlapping toxicities (most notably cytopenias, neuropathy, and fatigue), if anything the toxicities of nab-paclitaxel/pembrolizumab are expected to be less than the toxicities of carboplatin/nab-paclitaxel/pembrolizumab.

Based on the acceptable toxicity profile to date of triplet therapy in non-small cell lung cancer, the present study will be a phase II study using doublet nab-paclitaxel (dosed at 125 mg/m2 weekly, in accordance with prior efficacy data from the neoadjuvant GeparSepto study in breast cancer; see Section 2.3) plus pembrolizumab at 200 mg q3weeks. The rationale for switching to a fixed dose regimen for pembrolizumab is described in Section 2.2.

2.6 Rationale for the use of biomarker endpoints

There is growing evidence that signatures of tumor-associated immunologic activity are seen in breast cancer and are significant prognostic predictors. Many different groups have demonstrated that the amount of tumor-infiltrating lymphocytes (TILs) in a breast 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.[17-22] However, the majority of this work has been done on triple negative and HER2-positive (HER2+) breast cancers.

Compared to hormone receptor-negative tumors, ER+ breast cancers tend to have a significantly lower percentage of TILs in untreated specimens (median 10% versus median 12.5%, p=0.02, in the HER2-positive NeoALTTO cohort),[17] and a lower prevalence of lymphocyte predominant breast cancer (LPBC, commonly defined as breast cancer with >60% stromal TILs) (12.0% versus 36.5% LPBC in the GeparQuinto cohort).[19] By mRNA analysis, PD-1 expression is inversely correlated with ESR1 expression, and PD-1 expression is significantly higher in HER2+, triple negative, and grade 2/3 breast tumors. (Of note, PD-1 expression correlates significantly with PD-L1 expression across multiple datasets.)[23] In sum, therefore, ER+ breast tumors appear to have a generally less “immune active” profile at baseline, compared to the HER2+ and triple negative subsets.

While the value of TILs for predicting response to neoadjuvant and adjuvant chemotherapy is well established in triple negative and HER2+ breast cancer, the predictive value of tumor-associated immune signatures in ER+ tumors treated with chemotherapy is not yet clear. Analysis of some

datasets has failed to show an association between TIL percentage in baseline tumor tissue and disease outcome.[22] Conversely, other datasets (i.e. GeparQuinto) do demonstrate an association between more immune activity in ER+ tumors and improved clinical responses to therapy.[19] Our group's data suggest that gene expression signatures associated with "immune activation" identify a subset of ER+ breast cancers with higher rates of pCR to neoadjuvant chemotherapy. Moreover, these "immune activation" signatures appear to be proliferation-independent (Stover DG et al, data not yet published).

Overall, many unanswered questions remain about the significance of immunologic profile in ER+ breast cancers and, by proxy, the role of checkpoint inhibitor therapy in this disease subset. Though these tumors appear to start out less immune-activated than triple negative or HER2+ counterparts, it is not known how these characteristics change over the course of treatment. As outlined in the prior section, there is reason to believe that treatment with chemotherapy is immune-activating. It is also not known how use of chemotherapy in conjunction with immunotherapy affects the immune characteristics of ER+ tumors. The neoadjuvant setting is an ideal platform for serial tissue sampling to provide a high volume of information about dynamic changes in peri-tumoral immune markers. Therefore, the current trial proposes to examine changes in immune-related biomarkers over the course of neoadjuvant treatment for ER+ breast cancer. We have selected PD-L1 protein expression as the core immune marker to evaluate based on prior evidence across multiple malignancies that it is significantly associated with a tumor's clinical characteristics.

Regarding the requirement for 3 breast core biopsies on this protocol: Plentiful data from other malignancies, such as melanoma, indicate the utility of immune-related biomarkers for predicting response to single agent checkpoint inhibitor therapy.[24] However, early clinical data from 2 trials presented at San Antonio Breast Cancer Symposium 2015 suggest that PD-1/PD-L1 inhibition alone will not be effective therapy for the majority of ER+ breast cancers.[25,26] At the same time, our group's data demonstrate that RNA signatures of immune activation are predictive of response to neoadjuvant chemotherapy, as described above. Many chemotherapy agents are immunogenic,[27] therefore may synergize with checkpoint inhibitor partners, but an understanding of the distinct immunologic effects of chemo- versus checkpoint-inhibitor therapy will be an important precursor to ultimately evaluating the promise of such combination regimens. Taken together, these data indicate that in ER+ breast cancer in particular, it is important to separately evaluate potential immune-related biomarkers of response to (a) chemotherapy, (b) checkpoint inhibitor therapy, and (c) combined chemo-/checkpoint inhibitor therapy. The 3 biopsies proposed here are all low-risk core biopsies of breast tissue, have been deemed acceptable by the patient advocate advisor to this protocol, and will facilitate biomarker assessment in each of these separate categories.

2.7 PD-L1 expression in ER+ breast tumors

Prior data from 650 evaluable cases indicate that 20.1% of treatment-naïve ER+ primary breast tumors express PD-L1 with an H-score ≥100, while 22.1% of ER+ primary breast tumors express PD-L1 with H-score >0.[28] Further, PD-L1 expression on ER+ primary tumors does not follow a normal distribution (Muenst S, personal communication). There is no standard accepted definition of a threshold for PD-L1 positivity in ER+ breast cancers, or for breast cancers in general; a variety of different definitions are used in the literature.[28-30]

2.8 Correlative Studies Background

2.8.1 Blood and Tissue Analysis

The development of immune signatures in breast cancer that go beyond simple histology is at a very early stage. Given the promise of immune-based therapies in other solid malignancies such as melanoma and non-small cell lung cancer, with preliminary data demonstrating activity of immune checkpoint inhibition in advanced breast cancer participants,[31,32] there is a great need to better characterize the immune profile of breast tumors across multiple disease subtypes, and at different points in the course of therapy. 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.[18] The bulk of our correlative science in this trial focuses on characterizing a broad array of immune markers in early ER+ breast cancer before, during, and after therapy, exploring baseline levels and dynamic changes over time.

In melanoma, the solid malignancy currently at the forefront of understanding the anti-tumor immune response, investigation into expression of immune mediators in the peripheral blood is at an early phase.[33] As a correlative study to this trial, we will characterize the immune marker profile of peripheral blood mononuclear cells (PBMCs) in enrolled breast cancer Participants, both at baseline and while on therapy. Peripheral blood will be monitored for T cell, B cell, NK, and NK T cells markers by flow cytometry. These include CD45RA (naïve T cells), CD45RO (memory), CD16 and CD56 (NK), CD19 (B cell), CD25 and CD69 activation, CD44 and Inducible costimulator (ICOS). We will determine if "global" CD3/4/8 T cell activation occurs by flow cytometry. CD83 and CD86 subpopulations of cells will be assessed for markers of T cell activation.

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, or to a different immune marker in the tumor microenvironment. 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.

2.8.2 Microbiome Analysis

Breast Cancer (BC) is the most frequently diagnosed cancer and the second cause of cancer death in American women[42]. 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[43]. It is now recognized that a fraction of breast tumors, mainly triple-negative breast cancer (TNBC), have substantial lymphocyte infiltration, and that this pathologic feature has prognostic implications[44]. 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[45,46], and a 12% ORR among those with PDL1+ hormone receptor (HR)-positive BC[47]. 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[48]. Healthy individuals have microbial populations in their intestinal tract that vary markedly in composition[49,50]. 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 epithelium and the host immune system are associated with many disease, including cancer[51]. Recently, two preclinical studies provided to ICI, raising the possibility that stool microbiota could be used as biomarker predictors of efficacy to immunotherapy[52,53]. Interestingly, postmenopausal women with breast cancer have altered composition and low diversity of their gut microbiota compared to healthy controls[54].

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 [redacted]. Further details can be found in Section 9.

3. PARTICIPANT SELECTION

Participants must meet the following criteria on screening examination to be eligible to participate in this study.

3.1 Eligibility Criteria

3.1.1 Participants must have histologically or cytologically confirmed invasive breast cancer.

3.1.2 Participants must have operable breast cancer, with tumors greater than or equal to 2 cm in size; Participants must not have any evidence of distant metastatic disease. Inflammatory breast cancer is permitted.

3.1.3 All confirmed invasive disease must have been tested for ER, PR, and HER2 and participants must have hormone receptor-positive, HER2-negative breast cancer (ER>1% or PR>1%, AND HER2-negative per ASCO CAP guidelines, 2013).

3.1.4 Participants with multicentric, multifocal, and/or contralateral cancers are allowed as long as one lesion meets eligibility and no biopsied tumor is HER2+.

3.1.5 Prior systemic therapy: No prior chemotherapy, biologic therapy, hormonal therapy or investigational therapy for this operable breast cancer.

3.1.6 Prior radiation therapy: No prior radiation to the ipsilateral breast.

3.1.7 The participant is ≥18 years old

3.1.8 The participant has an Eastern Cooperative Oncology Group (ECOG) performance status ≤1 (see Appendix A)

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

- Absolute neutrophil count ≥1500/mm^3
- Platelets ≥100,000/mm^3
- Hemoglobin ≥9 g/dL
- Total Bilirubin ≤1.5 mg/dL (≤ 2.0 in participants with known Gilbert's syndrome)
- Serum creatinine ≤1.5 mg/dL OR calculated GFR ≥60mL/min
- Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 2.5 times the upper limit of normal.
- 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.

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

3.1.11 The participant must be willing to undergo the three required research biopsies over the course of protocol therapy. Participants who undergo an attempted research biopsy procedure for the purpose of this protocol, and in whom inadequate tissue is obtained, are not required to undergo a repeat biopsy in order to continue on protocol.

3.1.12 The effects of pembrolizumab on the developing human fetus are unknown. For this reason, both women and men of child-bearing potential must agree to use adequate contraception (Section 5.5.2) starting with the first dose of study therapy and for the duration of study participation, 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. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. While on the study, women may not breast-feed. Women of childbearing potential are defined as those who have not been surgically sterilized or have not been free from menses for > 1 year.

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

3.1.14 Participants on bisphosphonates may continue receiving bisphosphonate therapy during study treatment.

3.2 Exclusion Criteria

3.2.1 The participant has received prior pembrolizumab or any other anti-PD-1, anti-PD-L1, or anti-PD-L2 therapy, or has participated in any prior studies involving pembrolizumab

3.2.2 Hypersensitivity to pembrolizumab or any of its excipients.

3.2.3 The participant has any history or evidence of active, non-infectious pneumonitis or interstitial lung disease.

3.2.4 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; see Appendix B), active ischemic heart disease, myocardial infarction within the previous six months, uncontrolled diabetes mellitus, chronic liver or renal disease, or severe malnutrition.

3.2.5 Concurrent use of potent CYP3A4 inhibitors (see Appendix C), such as ketoconazole and erythromycin, should be avoided during the study treatment with nab-paclitaxel.

3.2.6 Pregnant women are excluded from this study because pembrolizumab has the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with pembrolizumab, breastfeeding should be discontinued if the mother is treated with pembrolizumab.

3.2.7 Active infection requiring intravenous antibiotics at week 1 day 1.

3.2.8 Individuals with a history of a second 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 5 years and are deemed by the investigator to be at low risk for recurrence of that malignancy. Individuals with the following cancers are eligible if diagnosed and treated within the past 5 years: cervical cancer *in situ*, and non-melanoma cancer of the skin. Participants with other cancers diagnosed within the past 5 years and felt to be at low risk of recurrence should be discussed with the study sponsor to determine eligibility.

3.2.9 The participant has a medical condition that requires chronic systemic steroid therapy or any other form of immunosuppressive medication including disease modifying agents. Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

3.2.10 The participant has an active autoimmune disease or a documented history of autoimmune disease or syndrome that requires systemic steroids or immunosuppressive agents.

3.2.11 The participant is known to be positive for Hepatitis B surface antigen, or Hepatitis C RNA. Testing for screening is not required.

3.2.12 Known HIV-positive participants. HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with pembrolizumab. In addition, these participants are at increased risk of lethal infections with bone marrow suppressive therapy, i.e. nab-paclitaxel. Appropriate studies will be undertaken in participants receiving combination antiretroviral therapy when indicated. Testing for screening is not required.

3.2.13 The participant has received a live vaccine within 28 days of planned start of study therapy. **Note:** seasonal influenza vaccines for infection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (i.e. Flu-Mist ®) are live attenuated vaccines, and are not allowed.

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.

The following registration/randomization procedures should be followed:

- An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.
- The eligibility checklist(s) and all pages of the consent form(s) will be faxed to the ODQ at [redacted].
- The ODQ Registrar will (a) review the eligibility checklist, (b) register the participant on the protocol, and (c) randomize the participant.
- An email confirmation of the registration and/or randomization will be sent to the Overall PI, study coordinator(s) from the Lead Site

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.

4.3 General Guidelines for Other Investigative Sites

N/A

4.4 Registration Process for Other Investigative Sites

N/A

5. TREATMENT PLAN

5.1 Treatment Regimen

This is a pilot study examining biomarker changes over the course of treatment with nab-paclitaxel, pembrolizumab, or both in hormone receptor-positive (HR+) breast cancer. A baseline research biopsy (biopsy 1) is required. After registration, participants will be randomized to receive a run-in of either two weeks of nab-paclitaxel (cohort A) or one dose of pembrolizumab (cohort B) monotherapy. A post-monotherapy biopsy (biopsy 2) will then be performed. Participants will then receive combination nab-paclitaxel (administered weekly) and pembrolizumab (administered every 3 weeks) for a total of 15 weeks (cohort A) or 14 weeks (cohort B). At completion, each patient will have received 12 doses of weekly nab-paclitaxel, and 5 doses of every-3-week pembrolizumab.

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7.1. 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

Regimen Description				
Agent	**Premedications; Precautions**	**Dose**	**Route**	**Schedule**
Pembrolizumab	Not routinely necessary unless prior infusion reaction.	200 mg, at a final concentration of 1 mg/mL to 10 mg/mL in NS or D5	IV over approximately 30 minutes (range: 25-40 minutes). Please refer to Section 8.1.4 for compatible infusion set materials including in-line filter. Infuse prior to starting nab-paclitaxel infusion.	Day 1, every 3 weeks

Nab-paclitaxel	Not routinely necessary unless prior infusion reaction	125 mg/m^2 in NS*	IV over 30 minutes.** Start nab-paclitaxel infusion after pembrolizumab infusion is complete.	Day 1, weekly
*Dose reductions may be made as per Table 4. Further details about dose reductions can be found in Sections 6.2 and 6.3. **Limiting the infusion of nab-paclitaxel to 30 minutes, as directed, decreases the likelihood of infusion-related reactions				

5.1.1 Treatment Schedule – Cohort A

Participants randomized to cohort A will undergo a baseline biopsy (biopsy 1), then start treatment with a 2-week run in of weekly nab-paclitaxel monotherapy, followed by a second biopsy post-monotherapy (biopsy 2). On week 3, nab-paclitaxel will be continued, and pembrolizumab will begin its every-3-week dosing. Weekly nab-paclitaxel will continue for a total of 12 weeks. Every-3-week pembrolizumab will be administered for 5 doses total (weeks 3, 6, 9, 12, 15). A biopsy on doublet therapy (biopsy 3) will be obtained between weeks 6 and 7. Neoadjuvant therapy will complete at week 15, with the fifth and final dose of pembrolizumab.

