DF/HCC Protocol #: 18-561

BMS Protocol #: CA209-8RP

TITLE: NIMBUS: A phase II study of nivolumab plus ipilimumab in metastatic hypermutated HER2-negative breast cancer

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SCHEMA

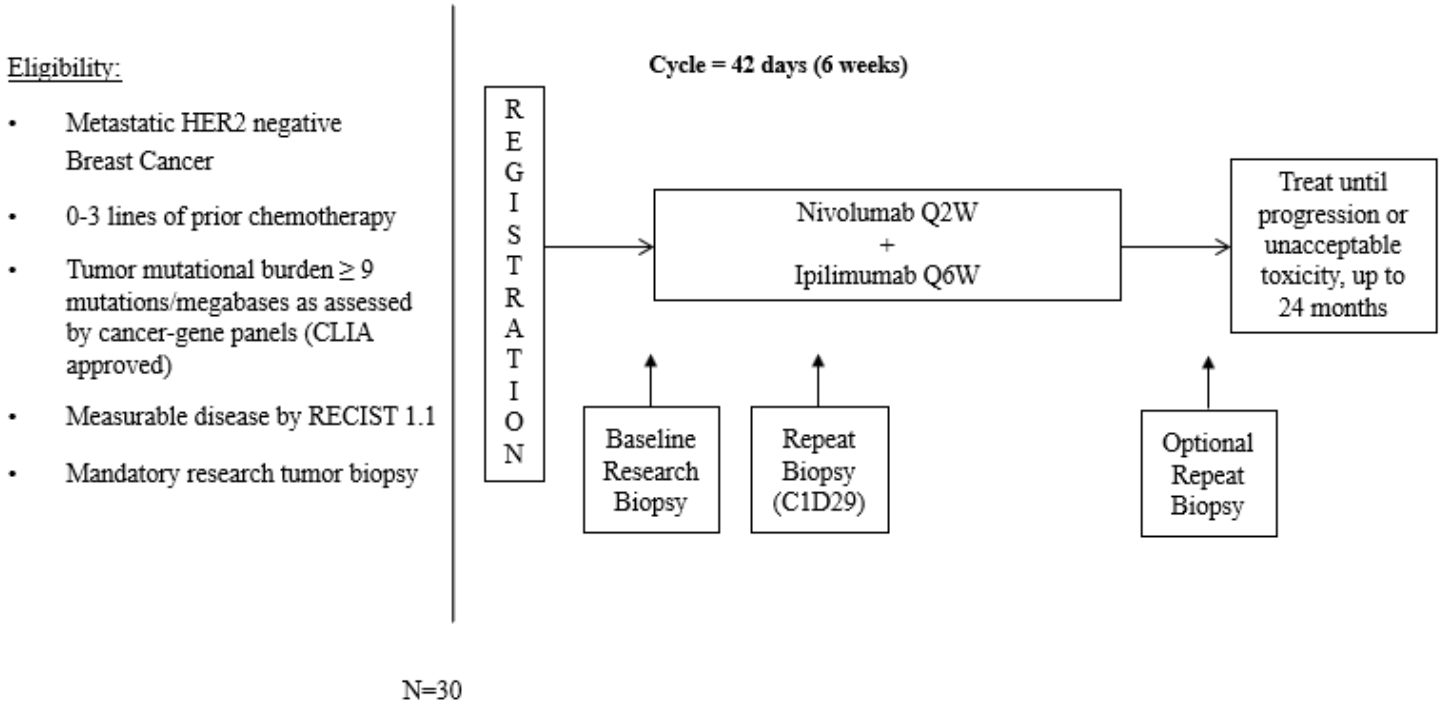

Eligibility:
• Metastatic HER2 negative Breast Cancer
• 0-3 lines of prior chemotherapy
• Tumor mutational burden ≥ 9 mutations/megabases as assessed by cancer-gene panels (CLIA approved)
• Measurable disease by RECIST 1.1
• Mandatory research tumor biopsy
Cycle = 42 days (6 weeks)
REGISTRATION
Nivolumab Q2W + Ipilimumab Q6W
Treat until progression or unacceptable toxicity, up to 24 months
Baseline Research Biopsy
Repeat Biopsy (C1D29)
Optional Repeat Biopsy
N=30

TABLE OF CONTENTS

APPENDIX E DF/HCC MULTI-CENTER DATA AND SAFETY MONITORING PLAN 104

1. OBJECTIVES

1.1 Study Design

1.1.1 This is an open-label, single-arm, multicenter, phase 2 study of nivolumab 3 mg/Kg intravenously (IV) every 2 weeks (14 days) plus Ipilimumab 1 mg/Kg IV every 6 weeks (cycle length= 6 weeks) in subjects with hypermutated metastatic HER-2 negative breast cancer previously treated with 0 to 3 chemotherapy regimens in the metastatic setting. Mandatory research biopsies will be performed at baseline (if tissue is safely accessible) and at day 29 of Cycle 1 (+14 day scheduling window).

1.2 Primary Objectives

1.2.1 To evaluate the efficacy of the nivolumab plus ipilimumab, as defined by objective response rate (ORR) according to RECIST 1.1 [Eisenhauer *et al.*, 2009] in patients with hypermutated HER2- metastatic breast cancer previously treated with 0 to 3 lines of systemic therapy in the metastatic setting.

1.3 Secondary Objectives

Safety objectives

1.3.1 To evaluate the safety and tolerability of the combination of nivolumab plus ipilimumab.

Efficacy objectives:

1.3.2 To evaluate the objective response rate of the combination according to immune-related response criteria (irRC) [Wolchok *et al.*, 2009]. (Section 11).
1.3.3 To evaluate the Clinical Benefit Rate (CR + PR + SD ≥ 24 weeks) of the combination according to RECIST 1.1.
1.3.4 To evaluate the progression-free survival (PFS) of the combination according to RECIST 1.1.
1.3.5 To evaluate the Clinical Benefit Rate (CR + PR + SD ≥ 24 weeks) of the combination according to irRC [Wolchok *et al.*, 2009]. (Section 11).
1.3.6 To evaluate the progression-free survival (PFS) of the combination according to irRC [Wolchok *et al.*, 2009]. (Section 11)
1.3.7 To evaluate the objective response rate of the combination according to RECIST 1.1 in the population with centrally confirmed (via Foundation Medicine panel) hypermutated tumors (≥ 9 mutations/megabase)
1.3.8 To evaluate the PFS of the combination according to RECIST 1.1 in the population with centrally confirmed hypermutated tumors (≥ 9 mutations/megabase)
1.3.9 To evaluate the overall survival (OS) of the combination.

1.4 Correlative Objectives

1.4.1 To characterize a broad array of immune markers in metastatic hypermutated HER2-negative breast cancers. (characterization will be based on histology, protein expression, and mRNA expression).

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

1.4.3 To characterize changes in tumor-infiltrating lymphocytes, PD-L1 expression and immune gene signatures in the tissue microenvironment (TME) from baseline to after 4 weeks of the experimental combination.

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

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

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

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

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

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

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

1.4.11 To characterize changes in the structure and function of the gut microbiome of patients with breast cancer after 4 weeks of therapy compared to baseline.

1.4.12 To determine whether changes in the overall diversity of the gut microbiome, estimated by the Shannon Index, of patients with breast cancer after after 4 weeks of therapy is associated with disease response (response assessed by RECIST 1.1, irRC and PFS).

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

1.4.14 To evaluate the functional pathways that may play a role as a predictive biomarker of disease response to therapy (response assessed by RECIST 1.1, irRC and PFS).

1.4.15 To determine whether pre-treatment characteristics of the structure and function of the gut microbiome in patients with breast cancer is associated with therapy-induced grade ≥2 diarrhea.

1.4.16 To explore whether the cut off of ≥ 14 mutations/megabase is associated with the efficacy of this investigational therapy, assessed by ORR per RECIST 1.1.

1.4.17 To explore whether the cut off of ≥ 14 mutations/megabase is associated with the efficacy of this investigational therapy, assessed by PFS per RECIST 1.1

1.4.18 To explore whether the cut off of ≥ 14 mutations/megabase is associated with the efficacy of this investigational therapy, assessed by OS per RECIST 1.1

1.5 Patient Reported Outcomes Objectives

1.5.1 To assess the participant's quality of life by the FACT-B questionnaire
1.5.2 To assess the participant's quality of life by the Rotterdam Symptom Checklist

2. BACKGROUND

2.1 Study Disease(s)

Breast cancer (BC) is the most frequently diagnosed cancer and the second cause of cancer death in women in the Unites States[Siegel *et al.*, 2016]. Approximately, 80% of these cancers are classified as HER2-negative, comprising those with absent overexpression of the human epidermal growth factor receptor 2 (HER2)[O'brien *et al.*, 2010]. Despite progress in the past years regarding the biology of breast cancer, metastatic disease is still incurable, independently of the hormonal receptor status. Therefore, new treatments are urgently needed.

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

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades[Schreiber *et al.*, Schreiber, 2012]. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies[Mlecnik *et al.*, 2014]. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors[Tosolini *et al.*, 2006, Adams *et al.*, 2014, Denkert *et al.*, 2015].

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene *Pdcd1*) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions

with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL). This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention[Intlekofer *et al.*, 2013].

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

Unlike melanoma and NSCLC, BC has not been intensively investigated for its susceptibility to immunotherapy in clinical settings. However, there is accumulating preclinical and clinical evidence suggesting that immune system is critical during natural history of breast cancer and the immune system can be modulated to improve outcomes in this disease[Kroemer *et al.*, 2015]. It has been recognized that BC is capable of stimulating the immune system, as many breast tumors have substantial lymphocyte infiltration [Denkert *et al.*, 2010, Denkert *et al.*, 2015]. Additionally, this pathologic feature has prognostic implications, as lymphocyte predominant breast cancers are associated with improved prognosis [Denkert *et al.*, 2010, Loi *et al.*, 2013]. However, the degree of immune infiltration differs by BC subtype; while a substantial proportion of triple negative BC can be richly infiltrated, hormone-receptor positive BC is poorly T-cell infiltrated[Dushyanthen *et al.*, 2015]. Recently, it has been demonstrated that the expression of PD-1 and PD-L1 differs among breast tumors subtype: HR-positive (30% PD-1; 33% PD-L1), triple-negative (70% PD-1; 59% PD-L1) and HER2-positive (60% PD-1; 20% PD-L1)[Gatalica *et al.*, 2014].

2.3 Ipilimumab (Yervoy)

Ipilimumab (BMS-734016, MDX010, MDX-CTLA4) is a fully human monoclonal immunoglobulin (Ig) G1κ specific for human cytotoxic T-lymphocyte antigen 4 (CTLA-4, cluster of differentiation [CD] 152), which is expressed on a subset of activated T cells. CTLA-4 is a negative regulator of T-cell activity. Ipilimumab is a monoclonal antibody (mAb) that binds to CTLA-4 and blocks the interaction of CTLA-4 with its ligands, CD80/CD86. Blockade of CTLA-4 has been shown to augment T-cell activation and proliferation, including the activation and proliferation of tumor infiltrating T-effector cells. Inhibition of CTLA-4 signaling can also reduce T-regulatory cell (Treg) function, which may contribute to a general increase in T-cell responsiveness, including the anti-tumor response.

Ipilimumab has been approved for use in over 47 countries including the United States (US, Mar-2011), the European Union (EU, Jul-2011), and Australia (Jul-2011).

2.3.1 Non-Clinical Summary

Ipilimumab has specificity and a high affinity for human CTLA-4. The calculated dissociation constant value from an average of several studies was 5.25 nM. Binding of ipilimumab to purified, recombinant human CTLA-4 antigen was also demonstrated by enzyme-linked

immunosorbent assay with half-maximal binding at 15 ng/mL, whereas saturation was observed at approximately 0.1 μg/mL. No cross-reactivity was observed against human CD28. Ipilimumab completely blocked binding of B7.1 and B7.2 to human CTLA-4 at concentrations higher than 6 and 1 μg/mL, respectively.

Please refer to the ipilimumab investigator's brochure (IB) for further information on pre-clinical development.

2.3.2 Clinical Studies

Bristol-Myers Squibb (BMS) and Medarex, Inc. (MDX, acquired by BMS in Sep-2009) have co-sponsored an extensive clinical development program for ipilimumab, encompassing more than 19,500 subjects (total number of subjects enrolled in ipilimumab studies) in several cancer types in completed and ongoing studies, as well as a compassionate use program. The focus of the clinical program is in melanoma, prostate cancer, and lung cancer, with advanced melanoma being the most comprehensively studied indication. Ipilimumab is being investigated both as monotherapy and in combination with other modalities such as chemotherapy, radiation therapy, and other immunotherapies.

Phase 3 programs are ongoing in melanoma, prostate cancer, and lung cancer. In melanoma, 2 completed phase 3 studies have demonstrated a clinically meaningful and statistically significant survival benefit in pretreated advanced melanoma and previously untreated advanced melanoma, respectively.

The safety profile of ipilimumab is generally consistent across these trials with a) the majority adverse events (AEs) being inflammatory in nature, which is consistent with the proposed mechanism of action of ipilimumab; b) the same types of such immune-mediated events in the gastrointestinal (GI) tract, skin, liver, and endocrine system being reported; and c) most of these events being manageable with immune suppressive therapies.

In melanoma, 2 BMS-sponsored phase 3 studies are ongoing in subjects with high-risk stage III melanoma and pretreated and treatment-naïve advanced melanoma (3 mg/kg versus 10 mg/kg ipilimumab).

The completed phase 3 study evaluated ipilimumab in subjects with metastatic castration-resistant prostate cancer (mCRPC) who had progressed during or following treatment with docetaxel. Eligible subjects were randomized to a single dose of bone-directed radiotherapy (RT), followed by either ipilimumab 10 mg/kg or placebo (799 randomized: 399 ipilimumab, 400 placebo). This study did not meet its primary endpoint of overall survival (OS). The hazard ratio (HR) of 0.85 (95% confidence interval [CI]: 0.72, 1.00) for survival favored ipilimumab but did not reach statistical significance with a p-value of 0.053. Planned sensitivity analyses favored ipilimumab, where the greatest benefit appeared to be in subgroups defined by good prognostic features and low burden of disease. Additional evidence of ipilimumab activity observed in the study included a reduced risk of disease progression relative to placebo (HR = 0.70), superior clinical outcomes compared to placebo in tumor regression, and declines in prostate specific antigen (PSA). The safety profile in this study was consistent with the previously defined AE profile at the same dose.

A second phase 3 study evaluated ipilimumab 10 mg/kg versus placebo in men with asymptomatic or minimally symptomatic, chemotherapy-naïve mCRPC with no visceral metastases. A total of 602 subjects were randomized in a 2:1 ratio (400 subjects to 10 mg/kg ipilimumab and 202 subjects to placebo). Preliminary data indicate the study did not meet its primary endpoint based on intent-to-treat analysis. The HR of 1.11 (95.87% CI: 0.88, 1.39; P value

= 0.3667) for OS did not favor ipilimumab. A longer median progression-free survival (PFS) interval was observed for the ipilimumab group than for the placebo group, which may be indicative of activity of ipilimumab in delaying disease progression. The safety profile in this study was generally consistent with the previously defined AE profile at the same dose.

In addition, a completed, large phase 2 study has investigated the addition of ipilimumab to carboplatin and paclitaxel using 2 different schedules (concurrent and phased) in subjects with NSCLC or small cell lung cancer (SCLC, a secondary endpoint). Ipilimumab, given in combination with paclitaxel/carboplatin in a phased schedule improved immune-related progression-free survival (irPFS) compared to the control treatment, but no improvement was seen when ipilimumab was given in a concurrent schedule. Phased ipilimumab also improved PFS according to modified World Health Organization (mWHO) criteria and showed a trend for improved OS.

The efficacy and safety of ipilimumab in a phased schedule with carboplatin/paclitaxel is also being investigated in a phase 3 study in subjects with advanced squamous NSCLC (CA184104). The efficacy and safety of ipilimumab in a phased schedule with etoposide/platinum in subjects with extensive stage disease SCLC is being investigated in a phase 3 study (CA184156). In Study CA184104, the last patient, last visit was achieved in June 2015, and database lock occurred on 01-Sep-2015. No final data are currently available, but preliminary data indicate that no new safety concerns were identified in the course of standard clinical safety monitoring of the study. In Study CA184156, preliminary data indicate the primary endpoint of prolonging survival was not achieved, but no new safety signals were identified.

The unique immune-based mechanism of action is reflected in the clinical patterns of anti-cancer activity in some patients. Ipilimumab induces an immunologic response, and measurable clinical effects emerge after the immunological effects. Tumor infiltration with lymphocytes and the associated inflammation (documented by biopsy in some subjects) is likely the cornerstone of the effect of ipilimumab and can manifest in various patterns of clinical activity leading to tumor control. In some cases, inflammation may not be noted by radiological examination, and objective response is observed with the first tumor assessment in a manner seen in patients receiving other types of anti-cancer treatments. In other cases, response may be preceded by an apparent increase in initial tumor volume and/or the appearance of new lesions, which may be mistaken for tumor progression on radiological evaluations. Therefore, in patients who are not experiencing rapid clinical deterioration, confirmation of progression is recommended (at the investigator's discretion) to better understand the prognosis, as well as to avoid unnecessarily initiating potentially toxic alternative therapies in subjects who might be benefiting from treatment. Immune-related response criteria were developed based on these observations to systematically categorize novel patterns of clinical activity and are currently being prospectively evaluated in clinical studies.

In metastatic diseases, stabilization is more common than response, and in some instances is associated with a slow, steady decline in tumor burden over many months, sometimes improving to partial and/or complete responses (CRs). Thus, the immune-based mechanism of action of ipilimumab results in durable disease control, sometimes with novel patterns of response, which contribute to its unique improvement in OS.

The unique immune-based mechanism of action is also reflected in the safety profile. The most common treatment-related AEs are inflammatory in nature, consistent with the mechanism of action of the drug and generally medically manageable with topical and/or systemic immunosuppressants. Such immunological safety events are described as immune-related adverse

events (irAEs) or immune-mediated adverse reactions (imARs). The irAEs are described as AEs of unknown etiology, which were consistent with an immune phenomenon and considered causally related to drug exposure by investigators. The irAEs primarily involve the GI tract and skin. Immune-related AEs in the liver were also observed, particularly in subjects receiving 10 mg/kg. Endocrinopathy and neuropathy were important irAEs that were observed less frequently. The imARs were adjudicated in a blinded fashion based on Sponsor-physician data review to exclude noninflammatory etiologies, such as infection or tumor progression, and to consider available evidence of inflammation, such as tumor biopsies or responsiveness to steroids, in an effort to determine whether specific AEs or abnormal hepatic laboratory values were likely to be immune mediated and associated with ipilimumab treatment.

The early diagnosis of inflammatory events is important to initiate therapy and minimize complications. Inflammatory events are generally manageable using symptomatic or immuno-suppressive therapy as recommended through detailed diagnosis and management guidelines.

A program-wide independent Data Monitoring Committee (DMC) reviews data from the ipilimumab studies, allowing for an ongoing safety and benefit/risk assessment in subjects receiving ipilimumab. The DMC charter includes explicit stopping rules for some studies, allowing the DMC to recommend discontinuing further treatment across the ipilimumab program, if necessary.

In summary, ipilimumab offers clinically meaningful and statistically significant survival benefit to patients with pretreated advanced melanoma (as 3 mg/kg monotherapy compared to the melanoma peptide vaccine gp100) and previously untreated advanced melanoma (at 10 mg/kg in combination with dacarbazine [DTIC] compared to DTIC alone) and evidence of clinical activity in randomized studies in other tumor types. These findings, together with evidence of a safety profile that is manageable with careful monitoring and appropriate intervention for treatment of immune-related toxicities, suggest an acceptable benefit to risk ratio.

Please also refer to the IB for the most comprehensive clinical background information for ipilimumab.

2.3.3 Clinical Pharmacokinetics

The pharmacokinetics (PK) of ipilimumab has been extensively studied in subjects with melanoma, at the 3 and 10 mg/kg doses administered as a 1.5-hour IV infusion. The PK of ipilimumab was characterized by population PK (PPK) analysis and determined to be linear and time invariant in the dose range of 0.3 to 10 mg/kg. The mean CL (±SD) value after IV administration of 10 mg/kg was 18.3 ± 5.88 mL/h, and the mean steady-state volume of distribution (Vss) [±SD] value was 5.75 ± 1.69 L.

