DF/HCC Protocol #: 17-512

TITLE: A randomized phase II trial of carboplatin with or without nivolumab in first-line metastatic triple-negative breast cancer

Coordinating Center: Dana-Farber/Partners Cancer Care (DF/PCC) on behalf of Dana Farber/Harvard Cancer Center

***Principal Investigator (PI):** Sara Tolaney, MD, MPH

[redacted]

Other Investigators:

[redacted]

Statistician:

[redacted]

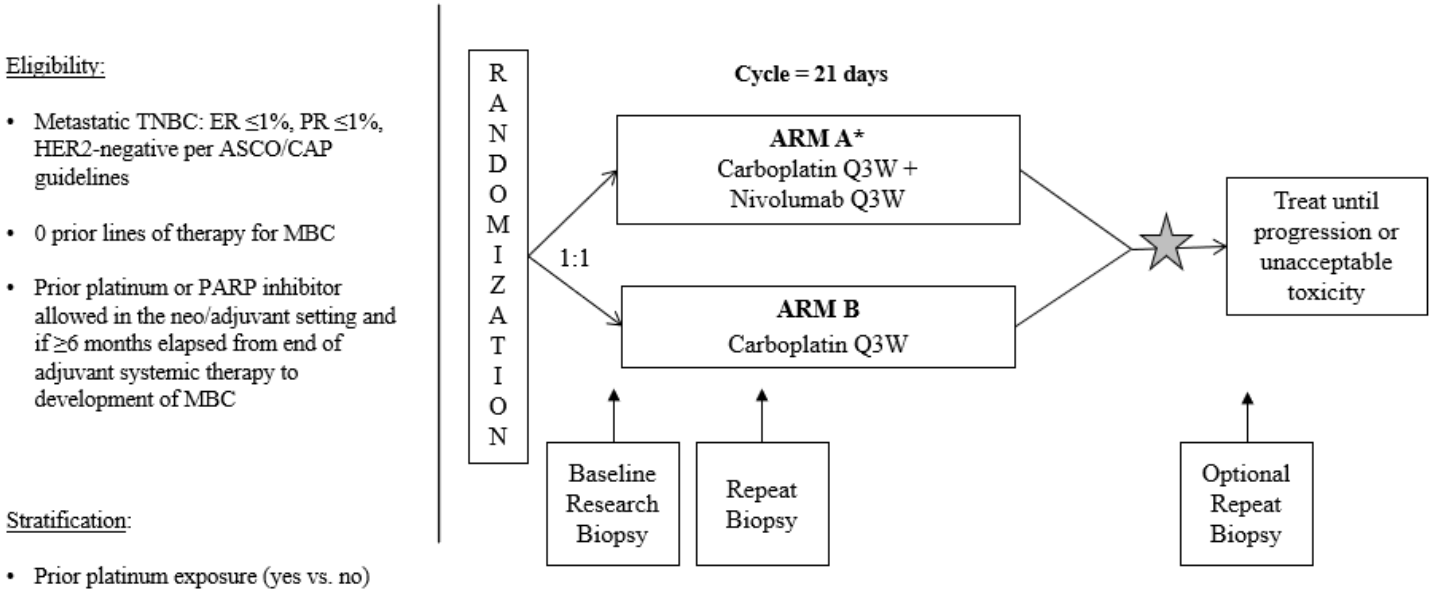
Project Manager:

[redacted]

IND #: 136600
IND Sponsor: Sara Tolaney, MD, MPH

Protocol Type / Version # / Version Date: Amendment 4/Version 3/02.02.21

SCHEMA



N=132

* Safety Run-In Analysis for the first 12 patients enrolled on Arm A
☆ If PD on Arm B, patient may crossover to receive **Nivolumab + nab-Paclitaxel.** A mandatory biopsy will be required (when safely accessible).

TABLE OF CONTENTS

1. OBJECTIVES

1.1 Study Design

This is an open-label, multi-institutional, randomized phase II trial of carboplatin given intravenously at AUC 6 on day 1 of every 21-day cycle, alone or in combination with nivolumab given intravenously at 360 mg on day 1 of every 21-day cycle, in subjects with metastatic triple-negative breast cancer (TNBC) treated with 0 prior lines of therapy in the metastatic setting.

1.2 Primary Objectives

To compare the efficacy of carboplatin in combination with nivolumab versus carboplatin alone, as defined by progression-free survival (PFS), as first-line therapy in the metastatic setting for patients with unselected (by PD-L1 status) metastatic TNBC.

1.3 Secondary Objectives

Efficacy objectives

- To compare the efficacy of carboplatin in combination with nivolumab versus carboplatin alone, as defined by overall response rate (ORR) according to RECIST 1.1 (1) and immune-related response criteria (irRC)(2), in patients with unselected metastatic TNBC.
- To compare the efficacy of carboplatin in combination with nivolumab versus carboplatin alone, as defined by overall survival (OS), in patients with unselected metastatic TNBC.
- To compare the efficacy of carboplatin in combination with nivolumab versus carboplatin alone, as defined by clinical benefit rate (CBR) according to RECIST 1.1, in patients with unselected metastatic TNBC.
- To evaluate the duration of response (DOR) and time to objective response (TTOR) of carboplatin in combination with nivolumab versus carboplatin alone, in patients with unselected metastatic TNBC.
- To compare the efficacy of carboplatin in combination with nivolumab versus carboplatin alone, as defined by PFS, ORR according to RECIST 1.1 and irRC, CBR, DOR, TTOR and OS, in patients with PD-L1-positive metastatic TNBC (defined as ≥1% of the tumor cell population demonstrating unequivocal staining for PD-L1).
- To explore the efficacy of carboplatin alone or in combination with nivolumab, in terms of PFS, ORR according to RECIST 1.1 and irRC, CBR, DOR, TTOR and OS, for metastatic TNBC in patients with germline *BRCA1* or *BRCA2* mutations.
- To explore the efficacy of nab-paclitaxel in combination with nivolumab, in terms of PFS, ORR according to RECIST 1.1 and irRC, CBR, DOR, TTOR and OS, as second-line therapy in patients with metastatic TNBC who crossover after progression on carboplatin alone, and explore outcomes by PD-L1 status.

Safety objectives

- To evaluate the safety and tolerability of carboplatin in combination with nivolumab, and compare to that of carboplatin alone, in patients with metastatic TNBC previously treated with 0 lines of chemotherapy in the metastatic setting.
- To evaluate the safety and tolerability of nab-paclitaxel in combination with nivolumab, as second-line therapy in patients with metastatic TNBC who crossover after progression on carboplatin alone.

1.4 Correlative Objectives

1.4.1 To explore tissue biomarkers of antitumoral immune activity and tumor genomic instability as predictors of response, or resistance, to carboplatin plus nivolumab, compared to carboplatin alone, in patients with metastatic TNBC.

- To characterize tumor-infiltrating lymphocytes (TILs), by histological assessment, at baseline and correlate with disease response to treatment (PFS, objective response assessed by RECIST 1.1 and irRC, OS).
- To characterize the expression of markers of immune cell subsets (i.e. CD8 for cytotoxic T cells, CD68 for macrophages), inhibitory checkpoint pathway molecules (i.e. PD-1, PD-L1, TIM3, LAG3), and co-stimulatory pathway molecules (i.e. GITR, OX40) by immunohistochemistry (IHC) and/or immunofluorescence (IF).
- To explore whether immunosuppressive and/or immune-stimulating immune marker profiles at baseline correlate with disease response to treatment (PFS, objective response assessed by RECIST 1.1 and irRC, OS).
- To characterize mutational load and neoantigen burden at baseline and correlate with disease response to treatment (PFS, objective response assessed by RECIST 1.1 and irRC, and OS).
- To characterize RNA expression signatures of immune pathway activation and DNA damage repair deficiency at baseline and correlate with disease response to treatment (PFS, objective response assessed by RECIST 1.1 and irRC, and OS).
- To explore whether changes in TILs, immunosuppressive and/or immune-stimulating immune marker profiles, mutational load, neoantigen burden, and RNA expression signatures, between paired biopsies from baseline and after 2 cycles of treatment, correlate with disease response to treatment (PFS, objective response assessed by RECIST 1.1 and irRC, OS).
- To explore mechanisms of resistance to carboplatin plus nivolumab, compared to carboplatin alone, in paired biopsies from baseline and at time of progression.

1.4.2 To explore blood biomarkers of antitumoral immune activity as predictors of response, or resistance, to carboplatin plus nivolumab, compared to carboplatin alone, in patients with metastatic TNBC.

- To characterize serial changes in immune marker profile in peripheral blood mononuclear cells (PBMCs) and in plasma over the course of study 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 (PFS, objective response assessed by RECIST 1.1 and irRC, OS).
- To investigate whether there is an immune marker (i.e. PD-L1) in circulating PBMCs that correlates to tumor infiltrating lymphocyte (TIL) percentage in baseline tumor.
- To characterize serial changes of neoantigen burden in circulating tumor DNA and correlate with disease response to treatment (PFS, objective response assessed by RECIST 1.1 and irRC, OS).
- To explore serial changes in blood biomarkers as mechanisms of resistance to carboplatin plus nivolumab, compared to carboplatin alone.

1.4.3 To explore the structure and function of the gut microbiome as predictors of response, or resistance, to carboplatin plus nivolumab, compared to carboplatin alone, in patients with metastatic TNBC.

- To characterize structure and function of the gut microbiome at baseline and correlate with disease response to treatment (PFS, objective response assessed by RECIST 1.1 and irRC, OS).
- To explore whether changes in the overall diversity of gut microbiome, estimated by Shannon index, correlate with disease response to treatment (PFS, objective response assessed by RECIST 1.1 and irRC, OS).
- To explore mechanisms of resistance to carboplatin plus nivolumab, compared to carboplatin alone, in paired samples from baseline and at time of progression.
- To explore correlations between diversity and taxa of the gut microbiome and 1) diet composition, 2) physical activity patterns, and 3) body mass index (BMI).

1.4.4 To explore tissue, blood and microbiome biomarkers as predictors of response, or resistance, to nab-paclitaxel plus nivolumab, in patients with metastatic TNBC who crossover after progression on carboplatin alone.

1.4.5 To explore the relationship between response to carboplatin plus nivolumab and diet composition, physical activity patterns and body mass index.

2. BACKGROUND

2.1 Overview of Triple-Negative Breast Cancer and Current Options

Breast cancer is the most frequently diagnosed cancer and the second cause of cancer death in women in the Unites States(3). The heterogeneity of breast cancer has been widely demonstrated by gene expression profiling, distinguishing intrinsic subtypes including luminal A, luminal B,

epidermal growth factor receptor type 2 (EGFR2, ERBB2 or HER2)-enriched, basal-like and claudin-low breast subtypes, with different prognostic and therapeutic implications for each type(4). Breast tumors that are immunohistochemically characterized by the lack of expression of estrogen receptor (ER), progesterone receptor (PR) and HER2, are classified as triple negative breast cancer (TNBC), accounting for 15-20% of all breast cancers(5). Despite the fact that most immunohistochemically defined TNBC (75%) have a basal-like phenotype according to gene expression, triple-negative tumors can less commonly fall into any of the other intrinsic subtypes. However, considering the overlap between both classifications, for daily clinical practice, TNBC is assumed to be equivalent to basal-like breast cancer.

TNBC has a highly aggressive clinical course, with an earlier age of onset, greater metastatic potential and poorer clinical outcomes, in terms of survival and higher rates of local relapse, compared to patients with receptor-positive cancers. Studies of neoadjuvant chemotherapy suggest that women with TNBC who obtain pathological complete response (pCR) with treatment achieve excellent outcomes(6). In addition, in patients with TNBC, pCR rates have been proven to correlate with higher scores of tumor-infiltrating lymphocytes (TIL)(7, 8). Unfortunately, residual disease is present in the majority of patients with early-stage TNBC treated with neoadjuvant chemotherapy, increasing the risk of relapse, predominantly in the first 3 years after treatment(6). Consequently, an elevated proportion of patients eventually develop visceral or central nervous system (CNS) metastases and treatment options are limited in these patients because standard chemotherapeutic regimens containing anthracyclines and taxanes have usually already been used in the neo-/adjuvant setting. After taxane- and anthracycline-based treament, there is no standard regimen for metastatic TNBC. With chemotherapy strategies selected from a number of currently recommended agents, ORR range from 26-48% in first-line TNBC, with a usually short duration of response, rapid relapse and median survival of 13-18 months(9).

Anthracyclines and taxanes have been suggested as rechallenge regimens in patients that relapse at least 6 to 12 months following completion of adjuvant chemotherapy. However, their use is often limited due to concerns of increased toxicity and, in the case of anthracyclines, exceeding cumulative dose levels. Moreover, there are few data to support this strategy in first- or second-line treatment for metastatic breast cancer. To date, one prospective phase III trial comparing docetaxel or pegylated liposomal doxorubicin (PLD) followed by docetaxel demonstrated a significant improvement in time to progression (median 7.0 vs. 9.8 months, respectively) and ORR (26% vs. 35%, respectively) favoring the combination arm(10). However, in subgroup analyses, PFS improvement was not statistically significant in the TNBC subtype.

Several chemotherapy regimens are felt to be appropriate, as single agents or in combination, for the treatment of metastatic TNBC. Although combination regimens generally provide higher rates of objective response and improved PFS, combination chemotherapy has been associated with increased toxicity and marginal overall survival (OS) benefit(11-13). Among preferred single agents, consensus guidelines include antimetabolites (capecitabine, gemcitabine), taxanes (docetaxel, paclitaxel or albumin-bound paclitaxel) and non-taxane microtubule inhibitors (vinorelbine and eribulin)(14). A recent phase III trial compared eribulin with capecitabine in patients with metastatic breast cancer. Although there was a significant survival advantage observed with eribulin compared to capecitabine in all subgroups, median OS was 14.4 months in the TNBC subgroup, highlighting the need for further efforts to improve clinical outcomes in these

patients(15). Increased PFS has been documented with the anti-angiogenic agent bevacizumab (monoclonal antibody targeting vascular endothelial growth factor; VEGF) in combination with taxanes, anthracyclines or capecitabine, albeit without OS benefit(16, 17). Other targeted therapies, including anti-VEGF (sorafenib, sunitinib) and anti-EGFR (cetuximab) drugs, have achieved ORR of only 10-20%(18, 19).

Overall, standard chemotherapy regimens may be considered effective for a subgroup of patients with early chemosensitive TNBC. However, patients with advanced disease typically respond poorly to current chemotherapeutic strategies and, when response is achieved, patients generally present rapid disease progression. Due to the modest progress in advanced TNBC, new strategies are warranted in order to improve survival and identify biomarkers that may predict response to treatment.

Role of Platinum Agents in Metastatic TNBC

Platinum agents have demonstrated activity in monotherapy, and in combination with other chemotherapy drugs, in patients with anthracycline-pretreated advanced breast cancer(20). Several phase II studies have evaluated the combination of carboplatin and paclitaxel in metastatic breast cancer with response rates reported from 43% to 62% (21-23). This regimen has also been found to be effective in phase III trials with response rates between 38% and 41% and manageable toxicity(24, 25). Other platinum-based combinations are also active in patients with metastatic TNBC(26), with response rates of approximately 32% observed in combination with gemcitabine(27, 28).

BRCA1/2 mutation-associated and some sporadic TNBC share impaired homologous recombination repair mechanisms (HR-deficiency), conferring sensitivity to platinums(29). The Translational Breast Cancer Research Consortium (TBCRC) conducted a single-arm phase II study (TBCRC009) of cisplatin (75 mg/m2) or carboplatin (AUC 6) every three weeks in patients with first- or second-line metastatic TNBC. A total of 86 patients were enrolled in the trial, and prior adjuvant chemotherapy had been administered in 86% of the population. An overall response rate of 25.6% was observed, 29.0% in first-line and 11.8% in second-line setting. Response with cisplatin was 32.6% compared to 18.6% with carboplatin, although this was not statistically different (p=0.22). At a median follow-up of 49.9 months, median PFS was 2.9 months and OS was 11 months. Germline *BRCA1/2* status was available in 77 patients. A total of 11 (14%) patients had germline *BRCA1/2* mutation and 66 (86%) were *BRCA1/2* wild type. The response rate was 54.5% in *BRCA* mutation carriers vs. 19.7% in non-carriers (p=0.02), although no differences in PFS or OS were noted between both groups. Single agent platinum was well-tolerated and active in this setting(30).

The TNT trial evaluated single agent carboplatin (AUC 6) or docetaxel 100 mg/m2, administered every 3 weeks for 6 cycles, in patients with advanced TNBC who had received no prior treatment or only anthracycline-based chemotherapy for metastatic disease(31). Similar efficacy was demonstrated with single agent carboplatin and docetaxel for patients with TNBC. The objective response rate was 31.4% for the patients receiving carboplatin and 35.6% for the patients receiving docetaxel. Median PFS and OS were 3.1 and 12.4 months, respectively, in patients receiving carboplatin, and 4.5 and 12.3 months, respectively, in patients treated with docetaxel. Although no

significant improvement in PFS, OS or ORR was noted in the global population, patients with *BRCA1* or *BRCA2* mutations had a significant increase in objective response rates (58% vs. 33.3%) and PFS (6.8 months vs. 4.8 months, p) with carboplatin compared to docetaxel. Consequently, platinum agents are increasingly being used in early therapeutic lines for metastatic TNBC, with particular interest in patients with tumors characterized by HRD.

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(32, 33). Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies(34). In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells appear to correlate with improved prognosis and long-term survival in many solid tumors(35-37).

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control(38). The normal function of PD-1, which is 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 a member of the Ig superfamily related to CD28 and CTLA-4, which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structures of murine PD-1 alone(39) and in complex with its ligands were first resolved(40, 41). More recently the NMR-based structure of the human PD-1 extracellular region and analyses of its interactions with its ligands were also reported(42). 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(43). The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4(44), 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(45). Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells(46), as well as subsets of macrophages(47) and dendritic cells(48).

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(49). 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(49). Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell

inhibitor(50, 51), which, via its interaction with the PD-1 receptor on tumor-specific T cells, plays a critical role in immune evasion by tumors(52). This suggests that the PD-1/PD-L1 pathway should be considered an attractive target for therapeutic intervention in cancer(53).

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

Unlike melanoma and NSCLC, breast cancer has not been intensively investigated for its susceptibility to immunotherapy in clinical settings. However, there are accumulating preclinical and clinical evidence suggesting that the immune system is critical during natural history of breast cancer and that the immune system can be modulated to improve outcomes in this disease(54). It has been recognized that breast cancer is capable of stimulating the immune system and many breast tumors have substantial lymphocyte infiltration(55, 56). Additionally, this pathologic feature has prognostic implications, as lymphocyte predominant breast cancers are associated with improved prognosis(55, 57). However, the degree of immune infiltration differs by breast cancer subtype; while a substantial proportion of TNBC are richly infiltrated, hormone-receptor positive breast cancer is poorly T-cell infiltrated(58). 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)(59).

Results from several clinical trials evaluating PD-1 or PD-L1 checkpoint inhibitors in the TNBC metastatic setting have been reported (**Table 1**)(60-63). However, comparisons are limited due to broad variations in the inclusion criteria of each study, including prior number of therapies allowed, pre-selection based on PD-L1 positivity and assays used to quantify PD-L1 expression.