5.1.2 Treatment Schedule – Cohort B

Participants randomized to cohort B will undergo a baseline biopsy (biopsy 1), then start treatment with a 2-week run in of pembrolizumab monotherapy, followed by a second biopsy post-monotherapy (biopsy 2). On week 3, weekly nab-paclitaxel will start, and continue for a total of 12 weeks. Every-3-week pembrolizumab will be administered for 5 doses total (weeks 1, 4, 7, 10, 13). A biopsy on doublet therapy (biopsy 3) will be obtained between weeks 6 and 7. Neoadjuvant therapy will complete at week 14, with the twelfth and final dose of nab-paclitaxel.

5.2 Pre-Treatment Criteria

5.2.1 Screening for trial eligibility

Day -28 to Day 1: Screening Visit
Eligibility and exclusion criteria are provided in Section 3. These criteria will be assessed within 28 days of registration to establish eligibility and baseline values.

Informed consent will be obtained after the study has been fully explained to the participant and before the conduct of any research screening procedures. If screening assessments occur within 3 days before start of study treatment, then they may serve as the Week 1 Day 1 visit labs.

Demographic information and baseline characteristics will be collected at the Screening Visit. Standard demographic parameters include age, sex, and race/ethnicity (recorded in accordance with prevailing regulations). Hormone receptor and HER2 status will also be collected.

Additional testing required, as per Section 3, is: CBC with differential, basic metabolic panel which includes liver function tests (LFTs), coagulation panel, urine or serum HCG (in women of childbearing potential), testing for Hepatitis B/C and HIV, and EKG. A urine or serum pregnancy test must be completed within 7 days prior to receiving the first dose of study medication.

A baseline tumor biopsy, obtained within 14 days before starting protocol therapy, is also required. Further details about collection and handling of tumor biopsy specimen can be found in Section 9.1.1.

All participants will also be asked to provide archival tumor tissue (either paraffin blocks or 15-20 unstained slides) from their diagnostic biopsy. However, if archival tissue is not available or not evaluable, that will not be a basis to exclude the participant from any portion of the trial or the planned analysis. This tissue can be obtained at any point during the study and does not need to be collected during the screening window.

5.2.2 On-Treatment Visits– Cohort A

Criteria to treat at week 1 day 1:

- **Absolute neutrophil count ≥1500/**mm^3
- **Platelets ≥100,000/**mm^3
- **ALT and AST ≤2.5 x ULN**
- **Total bilirubin ≤1.5 x ULN (2.0 x ULN in a patient with well documented Gilbert syndrome)**

Criteria to treat at day 1 of subsequent weeks:

- **Absolute neutrophil count ≥1000/**mm^3
- **Platelets ≥75,000/**mm^3
- **ALT and AST ≤3.0 x ULN**
- **Total bilirubin ≤1.5 x ULN (2.0 x ULN in a patient with well documented Gilbert syndrome)**

Reasonable effort should be made to conduct study visits on the day scheduled (+/- 3 days).

Any changes from screening clinical evaluation findings that meet the definition of an AE will be recorded on the AE page of the eCRF.

Weekly assessments, weeks 1-12 day 1
If screening assessments occur within 3 days before start of study treatment, then they may serve as the Week 1 Day 1 visit. Body weight should be documented on each treatment day such that drug dosing can be calculated accordingly.

Draw blood sample for:

- CBC with differential
- Basic metabolic panel
- LFTs (ALT, AST, alkaline phosphatase, total bilirubin)

Record:
- Height/weight (height at week 1 day 1 only)
- Vital signs

Review all laboratory results before administering study treatment.

Every-3-week assessments, weeks 3-15 day 1 (prior to pembrolizumab dosing; weeks 3, 6, 9, 12, 15)
The following assessments should be performed on the indicated weeks, *in addition to* the standard weekly assessments listed above. If a pembrolizumab dose(s) is held, the assessments below should move to coincide with the pembrolizumab infusions.

Draw blood sample for:
- TSH and free T4

Record:
- Weight
- Vital signs
- Physical exam
- Concomitant medications
- AEs or SAEs

Review all laboratory results (except TSH and T4) before administering study treatment.

The total dose of each drug will be calculated according to institutional standards.

5.2.3 On-Treatment Visits – Cohort B

Criteria to treat at week 1 day 1:
- **Absolute neutrophil count ≥1500/** mm^3
- **Platelets ≥100,000/** mm^3
- **ALT and AST ≤2.5 x ULN**
- **Total bilirubin ≤1.5 x ULN (2.0 x ULN in a patient with well documented Gilbert syndrome)**

Criteria to treat at day 1 of subsequent weeks:
- **Absolute neutrophil count ≥1000/**mm^3
- **Platelets ≥75,000/**mm^3
- **ALT and AST ≤3.0 x ULN**
- **Total bilirubin ≤1.5 x ULN (2.0 x ULN in a patient with well documented Gilbert syndrome)**

Reasonable effort should be made to conduct study visits on the day scheduled (+/- 3 days).

Any changes from screening clinical evaluation findings that meet the definition of an AE will be recorded on the AE page of the eCRF.

Every-3-week assessments, weeks 1-13 day 1 (prior to pembrolizumab dosing; weeks 1, 4, 7, 10, 13)
The following assessments should be performed on the indicated weeks, *in addition to* the standard weekly assessments listed below. If a pembrolizumab dose(s) is held, the assessments below should move to coincide with the pembrolizumab infusions.

If screening assessments occur within 3 days before start of study treatment, then they may serve as the baseline Week 1 Day 1 visit and screening tests do not need to be repeated. Body weight should be recorded on each treatment day such that drug dosing can be calculated accordingly.

Draw blood sample for:
- TSH and free T4

Record:
- Height/weight (height on week 1 day 1 only)
- Vital signs
- Physical exam
- Concomitant medications
- AEs or SAEs

Review all laboratory results (except TSH and T4) before administering study treatment.

Weekly assessments, weeks 3-14 day 1
Draw blood sample for:
- CBC with differential
- Basic metabolic panel
- LFTs (ALT, AST, alkaline phosphatase, total bilirubin)

Record:
- Weight
- Vital signs

Review all laboratory results before administering study treatment.

The total dose of each drug will be calculated according to institutional standards.

5.2.4 Additional On-Treatment Assessments – Both Cohorts

Biopsies
Three breast tumor biopsies should be collected at the following time points:
1. Biopsy 1: at baseline, within 14 days prior to week 1 day 1 of protocol therapy

2. Biopsy 2: after monotherapy, within 3 days prior to week 3 day 1 (while the allowed biopsy window is 3 days prior to week 3 day 1, it is preferred that the biopsy be performed as close to week 3 day 1 as possible—i.e. 0-1 days prior to week 3-day 1 treatment is most preferred)
3. Biopsy 3: after doublet therapy, within 3 days prior to week 7 day 1

A biopsy should also be obtained in participants who go off study for progressive disease, and in participants in whom treating physicians choose to give additional chemotherapy after protocol therapy, instead of proceeding to surgery. Additional instructions for obtaining a fourth tissue sample at the time of breast surgery are below.

Specific instructions for tumor biopsy handling are described in Section 9.1.1.

Physical Examinations
A physical examination, including breast examination, should be performed and documented at all specified protocol visits (see Section 10, Study Calendar).

Patients who go off study for progressive disease, and/or undergo additional non-trial chemotherapy before surgery
As above, tumor biopsies should be obtained in patients who go off study for progressive disease, and in patients in whom treating physicians choose to give additional chemotherapy after protocol therapy, instead of proceeding to surgery.

Tumor Staging - Breast MRI, mammogram, or breast ultrasound
All participants are required to have a MRI, mammogram, or ultrasound performed at screening (within 28 days prior to start of study treatment) and at pre-surgery (2-3 weeks after study treatment ends). Breast imaging must include imaging of the ipsilateral axilla. MRI is strongly recommended, although other imaging modalities (mammogram, ultrasound) are permitted if practical or financial considerations preclude MRI, as long as the target lesion can be adequately measured. This same imaging modality must be used at screening and prior to surgery to assess radiographic tumor response.

Whole-Body Staging - CT scans/bone scan
It is recommended, but not required that all participants with anatomic Stage IIa disease and above (by AJCC 8th edition) will have CT scans of chest, abdomen and pelvis and bone scans, or PET-CT scans, performed during screening to rule out metastatic disease.

Surgical Assessment
All participants will be seen and examined by the treating surgeon at Screening and at the Pre-Operative Visit. Each visit will include a clinical breast and lymph node examination and review of the imaging studies (mammogram, MRI, and any other radiographic method) of the breast and axilla. After examining the subject and reviewing the pertinent radiographic studies at the Screening visit, the surgeon will determine whether the subject is a candidate for potentially curative surgery. At both the Screening and Pre-Operative visits, the surgeon will also determine whether subject is eligible for breast conservation surgery. If the subject is not a breast

conservation candidate, the reason(s) will be documented in the CRF (multicentric tumor, tumor location, tumor size, other).

Axillary Assessment

An axillary assessment will be performed at screening. Ipsilateral axillary lymph nodes will be assessed as clinically normal or clinically suspicious by physical examination and will be assessed as clinically normal or clinically suspicious independently by imaging. Axillary imaging and/or biopsy do not need to be repeated if performed prior to the screening period. Participants with suspicious nodes documented by physical exam OR by imaging will have a biopsy of the nodes (fine needle aspirate or core needle biopsy). If clinical evaluation and biopsy results are discordant, the biopsy may be repeated at the discretion of the Investigator.

Immediate Post-Surgical Tumor Sampling

Formalin-fixed paraffin embedded tumor blocks as well as fresh tissue from the surgical specimen should be obtained. Details of this process are described in Section 9.1.2.

5.3 Agent Administration

5.3.1 Pembrolizumab

Pembrolizumab administration

Merck will provide the investigator with an adequate supply 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 participant's medical record.

Pembrolizumab will be administered in clinic on day 1 (+/- 3 days) of weeks 3, 6, 9, 12, and 15 (cohort A) or day 1 (+/- 3 days) of weeks 1, 4, 7, 10, and 13 (cohort B). 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 and + 10 minutes is permitted (i.e. infusion time is 30 minutes: -5 min/ +10 min).

Pembrolizumab should be administered prior to nab-paclitaxel administration. There should be no overlap in timing of the two administrations.

5.3.2 Nab-paclitaxel

Nab-paclitaxel administration

Please refer to the FDA-approved package insert for nab-paclitaxel for product information, extensive preparation instructions, and comprehensive list of adverse events.

Nab-paclitaxel will be administered as an intravenous infusion over 30 minutes. Filters are not required for preparation or administration of nab-paclitaxel. If filters are used as part of institutional procedure, the pore size must be ≥ 15 microns.

5.3.3 Other Modality(ies) or Procedures

N/A

5.3.4 Investigational Imaging Agent Administration

N/A

5.4 Definition of Dose-Limiting Toxicity (DLT)

Dose-limiting toxicity is defined as any of the following events occurring within 21 days (3 weeks_ after the first dose of therapy, if judged by the investigator to be possibly, probably, or definitely related to study drug administration:

- Death
- Asymptomatic grade 4 hematologic toxicity lasting ≥14 days unless deemed by the investigator to be clinically insignificant
- Grade 4 thrombocytopenia of any duration
- ≥ Grade 3 Febrile neutropenia
- ≥ Grade 3 Thrombocytopenia if associated with bleeding
- ≥ Grade 3 elevation in AST or ALT associated with a grade 2 elevation in bilirubin that is at least possibly related to study drug (Hy's Law)
- ≥ Grade 3 non-hematologic laboratory value if:
    - Medical intervention is required to treat the patient, or
    - The abnormality leads to hospitalization, or
    - The abnormality persists >7 days

  Excluding:
    - Alkaline phosphatase ≤10.0x ULN in a patient with grade 2 alkaline phosphatase elevation at baseline as a result of bone metastasis
    - Any laboratory values deemed by the investigator to be clinically insignificant
- ≥ Grade 3 pneumonitis of any duration
- ≥ Grade 3 Fatigue lasting >5 days
- ≥ Grade 3 other non-laboratory toxicity lasting >3 days despite optimal supportive care, excluding the following:
    - Alopecia of any grade

DLTs will be assessed during the DLT assessment window (lasting for 21 days or 3 weeks after initiation of study therapy).

Management and dose modifications associated with the above adverse events are outlined in Section 6.

Number of Participants with DLT in a Given Arm (Cohort A or B)	Enrollment Decision
0 -1 out of 6	Continue trial enrollment
≥2 out of 6	Halt trial enrollment

5.5 General Concomitant Medication, Supportive Care Guidelines, and Contraception 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 Principal Investigator should discuss any questions regarding this with the Merck Clinical team. The final decision on any supportive therapy or vaccination rests with the Principal Investigator and/or the participants treating physician.

Acceptable Concomitant Medications
All treatments that the investigator considers necessary for a participant's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care.

Prohibited Concomitant Medications
Participants 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
- Chemotherapy not specified in this protocol
- Biologic or targeted agents not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy
- 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 28 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.

- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor.

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

Care should be taken with concomitant use of strong CYP3A4 inhibitors/inducers (e.g., ketoconazole and itraconazole; see Appendix C) and nab-paclitaxel. An alternate medication with no or minimal potential to inhibit CYP3A4 should be considered. If a strong CYP3A4 inhibitor is co-administered with nab-paclitaxel, Participants should be closely monitored for adverse reactions.

The exclusion criteria section describes other medications which are prohibited in this trial.

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

5.5.1 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 below. 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.
- Hematopoietic growth factors (e.g., G-CSF, granulocyte macrophage colony stimulating factor) may be used at investigator's discretion for the primary prophylaxis and/or management of treatment-emergent neutropenia and/or for secondary prophylaxis as per NCCN/European Society for Medical Oncology guidelines [34,35] or local standard practice. However, treatment with granulocyte-colony stimulating factors will not permitted during the first two weeks of protocol therapy unless the patient has febrile neutropenia and the physician considers its use as clinically indicated. It will be left to the treating physician choice from week 3 onwards.
- Bisphosphonate or denosumab therapy for osteoporosis or osteopenia 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.

Participants 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.

5.5.2 Contraception Guidelines

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

For this trial, male participants 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 participants will be considered of non-reproductive potential if they are either:

(1) postmenopausal (defined as at least 12 months with no menses without an alternative medical cause; in women < 45 years of age a high follicle stimulating hormone (FSH) 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

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

OR

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

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

(1) practice abstinence† from heterosexual activity;

OR

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

activity.

Acceptable methods of contraception are‡:

Single method (one of the following is acceptable):

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

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

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

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

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

Participants 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 participants 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.