2.3.4 Pharmacodynamics

CTLA-4 is a key regulator of T-cell activity. Ipilimumab is a CTLA-4 immune checkpoint inhibitor that blocks T-cell inhibitory signals induced by the CTLA-4 pathway, increasing the number of tumor reactive T-effector cells that mobilize to mount a direct T-cell immune attack against tumor cells. Preclinical data indicate that CTLA-4 blockade can also reduce Treg function, which may lead to an increase in anti-tumor immune response. Ipilimumab may selectively deplete Tregs at the tumor site, leading to an increase in the intratumoral T-effector/Treg cell ratio which drives tumor response leading to cell death9.

2.3.4.1 Ipilimumab Effect on Circulating T Cells

CTLA-4 is a negative regulator of T-cell activation. By blocking CTLA-4, ipilimumab increases the percentage of peripheral activated T cells and central memory T cells. These changes are evidenced by Week 4 and generally remain sustained through Week 1. The sum of these changes in immune cell subsets may result in anti-tumor activity, as well as irAEs.

Table 1: Key T-cell Subsets following Ipilimumab Treatment

T-cell population	Ipilimumab Dose (mg/kg)	Fitted Mean Relative Frequency (%) Mean (95% CI)		
		Baseline[a]	Week 4[b]	Week 12[c]
Activated CD4+ / Total CD4+	3	17.0 (13.4, 20.6)	25.2 (21.3, 29.2)	24.7 (21.0, 28.3)
	10	14.9 (11.2, 18.6)	24.8 (20.8, 28.8)	24.7 (20.5, 29.0)
Activated CD8+ / Total CD8+	3	24.7 (18.9, 30.5)	31.1 (25.2, 37.1)	33.4 (27.5, 39.4)
	10	22.5 (16.5, 28.6)	25.7 (19.6, 31.9)	27.6 (21.0, 34.2)
Central Memory CD4+ / Total CD4+	10	54.5 (52.4, 56.7)	59.6 (57.5, 61.7)	62.0 (59.9, 64.1)
Central Memory CD8+ / Total CD8+	10	37.4 (33.8, 41.0)	41.0 (37.5, 44.6)	45.3 (41.7, 48.9)

a. Baseline is defined as the pre-dose measurement closest in time to first dose.
b. Nominal Week 4 is defined as visits between study Days 8 and 42, inclusive.
c. Nominal Week 12 is defined as visits between study Days 64 and 98, inclusive.
Note: Means and 95% CI are estimates from extended linear models.

2.3.4.2 Ipilimumab Effect on Absolute Lymphocyte Count

In clinical studies, ipilimumab increased absolute lymphocyte count (ALC) in peripheral blood.

In 214 subjects with advanced melanoma, ipilimumab increased ALC in a dose-dependent manner, with the largest increase observed at 10-mg/kg dose.

In phase 3 studies in advanced melanoma, high baseline ALC was found to be significantly associated with longer OS regardless of treatment. However, subjects with low baseline ALC treated with ipilimumab demonstrated longer OS relative to subjects not treated with ipilimumab.

2.2.5 Clinical Efficacy

Ipilimumab prolongs survival in subjects with pretreated and previously untreated advanced melanoma and has demonstrated anti-tumor activity in other malignancies, including lung, prostate cancer, and renal cell carcinoma. Please refer to the IB for detailed efficacy information regarding ipilimumab monotherapy.

2.2.6 Clinical Safety

Blockade of CTLA-4 by ipilimumab leads to T-cell activation, with the potential for clinical inflammatory adverse events (AEs) primarily involving the skin (dermatitis/pruritus), GI tract (diarrhea/colitis), liver (hepatitis), endocrine glands (e.g., hypophysitis and adrenal or thyroid

abnormalities), and other less frequent organs (e.g., uveitis/episcleritis). The majority of these inflammatory AEs initially manifested during treatment; however, a minority occurred weeks to months after discontinuation of ipilimumab. The majority of the inflammatory AEs are reversible with the guidance issued in **Section 6**. In rare cases, these inflammatory AEs may be fatal.

Patients should be assessed for signs and symptoms of enterocolitis, dermatitis, neuropathy, and endocrinopathy, and clinical chemistries (including liver function, adrenocorticotropic hormone [ACTH] level, and thyroid function tests) should be evaluated.

During evaluation of a suspected inflammatory AE, all efforts should be made to rule out neoplastic, infectious, metabolic, toxin, or other etiologic causes. Serological, immunological, imaging, and biopsy with histology (e.g., biopsy-proven lymphocytic) data should be used to support the diagnosis of an immune-mediated toxicity or support an alternative cause of the AE. In general, for severe inflammatory AEs, ipilimumab should be permanently discontinued, and systematic high-dose corticosteroid therapy should be initiated. For moderate immune-mediated AEs, ipilimumab should be held or delayed, and moderate-dose corticosteroids should be considered.

Based on limited current clinical experience, corticosteroids do not appear to adversely affect the anti-tumor response. For example, disease control was maintained in subjects with objective responses who received corticosteroid administration for concomitant serious inflammatory AEs.

2.4 Nivolumab

Nivolumab is a fully human, IgG4 (kappa) isotype monoclonal antibody that binds to PD-1 with nanomolar affinity (KD = 3.06 nM) and a high degree of specificity, thus precluding binding to its ligands PD-L1 and PD-L2. Nivolumab does not bind other related family members, such as BTLA, CTLA-4, ICOS or CD28. Pre-clinical testing of nivolumab demonstrated that blockade of PD-1 results in enhanced T cell proliferation and expression of interferon-gamma (IFN-).

2.4.1 Nivolumab nonclinical toxicology

Toxicology studies in cynomolgus monkeys revealed that nivolumab was well tolerated at doses up to 50 mg/kg given twice weekly for 27 doses. Drug related findings were limited to a reversible decrease in triiodothyronine (T3) by 28%, without concomitant abnormalities in other markers of thyroid function. Preliminary new non-clinical safety findings of adverse pregnancy outcomes and infant losses in the absence of overt maternal toxicity have been reported.66 The findings of increased late stage pregnancy loss and early infant deaths/euthanasia in nivolumab exposed pregnant monkeys suggest a potential risk to human pregnancy if there is continued treatment with nivolumab during pregnancy.

2.4.2 Clinical Experience with nivolumab

The overall safety experience with nivolumab, as monotherapy or in combination with other therapeutics, is based on experience in approximately 1,500 subjects treated to date. For monotherapy, the safety profile is similar across tumor types. The one exception is pulmonary inflammation AEs which may be numerically greater in subjects with NSCLC because in some cases it can be difficult to distinguish between nivolumab related and unrelated causes of pulmonary symptoms and radiographic changes. There was no pattern in the incidence, severity, or causality of AEs to nivolumab dose level.

In several ongoing clinical trials, the safety of nivolumab in combination with other therapeutics such as ipilimumab, cytotoxic chemotherapy, anti-angiogenics and targeted therapies

is being explored. Most studies are ongoing and as such, the safety profile of nivolumab combinations continues to evolve. The most advanced combination under development is nivolumab and ipilimumab in subjects with MEL. Thus far, the combination of both agents results in a safety profile with similar types of AEs as either agent alone, but in some cases with greater frequency.

Overall, the safety profile of nivolumab monotherapy as well as combination therapy is manageable and generally consistent across completed and ongoing clinical trials with no MTD reached at any dose tested, up to 10 mg/kg. There was no pattern in the incidence, severity, or causality of AEs to nivolumab dose level. Most AEs were low grade (grade 1 to grade 2) with relatively few related high grade (grade 3 to grade 4) AEs. Most high grade events were manageable with the use of corticosteroids or hormone replacement therapy for Endocrinopathies. Management algorithms including the use of immunosuppressive agents, such as corticosteroids, infliximab, etc., are provided in Section of this protocol. Nivolumab should not be used in subjects with active autoimmune disease given the mechanism of action of the antibody.

Nivolumab has demonstrated clinical activity in response evaluable subjects with a variety of solid tumor malignancies in the following studies:

1) completed Phase 1 single-dose (MDX1106-01): n = 37 subjects with prostate cancer, MEL, NSCLC, RCC, and CRC

2) ongoing Phase 1 multi-dose, dose escalation study with nivolumab monotherapy (CA209003/MDX1106-03): NSCLC n = 129, MEL n = 107, RCC n = 34 subjects

3) ongoing Phase 1b study with nivolumab in combination with ipilimumab (CA209004/MDX1106-04): MEL n = 82 subjects

4) ongoing Phase 1 study with nivolumab monotherapy or in combination with platinum-based chemotherapy or erlotinib (CA209012): NSCLC, monotherapy n = 20, combination therapy n = 77 subjects

In addition, monotherapy or combination trials with nivolumab are on-going for subjects with SCCHN,67 HCC,68 CRC69, GBM70, and NHL.71,72

Updated overall clinical experience for nivolumab is available in the current version of the Investigator's Brochure.

2.5 Ipilimumab with Nivolumab

2.5.1 Clinical Pharmacokinetics

The pharmacokinetics of nivolumab and ipilimumab were assessed using a PPK approach when nivolumab and ipilimumab were administered in combination. The %CV CL, Vss, and t1/2 of nivolumab were 10.0 mL/h (50.3%), 7.92 L (30.1%), and 24.8 days (94.3%), respectively. When ipilimumab 3 mg/kg (registrational dose) was administered in combination with nivolumab, the CL of nivolumab was increased by 24%, whereas there was no effect on the CL of ipilimumab. However, co-administration with ipilimumab 1 mg/kg did not appear to have an effect on nivolumab CL. This is unlikely to be clinically relevant given the observed efficacy with the nivolumab and ipilimumab combination regimen.

When administered in combination, the CL of nivolumab increased by 42% in the presence of anti-nivolumab antibodies. There was no effect of anti-ipilimumab antibodies on the CL of ipilimumab.

2.5.2 Immunogenicity

Of 394 patients who were treated with nivolumab in combination with ipilimumab and evaluable for the presence of anti-nivolumab antibodies, 149 patients (37.8%) tested positive for treatment-emergent anti-nivolumab antibodies by an ECL assay and 18 patients (4.6%) had neutralizing antibodies against nivolumab. Of the 391 patients evaluable for the presence of anti-ipilimumab antibodies, 33 patients (8.4%) tested positive for treatment-emergent anti-ipilimumab antibodies by an ECL assay and 1 patient (0.3%) had neutralizing antibodies against ipilimumab. There was no evidence of increased incidence of infusion reactions/hypersensitivity reactions with antinivolumab antibody development. There was no evidence of altered toxicity profile associated with anti-product antibody development and there was no apparent casual effect of neutralizing antibodies on loss of efficacy.

2.5.3 Clinical Efficacy

In trial CA209012 (NCT01454102; CheckMate 012), an ongoing multi-arm phase 1 safety study of nivolumab in chemotherapy-naive NSCLC subjects, 56 subjects were administered nivolumab in combination with chemotherapy (gemcitabine / cisplatin, pemetrexed / cisplatin, carboplatin / paclitaxel), 21 with nivolumab in combination with erlotinib, and 197 with nivolumab in combination with ipilimumab.

A summary of ORR and PFS rate at 12 months for subjects treated with nivolumab plus ipilimumab is provided in the table below. The original nivolumab plus ipilimumab combinations using the same dosing regimens as in the melanoma studies (n = 49) were found to be non-tolerable for the NSCLC population, and these regimens were not pursued further. The results from newer combination dosing regimens (arms N, O, P, Q; n = 148) with lower and less frequent dosing of ipilimumab are presented below. The cohorts containing the approved dose of nivolumab (3 mg/kg) demonstrated improved ORR and PFS compared to the cohorts containing the lower dose of nivolumab (1 mg/kg).

The regimens for these cohorts were:

- Arm N (n=31): nivolumab 1 mg/kg + ipilimumab 1 mg/kg every 3 weeks for 4 cycles, followed nivolumab 3 mg/kg every 2 weeks
- Arm O (n=40): nivolumab 1 mg/kg every 2 weeks + ipilimumab 1 mg/kg every 6 weeks
- Arm P (n= 38): nivolumab 3 mg/kg every 2 weeks + ipilimumab 1 mg/kg every 12 weeks
- Arm Q (n=39): nivolumab 3 mg/kg every 2 weeks + ipilimumab 1 mg/kg every 6 weeks

Table 2: Efficacy in Chemotherapy-Naive NSCLC Subjects Treated with Nivolumab and Ipilimumab

Treatment Group	N	Nivolumab (mg/kg)	ORR[a]		PFS at 12 months		Median OS	
			n (%)	95% CI[b]	%	95% CI[c]	Months	95% CI[b]
Nivo 1 + Ipi 1 ×4 (arm N)	31	1	6 (19)	8, 38	30	14, 47	NR	11.5, NR
Nivo 1 + Ipi 1 (arm O)	40	1	13 (33)	19, 49	25	12, 42	NR	11.0, NR
Nivo 3 + Ipi 1 q12w (arm P)	38	3	18 (47)	31, 64	48	31, 63	NR	14.1, NR
Nivo 3 + Ipi 1 q6w (arm Q)	39	3	15 (39)	23, 55	35	20, 50	18.1	13.3, 18.1

Database Lock Date 18-Feb-2016

a. CR + PR; assessed according to RECIST v.1.1
b. Based on Kaplan-Meier method
c. Based on Greenwoods formula

Abbreviations: CI = confidence interval; Ipi = ipilimumab; Nivo = nivolumab; NR: not reached; ORR = objective response rate; PFSR = progression-free survival rate; OS = overall survival

Hellmann et al published the results of the CheckMate 227, a multicenter randomized phase 3 trial that examined progression-free survival with nivolumab plus ipilimumab versus chemotherapy among patients with a high tumor mutational burden (defined as ≥10 mutations per megabase)[Hellmann *et al.*, 2018]. A total of 2877 patients were enrolled in part 1 of the trial from August 2015 through November 2016, and 1739 underwent randomization. Of the 1004 patients whose tumor mutational burden could be evaluated across all treatment groups, 444 (44.2%) had at least 10 mutations per megabase, including 139 patients assigned to nivolumab plus ipilimumab and 160 patients assigned to chemotherapy. Patients received nivolumab (3 mg per kilogram of body weight every 2 weeks) plus ipilimumab (1 mg per kilogram every 6 weeks) or platinum doublet chemotherapy. The progression-free survival among patients with a high tumor mutational burden was significantly longer with nivolumab plus ipilimumab than with chemotherapy. The 1-year progression-free survival rate was 42.6% with nivolumab plus ipilimumab versus 13.2% with chemotherapy, and the median progression-free survival was 7.2 months (95% confidence interval [CI], 5.5 to 13.2) versus 5.5 months (95% CI, 4.4 to 5.8) (hazard ratio for disease progression or death, 0.58; 97.5% CI, 0.41 to 0.81; P<0.001). The objective response rate was 45.3% with nivolumab plus ipilimumab and 26.9% with chemotherapy. The benefit of nivolumab plus ipilimumab over chemotherapy was broadly consistent within subgroups, including patients with a PD-L1 expression level of at least 1% and those with a level of less than 1%.

More recently, data from the TAPUR trial (ASCO 2019 presentation), demonstrated benefit from checkpoint inhibitor monotherapy (pembrolizumab) in patients with metastatic breast cancer and tumor mutational burden defined as ≥ 9 mutations per megabase [Alva *et al.,* 2019].

2.5.3.1 Clinical Safety

The original nivolumab plus ipilimumab combinations using the same dosing regimens as in the melanoma studies were found to be non-tolerable for the NSCLC population, so 4 additional

regimens were examined (arms N, O, P, Q), and the results from these newer cohorts are presented. The regimens for these cohorts were:

- Arm N (n=31): nivolumab 1 mg/kg + ipilimumab 1 mg/kg every 3 weeks for 4 cycles, followed nivolumab 3 mg/kg every 2 weeks
- Arm O (n=40): nivolumab 1 mg/kg every 2 weeks + ipilimumab 1 mg/kg every 6 weeks
- Arm P (n= 38): nivolumab 3 mg/kg every 2 weeks + ipilimumab 1 mg/kg every 12 weeks
- Arm Q (n=39): nivolumab 3 mg/kg every 2 weeks + ipilimumab 1 mg/kg every 6

The most frequently reported drug-related adverse events (AEs) in the newer nivolumab plus ipilimumab cohorts were fatigue (29.0%), diarrhea (25.0%), pruritus (23.7%), and fatigue (23.1%) in arms N, O, P, Q, respectively. Drug-related serious adverse events (SAEs) reported in more than 2 subjects/cohort treated in the newer nivolumab plus ipilimumab cohorts were adrenal insufficiency, hypophysitis, pneumonitis, autoimmune hepatitis, diarrhea, colitis, and acute kidney injury.

Drug-related AEs leading to discontinuation reported in more than 1 subject treated in the new nivolumab plus ipilimumab arms included ALT increased, AST increased, colitis, myalgia, pneumonitis, rash, autoimmune hepatitis, infusion-related reaction, facial nerve disorder, esophagitis, and transaminases increased.

Most deaths were due to disease progression. The only deaths reported due to study drug toxicity were in the nivolumab + ipilimumab original treatment groups (respiratory failure following Grade 3 colitis, pulmonary hemorrhage, and toxic epidermal necrolysis in a patient with history of ulcerative colitis).

In the above mentioned phase 3 trial CheckMate 227 the rate of grade 3 or 4 treatment-related adverse events was 31.2% with nivolumab plus ipilimumab and 36.1% with chemotherapy[Hellmann *et al.*, 2018]. Treatment-related adverse events leading to discontinuation were more common with nivolumab plus ipilimumab than with chemotherapy (17.4% vs. 8.9%); however, patients continued to receive nivolumab plus ipilimumab longer than chemotherapy. The most common treatment-related select adverse events (defined as adverse events of potential immunologic causes) with nivolumab plus ipilimumab and nivolumab monotherapy were skin reactions (33.9% and 20.7%, respectively); the most common grade 3 or 4 treatment-related select adverse events were hepatic events (8.0% and 3.3%, respectively) Overall, treatment-related deaths occurred in seven patients (1.2%) treated with nivolumab plus ipilimumab (three died from pneumonitis and one each died from myocarditis, acute tubular necrosis, circulatory collapse, and cardiac tamponade), six patients (1.1%) treated with chemotherapy (two died from sepsis and one each died from multiple brain infarctions, interstitial lung disease, thrombocytopenia, and febrile neutropenia with sepsis), and two patients (0.5%) treated with nivolumab (one each died from pneumonitis and neutropenia with sepsis).

2.6 Rationale

Breast cancer (BC) is the most frequently diagnosed cancer and the second cause of cancer death in women in the Unites States[Siegel *et al.*, 2016]. In the metastatic setting, despite available therapies, virtually all patients will die from their disease. Thus, new treatments, such as

immunotherapy, are needed. The recognition that overexpression of immune checkpoint molecules in the tumor microenvironment has a critical role for antitumor immunity evasion and for cancer progression has revolutionized cancer treatment[Pardoll, 2012]. In particular, anti-PD-1/PD-L1 antibodies have demonstrated clinical activity in more than 15 cancer types and two PD-1 inhibitors (nivolumab and pembrolizumab) are FDA approved for treatment of advanced melanoma, non-small-cell lung carcinoma and renal cell carcinoma[Brahmer *et al.*, 2012, Topalian *et al.*, 2012, Topalian *et al.*, 2015]. More than 1,000 clinical trials of checkpoint blockers are currently ongoing. In this context, preclinical and clinical evidence suggesting that the immune system can recognize and fight against breast cancer [Kroemer *et al.,* 2015, Savas *et al.,* 2015]. It is now recognized that a fraction of breast tumors, have substantial lymphocyte infiltration and that this pathologic feature has prognostic implications [Savas *et al.*, 2015]. Furthermore, the high rates of PD-L1 and PD-1 expression in patients with TNBCs led to clinical trials to address the role of PD-1 blockers in this population [Gatalica *et al.,* 2014].