Table 1. Results of PD-1/PD-L1 Blockade in Advanced Triple-Negative Breast Cancer in 2017

	Pembrolizumab in PD-L1+ Tumors: TNBC Cohort (KEYNOTE-012)	**Atezolizumab in Tumors Unselected for PD-L1: TNBC Cohort**	**Atezolizumab + *Nab*-Paclitaxel in Tumors Unselected for PD-L1: TNBC Cohort**	**Avelumab in Breast Cancer Unselected for PD-L1: TNBC Cohort (JAVELIN)**
Mechanism of action	Humanized IgG4 Anti-PD-1	Humanized IgG1 Anti-PD-L1	Humanized IgG1 Anti-PD-L1	Fully human IgG1 Anti-PD-L1
Dose and Schedule	10 mg/kg (Q2W)	15mg/kg or 20 mg/kg or 1200mg flat dose (Q3W)	Atezolizumab 800mg (D1,15); nab-Paclitaxel 125mg/kg (D 1,8,15) Q4W	10 mg/kg (Q2W)
PD-L1 positivity definition	≥1% TC or any staining in stroma	≥5% IC	≥1% IC; ≥1% TC	≥1% TC; ≥10% IC
PD-L1 clone assay	22C3 (Dako)	22C3 (Dako)	SP142 (Ventana)	NA (Dako-based test)
PD-L1 status inclusion criteria	Positive	All-comers †	All-comers	All-comers
No. PD-L1+ pts / No. PD-L1 evaluable cases, %	65/111 (58.6)	37/54 (68.5)	9/24 (37.5); 3/24 (12.5)	33/48 (68.8); 9/48 (18.8)
No. pts enrolled	32	54	32	58
No. pts included in efficacy analysis	27	21 †	24	58
No. prior therapies for metastatic disease, Median (range)	2 (0-9)	NA	5 (1-10)	NA

No. pts with ≥3 prior therapies for metastatic disease (%)	15 (46.9)	NA ‡	1 (4.2)	13 (22.4)
ORR, %	18.5	19.0	37.5	8.6
ORR in PD-L1+ cohort, %	18.5	19.0	36.3 ¶	6.1; 44.4
CBR, %	25.9	NA	81.3	31.0
Median PFS, mo	1.9 (1.7-5.5)	NA	NA	NA
PFS rate at 6 mo, %	24.4	27	NA	NA
Median OS, mo	11.2 (5.3-NR)	NA	NA	NA

TNBC: triple-negative breast cancer; no: number; pts: patients; TC: tumor cells; IC: immune cells; ORR: overall response rate; CBR: clinical benefit rate (defined as complete response, partial response or stable disease for ≥24 wks); wks: weeks; months; NR: not reached; NA: not available; PFS: progression-free survival; OS: overall survival.
† All evaluable patients were PD-L1 positive. Data for PD-L1 negative tumors: NA.
‡ No. Pts with ≥4 lines of prior therapy for advanced disease: 48 (88.9%).
¶ According to PD-L1 positivity in IC.

2.3 Rationale for Crossover Amendment and Clinical Trial Implications

2.3.1 Rationale for Crossover Arm to Nab-Paclitaxel plus Nivolumab

More recently, results from a large phase III randomized trial (IMpassion130) comparing nab-paclitaxel plus atezolizumab versus nab-paclitaxel alone as first-line therapy for advanced TNBC demonstrated a significant, though modest, clinical improvement in median PFS in the intent-to-treat (ITT) population (7.2 vs. 5.5 months, HR 0.80, p=0.0025) and PD-L1-positive study population (7.5 vs. 5.0 months, HR 0.62, p<0.001)(64). PD-L1 positivity was defined as PD-L1 staining on at least 1% of tumor-infiltrating immune cells as determined using the SP142 monoclonal antibody (Ventana Medical Systems).

A hierarchical statistical plan allowed analysis of OS in the PD-L1 population if a significant difference was noted in the ITT population. In the ITT population, median OS was not significantly different between treatment arms (21.3 vs. 17.6 months, HR 0.84, p=0.08). Thus, formal testing of OS in the PD-L1-positive population was not performed at the time of the interim analysis. However, OS in the PD-L1-positive population was explored; at a median follow up of 12.9 months, a significant 9.5-month increase in median OS was observed in patients treated with the combination versus chemotherapy alone (25.0 vs. 15.5 months, HR 0.62, 95% CI 0.45-0.86). This OS improvement led to the U.S. FDA accelerated approval of atezolizumab in combination with nab-paclitaxel for patients with locally advanced or metastatic TNBC whose tumors express PD-L1 (PD-L1 tumor-infiltrating immune cells of any intensity covering at least 1% of the tumor area), as determined by an FDA approved test (https://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm633065.htm).

In the first version of this protocol, patients randomized to receive carboplatin alone (Arm B) were offered the possibility of crossover to single-agent nivolumab at the time of progression on carboplatin. Considering the approval of the combination of a taxane with PD-L1 inhibition, and given the modest efficacy of PD-1/PD-L1 inhibitors as single-agents in metastatic TNBC, the Crossover phase has been changed from nivolumab alone to nab-paclitaxel plus nivolumab. This will allow all patients enrolled on the study to receive immune checkpoint inhibition plus

chemotherapy either as first- or second-line therapy for metastatic TNBC. Please note: At the time of the crossover amendment activation (protocol version 3), any active patients on the original crossover treatment (nivolumab monotherapy) will continue to receive nivolumab monotherapy rather than nivolumab + nab-paclitaxel.

2.3.2 Rationale for Modifications to First Course of Therapy

It remains unclear whether the synergistic effect observed with PD-1/PD-L1 inhibition depends on the type of cytotoxic agent that is combined with the immune checkpoint inhibitor. Considering preclinical data with platinum (see Section 2.6.1), we aim to identify whether there is benefit in both unselected and PD-L1-positive TNBC patients. In addition, taxanes remain a standard part of neo-/adjuvant therapy in TNBC and, for patients whose tumors progress rapidly in the neo-/adjuvant setting to paclitaxel, other chemotherapy options are needed. Thus, this study continues to address a clinically relevant question. Considering the characteristics of the study population included in IMpassion130, modifications to the following criteria will be made.

2.3.2.1 First-line metastatic setting only

Patients with previously untreated locally advanced or metastatic TNBC were allowed in IMpassion130(64). Thus, to compare carboplatin with or without nivolumab in metastatic TNBC, we will restrict eligibility to first-line patients only. Patients who have received prior platinum in the neo-/adjuvant setting will be eligible if at least 6 months have elapsed since the end of systemic therapy to the development of metastatic disease.

2.3.2.2 PD-L1 status as a stratification factor

Considering the current approval of PD-L1 inhibition plus nab-paclitaxel in patients with metastatic TNBC and positive PD-L1 status (as determined by the Ventana assay with SP142), known PD-L1 status will be required and included as a stratification factor in order to avoid selection bias toward PD-L1-negative TNBC. PD-L1-negative status will be restricted to 60% of the study population.

2.4 Nivolumab

2.4.1 Mechanism of Action of 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-γ). In syngeneic mouse tumor models, blocking PD-1 activity resulted in decreased tumor growth.

2.4.2 Nonclinical Toxicology of Nivolumab

<u>Carcinogenesis, Mutagenesis, Impairment of Fertility</u>

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. No studies have been performed to assess the potential of nivolumab for carcinogenicity or genotoxicity. Preliminary new non-clinical safety findings of adverse pregnancy outcomes and infant losses in the absence of overt maternal toxicity have been reported. 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.

Animal Toxicology and/or Pharmacology

In animal models, inhibition of PD-1 signaling increased the severity of some infections and enhanced inflammatory responses. M. tuberculosis–infected PD-1 knockout mice exhibit markedly decreased survival compared with wild-type controls, which correlated with increased bacterial proliferation and inflammatory responses in these animals. PD-1 knockout mice have also shown decreased survival following infection with lymphocytic choriomeningitis virus.

2.4.3 Pharmacodynamics of Nivolumab

Based on dose/exposure efficacy and safety relationships, there are no clinically significant differences in safety and efficacy between a nivolumab dose of 240 mg or 3 mg/kg every 2 weeks in patients with melanoma, NSCLC, RCC, and urothelial carcinoma. The FDA approved dose of nivolumab in these three settings is a flat dose of 240 mg every 2 weeks.

2.4.4 Pharmacokinetics of Nivolumab

Pharmacokinetics (PK) of nivolumab was studied in patients over a dose range of 0.1 to 20 mg/kg administered as a single dose or as multiple doses every 2 or 3 weeks. Nivolumab clearance decreases over time, with a mean maximal reduction (% coefficient of variation [CV%]) from baseline values of approximately 24.5% (47.6%) resulting in a geometric mean steady state clearance (CLss) (CV%) of 8.2 mL/h (53.9%); the decrease in CLss is not considered clinically relevant. The geometric mean volume of distribution at steady state (Vss) (CV%) is 6.8 L (27.3%), and geometric mean elimination half-life (t1/2) is 25 days (77.5%). Steady-state concentrations of nivolumab were reached by approximately 12 weeks when administered at 3 mg/kg every 2 weeks, and systemic accumulation was approximately 3.7-fold. The exposure to nivolumab increased dose proportionally over the dose range of 0.1 to 10 mg/kg administered every 2 weeks.

Specific Populations: The population PK analysis suggested that the following factors had no clinically important effect on the clearance of nivolumab: age (29 to 87 years), weight (35 to 160 kg), gender, race, baseline LDH, PD-L1 expression, solid tumor type, tumor size, renal impairment, and mild hepatic impairment.

Renal Impairment: The effect of renal impairment on the clearance of nivolumab was evaluated by a population PK analysis in patients with mild (eGFR 60 to 89 mL/min/1.73 m2; n=313), moderate (eGFR 30 to 59 mL/min/1.73 m2; n=140), or severe (eGFR 15 to 29 mL/min/1.73 m2;

n=3) renal impairment. No clinically important differences in the clearance of nivolumab were found between patients with renal impairment and patients with normal renal function.

Hepatic Impairment: The effect of hepatic impairment on the clearance of nivolumab was evaluated by population PK analyses in patients with mild hepatic impairment (total bilirubin [TB] less than or equal to the upper limit of normal [ULN] and AST greater than ULN or TB less than 1 to 1.5 times ULN and any AST; n=92). No clinically important differences in the clearance of nivolumab were found between patients with mild hepatic impairment and patients with normal hepatic function. Nivolumab has not been studied in patients with moderate (TB greater than 1.5 to 3 times ULN and any AST) or severe hepatic impairment (TB greater than 3 times ULN and any AST)(65).

2.4.5 Clinical Experience with Nivolumab

2.4.5.1 Safety of Nivolumab in Clinical Trials

The overall safety experience with nivolumab, as monotherapy or in combination with other therapeutics, is based on experience in over 6,000 subjects treated to date with nivolumab monotherapy in single-or multiple-dose Phase 1/2/3 studies, or studies with nivolumab in combination with other therapeutics. The AE profile has been consistent across multiple tumor types, with no maximum tolerated dose reached at any monotherapy dose tested up to 10 mg/kg. Treatment-related AEs have included pulmonary toxicity, renal toxicity (including acute renal failure), endocrine abnormalities, GI toxicity, dermatologic toxicity (including rash), and hepatotoxicity. In most cases, these AEs have been managed successfully with supportive care and, in more severe cases, a combination of dose delay, permanent discontinuation, and/or initiation of systemic corticosteroids. Preliminary safety and tolerability data remain consistent with the profile of nivolumab in monotherapy across tumor types, with the exception of pneumonitis which may be numerically greater in subjects with NSCLC, possibly due to difficulty in distinguishing between treatment-related and disease-related pulmonary symptoms or radiographic changes(66). 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 melanoma. Thus far, the combination of both agents has revealed overall a manageable toxicity profile.

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 adverse events (AEs) according 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 6 of this protocol. Nivolumab should not be used in subjects with active autoimmune disease given the mechanism of action of the antibody.

Updated overall safety data for nivolumab is available in the current version of the Investigator's Brochure. The Investigator's Brochure should be reviewed in conjunction with this study protocol.

2.4.5.2 Efficacy of Nivolumab in Clinical Trials

Nivolumab has demonstrated clinical activity, leading to U.S. Food and Drug Administration approval as a single agent or in combination, in a variety of solid tumor malignancies:

1) Unresectable or metastatic melanoma, regardless of BRAF mutational status: as a single-agent at a dose of 240 mg intravenously every 2 weeks or 480 mg every 4 weeks, or in combination with ipilimumab (nivolumab at 1mg/kg every 3 weeks for 4 doses, followed by nivolumab 240 mg every 2 weeks or 480 mg every 4 weeks).
2) Melanoma with lymph node involvement or metastatic disease after complete resection, in the adjuvant setting as single-agent at a dose of 240 mg intravenously every 2 weeks or 480 mg every 4 weeks.
3) Metastatic non-small cell lung cancer (NSCLC) with progression on or after platinum-based chemotherapy, at a dose of 240 mg intravenously every 2 weeks or 480 mg every 4 weeks. Patients with EGFR or ALK genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving nivolumab.
4) Metastatic small cell lung cancer (SCLC) after platinum-based chemotherapy and at least one other line of therapy, at a dose of 240 mg intravenously every 2 weeks.
5) Advanced renal cell carcinoma after progression of anti-angiogenic therapy, at a dose of 240 mg intravenously every 2 weeks or 480 mg every 4 weeks.
6) Untreated advanced renal cell carcinoma in patients with intermediate or poor risk, in combination with ipilimumab (nivolumab at 1mg/kg every 3 weeks for 4 doses, followed by nivolumab 240 mg every 2 weeks or 480 mg every 4 weeks).
   Classical Hodgkin lymphoma that has relapsed or progressed after autologous hematopoietic stem cell transplantation (HSCT) and post-transplantation brentuximab vedotin, or 3 or more lines of systemic therapy that includes HSCT, at a dose of 240 mg intravenously every 2 weeks or 480 mg every 4 weeks .
7) Recurrent or metastatic squamous cell carcinoma of the head and neck with progression on or after platinum-based therapy, at a dose of 240 mg intravenously every 2 weeks or 480 mg every 4 weeks.
8) Locally advanced or metastatic urothelial carcinoma after progression on platinum-based chemotherapy or within 12 months of neo-/adjuvant treatment with platinum-based chemotherapy, based on tumor response rate and duration of response, at a dose of 240 mg intravenously every 2 weeks or 480 mg every 4 weeks.
9) Adult and pediatric (12 years and older) patients with microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer that has progressed following treatment with a fluoropyrimidine, oxaliplatin and irinotecan, as a single-agent (240 mg intravenously every 2 weeks) or in combination with ipilimumab (nivolumab at 1mg/kg every 3 weeks for 4 doses, followed by nivolumab 240 mg every 2 weeks).
10) Hepatocellular carcinoma previously treated with sorafenib, at a dose of 240 mg every 2 weeks or 480 mg every 4 weeks.

Updated overall clinical experience for nivolumab is available in the current version of the Investigator's Brochure. The Investigator's Brochure should be reviewed in conjunction with this study protocol.

2.4.6 Ongoing Trials of Nivolumab in Breast Cancer

Nivolumab is currently being explored in many tumor types and in combination with various treatments, such as chemotherapy, radiotherapy, small molecule inhibitors (i.e tyrosine kinase inhibitors) and other immune checkpoint inhibitors. Safety and efficacy results of nivolumab alone or in combination with ipilimumab (anti-CTLA4) in breast cancer patients have not yet been published. A phase 1/2, open-label study of nivolumab in monotherapy (at a dose of 3mg/kg intravenously every 2 weeks) or nivolumab combined with ipilimumab (nivolumab at 1 mg/kg intravenously plus ipilimumab 3 mg/kg every 3 weeks for 4 doses followed by nivolumab 3 mg/kg every 2 weeks) in subjects with advanced or metastatic solid tumors (NCT01928394) recently closed to accrual; results from patients enrolled on the TNBC cohort are pending.

In recurrent HER2-negative metastatic breast cancer, a phase 1 study (NCT02309177) has been completed to determine the safety of the combination of nivolumab and nab-paclitaxel at two dose regimens (nab-paclitaxel at 100mg/m2 on days 1, 8 and 15 of each 28-day cycle, plus nivolumab on days 1 and 15 starting in cycle 3; nab-paclitaxel at 260mg/m2 on days 1 of each 21-day cycle, plus nivolumab on days 15 starting in cycle 3). In this trial, other dose regimens of this combination have been explored in additional cohorts for pancreatic and non-small cell lung cancer (NSCLC) (see section 2.6.5).

The TONIC trial (NCT02499367) is a phase II randomized non-comparative study evaluating the safety and efficacy of nivolumab at a dose of 3 mg/kg every 2 weeks after an induction treatment (radiation therapy on a metastatic lesion or doxorubicin 15 mg flat dose once weekly for 2 weeks or metronomic cyclophosphamide 50 mg daily orally for 2 weeks or cisplatin 40 mg/m2 weekly for 2 weeks, or no induction) in patients with metastatic TNBC. A ¨pick-the-winner¨ strategy was used in the first stage of the trial to discontinue early a cohort if the response rate (with n=10) was ≤30%, and to identify the most promising cohort for expansion in stage II. In stage I, a total of 70 patients were randomized to one of the 5 cohorts and 66 were evaluable for efficacy(67). Of note, 46 (72%) of patients were considered PD-L1-positive, defined as ≥5% of staining on immune cells. The overall response rate (ORR) was highest in the doxorubicin (35%) and cisplatin (23%) cohorts, and lowest in the radiation (8%) and cyclophosphamide (8%) cohorts, with an ORR of 17% in the arm without induction. Of patients who achieved a complete or partial response, 85% were alive at one-year, compared to 21% of those who did not respond to treatment. Compared to baseline, tumors treated with cisplatin had upregulation of several immune signatures – particularly CD8 and T-cell signatures after the 2-week induction phase, and CD8, cytotoxic cells, T-cells, PD-1, PD-L1, tumor-inflammation signature in biopsies after 8 weeks (while on nivolumab). In addition, in biopsies on nivolumab, more clonal T-cells were observed in responders (n=4) versus non-responders (n=25) across cohorts. Increased T-cells and T-cell clonality were observed after exposure to cisplatin and doxorubicin, and while on nivolumab, in these two cohorts(67).

2.5 Carboplatin

2.5.1 Mechanism of Action of Carboplatin

Carboplatin, like cisplatin, produces predominantly intrastrand and interstrand DNA cross-links(68). This effect is apparently cell-cycle nonspecific. The aquation of carboplatin, which is thought to produce the active species, occurs at a slower rate than in the case of cisplatin. Despite this difference, it appears that both carboplatin and cisplatin induce equal numbers of drug-DNA cross-links, causing equivalent lesions and biological effects. The differences in potencies for carboplatin and cisplatin appear to be directly related to the difference in aquation rates.

2.5.2 Nonclinical Toxicology of Carboplatin

Carcinogenesis, Mutagenesis, Impairment of Fertility
The carcinogenic potential of carboplatin has not been studied, but compounds with similar mechanisms of action and mutagenicity profiles have been reported to be carcinogenic. Secondary malignancies have been reported in association with multi-drug therapy. Carboplatin has been shown to be mutagenic both *in vitro* and *in vivo.* It has also been shown to be embryotoxic and teratogenic in rats receiving the drug during organogenesis.

2.5.3 Pharmacokinetics

In patients with creatinine clearances of about 60 mL/min or greater, plasma levels of intact carboplatin decay in a biphasic manner after a 30-minute intravenous infusion of 300 mg/m2 to 500 mg/m2 of carboplatin. The initial plasma half-life (alpha) was found to be 1.1 to 2 hours (n=6), and the postdistribution plasma half-life (beta) was found to be 2.6 to 5.9 hours (n=6). The total body clearance, apparent volume of distribution and mean residence time for carboplatin are 4.4 L/hour, 16 L and 3.5 hours, respectively. The Cmax values and areas under the plasma concentration versus time curves from 0 to infinity (AUC inf) increase linearly with dose, although the increase was slightly more than dose proportional. Carboplatin, therefore, exhibits linear pharmacokinetics over the dosing range studied (300 mg/m2 to 500 mg/m2).