5.6 Additional Preoperative Chemotherapy

If there is clinical or radiographic evidence of significant residual disease after completion of protocol treatment, after a mandatory biopsy the participant may receive additional preoperative chemotherapy. It is strongly encouraged that if additional preoperative chemotherapy is being considered, that the case is discussed with the overall study primary investigator prior to biopsy. The fourth mandatory biopsy collection marks the end of protocol mandated chemotherapy;

however additional data will be collected to document additional chemotherapy and response to the additional chemotherapy.

5.7 Criteria for Taking a Participant Off Protocol Therapy

Treatment will continue for 15 weeks (cohort A) or 14 weeks (cohort B), or until one of the following criteria applies:

- Disease progression (except as described above, in Section 5.2.4)
- 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
- 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 taken off treatment in OnCore. Alternative care options will be discussed with the participant.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Core Site PI, [redacted].

5.8 Duration of Follow Up

The first post-surgery follow-up visit will be considered the participant's final in-clinic study visit. If breast surgery is not performed the participant's final visit will be within 28 days after the last dose of protocol-specified therapy (i.e., nab-paclitaxel or pembrolizumab, whichever occurred later).

Simplified post-surgery follow-up information will be subsequently collected. Decisions regarding choice of post-surgical treatment and disease assessments will be at the discretion of the treating team and not mandated by the protocol. The investigator is not required to actively monitor participants for adverse events after the final visit. However, the Overall PI should be notified if the investigator becomes aware of any death or other serious adverse event that is considered related to the study medication.

Post-surgery follow-up information will be collected at assessments every 6 months until 5 years after surgery, or until a disease-free survival event.

Disease-free survival (DFS) will be defined from the time of randomization until the occurrence of the first of the following events:

- Local/regional recurrence: a recurrent or new invasive ipsilateral breast cancer, invasive breast cancer in the axilla, regional lymph nodes, chest wall, or skin of the ipsilateral breast.
- Contralateral invasive breast cancer,
- Distant recurrence: metastatic disease that has either been biopsy confirmed or clinically diagnosed as recurrent invasive breast cancer. A single new lesion on a bone scan without evidence of lytic disease on x-ray and without symptoms does not in and of itself constitute distant recurrence, but multiple new bone lesions, or increased isotope uptake associated with new bone symptoms are more likely due to metastases. Bone metastases must be documented with x-rays and clinical description.
- Death from any cause

In situ cancer is not included as DFS event. If a participant has in situ breast cancer (on the ipsilateral or contralateral side) diagnosed during follow-up before any of the DFS events above, then the participant should continue to be followed for DFS on study (even if she is given hormonal therapy after the in-situ diagnosis). These participants will be followed for survival.

If a participant is diagnosed with a non-melanoma skin cancer or a cervical carcinoma in situ, he/she will continue on this study and continue to be followed for DFS.

It is recommended that any disease-free survival event should be biopsied to confirm recurrent disease. Information on breast cancer status, new anti-cancer therapy, and new onset malignancy diagnoses will be collected via simplified CRFs. Following an IDFS event, survival information (i.e., date and cause of death or last known alive date if not deceased and new onset malignancy information) will be collected.

5.9 Criteria for Taking a Participant Off Study

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

- Patient completed required follow-up
- 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).

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.

For Decentralized Subject Registrations, the research team updates the relevant Off Treatment/Off Study information in OnCore.

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 [redacted]

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 should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Overall PI. The reason for interruption should be documented in the participant's study record.

Once doses are reduced, they may not be re-escalated.

General guidelines regarding dose modification:

- If, in the opinion of the investigator, a toxicity is considered to be due solely to one component of the study treatment (i.e. pembrolizumab or nab-paclitaxel) and the dose of that component is delayed or modified in accordance with the guidelines below, the other component may be administered if there is no contraindication
- If it is anticipated that nab-paclitaxel will be delayed by ≥3 weeks, then pembrolizumab should be given without the chemotherapy if there is no contraindication.
- Nab-paclitaxel doses may be delayed and/or reduced and pembrolizumab may be delayed as a result of toxicities. No dose reductions are allowed for pembrolizumab.
- It is recommended that participants receive all planned doses of pembrolizumab and nab-paclitaxel.
  - Nab-paclitaxel doses that are held should be made up at the completion of planned therapy, so as to not disturb the treatment schedule.
  - Pembrolizumab doses may be delayed or made up at the completion of planned therapy. This is at the investigator's discretion. If pembrolizumab doses are delayed, all other assessments assigned to specific Week numbers on the Required Data Table should remain the same.

6.1 Management of toxicities attributable to pembrolizumab alone

Hepatotoxicity has been previously described in participants on both single agent pembrolizumab

and single agent nab-paclitaxel; therefore, management of this toxicity is addressed separately, in Section 6.3.

For toxicities in this Section, which are attributable to pembrolizumab alone, only pembrolizumab should be held as directed. It is permissible to continue nab-paclitaxel despite discontinuation of pembrolizumab in these cases.

Treating physicians may discontinue or hold Pembrolizumab for intolerable AEs regardless of grade and duration if it is in the best interest of the patient.

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. As noted below the table, for **recurrence** of any **grade 3** or higher event (exception: **grade 2** or higher for pneumonitis), pembrolizumab must be permanently discontinued.

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 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
Participants 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 Section 6. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids.

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

Note: if after the evaluation the event is determined not to be related, the investigator is instructed to follow the ECI reporting guidance (see Section 7.7.2).

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

- **Pneumonitis**:
    - Pembrolizumab should be permanently discontinued following any event of pneumonitis **grade 2 or higher**.
    - For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
    - For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.

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

- **Diarrhea/Colitis**:
  Participants should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).

  - All participants who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
  - For **Grade 2 diarrhea/colitis** that persists greater than 3 days, administer oral corticosteroids.
  - For **Grade 3 or 4 diarrhea/colitis** that persists > 1 week, treat with intravenous steroids followed by high dose oral steroids.
  - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or ≥ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)**
  - For **T1DM** or **Grade 3-4** Hyperglycemia
    - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.
    - Evaluate Participants with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

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

- **Hyperthyroidism or Hypothyroidism**:
  Thyroid disorders can occur at any time during treatment. Monitor Participants for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.
  - **Grade 2** hyperthyroidism events (and **Grade 2-4** hypothyroidism):
    - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.

        - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyronine, is indicated per standard of care.
    - **Grade 3-4** hyperthyroidism
        - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hepatic**: (see associated dose modification guidelines for nab-paclitaxel in Section 6.3)
    - For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).
        - Treat with IV or oral corticosteroids
    - For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours.
    - When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

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

- **Management of Infusion Reactions**: Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Table 3 below shows treatment guidelines for participants 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 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. **Participants who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (pembrolizumab) with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
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.		

6.2 Management of toxicities attributable to nab-paclitaxel alone

Hepatotoxicity has been previously described in participants on both single agent pembrolizumab and single agent nab-paclitaxel; therefore, management of this toxicity is addressed separately, in Section 6.3.

For toxicities in this Section, which are attributable to nab-paclitaxel alone, concurrent holding of pembrolizumab is at the discretion of the treating physician, as described in the general guidelines at the start of this Section.

Dose reduction guidelines for nab-paclitaxel are shown in Table 4, below. In instances where the direction is to immediately dose reduce nab-paclitaxel, it is at the investigator's discretion if they would like to first hold nab-paclitaxel, followed by a dose reduction.

Table 4: Nab-paclitaxel dose reduction levels

Dose Level	Nab-paclitaxel Dose
0	125 mg/m^2 weekly
-1	100 mg/m^2 weekly
-2	75 mg/m^2 weekly
Further dose reductions warranted	Discontinue nab-paclitaxel. Continuation with pembrolizumab is at investigator discretion

Hematologic Toxicity
Absolute neutrophil count (ANC) must be ≥1000/µl and platelet count must be ≥75,000/ µl on day 1 of each week (after week 1) in order to treat. If these parameters are not met, treatment should be managed as listed below in Table 5.

At any time, if treatment is delayed or skipped for >3 weeks for hematologic toxicity (possibly, probably, or definitely related to nab-paclitaxel), discontinue nab-paclitaxel. Patients may continue

on study with pembrolizumab at the discretion of the treating investigator.

Table 5: Nab-paclitaxel dose reductions for hematologic toxicity

Event	Occurrence	Action to Be Taken
Grade 3 or 4 febrile neutropenia or Delay of next week by >7 days for nadir ANC <1000/µl or Nadir ANC<500/µl for >7 days	First	Decrease nab-paclitaxel by one dose level OR Add growth factor support to stay at current dose level. This decision is at the investigator's discretion.
	Second	Decrease nab paclitaxel by one dose level
	Third	Discontinue nab-paclitaxel. Continuation with pembrolizumab is at investigator discretion
Nadir platelet count <75,000/µl	First	Decrease nab-paclitaxel by one dose level
	Second	Decrease nab paclitaxel by one dose level
	Third	Discontinue nab-paclitaxel. Continuation with pembrolizumab is at investigator discretion

Neurotoxicity
Neurologic toxicity should be managed as instructed below, in Table 6.

At any time, if treatment is delayed or skipped for >3 weeks for neurologic toxicity (possibly, probably, or definitely related to nab-paclitaxel), discontinue nab-paclitaxel. Patients may continue on study with pembrolizumab at the discretion of the treating investigator.

Table 6: Nab-paclitaxel dose reductions for neurologic toxicity

Event	Occurrence	Action to Be Taken
Peripheral neuropathy grade 2	First	Decrease nab-paclitaxel by one dose level
	Second	Hold treatment until decreases to grade ≤1, then restart nab-paclitaxel at a reduction of one dose level
	Third	Discontinue nab-paclitaxel.

		Continuation with pembrolizumab is at investigator discretion
Peripheral neuropathy grade 3	First	Hold treatment until decreases to grade ≤1, then restart nab-paclitaxel at a reduction of one dose level.
	Second	Hold treatment until decreases to grade ≤1, then restart nab-paclitaxel at a reduction of one dose level
	Third	Discontinue nab-paclitaxel. Continuation with pembrolizumab is at investigator discretion
Peripheral neuropathy grade 4	Any	Discontinue nab-paclitaxel. Continuation with pembrolizumab is at investigator discretion

Other non-hematologic toxicity
Other toxicities not specifically outlined above should be managed as instructed below, in Table 7. Use of maximum supportive medical care is encouraged, within the parameters outlined in Section 5.5, concomitant medications and supportive care guidelines.

At any time, if treatment is delayed or skipped for >3 weeks for the same type of toxicity (possibly, probably, or definitely related to nab-paclitaxel), discontinue nab-paclitaxel. Patients may continue on study with pembrolizumab at the discretion of the treating investigator.

Table 7: Dose reductions for nab-paclitaxel for other toxicities

Grade	**Occurrence**	**Action to Be Taken**
3 or 4	First	Hold treatment until decreases to grade ≤2, then restart nab-paclitaxel at a reduction of one dose level.
	Second	Hold treatment until decreases to grade ≤2, then restart nab-paclitaxel at a reduction of one dose level
	Third	Discontinue nab-paclitaxel. Continuation with pembrolizumab is at investigator discretion

6.3 Management of hepatic toxicity attributable to both pembrolizumab and nab-paclitaxel

Hepatotoxicity has been previously described in participants on both single agent pembrolizumab and single agent nab-paclitaxel. Management of this toxicity is addressed in this Section.

Event	Grade	Action To Be Taken
AST/ALT elevation	2 – first occurrence	Hold both drugs until lab elevation is grade 0 or 1, then restart both drugs (at the previous doses). Recheck lab value weekly or as medically indicated. Recommendations for supportive care (steroid dosing) are above, in Section 6.1. If toxicity takes ≥12 weeks to resolve, permanently discontinue pembrolizumab.
	2- second occurrence and beyond	Hold both drugs until lab elevation is grade 0 or 1, then restart nab-paclitaxel only (at the previous dose). Permanently discontinue pembrolizumab. Recheck lab value weekly or as medically indicated. Recommendations for supportive care (steroid dosing) are above, in Section 6.1.
	3	Hold both drugs until lab elevation is grade 0 or 1, then restart nab-paclitaxel only, at dose level -1. Permanently discontinue pembrolizumab. Recheck lab value weekly or as medically indicated. Recommendations for supportive care (steroid dosing) are above, in Section 6.1.
	4	Discontinue all study treatment permanently. Recommendations for supportive care (steroid dosing) are above, in Section 6.1.
Total bilirubin elevation (see legend for special considerations for Gilbert's	2 – first occurrence	Hold both drugs until lab elevation is grade 0 or 1, then restart both drugs (at the

Syndrome)[1]		previous doses). Recheck lab value weekly or as medically indicated. Recommendations for supportive care (steroid dosing) are above, in Section 6.1. If toxicity takes ≥12 weeks to resolve, permanently discontinue pembrolizumab.
	2- second occurrence and beyond	Hold both drugs until lab elevation is grade 0 or 1, then restart nab-paclitaxel only (at the previous dose). Permanently discontinue pembrolizumab. Recheck lab value weekly or as medically indicated. Recommendations for supportive care (steroid dosing) are above, in Section 6.1.
	3 and 4	Discontinue all study treatment permanently. Recommendations for supportive care (steroid dosing) are above, in Section 6.1.
AST or ALT >3.0x upper limit of normal (ULN), AND total bilirubin >2.0x ULN		Discontinue all study treatment permanently. Recommendations for supportive care (steroid dosing) are above, in Section 6.1.

[1]In a patient with known Gilbert's Syndrome, an additional elevation of 0.5 mg/dL in total bilirubin is allowed in each case.

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 Event Lists

7.1.1 Adverse Event Lists (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 participants in

P012) to 100% (10 of 10 participants 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 participants 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 participants) in P001/P002. The most commonly reported Grade 3-5 DRAEs were anemia, alanine aminotransferase increased, and aspartate aminotransferase increased. Most participants who experienced an AE continued in the study, with the incidence of AEs leading to discontinuation ranging from 1.9% (8 of 430 participants in P028) to 12.3% (192 of 1562 participants 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 participants in P011) to 4.5% (4 of 88 participants in P013). The
most commonly reported DRAEs leading to discontinuation were pneumonitis, alanine aminotransferase increased, and aspartate aminotransferase increased.

List of AEs considered expected:

- Endocrine disorders: Adrenal insufficiency, Hyperthyroidism, Hypophysitis, Hypopituitarism, Hypothyroidism, Secondary adrenal insufficiency, Thyroid disorder
- Eye disorders: Uveitis
- Gastrointestinal disorders: Abdominal pain, Colitis, Diarrhea, 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

For more details regarding the safety profile of pembrolizumab, please refer to the Pembrolizumab *(*KEYTRUDA) investigator's brochure.

7.1.1.1 Adverse Event List (s) for Nab-Paclitaxel

In clinical studies, nab-paclitaxel has been associated with alopecia, myelosuppression (primarily neutropenia), sensory neuropathy, abnormal ECG, fatigue/asthenia, myalgia/arthralgia, AST elevation, alkaline phosphatase elevation, anemia, nausea, infections, and diarrhea.