However, while immunotherapy with PD-1/PD-1 inhibitors has revolutionized treatment for many cancers, the majority of patients with metastatic breast cancer are refractory to it. The KEYNOTE 086 trial, the largest study evaluating the efficacy of PD-1 inhibitors (Pembrolizumab given in monotherapy; flat dose of 200mg every 3 weeks) in patients with mTNBC to date showed different outcomes based on the number of previous lines of therapy: cohort A included 170 patients who had received ≥1 prior chemotherapy for metastatic disease, and showed an objective response rate of 4.7% (2% - 9%) [Adams *et al.,* 2015] ; Perhaps, more important is the duration of response with the PD-1 inhibitors in patients with mTNBC: 6.3 months; with more than 5 (63%) responders without progression of disease at study data cut off; cohort B included 52 patients no prior systemic anticancer therapy for metastatic disease, and showed an objective response rate of 23% (14% - 36%) [Adams *et al.,* 2017]. In patients with HR+ metastatic breast cancer (MBC) has been tested, and also only a fraction of patients have shown benefit [objective response rates (ORR) range from 2.8% to 12%].[Hugo *et al.*, 2015, Dirix *et al.*, 2018] Altogether, these results suggest that there must be additional immunosuppressive factors causing resistance to immunotherapy in this population, and other agents must be added to anti-PD-1/PD-L1 treatment in order to enhance the benefit of immunotherapy in TNBC. In addition, predictive biomarkers are needed to identify patients most likely to benefit from immunotherapy.

High tumor mutational burden (TMB) correlates with high neoantigen burden[Giannakis *et al.*, 2016] and high T-cell infiltration. Retrospective studies have been shown that high TMB could be predictive of response to immune checkpoint inhibitors (ICI) across different cancer types[Snyder *et al.*, 2014, Rizvi *et al.*, 2015, Van Allen *et al.*, 2015]. Moreover, surrogates genomic markers of high TMB, including mismatch repair deficiency (dMMR) or micro-satellite instability high (MSI-H), or POLE/POLD1 mutations, also have shown to be predictive of response to checkpoint blockade[Le *et al.*, 2015, Johanns *et al.*, 2016, Mehnert *et al.*, 2016, Gong *et al.*, 2017]. Finally, prospective clinical trials have recently confirmed the importance of high TMB to predict benefit of PD-1 inhibition in lung cancer, both as monotherapy[Carbone *et al.*, 2017] and in combination with ipilimumab[Hellmann *et al.*].

In the United States, both pembrolizumab and nivolumab given as monotherapy are approved for the treatment of adult and pediatric patients with dMMR/MSI-H metastatic colorectal cancer with progression after treatment with a fluoropyrimidine, oxaliplatin, and irinotecan. Preclinical and clinical data support the idea that nivolumab and ipilimumab act synergistically to promote T-cell antitumor activity through complementary mechanisms of action[Curran *et al.*, 2010, Larkin *et al.*, 2015, Motzer *et al.*, 2018]. Furthermore, recent data from

the CHECKMATE-142 suggests that the combination of a PD-1 inhibitor (Nivolumab) with ipilimumab has better efficacy outcomes and is associated with a numerically higher response rate (55%; 95% CI, 45 to 64) than nivolumab monotherapy (31%; 95% CI, 21 to 43) in a similar population of patients (n = 74) with a comparable median follow-up time[Overman *et al.*, 2017, Overman *et al.*, 2018]. Importantly, this study also showed that this combination had a manageable safety profile[Overman *et al.*, 2018].

High TMB is observed in approximately 2% of all breast cancers, and up to 3.5% in the metastatic setting (Barroso-Sousa et al, ASCO Annual Meeting 2018). Given that breast cancers are the most common malignancies in woman in the United States, this frequency represents a large number of individuals. Importantly, approximately 80% of these hypermutated tumors harbor an APOBEC mutational signature, and only approximately 18% have defective DNA mismatch repair (MMR) signature. Although pembrolizumab is approved for the treatment of MMR deficient tumors independently of cancer type, our data suggests that most hypermutated breast cancers would not be identified if using only the MMR status as a biomarker. Therefore, there is a critical need to evaluate if hypermutated tumors, independently of MMR status, are more likely to be responsive to immune checkpoint inhibitors.

Thus, we hypothesize that patients with hypermutated HER2-negative metastatic breast cancers are more likely to derive an objective response to the combination of nivolumab and ipilimumab. We propose to test this hypothesis by evaluating the combination of nivolumab 3mg/Kg every 2 weeks with ipilimumab 1mg/Kg every 6 weeks (1 cycle = 6 weeks) in patients with hypermutated metastatic HER2- breast cancer in an open-label, single arm phase II study. The primary endpoint will be to evaluate the efficacy of the nivolumab plus ipilimumab, as defined by objective response rate (ORR) according to RECIST 1.1 in patients with hypermutated HER2- metastatic breast cancer previously treated with 0 to 3 lines of systemic therapy in the metastatic setting. Additionally, we will perform correlative studies in order to explore the effect of treatment on immune-genomic markers in the peripheral blood and in the tumor microenvironment. Research biopsies will be performed at baseline (mandatory if the lesion is accessible) and and at day 29 of Cycle 1 (+14 day scheduling window). Optional biopsies will be performed at progression in patients who experienced an objective response during the study. Moreover, blood samples will be collected within 7 days before starting therapy and every 6 weeks for 24 weeks, and then every 9 weeks. Furthermore, stool samples will be collected within 14 days before starting therapy and and at day 29 of Cycle 1 (+14 day scheduling window).

2.7 Correlative Studies Background

2.7.1 Blood and Tissue Analysis

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

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

The T-cell inflamed phenotype has positive prognostic value for several types of early stage cancer, including breast cancer[Dushyanthen *et al.*, 2015, Perez *et al.*, 2015], suggesting that the attempt by the host to generate an anti-tumor immune response reflects a biologic process associated with improved patient outcomes[Gajewski, 2015]. In breast oncology, different groups have demonstrated that the amount of tumor-infiltrating lymphocytes (TILs) in a tumor specimen, commonly assessed simply by histological evaluation of a standard hematoxylin and eosin-stained slide by a trained pathologist, is a significant predictor of both response to therapy and overall disease outcomes in the neoadjuvant and adjuvant settings [Denkert *et al.*, 2010, Loi *et al.*, 2013, Adams *et al.*, 2014, Ali *et al.*, 2014, Salgado *et al.*, 2014, Denkert *et al.*, 2015, Denkert *et al.*, 2015]. Recently, more in-depth methods of immunologic profiling are being explored in breast cancer, for example mRNA expression of immune-activating and immunosuppressive factors, and these additional immune profiles also appear to have prognostic significance[Perez *et al.*, 2015]. Furthermore, in metastatic setting, the phenotype T-cell-inflamed appears to be associated with clinical response to several immunotherapies, including checkpoint blockade[Herbst *et al.*, 2014]. Patients with this tumor phenotype seem to be good candidates for immune checkpoint inhibitor therapy, alone or in combination. Thus, the bulk of our correlative science in this trial highlights our especial interest in characterize a broad array of immune markers in metastatic hypermutated HER2-negative breast cancer, investigating whether those markers predict disease response to therapy.

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

These correlative projects are made possible by collaboration with Drs. Elizabeth Mittendorf, Catherine Wu, Scott Rodig and Evisa Gjini, and Mariano Severgnini, all of whom are laboratory scientists with extensive experience with immune profiling in advanced tumors. Further details can be found in Section 9.

2.7.2 Microbiome Analysis

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

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

This correlative project is made possible by collaboration with Dr. Elizabeth Mittendorf of Dana-Farber Cancer Institute and Dr. Andrew Chan of Massachusetts General Hospital. Further details can be found in Section 9.

2.7.3 Tumor Genomic Profile

In addition to the immune microenvironment, intrinsic tumor factors may be associated with response to immune checkpoint inhibitors. Although some of the mechanisms related to de novo or acquired resistance to ICI have been recently described, including loss of function in beta-2- microglobulin or defects in the interferon signaling pathway[Gao *et al.*, 2016, Zaretsky *et al.*, 2016], the knowledge of immune resistance remains largely unknown. Several gene/pathways have been described as possible candidates of having an immunosuppressive role in different advanced solid tumor, including MYC amplification[Casey *et al.*, 2016], activation in WNT-β-catenin pathway[Spranger *et al.*, 2015], activation in MAPK pathway, loss of PTEN[Li *et al.*, 2016, Peng *et al.*, 2016, George *et al.*, 2017]. On the other hand, few possible biomarkers of response to ICI have emerged, including mutational load[Snyder *et al.*, 2014, Rizvi *et al.*, 2015], tumor aneuploidy[Davoli *et al.*, 2017], mismatch repair defects[Le *et al.*, 2015], and BRCA2 mutation[Hugo *et al.*, 2016]. Notably, there is no data on genomic mechanisms of de novo resistance to anti-PD-1 therapy in patients with breast cancer.

Therefore, as a correlative study to this trial, we will to explore whether the number and/or type of mutations identified using a next generation sequencing (NGS) panel – OncoPanel - is correlated with patient outcomes (PFS, ORR, and CBR). This tool is a cancer genomic assay to detect somatic mutations, copy number variations and structural variants in tumor DNA extracted

from fresh, frozen or formalin-fixed paraffin-embedded samples. The OncoPanel assay surveys exonic DNA sequences of 447 cancer genes and 191 regions across 60 genes for rearrangement detection. DNA is isolated from tissue containing at least 20% tumor nuclei and analyzed by massively parallel sequencing using a solution-phase Agilent SureSelect hybrid capture kit and an Illumina HiSeq 2500 sequencer. The targeted NGS assay (OncoPanel) will be performed at the Center for Advanced Molecular Diagnostics (Department of Pathology, Brigham and Women's Hospital). This assay has been extensively validated and is used as a CLIA-approved clinical molecular test in our institution without any additional sequencing assays to validate the findings [Wagle *et al.*, 2012].

3. PARTICIPANT SELECTION

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

3.1 Eligibility Criteria

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

3.1.2 Breast cancer must be HER2-negative by IHC or non-amplified as determined by the current ASCO-CAP criteria. If patient has more than one histological result, the most recent one will be usedfor inclusion. Participants may be ER/PR positive or negative.

3.1.3 Patients must harbor tumors with total mutational burden (TMB) of at least 9 mutations per megabase assessed by a cancer-gene panel containing more than 300 genes, and performed in a CLIA verified laboratory. Tests like Foundation One, Oncopanel (DFCI), or IMPACT (MSKCC) are acceptable for including patients on this trial.

3.1.4 Participants must have measurable disease by RECIST version 1.1.

3.1.5 Participants must agree to undergo a research biopsy, if tumor is safely accessible, at baseline and at day 29 cycle 1 (+14 scheduling window). Previously collected archival tissue will be obtained on all participants. Participants for whom newly-obtained samples cannot be provided (e.g. inaccessible or participant safety concern) may submit an archived specimen (block or if not possible, 20 unstained slides).

3.1.6 Prior chemotherapy: Participants may have received 0-3 prior chemotherapeutic regimens for metastatic breast cancer and must have been off treatment with chemotherapy for at least 14 days prior to study treatment initiation.

3.1.7 Patients with hormone receptor positive breast cancer must have progressed on at least one prior line of endocrine therapy in the metastatic setting or have disease recurrence while on adjuvant endocrine therapy.

NOTE: Participants should also be adequately recovered from acute toxicities of prior treatment, with the exception of alopecia and peripheral sensory neuropathy.

3.1.8 Prior biologic therapy: Patients must have discontinued all biologic therapy at least 14 days prior to study treatment initiation.

3.1.9 Prior radiation therapy: Patients may have received prior radiation therapy in either the metastatic or early-stage setting. Radiation therapy must be completed 14 days prior to study treatment initiation.

NOTE: In all cases, there must be no ongoing complications from prior radiotherapy.

3.1.10 The subject is ≥18 years old.

3.1.11 ECOG performance status ≤1(Karnofsky ≥70%, see Appendix A).

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

absolute neutrophil count	≥1,000/mcL
platelets	≥100,000/mcL
hemoglobin	≥ 8 g/dl
total bilirubin	≤1.5 × institutional upper limit of normal (ULN) (or ≤ 2.0 x ULN in patients with documented Gilbert's Syndrome)
AST(SGOT)/ALT(SGPT)	≤2.5 × institutional ULN or (≤ 3 × institutional ULN for participants with documented liver metastases)
creatinine	≤1.5 × institutional ULN OR creatinine clearance ≥ 40 mL/min (using Cockcroft-Gault formula) for participants with creatinine levels above institutional ULN.

3.1.13 Female subjects of childbearing potential must have a negative pregnancy test (serum or urine) at screening.

Note: Childbearing potential is defined as: participants who have not reached a postmenopausal state (≥ 12 continuous months of amenorrhea with no identified cause other than menopause) and has not undergone surgical sterilization (removal of ovaries and/or uterus).

3.1.14 Women participants of childbearing potential must agree to use an adequate method of contraception. For women, contraception is required starting with the first dose of study medication through 150 days (5 months) after the last dose of study medication. Examples of contraceptive methods with a failure rate of < 1% per year include bilateral tubal ligation, male sterilization, established and proper use of hormonal contraceptives that inhibit ovulation, hormone-releasing intrauterine devices, and copper intrauterine devices. The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or postovulation methods) and withdrawal are not acceptable methods of contraception.

3.1.15 Participants on bisphosphonates may continue receiving bisphosphonate therapy during study treatment. Initiation of bisphosphonate or RANKL agent is allowed on study.

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

3.2 Exclusion Criteria

3.2.1 Major surgery within 2 weeks before the first dose of study treatment.

3.2.2 Concurrent administration of other anti-cancer therapy within 14 days of starting protocol therapy and during the course of this study.

3.2.3 The participant has received another investigational agent within 14 days of the first dose of study drug.

3.2.4 Has received prior therapy with an anti-PD-1, anti-PD-L1, anti-PD-L2, or anti-Cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) antibody.

3.2.5 Known brain metastases that are untreated, symptomatic, or require therapy to control symptoms. Participants with a history of treated central nervous system (CNS) metastases are eligible. Treated brain metastases are defined as those having no evidence of progression for ≥ 2 weeks after treatment, and no ongoing requirement for corticosteroids, as ascertained by clinical examination and brain imaging (magnetic resonance imaging or CT scan) completed during screening. Subject must be either off corticosteroids, or on a stable or decreasing dose of ≤ 10 mg daily (or equivalent) for at least 7 days prior to first study treatment. Treatment for brain metastases may include whole brain radiotherapy, radiosurgery, or a combination as deemed appropriate by the treating physician. Participants with CNS metastases treated by neurosurgical resection or brain biopsy performed within 28 days before study treatment initiation will be excluded.

3.2.6 The subject has uncontrolled, significant intercurrent or recent illness. Individuals with a history of different malignancy are ineligible except for the following circumstances. Individuals with a history of other malignancies are eligible if they have been disease-free for at least 3 years or are deemed by the investigator to be at low risk for recurrence of that malignancy.

3.2.7 Participant has an active infection requiring IV antibiotics at initiation of study therapy.

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

3.2.9 Subjects with current pneumonitis, or requiring supplementary O2 therapy.

3.2.10 The participant is known to be positive for human immunodeficiency virus (HIV), HepBsAg, or HCV RNA. HIV-positive participants on combination antiretroviral therapy are ineligible

3.2.11 Participants with any other active malignancy requiring concurrent intervention.

3.2.12 Known hypersensitivity to any of the components of ipilimumab or nivolumab.

3.2.13 The participant has received a live vaccine 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. The use of the inactivated seasonal influenza vaccine (Fluzone®) is allowed.

3.2.14 The participant is pregnant or breastfeeding.

Inclusion of Women and Minorities

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

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

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

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

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

4.2 Registration Process for DF/HCC Institutions

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

4.3 General Guidelines for Other Investigative Sites

Eligible participants will be entered on study centrally at the coordinating center through the Study Coordinator or Project Manager. The list of required forms can be found in section 4.4. Following registration, participants should begin protocol therapy within 7 days. Issues that would cause treatment delays should be discussed with the Overall PI. If the subject does not receive protocol therapy following registration, the subject must be taken off-study in the CTMS (OnCore) with an appropriate date and reason entered.

4.4 Registration Process for Other Investigative Sites

To register a participant, the following documents should be completed by the participating site and faxed to 617-632-5152 or e-mailed to ctopm@dfci.harvard.edu to the project manager:

- Signed participant consent form
- HIPAA authorization form
- Eligibility Checklist
- Cancer gene panel showing ≥ 9 tumor mutations per megabase
- Baseline exam note including medical/surgical history, ECOG, vital signs
- Pathology reports including documentation of HER2 status
- EKG Report
- Laboratory report including hematology, chemistries, PT/PTT, and pregnancy test
- Tumor Assessment report (CAP CT and/or MRI)
- Brain MRI report (if applicable)

To complete the registration process, the Coordinator or Project manager will follow DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject protocol Registration* (SOP #: REGIST-101) and register the participant on the protocol. The coordinator will fax or e-mail the participant study number and confirmation of registration to the participating site.

NOTE: Registration can only be conducted during the business hours of 8:00 AM and 5:00 PM Eastern Standard Time, Monday through Friday. Same day treatment registrations will only be accepted with prior notice and discussion with the DF/HCC Lead Institution.

5. TREATMENT PLAN

5.1 Treatment Regimen

This is an open-label, single-arm, multi-center phase 2 study of nivolumab 3 mg/Kg given intravenously every 2 weeks, in combination with ipilimumab 1 mg/Kg given intravenously every 6 weeks, in subjects with hypermutated metastatic HER2- breast cancer previously treated with 0 to 3 chemotherapy regimens in the metastatic setting. Thirty patients will be enrolled to the study to evaluate the efficacy of the nivolumab plus ipilimumab, as defined by objective response rate (ORR) according to RECIST 1.1.

No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy. Agents may be administered within +/- 3 days of their expected date.

Table 1: Regimen Description

Agent	Premedication ; Precautions	Dose	Route	Schedule	Cycle Length
Nivolumab	Not routinely necessary unless prior infusion reaction.	3 mg/kg	IV over 60 minutes (+/- 5 minute infusion window)	Days 1, 15, 29	42 days (6 weeks)
Ipilimumab	Not necessary	1 mg/kg	IV over 60 minutes (+/- 5 minute infusion window)	Day 1	42 days (6 weeks)

5.2 Pre-Treatment Criteria

5.2.1 Cycle 1, Day 1

If screening laboratory values were completed ≤ 96 hours (4 days) before Cycle 1 Day 1, laboratory tests do not need to be repeated on Cycle 1 Day 1 and the screening laboratory values may be used as the Cycle 1 Day 1 values.

If screening laboratory values were completed > 96 hours (4 days) prior to Cycle 1 Day 1, laboratory tests must be repeated on Cycle 1 Day 1. Laboratory values drawn on Cycle 1 Day 1 must re-meet eligibility criteria, exceptions to this are possible following discussion with the principal investigator.

- absolute neutrophil count ≥1,000/mcL
- platelets ≥100,000/mcL
- hemoglobin ≥ 8 g/dl

- total bilirubin ≤1.5 × institutional upper limit of normal (ULN) (or 2.0 x ULN in patients with documented Gilbert's Syndrome)
- AST(SGOT)/ALT(SGPT) ≤2.5 × institutional ULN or ≤ 3 × institutional ULN for participants with documented liver metastases
- creatinine >1.5 ×within normal institutional ULN OR creatinine clearance ≥40 mL/min (using Cockcroft-Gault formula) for participants with creatinine levels above institutional ULN.

5.2.2 Subsequent Cycles, Day 1, and all cycles day 15 and day 29

- absolute neutrophil count ≥1,000/mcL
- platelets ≥75,000/mcL
- AST(SGOT)/ALT(SGPT) ≤ 2.5 × institutional ULN or ≤ 3 × institutional ULN for participants with documented liver metastases
- total bilirubin ≤1.5 × institutional ULN (or 2.0 x ULN in patients with documented Gilbert's Syndrome)

5.3 Agent administration

5.3.1 Nivolumab Administration

Nivolumab will be administered as an IV infusion over approximately 60 minutes (+/- 5 minute infusion window). Nivolumab will be administered every 2 weeks, on cycle days 1, 15, and 29. Weight for dosage calculations should be performed per institutional standards of practice. On days where both nivolumab and ipilimumab will be administered, nivolumab should be administered first. The ipilimumab infusion will start at least 30 minutes after completion of the nivolumab infusion.