Carboplatin is not bound to plasma proteins. No significant quantities of protein-free, ultrafilterable platinum-containing species other than carboplatin are present in plasma. However, platinum from carboplatin becomes irreversibly bound to plasma proteins and is slowly eliminated with a minimum half-life of 5 days.

The major route of elimination of carboplatin is renal excretion. Patients with creatinine clearances of approximately 60 mL/min or greater excrete 65% of the dose in the urine within 12 hours and 71% of the dose within 24 hours. All of the platinum in the 24-hour urine is present as carboplatin. Only 3% to 5% of the administered platinum is excreted in the urine between 24 and 96 hours. There are insufficient data to determine whether biliary excretion occurs.
In patients with creatinine clearances below 60 mL/min, the total body and renal clearances of carboplatin decrease as the creatinine clearance decreases. carboplatin dosages should therefore be reduced in these patients. The primary determinant of carboplatin clearance is glomerular

filtration rate (GFR) and this parameter of renal function is often decreased in elderly patients. Dosing formulas incorporating estimates of GFR to provide predictable carboplatin plasma AUCs should be used in elderly patients to minimize the risk of toxicity.

2.5.4 Clinical Experience with Carboplatin

2.5.4.1 Safety of Carboplatin in Metastatic TNBC

In TBCRC009, 43 patients with metastatic TNBC were treated with carboplatin in the first- or second-line setting (**Table 2**)(30). Single-agent carboplatin had generally mild toxicity, with fatigue, nausea, electrolyte abnormalities and hematologic toxicity among the most common. Grade 3 and 4 adverse events were rare; those occurring in at least 5% of all patients included fatigue, neutropenia, dyspnea, anemia, hyperglycemia and hyponatremia. No treatment-related deaths were reported.

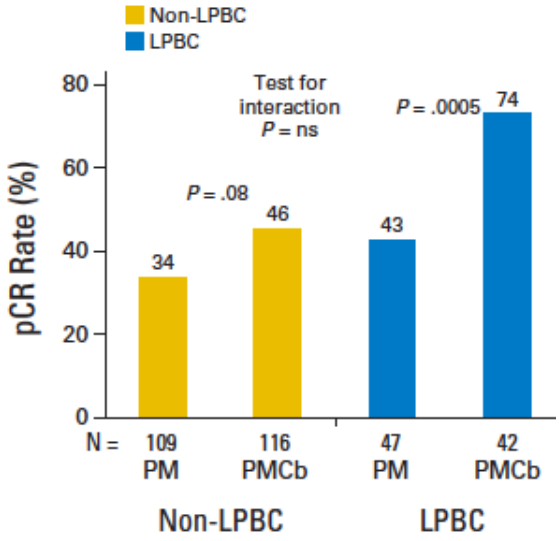
Table 2. Toxicity reported with carboplatin in TBCRC009

All Grade Toxicity in > 10% of Patients in the TBCRC 009 Trial

		Carboplatin (n=43)	
		No. of patients	%
Hematologic	Neutropenia	15	35
	Lymphopenia	9	21
	Anemia	28	65
	Thrombocytopenia	20	47
	Febrile neutropenia	4	9
Fatigue / Malaise		28	65
Decreased appetite		4	9
Peripheral neuropathy		15	35
Gastrointestinal	Diarrhea	3	7
	Constipation	11	26
	Nausea	27	63
	Vomiting	10	23
Hepatic	Increased ALT	9	21
	Increased AST	13	30
Pulmonary	Dyspnea	12	28
Metabolism / Electrolyte alterations	Hyperglycemia	19	44
	Hypomagnesemia	10	23
	Hyponatremia	6	14
	Hypokalemia	5	12
	Hypocalcemia	5	12
	Hypoalbuminemia	5	12
Skin/Mucosa	Rash	5	12

Grade 3 and 4 Adverse Events in > 5% of Patients in the TBCRC 009 Trial

	Carboplatin (n=43)	
	No. of patients	%
Fatigue	2	5
Neutropenia	4	9



Dyspnea	2	5
Anemia	4	9
Hyperglycemia	3	7
Hyponatremia	1	2

2.6 Combination of Carboplatin and Nivolumab

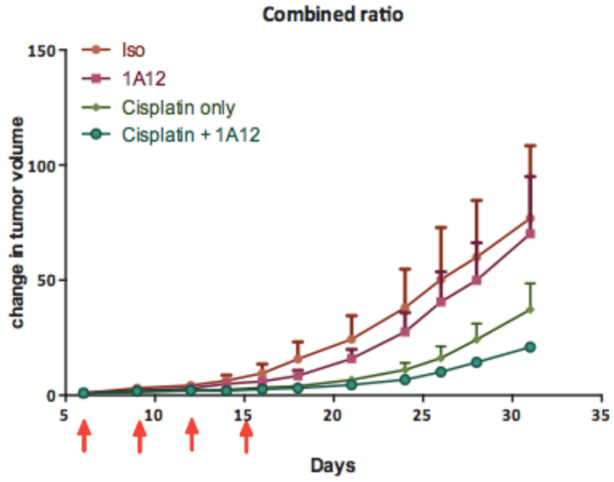
2.6.1 Rationale for the Combination of Carboplatin and Nivolumab

The discovery of the critical role that the overexpression of immune checkpoint molecules in the tumor microenvironment plays in antitumor immunity evasion and cancer progression has revolutionized cancer treatment(69). 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 the treatment of advanced melanoma, non-small-cell lung carcinoma and renal cell carcinoma(70-72). More than 1,000 clinical trials of checkpoint blockers are currently ongoing.

Although breast cancer has not been traditionally considered an immunogenic neoplasia, increasing preclinical and clinical data suggest that the interaction with the immune system is critical for disease outcome in this tumor type(54). It is now recognized that a fraction of breast tumors, especially TNBC, have substantial lymphocyte infiltration and that this pathologic feature has prognostic implications(56, 73). Neoadjuvant studies in TNBC have revealed that lymphocyte-predominant breast cancer (LPBC) is associated with higher pathologic complete response rates (pCR)(74) and that the presence of TILs in residual disease correlates with improved overall survival(75). In GeparSixto, patients with LPBC triple-negative tumors obtained significantly greater pCR with the addition of carboplatin to paclitaxel and non-pegylated liposomal doxorubicin, whereas no significant differences were noted between neoadjuvant regimens in patients with non-LPBC (**Figure 1**)(56).

Figure 1. Pathologic complete response (pCR) rates in the triple-negative subgroup of patients treated with neoadjuvant chemotherapy in GeparSixto. LPBC = lymphocyte-predominant breast cancer; Cb = Carboplatin; P= Paclitaxel; M = Non-pegylated liposomal doxorubicin. (Denkert et al. *J Clin Oncol* 2015)

The elevated rates of PD-L1 and PD-1 expression in TNBC(59) have led to the development of clinical trials to evaluate checkpoint inhibition in this population(76). Atezolizumab, a monoclonal antibody targeting PD-L1, was evaluated in patients with metastatic PD-L1-positive TNBC and demonstrated and ORR of 19% and 6-month PFS of 27%(61). Similar results were found among the TNBC cohort of the KEYNOTE-012 study, preselected for PD-L1 positivity, treated with the anti-PD-1 inhibitor pembrolizumab (ORR of 18% and 6-month PFS of 24%)(62). Data from the JAVELIN trial have revealed modest activity of avelumab, a PD-L1 antibody, in patients with metastatic breast cancer unselected for PD-L1 expression, with an ORR of 8.6% in the TNBC subgroup(63).



These results demonstrate that, although some patients with TNBC may derive clinical benefit from checkpoint blockade, in most cases single-agent PD-1/PD-L1 inhibitors are insufficient to control disease burden. Immunosuppressive mechanisms within the tumor microenvironment may play a role in *de novo* resistance to these therapies. Some of these mechanisms have been identified, and now provide targets for combination therapy(77). Considerably higher response rates have been noted with atezolizumab and albumin-bound paclitaxel in metastatic TNBC, particularly in the first-line setting in which a confirmed ORR of 46% was observed(78). Activity of the combination of immunotherapy and chemotherapy in this trial was evident regardless of PD-L1 positivity. This has encouraged further evaluation of immune checkpoint inhibitors in combination with chemotherapy in clinical trials, expecting to enhance the benefit of either strategy alone.

Furthermore, platinum agents are DNA-crosslinkers that cause the accumulation of genotoxic stress. Colleagues at Dana-Farber Cancer Institute (DFCI) have demonstrated that new neoantigens are generated in tumor tissue following cisplatin treatment (data not yet published). Genotoxic stress, in turn, stimulates immune activation via interferon-γ signaling. Accordingly, platinum chemotherapy has been shown to generate CD8-driven anti-tumor immune responses *in vitro* and *in vivo* murine models(79), making the combination with immunotherapy an attractive therapeutic strategy. Platinum chemotherapy also enhances T cell activation through downregulation of PD-L1 and PD-L2 on dendritic cells, increasing their T cell activation potential and sensitizing tumor cells to cytotoxic T cell-mediated attack(80). Preclinical work at DFCI has shown significant improvement in tumor burden control with the addition of a PD-1 antibody to platinum chemotherapy in the 4T1 mouse model of TNBC (**Figure 2**; 1A12 = anti-PD1 clone; iso = isotype antibody control).

Breast cancers that develop in patients that carry a germline *BRCA1* or *BRCA2* mutation are prone to genotoxic stress and, therefore, particularly sensitive to platinum chemotherapy(30, 81, 82). This may render them especially susceptible to treatment with platinum plus PD-1 blockade. The clinical efficacy of immune stimulation in genomically unstable tumors has been

proven in microsatellite instability-high colon cancer(83). Here, this randomized phase II study will evaluate the efficacy of combined therapy with carboplatin plus nivolumab in metastatic TNBC, with sub-analyses specifically focused on activity in the *BRCA1/2*-mutant subgroup. Carboplatin will be administered at area under the curve (AUC) 6 every 21 days, as is the clinical standard in metastatic breast cancer, mimicking the dose and schedule used in the phase III TNT and phase II TBCRC009 TNBC trials referenced previously. Nivolumab will be administered at a flat dose of 360 mg every 21 days, in order to maintain consistency between cycles.

2.6.2 Rationale for Dose of Nivolumab

The U.S. FDA has approved nivolumab at a flat dose of 240 mg every 2 weeks, as a single agent, for NSCLC, melanoma and renal cell carcinoma. This was based on pharmacokinetic data [redacted] data on file) that demonstrated that the 240 mg dose corresponded to the exposures observed with a dose of 3 mg/kg in an 80-kg patient, which was the approximate median body weight for patients in the nivolumab clinical trials. The range of predicted exposure with 240 mg overlapped with the range observed with 3 mg/kg across subjects with a wide range of body weights. Model-based simulation results indicated that systemic exposure of nivolumab was similar between 3 mg/kg or 240 mg flat dose, with a median percent difference in exposures of approximately 5%. Based on dose/exposure efficacy and safety relationships, there are no clinically significant differences in safety and efficacy between a nivolumab dose of 240 mg and 3 mg/kg every 2 weeks in patients with unresetable or metastatic melanoma, previously treated metastatic NSCLC and advanced renal cell carcinoma who have received prior antiangiogenic therapy.

Nivolumab demonstrated safety and linear pharmacokinetics with a dose range of 0.1mg/kg to 10mg/kg across solid tumor types in the phase 1 study CA209-003(71). No relationship was noted between nivolumab exposure and risk of adverse events leading to discontinuation of therapy or death. The subgroup safety analysis did not demonstrate a relationship between body weight and frequency or severity of adverse events. To select the appropriate flat dose of nivolumab, a body weight distribution assessment was conducted using observed baseline body weights from melanoma, NSCLC and renal cell carcinoma across nivolumab clinical trials (solid tumor study: CA209-003; NSCLC studies: CA209-017, CA209-063, CA209-057; melanoma studies: CA209-037, CA209-066, CA209-067; renal cell carcinoma: CA209-010, CA209-025). The approximate median body weight of these subjects was 80 kg (body weight range 35-160 kg in melanoma and NSCLC patients; 40-168 kg in renal cell carcinoma). Utilizing a dose of 3mg/kg, the corresponding flat dose was calculated to be 240mg.

A model-based simulation was conducted to evaluate the predicted exposure of nivolumab administered 240 mg every 2 weeks. These simulation results were then compared to exposures in patients treated with nivolumab 3mg/kg every 2 weeks in the nine previously mentioned trials across melanoma, NSCLC and renal cell carcinoma. The percent difference in geometric mean of all measures after weight-based dosing and flat-dosing was approximately 5%. Flat dosing resulted in higher nivolumab exposure in lighter patients and lower exposures in higher body weight patients compared to body-weight dosing. However, the median and 95th percentile of nivolumab exposures across the weight range were below exposures observed with nivolumab 10mg/kg every 2 weeks that was considered safe and tolerable.

Exposure-response analyses for both, efficacy and safety, were also conducted. The hazard ratios (HRs) for risk of death were estimated for nivolumab exposure following body-weight adjusted and flat dosing, and the HRs were similar between 3 mg/kg and 240 mg every 2 weeks across the three tumor types. The 95% confidence intervals (CI) of HRs for risk of death crossed 1.0, suggesting that a flat exposure-response relationship exists for nivolumab. Based on dose/exposure efficacy relationships, there are no clinically significant differences in efficacy between a nivolumab dose of 240 mg and 3 mg/kg every 2 weeks for these patients. In addition, no relationship was noted between nivolumab exposure and risk of adverse events leading to discontinuation of therapy or death. The HR was similar between nivolumab 3 mg/kg and 240 mg every 2 weeks. Based on dose/exposure efficacy relationships, there are no clinically significant differences in safety between a nivolumab dose of 240 mg and 3 mg/kg every 2 weeks for these patients ([redacted] data on file).

Nivolumab has been explored in an every 3-week schedule in a phase 1 study (CheckMate 012) in combination with platinum-based doublet chemotherapy (PT-DC) in first-line advanced NSCLC(84). Patients (n=56) received nivolumab (intravenously) plus PT-DC concurrently every 3 weeks for four cycles followed by nivolumab alone until progression or unacceptable toxicity. Regimens were nivolumab 10 mg/kg plus gemcitabine-cisplatin (squamous) or pemetrexed-cisplatin (nonsquamous) or nivolumab 5 or 10 mg/kg plus paclitaxel 200mg/m2 – carboplatin AUC 6 (all histologies) every 3 weeks. The study used a dose de-escalation design on the basis of the modified toxicity probability interval method, guided by the number of dose-limiting toxicities (DLTs) observed during the first two cycles (6 weeks) of therapy. Initially, six patients per arm were treated with nivolumab 10 mg/kg. Whereas the nivolumab 10 mg/kg dose was based on results from the phase 1 study CA209-003, the 5 mg/kg dose was added after a protocol amendment to assess the safety of a lower nivolumab dose in combination with paclitaxel-carboplatin (which can be used for all histologic subtypes). Addition of this arm was not driven by safety, because no DLTs were observed with nivolumab 10 mg/kg, but rather, it was added on the basis of pharmacokinetic modeling showing that nivolumab 5 mg/kg every 3 weeks provides a steady-state trough concentration equivalent to nivolumab 3 mg/kg every 2 weeks(85).

Median OS was longer than expected for PT-DC alone (8.1 to 10.3 months), ranging from 11.6 to 19.2 months across the nivolumab 10 mg/kg plus PT-DC arms. OS for the nivolumab 5 mg/kg plus paclitaxel-carboplatin arm was particularly notable; median OS was not reached (range, 8.8 to 30.1+ months), and 57% of patients (eight of 14 patients) were still alive after a median follow-up time of more than 2 years. Although sample sizes were small, 1-year OS rates (50% to 87%) seemed similar to that reported with nivolumab monotherapy (73%) in CheckMate 012. It is unclear whether nivolumab plus PT-DC offers improved long-term OS benefit compared with nivolumab monotherapy. However, survival data with nivolumab 5 mg/kg every 3 weeks in combination with chemotherapy are encouraging(84) and are being further explored in a phase III trial (NCT02477826).

Another phase I trial conducted in Japan demonstrated that nivolumab at 10 mg/kg every 3 weeks was a safe and tolerable regimen in combination with carboplatin (AUC 6), paclitaxel (200 mg/m2) and bevacizumab (15 mg/kg), with the lowest rate of discontinuation of therapy due to adverse events (0 of 6 patients) compared to the other arms of nivolumab plus cisplatin- or docetaxel-based chemotherapy(86). In addition, in this study serum concentrations of nivolumab on cycle 1 day 22

were similar to those reported in monotherapy, suggesting that cytoxic chemotherapy does not influence serum concentration of nivolumab when the agents are given in combination.

In addition, clinical safety of flat dosing at 480 mg every 4 weeks has been compared to 3mg/kg Q2W(87). Across four phase 3 clinical trials in which patients with multiple tumor types who were treated with nivolumab 3mg/kg Q3W transitioned to flat dosing of 480 mg Q4W, time-averaged steady-state exposure and safety profile of nivolumab 480 mg Q4W were consistent with that of 3mg/kg Q3W. Treatment-related adverse events that started after transitioning to the flat dose Q4W schedule were reported in 14.8% of patients (grade 3-4 toxicity in 1.6% of patients). No new safety signals were observed in this analysis.

Considering the linear pharmacokinetics of nivolumab at doses ranging from 0.1 to 10 mg/kg (every 2 weeks), in addition to data suggesting that this is not influenced by cytotoxic chemotherapy, a 3-week schedule estimated to correspond to a flat dose of 360 mg will be administered in the First Course of study treatment. For eligible patients randomized to Arm B (carboplatin alone) who elect to crossover to nab-Paclitaxel plus nivolumab after progression on carboplatin, a 4-week schedule estimated to correspond to a flat dose of 480 mg will be administered in the Crossover Phase.

2.6.3 Rationale for Shorter Infusion Time for Nivolumab

Long infusion times place a burden on patients and treatment centers. Establishing that nivolumab and ipilimumab can be safely administered using shorter infusion times of 30 minutes' duration will diminish the burden, provided that there is no change in the safety profile. Previous clinical studies of nivolumab and ipilimumab monotherapies and the combination of nivolumab and ipilimumab have used a 60-minute infusion duration for nivolumab and a 90-minute infusion duration for ipilimumab (1 - 3 mg/kg dosing for both). However, both nivolumab and ipilimumab have been administered at up to 10 mg/kg with the same infusion duration (ie, 60 minutes).

Nivolumab has been administered safely over 60 minutes at doses ranging up to 10 mg/kg over a long treatment duration. In subjects with advanced/metastatic clear cell RCC, a dose association was observed for infusion site reactions and hypersensitivity reactions (1.7% at 0.3 mg/kg, 3.7% at 2 mg/kg and 18.5% at 10 mg/kg). All the events were Grade 1/2 and were manageable. An infusion duration of 30 minutes for 3 mg/kg nivolumab (30% of the dose provided at 10 mg/kg) is not expected to present any safety concerns compared to the prior experience at 10 mg/kg nivolumab dose infused over a 60-minute duration.