Participants will be monitored for nab-paclitaxel related adverse events, including hematologic, GI and hepatic toxicities, and peripheral neuropathy.

For more details regarding the safety profile of nab-paclitaxel, please refer to the ABRAXANE®

Package Insert.

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 [redacted]

- **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.
  - Other AEs for the protocol that do not require expedited reporting are outlined in the next section (Expedited Adverse Event Reporting) under the sub-heading of Protocol-Specific Expedited Adverse Event Reporting Exclusions.

- **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.

7.3.3 Protocol-Specific Expedited Adverse Event Reporting Exclusions

For this protocol only, the AEs/grades listed below do not require expedited reporting to the DFCI IRB. However, they still must be reported through the routine reporting mechanism (i.e. case report form) and may require reporting to the FDA and/or Merck.

CTCAE SOC	Adverse Event	Grade	Expectedness	Attribution
Investigations	Neutrophil count	2 or 3	Unexpected	Possibly,

	decreased (neutropenia)			probably, or definitely related to study treatment
Blood and lymphatic system disorders	Anemia	2 or 3	Unexpected	Possibly, probably, or definitely related to study treatment
Investigations	Platelet count decreased (thrombocytopenia)	2 or 3	Unexpected	Possibly, probably, or definitely related to study treatment
Blood and lymphatic system disorders	Blood and lymphatic system disorders (other)	2 or 3	Unexpected	Possibly, probably, or definitely related to study treatment
Nervous system disorders	Peripheral motor/sensory neuropathy	2	Unexpected	Possibly, probably, or definitely related to study treatment

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 Routine Adverse Event Reporting

Only ≥ grade 2 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 <u>also</u> be reported in routine study data submissions.**

7.7 Immediate Reporting of Adverse Events and Events of Clinical Interest (ECI) to Merck

7.7.1 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 participant 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 within 2 working days to Merck Global Safety.

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

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

SAE reports and any other relevant safety information should be forwarded to the Merck Global Safety facsimile number: [redacted]

All participants with serious adverse events must be followed up for outcome until resolution of event to grade ≤1. If resolution to grade ≤1 has not occurred within 30 days of the initial event, the participant must be followed until stabilization of the event (at a constant grade) for a period of at least 2 weeks.

7.7.2 Events of Clinical Interest (ECIs)

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported as such on the Adverse Event case report forms/worksheets within 2 working days to Merck Global Safety. ([redacted]). 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 participant should be observed closely for signs of toxicity.

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 ALT 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.

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 within 2 working days to Merck Global Safety. ([redacted], regardless of attribution to study treatment, consistent with standard SAE reporting guidelines.

Participants 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. Participants 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.

7.7.3 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 participant (spontaneously reported to them) that occurs during the trial.

Pregnancies and lactations that occur after the consent form is signed but before treatment allocation must be reported by the investigator if they cause the participant to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

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

Such events must be reported within 24 hours to the Core Site PI, [redacted], and within 2 working days to Merck Global Safety. ([redacted])

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or 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 names: 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 7.

Table 7: 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 up to 20 hours. Pembrolizumab solutions may be stored at room temperature for a cumulative time of up to 4 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 by 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 per Institutional policies.

8.2 Nab-Paclitaxel

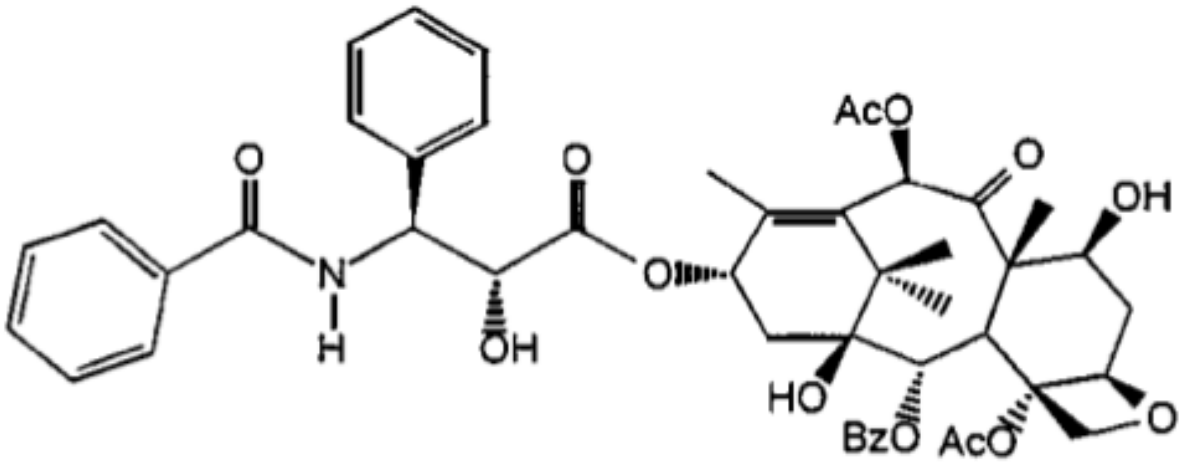
Please refer to the FDA-approved package insert for nab-paclitaxel for product information, extensive preparation instructions, and a comprehensive list of adverse events.

8.2.1 Description

ABRAXANE® for Injectable Suspension (paclitaxel protein-bound particles for injectable suspension) (albumin-bound) is an albumin- bound form of paclitaxel with a mean particle size of approximately 130 nanometers. Paclitaxel exists in the particles in a non crystalline, amorphous state. ABRAXANE® is free of solvents.

The active agent in ABRAXANE® is paclitaxel, a microtubule inhibitor. The chemical name for paclitaxel is 5β,20-Epoxy 1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine.

Paclitaxel has the following structural formula:



Paclitaxel is a white to off-white crystalline powder with the empirical formula C47H51NO14 and a molecular weight of 853.91. It is highly lipophilic, insoluble in water, and melts at approximately 216°C to 217°C.

8.2.2 **Form**

Nab-paclitaxel is available in single use vials containing 100 mg of paclitaxel as a lyophilized powder.

8.2.3 **Storage and Stability**

Store vials in original cartons at room temperature (20°C-25°C; 68°F-77°F). Retain the original package to protect from bright light. Unopened vials of albumin-bound paclitaxel are stable until the date indicated on the package when stored at the above temperature in the original package.

Reconstituted vials of nab-paclitaxel may be refrigerated at (2°C-8°C; 38°F-46°F) for a maximum of 8 hours and should be protected from bright light.

8.2.4 **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.

8.2.5 **Availability**

Nab-paclitaxel is commercially available and will be obtained from the Institutional pharmacy.

8.2.6 **Preparation**

Reconstitute each vial with 20 mL of 0.9% Sodium Chloride Injection, USP injected over at least 1 minute. Direct the NaCl onto the inside wall of the vial, and not directly onto the lyophilized cake, as this will result in foaming. Following reconstitution, allow the vial to sit for a minimum of 5 (five) minutes to ensure proper wetting of the lyophilized cake/powder. Gently swirl and/or invert the vial slowly for at least 2 minutes until complete dissolution of any cake/powder occurs. Rapid agitation or shaking will result in foaming. If foaming or clumping occurs, stand solution for at least 15 minutes until foam subsides. The reconstituted suspension should appear milky and homogeneous without visible particulates. If unsuspended powder is visible, the vial should be gently inverted again to ensure complete resuspension, prior to use. Each mL of reconstituted product will contain 5 mg of paclitaxel. Withdraw the desired volume and inject the suspension into an empty sterile PVC container.

Parenteral drug products should be inspected visually for particulate matter and discoloration before administration whenever the solution and container permit. The reconstituted sample should be milky and homogenous, without visible particulates. If particulates are visible or settling occurs, the vial should be gently inverted to ensure complete resuspension before use.

8.2.7 **Administration**

Nab-paclitaxel will be administered intravenous infusion over 30 minutes. Filters are not required for preparation or administration of nab-paclitaxel. If filters are used as part of institutional procedure, the pore size must be $\geq$ 15 micron.

8.2.8 **Ordering**

Nab-paclitaxel is commercially available and will be ordered per the standard practices of the Institutional pharmacy.

8.2.9 **Accountability**

Nab-paclitaxel is a commercially available product. Accountability will be handled per institutional polices regarding commercial products.

8.2.10 **Destruction and Return**

Nab-paclitaxel is a commercially available product. Destruction and return will be handled per institutional policies regarding commercial products.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

A baseline research biopsy (biopsy 1) prior to study therapy is required on this trial, in addition to two on-treatment biopsies (biopsy 2 and biopsy 3). Tissue will be collected at the time of surgery for research purposes. For patients who are determined to have evidence of clinically

significant residual disease by physical exam or imaging after completion of protocol treatment and who wish to continue with additional chemotherapy, an image-guided biopsy is mandatory prior to the initiation of additional therapy. In these cases, fresh and archival tissue at surgery will also be collected. All patients will also submit an archived tissue sample from their diagnostic biopsy and breast surgery. We plan to use this tissue to perform a number of immune profiling assays, detailed below. Background information on the pre-clinical and clinical rationale for these investigations is discussed in Section 2.5 and Section 2.6. On tumor tissue, we will perform characterization based on histology (TILs), protein expression, mRNA expression, and single cell RNA sequencing. Additionally, we will bank specimens for possible future DNA analysis, and other further testing.

If any of the biomarkers are inevaluable based on testing of tissue from biopsy 1, the assay may be attempted on the patient's archival tumor tissue, if feasible.

Four blood draws for correlative science are required on this trial; blood draws will be performed just prior to week 1 day 1 infusion of study drugs, just prior to week 3 day 1 infusion of study drugs, just prior to week 7 day 1 infusion of study, and at the time of the last dose of chemotherapy (or up to 2 weeks after the last dose of chemotherapy). On each blood draw, we will perform flow cytometry to characterize protein expression of immune mediators, detailed below, and additional blood will be stored for future testing.

All patients will additionally be asked to provide a stool sample at three separate timepoints: prior to treatment, after exposure to either nab-paclitaxel or pembrolizumab alone (after W1, both arms), and after exposure to both nab-paclitaxel and pembrolizumab together (after W4). 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 Requirements

All participants will have archival tissue collected at baseline from their diagnostic biopsy as well as from their surgical procedure.

9.1.1 Collection, handling, and shipping of archival tissue

Diagnostic biopsy and breast surgery archival tissue requirements: 20 unstained slides at 4-5um are required. For the surgical sample, a sample containing residual tumor or tissue from the tumor bed (in the case of a pathologic complete response) should be collected.

Tissue will be stored with the current CRC for this trial, but may at some point, be transitioned to storage within the DF/HCC Clinical Trials Core Laboratory or the Breast Tumor Immunology Lab (BTIL). Any samples leftover after correlative projects for this trial have been completed will become property of the DF/HCC Clinical Trials Core Laboratory (Dr. Deborah Dillon).

9.2 Procedures for obtaining breast tissue for study

9.2.1 Collection, handling, and shipping of non-surgical biopsy specimens

Research core biopsies of the primary breast lesion will be obtained at the indicated timepoints from all participants.

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 in advance to ensure that there will be adequate time for processing fresh tissue, since fresh tissue cannot be stored over the weekend. Specimens in RNALater and formalin should be stored at room temperature until shipment. Specimens in RNALater and formalin may be stored over the weekend and shipped on Monday.

It is mandatory that core biopsies be image-guided. A clip should be placed in the biopsy site at the time of the research biopsy if a clip was not placed during diagnostic biopsy. Pre- and post-procedure 90-degree lateral and craniocaudal mammogram is recommended to ensure that the correct lesion has been biopsied and to determine the relationship of the clip to the lesion that was visualized prebiopsy. Clip migration following biopsy has been reported and the distance from the original biopsy cavity can be measured[36]. If sufficiently far away from the biopsy cavity, then an addendum should be made to the report documenting that the clip should NOT be used to guide post-treatment tumor sampling. Experience with NeoALTTO and I-SPY1 suggest that taking four core biopsy samples from one area is feasible and acceptable to participants and ethics committees[37].

Ideally five core biopsies will be obtained:

- Two cores should be placed in 10% neutral buffered formalin tube.
- 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

For up to the next 12 patients enrolled, the order of specimen collection will be:

- First core: 10% neutral buffered formalin
- Second core: RPMI with Hepes
- Third core: RPMI with Hepes
- Fourth core: RNA later
- Fifth core: RPMI with Hepes

If additional cores are obtained, they should be processed as follows:

- Sixth core: RNAlater
- Seventh core: 10% neutral buffered formalin

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.
- Cores in sterile DMEM should be brought as fresh tissue immediately to the lab of [redacted]

  [redacted]

  This core must arrive to the lab to be processed for TILs (as described below) **within 1.5 hours of its collection, ideally** (though an additional 2 hour window is allowed). In addition, a small piece of this core will be immediately frozen in liquid nitrogen upon arrival to [redacted], for later use for RNA sequencing. [redacted] lab should be contacted ahead of time, ideally by approximately one week, to advise them of planned specimen delivery.

- Cores in formalin should be brought to the [redacted] (with the appropriate work order submitted and printed), where a block will be made. 5 positively charged, 75mm x 25mm x 1mm slides should be cut from the block at 4micron thickness to be sent to QualTek for PD-L1 IHC assay. Shipping details will be provided in a lab manual.

- Cores in RNAlater should be delivered or shipped to the [redacted] at the address provided here:

  [redacted]

  Please email the [redacted] with participant name, study ID, date of collection, approximate time of collection, and study time point the day prior to collection. Any tissue remaining after study-specific protocol testing occurs will be banked in the DF/HCC Clinical Trial Core Laboratory and may be used for additional or future analyses as needed.

Specific to the DFCI patients undergoing single-cell RNA sequencing:

- Three cores in RPMI with Hepes: should be brought immediately to the lab [redacted] for processing. Members of the [redacted] will collect these cores to deliver them to t[redacted] with study ID, study timepoint, tissue site, and collection date, location, and approximate time at least 24 hours prior to the collection.

9.2.2 Collection, handling, and shipping of surgical biopsy specimen

Obtaining fresh tissue from the surgical specimen

At the time of definitive surgery, the pathologist or pathology assistant will take core-sized pieces of tissue from the tumor or residual tumor bed (goal is at least two core-sized pieces of tissue; more or less are allowable per protocol, though a minimum of two is strongly preferred). Every effort should be made to obtain the sample as soon as possible after the time of resection.

- All samples should be de-identified and labeled with the Participant initials, Participant Study ID number and date of procedure.
- Obtain sample from the largest area of residual tumor whenever possible.

Surgical tissue should be placed in sterile DMEM in a specimen tube, and brought as fresh tissue immediately to the lab [redacted]

[redacted]

This sample must arrive to the lab to be processed for TILs (as described below) **within 1.5 hours of its collection, ideally** (though an additional 2 hour window is allowed). In addition, a small piece of this sample will be immediately frozen in liquid nitrogen upon arrival to Mariano Severgnini, for later use for RNA sequencing. If a surgical tissue biopsy must be performed on a Friday, 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

For up to the next 12 patients enrolled, the first, second, and fourth core-sized pieces of surgical tissue will be collected in RPMI with Hepes for single-cell RNA sequencing instead of the preparation listed above. The third and fifth core-sized pieces will be processed in RNA later, and any remaining core-sized pieces will be processed in sterile DMEM.