5.3.2 Ipilimumab administration

Ipilimumab will be administered as an IV infusion over approximately 60 minutes (+/- 5 minute infusion window). Ipilimumab will be administered every 6 weeks, on cycle day 1. Weight for dosage calculations should be performed per institutional standards of practice. Re-calculation of dosage for fluctuations in body weight should be made in accordance with institutional guidelines. On days where both nivolumab and ipilimumab will be administered, nivolumab should be administered first. The ipilimumab infusion will start at least 30 minutes after completion of the nivolumab infusion.

5.4 General Concomitant Medication and Supportive Care Guidelines

5.4.1 Concomitant Medication Guidelines –

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or

vaccination may be required. The investigator should discuss any questions regarding this with the overall PI.

Acceptable Concomitant Medications
All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care and documented in the medical record. All medications being taken by the patient including dosage, frequency, route, and dates of administration will be recorded in the electronic medical record.

Palliative radiotherapy is permitted to areas not followed for response, but presence of new or worsening metastases will be considered progression; however, if there is clear evidence of clinical benefit, treatment may be continued after completion of palliative radiotherapy. Protocol therapy should be interrupted one week before radiotherapy and held until radiotherapy is completed. Patients can restart protocol therapy once clinically stable as deemed by the treating investigator, but no earlier than one week after completing radiotherapy.

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

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Investigational agents other than nivolumab and ipilimumab
- Radiation therapy (except palliative radiotherapy as noted above)
- 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. The use of the inactivated seasonal influenza vaccine (Fluzone ®) is allowed.

- Systemic glucocorticoids should be avoided for any purpose other than to modulate symptoms from radiation or an event of clinical interest of suspected immunologic etiology. If corticosteroids are required for this purpose, the minimum effective dose should be used. The use of physiologic doses of corticosteroids (10 mg prednisone daily or equivalent) can be used without principal investigator (PI) authorization.
- Subjects may receive other medications that the investigator deems to be medically necessary.

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

5.4.2 Supportive Care Guidelines – General Medications

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

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

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

Potential Drug Interactions:

- Drug-Drug Interaction Potential with Nivolumab
    - Although monoclonal antibodies are not direct inhibitors/inducers of metabolizing enzymes, recent literature reports suggest that therapeutic proteins that are modulators of cytokines may indirectly affect expression of cytochrome (CYP) enzymes. The indirect drug-drug interaction potential of nivolumab was assessed using systematic cytokine modulation data for cytokines known to modulate CYP enzymes, at single and multiple doses of 0.3 to 10 mg/kg Q3 weeks.
    - There were no meaningful changes in cytokines known to have indirect effects on CYP enzymes across all dose levels of nivolumab (0.3, 2 and 10 mg/kg) during the course of treatment. This lack of cytokine modulation suggests that nivolumab has no or low potential for modulating CYP enzymes, thereby indicating a low risk of therapeutic protein-drug interaction.
    - Nivolumab is an IgG4 monoclonal antibody, which is eliminated by mechanisms similar to that of other antibodies, namely by non-specific catabolism (mainly by enzymes in the reticuloendothelial system). These enzymes are not known to be inhibited or induced by drugs, and therefore it is unlikely that other drugs will have an impact on the PK of nivolumab.
- Drug-Drug Interaction Potential with Ipilimumab
    - Ipilimumab is a human monoclonal antibody that is not metabolized by cytochrome P450 enzymes (CYP2) or other drug metabolizing enzymes.
    - The use of anticoagulants is known to increase the risk of gastrointestinal hemorrhage. Since gastrointestinal hemorrhage is an adverse reaction with ipilimumab, patients who require concomitant anticoagulant therapy should be monitored closely.

5.5 Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse event(s), treatment may continue for 24 months, or until one of the following criteria applies:

- Disease progression by RECIST 1.1 criteria. Please note that although the primary endpoint is ORR as defined by RECIST 1.1, patients may remain on protocol therapy until the time of disease progression by irRC criteria. The immune criteria allows treatment beyond initial radiographic worsening of disease in order to distinguish between pseudoprogression and true disease progression.
- Participants who have attained a confirmed complete response (CR) that have been treated for at least 24 weeks on protocol therapy and had at least two treatments with nivolumab beyond the date when the initial CR was declared. Participants who stop nivolumab with CR may be eligible for additional nivolumab therapy if they progress after stopping study treatment. This retreatment is termed the Second Course Phase Therapy. See additional details below.
- 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 in the CTMS system (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 Overall PI, Sara Tolaney, [redacted]

Second Course Phase (Retreatment Period)

Participants may elect to stop nivolumab and ipilimumab with confirmed CR after at least 24 weeks of treatment.

Subjects who stop nivolumab with CR may be eligible for additional nivolumab therapy if they progress after stopping study treatment. This retreatment is termed the Second Course Phase of this study and is only available if the study remains open and the subject meets the following conditions:

- Stopped initial treatment with nivolumab and ipilimumab after attaining an investigator-determined confirmed CR according to RECIST 1.1, was treated for at

least 24 weeks with nivolumab before discontinuing therapy, and received at least two treatments with nivolumab beyond the date when the initial CR was declared

AND

- experienced an investigator-determined confirmed radiographic disease progression after stopping their initial treatment with nivolumab
- did not receive any anti-cancer treatment other than ipilimumab since the last dose of nivolumab

Non-DF/HCC sites will fax or email documentation to the Coordinating Center at 617-632-5152 or ctopm@dfci.harvard.edu for review. Provided the patient meets criteria per protocol for retreatment, the Coordinating Center will process the request and the participating site will be notified that the patient may proceed to retreatment.

Subjects who restart treatment will be retreated at the same dose and dose interval as when they last received nivolumab. Visit requirements are as outlined for subjects per the initial treatment phase of the trial.

5.6 Duration of Follow Up

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

Participants who are alive and free of disease progression at the time of removal from protocol therapy will be followed for first disease progression event after removal from protocol therapy. Participants will be followed for survival until death. This will be performed by checking the medical record or reaching out to local provider offices as needed, annually.

Tumor assessments should continue to be performed every 6-12 weeks on these participants until radiographic disease progression or initiation of another anti-cancer therapy; however, it will not constitute a protocol violation if restaging at this interval is not possible.

After first disease progression, participants will continue to be followed for survival until death. This will be performed by checking the medical record or reaching out to local provider offices as needed, annually.

5.7 Criteria for Taking a Participant Off Study

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

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

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

6. DOSING DELAYS/DOSE MODIFICATIONS

Dose delays will be made as indicated in the following tables. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

Given that it may be difficult to tell which agent is causing which toxicity due to overlapping AE profiles, it is expected that both agents will be concurrently held or delayed per Table 6.1.

6.1 Toxicity Management

Table 4: Gastrointestinal Adverse Event Management

Note: Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue protocol therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.

Grade of Diarrhea / Colitis	Management	Follow-Up
Grade 1	Continue protocol therapy. Treat symptoms as clinically appropriate.	Monitor patient closely for worsening symptoms. Educate patient to report worsening immediately. **If worsens:** Treat as Grade 2 or ≥ Grade 3.
Grade 2	Delay protocol therapy. Treat symptoms as clinically appropriate.	**If improves to Grade 1:** Resume protocol therapy **If persists > 5-7 days or recurs:** Administer 0.5-1.0 mg/kg/day methylprednisolone or oral equivalent When symptoms improve to Grade 1, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume therapy per protocol. **If worsens or persists > 3-5 days with oral steroids:** Treat as ≥ Grade 3.
≥ Grade 3	Permanently discontinue or delay protocol therapy * Administer 1.0 to 2.0 mg/kg/day	**If improves:** Continue steroids until grade 1, then taper over at least 1 month

Table 4: Gastrointestinal Adverse Event Management		
Note: Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue protocol therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.		
Grade of Diarrhea / Colitis	**Management**	**Follow-Up**
	methylprednisolone IV or IV equivalent Add prophylactic antibiotics for opportunistic infections Consider lower endoscopy as clinically appropriate	**If persists > 3-5 days, or recurs after improvement:** Add infliximab 5 mg/kg (if no contraindication) Consider testing CMV for steroid refractory colitis **Note:** Infliximab should not be used in cases of perforation or sepsis
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids. *Discontinue for Grade 4 diarrhea or colitis. For Grade 3 diarrhea or colitis, 1) Nivolumab monotherapy: Nivolumab can be delayed. 2) Nivolumab+ Ipilimumab combination: Ipilimumab should be discontinuedwhile nivolumab can be delayed. Nivolumab monotherapy can be resumed when symptoms improve to Grade 1.		

Table 5: Renal Adverse Event Management		
Note: Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue protocol therapy.		
Grade of Creatinine Elevation	**Management**	**Follow-Up**
Grade 1	Continue protocol therapy. Monitor creatinine weekly.	**If returns to baseline:** Resume routine creatinine monitoring per protocol **If worsens:** Treat as Grade 2-3 or 4
Grade 2-3	Delay protocol therapy Monitor creatinine every 2-3 days Administer 0.5 to 1.0 mg/kg/day methylprednisolone IV or oral equivalent Consider renal biopsy with nephrology consult as clinically appropriate	**If returns to Grade 1:** Taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume protocol therapy and routine creatinine monitoring per protocol **If elevations persist > 7 days or worsens:** Treat as Grade 4

Table 5: Renal Adverse Event Management		
Note: Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue protocol therapy.		
Grade of Creatinine Elevation	**Management**	**Follow-Up**
Grade 4	Permanently discontinue protocol therapy Monitor creatinine daily Administer 1.0-2.0 mg/kg/day methylprednisolone IV or IV equivalent Consult nephrologist and consider renal biopsy as clinically appropriate	**If returns to Grade 1:** Taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections **If not improvement or worsening,** add additional immunosuppression
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.		

Table 6: Pulmonary Adverse Event Management		
Note: Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue protocol therapy. Evaluate with imaging and pulmonary consultation as clinically appropriate.		
Grade of Pneumonitis	**Management**	**Follow-Up**
Grade 1	Consider delay of protocol therapy Monitor for symptoms every 2-3 days Consider Pulmonary and ID consults	Re-image at least every 3 weeks **If worsens:** Treat as Grade 2 or ≥ Grade 3.
Grade 2	Delay protocol therapy Obtain Pulmonary and ID consults as clinically appropriate Monitor symptoms daily, consider hospitalization Administer 1.0 mg/kg/day methylprednisolone IV or oral equivalent Consider bronchoscopy, lung biopsy as clinically appropriate	Consider reimaging weekly **If improves:** Re-imaging every 1-3 weeks, monitoring symptoms every 2-3 days. When symptoms return to near baseline, taper steroids over at least 1 month and then resume protocol therapy and consider prophylactic antibiotics **If not improving after 2 weeks or worsening:**

Table 6: Pulmonary Adverse Event Management		
Note: Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue protocol therapy. Evaluate with imaging and pulmonary consultation as clinically appropriate.		
Grade of Pneumonitis	**Management**	**Follow-Up**
		Treat as ≥ Grade 3.
≥ Grade 3	Permanently discontinue protocol therapy Hospitalize as clinically indicated Obtain pulmonary and ID consults as appropriate Administer 2-4 mg/kg/day methylprednisolone IV or IV equivalent Add prophylactic antibiotics for opportunistic infections Consider bronchoscopy, lung biopsy	**If improves to baseline:** Taper steroids over at least 6 weeks. **If not improving after 48 hours or worsening:** Add additional immunosuppression.
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.		

Table 7: Hepatic Adverse Event Management		
Note: Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue protocol therapy. Consider imaging for obstruction.		
Grade of Liver Test Function	**Management**	**Follow-Up**
Grade 1	Continue protocol therapy	Continue LFT monitoring per protocol **If worsens:** Treat as Grade 2 or ≥ Grade 3.
Grade 2	Delay protocol therapy Increase frequency of monitoring to every 3 days	**If returns to baseline:** Resume routine monitoring, resume protocol therapy **If elevations persist > 5-7 days or worsen:** Administer 0.5-1 mg/kg/day

Table 7: Hepatic Adverse Event Management		
Note: Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue protocol therapy. Consider imaging for obstruction.		
Grade of Liver Test Function	**Management**	**Follow-Up**
		methylprednisolone or oral equivalent and when LFT returns to grade 1 or baseline, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume protocol therapy
≥ Grade 3	Permanently discontinue protocol therapy[A] Increase frequency of monitoring to every 1-2 days Administer 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent[B] Add prophylactic antibiotics for opportunistic infections Consult gastroenterologist or hepatologist as clinically appropriate	**If returns to grade 2:** Taper steroids over at least 1 month **If does not improve in >3-5 days, worsens or rebounds:** Add mycophenolate mofetil 1 g BID If no response within an additional 3-5 days, consider tacrolimus or other immunosuppressants per local guidelines Note: avoid infliximab due to potential risk of liver failure
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids. A. Protocol therapy may be delayed rather than discontinued if AST/ALT ≤ 8 × institutional ULN or total bilirubin ≤ 5 × institutional ULN. B. The recommended starting dose for Grade 4 hepatitis is 2 mg/kg/day methylprednisolone IV.		

Table 8: Endocrinopathy Adverse Event Management		
Note: Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue protocol therapy. Consider visual field testing, endocrinology consultation, and imaging.		
Event	**Management**	**Follow-Up**
Asymptomatic TSH Elevation Or Hyperglycemia requiring no medical intervention	Continue protocol therapy. If TSH < 0.5 × lower limit of normal (LLN), or TSH > 2 × ULN, or consistently out of range in 2 subsequent measurements: include free T4 at subsequent cycles as clinically indicated; consider endocrinology consult.	Continue to follow thyroid function per protocol. Monitor blood sugar If symptomatic or blood sugar requiring initiation of treatment or change in management, manage as below

<table>
<tr><th colspan="3">Table 8: Endocrinopathy Adverse Event Management</th></tr>
<tr><td colspan="3">Note: Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue protocol therapy. Consider visual field testing, endocrinology consultation, and imaging.</td></tr>
<tr><td>Symptomatic Endocrinopathy
Or
Hyperglycemia requiring initiation of treatment or change in daily management or work up</td><td>Evaluate endocrine function

Consider pituitary scan

Symptomatic with abnormal lab/pituitary scan:

Delay protocol therapy

Administer 1-2 mg/kg/day methylprednisolone IV or PO equivalent

Initiate appropriate hormone therapy

No abnormal lab/pituitary MRI scan but symptoms persist:

Continue therapy, Repeat labs in 1-3 weeks / MRI in 1 month

Hyperglycemia:
Screen for diabetic ketoacidosis (DKA). If negative, continue IO and treat diabetes. If DKA, hold IO, admit, manage DKA. Steroids not recommended for DKA/hyperglycemia</td><td>If improves (with or without hormone replacement):

Taper steroids over at least 1 month and consider prophylactic antibiotics for opportunistic infections

Resume protocol therapy

Patients with adrenal insufficiency may need to continue steroids with mineralocorticoid component</td></tr>
<tr><td>Suspicion of adrenal crisis (e.g. severe dehydration, hypotension, shock out of proportion to current illness)</td><td>Delay protocol therapy

Rule out sepsis

Stress dose of IV steroids with mineralocorticoid activity

Administer IV fluids

Consult endocrinologist as clinically appropriate

If adrenal crisis ruled out, then treat as above for symptomatic endocrinopathy</td><td>Follow until resolution.

If adrenal crisis resolves and patient is adequately controlled with hormone repclacement, patient can resume therapy per protocol.</td></tr>
<tr><td colspan="3">Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.</td></tr>
</table>

Table 9: Skin Adverse Event Management

Note: Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue protocol therapy.

Grade of Rash[A]	Management	Follow-Up
Grade 1 – 2	Continue protocol therapy. Administer symptomatic therapy (e.g. antihistamines, topical steroids)	**If persists > 1-2 weeks or recurs:** Consider skin biopsy Delay protocol therapy Consider 0.5-1.0 mg/kg/day methylprednisolone IV or oral equivalent. Once improving, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume protocol therapy **If worsens:** Treat as Grade 3-4
Grade 3-4	Delay or discontinue protocol therapy Consider skin biopsy Dermatology consult as clinically appropriate Administer 1.0-2.0 mg/kg/day IV methylprednisolone IV or IV equivalent	**If returns to Grade 1:** Taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections. Resume protocol therapy if appropriate.

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

A. If Stevens-Johnson Syndrome (SJS) ,Toxic Epidermal Necrolysis (TEN), Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS)is suspected, withhold protocol therapy and refer patient for specialized care for assessment and treatment. If SJS or TEN is diagnosed, permanently discontinue protocol therapy.

Table 10: Neurological Adverse Event Management		
Note: Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue protocol therapy.		
Grade of Neurological Toxicity	**Management**	**Follow-Up**
Grade 1	Continue protocol therapy Discontinue therapy for select AEs*	Continue to monitor the patient. **If worsens:** Treat as Grade 2 or ≥ Grade 3.
Grade 2	Delay protocol therapy Discontinue therapy for select AEs* Treat symptoms per local guidelines Consider neurology consult Consider 0.5 to 1.0 mg/kg/day methylprednisolone IV or oral equivalent	**If improves to baseline:** Resume protocol therapy. **If worsens:** Treat as ≥ Grade 3.
≥ Grade 3	Permanently discontinue protocol therapy Obtain neurology consult as clinically appropriate Treat symptoms per local guidelines Administer 1.0-2.0 mg/kg/day methylprednisolone IV or IV equivalent Add prophylactic antibiotics for opportunistic infections	**If returns to Grade 2:** Taper steroids over at least 1 month. **If worsens or atypical presentation:** Consider IVIG or other immunosuppressive therapies per local guidelines.
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids. * Discontinue for any grade myasethenia gravis, Guillain-Barre syndrome, treatment-related myelitis, or encephalitis		

Table 11: Pancreatic Adverse Event Management		
Note: Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue protocol therapy.		
Grade of Amylase or Lipase Elevation, Pancreatitis	**Management[B]**	**Follow-Up**
	Continue protocol therapy.	Resume routine protocol monitoring.

Table 11: Pancreatic Adverse Event Management		
Note: Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue protocol therapy.		
Grade of Amylase or Lipase Elevation, Pancreatitis	**Management[B]**	**Follow-Up**
Asymptomatic Grade 1[A]	Monitor amylase and lipase weekly until resolution to baseline.	**If worsens:** Treat as Grade 2-3 or 4.
Asymptomatic Grade 2-3[A]	Delay protocol therapy. Monitor amylase and lipase weekly until resolution to baseline.	**If improves to baseline:** Resume protocol therapy and routine protocol monitoring. **If worsens:** Treat as Grade 4.
Asymptomatic Grade 4[A]	Permanently discontinue protocol therapy. Monitor amylase and lipase weekly until resolution.	Monitor patient until resolution to baseline.
Any Symptomatic Elevations in Amylase/Lipase, Pancreatitis	Permanently discontinue protocol therapy. Consider GI consult as clinically appropriate. Administer supportive care.	Monitor patient until resolution to baseline.
A. Patients should be monitored for signs/symptoms consistent with pancreatitis, including abdominal pain and vomiting. B. Corticosteroids do not seem to alter the natural course of amylase/lipase elevations.		

Table 12: Ocular Adverse Event Management		
Note: Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue protocol therapy. Work-up for ocular adverse events should also consider pituitary inflammation as a potential cause.		
Event Grade	**Management**	**Follow-Up**
Grade 1	Continue protocol therapy. Monitor patient for worsening symptoms. Consider ophthalmologist consult as clinically appropriate. Administer topical	**If worsens:** Treat as Grade 2 or ≥ Grade 3.

Table 12: Ocular Adverse Event Management		
Note: Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue protocol therapy. Work-up for ocular adverse events should also consider pituitary inflammation as a potential cause.		
Event Grade	**Management**	**Follow-Up**
	corticosteroids as clinically indicated.	
Grade 2	Delay protocol therapy. Obtain ophthalmologist consult. Administer topical corticosteroids as clinically indicated.	**If improves to baseline:** Resume protocol therapy. **If worsens:** Treat as ≥ Grade 3.
≥ Grade 3	Permanently discontinue protocol therapy. Obtain ophthalmologist consult. Administer systemic corticosteroids as clinically indicated.	Monitor patient until resolution to baseline. Taper systemic corticosteroids as clinically appropriate.
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.		