Similarly, ipilimumab at 10 mg/kg has been safely administered over 90 minutes. In subjects with advanced Stage II or Stage IV melanoma, where ipilimumab was administered up to a dose of 10 mg/kg, on-study drug related hypersensitivity events (Grade 1/2) were reported in 1 subject (1.4%) in the 0.3 mg/kg and in 2 subjects (2.8%) in the 10 mg/kg group. There were no drug-related hypersensitivity events reported in the 3 mg/kg group. Across the 3 treatment groups, no Grade 3/4 drug-related hypersensitivity events were reported and there were no reports of infusion reactions. Ipilimumab 10 mg/kg monotherapy has also been safely administered as a 90-minute infusion in a large Phase 3 study in prostate cancer and as adjuvant therapy for Stage III melanoma, with infusion reactions occurring in subjects. Administering 3 mg/kg of ipilimumab represents

approximately one-third of the 10 mg/kg dose.

Overall, infusion reactions including high-grade hypersensitivity reactions have been uncommon across clinical studies of nivolumab. Furthermore, a 30-minute break after the first infusion for the combination cohort will ensure the appropriate safety monitoring before the start of the second infusion. Overall, a change in safety profile is not anticipated with 30-minute infusions of nivolumab.

2.6.4 Safety of the Combination of Carboplatin and Nivolumab

The safety of the combination of nivolumab and carboplatin has only been reported with doublet platinum-based regimens that include paclitaxel (**Table 3**). In CheckMate 012, the most common toxicities reported for the combination were those anticipated with PT-DC alone(84). The observed frequencies of treatment-related grade 3 or 4 nonhematologic toxicities, such as fatigue and nausea (0% to 13% and 0% to 7%, respectively), were consistent with those previously reported for paclitaxel-carboplatin (8% and 0% to 9%, respectively). The observed frequencies of immune-related adverse events affecting the skin, GI, renal, and pulmonary organs were greater than expected with single-agent nivolumab (combination therapy vs. monotherapy: 36% vs. 25%, 23% vs. 12%, 14% vs. 0%, and 13% vs. 6%, respectively). However, these treatment-related AEs, including pneumonitis, were effectively managed with corticosteroids or infliximab and did not lead to any deaths. No treatment-related adverse events led to discontinuation of treatment during concurrent nivolumab and PT-DC therapy in the carboplatin-containing arms.

In the Japanese phase I study, no DLTs were observed in the nivolumab-carboplatin-paclitaxel-bevacizumab arm(86). Hematological grade 3 or higher adverse events were present in all 6 patients in this arm, although no non-hematological adverse events of grade 3 or worse were reported. There were no treatment-related deaths and there were no adverse events leading to discontinuation of nivolumab in the carboplatin arm. In 2 (33.3%) patients, chemotherapy was discontinued due to adverse events – epistaxis and peripheral neuropathy.

Table 3. Treatment-Related Adverse Events with Nivolumab in Combination with Carboplatin-Based Regimens in NSCLC

		Carboplatin / Paclitaxel / Nivolumab 10mg/kg Q3W (n=15)*		Carboplatin / Paclitaxel / Nivolumab 5mg/kg Q3W (n=14)*		Carboplatin/ Paclitaxel / Bevacizumab / Nivolumab 10mg/kg Q3W (n=6) **	
		Any Grade	Grade 3-4	Any Grade	Grade 3-4	Any Grade	Grade 3-4
All adverse events		15 (100)	11 (73)	14 (100)	4 (29)	6 (100)	6 (100)
Hematologic	Neutropenia	2 (13)	1 (7)	0 (0)	0 (0)	6 (100)	6 (100)
	Lymphopenia	3 (20)	0 (0)	1 (7)	0 (0)	4 (67)	1 (17)
	Anemia	5 (33)	1 (7)	2 (14)	0 (0)	4 (67)	1 (17)
	Thrombocytopenia	2 (13)	0 (0)	0 (0)	0 (0)	6 (100)	2 (33)
	Febrile neutropenia	NR	NR	NR	NR	1 (17)	1 (17)
Fatigue / Malaise		10 (67)	2 (13)	10 (71)	0 (0)	3 (50)	0 (0)
Decreased appetite		5 (33)	1 (7)	2 (14)	0 (0)	5 (83)	0 (0)
Pyrexia		2 (13)	0 (0)	1 (7)	0 (0)	1 (17)	0 (0)
Arthralgia		7 (47)	0 (0)	2 (14)	0 (0)	4 (67)	0 (0)
Peripheral neuropathy		1 (7)	0 (0)	7 (50)	0 (0)	4 (67)	0 (0)
Gastrointestinal	Diarrhea	5 (33)	1 (7)	3 (21)	0 (0)	2 (33)	0 (0)

	Constipation	3 (20)	0 (0)	3 (21)	0 (0)	4 (67)	0 (0)
	Nausea	2 (13)	0 (0)	6 (43)	0 (0)	4 (67)	0 (0)
	Vomiting	0 (0)	0 (0)	2 (14)	0 (0)	1 (17)	0 (0)
Endocrine	Hypothyroidism	0 (0)	0 (0)	1 (7)	0 (0)	0 (0)	0 (0)
	Hypophysitis	NR	NR	NR	NR	0 (0)	0 (0)
Hepatic	Increased ALT	0 (0)	0 (0)	0 (0)	0 (0)	3 (50)	0 (0)
	Increased AST	0 (0)	0 (0)	0 (0)	0 (0)	2 (33)	0 (0)
Pulmonary	Pneumonitis	0 (0)	0 (0)	2 (14)	1 (7)	0 (0)	0 (0)
Renal	Increased serum creatinine	1 (7)	0 (0)	1 (7)	0 (0)	1 (17)	0 (0)
Skin/Mucosa	Pruritus	4 (27)	0 (0)	0 (0)	0 (0)	5 (83)	0 (0)
	Rash	4 (27)	0 (0)	5 (36)	1 (7)	3 (50)	0 (0)
	Maculopapular rash	2 (13)	2 (13)	0 (0)	0 (0)	1 (17)	0 (0)
	Stomatitis	1 (7)	0 (0)	3 (21)	0 (0)	2 (33)	0 (0)
Epistaxis		2 (13)	0 (0)	0 (0)	0 (0)	4 (67)	0 (0)
Hypersensitivity		4 (27)	0 (0)	0 (0)	0 (0)	1 (17)	0 (0)
Infusion-related reaction		2 (13)	0 (0)	0 (0)	0 (0)	1 (17)	0 (0)

*CA209-012 (Rizvi, JCO 2016); ** JapicCTI-132071 (Randa, Ann Oncol 2016)

2.6.5 Safety of the Combination of Nab-Paclitaxel and Nivolumab

For patients who are randomized to Arm B, carboplatin monotherapy as first-line metastatic treatment, the option of crossover to receive nab-paclitaxel plus nivolumab may be considered at the time of progression on carboplatin. Several trials have explored nab-paclitaxel-based regimens in combination with nivolumab across different tumor types.

In a phase I trial assessing nivolumab (3mg/kg Q2W) with nab-paclitaxel (125mg/m^2 on days 1,8 and 15, every 28-day cycle) plus or minus gemcitabine in advanced pancreatic cancer (NCT02309177), safety data from a total of 50 treatment-naïve patients were reported(88). One DLT was identified (hepatitis, evidenced by grade 3 elevated liver function tests that were considered to be related to nab-paclitaxel plus gemcitabine). Across all treated patients, 48 (96%) had at least one grade 3-4 treatment-related adverse event; the most common G3-4 events were anemia (36%), neutropenia (36%), gastrointestinal events (24%), hepatotoxicity (22%), peripheral neuropathy (16%), thrombocytopenia (12%) and colitis (12%). One grade 5 event, respiratory failure (considered most likely pneumonitis) was observed.

In a separate cohort of this phase I trial (NCT02309177) for stage IIIB/IV NSCLC, patients received nab-paclitaxel (100mg/m^2 on days 1, 8 and 15) plus carboplatin plus nivolumab (5mg/kg day 15 of each 21-day cycle), followed by single-agent nivolumab as maintenance therapy starting in cycle 5(89). In the first 6 patients treated with the triplet combination, no DLTs were observed. Of the total 20 patients who received the triplet, the most common grade 3-4 treatment-related adverse events were neutropenia (45%) and anemia (40%). Grade 3 or 4 colitis or pneumonitis was not observed.

These results suggest that nivolumab plus nab-paclitaxel and another chemotherapeutic agent is tolerable; thus, the combination of only nab-paclitaxel and nivolumab is anticipated to have an acceptable safety profile.

2.7 Correlative Studies Background

2.7.1 Immune Marker Profiling

The importance of the tumor microenvironment and immunosurveillance in the natural history of cancer and response to therapy has become patent over the last years. This has led to improvement of clinical outcomes and U.S. FDA approval of immune checkpoint inhibitors for the treatment of various tumor types(90). However, less than half of patients with solid tumors derive benefit from these drugs (91, 92). Thus, it is crucial to elucidate the exact mechanisms of antitumor immunity evasion ongoing in the tumor microenvironment, in order to successfully develop new strategies that may enhance the effect of immunotherapeutic drugs.

Growing evidence suggests that patients with advanced solid tumors show differences in tumor microenvironment regarding the presence or absence of a gene expression profile indicative of a pre-existing T-cell–inflamed tumor microenvironment(93). Tumors classified as T-cell inflamed present a significant infiltration of CD8+ T cells and a type-I interferon (IFN) signature. In this group, the main mechanisms of immune evasion are the overexpression of immunosupressive molecules acting at the level of the tumor microenvironment, such as immune checkpoint molecules (CTLA-4, PD-1/PD-L1, TIM-3, LAG-3), indoleamine-2,3-dioxygenase (IDO), and FoxP3. Interestingly, these immunosuppressive molecules seem to be upregulated after deflagration of a type-I IFN antitumor response, resulting in T-cell exhaustion, and adaptive immune resistance(93, 94). In contrast, other tumors are characterized by low or absent intratumoral CD8 T cells and no evidence of a type-I IFN transcriptional signature; this tumor phenotype is non-T-cell-inflamed(93).

The T-cell inflamed phenotype has positive prognostic value for several types of early stage cancer, including breast cancer(58, 95), suggesting that an attempt by the host to generate an anti-tumor immune response reflects a biologic process associated with improved patient outcomes(93). In breast oncology, different groups have demonstrated that the amount of TILs in a tumor specimen, commonly assessed 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 (36, 37, 55, 57, 96-98). 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(95). Furthermore, in the metastatic setting, the phenotype T-cell-inflamed appears to be associated with clinical response to several immunotherapies, including checkpoint blockade(99). Patients with this tumor phenotype seem to be good candidates for immune checkpoint inhibitor therapy, alone or in combination. Thus, one of the main objectives of our correlative science in this trial is the characterization of a broad array of immune markers in metastatic TNBC, investigating whether these markers may predict disease response to therapy.

Considering the mechanism of action of anti-PD-1/anti-PD-L1 drugs, the absence of significant T-cell infiltrate, in addition to low expression of immune checkpoint molecules, may correlate with a non-inflamed tumor phenotype that is associated with *de novo* resistance to these agents. For this group of patients, therapeutic strategies that promote an increase in cytotoxic T-cell infiltration,

such as chemotherapy, may be crucial to successfully overcoming T-cell exclusion and improve the likelihood of benefit of PD-1 blockade.

Additionally, as part of the correlative work in this study, 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 infiltration. We will also evaluate whether there is a correlation between changes in PBMC immune profiles and response to treatment. Evidence of a correlation would be of significant interest as it would suggest the potential presence of a predictive biomarker of response or resistance in the peripheral blood.

2.7.2 Microbiota and Response to Immune Checkpoint Inhibitors

The gut microbiota has been recognized as a modulator of immune system development(100). Healthy individuals have microbial populations in their intestinal tract that vary markedly in composition(101, 102). The diversity of intestinal microbiota represents a significant challenge to the host's immune system, responsible for the balance between immune tolerance of beneficial microbes and inflammatory responses against pathogens. Alterations in the gut microbiota and their resulting interactions with intestinal epithelium and the host immune system are associated with many diseases, including cancer(103). Preclinical studies have provided strong evidence for the role of gut microbiota in modulating response and resistance to immune checkpoint inhibitors, raising the possibility that stool microbiota could be used as a predictive biomarker of efficacy to immunotherapy(104, 105). Interestingly, postmenopausal women with breast cancer have altered composition and low diversity of their gut microbiota compared to healthy controls, as measured by the Shannon index(106).

Emerging evidence suggests that the gut microbiome may influence response to checkpoint inhibitors in a number of malignancies(14, 107-109). Preclinical studies in murine models have demonstrated that microbiome composition is associated with response to PD-L1 inhibitors, with mice exhibiting a "favorable" microbiome having a greater likelihood of responding to treatment as compared to mice with an "unfavorable" microbiome. Studies also demonstrate that transplanting feces from mice with favorable microbiota to those with unfavorable microbiota increases response rates, suggesting that modification of the microbiome could have a therapeutic effect in patients receiving checkpoint inhibitors(14). Translational work demonstrates that fecal transplants from patients responding to checkpoint inhibitors to nude mice increases the likelihood of response to immunotherapy in these animals, whereas transplants from human non-responders leads to a lower likelihood of response(14, 108). The evidence linking the gut microbiome to checkpoint inhibitor efficacy in humans has been relatively limited to date, but a number of recent publications have demonstrated a relationship between composition of the microbiome and response to immunotherapy in melanoma, non-small cell lung carcinoma and renal cell cancer(14, 107-109). Notably, the bacterial species associated with a favorable response to treatment have differed across disease sites and studies(107, 109).

Microbiome composition is influenced by many factors, including age, genetics, and use of antibiotics and probiotics.(110-112). Studies demonstrate that dietary composition is one of the

primary drivers of microbiome diversity and taxa(110, 111). Other factors related to energy balance, the balance of calories ingested versus expended, such as physical activity and body weight also impact microbiome composition(111, 112). Limited data in humans suggest that factors impacting microbiome composition may also be related to checkpoint inhibitor response. An analysis of 113 patients with metastatic melanoma found significant associations between dietary factors (ingestion of red meat [p=0.006], sugar-sweetened beverages [p=0.048] and fruits/vegetables [p=0.049]) and microbiome composition(113). Use of antibiotics (p=0.05) and probiotics (p=0.02) was also associated with lower microbiome α-diversity. Exploratory analyses of 46 of these patients who were initiating treatment with anti-PD-1 therapy suggested that individuals in this cohort with a higher consumption of dietary fiber had a 5-fold likelihood of response to anti-PD-1 therapy as compared to individuals with low fiber consumption (personal communication, Wargo). These findings are provocative, but were based on a dietary screening tool that provides a relative crude assessment of dietary intake. The study also did not assess other modifiable factors that can influence microbiome composition, such as physical activity, precluding identification of an optimal strategy of lifestyle modification to enhance immunotherapy response.

Identification of biomarkers that predict response to immunotherapies could potentially 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.

In an effort to identify predictive biomarkers of response or resistance to immune checkpoint inhibitors, stool samples will be collected to determine whether baseline characteristics of the structure of the gut microbiome or changes in the overall diversity of gut microbiome are associated with efficacy of nivolumab in combination with carboplatin, in patients with metastatic TNBC. Additionally, to identify potentially modifiable drivers of microbiome diversity and composition, participants will undergo assessment of dietary composition, physical activity and body mass index at the same time points.

These correlative projects are made possible by collaboration with Drs. Scott Rodig, Eliezer Van Allen, Peter Sorger and Mariano Severgnini, all of whom are laboratory scientists with extensive experience with immune profiling in melanoma. Further details can be found in Section 9.

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 unresectable locally advanced or 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 Estrogen-receptor and progesterone-receptor expression both ≤ 1% by immunohistochemistry (IHC), and HER2-negative status as determined by the current ASCO/CAP guidelines. If a patient has more than one histological result, the most recent sample will be considered for inclusion.

3.1.3 Participants must have PD-L1 status available at the time of registration. Standard local testing with any PD-L1 antibody that has been validated in a CLIA-certified environment will be acceptable for including patients on trial. Primary or metastatic samples may be tested for PD-L1 status.

3.1.4 Participants must have measurable or evaluable disease by RECIST version 1.1.

3.1.5 Participants must agree to undergo a research biopsy, if tumor is safely accessible, at baseline. Previously collected archival tissue will also be obtained on all participants. For participants for whom newly-obtained samples cannot be provided (e.g. inaccessible or participant safety concern) the archival tissue alone will be acceptable. Tissue needs to be located and availability confirmed at time of registration (See Section 9 for more details). Participants must agree to a mandatory repeat biopsy 3-6 weeks after starting treatment, if tumor is safely accessible. For patients randomized to carboplatin alone who decide to crossover to nivolumab + nab-paclitaxel at time of progression, a mandatory biopsy will be required, if tumor is safely accessible, prior to initiating crossover treatment; participants must also agree to undergo this biopsy, if applicable.

3.1.6 Prior chemotherapy: Participants must have received 0 prior chemotherapeutic regimens for metastatic breast cancer. Prior platinum in the neo/adjuvant setting is permissible, if at least 6 months elapsed since the end of adjuvant systemic therapy to the development of metastatic disease. All toxicities related to prior chemotherapy must have resolved to CTCAE v4.0 grade 1 or lower, unless otherwise specified in 3.1.10.

3.1.7 Prior biologic therapy: Prior poly-ADP ribose polymerase (PARP) inhibitors are not allowed in the metastatic setting. Prior PARP inhibitors in the neo/adjuvant setting are permissible, if at least 6 months elapsed since the end of adjuvant systemic therapy to the development of metastatic disease. All toxicities related to prior biologic therapy must have resolved to CTCAE v4.0 grade 1 or lower, unless otherwise specified in 3.1.11.

3.1.8 Prior radiation therapy: Patients may have received prior radiation therapy. Radiation therapy must be completed at least 7 days prior to registration, and all toxicities related to prior radiation therapy must have resolved to CTCAE v4.0 grade 1 or lower, unless otherwise specified in 3.1.11. Patients may not have had >25% of their bone marrow radiated.

3.1.9 The subject is ≥18 years old.

3.1.10 ECOG performance status ≤1 (Karnofsky >60%, see Appendix A).

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

- Absolute neutrophil count ≥1,500/mcL
- Platelets ≥100,000/mcL
- Hemoglobin ≥ 9.0 g/dl
- Total bilirubin ≤1.5 × institutional upper limit of normal (ULN) (or ≤2.0 x ULN in patients with documented Gilbert's Syndrome)
- AST(SGOT)/ALT(SGPT) ≤2.5 × institutional ULN or ≤5 × institutional ULN for participants with documented liver metastases
- Serum creatinine ≤1.5 × institutional ULN OR creatinine clearance ≥ 45 mL/min/ 1.73m^2 for participants with creatinine levels above institutional ULN.

Note: Supportive care (e.g. transfusion of red blood cells) is allowed to meet eligibility criteria.

3.1.12 Female subjects of childbearing potential must have a negative serum or urine pregnancy test within 2 weeks prior to registration.

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 have not undergone surgical sterilization (removal of ovaries and/or uterus).

3.1.13 Women of childbearing potential (WOCBP) must agree to use an adequate method of contraception. 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. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or postovulation methods) and withdrawal are not acceptable methods of contraception.

3.1.14 Males who are sexually active with WOCBP must agree to follow instructions for method(s) of contraception for the duration of study treatment with nivolumab and 7 months after the last dose of study treatment (i.e., 90 days (duration of sperm turnover) plus the time required for the investigational drug to undergo approximately five half-lives.)

3.1.15 Participants on bisphosphonates or RANK ligand inhibitors may continue receiving therapy during study treatment.

3.1.16 The participant must be capable of understanding and complying with the protocol and willing to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.1 Concurrent administration of any other anti-cancer therapy during the course of this study (bisphosphonates and RANK ligand inhibitors are allowed).