- First piece: RPMI with Hepes
- Second piece: RPMI with Hepes
- Third piece: RNA later
- Fourth piece: RPMI with Hepes

- Fifth piece: RNA later
- Remaining pieces: sterile DMEM

9.3 Tissue banking

All leftover tissue will be banked in the [redacted] as per standard lab protocol, such that it can be used for additional or future analyses as needed.

9.4 Procedures for obtaining blood specimens for study

Research blood collection is mandatory for all participants for flow cytometry and DNA isolation, both from cells and from cell-free DNA (cfDNA). The samples will be banked in the [redacted] 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 [redacted] 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 [redacted]

The following research blood samples are required:

Week 1 Day 1 (as close to administration of therapy as possible):

- 1-10 mL Streck Tube for whole blood
- 5- 10mL green top tubes for whole blood*

Week 3 Day 1 (within 3 days prior, ideally as close to Week 3 Day 1 as possible):

- 1-10 mL Streck Tube for whole blood
- 5- 10mL green top tubes for whole blood*

Week 7 Day 1 (within 3 days prior, ideally as close to Week 7 Day 1 as possible):

- 1-10 mL Streck Tube for whole blood
- 5- 10mL green top tubes for whole blood*

Preoperative Visit:

- 1-10 mL Streck Tube for whole blood
- 5- 10mL green top tubes for whole blood*

*If green top tubes are not available, purple top or CPT tubes can be substituted.

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," "Week 3," "Week 7," or "Preoperative visit").

Blood in green top tubes will then be hand carried at ambient temperature to [redacted]

[redacted]

Blood must be processed within 3-4 hours of its being drawn. The lab of [redacted] should be notified ahead of time (ideally by approximately a week) of blood deliveries.

Blood in Streck tubes will be handled as follows:

Fill the Streck tube completely and immediately mix by gentle inversion 8 to 10 times. Inadequate or delayed mixing may result in accurate results.

Tube precautions:

- 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.

Blood in Streck tubes should be brought (or shipped if applicable) to the [redacted]

Shipping Note (if applicable): Streck tubes are to be sent ambient. Frozen and ambient specimens obtained and shipped on the same day to the [redacted] 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, it is recommended that two shipping boxes should be used instead.

Every effort will be made to coordinate research blood draws with clinically-indicated blood tests to minimize additional venipuncture. Research coordinators will track participants closely and make every effort to collect blood at required timepoints. However, if research blood draws are missed or fall outside window due to the following circumstance they will not be considered deviations from the protocol.Unanticipated changes in treatment regimens and last-minute clinic visits. There are no plans to bring participants back to clinic solely for the purposes of a research blood draw. Instead, the study team should attempt to coordinate a missed research blood draw with the participant's next clinically indicated blood draw unless otherwise specified by the Overall PI.

9.5 Procedures for obtaining stool for specimens for study

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 (within 28 days of first dose)
- After exposure to Nab-paclitaxel or Pembrolizumab alone (after W1 dosing, but prior to W2 dosing)
- After exposure to Nab-paclitaxel and Pembrolizumab together (after W4 dosing, but prior to W5 dosing)
- 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 reference sheet for collection and processing instructions.

Samples will be stored at the [redacted] and will be shipped in batches by the biorepository to [redacted] for analysis.

9.6 Cell-free DNA (cfDNA) analysis

Blood will be collected in Streck tubes for evaluation of cell-free DNA (cfDNA), at timepoints described in Section 9.3. The cfDNA will be banked in the [redacted] laboratory for future research purposes. The banked samples will be used to analyze DNA, RNA and protein in future studies.

9.6.1.1 Collection of cfDNA specimen(s)

One 10 ml of whole blood will be collected in a Streck Tube. Fill the Streck tube completely and immediately mix by gentle inversion 8 to 10 times. Inadequate or delayed mixing may result in accurate results.

9.6.1.2 Handling and shipping of cfDNA specimens

Please follow the same tube precautions and delivery/shipping notes for Streck tubes provided in Section 9.3.

9.7 Blood banking

Any leftover blood will be banked in the lab [redacted] as per standard lab protocol respectively, such that it can be used for additional or future analyses as needed.

9.8 Hypotheses for secondary/correlative objectives

- We hypothesize that all ER+ breast tumors can be placed in a category of general "immune activity" level based on histologic, protein-based, and RNA-based analyses. We will assess this with (1) a "core" set of markers (PD-L1 expression; PD-L1 expression; PD-L2 expression; CD8 expression; and stromal TILs) and, (2) an expanded, exploratory set of markers.
- We hypothesize that an ER+ tumor's "immune activity" will increase over the course of treatment with nab-paclitaxel/pembrolizumab monotherapy, followed by combination therapy, but that the magnitude of this increase will be greatest when nab-paclitaxel therapy precedes combination therapy.
- We hypothesize that breast tumors with a higher level of "immune activity" at baseline, or a greater degree of increase from baseline to on-treatment tissue sampling, will have a higher likelihood of achieving more favorable RCB as well as pCR after treatment with both nab-paclitaxel and pembrolizumab.
- We hypothesize that the immune marker profile in the peripheral blood will change from baseline to surgery on nab-paclitaxel/pembrolizumab monotherapy or combination therapy.
- We hypothesize that a serially measured immune marker or composite of markers in the peripheral blood will correspond to changes in peri-tumoral immune activity as assessed on serial tumor biopsies.
- The **structure and function** of the gut microbiome before starting any treatment can be predictive of response to therapy, with a greater microbial diversity, estimated by Shannon index, being predictive of efficacy.
- The **structure and function** of gut microbiome changes in response to each treatment alone, and also in response to the combination of both Nab-paclitaxel and pembrolizumab therapy and can predict the likelihood of response to therapy.
- The abundance and functional profile of specific gut bacteria is associated with response to therapy.

9.9 Immunohistochemistry for primary endpoint (PD-L1 expression) and secondary/exploratory endpoints

Immunohistochemistry will be used to evaluate biomarkers for purposes of all primary and secondary objectives (see Section 1.2 and 1.3). Assessment of TILs by histology will also be a part of analyses of secondary objectives. The primary objective will be assessed based on immunohistochemistry for PD-L1.

PD-L1, as measured by immunohistochemistry (IHC), is an integral biomarker on this trial. The procedure for quantifying PD-L1 expression by IHC, as well as quantifying other exploratory biomarkers by IHC, is described below.

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 [redacted].

Formalin fixed-paraffin embedded (FFPE) tumor slides will be prepared and H&E stained for assessment of TIL in pre- and on-/post-treatment tumor samples. For the primary endpoint, PD-L1 expression will be assessed. 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)), IHC staining will be performed on FFPE tumor slices using some or all of the following antibodies:

Primary endpoint: PD-L1
Core set: CD8, PD-1, PD-L2
Others: CD3, CD4, CD25, FoxP3, Indoleamine 2,3 deoxygenase-1 (IDO1), CD11c, CD83, CD86, CD56, CD14, CD16, Tie2 (See also protocol lab manual)

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. PD-L1 will be tested centrally on each biopsy specimen for determination of the primary endpoint at a CLIA-approved laboratory (QualTek). Any additional IHC staining and panels used for correlative endpoints will be performed at Brigham and Women's Hospital.

Institutional 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.[38,39]

As part of the validation assays in a CLIA-certified laboratory, identical cases were stained multiple times and under a variety of staining conditions and the results reviewed by two certified pathologists. A positive control sample (classical Hodgkin Lymphoma for PD-L1 expression; primary mediastinal large B-cell lymphoma for PD-L2 expression) and negative control sample (benign lymph node) is stained with each experimental tissue biopsy sample. The controls are reviewed by a certified pathologist at the time of review of the experimental sample.

An IHC assay for PD-1 (CD279, clone NAT105, Cell Marque Inc.) expression has been in standard surgical pathology diagnostic practice for several years, used to confirm the diagnosis of antioimmunoblastic T-cell lymphoma (AITL).

PD-1 IHC is performed routinely in the CLIA-certified laboratory and interpreted by a certified pathologist with an appropriate control (reactive lymph node, intra-follicular T cells are positive for PD-1) as described above.

Chen et al[38] describe a semi-quantitative scoring method, which is in accordance with typical

biomarker scoring in anatomic and surgical pathology. Briefly, staining is scored according to intensity (0=no staining, 1=weak staining, 2=moderate staining, 3=strong staining), staining patter (M=predominantly cell membrane; C=predominantly cell cytoplasm), and the percentage of cells showing positive staining (0-100%). The product of the intensity and the percent of positive cells for a given marker is that marker's H-score. The semi-quantitative scoring is performed for: 1) the neoplastic tumor cells and 2) the non-neoplastic infiltrating immune cells. In the research setting, all cases are reviewed by two pathologists and any discordant results resolved by consensus review. Significant discordant results have been rare during case evaluations.[38]

Digital, quantitative scoring of stained tissue is performed using the Aperio slide scanning analysis platform. Quantitative assessment of positive staining uses the commercially provided algorithm for cell identification and positive pixels counted within a predefined DAB (brown, chromogenic) channel. It has been shown that this method of analysis showed good correlation with pathologists' scoring.[40] This method has been used to score PD-L1 expression in tumor cells.[41]

The scoring for markers (such as the PD-Ligands) that stain macrophages, dendritic cells, and other cells of heterogeneous morphology will be semi-quanititative and performed by a pathologist using a modified H-score to capture: 1) the percentage of neoplastic cells positive for biomarker expression, intensity of expression, and membrane or cytoplasmic expression, and 2) the percentage of non-neoplastic cells (macrophages, dendritic cells, endothelial cells) positive for biomarker expression, intensity of expression, and membrane or cytoplasmic expression.

Scoring for PD-1 and other markers that stain lymphoid cells (CD3, CE4, CD8, CD25, FOXP3, IDO, CD16, CD56, LAG-3, TIM-3) will primarily be performed by automated analysis using the Aperio system.

Aperio scoring for PD-1+ (and other lymphoid markers) lymphocytes will be accomplished using a standard Aperio algorithm, developed for quantifying nuclear stains, but found to be applicable to quantifying membrane staining of cells with a very high N:C ratio, such as lymphocytes (Nuclear algorithm). The output is number of positive-staining cells per unit area (micron^2).

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

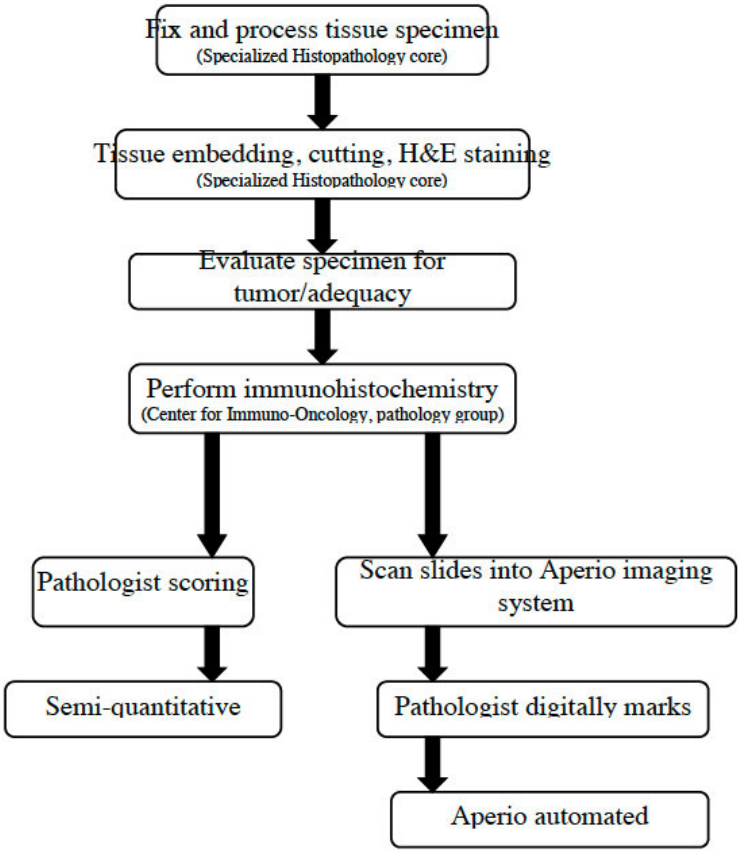
For image analysis:

1. IHC stained slides will be digitally scanned using the Aperio ScanScope XT ([redacted]). The instrumentation is housed in the [redacted]. This facility is located adjacent to the office of [redacted] All digital images are stored on servers owned by the TMI core facility and accessed via the internet using a password-protected login.
2. Digital images are viewed using ImageScope Software (version 10.0.35.1800; Lecia) on standard PCs. Slides are digitally annotated by the pathologist to identify the region of interest and analysis.
3. Quantitative analysis is performed using analytical software associated with ImageScope, specifically Aperio Color Deconvolution V.9 (for PD-Ligands) and nuclear algorithm (for PD-1+ lymphocytes) and the results given as the percentage of positive pixels per unit area

(for PD-Ligands) or number of positive cells per unit area (for PD-1+ lymphocytes). Intensity of staining is also captured automatically using the above algorithms and assigned a score (0, 1, 2, or 3) based upon the average optical density of the region or cells. All results are exported into an Excel spreadsheet.

4. Individual scoring data will be compared to clinical parameters to determine if there is an association with outcome. Scores using a combination of biomarker data will also be considered.

For overall biomarker analysis:



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 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.

Overall, for purposes of PD-L1 assessment for the primary objective of this trial: PD-L1 staining will be quantified as described, via visual evaluation by two separate pathologists to calculate the H-score for PD-L1 on tumor cells.

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

manual.

It should be noted that the above staining protocols are based on standard methods used at the time of protocol writing. It is possible that at the time protein expression assays are conducted, novel and improved methods for staining or its quantification will exist. In this case, we plan to use the best available, best validated experimental method available at the time for all secondary and exploratory analyses. This does not apply to the analysis of PD-L1 expression by H-score for purposes of the primary endpoint, which will be carried out as described above.

9.10 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.[22]

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.

Again, it should be noted that the above TIL assessment methods are based on standard methods used at the time of protocol writing. It is possible that at the time assessments are conducted, there will be a different, accepted method of TIL quantification. In this case, we plan to use the best available, best validated method available at the time for all secondary and exploratory analyses.

9.11 Exploratory correlative analyses of tumor tissue

9.11.1 Flow cytometry of tumor infiltrating lymphocytes

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

Surface staining followed by flow cytometry on the resultant TILs will then be performed as described in the protocol 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
(Other antibodies as listed in the protocol lab manual)

9.11.2 RNA and DNA analysis

RNA analysis will be performed, and tissue for RNA analysis will be stored, in the [redacted]

Messenger RNA (mRNA) expression within tumor biopsy specimens will 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,[18] include CXCL9, CCL5, CD8ACD80, CXCL13, IGKC, CD21, IDO1, PD-1, PD-L1, PD-L2, CTLA4, and FOXP3.