Table 13: Infusion-Related Reactions		
Event Grade	**Management**	**Follow-Up**
Grade 1	Monitor patient until recovery of symptoms to baseline.	Prophylactic premedications are recommended for future infusions: diphenhydramine 25 – 50 mg (or equivalent) and/or acetaminophen 325 to 1000 mg (or equivalent) at least 30 minutes before additional infusions.
Grade 2	Stop infusion. Administer diphenhydramine 50 mg IV (or equivalent) and/or acetaminophen 325 – 1000 mg PO (or equivalent). Corticosteroids or bronchodilators may also be administered as clinically appropriate. Monitor patient until symptoms	Prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or acetaminophen 325 to 1000 mg (or equivalent), administered at least 30 minutes prior to the infusion. If necessary per the judgment of the treating investigator, corticosteroids (up to 25 mg SoluCortef or equivalent) may be used as well.

Table 13: Infusion-Related Reactions		
Event Grade	**Management**	**Follow-Up**
	have resolved to baseline. After resolution of symptoms, the infusion may be restarted at 50% of the original infusion rate. If no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. If symptoms recur, no further administration of nivolumab or ipilimumab should occur at that visit. Administer 50 mg IV diphenhydramine (or equivalent) and monitor patient until the symptoms resolve to baseline.	
≥ Grade 3	Permanently discontinue protocol therapy. Emergency care should be implemented as clinically appropriate and per local institutional standards of practice.	Monitor patient until resolution to baseline.

6.2 Dose Delays

Dosing of ipilimumab or nivolumab may be held at the treating investigator's discretion for any toxicity of any grade. Additionally, holding of the study agents may be required as indicated in the tables in **Section 6.1**.

A treatment delay of up to six weeks for nivolumab and up to 12 weeks for ipilimumab is allowable. Participants requiring a longer hold should be removed from protocol therapy. Exceptions for participants exhibiting clinical benefit may be possible with approval from the principal investigator.

If study agent dosing is held due to toxicity, the counting of cycle days and assessment schedule will continue without interruption. For example, a participant who does not receive their Cycle 3 Day 15 dose of nivolumab due to toxicity will proceed with their next regularly scheduled clinic visit (Cycle 3 Day 29) as previously planned. Alternatively, if ipilimumab is held on C3D1 and nivolumab is given, the ipilumumab dose would be omitted for that cycle. Additional interim clinic visits may be scheduled to manage toxicity, however the cycle will not restart due to dosing delays due to adverse events and the assessment schedule (including tumor imaging evaluations) will continue as originally planned.

6.3 Dose Reductions

There will be no dose reductions for nivolumab or ipilimumab allowed. Participants who cannot tolerate the assigned doses of nivolumab or ipilimumab should be removed from protocol therapy.

6.4 Overdose Management

There is no available information concerning overdose with nivolumab or ipilimumab. In case of overdose, patients must be closely monitored for signs or symptoms of adverse reactions and appropriate symptomatic treatment instituted. There are no specific antidotes. Any overdoses of either drug should be reported as an SAE to BMS. Please see section 7.3.4 for information on reporting.

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 Expected Toxicities

7.1.1 Adverse Events List

In addition to the list below, please also refer to the investigator's brochure (IB) for both nivolumab and ipilimumab for comprehensive adverse event information.

7.1.1.1.1. Adverse Event List for Nivolumab combined with Ipilimumab

Common adverse events associated with combination treatment include:

- Fatigue
- Fever
- Chills
- Flu-like symptoms
- Infusion-related reactions
- Headache
- Dizziness
- Hyperthyroidism or hypothyroidism
- Cough
- Dyspnea
- Pneumonitis

- Hypotension
- Dry mouth
- Anorexia
- Diarrhea
- Nausea
- Vomiting
- Dehydration
- Abdominal pain
- Colitis
- Elevated liver transaminases
- Elevated lipase and/or amylase
- Rash
- Pruritus
- Vitiligo
- Joint pain or stiffness
- Muscle soreness, weakness, stiffness, or spasms

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 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

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

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

7.3 Expedited Adverse Event Reporting

In the event of an unanticipated problem or life-threatening complications treating investigators must immediately notify the Overall PI.

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 100 days of the last dose of Nivolumab and 30 days of the last dose of Ipilimumab. SAEs that occur after 30 days post-treatment must only be reported to the Overall PI if they are determined to be related to Nivolumb or Ipilimumab.

For Multi-Center Trials where a DF/HCC investigator is serving as the Sponsor, each participating institution **must** abide by the reporting requirements set by the DF/HCC. This applies to any medical event equivalent to an unexpected grade 2 or 3 with a possible, probable or definite attribution, unexpected grade 4 toxicities, and grade 5 (death) regardless of study phase or attribution.

7.3.1 Reporting to the Institutional Review Board (IRB)

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

Other investigative sites will report to their respective IRBs according to the local IRB's policies and procedures in reporting adverse events. A copy of the submitted institutional AE form should be forwarded to the Overall PI within the timeframes detailed in the table below.

Attribution	**DF/HCC Reportable AEs**				
	Gr. 2 & 3 AE Expected	**Gr. 2 & 3 AE Unexpected**	**Gr. 4 AE Expected**	**Gr. 4 AE Unexpected**	**Gr. 5 AE Expected or Unexpected**
Unrelated Unlikely	Not required	Not required	5 calendar days[#]	5 calendar days	24 hours*
Possible Probable Definite	Not required	5 calendar days	5 calendar days[#]	5 calendar days	24 hours*
# If listed in protocol as expected and not requiring expedited reporting, event does not need to be reported.					
* For participants enrolled and actively participating in the study ***or*** for AEs occurring within 30 days of the last intervention (100 days from last dose of nivolumab), the AE should be reported within 1 business day of learning of the event.					

The Overall PI will submit AE reports from outside institutions to the DFCI OHRS according to DFCI IRB policies and procedures for reporting adverse events.

7.3.2 **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.

Any report that meets FDA reporting requirements should also be submitted to BMS per section 7.3.4. These events are generally those that are both serious, at least possibly related to the investigational product(s), and are unexpected.

7.3.3 **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.

7.3.4 **Expedited Reporting to BMS**

SAEs, whether related or not related to study drug, and pregnancies must be reported to BMS within 24 buiness hours of the awareness of the event. SAEs must be recorded on a Medwatch Form 3500A.

SAE Email Address: Worldwide.Safety@BMS.com

SAE Facsimile Number: 609-818-3804

Adverse events generally considered serious are those that occur at any dose during any use of BMS product that:

- Results in death
- Is life-threatening (defined as an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or causes prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [eg, medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)
- Suspected transmission of an infectious agent (eg, pathogenic or nonpathogenic) via the study drug is an SAE.

A planned medical or surgical procedure is not, in itself, an SAE. Generally, any events that meet any of the seriousness criteria listed above and that are caused by progression of the disease under the study (metastatic breast cancer) are not reportable. Any instance of pregnancy, overdose, potential drug-induced liver injury (DILI), and secondary malignancies, though not necessarily considered serios per these definitions should be reported as SAEs.

If only limited information is initially available, a follow-up report is required and should include the same investigator term(s) initially reported.

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours of awareness to BMS (or designee) using the same procedure used for transmitting the initial SAE report.

All SAEs should be followed to resolution or stabilization.

7.3.5 Reporting of Pregnancy

If, following initiation of the investigational product, it is subsequently discovered that a study participant is pregnant or may have been pregnant at the time of investigational product exposure, including during at least 5 half-lives after product administration, the investigational product will be permanently discontinued in an appropriate manner (eg, dose tapering if necessary for participant).

The investigator must immediately notify Worldwide.Safety@bms.com of this event via either the CIOMS, MedWatch or appropriate Pregnancy Surveillance Form in accordance with SAE reporting procedures.

Protocol-required procedures for study discontinuation and follow-up must be performed on the participant.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the CIOMS, MedWatch, BMS Pregnancy Surveillance Form, or approved site SAE form. A BMS Pregnancy Surveillance Form may be provided upon request.

Any pregnancy that occurs in a female partner of a male study participant should be reported to BMS. Information on this pregnancy will be collected on the Pregnancy Surveillance Form. In order for Sponsor or designee to collect any pregnancy surveillance information from the female partner, the female partner must sign an informed consent form for disclosure of this information.

7.4 Routine Adverse Event Reporting

All Grade 2 or higher Adverse Events (excluding non-clinically significant laboratory values) **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.**

8. PHARMACEUTICAL INFORMATION

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

8.1 Nivolumab

8.1.1 Description

Nivolumab is also referred to as BMS-936558-01 or BMS-936558. Nivolumab is a soluble protein consisting of 4 polypeptide chains, which include 2 identical heavy chains and 2 identical light chains. The physical and chemical properties of nivolumab are provided in Table 8.1.1 below. The geometric mean of terminal T-HALF was 25.6 days and the typical clearance was 8.8 mL/h, which are consistent with those of full human immunoglobulin antibodies.

Table 14: Nivolumab Physical and Chemical Properties

BMS Number	BMS-936558-01
Other Names	Nivolumab, BMS-936558, MDX1106, ONO-4538, anti-PD-1
Molecular Weight	146,221 daltons
Appearance	Clear to opalescent, colorless to pale yellow liquid, light (few) particulates may be present
Solution pH	5.5 to 6.5

8.1.2 Storage and Stability

Vials of Nivolumab injection must be stored at 2°-8°C (36°-46°F) and protected from light, freezing and shaking. Shelf-life surveillance of the intact vials is ongoing.

The administration of undiluted and diluted solutions of Nivolumab must be completed within 24 hours of preparation. If not used immediately, the infusion solution may be stored up to 24 hours in a refrigerator at 2°-8°C (36°-46°F) and a maximum of 8 hours of the total 24 hours can be at room temperature (20°-25°C, 68°-77°F) and room light. The maximum 8-hour period under room temperature and room light conditions includes the product administration period.

CAUTION: The single-use dosage form contains no antibacterial preservative or bacteriostatic agent. Therefore, it is advised that the product be discarded 8 hours after initial entry.

8.1.3 Compatibility

No incompatibilities between Nivolumab injection and polyvinyl chloride (PVC), non-PVC/non-DEHP (di(2-ethylhexyl)phthalate) IV components, or glass bottles have been observed.

8.1.4 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.

8.1.5 Availability

Nivolumab is available as 100 mg vials (10 mg/mL) with a 0.7mL overfill. It is supplied in 10 mL type I flint glass vials, with butyl rubber stoppers and aluminum seals.

8.1.6 Preparation

Nivolumab is available as 100 mg vials (10 mg/mL), which include an overfill. It is supplied in 10 mL type I flint glass vials, with butyl stoppers and aluminum seals. Nivolumab injection can be infused undiluted (10 mg/mL) or diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose, USP to concentrations no less than 0.35 mg/mL.

8.1.7 Administration

Nivolumab will be delivered in infusion bags with IV infusion lines over 60 minutes (+/- 5 minutes) using a volumetric pump with 0.2 to 1.2 micron pore size, low-protein binding polyethersulfone membrane in-line filter. Weight for dosage calculations should be done per institutional standards of practice. Re-calculation of dosage for fluctuations in body weight should be made in accordance with institutional guidelines.

8.1.8 Ordering

Nivolumab will be provided by BMS. Each participating institutions is responsible for completing the applicable drug supply forms to receive re-supply of Nivolumab for the duration of the study. Except in very unusual circumstances, each participating institution will order the study agent(s). A participating site may order the agent(s) only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the supplier.

8.1.9 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.)

8.1.10 Destruction and Return

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

8.2 Ipilimumab

8.2.1 Description

Ipilimumab (BMS-734016, MDX-010) is a fully human IgG1κ consisting of 4 polypeptide chains; 2 identical heavy chains primarily consisting of 447 amino acids each with 2 identical kappa light chains consisting of 215 amino acids each linked through inter-chain disulfide bonds.

Table 15: IpilimumabPhysical and Chemical Properties

BMS Number	BMS-734016
Other Names	BMS-734016, MDX-010, YERVOY
Molecular Weight	147, 991 Daltons
Appearance	Clear to slightly opalescent, colorless to pale yello liquid, may contain particiles
Solution pH	7.0
pI	The isoelectric focusing analysis generates a banding batter in the pI range of 8.5 to 8.8, with the major isoform at an approcimate pI of 8.7

8.2.2 Form

Ipilimumab injection, 200 mg/40 mL (5 mg/mL), is formulated as a clear to slightly opalescent, colorless to pale yellow, sterile, nonpyrogenic, single-use, isotonic aqueous solution that may contain particles. Ipilimumab injection, 200 mg/40 mL, is supplied in 50-cc Type I flint glass vials, respectively, stoppered with gray butyl stoppers and sealed with aluminum seals. The drug product is formulated at a concentration of 5 mg/mL at a pH of 7.0.

8.2.3 Storage and Stability

Ipilimumab Injection, 200 mg/40 mL (5 mg/mL), must be stored refrigerated (2°C to 8°C) and protected from light. Ipilimumab injection must not be frozen.

Partially used vials or empty vials of ipilimumab injection should be discarded at the site according to appropriate drug disposal procedures.

Ipilimumab injection may be stored undiluted (5 mg/mL) or following dilution in 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection in PVC, non-PVC/non-DEHP or glass containers for up to 24 hours (at 2°C to 8°C) or room temperature/room light.

8.2.4 Compatibility

Ipilimumab injection may be diluted in 0.9% Sodium Chloride Injection, United States Pharmacopeia (USP) or 5% Dextrose Injection.

8.2.5 Availability

Ipilimumab will be supplied as an investigational agent by Bristol-Myers Squibb (BMS).

8.2.6 Preparation

Ipilimumab injection (5 mg/mL) can be used for intravenous (IV) administration after transferring to a polyvinyl chloride (PVC), non-PVC/non-di-(2-ethylhexyl)phthalate (DEHP), or glass container and is stable for 24 hours at 2°C to 8ºC or room temperature/room light (RT/RL). Ipilimumab injection may be diluted in 0.9% Sodium Chloride Injection, United States Pharmacopeia (USP) or 5% Dextrose Injection. Preparation will follow institutional standard. The product may be infused using a volumetric pump at the protocol-specific dose(s) and rate(s) through a infusion set with an inline, sterile, nonpyrogenic, low-protein-binding filter (pore size of 0.2 to 1.2 micrometer).

Ipilimumab injection must not be administered as an IV push or bolus injection. Care must be taken to assure sterility of the prepared solutions since the drug product does not contain any antimicrobial preservatives or bacteriostatic agents.

8.2.7 Administration

Ipilimumab will be administered as an IV infusion over approximately 60 minutes (± 5 minute infusion window). Ipilimumab will be administered every 6 weeks, on cycle day 1. Calculation of weight should be done per institutional standards of practice. Re-calculation of dosage for fluctuations in body weight should be made in accordance with institutional guidelines. On days where both nivolumab and ipilimumab will be administered, nivolumab should be administered first. The ipilimumab infusion will start at least 30 minutes after completion of the nivolumab infusion.

8.2.8 Ordering

Ipilimumab will be ordered by site pharmacy personnel from BMS. Each participating institution is responsible for completing the applicable drug supply forms to receive re-supply of Ipilimumab for the the duration of the study. Except in very unusual circumstances, each participating institution will order the study agent(s). A participating site may order the agent(s) only after the initial IRB approval for the site has been forwarded by the Coordinatin Center to the supplier.

8.2.9 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.)

8.2.10 Destruction and Return

Expired or unused supplies of ipilimumab should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

In all patients in whom a tumor safely accessible, two biopsies will be required: baseline biopsy and on-treatment biopsy and at day 29 of cycle 1 (+14 day scheduling window). Optional biopsies will be performed at progression in patients who experienced an objective response during the study. We plan to use baseline biopsy tissue to perform a number of immune profiling assays, detailed below. On baseline tumor biopsies, we will perform characterization based on histology (TILs), protein expression, and mRNA expression. Additionally, we will bank specimens for possible future DNA analysis, and other further testing.

All participants will be asked to provide a sample of their archival tissue. For participants in whom tumor biopsies are not safely accessible, an archival sample will be required in order to enroll. In these cases, teams should ensure that an archival sample is available, but it does not need to be at the site in order for the patient to start treatment. Though listed as a screening procedure, archival tissue may be requested and obtained at any point throughout the trial.

Serial blood draws for correlative science are required on this trial; blood samples will be collected within 7 days before starting therapy; blood draws will be obtained every 6 weeks for 24 weeks, and then every 12 weeks, at the end-of-treatment visit in patients who are removed from treatment for progressive disease, and all efforts will be made to obtain an additional blood draw at the time of progressive disease, in patients who went off treatment for anything other than progressive disease. On each blood draw, we will perform flow cytometry to characterize protein expression of immune mediators, detailed below, and additional blood will be banked for future testing.

All patients will additionally be asked to provide a stool sample at three timepoints: within 14 days prior to starting treatment, during treatment at day 29 Cycle 1 (+14 day scheduling window), and at the end of treatment. A fourth collection may be requested from patients who experience grade ≥ 2 diarrhea related to study treatment after discussion with the PI. This collection is not required, but is strongly encouraged. These samples will be analyzed for microbiota content.

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

Please note that some of the downstream correlative plans for blood and tissue include whole exome and single cell sequencing, plus analysis of the data generated, that will be performed by Foundation Medicine, Nanostring Technologies, and the Broad Institute of MIT.. All samples will be sent in a completely de-identified fashion and staff involved at the Broad Institute, Foundation Medicine, and Nanostring Technologies will not have any access to patient health information (PHI). The Broad Institute will provide barcode labels for all samples that are generated at random by their laboratory software.

Summary of Specimens

Research Sampling	Timepoint	Contents	Destination
Archival Tissue	Anytime on study	1 FFPE Block or 10-20 5 micron unstained slides	Current DFCI CRC
Fresh Tissue	Baseline* (within 28 days of C1D1, prior to treatment start)	5-7 cores	RNAlater Cores – Breast Bank
	C1D29 (+14 day scheduling window)	5-7 cores	Formalin Cores – Breast Bank
	End of Treatment (Optional)	5-7 cores	External Sites Only: OCT Core – Breast Bank
Blood	Baseline*, Restaging Visits, Time of Progression	1 – 10mL Streck Tube (cfDNA)	Breast Bank
		5 – 10mL CPT tubes (PBMCs)*	Mayer 308 / Mariano Severignini
Stool	Baseline*	Home Collection Kit (DNA Genotek)	Breast Tumor Immunology Lab/ Elizabeth Mittendorf, MD, PhD
	At C1D29 (any time between C1D29 and C2D1)		
	End of treatment		
	Optional collection at the time of grade ≥ 2 diarrhea		

Note: The "Breast Bank" and Core Blood and Tissue Bank are the same lab and interchangeable

*Baseline samples preferably completed after registration or on Cycle 1 Day 1 prior to treatment start (once a study ID is assigned). Baseline samples completed during screening will not be a violation.

9.1 Archival Tissue

Archival tissue will be obtained on all participants. One block or 10-20 5 micron unstained, charged slides will be collected to centrally test for hypermutation status for participants that are unable to undergo a baseline biopsy. Local tests will be used for eligibility and if the participant had prior Foundation One testing, it will not need to be centrally repeated. Any leftover tissue or tissue from participants who had a baseline biopsy will be banked and stored for future research as a part of correlatives for this clinical trial or otherwise.

9.2 Fresh Tissue Biopsy

9.2.1 Objectives:

- Characterizing immune markers in hypermutated HER2- metastatic breast cancer
- To evaluate MET and phospho MET expression in tumor tissue at baseline by immunohistochemistry

9.2.2 Collection

Biopsies will be performed at the below timepoints, if tumor is safely accessible.

Mandatory:

- Baseline (preferably after registration or on Cycle 1 Day 1 prior to dosing):
- Around C1D29 (Any time from C1D29-C2D1 is allowed)

Optional:

- At time of progression or end of treatment

For patients proceeding to the retreatment phase, optional biopsies will be performed at the below timepoints:

- C1D1 of retreatment, prior to dosing
- At the time of progression after retreatment with nivolumab

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 and Cathy Wu must be notified ahead of time to ensure that there will be adequate time for processing fresh tissue, since fresh tissue cannot be stored over the weekend. The specimens in RNALater, OCT, and formalin may be stored over the weekend and shipped on Monday. Specimens in RNA Later and formalin should be stored at room temperature until shipment.