3.2.2 Prior hypersensitivity to platinum chemotherapy or to any of the excipients of platinum or nivolumab therapy.

3.2.3 Prior therapy with an anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, or anti-Cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) antibody (including pembrolizumab, ipilimumab, and any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways).

3.2.4 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 without ongoing requirement for corticosteroids, as ascertained by clinical examination and brain imaging (magnetic resonance imaging or CT scan) completed during screening. Any corticosteroid use for brain metastases must have been discontinued without the subsequent appearance of symptoms for ≥ 7 days prior to registration. Treatment for brain metastases may include whole brain radiotherapy, radiosurgery, surgery or a combination as deemed appropriate by the treating physician. Radiation therapy must be completed at least 7 days prior to registration, as specified in Section 3.1.8.

3.2.5 Major surgery within 2 weeks prior to registration. Patients must have recovered from any effects of any major surgery.

3.2.6 Uncontrolled, significant intercurrent or recent illness including, but not limited to, ongoing or active infection, uncontrolled non-malignant systemic disease, uncontrolled seizures, or psychiatric illness/social situation that would limit compliance with study requirements in the opinion of the treating investigator.

3.2.7 Participant has a medical condition that requires chronic systemic steroid therapy (> 10 mg of prednisone daily or equivalent) or any other form of immunosuppressive medication (including disease modifying agents) and has required such therapy in the last 2 years. Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic therapy.

3.2.8 Participant has documented history of autoimmune disease or syndrome that currently requires systemic steroids or immunosuppressive agents.

3.2.9 History or evidence of active, non-infectious pneumonitis or interstitial lung disease.

3.2.10 Individuals with a history of a second malignancy are ineligible except for the following circumstances. Individuals with a history of other malignancies are eligible if they have been disease-free for at least 3 years or are deemed by the investigator to be at low risk for recurrence of that malignancy. Individuals with the following cancers that have been diagnosed and treated within the past 3 years are eligible: cervical/prostate carcinoma *in situ*, superficial bladder cancer, non-melanoma cancer of the skin. Patients with other cancers diagnosed within the past 3 years and felt to be at low risk of recurrence should be discussed with the study sponsor to determine eligibility.

3.2.11 Participant is known to be positive for the human immunodeficiency virus (HIV), HepBsAg, or HCV RNA. HIV-positive participants are ineligible because of the potential for pharmacokinetic interactions of combination antiretroviral therapy with study drugs. In addition, these participants are at increased risk of fatal infections when treated with marrow-suppressive therapy.

3.2.12 The participant has received a live vaccine within 28 days prior to registration. 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 is allowed.

3.2.13 Women who are pregnant or breastfeeding or adults of reproductive potential not employing an adequate method of contraception.

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 have not undergone surgical sterilization (removal of ovaries and/or uterus).

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 via the Office of Data Quality. 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, it is recommended that participants begin protocol therapy within 7 days. Issues that would cause treatment delays should be discussed with the Overall PI. If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

4.4 Registration Process for Other Investigative Sites

To register a participant, the following documents should be completed by the research nurse or data manager and faxed to 617-632-5152 or e-mailed to ctopm@dfci.harvard.edu to the Study Coordinator:

- Signed participant consent form
- HIPAA authorization form
- Eligibility Checklist
- Pathology reports documenting receptor status and PD-L1 status
- Baseline exam note including medical/surgical history, ECOG, vital signs
- EKG report
- Laboratory report including hematology, chemistries, PT/PTT, and pregnancy test
- Tumor Assessment report (C/A/P CT and/or MRI)
- Brain MRI report (if applicable)
- Documentation of tissue availability (per eligibility criteria 3.1.4)

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 if applicable the dose treatment level, to the participating site.

NOTE: Registration and randomization 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, randomized, phase II study of carboplatin at a dose of AUC 6 given with or without nivolumab 360 mg intravenously, every 3 weeks. One hundred and thirty-two participants will be enrolled to the study to compare the efficacy of the combination to carboplatin alone, defined by PFS, as first-line therapy in patients with metastatic TNBC.

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents of therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Regimen Description							
Agent	**Premedication; Precautions**	**Dose**	**Route**	**Schedule**	**Cycle length**	**Infusion**	**Observation period post-infusion**
Arm A							
Nivolumab (BMS-936558-01)	Not routinely necessary unless prior infusion reaction.	360 mg	IV	Every 3 weeks	21 days (3 weeks)	Over 30 min (+/- 10 min)	30 min (+/- 10 min)
Carboplatin	Dexamethasone 4 mg IV is recommended (See Section 5.3).	AUC 6	IV	Every 3 weeks	21 days (3 weeks)	Per institutional guidelines (Recommended : 30-60min).	Per institutional guidelines.
Arm B							
Carboplatin	Dexamethasone 4 mg IV is recommended (See Section 5.3).	AUC 6	IV	Every 3 weeks	21 days (3 weeks)	Per institutional guidelines (Recommended : 30-60min).	Per institutional guidelines.

5.2 Pre-Treatment Criteria

5.2.1 Cycle 1, Day 1

Participants do not need to re-meet eligibility criteria on Cycle 1, Day 1.

- Absolute neutrophil count ≥ 1,000/mcL
- Platelets ≥ 75,000/mcL
- Hemoglobin ≥ 9 g/dl
- Total bilirubin ≤ 1.5 × institutional upper limit of normal (ULN) (or ≤ 2.0 x ULN in patients with documented Gilbert's Syndrome)
- AST(SGOT)/ALT(SGPT) ≤ 2.5 × institutional ULN or ≤ 5 × institutional ULN for participants with documented liver metastases
- Creatinine ≤1.5 × institutional ULN OR creatinine clearance ≥ 45 mL/min/1.73 m^2 for participants with creatinine levels above institutional ULN.

5.2.2 Subsequent Cycles, Day 1

- Absolute neutrophil count ≥ 1,000/mcL
- Platelets ≥ 75,000/mcL
- Total bilirubin ≤ 1.5 × institutional ULN (or ≤ 2.0 x ULN in patients with documented Gilbert's Syndrome)
- AST(SGOT)/ALT(SGPT) ≤ 3.0 × institutional ULN or ≤ 5 × institutional ULN for participants with documented liver metastases
- Creatinine ≤2.0 × institutional ULN OR creatinine clearance ≥ 30 mL/min/1.73 m^2 for participants with creatinine levels above institutional ULN.

Please see Section 6.1 regarding dose delays and discontinuation.

5.3 Agent Administration

Nivolumab infusion and observation period after nivolumab should be completed prior to the administration of carboplatin when both drugs are administered on the same day of each cycle.

5.3.1 Nivolumab Administration

Nivolumab will be administered every three weeks +/- 4 days at a dose of 360 mg given intravenously over approximately 30 minutes (+/- 10 minutes) using a volumetric pump with 0.2 to 1.2 micrometer pore size, low-protein binding polyethersulfone membrane in-line filter. There will be an observation period of 30 minutes after the end of the nivolumab infusion. Dosing of nivolumab should be until progression or unacceptable toxicity. Please see section 2.5.2 for information regarding the rationale for the dose of nivolumab, and 2.5.3 for the rationale for shorter infusion time for nivolumab.

Please see section 5.7 for information on Second Course Phase: Retreatment and Crossover therapy. For patients randomized to Arm B (carboplatin alone) who elect to crossover at the time of progression to receive nab-paclitaxel plus nivolumab, nivolumab will be administered every 4 weeks at a dose of 480 mg intravenously over approximately 30 minutes (+/- 10 minutes). There will be an observation period of 30 minutes after the end of the nivolumab infusion. Nivolumab will be administered prior to nab-paclitaxel.

5.3.2 Carboplatin Administration

The dose of carboplatin in this study is AUC of 6 mg/mL*min administered by IV infusion per institutional standard practice (recommended infusion time of approximately 30 to 60 minutes). Observation period after carboplatin administration will follow institutional guidelines.

Dose will be based on the Calvert formula:

$$\text{Carboplatin dose (mg)} = \text{AUC} \times (\text{GFR} + 25) \text{ where GFR is glomerular filtration rate}$$

$$\text{Maximum carboplatin dose (mg)} = \text{target AUC (mg/mL} \bullet \text{min)} \times (150 \text{ mL/min})$$

GFR is estimated using the Cockcroft–Gault formula for creatinine clearance:

$$\frac{(140 - \text{age}) \times (\text{weight in kg}) \times (0.85 \text{ if female})}{72 \times (\text{serum creatinine in mg/dL})}$$

The total dose of carboplatin for each patient will be per institutional guidelines. Carboplatin should be administered after nivolumab.

No premedication is usually required although 4 mg of dexamethasone IV (administered 30 min prior to carboplatin) are recommended to avoid hypersensitivity reactions, at physician´s discretion. If a patient was previously pretreated with carboplatin and there has been more than a 6 month gap between courses of treatment, chlorphenamine 10mg IV + hydrocortisone 100mg IV should be given due to a possible reaction to carboplatin antibodies.

Carboplatin as well as premedication is to be administered and stored in accordance with local prescribing information and local institutional guidelines. For further details, see the carboplatin Package Insert or Summary of Product Characteristics.

NOTE: Aluminum reacts with carboplatin causing precipitate formation and loss of potency, therefore, needles or intravenous sets containing aluminum parts that may come in contact with the drug must not be used for the preparation or administration of carboplatin.

5.3.3 Nab-paclitaxel Administration

Nab-paclitaxel is administered in the Crossover phase of second course treatment (See Section 5.7).

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

Nab-paclitaxel will be administered on Days 1, 8, and 15 of every 28 day (4 week)-cycle. Nab-paclitaxel will be administered as an intravenous infusion over 30 minutes after the nivolumab infusion and observation window is complete. Filters are not required for preparation or administration of nab-paclitaxel. If filters are used as part of institutional procedure, the pore size must be ≥ 15 microns.

5.4 Definition of Dose-Limiting Toxicity (DLT)

We will perform a safety run-in analysis on the first 12 participants enrolled to the experimental arm (Arm A) of the trial. If there are 4 or more dose-limiting toxicity (DLTs) in the first 12 participants, enrollment will be halted to discuss whether the study will be amended, with re-evaluation of the appropriate dosing schedule and study design, or closed.

Additionally, we will perform a safety run-in analysis in the first 12 participants who crossover to nivolumab + nab-paclitaxel. If there are 4 or more dose-limiting toxicity (DLTs) in the first 12 participants treated with nivolumab + nab-paclitaxel, crossover will be halted to discuss whether the study will be amended, with re-evaluation of the appropriate crossover dosing schedule and design, or the option to crossover closed.

A DLT is defined as an IP-related AE(s) (attributed to nivolumab and/or the combination of nivolumab plus carboplatin) occurring during the DLT assessment period (the first 21-day cycle of concurrent carboplatin and nivolumab in Arm A; the first 28-day cycle of nivolumab + nab-paclitaxel crossover treatment), if judged by the investigator to be possibly, probably, or definitely related to study drug administration, that meets one of the following criteria:

- Any grade 5 toxicity
- Grade 3 thrombocytopenia if associated with clinically significant bleeding requiring medical intervention or
- Grade 4 thrombocytopenia of any duration
- Grade 4 neutropenia lasting ≥ 7 days
- ≥ Grade 3 febrile neutropenia
- Any other grade 4 hematologic toxicity lasting ≥ 14 days, unless deemed by the investigator to be clinically insignificant
- ≥ Grade 3 AST or ALT elevation of any duration, or ≥ Grade 3 AST or ALT elevation lasting ≥ 7 days in patients with documented liver metastases
- Grade 2 bilirubin elevation (except bilirubin > 3 x ULN in patients with documented Gilbert´s syndrome)
- Cases of Hy's Law
- Any ≥ Grade 3 non-hematologic toxicity:
  Excluding:

  - ≥ Grade 3 electrolyte abnormalities that lasts <24 to 72 hours, are not clinically complicated, and resolve spontaneously or respond to conventional medical interventions
  - ≥ Grade 3 amylase or lipase that is not associated with symptoms or clinical manifestations of pancreatitis
  - Grade 3 nausea/vomiting or diarrhea < 72 hours with adequate antiemetic and other supportive care
  - Grade 3 fatigue < 7 days
  - Alopecia of any grade
- Any Grade 2 treatment-related pneumonitis or interstitial lung disease that did not resolve with dose delay and systemic steroids to ≤ Grade 1 within 14 days.
- ≥ Grade 3 other non-laboratory toxicity lasting > 5 days despite optimal supportive care, excluding alopecia of any grade

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

5.5 Definition of Potential Drug Induced Liver Injury (DILI)

Definition of drug induced liver injury (DILI) criteria is mandatory for all pre-marketed asset protocols enrolling participants without known abnormalities in liver function at baseline AND for protocols involving participants with known liver abnormalities at baseline or with other clinical confounders where asset specific criteria for potential drug induced liver injury have been defined. All occurrences of potential DILIs, meeting the defined criteria, must be reported as SAEs.

In participants without known abnormalities in liver function at baseline, potential DILI is defined as:
1) ALT or AST elevation > 3 times upper limit of normal (ULN)
AND
2) Total bilirubin > 2 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase)
AND
3) No other immediately apparent possible causes of AST or ALT elevation and hyperbilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drug(s) known to be hepatotoxic.

In participants with known abnormalities in liver function at baseline (e.g. liver metastases), or with other clinical counfounders (e.g. Gilbert´s disease), potential DILI is defined as:
1) ALT or AST elevation > 5 times upper limit of normal (ULN)
AND
2) Total bilirubin > 3 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase)
AND
3) No other immediately apparent possible causes of increase in AST or ALT elevation and hyperbilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute

liver disease, or the administration of other drug(s) known to be hepatotoxic.

5.6 General Concomitant Medication and Supportive Care Guidelines

5.6.1 Concomitant Medication Guidelines

Medications or vaccinations specifically prohibited in the exclusion criteria (3.2.12) 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 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 prior treatment or medication administered during the 28 days preceding the first dose of trial treatment and any concomitant therapy administered to the subject throughout the study until 30 days after the last dose of trial treatment, including medication name and indication, will be captured.

Prohibited Concomitant Medications
Subjects are prohibited from receiving the following therapies during the Screening (14 days prior to trial registration) and Treatment Phase(s) of this trial:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Investigational agents other than nivolumab
- Radiation therapy (7 days prior to screening and during treatment)
- 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 vaccine is allowed.

- Systemic glucocorticoids should be avoided for any purpose other than to modulate symptoms from radiation, prevent hypersensitivity reactions 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.

For eligible patients who elect to crossover to nab-paclitaxel plus nivolumab, care should be taken with concomitant use of strong CYP3A4 inhibitors/inducers (e.g., ketoconazole and itraconazole) and nab-paclitaxel. An alternate medication with no or minimal potential to inhibit CYP3A4 should be considered. If a strong CYP3A4 inhibitor is co-administered with nab-paclitaxel, participants should be closely monitored for adverse reactions.

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

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

5.6.2 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:
    - Recommended prophylactic anti-emetic therapy for carboplatin include:
        - -Aprepitant 125 mg oral (1 capsule on the same day, to be administered approximately 60 minutes prior to carboplatin)
        - -Aprepitant 80 mg oral (1 capsule daily for 2 days, starting 24 hours after the administration of carboplatin)
        - -Palonosetron 0.25 mg IV (1 dose to be administered 30 minutes prior to carboplatin)
        - -Lorazepam 0.5-1.0 mg oral (every 4-6 hours if needed for anticipatory nausea or vomiting, or anxiety)
        - -Dexamethasone: Corticosteroids should be avoided unless deemed necessary to control refractory nausea or vomiting. If needed, administer 4 mg oral (at the start of treatment) or 4mg IV (dose to be administered 30 minutes prior to carboplatin)
- Hematopoietic growth factors (e.g., G-CSF, granulocyte macrophage colony stimulating factor) may be used at investigator's discretion for the primary prophylaxis and/or management of treatment-emergent neutropenia and/or for secondary prophylaxis as per NCCN/European Society for Medical Oncology guidelines or local standard practice.
- 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:

No formal pharmacokinetic drug-drug interaction studies have been conducted with nivolumab. The renal effects of nephrotoxic compounds (e.g., aminoglycoside antibiotics, diuretics) may be potentiated by carboplatin. Drugs that increase the risk of agranulocytosis (e.g., clozapine) should be avoided.

The following significant drug interactions have been reported with carboplatin:

- Warfarin/cumarin anticoagulants: (unknown mechanism) Avoid if possible as use often causes an elevation or fluctuation in the INR – in the first instance, consider switching to a low molecular weight heparin during treatment or, if the patient continues taking an oral anticoagulant, monitor the INR at least once a week and adjust dose accordingly.
- Clozapine: (additive) increased risk of agranulocytosis, avoid concomitant use
- Phenytoin: reduced absorption of the antiepileptic
- Diuretics, aminoglycoside antibiotics: (additive) increased risk of nephrotoxicity and ototoxicity

For eligible patients who elect to crossover to nab-paclitaxel plus nivolumab, caution should be exercised when administering nab-paclitaxel concomitantly with medicines known to inhibit or induce either CYP2C8 or CYP3A4.

5.7 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 until one of the following criteria applies:

1. Disease progression by RECIST 1.1 criteria:

A. Please note that, although the primary endpoint is PFS as defined by RECIST 1.1, patients may remain on protocol therapy until the time of disease progression by irRC criteria. The immune criteria allow treatment beyond initial radiographic worsening of disease in order to distinguish between pseudoprogression and true disease progression.

B. 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.

2. Intercurrent illness that prevents further administration of treatment.

3. Unacceptable adverse event(s).

4. Participant demonstrates an inability or unwillingness to comply with the medication regimen and/or documentation requirements.

5. Participant decides to withdraw from the protocol therapy.

6. 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, [redacted]

Second Course Phase:
A) Retreatment Phase

A1. Participants randomized to the experimental arm (Arm A) may elect to stop nivolumab and/or carboplatin 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 carboplatin after attaining an investigator-assessed CR according to RECIST 1.1, confirmed in at least two consecutive restaging scans, 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-assessed confirmed radiographic disease progression after stopping their initial treatment with nivolumab

- Did not receive any anti-cancer treatment other than carboplatin since the last dose of nivolumab

The retreatment section of the eligibility checklist should be completed and signed by the treating

investigator. DF/HCC sites will re-fax the checklist to ODQ for processing. 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 checklist 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 on the initial treatment phase of the trial.

A2. Eligible participants who receive nab-paclitaxel and nivolumab during the Crossover phase (please see below) may elect to stop nivolumab and/or nab-paclitaxel with confirmed CR after at least 24 weeks of treatment. Subjects in the Crossover phase 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 nab-paclitaxel after attaining an investigator-assessed CR according to RECIST 1.1, confirmed in at least two consecutive restaging scans, 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-assessed confirmed radiographic disease progression after stopping their initial treatment with nivolumab

- Did not receive any anti-cancer treatment other than nab-paclitaxel since the last dose of nivolumab

The retreatment section of the eligibility checklist should be completed and signed by the treating investigator. DF/HCC sites will re-fax the checklist to ODQ for processing. 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 checklist 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 on the crossover treatment phase of the trial.

<u>B) Standard arm: Crossover Phase</u>
If treatment is permanently discontinued due to disease progression (i.e. not due to toxicity) in a participant randomized to the standard arm of carboplatin alone, the patient will be offered the option to crossover to receive nivolumab at a dose of 480 mg intravenously every 4 weeks in

combination with nab-paclitaxel (Days 1, 8, 15 of every 28-day cycle) at a dose of 100 mg/m2, until documented disease progression per RECIST 1.1 criteria or unacceptable toxicity. This phase is considered part of the protocol therapy.