Additional RNA analysis, and specifically use of single cell RNA sequencing techniques, will be performed in collaboration with Dr. Eliezer Van Allen at the Broad Institute. DNA analysis may also be performed to assess parameters such as mutational load and neoantigen burden.

For a subset of patients, single-cell RNA sequencing will be performed on fresh tumor biopsies and surgical tissue. These studies will determine whether changes in RNA expression signatures at the level of individual tumor and immune cells correlate with treatment response and resistance. The analyses will also investigate whether particular immune cell subsets are enriched in responders compared to non-responders, as suggested by studies in other tumors treated with checkpoint inhibitors.

9.12 Exploratory correlative analyses of peripheral blood: flow cytometry to characterize immune markers in peripheral blood mononuclear cells (PBMCs) prior to, during, and after therapy with nab-paclitaxel and pembrolizumab

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

Surface staining with a panel of antibodies (see below, and protocol lab manual) and flow cytometry on PBMCs will then be performed as described in the protocol 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

9.13 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. Some of the above-mentioned markers for secondary/correlative objectives may be eliminated, if they are no longer deemed biologically relevant at the time analyses are performed.

9.14 Analysis of DNA extraction from stool samples

Under the supervision of [redacted], total genomic DNA will be isolated from 0.25g of feces using the PowerSoil DNA isolation kit (Mo Bio, USA) [redacted]. Purified DNA will be separated on a 1% agarose gel and quantified by densitometry and spectrophotometry (NanoDrop 1000; Thermo Scientific, USA).

9.15 Analysis of RNA Extraction from stool samples

Under [redacted] supervision, 16S rRNA analysis will be performed at The V4–V5 region of the 16S rRNA gene will be amplified and sequenced on an Illumina MiSeq platform at [redacted] For each stool sample, replicate PCR reactions will be performed using modified universal bacterial primers designed to amplify the V4-V5 16S rRNA region: 563F (59-nnnnnnnn-NNNNNNNNNNNN-AYTGGGYDTAAAGN G-39) and 926R (59-nnnnnnnn-NNNNNNNNNNNN-CCGTCAATTYHTTTR AGT-39).

Using the Illumina TruSeq Sample Preparation procedure, PCR products will be quantified and pooled at equimolar amounts before Illumina barcodes and adaptors will be ligated on. The completed library will be sequenced on an Ilumina Miseq platform according to the Illumina recommended protocol. Sequences will be analysed using mothur version 1.31.115. Sequences were aligned using the Silva reference alignment as a template and potentially chimeric sequences were eliminated using the UChime algorithm16. Five thousand sequences per patient were selected (mean 4,974, s.d. 150) and sequences with a distance-based similarity of Z97% were grouped into OTUs using the furthest-neighbour algorithm. OTUs were classified using the Greengenes 16S rRNA reference database. OTU-based microbial diversity was estimated by calculating two diversity indices, Shannon and Inverse Simpson. OTU-based richness was determined by calculating the Chao richness estimate and constructing rarefaction curves. OTUs were grouped at different levels of classification (phylum, class, order, family and genus); at each level, OTUs that did not have a classification were grouped together by the highest available resolution. Feature selection of the intestinal microbia's composition was performed on OTUs with an average abundance 40.01% in either patient group and grouped by phylotype.

9.16 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.

9.17 Site Performing Correlative Studies

The IHC staining will be conducted in a research laboratory by [redacted] is a hematopathologist at [redacted] with prior expertise evaluating PD-1, PD-L1, and other immunologic markers in paraffin embedded tumor samples, and he has many prior publications in this area. Flow cytometry assessment of both tissue and blood will be performed by [redacted], who has performed similar assays on many specimens collected on clinical trials of melanoma. The [redacted] will be receiving, processing, and storing stool samples. They will eventually be shipped in batches to [redacted] laboratory ([redacted] for analysis.

10. STUDY CALENDAR

Baseline evaluations are to be conducted within **28 days** prior to treatment start (except for pregnancy test and baseline tumor biopsy, as detailed below). If these screening assessments occur within 3 days before start of study treatment, then they may serve as the baseline Week 1 Day 1 values. Baseline radiographic assessment is required within **28 days** prior to starting protocol therapy.

As detailed in the Study Calendar, a negative pregnancy test in women of child-bearing potential must be documented within **7 days** before the first dose of study medication.

A baseline tumor biopsy, obtained within **14 days** before starting protocol therapy, is also 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 week 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.

COHORT A CALENDAR

	Pre-study[a]	W1 D1	W2 D1	W3 D1	W4 D1	W5 D1	W6 D1	W7 D1	W8 D1	W9 D1	W10 D1	W11 D1	W12 D1	W15 D1	Surgery[m]	Post-surgery	Follow-up
Informed consent	X																
Demographics	X																
Medical history	X																
Performance status[b]	X																
Concomitant medications	X	X		X			X			X			X	X		X	
Adverse event evaluation		X		X			X			X			X	X		X	
Physical exam[f]	X	X		X			X			X			X	X		X	
Vital signs (including weight/height)[p]	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	
Hematology (CBC with diff) and, chemistry panel[q]	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	
PT/PTT	X																
TSH/free T4		X		X			X			X			X	X			
Cortisol[s]	X			X			X			X			X	X			
Pregnancy test[c]	X																
EKG	X																
Mammogram, breast US, or breast MRI[e]	X													X[r]			
CT scan (chest/abd/pelvis) and bone scan or PET-CT scan[g]	X																
Surgical assessment[h]	X													X[r]			
Axillary assessment[i]	X																
Nab-paclitaxel administration		X	X	X	X	X	X	X	X	X	X	X	X				
Pembrolizumab administration				X			X			X			X	X			
Image-guided Research biopsy[d,n]	X[d]			X[d]				X[d]							X[n]		
Research Blood collection[j]	X			X				X						X[r]			
Research Stool Collection[t]	X	X		X													
Stool Questionnaire[u]	X	X		X													
Archival tumor tissue[o]	X														X		
Assessment for residual cancer burden[k]															X		
Assessment for progressive disease/survival[i]																	X

COHORT B CALENDAR

	Pre-study[a]	W1 D1	W3 D1	W4 D1	W5/6 D1	W7 D1	W8/9 D1	W10 D1	W11/12 D1	W13 D1	W14 D1	Surgery[m]	Post-surgery	Follow-up
Informed consent	X													
Demographics	X													
Medical history	X													
Performance status[b]	X													
Concomitant medications	X	X		X		X		X		X			X	
Adverse event evaluation		X		X		X		X		X			X	
Physical exam[f]	X	X		X		X		X		X			X	
Vital signs (including weight/height)[p]	X	X	X	X	X	X	X	X	X	X	X		X	
Hematology (CBC with diff) and chemistry panel[q]	X	X	X	X	X	X	X	X	X	X	X		X	
PT/PTT	X													
TSH/free T4		X		X		X		X		X				
Cortisol[s]	X		X		X		X		X		X			
Pregnancy test[c]	X													
EKG	X													
Mammogram, breast US, or breast MRI[e]	X										X[r]			
CT scan (chest/abd/pelvis) and bone scan or PET-CT scan[g]	X													
Surgical assessment[h]	X										X[r]			
Axillary assessment[i]	X													
Nab-paclitaxel administration			X	X	X	X	X	X	X	X	X			
Pembrolizumab administration		X		X		X		X		X				
Image-guided Research biopsy[d,n]	X[d]		X[d]			X[d]						X[n]		
Research Blood collection[j]	X		X			X					X[r]			
Research Stool Collection[t]	X	X		X										
Stool Questionnaire[u]	X	X		X										
Archival tumor tissue[o]	X											X		
Assessment for residual cancer burden[k]												X		
Assessment for progressive disease/survival[l]														X

Abbreviations: W: Week; D: Day

a. Baseline evaluations are to be conducted within 28 days prior to start of protocol therapy unless otherwise noted. If screening assessments occur within 3 days before start of study treatment, then they may serve as the Week 1 Day 1 values.
b. See Appendix A.
c. In female participants of child-bearing potential, urine or serum pregnancy test must be performed within **7 days** before the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, then a serum test is required.
d. Baseline research biopsy obtained within 14 days before starting protocol therapy is required. See Section 5.2.4 for more information regarding the timing of the biopsies and Section 9.1.1 for biopsy handling and processing instructions. A biopsy should also be obtained in participants who go off study for progressive disease, or in whom the treating physician chooses to administer additional non-protocol therapy prior to surgery.
e. See Section 5.2.4 regarding breast imaging recommendations. Affected breast imaging that measures the tumor must be done within **28 days** prior to enrollment; MRI is strongly recommended, although other imaging modalities (mammogram, ultrasound) are permitted if practical or financial considerations preclude MRI, as long as the target lesion can be adequately measured. This same imaging modality should be performed again prior to surgery (**2-3 weeks** after the last dose of study treatment) to assess tumor response.
f. Tumor measurements should be ascertained by physical exam (not required at Post-Surgery visit)..
g. It is recommended, but not required that participants with anatomic Stage IIa disease and above (AJCC 8th edition) will have CT scans of chest, abdomen and pelvis and bone scans or PET-CT scans performed during screening to rule out metastatic disease.
h. All participants will be seen and examined by the treating surgeon at Screening and at the Pre-Operative visit. See Section 5.2.4 for further details of the surgical assessment.
i. See guidelines for axillary assessment in Section 5.2.4.
j. See Section 9.3 for specific timing of research blood draws and handling and processing instructions.
k. Assessment of residual cancer burden (RCB) is described in Section 11.5.

l. Post-surgical follow-up information will be collected at assessments every 6 months until 5 years after surgery. See Section 5.7. No tests or procedures are required during follow-up; however clinical data will be collected on CRFs. These data should be based on exams, tests, or procedures done at the registering institution or at a local facility.
m. Surgery should take place no more than **42 days** from the last dose of study treatment.
n. The procedure for surgical collection is described in Section 9.1.2.
o. Patients will submit an archived sample from their diagnostic biopsy and from their surgery.See Section9.0. Can be obtained at any time during the life of the trial and does not have to be performed as a screening or surgery assessment.
p. Height needed at week 1 day 1 only. Weight not required at Pre-Surgery, Surgery, or Follow-up visits. See sections 5.2.2 and 5.2.3 regarding dosing changes for weight fluctuation while on therapy.
q. Sodium, potassium, chloride, bicarbonate, BUN, creatinine, total protein, albumin, total bilirubin, SGOT (AST), SGPT (ALT), Alkaline Phosphatase. PT and PTT at screening only.
r. Pre-surgical assessments to be done at the time of the last dose of chemotherapy or within 2 weeks after the last dose of protocol therapy.
s. Cortisol to be drawn at baseline, W3D1, W6D1, W9D1, W12D1, and W14D1 on Cohort B.
t. Baseline stool collection should be obtained within 28 days before starting protocol therapy. The W1 stool collection should be performed after dosing on W1, but prior to dosing on W2. The W4 stool collection should be performed after dosing on W4, but prior to dosing on W5. 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 stool collection guidance document for more details.
u. 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.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

A baseline and pre-surgical radiographic study of the breast is required; MRI is recommended. The same radiographic modality should be used consistently. The baseline scan must be obtained within 28 days of beginning therapy. The pre-surgical imagining should occur at the time of the last dose ofchemotherapy or within 2 weeks after the last dose of chemotherapy. If the participant clinically progresses, repeat imaging is required. If there is discordance (clinical progression, but radiographic stable disease or response), contact the study chair.

11.2 Radiographic assessment

Each participant will have pre- and post-therapy radiographic tumor measurements, preferably by MRI, however if logistic or practical issues preclude MRI use, mammogram or ultrasound may be substituted. The longest diameter (LD) of the target lesion at the time of study initiation will be reported as the baseline LD. The baseline LD of the target lesion will be used as reference to further characterize the objective tumor response of the measureable dimension of the disease.

Response criteria are based on the RECIST 1.1 criteria:

Radiographic Complete Response (CR): Complete disappearance of the target lesion

Radiographic Partial Response (PR): Greater than or equal to 30% decrease in the longest diameter (LD) of the target lesion taking as reference the baseline LD.

Radiographic Progressive Disease (PD): Greater than or equal to 20% increase in the LD of target lesion taking as reference the baseline LD or the appearance of one or more new lesions.
Radiographic Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as reference the baseline LD

11.3 Other radiographic response parameters: Immune-Related Response Criteria (irRECIST)

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

irRECIST is RECIST 1.1 adapted as described below to account for the unique tumor response seen with immuno-therapeutics.

irRECIST may be used by site investigators and local radiology review to assess tumor response and progression, and make treatment decisions. This data will be collected in the clinical database.

irRECIST takes into account the clinical condition/stability of participants, as described in the table below, in addition to response or progression via tumor imaging.

Clinically stable is defined by the following criteria:

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

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

	Clinically Stable		**Clinically Unstable**	
	Tumor Imaging	**Treatment**	**Tumor Imaging**	**Treatment**
1st radiologic evidence of PD	Repeat tumor imaging at ≥ 4 weeks at site to confirm PD	May continue study treatment at the site Investigator's discretion while awaiting confirmatory scan by site	Repeat tumor imaging at ≥ 4 weeks to confirm PD per physician discretion only	Discontinue treatment
Repeat tumor imaging confirms PD	No additional tumor imaging required	Discontinue treatment (exception is possible upon consultation with Sponsor).	No additional tumor imaging required	N/A
Repeat tumor imaging shows SD, PR or CR	Continue regularly scheduled tumor imaging assessments	Continue study treatment at the siteInvestigator's discretion	Continue regularly scheduled tumor imaging assessments	May restart study treatment if condition has improved and/or clinically stable per site Investigator's discretion. Next tumor imaging should occur according to the every 9 week (63 ± 7 days) imaging schedule in the first year or every 12 weeks after one year.

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

Any subject deemed clinically unstable should be discontinued from trial treatment at first

evidence of progressive disease by tumor imaging and is not required to have repeat tumor imaging for confirmation.

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

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

The same imaging modality (i.e., CT or MRI), acquisition and technical parameters should be used throughout the study for a given subject.

11.4 Clinical assessments

Both target and, in the event of multifocal or multicentric invasive cancer, nontarget lesions should be followed clinically and their clinical size recorded at baseline. Measurements thereafter are required; these lesions should be categorized at subsequent visits regarding whether there is evidence of progression. If "yes", the study chair should be notified in order to determine whether the participant should come off protocol treatment.

11.5 Pathologic Response

Pathologic response will be reported using the Residual Cancer Burden[11] calculator from M.D Anderson: [redacted]

The following parameters are required from pathologic examination in order to calculate Residual Cancer Burden (RCB) after neoadjuvant treatment:

- The largest two dimensions (mms) of the residual tumor bed in the breast (largest tumor bed if multicentric disease)
- Histologic assessment of the percentage of the tumor bed area that contains carcinoma (all carcinoma, i.e. invasive and in situ), select one of the following: 0%, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%
- To assess cellularity it is helpful to scan across the sections of tumor bed and then estimate the average cellularity from the different microscopic fields.
- When estimating percentage cancer cellularity in any microscopic field, compare the involved area with obvious standards, e.g. more or less than half, one quarter, one fifth, one tenth, one twentieth, etc.