DFCI Participants:

Ideally five core biopsies will be obtained:

- Three cores should be placed in 10% neutral buffered formalin tube supplied by the study.
- Two cores should be placed in RNAlater

The order of specimen collection should be:

- First core: 10% neutral buffered formalin
- Second core: 10% neutral buffered formalin
- Third core: RNAlater
- Fourth core: RNAlater
- Fifth core: 10% neutral buffered formalin

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

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

External Site Participants:

Ideally five core biopsies will be obtained:

- Three cores should be placed in 10% neutral buffered formalin tube supplied by the study.
- One core should be placed in RNAlater
- One core should be placed in OCT

The order of specimen collection should be:

- First core: 10% neutral buffered formalin
- Second core: RNAlater
- Third core: OCT
- Fourth core: 10% neutral buffered formalin
- Fifth core: 10% neutral buffered formalin

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

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

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

9.2.3 Handling and Shipping

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

- All samples should be de-identified and labeled with the Participant initials, Participant Study ID number and date of procedure.

DFCI Participants Only:

- Cores in formalin should be brought to the Brigham and Women's SHL lab (with appropriate work order submitted and printed) on the 6th floor of the Thorn building, where a block will be made. An email will be sent to the CRC within 2-3 days to confirm that the block has been made.
- Cores in RNAlater should be brought to the [redacted] with patient name/initials, study ID, date of collection, approximate time of collection, and study time point the day prior to collection.

[redacted]

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

External Site Participants Only:

- Cores in formalin may either: 1) be processed into FFPE at local participating sites and shipped in batches to the DF/HCC Core Blood and Tissue Bank or 2) be shipped same day, overnight to the DF/HCC Core Blood and Tissue Bank as tissue in formalin and processed into FFPE at the coordinating center. Please email dfcibreastbank@partners.org with patient name/initials, study ID, date of collection, approximate time of collection, and study time point the day prior to collection and shipment.

- Cores in OCT should be shipped to the DF/HCC Core Blood and Tissue Bank (Deborah Dillon, MD) overnight on dry ice. Alternatively, sites may store samples locally at -80°C and batch ship samples to the DF/HCC Core Blood and Tissue Bank overnight on dry ice. Please email dfcibreastbank@partners.org with patient name/initials, study ID, date of collection, approximate time of collection, and study time point the day prior to collection and shipment.

- Cores in RNAlater should be shipped ambient overnight to the [redacted] with patient name/initials, study ID, date of collection, approximate time of collection, and study time point the day prior to collection and shipment.

Tissue remaining after specific protocol testing described below will be banked in the Core Blood and Tissue Bank [redacted] and may be used for additional or future analyses as needed.

9.2.4 Potential Testing

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

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

recommendations from the International TILs Working Group 2014.[Salgado *et al.*, 2015]

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

Assay 2: Immunohistochemistry

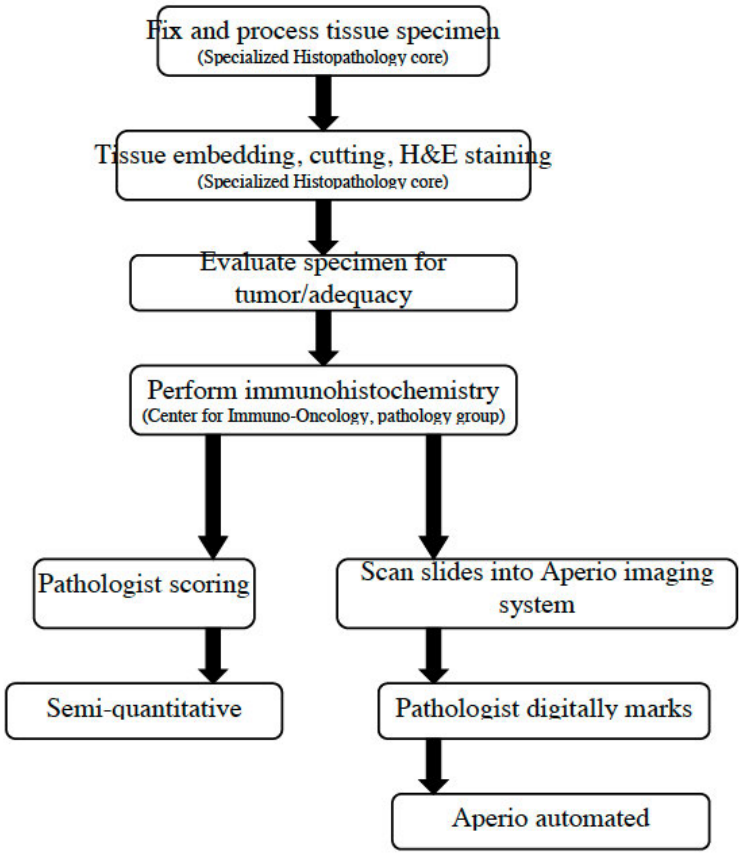
Tissue will be collected and fixed by 10% neutral buffered formalin overnight, dehydrated, and paraffin embedded. Four micrometer-thick sections will be cut. The paraffin blocks and unstained slides will be stored at room temperature. All immunohistochemical staining will be performed in the Center for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

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

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

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

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



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

The semi-quantitative scoring for this study is in accordance with those published previously and, as described above, will include scores for both the neoplastic and non-neoplastic cells within the tumor microenvironment. Data derived from pathologist visual scoring (semi-quantitative, but with increased specificity for delineating neoplastic and non-neoplastic cells) and pathologist-assisted, automated scoring (quantitative, but without accurately delineating neoplastic and non-neoplastic cells) for each marker of interest will be assessed for its clinical value. As necessary, the data from combinations of markers 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.

Assay 3: Flow cytometry

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

Surface staining followed by flow cytometry on the resultant TILs will then be

performed as described in the lab manual. The following antibodies may be used on all specimens: (core set)
CD8
PD-1
PD-L1
PD-L2

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

Assay 4: RNA analysis

RNA analysis may be performed, and tissue for RNA analysis will be stored, in the Core Blood and Tissue Bank (Deborah Dillon, MD).

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

Assay 5: Single cell sequencing

1. Tumor biopsies in DMEM media will be delivered to Dr. Derin Keskin (Immunology Lead, TIGL*), Wandi Zhang (Research Technician), or Phuong Le (Research Technician) at the Tissue Immunogenomics Lab, TIGL (Dana 520).
2. The tumor will be processed using collagenase, hyalurinidase and DNase to generate single cell suspension.
3. These cells will be stained with CD45 antibody. CD45 positive vs negative single cells will be sorted into 96 wells plates at the Hematology/Oncology flow cytometry core facility (Mayer 584).
4. Single cell sorted plates will be transferred to Dr. Kenneth Livak (Technology Lead, TIGL) and Dr. Shuqiang Li (Senior Scientist) at the Genomics Lab, TIGL (Smith 1048, Dana Farber), who will generate single cell RNA-seq libraries. For single-cell TCR analysis, the RNA-seq libraries will be sequenced in the TIGL Genomics Lab using next-generation sequencing (NGS). For single-cell transcriptome analysis, Dr. Li will drop off the RNA-seq libraries at the Broad Institute walk-up sequencing station (6th floor, 415 Main St, Cambridge) for sequencing using NGS.

*Dr. Catherine Wu is the faculty mentor of TIGL (Translational Immunogenomics Laboratory), which was established in 2017.

Assay 6: Tumor Genomic Profiling

Next generation sequencing with tumor biopsy specimens may be assessed using the most current and informative methodologies at the time that correlative science is performed on all specimens. This may be at the Center for Advanced Molecular Diagnositics (Department of Pathology, Brigham and Women's Hospital) or Foundation Medicine, Inc.

9.2.5 Sites Performing Correlatives
BWH
DFCI CIO
DF/HCC Core Blood and Tissue Bank
Translation Immunogenomics Laboratory (TIGL)
The Broad Institute of MIT
Foundation Medicine, Inc
Nanostring Technologies, Inc

9.3 Blood Collection

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

The following research blood samples are required:

Baseline (preferably on Cycle 1 Day 1 prior to dosing):

- 1-10 mL Streck Tube for cfDNA
- 5-10mL CPT tubes for PBMCs

Every Restaging Visit (C2D1, C3D1, C4D1, C6D1. C8D1, etc.):

- 1-10mL Streck Tube for cfDNA
- 5-10mL CPT tubes for PBMCs

Off Treatment (if for progressive disease):

- 1-10 mL Streck Tube for cfDNA
- 5- 10mL CPT tubes for PBMCs

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

- 1-10 mL Streck Tube for cfDNA
- 5-10mL CPT tubes for PBMCs

For retreatment patients, research blood samples will be drawn with every restaging visit:

- 1-10 mL Streck Tube for cfDNA
- 5-10mL CPT tubes for PBMCs

9.3.1 Handling and Shipping

All samples should be de-identified and labeled with the Participant initials, Participant Study ID number and date of collection and time point (e.g., "Baseline" or "Cycle 1" or "Progressive Disease").

- PBMCs (CPT):

  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.

  DFCI Participants Only:
  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.

  Must be processed within 3-4 hours of being drawn. Will be hand carried at ambient temperature to [redacted] Please contact the lab approximately one week in advance to notify of upcoming specimen drop off. Please deliver to:

  [redacted]

  External Participants Only:
  PBMCs will be processed locally by site laboratories using the processing instructions provided in the laboratory manual.

- cfDNA (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.

  Tube precautions:
  - DO NOT FREEZE OR REFRIGERATE 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/shipped to the [redacted] for processing.

Email [redacted] and the current Dana-Farber CRC with the sample information and tracking information the day before transporting specimens.

In small batches or at the end of the trial, samples will be shipped to the Broad Institute under the care of [redacted] for genomic sequencing.

9.3.2 Potential Testing

Assay 1: Flow cytometry

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

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

Assay 2: Cell-free DNA (cfDNA) analysis

Blood will be collected at baseline, restaging visits and at time of progression for evaluation of cell-free DNA (cfDNA). The banked samples will be used to analyze DNA, RNA and protein in future studies.

Assay 3: Tumor Genomic Profiling

Next generation sequencing with blood specimens may be assessed using the most current and informative methodologies at the time that correlative science is performed on all specimens. This may be performed at Foundation Medicine, Inc using the latest

FoundationOne Liquid assay.

9.3.3 Sites Performing Correlatives

DF/HCC Core Blood and Tissue Bank
The Broad Institute
DFCI CIO Laboratories
Foundation Medicine, Inc

9.4 Stool Collection

9.4.1 Handling and shipping

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

- Baseline
- At day C1D29 (+14 day window)
- At the end of treatment
- Optional collection at the time of grade ≥ 2 diarrhea

Retreatment patients will collect optional samples at the below timepoint:

- C1D1 of retreatment, prior to dosing
- At the time of progression after retreatment, if response was again observed

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. The questionnaires will be part of the kit and should not be administered in clinic. Failure for patients to comply with stool collection or completion of questionnaires at the required or optional timepoints will not constitute protocol violations.

Please refer to the separate laboratory manual for more information regarding this.

Samples will be shipped to and stored at the laboratory of [redacted] and will be shipped in batches by the biorepository to Dr. [redacted] lab at Massachusetts General Hospital for analysis.

[redacted]

(Jennifer_Guerriero@dfci.harvard.edu; emittendorf@bwh.harvard.edu)

9.4.2 Analysis of DNA extraction from stool samples

Under the supervision of Dr. Andrew T. Chan, total genomic DNA will be isolated from 0.25g of feces using the PowerSoil DNA isolation kit (Mo Bio, USA) at the Clinical and Translation Epidemiology Unit at Massachusetts General Hospital. Purified DNA will be separated on a 1% agarose gel and quantified by densitometry and spectrophotometry (NanoDrop 1000; Thermo Scientific, USA).

9.4.3 Analysis of RNA extraction from stool samples

Under Dr. Chan's 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 the Broad Institute. 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-NNNNNNNNNNN-AYTGGGYDTAAAGN G-39) and 926R (59-nnnnnnnn-NNNNNNNNNNN-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.

9.4.4 Shotgun sequencing and metabolic pathway reconstruction of stool samples

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

9.4.5 Sites performing correlative analysis

DF/HCC Core Blood and Tissue Bank
MGH Clinical and Translational Epidemiology Unit

9.5 Central confirmation of the hypermutated status

All patients with safely accessible tumors need to undergo a research biopsy at baseline, and tumor will be centrally sequenced with the FoundationOne medicine panel. The in vitro diagnostic (IVD) device, the FoundationOne panel, will be used in accordance with the instructions for use (IFU) that are provided in the device's approved labeling. If participant's previously had testing performed by Foundation One, it will not need to be repeated. Patients in which a baseline biopsy was not performed need to provide archival tissue for performing this central test. Eligibility will be determined by local test results.

9.6 Additional analysis

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

10. STUDY CALENDAR

Screening evaluations are to be conducted within 28 days prior to start of protocol therapy unless otherwise specified. Screening assessments occurring within 4 days prior to initiating study treatment do not need to be repeated on Cycle 1 Day 1.

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

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

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

	Screening[a]	Cycle 1			Cycle 2-4			Cycle 5+			End of Treatment[q]	Follow-Up
		Day 1	Day 15	Day 29	Day 1	Day 15	Day 29	Day 1	Day 15	Day 29		
Medical History[b]	X											
Physical exam[c]	X	X	X	X	X			X			X	
Concurrent medications[d]	X	X			X			X			X	
ECOG	X	X			X			X			X	
Adverse event evaluation		X	X	X	X	X	X	X	X	X	X[s]	
Vital signs[e]	X	X	X	X	X	X	X	X	X	X	X	
Hematology panel[f]	X	X	X	X	X	X	X	X	X	X	X	
Chemistry panel[g]	X	X	X	X	X	X	X	X	X	X	X	
Urine Protein Creatinine Ratio	X				X			X			X	
TSH/fT4	X	X			X			X[h]			X	
ACTH/Cortisol	X	X			X			X[h]				
Amylase and Lipase	X	X			X			X[h]				
Coagulation panel (PT/PTT)	X											
Pregnancy test[i]	X[i]	X[i]		X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]		
12- lead EKG	X											
Tumor Assessment[j]	X				X			X			X[k]	X[k]
Research Blood[l]		X			X			X			X	X
Research Biopsy[m]	X			X							X	
Archival Tumor Retrieval[n]	X											
Quality of Life[r]	X				X			X				
Research Stool Collection & Questionnaire[o, p] (Section 9.4)	X			X							X	
Survival (Section 5.6)												X

a. Screening assessments are to be conducted within 28 days prior to start of protocol therapy unless otherwise specified. If these screening assessments occur within 4 days before start of study treatment, then they may serve as the baseline Cycle 1 Day 1 values.

b. Medical history includes clinically significant diseases, surgeries, and cancer history (including prior cancer therapies and procedures).

c. A complete physical examination will be performed at baseline. A limited physical exam will be completed prior to therapy on Days 1,15 and 29 for Cycles 1 and on Day 1 of every cycle beginning with Cycle 2.

d. All medications taken from the time informed consent is signed through 30 days after the last dose of study therapy will be captured in the participant's electronic medical record.

e. Vital sign assessments include measurements of heart rate, systolic and diastolic blood pressures, respiratory rate, temperature and weight.
f. Hematology includes: hemoglobin, hematocrit, platelet count, RBC count, WBC count, and percent and absolute differential count. Results must be available prior to the administration of study drug.
g. Chemistry testing includes: sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, calcium, total bilirubin, total protein, albumin, ALT, AST, and alkaline phosphatase. LDH will be drawn at baseline only (during screening or on C1D1). Results must be available prior to the administration of study drug.
h. TSH, free T4, ACTH, cortisol, amylase, and lipase will be performed during screening, on Day 1 of Cycles 1-4 and then every other cycle on Day 1. These lab tests should be drawn prior to treatment, however, treatment can be administered prior to the results of these tests.
i. In female subjects of child-bearing potential as defined in the eligibility criteria, the screening pregnancy test (urine preferred, serum acceptable) must be performed within **7 days** before the first dose of study medication. A urine pregnancy test should be repeated within 24 hours of the first dose of study treatment on C1D1. Urine pregnancy tests should be repeated every 4 weeks (+/- 1 week) while receiving study treatment (this is approximately every other Nivolumab infusion). Any urine pregnancy test that comes back as positive should be confirmed with a serum hCG.
j. Tumor assessments should consist of 1) CT and/or MRI of the chest/abdomen/pelvis, 3) any other imaging studies (CT neck, plain films, etc.) as clinically indicated by the treating physician. The same radiographic procedures and technique must be used throughout the study for each patient (e.g., if the patient had CT chest/abdomen/pelvis performed during screening, then she should subsequently undergo CT performed using the same radiologic protocol throughout the remainder of the study). Tumor assessments will be performed at baseline, at day 1 (±7 day scheduling window) of Cycles 2, 3, and 4 and then every 12 week [at day 1 (±7 day scheduling window) of every odd cycle: C6, C8, C10, etc.]. Additional scans are permitted as clinically indicated. All known sites of disease documented at screening should be re-assessed at each subsequent tumor evaluation.
k. For those taken off the study for reasons other than progressive disease, tumor measurements should continue to be repeated every 6-12 weeks until first disease progression or start of a new anti-cancer therapy. Every effort should be made to maintain this scheduled, but it is understood that it may not be possible in all cases. Failure to complete scans at this interval will not be deemed protocol violations.
l. Collected at screening, every restaging, end of treatment (if removed for progressive disease) and optionally at time of progression. See section 9.0
m. Baseline tumor biopsy should be obtained within 28 days prior to initiating protocol therapy. On treatment biopsies should be obtained at C1D29 (+14 day scheduling window). A tumor biopsy may be performed at the end of treatment. See Section 9.2.
n. Archival tumor sample should be collected (block or if not possible, 10-20 5mincron, unstained positively charged slides). This sample may be collected at any time throughout the lifetime of the study.
o. Baseline stool collection should be obtained within 14 days before starting protocol therapy. On treatment stool collection should be obtained at C1D29 (anytime from C1D29-C2D1). A stool collection should be obtained at the end of treatment. As these collections are for exploratory correlative purposes, failure to provide a sample at these timepoints will not constitute a protocol violation. See section 9 and/or lab manual for stool collection and processing instructions.
p. 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 or complete the stool questionnaires at these timepoints will not constitute a protocol violation. See section 9 and/or lab manual for stool collection and processing instructions.
q. End of treatment visit is to occur within 30 days of final administration of study treatment. End of treatment assessments do not have to be repeated if the same assessments were performed within 7 days (28 days for tumor assessments) prior to the visit.

r. FACT B and the Rotterdam Symptom Checklist should be completed baseline or C1D1 (any time prior to first dose), D1 of C2-C4, and then on D1 of every other cycle (C6D1, C8D1, etc.) These questionnaires may be found in Appendices C and D.

s. An adverse event assessment is performed 100 days (-15/+30 days) after the last dose of nivolumab. This may be conducted in person or by phone by the investigator or suitably trained and qualified delegate (i.e. MD, RN, NP, etc).

11. MEASUREMENT OF EFFECT

For the purposes of this study, participants should be re-evaluated for response approximately every 6 weeks for the first 24 weeks and then every 12 weeks thereafter. If cycles are delayed for any reason, the scan schedule should be moved to align with cycles of treatment received.

11.1 Antitumor Effect – Solid tumors

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

11.1.1 RECIST 1.1 Definitions

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

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

11.1.2 Disease Parameters

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

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

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

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis),

are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all considered non-measurable.

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

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

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

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow up.

11.1.3 Methods for Evaluation of Disease

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

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

Clinical lesions. Clinical lesions will only be considered measurable when they are

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

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

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

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

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

(a) Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.

(b) No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

(c) FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

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

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

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

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

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

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

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

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

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

CT, MRI may be used instead of CT in selected instances.

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

Tumor markers. Tumor markers alone cannot be used to assess response.

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

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

11.1.3.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

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

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

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

11.1.3.2 Evaluation of Non-Target Lesions

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

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

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance

of tumor marker level above the normal limits.