Regimen Description							
Agent	**Premedication ; Precautions**	**Dose**	**Route**	**Schedule**	**Cycle length**	**Infusion**	**Observation period post-infusion**
Crossover							
Nivolumab (BMS-936558-01)	Not routinely necessary unless prior infusion reaction.	480 mg	IV	Every 4 weeks	28 days (4 weeks)	Over 30 min (+/- 10 min)	30 min (+/- 10 min)
nab-Paclitaxel	Not routinely necessary unless prior infusion reaction.	100 mg/m2 in NS*	IV	Day 1, 8, 15	28 days (4 weeks)	30 minutes (+/- 10 min) Start nab-paclitaxel after nivolumab infusion + observation window completed	Per institutional guidelines.
*Dose reductions may be made per Section 6.1. Further details about dose reductions can be found in Sections 6.4 **Limiting the infusion of nab-paclitaxel to 30 minutes, as directed, decreases the likelihood of infusion-related reactions.							

Screening does not need to be repeated, however, patients must meet the following criteria to treat Day 1 of Crossover Therapy, and eligibility must be confirmed by the treating investigator:

- ECOG performance status ≤ 2 (or Karnofsky ≥ 50%)
- Absolute neutrophil count ≥ 1,000/mcL
- Platelets ≥ 75,000/mcL
- Peripheral neuropathy ≤ Grade 2
- 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) ≤ 3.0 × institutional ULN or ≤ 5 × institutional ULN for participants with documented liver metastases
- Negative pregnancy test within 7 days of the first dose of nivolumab
- No clinically significant ECG abnormalities
- No history or evidence of active, noninfectious pneumonitis that required treatment for steroids
- Willing to undergo a mandatory research biopsy prior to initiation of nivolumab, if tumor is safely accessible for biopsy

Documents to support the above criteria, including the crossover section of the eligibility

checklist, should be completed and signed by the treating investigator. DF/HCC sites will re-fax the checklist to ODQ for processing. Non-DF/HCC 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 Crossover Therapy, the Coordinating Center will process the checklist and the participating site will be notified that the patient may proceed to Crossover Therapy. Please see the Crossover Study Calendar in Section 10 for guidance on visit requirements.

5.8 Duration of Follow-Up

Participants will be followed on-study until death.

Participants removed from protocol therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event. An additional adverse event assessment will be performed 100 days (-15/+30 days) after the last dose of treatment for participants who receive nivolumab. This may be conducted by phone.

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. Tumor assessments should continue to be performed every 6-12 weeks in these participants until first disease progression event or death, whichever occurs first. Research blood draws are optional at the time of tumor assessments.

In follow-up, all participants, including those who came off protocol therapy due to progression, will be contacted every 6 months for survival and progression. Follow up visits start 6 months from the end of treatment visit.

5.9 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). In addition, the study team will ensure Off Treatment/Off Study information is updated in OnCore in accordance with DF/HCC policy REGIST-101.

6. DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made as indicated in the following table(s). The descriptions and grading scales found in the revised NCI Common Terminology Criteria for

Adverse Events (CTCAE) version 4.0 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website:
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

6.1 Dosing Delays/Omission/Modifications

Dosing interruptions are permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Participants held for these reasons require prior approval from the PI and should resume therapy within 6 weeks of the scheduled interruption. The reason for interruption should be documented in the participant's study record.

In the absence of an unacceptable therapy-related toxicity and/or clinical signs of disease progression, subjects may continue treatment at the discretion of the investigator. Subjects must be instructed to notify their physician immediately for any and all toxicities.

Guidelines for the management of AEs (ie, dose interruptions and dose reductions) are presented in the next sections. For management of AEs which can be clearly attributed to chemotherapy (i.e. carboplatin, nab-paclitaxel) or nivolumab, independent dose modification for either agent is allowed. For AEs without clear attribution to either study treatment, management of toxicity should include dose modifications of both agents.

Each dose reduction of carboplatin should be to one dose level lower than the current dose (see Table below). Dose reductions of more than one dose level are acceptable if agreed to by the Investigator. Once a dose level reduction has been made, dose re-escalation of carboplatin is not allowed in subsequent treatment cycles. In subjects unable to tolerate carboplatin at a dose of AUC 4 mg/ml*min, carboplatin should be discontinued permanently.

Starting Dose of Carboplatin	AUC 6 mg/mL*min
First Dose Level Reduction	AUC 5 mg/mL*min
Second Dose Level Reduction	AUC 4 mg/mL*min

In the Crossover phase, each dose reduction of nab-paclitaxel should be to one dose level lower than the current dose (see Table below). Dose reductions of more than one dose level are acceptable if agreed to by the Investigator. Once a dose level reduction has been made, dose re-escalation of nab-paclitaxel is not allowed in subsequent treatment cycles. In subjects unable to tolerate nab-paclitaxel at a dose of 50 mg/m^2, nab-paclitaxel should be discontinued permanently.

Starting Dose of nab-paclitaxel	100 mg/m^2
First Dose Level Reduction	75 mg/m^2
Second Dose Level Reduction	50 mg/m^2

There are no dose reductions for nivolumab.

Nivolumab should be held (delayed) or omitted for toxicities that are considered possibly, probably or definitely related to nivolumab. Carboplatin should be held (delayed) or omitted for toxicities that are considered possibly, probably or definitely related to carboplatin. Nab-paclitaxel should be held (delayed) or omitted for toxicities that are considered possibly, probably or definitely related to nab-paclitaxel.

- If a subject receiving combination therapy (Arm A: carboplatin plus nivolumab; Arm B Crossover phase: nab-paclitaxel plus nivolumab) does not meet criteria for treatment (Section 5.2) due to a toxicity that is considered attributable to only one of the drugs of the combination (e.g., platelets 60,000/mm^3 attributed to carboplatin), the drug causing the toxicity should be held, and the second drug may be administered (unless specified below in the corresponding AE table of the second drug).

- There must be a minimum of 21 days ± 4 days between administration of each dose of carboplatin and, for patients in Arm A, between administration of each dose of nivolumab. For patients in Arm B who elect to crossover to nab-paclitaxel plus nivolumab, there must be a minimum of 7 days ± 1 day between administration of each dose of nab-paclitaxel and a minimum of 28 days ± 4 days between administration of each dose of nivolumab during the Crossover phase.

- If treatment with chemotherapy (i.e. carboplatin or nab-paclitaxel) is held or omitted due to an AE and the patient cannot be retreated with chemotherapy within 6 weeks from the last dose, carboplatin or nab-paclitaxel should be permanently discontinued. Nivolumab can be continued per protocol (if the AE is unrelated).

- If treatment with nivolumab is held or omitted due to an AE and the patient cannot be retreated with nivolumab within 9 weeks from the last dose, nivolumab should be permanently discontinued. Carboplatin or nab-paclitaxel can be continued per protocol (if the AE is unrelated).

Recommendations for treating the expected toxicities of nivolumab are provided below. Consultation with a medical or surgical specialist, especially prior to an invasive diagnostic or therapeutic procedure, is recommended.

Doses of nivolumab **should be omitted (not delayed)** for the following treatment-related toxicities:

- Grade 3 skin, drug-related adverse event
- Grade 2 pneumonitis
- Grade 2 and 3 serum creatinine increase (>1.5 – 6.0 x ULN)
- Grade 2 or 3 neurologic signs or symptoms
- Elevated AST or ALT (>3.0 – 5.0 x ULN; except in patients with documented liver metastases at baseline in which case AST or ALT > 5.0 – 8.0 x ULN)

- Elevated total bilirubin (>1.5 – 3.0 x ULN; except in patients with documented Gilbert´s syndrome in which case total bilirubin > 2.0 – 3.0 x ULN)
- Grade 2 diarrhea
- Grade 2 or 3 hypophysitis
- Grade 2 adrenal insufficiency
- Any other Grade 3 adverse event (unless otherwise specified in the tables below), with the exception of electrolyte imbalances/abnormalities considered correctable within 72 hours:
    - First occurrence: omit dose
    - Recurrence of same Grade 3 AE: discontinue nivolumab permanently

After omitting a dose, in order to resume treatment with nivolumab, AE must have resolved to grade ≤ 1 or baseline value (unless otherwise specified in the tables below), with the following exceptions:

- Subjects may resume treatment in the presence of grade 2 fatigue
- Drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment

Nivolumab treatment should be **permanently discontinued** for the following treatment-related toxicities:

- Any Grade 2 drug-related uveitis, eye pain or blurred vision that does not respond to topical therapy and does not improve to grade 1 severity within re-treatment period OR requires systemic treatment
- Grade 4 serum creatinine increase (> 6.0 x ULN)
- Grade 3 or 4 diarrhea or colitis (except if a patient has experienced a first occurrence of G3 diarrhea that has resolved within 14 days and the daily dose of prednisone is ≤ 10 mg/day)
- Grade 3 or 4 pneumonitis
- Elevated AST or ALT (> 5.0 x ULN; > 8.0 x ULN for patients with documented liver metastases at baseline)
- Grade 3 or 4 elevated total bilirubin (> 3.0 x ULN)
- Grade 3 or 4 hypophysitis
- Grade 3 or 4 adrenal insufficiency
- Grade 4 hyperglycemia (related to study drug)
- Grade 4 skin-related AE

- Any Grade symptomatic elevation of amylase and/or lipase
- Immune-mediated encephalitis
- Grade 3 or 4 nivolumab infusion-related reaction
- Recurrent Grade 3 event (unless otherwise specified in tables below)
- Any severe or Grade 3 immune-mediated adverse reaction that recurs on reintroduction of nivolumab, or an inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks
- Any Grade 4 drug-related adverse event or laboratory abnormality, except for the following events which do not require discontinuation:
    - Asymptomatic Grade 4 hematologic adverse events considered unrelated to nivolumab
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the Investigator, presents a substantial clinical risk to the subject with continued nivolumab dosing.

6.2 Management of Toxicities Attributable to Nivolumab

Immunosuppressive agents and the use of systemic corticosteroids are permitted in the context of treating adverse events. Subjects receiving corticosteroids for treatment of drug-related adverse events must be at $\leq$ 10 mg/day prednisone or equivalent prior to re-initiation of study therapy.

6.2.1 Gastrointestinal Disorders: Diarrhea/Colitis

Subjects should be instructed to notify their physician immediately at the first signs of poorly formed or loose stool or an increased frequency of bowel movements as nivolumab can be associated with severe diarrhea/colitis. General supportive measures should be implemented including continuous oral hydration, correction of fluid and electrolyte abnormalities, small frequent meals, and stopping lactose-containing products, high fat meals, and alcohol.

Causes of gastrointestinal AE that are unrelated to study treatment (either carboplatin or nivolumab) should be ruled out. Participants experiencing intolerable grade 2 or grade 3 diarrhea unable to be managed with standard antidiarrheal treatments should consult a gastrointestinal (GI) doctor for potential endoscopy and biopsy to help confirm immune-mediated toxicity. If a cause that is unrelated to study treatment (either carboplatin/nab-paclitaxel or nivolumab) is identified, treat accordingly and continue nivolumab therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.

Treatment may be restarted following the resolution of colitis. In addition, if the patient is being managed with corticosteroids, treatment should not be restarted until the steroids have been tapered down to a prednisone dose $\leq$ 10 mg/day. Patients with grade 3 or 4 diarrhea should discontinue nivolumab with the following exception: if a patient has experienced a first occurrence of G3 diarrhea that has resolved within 14 days and the daily dose of prednisone is

currently ≤ 10 mg/day, nivolumab can be resumed per protocol. Patients who resume treatment should be monitored closely for signs of renewed diarrhea.

Table 4: Diarrhea and Colitis Management Algorithm

Grade of Diarrhea/Colitis (NCI-CTCAE v4)	**Management**	**Follow-up**
Grade 1	• Continue nivolumab per protocol • Symptomatic treatment: start anti-diarrheal agent (e.g., Imodium®) – up to 3 agents are permitted	• Close monitoring for worsening symptoms • Educate subjects to report worsening immediately If worsening: • Treat as Grade 2 or 3/4
Grade 2	• Omit nivolumab per protocol • Symptomatic treatment: start anti-diarrheal agent (e.g., Imodium®) – up to 3 agents are permitted	If improved to Grade 1: • Resume nivolumab per protocol If persists > 5-7 days or recurs: • 0.5-1.0 mg/kg/day methylprenisolone or oral equivalent • Consider GI consult and lower endoscopy • When symptoms improve to Grade 1, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume nivolumab when corticosteroids have been reduced to the equivalent of prednisone ≤ 10 mg/day. If worsens or persists > 5 days with oral steroids: • Treat as Grade 3 or 4
Grade 3-4	• Discontinue nivolumab per protocol* • 1.0-2.0 mg/kg/day methylprednisolone IV or equivalent • Add prophylactic antibiotics for opportunistic infections • Consider GI consult and lower endoscopy	If improves: • Continue steroids until Grade 1, then taper over at least 1 month If persists > 3-5 days or recurs after improvement: • Add infliximab 5 mg/kg (if no contraindication) Note: infliximab should not be used in cases of perforation or sepsis

ADL = activities of daily living; h = hour(s); IV = intravenous; NCI-CTCAE v4 = National Cancer Institute Common Terminology Criteria version 4.

Note: Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, 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.
*If a patient has experienced a first occurrence of G3 diarrhea that has resolved within 14 days and the daily dose of prednisone is currently ≤ 10 mg/day, nivolumab can be resumed per protocol

6.2.2 Pulmonary Adverse Event Management

Dyspnea, cough, fatigue, hypoxia, pneumonitis, and pulmonary infiltrates have been associated with the administration of nivolumab and have primarily been observed in patients with underlying NSCLC.

Mild-to-moderate events of pneumonitis have been reported with nivolumab. All pulmonary events should be thoroughly evaluated for other commonly reported etiologies such as pneumonia/infection, lymphangitic carcinomatosis, pulmonary embolism, heart failure, chronic obstructive pulmonary disease (COPD), or pulmonary hypertension and the following should be performed:

- Measurement of oxygen saturation (i.e., arterial blood gas)
- High-resolution CT scan of the chest
- Bronchoscopy with bronchoalveolar lavage and biopsy
- Pulmonary function tests (with diffusion capacity of the lung for carbon monoxide [DL_{CO}])

Patients will be assessed for pulmonary signs and symptoms throughout the study. Patients will also have CT scans of the chest at every tumor assessment (see Section 11). If the cause of a pulmonary AE is considered to be unrelated to study treatment, treat accordingly and continue nivolumab therapy. Evaluate with imaging and pulmonary consultation.

Table 5. Management of Pulmonary Adverse Event

Grade of Pneumonitis (NCI-CTCAE v4)	**Management**	**Follow-up**
Grade 1	• Dose of nivolumab may be held for further evaluation per investigator discretion • Monitor for symptoms every 2-3 days • Consider Pulmonary and ID consults	• Re-image at least every 3 weeks If worsening: • Treat as Grade 2 or 3/4
Grade 2	• Omit dose of nivolumab per protocol • Pulmonary and ID consults • Monitor symptoms daily, consider hospitalization	• Re-image at least every 1-3 days If improves: • When symptoms return to near baseline, taper steroids over at least 1 month and then resume nivolumab

	• 1.0 to 2.0 mg/kg/day methylprednisolone IV or oral equivalent • Consider bronchoscopy, lung biopsy	per protocol and consider prophylactic antibiotics If not improving after 2 weeks or worsening: • Treat as Grade 3/4 (discontinue nivolumab unless an alternative etiology of findings is identified)
Grade 3-4	• Discontinue nivolumab per protocol • Hospitalize • Pulmonary and ID consults • 2.0-4.0 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 (e.g. infliximab, cyclophosphamide, IVIG or mycophenolate mofetil)

ID = infectious disease; IV = intravenous; IVIG = intravenous immunoglobulin; NCI-CTCAE v4 = National Cancer Institute Common Terminology Criteria version 4; ULN = upper limit of normal.

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

6.2.3 Hepatobiliary Disorders

Elevations of ALT, AST, and bilirubin have been observed during treatment with nivolumab. It is recommended that participants with elevation of ALT, AST, and/or bilirubin have more frequent laboratory monitoring of these parameters. If possible, hepatotoxic concomitant medications should be discontinued in participants who develop increased values of ALT, AST, or bilirubin. Causes of hepatobiliary disorders that are unrelated to study treatment (either carboplatin or nivolumab) should be ruled out. If a cause that is unrelated to treatment is identified, treat accordingly and continue nivolumab therapy. Consider imaging to rule out obstruction.

Table 6. Hepatic Adverse Event Management Algorithm

Grade of Elevated AST, ALT or Bilirubin (NCI-CTCAE v4)	**Management**	**Follow-up**
Grade 1	• Continue nivolumab per protocol	• Continue LFT monitoring protocol every 7-10 days for at least 4 weeks [a] If worsening: • Treat as Grade 2 or 3/4

Grade 2	• Omit nivolumab per protocol with the following exceptions: a) Documented liver metastases and baseline AST or ALT > 3.0 to ≤ 5.0 x ULN b) Documented Gilbert´s syndrome and total bilirubin > 1.5 to ≤ 2.0 x ULN • Increase frequency of LFT monitoring to every 3-5 days [a] • Consider referral to a hepatologist	If returns to baseline: • Resume routine monitoring, resume nivolumab per protocol If elevations persist > 5-7 days or worsen: • 0.5-1.0 mg/kg/day 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 nivolumab per protocol
Grade 3-4	• Discontinue nivolumab [b] • Increase frequency of monitoring to every 1-2 days • Consider referral to hepatologist and liver biopsy to establish etiology of hepatic injury • 1.0-2.0 mg/kg/day methylprednisolone IV or IV equivalent [c] • Add prophylactic antibiotics for opportunistic infections	If returns to Grade 2: • Taper steroids over at least 1 month If does not improve in > 3-5 days, or worsens or rebounds: • Add mycophenolate mofetil 1g BID • If no response within an additional 3-5 days, consider other immunosuppressants per local guidelines

ALT = alanine aminotransferase; AST = aspartate aminotransferase; IV = intravenous; LFT = liver function test; NCI-CTCAE v4 = National Cancer Institute Common Terminology Criteria version 4; ULN = upper limit of normal.

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

[a] LFT monitoring does not to be increased in patients with documented liver metastases and baseline AST or ALT > 3.0 to ≤ 5.0 x ULN or patients with documented Gilbert´s syndrome and total bilirubin > 1.5 to ≤ 2.0 x ULN.
[b] Nivolumab therapy may be omitted rather than permanently discontinued if AST/ALT > 5.0 to ≤ 8.0 x ULN and patient has documented liver metastases at baseline.
[c] The recommended starting dose for Grade 4 hepatitis is 2 mg/kg/day methylprednisolone IV.

6.2.4 Elevated Amylase and/or Lipase

Patients may develop symptomatic and/or radiographic evidence of pancreatitis or pancreatic dysfunction. Amylase and lipase should be checked if there is clinical suspicion for pancreatitis. Causes of pancreatitis that are unrelated to study treatment (e.g. gallstones, alcohol consumption, infections) should be ruled out. If a cause that is unrelated to treatment is identified, treat

accordingly and continue nivolumab therapy. Corticosteroids do not seem to alter the natural history of lipase/amylase elevations. Patients with symptomatic or radiographic pancreatitis suspected to be nivolumab-related should discontinue nivolumab and consider consulting a gastroenterologist for management.

Table 7. Management of Elevated Amylase and/or Lipase

Grade of Elevated Amylase and/ or Lipase (NCI-CTCAE v4)	Management	Follow-up
Any grade	• Patients with symptomatic (e.g. abdominal pain, hyperglycemia) or radiographic pancreatitis considered to be nivolumab-related should discontinue nivolumab • Consider GI consultation	• Elevations should be monitored approximately every 7-10 days until ≤ Grade 1

NCI-CTCAE v4 = National Cancer Institute Common Terminology Criteria version 4.