- Expect there to be variable cellularity within the cross section of any tumor bed, but estimate the overall cellularity from the average of the estimates in different microscopic fields of the tumor bed. E.g., if cellularity in different fields of the tumor bed were estimated as 20%, 10%, 20%, 0%, 20%, 30%, then an average estimate of overall cellularity would be 20%.
- Histologic estimate of the percentage of the carcinoma in the tumor bed that is in situ, select one of the following: 0%, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%
- The number of positive (metastatic) lymph nodes
- The largest diameter (mm) of the largest nodal metastasis

For the purpose of this study pCR will be defined as RCB=0.

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

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 according to the schedule set by ODQ.

12.2 Data Safety Monitoring

The [redacted] 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 Research and Future Use of Data and Biospecimens

Tissue, blood, stool, bodily fluids, and other materials derived from these will be collected in this study to analyze genes, DNA, RNA, proteins and cells for the study's correlative endpoints and potential future research, utilizing new types of biomarker testing as it becomes available.

These samples and any data generated as a part of these clinical trials may be used for future research studies and may be provided to collaborating investigators both within and outside of the DF/HCC for either correlative endpoints or secondary use. Samples and data may be shared with outside non-profit academic investigators, as well as with for-profit pharmaceutical investigators or commercial entities, with whom we collaborate. When samples or data are sent to collaborators and when any research is performed on them, all information will be identified with a code, and will not contain any PHI, such as name, birthday, or MRNs.

In order to allow the greatest amount of research to be performed on the specimens and information generated as a part of this trial, researchers in this study may share results of genetic sequencing with other scientists. De-identified specimen or genetic data may be placed into one of more publicly-accessible scientific databases, such as the National Institutes of Health's Database for Genotypes and Phenotypes (dbGaP). The results from the correlative research on this study will be shared with these public databases. Through such databases, researchers from around the world will have access to de-identified samples or data for future research. More detailed information, beyond the public database, may only be accessed by scientists at other research centers who have received special permission to review de-identified data.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

This is a randomized open label study examining biomarker changes over the course of treatment with nab-paclitaxel, pembrolizumab, or both in HR+ breast cancer. Participants will be accrued in one stage with completion of a safety analysis of the first 6 patients per arm. Participants will be registered and randomized by the Office of Data Quality (ODQ). Participants will be randomized 1:1 to receive a run-in of either two weeks of nab-paclitaxel (cohort A) or one dose of pembrolizumab (cohort B) monotherapy. Participants will then receive combination nab-paclitaxel (administered weekly) and pembrolizumab (administered every 3 weeks) for a total of 15 weeks (cohort A) or 14 weeks (cohort B). At completion, each patient will have received 12 doses of weekly nab-paclitaxel, and 5 doses of every-3-week pembrolizumab. Three research biopsies are required over the course of therapy: one at pre-treatment baseline (biopsy 1), one post-monotherapy (biopsy 2), and one post-doublet therapy (biopsy 3). Additional tissue collection at surgery will be performed if residual disease is present.

All participants who receive at least one dose of either study drug will be evaluated for the biomarker and clinical endpoints.

Primary endpoint:

- Change in PD-L1 expression by immunohistochemistry from baseline biopsy (biopsy 1) to biopsy after 2-week treatment with either nab-paclitaxel or pembrolizumab (biopsy 2), assessed as a dichotomous variable (H-score ≥ 100 versus 0-99). Secondary analysis of the endpoint will evaluate change in PD-L1 expression as a continuous variable (absolute change in H-score).

Secondary endpoints:

Secondary biomarker endpoints

- The absolute change in expression of core immune biomarkers (stromal TILs;) from baseline biopsy (biopsy 1) to biopsy after 2-week treatment with either nab-paclitaxel or pembrolizumab (biopsy 2).

Secondary safety endpoint

- Safety and tolerability profile: maximum grade of all treatment-related adverse events using CTCAE v4.0

Secondary efficacy endpoints

- Pathologic complete response rate, defined as RCB 0,[11] following treatment with combination nab-paclitaxel and pembrolizumab in the neoadjuvant setting for HR+ breast cancer.
- Overall response rate, assessed radiographically by both RECIST 1.1 and irRECIST, following treatment with combination nab-paclitaxel and pembrolizumab in the neoadjuvant setting
- Disease-free survival following treatment with combination nab-paclitaxel and pembrolizumab in the neoadjuvant setting for HR+ breast cancer.

Blood and Tissue correlative science endpoints

- The absolute change in PD-L1 expression by immunohistochemistry from baseline biopsy (biopsy 1) to biopsy after treatment with nab-paclitaxel or pembrolizumab monotherapy, followed by doublet therapy (biopsy 3, and surgery in participants with residual disease) , assessed as a dichotomous variable (H-score ≥ 100 versus 0-99) and as a continuous variable (absolute change in H-score)
- The absolute change in expression of core immune biomarkers (stromal TILs) from baseline biopsy (biopsy 1) to biopsy after treatment with nab-paclitaxel or pembrolizumab monotherapy, followed by doublet therapy (biopsy 3, and surgery in participants with residual disease).
- Characterization of an expanded set of immune biomarkers (based on histology, protein expression, and mRNA expression) in HR+ breast tumors both pre-treatment and on serial biopsies.
- Characterization of the immune biomarker profile in PBMCs both pre-treatment and on

serial blood draws.

Stool and Microbiome correlative science endpoints

Overall, we plan to describe the landscape of gut microbiota in patients with BC who will receive pembrolizumab plus Nab-paclitaxel, and the changes in their gut microbiota after each treatment alone, and after the combined regimen. 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 PD-L1 change 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.

An initial safety review is planned for each cohort. Accrual will be paused after 6 participants are enrolled on each arm and a formal monitoring of safety will be conducted when the 6 participants per arm have completed the period of DLT evaluation. If >1 patient in either arm develops a dose limiting toxicity, enrollment will be halted and there will be a discussion with the DSMC to determine whether modifications are required to proceed with the study. The following table gives the probability of continuing after 6 patients under varying true rates of dose-limiting toxicity.

	Dose acceptance using 6 subjects						
True DLT rate	0.1	0.2	0.3	0.4	0.5	0.6	0.7
Probability	89%	66%	42%	23%	11%	4%	1%

13.2 Sample Size, Accrual Rate and Study Duration

The target accrual is 50 participants (25 per arm). The sample size was chosen to have adequate

power for detecting changes in PD-L1 expression within each arm. The null hypothesis is no change in the amount of PD-L1 expression from baseline to after a two-week run in of nab-paclitaxel or pembrolizumab, and the primary analysis will be tested in each arm using one-sided alpha (type I error) of 0.05 using a McNemar's test (changes from H-score 0-99 to ≥100). As a secondary analysis, a Wilcoxon signed rank test will be used to evaluate absolute change in H-score using an alpha (type I error) of 0.05. With an anticipated 15% assay failure rate, 25 patients per arm will provide 21 evaluable paired specimens for each treatment. Under the assumption that positive PD-L1 expression occurs in 20.1% of cases,[28] and that there is a 0.1% probability of PD-L1 expression changing from positive to negative, there will be 80% power to see an increase in PD-L1 positive expression to 50% of the cases after the two-week run-in of monotherapy. Power calculations for the exact non-parametric test for small sample sizes was performed in East v6.3 (Cytel Inc.).

Expected accrual is 0-1 participants per month; therefore, it will take approximately 75 months to complete accrual. An additional 6 months of follow-up will be required after the last participant is accrued to cover the time from initial enrollment to the first post-surgical follow-up visit, for a total study duration of approximately 80 months.

13.3 Stratification Factors

No stratification factors are planned.

13.4 Interim Monitoring Plan

During the safety run-in phase, the first 6 patients within a dosing cohort need to complete 21 days of treatment and the safety data will be reviewed before enrolling additional patients. Toxicity will also be monitored by the DF/HCC DSMC as described in Section 12.2

13.5 Analysis of Primary Endpoints

Descriptive statistics will be used to summarize the distribution of H-scores of PD-L1 expression by immunohistochemistry that are observed at baseline and after 2 weeks of monotherapy (e.g. mean, standard deviation, median, and inter-quartile range). The primary evaluation of change in PD-L1 expression within each arm will be based on an exact McNemar's test (H-score 0-99 versus 100) and Wilcoxon signed rank test (absolute change in H-score) using a one-sided alpha = 0.05 for each test. No statistical inferences will made to compare the change in PD-L1 expression across arms.

13.6 Analysis of Secondary Endpoints

Secondary endpoints of additional core immune biomarkers and of changes in all markers from baseline to post-neoadjuvant treatment will similarly use descriptive statistics and test for changes within arm using the Wilcoxon signed rank test with two-sided alpha = 0.05. Exploratory analyses will evaluate whether log fold-change in marker levels removed skewness in the empirical distribution of changes from baseline. In addition, exploratory analysis will evaluate the Spearman

correlation in marker levels at each timepoint and changes from baseline. With an anticipated 15% assay failure rate, 42 evaluable paired specimen are anticipated for detecting changes in biomarker levels from baseline to post-neoadjuvant treatment. This will provide 90% power to detect a 0.47 shift in standardized units under an assumption of an additive shift model with a Gaussian common density function.

For the secondary safety endpoint, treatment-related toxicities will be summarized by maximum grade and by term using CTCAE v4.0 and reported with 90% binomial exact confidence intervals.

The overall pCR rate will be reported using 95% Binomial exact confidence intervals, and RCB levels will be summarized for all patients using frequency tables. With 50 patients receiving protocol treatment, the maximum width of any confidence interval will be 0.21. To explore the association of pCR to baseline marker levels, and changes from baseline in biomarkers, 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.

Disease-free survival will be summarized using Kaplan-Meier estimates and 95% confidence bands.

Single-cell RNA sequencing will be performed on fresh tumor biopsies and surgical tissue for a subset of patients to assess the relationship of timepoint-specific tumor and immune cell subpopulations with clinical outcomes in exploratory analyses. In addition, changes in single-cell RNA expression signatures from baseline will be correlated with response. Standard preprocessing and quality control algorithms will be performed to filter out low quality cells and to normalize the expression data. Differentially expressed genes will be identified using the Benjamini-Hochberg procedure to control the false discovery rate for multiple comparisons. Descriptive statistics and clustering techniques, including principal component analysis and t-distributed stochastic neighbor embedding, will be employed to summarize the distributions of the expression data and identify tumor and immune cell subpopulations. Changes in cell subpopulations between timepoints will be assessed with Wilcoxon signed-rank tests, while differences in cell subpopulations between responders and non-responders will be compared with Mann-Whitney tests. Preliminary associations of tumor and immune cell subpopulations with disease-free survival will be explored using Cox proportional hazard models.

13.7 Reporting and Exclusions

13.7.1 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time of their first treatment. Participants who never start protocol therapy will be considered inevaluable.

13.7.2 Evaluation of the Primary Efficacy Endpoint

All Participants who receive at least one dose of either study drug will be evaluable for efficacy endpoints. Participants who never start protocol therapy will be considered inevaluable and will be replaced. Biomarker analyses will be conducted in all Participants with evaluable biospecimen

within arm that is defined by the treatment received (per protocol).

14. PUBLICATION PLAN

The Primary Investigator will be the final arbiter of the manuscript content.

REFERENCES

1. Fisher B, Bryant J, Wolmark N, Mamounas E, Brown A, Fisher ER, Wickerham DL, Begovic M, DeCillis A, Robidoux A, Margolese RG, Cruz AB, Jr., Hoehn JL, Lees AW, Dimitrov NV, Bear HD. Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. J Clin Oncol 1998;16:2672-85.

2. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, Patnaik A, Aggarwal C, Gubens M, Horn L, Carcereny E, Ahn MJ, Felip E, Lee JS, Hellmann MD, Hamid O, Goldman JW, Soria JC, Dolled-Filhart M, Rutledge RZ, Zhang J, Lunceford JK, Rangwala R, Lubiniecki GM, Roach C, Emancipator K, Gandhi L, Investigators K-. Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med 2015;372:2018-28.

3. Hamid O, Chasalow SD, Tsuchihashi Z. Association of baseline and on-study tumor biopsy markers with clinical activity in patients (pts) with advanced melanoma treated with ipilimumab. J Clin Oncol 2009;27:A9008.

4. Berman DM, Wolchok J, Weber J. Association of peripheral blood absolute lymphocyte count (ALC) and clinical activity in patients (pts) with advanced melanoma treated with ipilimumab. J Clin Oncol 2009;27:A3020.

5. Yardley DA. nab-Paclitaxel mechanisms of action and delivery. J Control Release 2013;170:365-72.

6. Gradishar WJ, Tjulandin S, Davidson N, Shaw H, Desai N, Bhar P, Hawkins M, O'Shaughnessy J. Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer. J Clin Oncol 2005;23:7794-803.

7. Untch M, Jackisch C, Schneeweib A, Conrad B, Aktas B, Denkert C, Eidtmann H, Wiebringhaus H, Kummel S, Hilfrich J, Warm M, Paepke S, Just M, Hanusch C, Hackmann J, Blohmer J-U, Clemens M, Costa SD, Gerber B, Nekljudova V, Loibl S, von Minckwitz G. A randomized phase III trial comparing neoadjuvant chemotherapy with weekly nanoparticle-based paclitaxel with solvent-based paclitaxel followed by anthracycline/cyclophosphamide for patients with early breast cacner (GeparSepto). 2014:abstr PD2-6.

8. Berry DA, Cirrincione C, Henderson IC, Citron ML, Budman DR, Goldstein LJ, Martino S, Perez EA, Muss HB, Norton L, Hudis C, Winer EP. Estrogen-receptor status and outcomes of modern chemotherapy for patients with node-positive breast cancer. JAMA 2006;295:1658-67.

9. Colleoni M, Montagna E. Neoadjuvant therapy for ER-positive breast cancers. Ann Oncol 2012;23 Suppl 10:x243-8.

10. Martin M, Romero A, Cheang MC, Lopez Garcia-Asenjo JA, Garcia-Saenz JA, Oliva B, Roman JM, He X, Casado A, de la Torre J, Furio V, Puente J, Caldes T, Vidart JA, Lopez-Tarruella S, Diaz-Rubio E, Perou CM. Genomic predictors of response to doxorubicin versus docetaxel in primary breast cancer. Breast Cancer Res Treat 2011;128:127-36.

11. Symmans WF, Peintinger F, Hatzis C, Rajan R, Kuerer H, Valero V, Assad L, Poniecka A, Hennessy B, Green M, Buzdar AU, Singletary SE, Hortobagyi GN, Pusztai L. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol 2007;25:4414-22.

12. Zitvogel L, Apetoh L, Ghiringhelli F, Andre F, Tesniere A, Kroemer G.

The anticancer immune response: indispensable for therapeutic success? J Clin Invest 2008;118:1991-2001.