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

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

11.1.3.3 Evaluation of New Lesions

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

11.1.3.4 Evaluation of Best Overall Response

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

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

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

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

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

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

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

11.1.4 Duration of Response

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

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

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

11.1.5 Clinical Benefit rate

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

11.2 Antitumor Effect – Hematologic Tumors

N/A

11.3 Other Response Parameters

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

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

11.3.1.1 Impact of New Lesions on irRC

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

11.3.1.2 Definition of Target Lesions Response Using irRC

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

11.3.1.3 Definition of Non-Target Lesions Response Using irRC

- **irComplete Response (irCR):** Complete disappearance of all non-target lesions. This category encompasses exactly the same subjects as "CR" by the mWHO criteria.
- **irPartial Response (irPR) or irStable Disease (irSD):** Non-target lesion(s) are not considered in the definition of PR; these terms do not apply.
- **irProgressive Disease (irPD):** Increases in number or size of non-target lesion(s) does not constitute progressive disease unless/until the Percentage Change in Tumor Burden increases by 25% (i.e. the SPD at nadir of the target lesions increases by the required amount).

11.3.1.4 Definition of Overall Response Using irRC

Overall response using irRC will be based on these criteria:

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

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

Immune-Related Response Criteria Definitions

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

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

irBOR is the best confirmed overall response over the study as a whole, recorded between the date of first dose until the last tumor assessment before subsequent therapy (except for local palliative radiotherapy for painful bone lesions) for the individual subject in the study. For the assessment of irBOR, all available assessments per subject are considered.

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

11.3.1.6 Patient Reported Outcome Assessments

The PRO outcome measure for this study is as follows: Scores from the FACT-B assessment (Appendix C) and Rotterdam Symptom Checklist (Appendix D).

12. DATA REPORTING / REGULATORY REQUIREMENTS

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

12.1 Data Reporting

12.1.1 Method

The Office of Data Quality (ODQ) will collect, manage, and perform quality checks on the data for this study.

12.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the Office of Data Quality in accordance with DF/HCC SOPs.

12.2 Data Safety Monitoring

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

The DSMC will review each protocol up to four times a year or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring with 30 days

of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Multicenter Guidelines

This protocol will adhere to the policies and requirements of the DF/HCC Multi-Center Data and Safety Monitoring Plan. The specific responsibilities of the Overall PI, Coordinating Center, and Participating Institutions and the procedures for auditing are presented in Appendix E.

- The Overall PI/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports to all participating institutions for submission to their individual IRBs for action as required.
- Mechanisms will be in place to ensure quality assurance, protocol compliance, and adverse event reporting at each site.
- Except in very unusual circumstances, each participating institution will order the study agent(s) directly from supplier. A participating site may order the agent(s) only after the initial IRB approval for the site has been forwarded to the Coordinating Center.

12.4 Collaborative Research and Future Use of Data and Samples

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 entitites, 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 toe 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 an open-label, single-arm, multicenter, phase 2 study of nivolumab 3 mg/Kg intravenously (IV) on day 1 of each 14 days plus Ipilimumab 1 mg/Kg IV every 6 weeks in subjects with hypermutated metastatic HER2- breast cancer previously treated with 0 to 3 chemotherapy regimens in the metastatic setting. The target enrollment is 30 evaluable patients. Patients that do not start protocol therapy will be considered unevaluable and will be replaced, therefore total enrollment may exceed 30 pateints.

Primary Endpoint
The primary endpoint is ORR of nivolumab in combination with ipilimumab, according to RECIST 1.1 (Section 11), in patients with with hypermutated HER2- metastatic breast cancer previously treated with 0 to 3 lines of chemotherapy in the metastatic setting.

Secondary endpoints include:
Secondary endpoints include the ORR of the combination according to immune-related response criteria (irRC) [Wolchok *et al.*, 2009] (Section 11), the clinical benefit rate according to RECIST 1.1. and irRC, PFS according to RECIST 1.1. and irRC, the OS, the ORR, PFS and OS of the combination according to RECIST 1.1 in the population with centrally confirmed hypermutated tumors (≥ 9 mutations/megabase); and whether the cut off of ≥ 14 mutations/megabase is associated with the efficacy of this investigational therapy, assessed by ORR, PFS and OS.

Blood and Tissue correlative science objectives include:
- To characterize a broad array of immune markers in metastatic hypermutated HER2-negative breast cancers. (characterization will be based on histology, protein expression, and mRNA expression).
- To explore how different immunosuppressive and/or immune-stimulating immune marker profiles at baseline correlate with disease response to therapy (PFS, objective response assessed by RECIST 1.1 and immune-related response criteria).
- To characterize changes in tumor-infiltrating lymphocytes, PD-L1 expression and immune gene signatures in the tissue microenvironment (TME) from baseline to after 4 weeks of the experimental combination.
- To explore whether induction of changes in the immunosuppressive and/or immune-stimulating immune marker profile in TME correlates with disease response to therapy (response assessed by RECIST 1.1 and immune-related response criteria).
- To characterize serial changes in immune marker profile in peripheral blood mononuclear cells (PBMCs) and in plasma over the course of the trial treatment.
- To explore whether induction of changes in the immunosuppressive and/or immune-stimulating immune marker profile in PBMCs correlates with disease response to therapy (response assessed by RECIST 1.1 and immune-related response criteria).
- To investigate whether there is an immune marker in circulating PBMCs that corresponds to

tumor infiltrating lymphocyte (TIL) percentage in baseline tumor.

- To collect blood to study cell-free DNA for comparison to tumor specimens before and after immunotherapy.
- To explore whether the cut off of ≥ 14 mutations/megabase is associated with the efficacy of this investigational therapy, assessed by ORR per RECIST 1.1.
- To explore whether the cut off of ≥ 14 mutations/megabase is associated with the efficacy of this investigational therapy, assessed by PFS per RECIST 1.1
- To explore whether the cut off of ≥ 14 mutations/megabase is associated with the efficacy of this investigational therapy, assessed by OS per RECIST 1.1

Stool and microbiome correlative science endpoints:

Overall, we plan to describe the landscape of gut microbiota in patients with BC who will receive the combination of ipilimumab plus nivolumab, and the changes in their gut microbiota after 4 weeks of the combination. Statistical analyses of intestinal microbiota samples will be performed using R Statistical Language (v3.1.1) and GraphPad Prism (version 6.0e) software packages. Unpaired Mann–Whitney rank sum test (two-tailed) will be used for comparisons of continuous variables between two groups. Bar plots will be used to represent the data's mean at the center values, with error bars to indicate standard deviation. In order to explore the association of response (objective response according RECIST 1.1 and PFS) 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.

13.2 Sample Size, Accrual Rate and Study Duration

Based on data of recent trials with a population of individuals with metastatic breast cancer treated with anti-PD-1/PD-1 agents in monotherapy, and considering that our population will not be previously selected by PD-L1 expression status a true rate of 5% or less would not be of clinical interest. The study will follow a two-stage design in the first stage 14 patients will be enrolled. If in this first stage there is at least 1 patient with objective response (complete or particl response), accrual will continue to the second stage where an additional 16 patients will be

enrolled. If there are at least 4 patients with ORR among the 30 evaluable patients, the regimen will be considered worthy of further study. If the true response rate is 5%, the chance the regimen is declared worthy of further study is less than 5%. If the true response rate is 25%, the chance that the regimen is declared worthy of further study is >90%. The design has been evaluated under departures from the assumption that 100% of the enrollments will be confirmed as hypermutated tumors (>9mut/Mb). The rational for this evaluation is represented by multiple platforms to identify hypermutated tumors and validation of the hypermutation status after enrollment. We considered power based on exact conditional testing (type I error <0.05), with frequencies of non validated hypermutation status equal to 5%, 10%, 15% or 20% in the enrolled population, and analyses restricted to patients with validated hypermutation status. Under these parameters, restricting continuation to the second stage with OR for one or more patients with validated hypermutation status, power is equal to 0.90, 0.90, 0.88, 0.86.

13.3 Analysis of Primary Endpoint

The primary endpoint is ORR of the experimental combination, which will be assessed among all patients who initiated protocol therapy. Radiographic response will be assessed using RECIST 1.1 criteria as defined in section 11. Objective response will require confirmatory scans as indicated. The ORR (CR + PR) will be reported with 90% exact confidence intervals. (per RECIST 1.1 criteria; Section 11).

13.4 Analysis of Secondary Endpoints

Efficacy Endpoints
All patients who initiated protocol therapy will also be evaluated for ORR according irRC, and for CBR and PFS, according RECIST. Clinical benefit is defined as CR, PR or SD $\geq$ 24 weeks according RECIST 1.1. ORR according to irRC will be reported with 90% exact confidence intervals. CBR will be reported respectively with 95% exact confidence intervals. PFS will be analyzed using Kaplan–Meier product-limit estimates and will be plotted using Kaplan-Meier plots. PFS is defined as the time from study randomization to disease progression according RECIST 1.1, medical judgment or death due to any cause, whichever occurred first. Patients alive without disease progression are censored at the date of last disease evaluation. The hazard ratio for each time-to-event endpoint will be estimated with 95% confidence intervals derived from the Cox proportional hazard model, but no hypothesis testing will be conducted.

Safety and tolerability
All patients will be evaluable for toxicity from the time of their first treatment with any study agent. Toxicity will be graded according to NCI CTCAE, Version 5.0. Toxicities will be summarized by maximum grade and by treatment arm. Incidence rate of each toxicity will be reported with 95% exact CI. The incidence rates of any grade 3+ toxicity will be compared between two arms using Fisher's exact test.

Correlative endpoints
Recently, Herbst et al have demonstrated that patients who presented an increase of at least 5% in expression of PD-L1 in tumor microenvironment experienced a bigger likelihood to respond to treatment with the anti-PD-L1 Atezolizumab[Herbst *et al.*, 2014]. Also, modifications in molecular

signature of tumor microenvironment also correlated with response rate to this drug. Because of this rationale, we plan to perform two research biopsies in tumor lesion: one at baseline and the other one at day 29 cycle 1 of the experimental treatment.

With a sample size of 30 and assuming that there will be patients without accessible lesions, the table below indicates the power available to detect 20%, 30%, 40%, or 50% increases of PD-L1 positivity rate. The power calculation is based on McNemar's test with 1-sided alpha of 0.05 and assuming 2% of patients unexpectedly show PD-L1 positivity only in the baseline assessment. The calculation was done using East v6.3 (Cytel Inc).

Increase of PD-L1 positivity rate post treatment	# of paired biopsies available	Power
20%	15	54%
	20	64%
30%	15	75%
	20	85%
40%	15	90%
	20	96%
50%	15	97%
	20	> 99%

PD-L1 positivity seen at baseline and C3D1 samples will be summarized using contingency tables. An exploratory analysis is planned to evaluate PD-L1 change in continuous scale.

13.5 Central confirmation of the tumor mutational burden

All patients who initiated protocol therapy will need to agree to undergo a research biopsy, if tumor is safely accessible, at baseline. Participants for whom newly-obtained samples cannot be provided (e.g. inaccessible or participant safety concern) may submit an archived specimen (block or if not possible, 20 unstained slides). The tumor mutational burden will be accessed by Foundation One panel that will be used as a central confirmation test for the cutoff of ≥ 9 Mut/Mb.

13.6 Reporting and Exclusions

13.6.1 Evaluation of Efficacy

For this Phase II trial, the efficacy evaluable population is a modified intent-to-treat (ITT) population. The modified ITT population consists of all patients who initiate protocol therapy, even if there are major protocol therapy deviations. Patients that do not start protocol therapy will be considered unevaluable and will be replaced.

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

reported. If applicable to the endpoint, the 95% confidence intervals should also be provided.

13.6.2 Evaluation of Safety

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

14. PUBLICATION PLAN

The results should be made public within 1 year of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

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APPENDIX A PERFORMANCE STATUS CRITERIA

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

APPENDIX B GUIDELINES FOR COLLECTING RESEARCH BIOPSY TISSUE

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

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

Lymph node: A goal of 3-6 core biopsy specimens will be obtained using an 18-gauge needle.

Liver: A goal of 3-6 core biopsy specimens will be obtained using an 18-gauge needle.

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

Bone: Because the yield of malignant tissue from bone biopsies tends to be relatively low, if a patient has another accessible site of disease (i.e. skin, lymph node, liver), that site should be biopsied preferentially. If bone is the only biopsy-accessible site, then a goal of 3-6 core biopsy specimens will be obtained using an 11-13 gauge needle.

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

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

Risks of Research Biopsy and Procedures for Minimizing Risk

Potential risks according to site are:

Skin/chest wall (punch biopsy):

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

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

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

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

Breast (core biopsy):

- Likely: local discomfort and minor bleeding.

- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due

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

Pleural fluid (thoracentesis):

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

Ascites fluid (paracentesis):

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

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

Risks of Anesthesia

Local Anesthesia

All biopsy procedures require local anesthesia using lidocaine, xylocaine, or related compounds. There is a small risk of an allergic reaction associated with these drugs. In order to minimize the risk of local anesthesia, only qualified personnel will perform the biopsy procedure. Patients will be queried if they have had previous allergic reactions to local anesthetics.

Intravenous Conscious Sedation

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

The risks of intravenous conscious sedation include: inhibition of the gag reflex and concomitant risk of aspiration, cardiopulmonary complications (myocardial infarction, cardiac arrhythmias, hypoxemia), and allergic reactions to the sedative or analgesic medications. These risks are small

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

General Anesthesia

Because of the higher risk of general anesthesia compared with local anesthesia or intravenous conscious sedation, biopsies that would require general anesthesia in order to be performed *are not permitted* on this protocol.

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

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

For Effusions and Ascites

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

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

For Fine Needle Aspiration Samples

A goal of 3 passes:

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

APPENDIX C **FACT-B Questionnaire**

Participant ID_____ Cycle#_____

Below is a list of statements that other people with your illness have said are important. Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	PHYSICAL WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4

Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please mark this box and go to the next section.*					
GS7	I am satisfied with my sex life	0	1	2	3	4

EMOTIONAL WELL-BEING		**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

FUNCTIONAL WELL-BEING		**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling	0	1	2	3	4
GF3	I am able to enjoy life	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4

GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right now	0	1	2	3	4

ADDITIONAL CONCERNS		**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
B1	I have been short of breath	0	1	2	3	4
B2	I am self-conscious about the way I dress	0	1	2	3	4
B3	One or both of my arms are swollen or tender	0	1	2	3	4
B4	I feel sexually attractive	0	1	2	3	4
B5	I am bothered by hair loss	0	1	2	3	4
B6	I worry that other members of my family might someday get the same illness I have	0	1	2	3	4
B7	I worry about the effect of stress on my illness	0	1	2	3	4
B8	I am bothered by a change in weight	0	1	2	3	4
B9	I am able to feel like a woman	0	1	2	3	4
P2	I have certain parts of my body where I experience pain	0	1	2	3	4

APPENDIX D Rotterdam Symptom Checklist

Participant ID_____ Cycle#_____

Rotterdam Symptom Checklist Confidential

[redacted]

In this questionnaire you will be asked about your symptoms. Would you please, for all symptoms mentioned, indicate to what extent you have been bothered by it, by circling the answer most applicable to you. The questions are related to the past week.

Example: Have you been bothered, during the past week, by

headaches	not at all	a little	quite a bit	very much

Have you, during the past week, been bothered by

lack of appetite	not at all	a little	quite a bit	very much
irritability	not at all	a little	quite a bit	very much
tiredness	not at all	a little	quite a bit	very much
worrying	not at all	a little	quite a bit	very much
sore muscles	not at all	a little	quite a bit	very much
depressed mood	not at all	a little	quite a bit	very much
lack of energy	not at all	a little	quite a bit	very much
low back pain	not at all	a little	quite a bit	very much
nervousness	not at all	a little	quite a bit	very much
nausea	not at all	a little	quite a bit	very much
despairing about the future	not at all	a little	quite a bit	very much
difficulty sleeping	not at all	a little	quite a bit	very much
headaches	not at all	a little	quite a bit	very much
vomiting	not at all	a little	quite a bit	very much
dizziness	not at all	a little	quite a bit	very much
decreased sexual interest	not at all	a little	quite a bit	very much
tension	not at all	a little	quite a bit	very much
abdominal (stomach) aches	not at all	a little	quite a bit	very much
anxiety	not at all	a little	quite a bit	very much
constipation	not at all	a little	quite a bit	very much

diarrhoea	not at all	a little	quite a bit	very much
acid indigestion	not at all	a little	quite a bit	very much
shivering	not at all	a little	quite a bit	very much
tingling hands or feet	not at all	a little	quite a bit	very much
difficulty concentrating	not at all	a little	quite a bit	very much
sore mouth/pain when swallowing	not at all	a little	quite a bit	very much
loss of hair	not at all	a little	quite a bit	very much
burning/sore eyes	not at all	a little	quite a bit	very much
shortness of breath	not at all	a little	quite a bit	very much
dry mouth	not at all	a little	quite a bit	very much

A number of activities is listed below. We do not want to know whether you actually do these, but only whether you are able to perform them presently. Would you please mark the answer that applies most to your condition of the past week.

	unable	only with help	without help, with difficulty	without help
care for myself (wash etc.)	0	0	0	0
walk about the house	0	0	0	0
light housework/household jobs	0	0	0	0
climb stairs	0	0	0	0
heavy housework/household jobs	0	0	0	0
walk out of doors	0	0	0	0
go shopping	0	0	0	0
go to work	0	0	0	0

All things considered, how would you describe your quality of life during the past week?

0 excellent
0 good
0 moderately good
0 neither good nor bad
0 rather poor
0 poor
0 extremely poor

Would you please check whether you answered all questions?

Thank you for your help.

patient number _____

APPENDIX E DF/HCC MULTI-CENTER DATA AND SAFETY MONITORING PLAN

DFCI IRB Protocol #: 18-561

TABLE OF CONTENTS

1. INTRODUCTION

The Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (DF/HCC DSMP) outlines the procedures for conducting a DF/HCC Multi-Center research protocol. The DF/HCC DSMP serves as a reference for any sites external to DF/HCC that are participating in a DF/HCC clinical trial.

1.1 Purpose

To establish standards that will ensure that a Dana-Farber/Harvard Cancer Center Multi-Center protocol will comply with Federal Regulations, Health Insurance Portability and Accountability Act (HIPAA) requirements and applicable DF/HCC Standard Operating Procedures.

1.2 Multi-Center Data and Safety Monitoring Plan Definitions

DF/HCC Multi-Center Protocol: A research protocol in which one or more outside institutions are collaborating with Dana-Farber/Harvard Cancer Center where a DF/HCC investigator is the sponsor. DF/HCC includes Dana-Farber/Partners Cancer Care (DF/PCC) Network Clinical Trial Affiliates.

Lead Institution: One of the Dana-Farber/Harvard Cancer Center consortium members (Dana-Farber Cancer Institute (DFCI), Massachusetts General Hospital (MGH), Beth Israel Deaconess Medical Center (BIDMC), Boston Children's Hospital (BCH), Brigham and Women's Hospital (BWH) responsible for the coordination, development, submission, and approval of a protocol as well as its subsequent amendments per the DFCI IRB and applicable regulatory guidelines (CTEP, Food and Drug Administration (FDA), Office of Biotechnology Activities (OBA) etc.). The *Lead Institution is typically the home of the DF/HCC Sponsor. The Lead Institution also typically serves as the Coordinating Center for the DF/HCC Multi-Center Protocol.*

DF/HCC Sponsor: The person sponsoring the submitted Multi-Center protocol who takes responsibility for initiation, management and conduct of the protocol at all research locations. In applicable protocols, the DF/HCC Sponsor will serve as the single liaison with any regulatory agencies (i.e. FDA*)*. The DF/HCC Sponsor has ultimate authority over the protocol and is responsible for the conduct of the study at DF/HCC and all Participating Institutions. In *most cases the DF/HCC Sponsor is the same person as the DF/HCC Overall Principal Investigator; however, both roles can be filled by two different people.*

Participating Institution: An institution that is outside the DF/HCC and DF/PCC consortium that is collaborating with DF/HCC on a protocol where the sponsor is a DF/HCC Investigator. The Participating Institution acknowledges the DF/HCC Sponsor as having the ultimate authority and responsibility for the overall conduct of the study.