6.2.5 Endocrinopathy Management

Changes in thyroid function tests (TFTs) and hypothyroidism have been reported with nivolumab. Non-inflammatory causes should be ruled out. If a non-inflammatory cause is identified, treat accordingly and continue nivolumab therapy. Management of thyroid dysfunction (e.g., symptomatic hypothyroidism) should follow accepted clinical practice guidelines. Nivolumab does not need to be held for asymptomatic participants. Visual field testing, endocrinology consultation, and imaging should be considered.

Note: all patients with symptomatic pituitary enlargement, regardless of hormone deficiency, including severe headache or enlarged pituitary on MRI or aseptic meningitis or encephalitis, should be considered grade 3 events. Isolated thyroid or testosterone deficiency may be treated as grade 2 if there are no other associated deficiencies and adrenal function is monitored.

Table 8. Endocrinopathy Management Algorithm

Grade of Endocrinopathy (NCI-CTCAE v4)	Management	Follow-up
Grade 1	• Continue nivolumab per protocol • If TSH < 0.5 x LLN, or TSH > 2.0 x ULN, or consistently out of range in 2 subsequent measurements: include fT4 at subsequent cycles as clinically indicated. • Consider endocrinology consult.	-
Grade 2 [a]	• Evaluate endocrine function • Consider pituitary scan If symptomatic with abnormal lab and/or pituitary scan: [b]	If improves with or without hormone replacement: • Taper steroids over at least 1 month and consider prophylactic antibiotics

	• Delay/omit nivolumab per protocol [c] • 1-2 mg/kg/day methylprednisolone IV or PO equivalent • Initiate appropriate hormone therapy No abnormal lab/pituitary MRI scan but symptoms persist: • Repeat labs in 1-3 weeks / MRI in 1 month	for opportunistic infections and resume nivolumab per protocol • Subjects with adrenal insufficiency may need to continue steroids with mineralocorticoid component
Grade 3 **Includes suspicion of adrenal crisis (e.g. severe dehydration, hypotension, shock out proportion to current illness)**	• Omit nivolumab per protocol • Consult endocrinologist • 1-2 mg/kg/day methylprednisolone IV or IV equivalent If suspicion of adrenal crisis: • Rule out sepsis • Stress dose of IV steroids with mineralocorticoid activity • IV fluids If Grade 3 hypophysitis or adrenal insufficiency is confirmed: • Discontinue nivolumab permanently	If improves with or without hormone replacement to ≤ Grade 1: • Taper steroids over at least 1 month and consider prophylactic antibiotics for opportunistic infections and resume nivolumab per protocol
Grade 4	• Discontinue nivolumab per protocol • Consult endocrinologist • 1-2 mg/kg/day methylprednisolone IV or IV equivalent If suspicion of adrenal crisis: • Rule out sepsis • Stress dose of IV steroids with mineralocorticoid activity • IV fluids	If improves with or without hormone replacement to ≤ Grade 1: • Taper steroids over at least 1 month and consider prophylactic antibiotics for opportunistic infections

TSH = thyroid stimulating hormone; fT4 = free thyroxine; IV = intravenous; PO = oral administration; ULN = upper limit of normal; LLN = lower limits of normal; MRI = magnetic resonance imaging; NCI-CTCAE v4 = National Cancer Institute Common Terminology Criteria version 4

Note: Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, 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] Excludes Grade 2 hypo- or hyperthyroidism. These patients should be treated with hormone replacement therapy; no dose modifications of nivolumab unless worsening to Grade 3 or 4.
[b] Patients must be evaluated to rule out pituitary disease prior to initiating thyroid replacement and steroids, including baseline serum: cortisol, ACTH, TSH and free T4.

[c] Nivolumab therapy should be omitted, rather than delayed, if Grade 2 hypophysitis or adrenal insufficiency.

6.2.6 Dermatologic Adverse Event Management

Treatment-emergent rash has been associated with nivolumab. The majority of cases of rash were mild in severity and self-limited, with or without pruritus. A dermatologist should evaluate persistent and/or severe rash or pruritus. Causes of dermatologic AE that are unrelated to study treatment (either carboplatin or nivolumab) should be ruled out. A biopsy should be performed unless contraindicated.

If a cause that is unrelated to treatment is identified, treat accordingly and continue nivolumab therapy. Low-grade immune-related rash and pruritus have been treated with symptomatic therapy (e.g., antihistamines). Pruritus may occur with or without skin rash and should be treated symptomatically. Topical or parenteral corticosteroids may be required for more severe symptoms. Skin rash typically occurs early and may be followed by additional events particularly during steroid taper.

Table 9: Dermatologic Adverse Event Management Algorithm

Grade of Dermatologic Adverse Event (NCI-CTCAE v4)	Management	Follow-up
Grade 1-2	• Continue nivolumab per protocol • Symptomatic therapy (e.g. antihistamines, topical steroids)	If persists > 1-2 weeks or recurs: • Consider skin biopsy • Interrupt nivolumab therapy per protocol • Consider 0.5-1.0 mg/kg/day methylprednisone IV or oral equivalent. Once improving, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume nivolumab therapy per protocol If worsens: • Treat as Grade 3-4
Grade 3-4	• Omit (if grade 3) or discontinue (if grade 4) nivolumab therapy per protocol • Consider skin biopsy • Dermatology consult • 1.0-2.0 mg/kg/day IV methylprednisone IV or IV equivalent	If improves to grade 1: • Taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections • Resume nivolumab per protocol

BSA = body surface area; IV = intravenous. NCI-CTCAE v4 = National Cancer Institute Common Terminology Criteria version 4

Note: Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, 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.

6.2.7 Renal Adverse Event Management

Causes of renal AE that are unrelated to study treatment (either carboplatin or nivolumab) should be ruled out. If a cause that is unrelated to treatment is identified, treat accordingly and continue nivolumab therapy.

Table 10. Renal Adverse Event Management Algorithm

Grade of Creatinine Elevation (NCI-CTCAE v4)	Management	Follow-up
Grade 1	• Continue nivolumab per protocol	• Routine creatinine monitoring per protocol
Grade 2-3	• Omit nivolumab per protocol • Monitor creatinine weekly • Consider nephrologist consult and renal biopsy • 0.5-1.0 mg/kg/day prednisone IV or PO equivalent if considered nivolumab-related	If improves to ≤ Grade 1: • Taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume nivolumab therapy per protocol and routine creatinine monitoring per protocol If elevations persist > 7 days or worsen: • Treat as Grade 4
Grade 4	• Discontinue nivolumab • Monitor creatinine daily • Consult nephrologist and consider renal biopsy • 1.0-2.0 mg/kg/day methylprednisolone IV or IV equivalent if considered nivolumab-related	If improves to ≤ Grade 1: • Taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections

ADL = activities of daily living; IV = intravenous; PO = oral administration; NCI-CTCAE v4 = National Cancer Institute Common Terminology Criteria version 4; ULN = upper limit of normal.

Note: Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, 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.

6.2.8 Hematologic Adverse Event Management

Hematologic AE (e.g., anemia, neutropenia, febrile neutropenia, thrombocytopenia) related to nivolumab are rare. Non-inflammatory causes of hematologic AE should be ruled out, particularly related to carboplatin. If a non-inflammatory cause is identified, treat accordingly and continue nivolumab therapy unless otherwise specified in the table below. Consider peripheral blood smear and hematology consultation.

Table 11. Hematologic Adverse Event Management Algorithm

Grade of Hematologic Adverse Event (NCI-CTCAE v4)	**Management**	**Follow-up**
Grade ≤ 3	• Continue nivolumab per protocol • Supportive care per local guidelines <u>If Grade 3 hematologic AE recurr despite two dose level reductions of carboplatin:</u> • Consider hematology consult, antibody panel and bone marrow biopsy [a] • 0.5-1.0 mg/kg/day prednisone IV or PO equivalent if considered nivolumab-related	If improves to ≤ Grade 2: • Taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections
Grade 4	• Delay/omit nivolumab until resolves to ≤ Grade 2 • Supportive care per local guidelines <u>If Grade 4 hematologic AE recurr despite two dose level reductions of carboplatin:</u> • Consider hematology consult, antibody panel and bone marrow biopsy [a] • 1.0-2.0 mg/kg/day prednisone IV or PO equivalent if considered nivolumab-related and permanently discontinue nivolumab.	If improves to ≤ Grade 2: • Taper steroids over at least 1 month, resume nivolumab per protocol and add prophylactic antibiotics for opportunistic infections If symptomatic or Grade 4 persists despite steroids > 14 days: • Consider IVIG or other immunosuppressive therapies per local guidelines • Discontinue nivolumab if considered nivolumab-related
Febrile neutropenia	• Hold nivolumab until resolved (ANC ≥ 1000/mm^3, fever < 38ºC) • Supportive care per local guidelines	

NCI-CTCAE v4 = National Cancer Institute Common Terminology Criteria version 4; PO = oral administration; IV = intravenous; IVIG = intravenous immunoglobulin.

Note: Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, 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 immune-mediated hemolytic anemia or thrombocytopenia are suspected, Coombs test, anti-platelet antibodies, peripheral blood smear and/or bone marrow aspiration or biopsy should be considered.

6.2.9 Neurologic Adverse Event Management

Causes of neurologic AE that are unrelated to treatment (either carboplatin or nivolumab) should be ruled out. If a cause that is unrelated to treatment is identified, treat accordingly and continue nivolumab therapy. Consider visual field testing, endocrinology consultation, and imaging.

Patients that experience nivolumab-related aseptic meningitis, encephalitis, myopathy, peripheral demyelinating neuropathy, cranial neuropathy (other than peripheral n. VII), Guillain-Barre syndrome, and myasthenia gravis should discontinue nivolumab therapy.

Table 12. Neurologic Adverse Event Management Algorithm

Grade of Neurologic Adverse Event (NCI-CTCAE v4)	Management	Follow-up
Grade 1	• Continue nivolumab per protocol	• Continue to monitor the patient If worsening: • Treat as Grade 3/4
Grade 2-3	• Omit nivolumab per protocol • Treat symptoms per local guidelines • Consider 0.5-1.0 mg/kg/day prednisone IV or PO equivalent if considered nivolumab-related	If improves to baseline: • Taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections and resume nivolumab therapy per protocol
Grade 4	• Discontinue nivolumab • Obtain neurology consult • Treat symptoms per local guidelines • 1.0-2.0 mg/kg/day methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections	If improves to Grade 2: • Taper steroids over at least 1 month If worsens or atypical presentation: • Consider IVIG or other immunosuppressive therapies per local guidelines

ADL= activities of daily living; NCI-CTCAE v4 = National Cancer Institute Common Terminology Criteria version 4; PO = oral administration; IV = intravenous; IVIG = intravenous immunoglobulin;

Note: Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, 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.

6.2.10 Nivolumab Infusion-Related Reactions

Nivolumab contains only human immunoglobulin protein sequences and, therefore, it is unlikely to be immunogenic and induce infusion or hypersensitivity reactions. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash, pruritis, arthralgias, hypo- or hypertension, bronchospasm, or other symptoms. All Grade 3 or 4 infusion reactions should be reported within 24 hours to the study PI and reported as a SAE if criteria are met. Infusion reactions should be graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE version 4.0) guidelines. Treatment recommendations are provided below and may be modified based on local treatment standards and guidelines as appropriate:

Table 13: Management of Nivolumab Infusion-Related Reactions

Grade of Nivolumab Infusion-Related Reaction (NCI-CTCAE v4)	Management	Follow-up
Grade 1	• Continue nivolumab per protocol. Remain at beside.	• Institute prophylactic medications with subsequent infusions
Grade 2	• Hold nivolumab until resolution to ≤ Grade 1	• Refer to management and rechallenging instructions below
Grade 3-4	• Discontinue nivolumab therapy	

NCI-CTCAE v4 = National Cancer Institute Common Terminology Criteria version 4

For Grade 1 symptoms:
Mild reaction; infusion interruption not indicated; intervention not indicated.

Remain at bedside and monitor subject until recovery from symptoms. The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) at least 30 minutes before additional nivolumab administrations.

For Grade 2 symptoms:
Moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [eg, antihistamines, non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; prophylactic medications indicated for ≤ 24 hours).

Stop the nivolumab infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen); remain at bedside and monitor subject until resolution of symptoms. Corticosteroid or bronchodilator therapy may also be administered as appropriate. Restart the infusion at 50% of the

original infusion rate when symptoms resolve to Grade ≤ 1; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor participant closely. If symptoms recur, then no further nivolumab will be administered at that visit. Administer diphenhydramine 50 mg IV, and remain at bedside and monitor the participant until resolution of symptoms. The amount of study drug infused must be recorded on the electronic case report form (eCRF). The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) should be administered at least 30 minutes before additional nivolumab administration. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.

For Grade 3 or Grade 4 symptoms:
Grade 3: prolonged [e.g. not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae [e.g. renal impairment, pulmonary infiltrates).
Grade 4: life-threatening; pressor or ventilatory support indicated.

Immediately discontinue infusion of nivolumab. Begin an IV infusion of normal saline, treat with bronchodilators, epinephrine 0.2 to 1 mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Participant should be monitored until the investigator is comfortable that the symptoms will not recur.

Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery from symptoms. In the case of late-occurring hypersensitivity symptoms (e.g., appearance of a localized or generalized pruritis within 1 week after treatment), symptomatic treatment may be given (e.g., oral antihistamine, or corticosteroids).

6.3 Management of Toxicities Attributable to Carboplatin

6.3.1 Hematological Adverse Event Management

Hematological toxicities (ie, neutropenia and thrombocytopenia) and associated complications have been observed with carboplatin and may be managed with dose delays and/or dose reductions. Use of granulocyte colony-stimulating factor support for neutrophil recovery is allowed per investigator discretion and in accordance with accepted guidelines after the first incidence of clinically relevant cytopenia.

Complete blood counts with differentials and platelets should be performed regularly. Participants with hematological toxicities may require additional or more frequent laboratory tests according to institutional guidelines. Febrile neutropenia or evidence of infection associated with neutropenia must be assessed immediately and treated appropriately and in a timely manner according to institutional guidelines.

Dose reductions or dose interruptions for anemia are not mandated but can be applied as clinically indicated. Supportive care (i.e. red blood cell transfusions) may be managed according to institutional guidelines.

Table 14. Hematological Adverse Event Management Algorithm

Grade of Hematological Adverse Event (NCI-CTCAE v4)	Management	Follow-up
NEUTROPENIA		
Grade 1-2	• Continue carboplatin at same dose	
Grade 3-4	• Hold carboplatin until ANC ≥ 1000/mm^3 First occurrence: • Resume carboplatin with a dose reduction by 1 dose level (-1 AUC) for all subsequent cycles (unless initiating G-CSF) Second occurrence: • Resume carboplatin with another dose reduction by 1 dose level (-2 AUC) for all subsequent cycles (unless initiating G-CSF)	• G-CSF may be used between days 2-6 at physician discretion, and to avoid dose reduction • If Grade 3-4 toxicity persists despite 2 dose level reductions, discontinue carboplatin
FEBRILE NEUTROPENIA		
ANC < 1000/mm^3 with a single temperature > 38.3ºC (101ºF) or a sustained temperatuire of ≥ 38ºC (100.4ºF) for > 1 hour	• Hold carboplatin until resolved (ANC ≥ 1000/mm^3, fever < 38ºC) First occurrence: • Resume carboplatin with a dose reduction by 1 dose level (AUC 5) for all subsequent cycles (unless initiating G-CSF) Second occurrence: • Resume carboplatin with another dose reduction by 1 dose level (AUC 4) for all subsequent cycles (unless initiating G-CSF)	• G-CSF may be used between days 2-6 at physician discretion, and to avoid dose reduction • If febrile neutropenia despite 2 dose level reductions, discontinue carboplatin
THROMBOCYTOPENIA		
≥ 75,000/mm^3	• No change to carboplatin	
50,000 - 75,000/mm^3	First occurrence: • Hold carboplatin until recovery to 75,000/mm3 • Resume carboplatin with a 1 dose level reduction (AUC 5) for all subsequent cycles	• Supportive platelet transfusion and/or romiplostim may be used to meet criteria for treatment

	Second occurrence: • Hold carboplatin until recovery to 75,000/mm3 • Resume carboplatin with another dose reduction by 1 dose level (AUC 4) for all subsequent cycles*	
< 50,000/mm^3	First occurrence (of any thrombocytopenia ≤75,000): • Hold carboplatin until recovery to 75,000/mm^3 • Resume carboplatin with a 1 dose level reduction (AUC 5) for all subsequent cycles Second occurrence (of any thrombocytopenia <75,000): • Hold carboplatin until recovery to 75,000/mm^3 • Resume carboplatin with another dose reduction by 1 dose level (AUC 4) for all subsequent cycles*	• Supportive platelet transfusion and/or romiplostim may be used to meet criteria for treatment • If Grade 3-4 toxicity persists despite 2 dose level reductions, discontinue carboplatin
ANEMIA		
All grades	• For all anemia events related to carboplatin, regardless of grade, iron studies should be checked and iron should be replaced as indicated.	• Red blood cell transfusions can be given at the investigator´s discretion as needed for symptom control.

*If held at AUC4, restarting carboplatin at the same dose is per investigator discretion. Carboplatin should be discontinued after three mandated dose holds at this dose level, unless approval is granted from the study sponsor.

6.3.2 Nausea and Vomiting Adverse Event Management

Carboplatin has moderate to high emetic potential, although manageable with adequate anti-emetic therapy. Prophylactic anti-emetic therapy (e.g., aprepitant, ondansetron, palonosteron, etc.) should be administered to **all** patients and specific agents are at the discretion of the treating physician.

Table 15. Nausea and Vomiting Adverse Event Management Algorithm

Grade of Nausea and/or Vomiting Adverse Event (NCI-CTCAE v4)	Management	Follow-up
Grade 1-2	• Continue carboplatin at same dose • Nausea and/or vomiting should be controlled with adequate anti-emetic therapy. • Patients are encouraged to take plenty of oral fluids	• Prophylactic anti-emetic therapy should be administered to all patients; specific agents are at the discretion of the treating physician (please see recommended regimens below)
Grade 3-4	• Hold carboplatin until resolved to ≤ Grade 1 • Resume carboplatin at previous administered dose with modification of premedications.	• Persistent toxicity ≥ Grade 3 despite maximal supportive care, dose reduce carboplatin by one dose level (AUC 5) at the next chemotherapy cycle for all subsequent cycles

NCI-CTCAE v4 = National Cancer Institute Common Terminology Criteria version 4

Recommended prophylactic anti-emetic therapy includes:
-Aprepitant 125 mg oral (1 capsule on the same day, to be administered approximately 60 minutes prior to carboplatin)
-Aprepitant 80 mg oral (1 capsule daily for 2 days, starting 24 hours after the administration of carboplatin)
-Palonosetron 0.25 mg IV (1 dose to be administered 30 minutes prior to carboplatin)
-Lorazepam 0.5-1.0 mg oral (every 4-6 hours if needed for anticipatory nausea or vomiting, or anxiety)
-Dexamethasone: Corticosteroids should be avoided unless deemed necessary to control refractory nausea or vomiting. If needed, administer 4 mg oral (at the start of treatment) or 4mg IV (dose to be administered 30 minutes prior to carboplatin)

6.3.3 Renal Adverse Event Management

Table 16. Renal Adverse Event Management Algorithm

Serum Creatinine Elevation or Abnormal Creatinine Clearance	Management	Follow-up
Creatinine > ULN to ≤ 1.5 x ULN or CrCL ≥ 45 ml/min	• Continue carboplatin at same dose	• Patients are encouraged to take plenty of oral fluids
Creatinine > 1.5 to ≤ 2.0 x ULN or CrCL ≥ 30 to < 45 ml/min	• Reduce carboplatin one dose level (-1 AUC) for all subsequent cycles	• Patients are encouraged to take plenty of oral fluids

Creatinine > 2.0 to ≤ 6.0 x ULN or CrCL ≥ 10 to < 30 ml/min	• Hold carboplatin until ≤ 2.0 x ULN or CrCL ≥ 30 ml/min. • Reduce carboplatin one dose level (-1 AUC) for all subsequent cycles • Consider nephrologist consult	• Once resolved and carboplatin resumed, weekly creatinine monitoring is recommended • Patients are encouraged to take plenty of oral fluids
Creatinine > 6.0 x ULN or CrCL < 10 ml/min	• Discontinue carboplatin • Monitor creatinine daily • Consult nephrologist	

CrCL = Creatinine clearance calculated using Calvert formula; mL/min= milliliters/minute

Note: If carboplatin is already reduced to AUC 4 and the patient requires another dose level reduction, carboplatin therapy will be discontinued.