13. Carson WE, 3rd, Shapiro CL, Crespin TR, Thornton LM, Andersen BL. Cellular immunity in breast cancer patients completing taxane treatment. Clin Cancer Res 2004;10:3401-9.

14. Lu L, Xu X, Zhang B, Zhang R, Ji H, Wang X. Combined PD-1 blockade and GITR triggering induce a potent antitumor immunity in murine cancer models and synergizes with chemotherapeutic drugs. J Transl Med 2014;12:36.

15. Demaria S, Volm MD, Shapiro RL, Yee HT, Oratz R, Formenti SC, Muggia F, Symmans WF. Development of tumor-infiltrating lymphocytes in breast cancer after neoadjuvant paclitaxel chemotherapy. Clin Cancer Res 2001;7:3025-30.

16. Zitvogel L, Galluzzi L, Smyth MJ, Kroemer G. Mechanism of action of conventional and targeted anticancer therapies: reinstating immunosurveillance. Immunity 2013;39:74-88.

17. Salgado R, Denkert C, Campbell C, Savas P, Nucifero P, Aura C, de Azambuja E, Eidtmann H, Ellis CE, Baselga J, Piccart-Gebhart MJ, Michiels S, Bradbury I, Sotiriou C, Loi S. Tumor-Infiltrating Lymphocytes and Associations With Pathological Complete Response and Event-Free Survival in HER2-Positive Early-Stage Breast Cancer Treated With Lapatinib and Trastuzumab: A Secondary Analysis of the NeoALTTO Trial. JAMA Oncol 2015;1:448-454.

18. Denkert C, von Minckwitz G, Brase JC, Sinn BV, Gade S, Kronenwett R, Pfitzner BM, Salat C, Loi S, Schmitt WD, Schem C, Fisch K, Darb-Esfahani S, Mehta K, Sotiriou C, Wienert S, Klare P, Andre F, Klauschen F, Blohmer JU, Krappmann K, Schmidt M, Tesch H, Kummel S, Sinn P, Jackisch C, Dietel M, Reimer T, Untch M, Loibl S. Tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2-positive and triple-negative primary breast cancers. J Clin Oncol 2015;33:983-91.

19. Issa-Nummer Y, Darb-Esfahani S, Loibl S, Kunz G, Nekljudova V, Schrader I, Sinn BV, Ulmer HU, Kronenwett R, Just M, Kuhn T, Diebold K, Untch M, Holms F, Blohmer JU, Habeck JO, Dietel M, Overkamp F, Krabisch P, von Minckwitz G, Denkert C. Prospective validation of immunological infiltrate for prediction of response to neoadjuvant chemotherapy in HER2-negative breast cancer--a substudy of the neoadjuvant GeparQuinto trial. PLoS One 2013;8:e79775.

20. Dieci MV, Criscitiello C, Goubar A, Viale G, Conte P, Guarneri V, Ficarra G, Mathieu MC, Delaloge S, Curigliano G, Andre F. Prognostic value of tumor-infiltrating lymphocytes on residual disease after primary chemotherapy for triple-negative breast cancer: a retrospective multicenter study. Ann Oncol 2014;25:611-8.

21. Denkert C, Loibl S, Noske A, Roller M, Muller BM, Komor M, Budczies J, Darb-Esfahani S, Kronenwett R, Hanusch C, von Torne C, Weichert W, Engels K, Solbach C, Schrader I, Dietel M, von Minckwitz G. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol 2010;28:105-13.

22. Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, Wienert S, Van den Eynden G, Baehner FL, Penault-Llorca F, Perez EA, Thompson EA, Symmans WF, Richardson AL, Brock J, Criscitiello C, Bailey H, Ignatiadis M, Floris G, Sparano J, Kos Z, Nielsen T, Rimm DL, Allison KH, Reis-Filho JS, Loibl S, Sotiriou C, Viale G,

Badve S, Adams S, Willard-Gallo K, Loi S. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol 2015;26:259-71.

23. Vidula N, Yau C, Goga A, Rugo HS. Programmed cell death 1 (PD-1) receptor and prograamed death ligand 1 (PD-L1) expression in primary breast cancer (BC); correlations with clinical characteristics and patient outcomes. J Clin Oncol 2015;33:suppl; abstr 1090.

24. Van Allen EM, Miao D, Schilling B, Shukla SA, Blank C, Zimmer L, Sucker A, Hillen U, Geukes Foppen MH, Goldinger SM, Utikal J, Hassel JC, Weide B, Kaehler KC, Loquai C, Mohr P, Gutzmer R, Dummer R, Gabriel S, Wu CJ, Schadendorf D, Garraway LA. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. Science 2015;350:207-11.

25. Dirix LY, Takacs I, Nikolinakos P, Arkenau H-T, Hamilton EP, von Heydebreck A, Grote H-J, Chin K, Lippman ME. Avelumab (MSB0010718C), an anti-PD-L1 antibody, in patients with locally advanced or metastatic breast cancer: A phase Ib JAVELIN solid tumor trial. San Antonio Breast Cancer Symposium 2015:abstr S1-04.

26. Rugo HS, Delord J-P, Im S-A, Ott PA, Piha-Paul SA, Bedard PL, Sachdev J, Le Tourneau C, van Brummelen E, Varga A, Saraf S, Petrangelo D, Karantza V, Tan A. Preliminary efficacy and safety of pembrolizumab (MK-3475) in patients with PD-L1-positive, estrogen receptor-positive (ER+)/HER2-negative advanced breast cancer enrolled in KEYNOTE-028. San Antonio Breast Cancer Symposium 2015;December 8-12, 2015:abstr S5-07.

27. Bracci L, Schiavoni G, Sistigu A, Belardelli F. Immune-based mechanisms of cytotoxic chemotherapy: implications for the design of novel and rationale-based combined treatments against cancer. Cell Death Differ 2014;21:15-25.

28. Muenst S, Schaerli AR, Gao F, Daster S, Trella E, Droeser RA, Muraro MG, Zajac P, Zanetti R, Gillanders WE, Weber WP, Soysal SD. Expression of programmed death ligand 1 (PD-L1) is associated with poor prognosis in human breast cancer. Breast Cancer Res Treat 2014;146:15-24.

29. Basu GD, Ghazalpour A, Vader R, Reddy S, Anderson K, McCullough A, Pockaj B. Expression of novel immunotherapeutic targets in luminal breast cancer patients. Breast Cancer Res Treat 2014:abstr 04-08.

30. Ali HR, Provenzano E, Dawson SJ, Blows FM, Liu B, Shah M, Earl HM, Poole CJ, Hiller L, Dunn JA, Bowden SJ, Twelves C, Bartlett JM, Mahmoud SM, Rakha E, Ellis IO, Liu S, Gao D, Nielsen TO, Pharoah PD, Caldas C. Association between CD8+ T-cell infiltration and breast cancer survival in 12,439 patients. Ann Oncol 2014;25:1536-43.

31. Nanda R, Chow LQ, Dees EC, Berger R, Gupta S, Geva R, Pusztai L, Dolled-Filhart M, Emancipator K, Gonzalez EJ, Houp J, Pathiraja K, Karantza V, Iannone R, Gause CK, Cheng JD, Buisseret L. A phase Ib stufy of pembrolizumab (MK-3475) in patients with advanced triple-negative breast cancer. Breast Cancer Res Treat 2014:abstr S1-09.

32. Emens LA, Braiteh FS, Cassier P, DeLord J-P, Eder JP, Shen X, Xiao Y, Wang Y, Hegde PS, Chen DS, Krop I. Inhibition of PD-L1 by MPDL3280A leads to clinical activity in patients with metastatic triple-negative breast cancer. 2014:abstr PD1-6.

33. Chevolet I, Speeckaert R, Schreuer M, Neyns B, Krysko O, Bachert C, Hennart B, Allorge D, van Geel N, Van Gele M, Brochez L. Characterization of the immune network of IDO, tryptophan metabolism, PD-L1, and in circulating immune cells in melanoma.

Oncoimmunology 2015;4:e982382.

34. Crawford J, Caserta C, Roila F, Group EGW. Hematopoietic growth factors: ESMO Clinical Practice Guidelines for the applications. Ann Oncol 2010;21 Suppl 5:v248-51.

35. Crawford J, Armitage J, Balducci L, Becker PS, Blayney DW, Cataland SR, Heaney ML, Hudock S, Kloth DD, Kuter DJ, Lyman GH, McMahon B, Rugo HS, Saad AA, Schwartzberg LS, Shayani S, Steensma DP, Talbott M, Vadhan-Raj S, Westervelt P, Westmoreland M, Dwyer M, Ho M. Myeloid growth factors. J Natl Compr Canc Netw 2013;11:1266-90.

36. Esserman LE, Cura MA, DaCosta D. Recognizing pitfalls in early and late migration of clip markers after imaging-guided directional vacuum-assisted biopsy. Radiographics 2004;24:147-56.

37. Loi S, Symmans WF, Bartlett JM, Fumagalli D, Van't Veer L, Forbes JF, Bedard P, Denkert C, Zujewski J, Viale G, Pusztai L, Esserman LJ, Leyland-Jones BR. Proposals for uniform collection of biospecimens from neoadjuvant breast cancer clinical trials: timing and specimen types. Lancet Oncol 2011;12:1162-8.

38. Chen BJ, Chapuy B, Ouyang J, Sun HH, Roemer MG, Xu ML, Yu H, Fletcher CD, Freeman GJ, Shipp MA, Rodig SJ. PD-L1 expression is characteristic of a subset of aggressive B-cell lymphomas and virus-associated malignancies. Clin Cancer Res 2013;19:3462-73.

39. Shi M, Roemer MG, Chapuy B, Liao X, Sun H, Pinkus GS, Shipp MA, Freeman GJ, Rodig SJ. Expression of programmed cell death 1 ligand 2 (PD-L2) is a distinguishing feature of primary mediastinal (thymic) large B-cell lymphoma and associated with PDCD1LG2 copy gain. Am J Surg Pathol 2014;38:1715-23.

40. Mino-Kenudson M, Chirieac LR, Law K, Hornick JL, Lindeman N, Mark EJ, Cohen DW, Johnson BE, Janne PA, Iafrate AJ, Rodig SJ. A novel, highly sensitive antibody allows for the routine detection of ALK-rearranged lung adenocarcinomas by standard immunohistochemistry. Clin Cancer Res 2010;16:1561-71.

41. Green MR, Monti S, Rodig SJ, Juszczynski P, Currie T, O'Donnell E, Chapuy B, Takeyama K, Neuberg D, Golub TR, Kutok JL, Shipp MA. Integrative analysis reveals selective 9p24.1 amplification, increased PD-1 ligand expression, and further induction via JAK2 in nodular sclerosing Hodgkin lymphoma and primary mediastinal large B-cell lymphoma. Blood 2010;116:3268-77.

42. DeSantis CE, Fedewa SA, Goding Sauer A, Kramer JL, Smith RA, Jemal A. Breast cancer statistics, 2015: Convergence of incidence rates between black and white women. *CA: a cancer journal for clinicians.* Jan-Feb 2016;66(1):31-42.

43. Kroemer G, Senovilla L, Galluzzi L, Andre F, Zitvogel L. Natural and therapy-induced immunosurveillance in breast cancer. *Nature medicine.* Oct 2015;21(10):1128-1138.

44. Stanton SE, Disis ML. Clinical significance of tumor-infiltrating lymphocytes in breast cancer. *J Immunother Cancer.* 2016;4:59.

45. Dirix L, Takacs I, Nikolinakos P, et al. Abstract S1-04: Avelumab (MSB0010718C), an anti-PD-L1 antibody, in patients with locally advanced or metastatic breast cancer: A phase Ib JAVELIN solid tumor trial. *Cancer Research.* 2016;76(4 Supplement):S1-04-S01-04.

46. Nanda R, Chow LQ, Dees EC, et al. Pembrolizumab in Patients With

Advanced Triple-Negative Breast Cancer: Phase Ib KEYNOTE-012 Study. *J Clin Oncol.* Jul 20 2016;34(21):2460-2467.

47. Rugo H, Delord J-P, Im S-A, et al. Abstract S5-07: Preliminary efficacy and safety of pembrolizumab (MK-3475) in patients with PD-L1–positive, estrogen receptor-positive (ER+)/HER2-negative advanced breast cancer enrolled in KEYNOTE-028. *Cancer Research.* 2016;76(4 Supplement):S5-07-S05-07.

48. Trinchieri G. Cancer Immunity: Lessons From Infectious Diseases. *J Infect Dis.* Jul 15 2015;212 Suppl 1:S67-73.

49. Human Microbiome Project C. Structure, function and diversity of the healthy human microbiome. *Nature.* Jun 13 2012;486(7402):207-214.

50. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature.* Mar 04 2010;464(7285):59-65.

51. Roy S, Trinchieri G. Microbiota: a key orchestrator of cancer therapy. *Nat Rev Cancer.* Mar 17 2017.

52. Sivan A, Corrales L, Hubert N, et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science.* Nov 27 2015;350(6264):1084-1089.

53. Vetizou M, Pitt JM, Daillere R, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science.* Nov 27 2015;350(6264):1079-1084.

54. Goedert JJ, Jones G, Hua X, et al. Investigation of the association between the fecal microbiota and breast cancer in postmenopausal women: a population-based case-control pilot study. *J Natl Cancer Inst.* Aug 2015;107(8).

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 NEW YORK HEART ASSOCIATION (NYHA) CLASSIFICATIONS

The New York Heart Association (NYHA) Cardiac Disease Classification provides a functional and therapeutic classification for the prescription of physical activity for cardiac participants. Based on NYHA definitions, participants are to be classified as follows:

Class	Definition
Class I	Subjects with no limitation of activities; they suffer no symptoms from ordinary activities.
Class II	Subjects with slight, mild limitation of activity; they are comfortable with rest or with mild exertion.
Class III	Subjects with marked limitation of activity; they are comfortable only at rest.
Class IV	Subjects who should be at complete rest, confined to bed or chair; any physical activity brings on discomfort and symptoms occur at rest.

APPENDIX C STRONG CYP3A INDUCERS/INHIBITORS

The list provided below is not exhaustive. For a more comprehensive, frequently updated list, please visit: [redacted]

Medications that strongly inhibit CYP3A:
Amprenavir
Atazanavir
Boceprevir
Clarithromycin
Conivaptan
Delavirdine
Diltiazem
Erythromycin
Fosamprenavir
Indinavir
Itraconazole
Ketoconazole
Lopinavir
Mibefradil
Miconazole
Nefazodone
Nelfinavir
Posaconazole
Ritonavir
Saquinavir
Telaprevir
Telithromycin
Verapamil
Voriconazole
Grapefruit, grapefruit juice, or any product containing grapefruit

Medications that strongly induce CYP3A:
Carbamazepine
Felbamate
Nevirapine
Phenobarbital
Phenytoin
Primidone
Rifabutin
Rifampin
Rifapentin
St. John's wort