Coordinating Center: The entity (i.e. Lead Institution, Medical Monitor, Contract Research Organization (CRO), etc) that provides administrative support to the DF/HCC Sponsor in order that he/she may fulfill the responsibilities outlined in the protocol document and DSMP, and as specified in applicable regulatory guidelines (i.e. CTEP Multi-Center Guidelines). In general, the

Lead Institution is the Coordinating Center for the DF/HCC Multi-Center Protocol.

DF/HCC Office of Data Quality (ODQ): A group within DF/HCC responsible ensuring high-quality standards are used for data collection and the ongoing management of clinical trials, auditing, and data and safety monitoring. ODQ also coordinates quality assurance efforts related to multi-center clinical research.

DF/HCC Research Informatics Office (RIO): A group within DF/HCC responsible for providing a comprehensive data management platform for managing clinical trial data.

2 GENERAL ROLES AND RESPONSIBILITIES

For DF/HCC Multi-Center Protocols, the DF/HCC Sponsor, the Coordinating Center, and the Participating Institutions are expected to adhere to the following general responsibilities:

2.1 DF/HCC Sponsor

The DF/HCC Sponsor, Sara Tolaney, MD, MPH will accept responsibility for all aspects of conducting a DF/HCC Multi-Center protocol which includes but is not limited to:

- Oversee the coordination, development, submission, and approval of the protocol as well as subsequent amendments.
- Ensure that the investigators, study team members, and Participating Institutions are qualified and appropriately resourced to conduct the protocol.
- Include the Multi-Center Data and Safety Monitoring Plan as an appendix to the protocol.
- Ensure all Participating Institutions are using the correct version of the protocol.
- Ensure that each participating investigator and study team member receives adequate protocol training (and/or a Site Initiation Visit prior to enrolling participants) and throughout trial's conduct as needed.
- Ensure the protocol will be provided to each participating site in a language understandable to all applicable site personnel when English is not the primary language.
- Monitor progress and overall conduct of the study at all Participating Institutions.
- Ensure all DFCI Institutional Review Board (IRB), DF/HCC and other applicable (i.e. FDA) reporting requirements are met.
- Review data and maintain timely submission of data for study analysis.
- Act as the single liaison with FDA (investigator-held IND trials) as applicable.
- Ensure compliance with all requirements as set forth in the Code of Federal Regulations, applicable DF/HCC requirements, HIPAA requirements, and the approved protocol.
- Commit to the provision that the protocol will not be rewritten or modified by anyone other than the DF/HCC Sponsor.
- Identify and qualify Participating Institutions and obtain accrual commitments prior to extending the protocol to that site.
- Monitor accrual and address Participating Institutions that are not meeting their accrual requirements.

2.2 Coordinating Center

The general responsibilities of the Coordinating Center may include but are not limited to:

- Assist in protocol development.
- Maintain FDA as applicable.
- Review registration materials for eligibility and register participants from Participating Institutions in the DF/HCC clinical trial management system (CTMS).
- Distribute protocol and informed consent document updates to Participating Institutions as needed.
- Oversee the data collection process from Participating Institutions.
- Maintain documentation of Serious Adverse Event (SAE) reports and deviations/violation submitted by Participating Institutions and provide to the DF/HCC Sponsor for timely review and submission to the DFCI IRB, as necessary.
- Distribute serious adverse events reported to the DF/HCC Sponsor that fall under the DFCI IRB Adverse Event Reporting Policy to all Participating Institutions.
- Provide Participating Institutions with information regarding DF/HCC requirements that they will be expected to comply with.
- Carry out plan to monitor Participating Institutions either by on-site or remote monitoring.
- Maintain Regulatory documents of all Participating Institutions which includes but is not limited to the following: local IRB approvals/notifications from all Participating Institutions, confirmation of Federalwide Assurances (FWAs) for all sites, all SAE submissions, Screening Logs for all sites, IRB approved consents for all sites
- Conduct regular communications with all Participating Institutions (conference calls, emails, etc) and maintain documentation all relevant communications.

2.3 Participating Institution

Each Participating Institution is expected to comply with all applicable federal regulations and DF/HCC requirements, the protocol and HIPAA requirements.

The general responsibilities for each Participating Institution may include but are not limited to:

- Document the delegation of research specific activities to study personnel.
- Commit to the accrual of participants to the protocol.
- Submit protocol and/or amendments to their local IRB.
- Maintain regulatory files as per sponsor requirements.
- Provide the Coordinating Center with regulatory documents or source documents as requested.
- Participate in protocol training prior to enrolling participants and throughout the trial as required (i.e. teleconferences).
- Update Coordinating Center with research staff changes on a timely basis.
- Register participants through the Coordinating Center prior to beginning research related activities.
- Submit Serious Adverse Event (SAE) reports to local IRB per institutional requirements and to the Coordinating Center, in accordance with DF/HCC requirements.

- Submit protocol deviations and violations to local IRB per institutional requirements and to the DF/HCC Sponsor in accordance with DF/HCC requirements.
- Order, store and dispense investigational agents and/or other protocol mandated drugs per federal guidelines and protocol requirements.
- Have office space, office equipment, and internet access that meet HIPAA standards.
- Participate in any quality assurance activities and meet with monitors or auditors at the conclusion of a visit to review findings.
- Promptly provide follow-up and/or corrective action plans for any monitoring queries or audit findings.

3 DF/HCC REQUIREMENTS FOR MULTI-CENTER PROTOCOLS

The following section will clarify DF/HCC Requirements and further detail the expectations for participating in a DF/HCC Multi-Center protocol.

3.1 Protocol Distribution

The Coordinating Center will distribute the final DFCI IRB approved protocol and any subsequent amended protocols to all Participating Institutions.

3.2 Protocol Revisions and Closures

The Participating Institutions will receive notification of protocol revisions and closures from the Coordinating Center. It is the individual Participating Institution's responsibility to notify its IRB of these revisions.

- **Non life-threatening revisions:** Participating Institutions will receive written notification of protocol revisions regarding non life-threatening events from the Coordinating Center. Non-life-threatening protocol revisions must be IRB approved and implemented within 90 days from receipt of the notification.

- **Revisions for life-threatening causes:** Participating Institutions will receive immediate notification from the Coordinating Center concerning protocol revisions required to protect lives with follow-up by fax, mail, e-mail, etc. Life-threatening protocol revisions will be implemented immediately followed by IRB request for approval.

- **Protocol closures and temporary holds:** Participating Institutions will receive notification of protocol closures and temporary holds from the Coordinating Center. Closures and holds will be effective immediately. In addition, the Coordinating Center, will update the Participating Institutions on an ongoing basis about protocol accrual data so that they will be aware of imminent protocol closures.

3.3 Informed Consent Requirements

The DF/HCC approved informed consent document will serve as a template for the informed consent for Participating Institutions. The Participating Institution consent form must follow the

consent template as closely as possible and should adhere to specifications outlined in the DF/HCC Guidance Document on Model Consent Language for PI-Initiated Multi-Center Protocols. This document will be provided separately to each Participating Institution upon request.

Participating Institutions are to send their version of the informed consent document and HIPAA authorization, if a separate document, to the Coordinating Center for review and approval prior to submission to their local IRB. The approved consent form must also be submitted to the Coordinating Center after approval by the local IRB for all consent versions.

The Principal Investigator (PI) at each Participating Institution will identify the physician members of the study team who will be obtaining consent and signing the consent form for therapeutic protocols. Participating institutions must follow the DF/HCC requirement that only attending physicians obtain informed consent and re-consent for all interventional drug, biologic, or device research.

3.4 IRB Documentation

The following must be on file with the Coordinating Center:

- Initial approval letter of the Participating Institution's IRB.
- Copy of the Informed Consent Form(s) approved by the Participating Institution's IRB.
- Participating Institution's IRB approval for all amendments.
- Annual approval letters by the Participating Institution's IRB.

3.5 IRB Re-Approval

Verification of IRB re-approval from the Participating Institutions is required in order to continue research activities. There is no grace period for continuing approvals.

The Coordinating Center will not register participants if a re-approval letter is not received from the Participating Institution on or before the anniversary of the previous approval date.

3.6 Participant Confidentiality and Authorization Statement

In 1996, congress passed the first federal law covering the privacy of health information known as the Health Insurance Portability and Accountability Act (HIPPA). Any information, related to the physical or mental health of an individual is called Protected Health Information (PHI). HIPAA outlines how and under what circumstances PHI can be used or disclosed.

In order for covered entities to use or disclose protected health information during the course of a study, the study participant must sign an authorization statement. This authorization statement may or may not be separate from the informed consent document. The Coordinating Center, with the approval from the DFCI IRB will provide a consent template, with information regarding authorization for the disclosure of protected health information.

The DF/HCC Sponsor will use all efforts to limit its use of protected health information in its

trials. However, because of the nature of these trials, certain protected health information must be collected. DF/HCC has chosen to use authorizations, signed by the participant in the trial, rather than limited data sets with data use agreements.

3.6.1 DF/HCC Multi-Center Protocol Confidentiality

All documents, investigative reports, or information relating to the participant are strictly confidential. Whenever reasonably feasible, any participant specific reports (i.e. Pathology Reports, MRI Reports, Operative Reports, etc.) submitted to the Coordinating Center should be de-identified. It is recommended that the assigned protocol case number (as described below) be used for all participant specific documents. Participant initials may be included or retained for cross verification of identification.

3.7 DF/HCC Multi-Center Protocol Registration Policy

3.7.1 Participant Registration and Randomization
Please refer to Protocol Section 4.3 and 4.4 for participant registration information. Treatment cannot begin until site has received confirmation that participant has been registered with DF/HCC CTMS.

3.7.2 Initiation of Therapy

Participants must be registered with the DF/HCC CTMS before the initiation of treatment or other protocol-specific interventions. Treatment and other protocol-specific interventions may not be initiated until the Participating Institution receives confirmation of the participant's registration from the Coordinating Center. The DF/HCC Sponsor and DFCI IRB must be notified of any violations to this policy.

3.7.3 Eligibility Exceptions

No exceptions to the eligibility requirements for a protocol without DFCI IRB approval will be permitted. All Participating Institutions are required to fully comply with this requirement. The process for requesting an eligibility exception is defined below.

3.8 DF/HCC Protocol Case Number

At the time of registration, the following identifiers are required for all subjects: initials, date of birth, gender, race and ethnicity. Once eligibility has been established and the participant successfully registered, the participant is assigned a unique protocol case number. Participating Institutions should submit all de-identified subsequent communication and documents to the Coordinating Center, using this case number to identify the subject.

3.8.1 Protocol Deviations, Exceptions and Violations

Federal Regulations require an IRB to review proposed changes in a research activity

to ensure that researchers do not initiate changes in approved research without IRB review and approval, except when necessary to eliminate apparent immediate hazards to the participant. DF/HCC requires all departures from the defined procedures set forth in the IRB approved protocol to be reported to the DF/HCC Sponsor, who in turn is responsible for reporting to the DFCI IRB.

For reporting purposes, DF/HCC uses the terms "violation", "deviation" and "exception" to describe departures from a protocol. All Participating Institutions must adhere to these requirements for reporting to the DF/HCC Sponsor and will follow their institutional policy for reporting to their local IRB.

3.8.2 Definitions

Protocol Deviation: Any departure from the defined procedures set forth in the IRB-approved protocol which is *prospectively approved* prior to its implementation.

Protocol Exception: Any protocol deviation that relates to the eligibility criteria, e.g. enrollment of a participant who does not meet all inclusion/exclusion criteria.

Protocol Violation: Any protocol departure that was not *prospectively approved* by the IRB prior to its initiation or implementation.

3.8.3 Reporting Procedures

DF/HCC Sponsor: is responsible for ensuring that clear documentation is available in the medical record and/or regulatory documents to describe all protocol exceptions, deviations and violations. The DF/HCC Sponsor will also be responsible for ensuring that all protocol violations/deviations are promptly reported per DFCI IRB guidelines.

Participating Institutions: Protocol deviations require prospective approval from the DFCI IRB. The Participating Institution must submit the deviation request to the Coordinating Center who will then submit the deviation request to the DFCI IRB. Upon DFCI IRB approval the deviation is submitted to the Participating Institution IRB, per institutional policy. A copy of the Participating Institution's IRB report and determination will be forwarded to the Coordinating Center within 10 business days after the original submission. The deviation may not be implemented without all required approvals.

All protocol violations must be sent to the Coordinating Center in a timely manner. The Coordinating Center will provide training for the requirements for the reporting of violations.

Coordinating Center: Upon receipt of the violation/deviation report from the Participating Institution, the Coordinating Center will submit the report to the DF/HCC Sponsor for review. Subsequently, the Participating Institution's IRB violation/deviation report will be submitted to the DFCI IRB for review per DFCI

IRB reporting guidelines.

3.9 Safety Assessments and Toxicity Monitoring

The study teams at all participating institutions are responsible for protecting the safety, rights and well-being of study participants. Recording and reporting of adverse events that occur during the course of a study help ensure the continuing safety of study participants.

All participants receiving investigational agents and/or other protocol mandated therapy will be evaluated for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical examination findings, and spontaneous reports of adverse events reported by participants. All toxicities encountered during the study will be evaluated according to the NCI criteria specified in the protocol. Life-threatening toxicities must be reported immediately to the DF/HCC Sponsor via the Coordinating Center.

Additional safety assessments and toxicity monitoring will be outlined in the protocol.

3.9.1 Guidelines for Reporting Serious Adverse Events

Guidelines for reporting Adverse Events (AEs) and Serious Adverse Events (SAEs) are detailed in protocol section 7.

Participating Institutions must report the SAEs to the DF/HCC Sponsor and the Coordinating Center following the DFCI IRB Adverse Event Reporting Policy.

The Coordinating Center will maintain documentation of all Participating Institution Adverse Event reports and be responsible for communicating to all participating investigators, any observations reportable under the DFCI IRB Reporting Requirements. Participating Institutions will review and submit to their IRB according to their institutional policies and procedures

3.9.2 Guidelines for Processing IND Safety Reports

The DF/HCC Sponsor will review all IND Safety Reports and ensure that all IND Safety Reports are distributed to the Participating Institutions. Participating Institutions will review/submit to their IRB according to their institutional policies and procedures.

3.10 Data Management

DF/HCC RIO develops case report forms (CRF/eCRFs), for use with the protocol. These forms are designed to collect data for each study. DF/HCC RIO provides a web based training for all eCRF users.

3.10.1 Data Forms Review

Data submissions are monitored for timeliness and completeness of submission. If study forms are received with missing or questionable data, the submitting institution will receive a written or electronic query from the DF/HCC Office of Data Quality, Coordinating Center, or designee.

Responses to all queries should be completed and submitted within 14 calendar days.

Responses may be returned on the written query or on an amended paper case report form, or in the case of electronic queries, within the electronic data capture (eDC) system. In the case of a written query for data submitted on a paper case report form, the query must be attached to the specific data being re-submitted in response.

If study forms are not submitted on schedule, the Participating Institution will periodically receive a Missing Form Report from the Coordinating Center noting the missing forms.

4 REQUISITIONING INVESTIGATIONAL DRUG

The ordering of investigational agent is specified in the protocol section 8.

Participating Institutions should order their own agent regardless of the supplier.

If the agent is commercially available, check with the local Director of Pharmacy and/or the Research Pharmacy to ensure that the agent is in stock. If the agent is not stocked, ensure that the agent can be ordered once the protocol is approved by the local IRB.

If the agent is investigational, ensure that the pharmacy will be able to receive and store the agent according to state and federal requirements. The local IRB should be kept informed of who will supply the agent (i.e., NCI or a pharmaceutical company) so that any regulatory responsibilities can be met in a timely fashion.

5 MONITORING: QUALITY CONTROL

The quality control process for a clinical trial requires verification of protocol compliance and data accuracy. The Coordinating Center, with the aid of the DF/HCC Office of Data Quality, provides quality control oversight for the protocol.

5.1 Ongoing Monitoring of Protocol Compliance

The Participating Institutions may be required to submit participant source documents to the Coordinating Center for monitoring. Participating Institution may also be subject to on-site monitoring conducted by the Coordinating Center.

The Coordinating Center will implement ongoing monitoring activities to ensure that Participating Institutions are complying with regulatory and protocol requirements, data quality, and participant safety. Monitoring practices may include but are not limited to source data

verification, and review and analysis of eligibility requirements, informed consent procedures, adverse events and all associated documentation, review of study drug administration/treatment, regulatory files, protocol departures reporting, pharmacy records, response assessments, and data management.

Additionally, a plan will be formulated to provide regular and ongoing communication to Participating Institutions about study related information which will include participation in regular Lead Institution initiated teleconferences. Teleconferences will occur every 2 weeks and will continue regularly until completion of accrual. Upon completion of accrual, teleconferences will occur monthly until all patients complete protocol therapy. Upon completion of protocol therapy, teleconferences will occur every 3 months until study completion. Additional communication may be distributed via "Newsletter" or email as deemed appropriate by DF/HCC Sponsor.

On-Site Monitoring: On-site monitoring will occur on an as-needed basis. Participating Institutions will be required to provide access to participants' complete medical record and source documents for source documentation verification during the visit. In addition, Participating Institutions should provide access to regulatory documents, pharmacy records, local policies related to the conduct of research, and any other trial-related documentation maintained by the Participating Site. On-site monitoring visits can be substituted with remote (virtual) monitoring visits at the discretion of the Principal Investigator.

Remote Monitoring: Remote monitoring will be performed on an as-needed basis by the Clinical Trial Monitor. Sites will be asked to provide source documentation via fax, email, or mail as specified by the Clinical Trial Monitor for virtual monitoring.

5.2 Monitoring Reports

The DF/HCC Sponsor will review all monitoring reports to ensure protocol compliance. The DF/HCC Sponsor may increase the monitoring activities at Participating Institutions that are unable to comply with the protocol, DF/HCC Sponsor requirements or federal and local regulations.

5.3 Accrual Monitoring

Prior to extending a protocol to an external site, the DF/HCC Sponsor will establish accrual requirements for each participating institution. Accrual will be monitored for each participating institution by the DF/HCC Sponsor or designee. Sites that are not meeting their accrual expectations may be subject to termination. Sites are expected to accrue at least 3 patients per year.

6 AUDITING: QUALITY ASSURANCE

Auditing is a method of Quality Assurance and involves the systematic and independent examination of all trial related activities and documents. Audits determine if evaluated activities were appropriately conducted and whether data was generated, recorded and analyzed, and

accurately reported per the protocol, applicable Standard Operating Procedures (SOPs), and the Code of Federal Regulations (CFR).

6.1 DF/HCC Internal Audits

All Participating Institutions are subject to audit by the DF/HCC Office of Data Quality (ODQ). Typically, approximately 3-4 participants would be audited at the site over a 2-day period. If violations which impact participant safety or the integrity of the study are found, more participant records may be audited.

6.2 Audit Notifications

It is the Participating Institution's responsibility to notify the Coordinating Center of all external audits or inspections (e.g., FDA, EMA, NCI) that involve this protocol. All institutions will forward a copy of final audit and/or re-audit reports and corrective action plans (if applicable) to the Coordinating Center, within 12 weeks after the audit date.

6.3 Audit Reports

The DF/HCC Sponsor will review all final audit reports and corrective action plans, if applicable. The Coordinating Center, must forward any reports to the DF/HCC ODQ per DF/HCC policy for review by the DF/HCC Audit Committee. For unacceptable audits, the DF/HCC Audit Committee would forward the final audit report and corrective action plan to the DFCI IRB as applicable.

6.4 Participating Institution Performance

The DF/HCC Sponsor and the DFCI IRB are charged with considering the totality of an institution's performance in considering institutional participation in the protocol.

Participating Institutions that fail to meet the performance goals of accrual, submission of timely and accurate data, adherence to protocol requirements, and compliance with state and federal regulations, may be recommended for a six-month probation period. Such institutions must respond with a corrective action plan and must demonstrate during the probation period that deficiencies have been corrected, as evidenced by the improved performance measures. Participating Institutions that fail to demonstrate significant improvement will be considered by the DF/HCC Sponsor for revocation of participation. A DF/HCC Sponsor and/or the DFCI IRB may terminate a site's participation if it is determined that a site is not fulfilling its responsibilities as described above.