6.3.4 Neurologic Adverse Event Management

Table 17. Neurologic Adverse Event Management Algorithm

Grade of Sensory Neuropathy (NCI-CTCAE v4)	**Management**
Grade 1	• Continue carboplatin at same dose
Grade 2	• First occurrence: reduce carboplatin one dose level (AUC 5) • Second occurrence: delay carboplatin until resolved to ≤ Grade 1 and resume with another dose level reduction (AUC 4)
Grade 3-4	• Discontinue carboplatin

ADL = activities of daily living; NCI-CTCAE v4 = National Cancer Institute Common Terminology Criteria version 4

6.3.5 Hepatobiliary Disorders

Table 18. Hepatobiliary Adverse Event Management Algorithm

Grade of Elevated AST, ALT or Bilirubin (NCI-CTCAE v4)	**Management**	**Follow-up**
Grade 1	• Continue carboplatin at same dose	
Grade 2	• Delay/omit carboplatin until it resolves to ≤ Grade 1 or baseline, with the following exceptions: a) Documented liver metastases and baseline AST or ALT > 3.0 to ≤ 5.0 x ULN	If returns to ≤ Grade 1 or baseline: • Resume carboplatin at same dose

	b) Documented Gilbert´s syndrome and baseline total bilirubin > 1.5 to ≤ 3.0 x ULN	
Grade 3	• Delay/omit carboplatin until it resolves to ≤ Grade 1 or baseline	If returns to ≤ Grade 1 or baseline: • Resume carboplatin at one dose level reduction (-1 AUC) for all subsequent cycles
Grade 4	• Discontinue carboplatin	

ALT = alanine aminotransferase; AST = aspartate aminotransferase; LFT = liver function test; NCI-CTCAE v4 = National Cancer Institute Common Terminology Criteria version 4; ULN = upper limit of normal

6.3.6 Management of Carboplatin Infusion-Related Reactions and Hypersensitivity

Patients should be observed closely for hypersensitivity reactions, particularly during the first and second infusions. Hypersensitivity reactions may occur within a few minutes following the initiation of the infusion of carboplatin. If hypersensitivity reactions occur, minor symptoms such as flushing or localised cutaneous reactions do not require discontinuation of therapy. The infusion may be temporarily interrupted and when symptoms improve re-started at a slower infusion rate. Chlorphenamine 10mg iv may be administered. Severe reactions, such as hypotension, bronchospasm or generalised rash/erythema require immediate discontinuation of carboplatin and appropriate therapy.

Table 19. Carboplatin Infusion-Related Reactions and Hypersensitivity Management

Grade of Carboplatin Infusion-Related Reaction (NCI-CTCAE v4)	Management	Follow-up
Grade 1	• Continue carboplatin per protocol. Remain at beside.	• Institute prophylactic medications with subsequent infusions
Grade 2	• Hold carboplatin until resolution to ≤ Grade 1	• Refer to management and rechallenging instructions below
Grade 3	• For patients on carboplatin monotherapy, infusion should be held until resolution to ≤ Grade 1	• If patient is not rechallenged, desensitization may be considered per physician discretion
Grade 4	• Discontinue carboplatin therapy	

NCI-CTCAE v4 = National Cancer Institute Common Terminology Criteria version 4

For Grade 1 symptoms:
Mild reaction; infusion interruption not indicated; intervention not indicated.

Remain at bedside and monitor subject until recovery from symptoms. The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) at least 30 minutes before additional carboplatin administrations.

For Grade 2 symptoms:
Moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [eg, antihistamines, non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; prophylactic medications indicated for ≤ 24 hours).

Stop the carboplatin infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen); remain at bedside and monitor subject until resolution of symptoms. Corticosteroid or bronchodilator therapy may also be administered as appropriate. Restart the infusion at 50% of the original infusion rate when symptoms resolve to Grade ≤ 1; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor participant closely. If symptoms recur, then no further carboplatin will be administered at that visit. Administer diphenhydramine 50 mg IV, and remain at bedside and monitor the participant until resolution of symptoms. The amount of study drug infused must be recorded on the electronic case report form (eCRF). The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) should be administered at least 30 minutes before additional carboplatin administration. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.

For Grade 3 or Grade 4 symptoms:
Grade 3: prolonged [e.g. not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae [e.g. renal impairment, pulmonary infiltrates).
Grade 4: life-threatening; pressor or ventilatory support indicated.

Immediately discontinue infusion of carboplatin. Begin an IV infusion of normal saline, treat with bronchodilators, epinephrine 0.2 to 1 mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Participant should be monitored until the investigator is comfortable that the symptoms will not recur.

Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery from symptoms. In the case of late-occurring hypersensitivity symptoms (e.g., appearance of a localized or generalized pruritis within 1 week after treatment), symptomatic treatment may be given (e.g., oral antihistamine, or corticosteroids).

Desensitization is allowed for patients on carboplatin monotherapy and may be considered per physician discretion.

6.3.7 Management of All Other Adverse Events

Table 20: General Guidance for All Other Adverse Events

Grade of Adverse Event (NCI-CTCAE v4)	Management
Grade 1-2	• Continue carboplatin at same dose.
Grade 3	• Delay/omit carboplatin until resolution to ≤ Grade 1 and resume carboplatin at one dose level reduction (AUC 5) [a]
Grade 4	• Discontinue carboplatin therapy

NCI-CTCAE v4 = National Cancer Institute Common Terminology Criteria version 4
[a] If a patient has persistent Grade 3 AE, a further dose level reduction (to AUC 4) is allowed at physician´s discretion.

6.4 Management of Toxicities Attributable to nab-Paclitaxel Alone

For toxicities in this section, which are attributable to nab-paclitaxel alone, concurrent holding of nivolumab is at the discretion of the treating physician, as described in the general guidelines at the start of Section 6.1.

6.4.1 Hematological Adverse Event Management

Hematological toxicities (ie, neutropenia and thrombocytopenia) and associated complications have been observed with nab-paclitaxel and may be managed with dose delays and/or dose reductions. Use of granulocyte colony-stimulating factor support for neutrophil recovery is allowed per investigator discretion and in accordance with accepted guidelines after the first incidence of clinically relevant cytopenia.

Complete blood counts with differentials and platelets should be performed regularly. Participants with hematological toxicities may require additional or more frequent laboratory tests according to institutional guidelines. Febrile neutropenia or evidence of infection associated with neutropenia must be assessed immediately and treated appropriately and in a timely manner according to institutional guidelines.

Dose reductions or dose interruptions for anemia are not mandated but can be applied as clinically indicated. Supportive care (i.e. red blood cell transfusions) may be managed according to institutional guidelines.

Table 21. Hematological Adverse Event Management Algorithm

Grade of Hematological Adverse Event (NCI-CTCAE v4)	Management	Follow-up
NEUTROPENIA		
Grade 1-2	• Continue nab-paclitaxel at same dose	
Grade 3-4	• Hold nab-paclitaxel until ANC ≥ 1000/mm^3. If delay of next dose by >7 days for nadir ANC <1000/mm^3 or nadir ANC <500/mm^3 for >7 days: First occurrence: • Resume nab-paclitaxel with a dose reduction by 1 dose level (75 mg/m^2) for all subsequent cycles (unless initiating G-CSF) Second occurrence: • Resume nab-paclitaxel with another dose reduction by 1 dose level (50 mg/m^2) for all subsequent cycles Third occurrence: • Discontinue nab-paclitaxel permanently. Continuation of nivolumab is at physician´s discretion.	• Growth factor support may be used at physician´s discretion, and to avoid dose reduction • If Grade 3-4 toxicity persists despite 2 dose level reductions, discontinue nab-paclitaxel
FEBRILE NEUTROPENIA		
ANC < 1000/mm^3 with a single temperature > 38.3ºC (101ºF) or a sustained temperatuire of ≥ 38ºC (100.4ºF) for > 1 hour	• Hold nab-paclitaxel until resolved (ANC ≥ 1000/mm^3, fever < 38ºC) First occurrence: • Resume nab-paclitaxel with a dose reduction by 1 dose level (75 mg/m^2) for all subsequent cycles (unless initiating G-CSF) Second occurrence: • Resume nab-paclitaxel with another dose reduction by 1 dose level (50 mg/m^2) for all subsequent cycles Third occurrence:	• Growth factor support may be used at physician´s discretion, and to avoid dose reduction • If febrile neutropenia despite 2 dose level reductions, discontinue nab-paclitaxel

	• Discontinue nab-paclitaxel permanently. Continuation of nivolumab is at physician´s discretion.	
THROMBOCYTOPENIA		
≥ 75,000/mm^3	• No changes to nab-paclitaxel	
< 75,000/mm^3	First occurrence (of any thrombocytopenia <75,000): • Hold nab-paclitaxel until recovery to 75,000/mm^3 • Resume nab-paclitaxel with 1 dose level (75 mg/m^2) reduction for all subsequent doses Second occurrence (of any thrombocytopenia <75,000): • Hold nab-paclitaxel until recovery to 75,000/mm^3 • Resume nab-paclitaxel with another dose reduction by 1 dose level (50 mg/m^2) for all subsequent cycles* Third occurrence (of any thrombocytopenia <75,000): • Discontinue nab-paclitaxel permanently. Continuation of nivolumab is at physician´s discretion.	• Supportive platelet transfusion and/or romiplostim may be used to meet criteria for treatment • If Grade 3-4 toxicity persists despite 2 dose level reductions, discontinue nab-paclitaxel
ANEMIA		
All grades	• For all anemia events related to nab-paclitaxel, regardless of grade, iron studies should be checked and iron should be replaced as indicated.	• Red blood cell transfusions can be given at the investigator´s discretion as needed for symptom control.

*If held at 50mg/m^2, restarting nab-paclitaxel at the same dose is per investigator discretion. Nab-paclitaxel should be discontinued after three mandated dose holds at this dose level, unless approval is granted from the study sponsor.

6.4.2 Neurologic Adverse Event Management

Table 22. Neurologic Adverse Event Management Algorithm

At C1D1 of Crossover phase, baseline neuropathy: Grade ≤1

Grade of Neuropathy (NCI-CTCAE v4)	**Management**
Grade 1	• Continue nab-paclitaxel at same dose
Grade 2	• Reduce nab-paclitaxel one dose level (75 mg/m^2)*
Grade 3	• First occurrence: Delay nab-paclitaxel until resolved to ≤ Grade 2 and: • If prior dose 100 mg/m^2: resume nab-paclitaxel with one dose level reduction (75 mg/m^2). • If prior dose 75 mg/m^2: resume nab-paclitaxel with another dose level reduction (50 mg/m^2) • Second occurrence: • If prior dose 75 mg/m^2: delay nab-paclitaxel until resolved to ≤ Grade 2 and resume nab-paclitaxel with another dose level reduction (75 mg/m^2). If there is a third occurrence, discontinue nab-paclitaxel permanently. Continuing nivolumab is at physician´s discretion. • If prior dose 50 mg/m^2: permanently discontinue nab-paclitaxel. Continuing nivolumab is at physician´s discretion.
Grade 4	Discontinue nab-paclitaxel permanently. Continuing nivolumab is at physician´s discretion.

ADL = activities of daily living; NCI-CTCAE v4 = National Cancer Institute Common Terminology Criteria version 4.

At C1D1 of Crossover phase, baseline neuropathy: Grade 2

If prior to starting Crossover phase a patient has residual grade 2 neuropathy attributed to carboplatin, treatment with nab-paclitaxel should be initiated at one dose level reduction (75 mg/m^2).

Grade of Neuropathy (NCI-CTCAE v4)	**Management**
Grade 1	• Continue nab-paclitaxel at same dose
Grade 2	• Continue nab-paclitaxel at same dose
Grade 3	• First occurrence: delay nab-paclitaxel until resolved to ≤ Grade 2 and resume with another dose level reduction (50 mg/m^2)

	• Second occurrence: Discontinue nab-paclitaxel permanently. Continuing nivolumab is at physician´s discretion.
Grade 4	Discontinue nab-paclitaxel permanently. Continuing nivolumab is at physician´s discretion.

ADL = activities of daily living; NCI-CTCAE v4 = National Cancer Institute Common Terminology Criteria version 4.

6.4.3 Hepatobiliary Disorders

Since the exposure and toxicity of paclitaxel can be increased with hepatic impairment, administration of nab-paclitaxel in patients with hepatic impairment should be performed with caution. Patients with hepatic impairment may be at increased risk of toxicity, particularly from myelosuppression; such patients should be closely monitored for development of profound myelosuppression.

Table 23. Hepatobiliary Adverse Event Management Algorithm

Grade of Elevated AST, ALT or Bilirubin (NCI-CTCAE v4)	**Management**	**Follow-up**
Grade 1	• Continue nab-paclitaxel at same dose	
Grade 2	• Delay/omit nab-paclitaxel until it resolves to ≤ Grade 1 or baseline, with the following exceptions: a) Documented liver metastases and baseline AST or ALT > 3.0 to ≤ 5.0 x ULN b) Documented Gilbert´s syndrome and baseline total bilirubin > 1.5 to ≤ 3.0 x ULN	If returns to ≤ Grade 1 or baseline: • Resume nab-paclitaxel at same dose
Grade 3	• Delay/omit nab-paclitaxel until it resolves to ≤ Grade 1 or baseline	If returns to ≤ Grade 1 or baseline: • Resume nab-paclitaxel at one dose level reduction (75 mg/m^2) for all subsequent cycles[a]
Grade 4	• Discontinue nab-paclitaxel	

ALT = alanine aminotransferase; AST = aspartate aminotransferase; LFT = liver function test; NCI-CTCAE v4 = National Cancer Institute Common Terminology Criteria version 4; ULN = upper limit of normal
[a] If a patient has persistent Grade 3 AE, a further dose level reduction (75 mg/m^2) is allowed at physician´s discretion.

6.4.4 Management of Nab-Paclitaxel Infusion-Related Reactions and Hypersensitivity

Severe and sometimes fatal hypersensitivity reactions, including anaphylactic reactions, have been reported. Patients who experience a severe hypersensitivity reaction to nab-paclitaxel **should not be rechallenged** with this drug (please see management and rechallenging instructions below). Cross-hypersensitivity between nab-paclitaxel and other taxane products has been reported and may include severe reactions such as anaphylaxis. Patients with a previous history of hypersensitivity to other taxanes should be closely monitored during initiation of nab-paclitaxel therapy, and premedication with diphenhydramine and corticosteroids may be considered per physician discretion.

Hypersensitivity reactions may occur within a few minutes following the initiation of the infusion of nab-paclitaxel. If hypersensitivity reactions occur, minor symptoms such as flushing or localised cutaneous reactions do not require discontinuation of therapy. The infusion may be temporarily interrupted and when symptoms improve re-started at a slower infusion rate. Chlorphenamine 10mg iv may be administered. Severe reactions, such as hypotension, bronchospasm or generalized rash/erythema require immediate discontinuation of nab-paclitaxel and appropriate therapy.

Table 24. Nab-Paclitaxel Infusion-Related Reactions and Hypersensitivity Management

Grade of nab-Paclitaxel Infusion-Related Reaction (NCI-CTCAE v4)	Management	Follow-up
Grade 1	• Continue nab-paclitaxel per protocol. Remain at beside.	• Institute prophylactic medications with subsequent infusions
Grade 2	• Hold nab-paclitaxel until resolution to ≤ Grade 1	• Refer to management and rechallenging instructions below
Grade 3	• For patients, infusion should be held until resolution to ≤ Grade 1	• If patient is not rechallenged, desensitization may be considered per physician discretion
Grade 4	• Discontinue nab-paclitaxel therapy	

NCI-CTCAE v4 = National Cancer Institute Common Terminology Criteria version 4

For Grade 1 symptoms:
Mild reaction; infusion interruption not indicated; intervention not indicated.

Remain at bedside and monitor subject until recovery from symptoms. The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) at least 30 minutes before additional nab-paclitaxel administrations.

For Grade 2 symptoms:
Moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [eg, antihistamines, non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; prophylactic medications indicated for ≤ 24 hours).

Stop the nab-paclitaxel infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen); remain at bedside and monitor subject until resolution of symptoms. Corticosteroid or bronchodilator therapy may also be administered as appropriate. Restart the infusion at 50% of the original infusion rate when symptoms resolve to Grade ≤ 1; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor participant closely. If symptoms recur, then no further nab-paclitaxel will be administered at that visit. Administer diphenhydramine 50 mg IV, and remain at bedside and monitor the participant until resolution of symptoms. The amount of study drug infused must be recorded on the electronic case report form (eCRF). The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) should be administered at least 30 minutes before additional nab-paclitaxel administration. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.

For Grade 3 or Grade 4 symptoms:
Grade 3: prolonged [e.g. not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae [e.g. renal impairment, pulmonary infiltrates).
Grade 4: life-threatening; pressor or ventilatory support indicated.

Immediately discontinue infusion of nab-paclitaxel. Begin an IV infusion of normal saline, treat with bronchodilators, epinephrine 0.2 to 1 mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Participant should be monitored until the investigator is comfortable that the symptoms will not recur.

Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery from symptoms. In the case of late-occurring hypersensitivity symptoms (e.g., appearance of a localized or generalized pruritis within 1 week after treatment), symptomatic treatment may be given (e.g., oral antihistamine, or corticosteroids). Desensitization is allowed for patients on nab-paclitaxel and may be considered per physician discretion.

6.4.5 Management of All Other Adverse Events

Table 25: General Guidance for All Other Adverse Events

Grade of Adverse Event (NCI-CTCAE v4)	Management
Grade 1-2	• Continue nab-paclitaxel at same dose

Grade 3	• Delay/omit nab-paclitaxel until resolution to ≤ Grade 1 or baseline and resume nab-paclitaxel at one dose level reduction (75 mg/m^2)[a]
Grade 4	• Discontinue nab-paclitaxel therapy

NCI-CTCAE v4 = National Cancer Institute Common Terminology Criteria version 4
[a] If a patient has persistent Grade 3 AE, a further dose level reduction (50 mg/m^2) is allowed at physician´s discretion.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

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

7.1 Adverse Events List

7.1.1 Expected Adverse Events for Nivolumab

The PD-L1/PD-1 pathway is involved in peripheral tolerance and, therefore, its inhibition may increase the risk of immune-related AEs, specifically the induction or enhancement of autoimmune conditions. AEs with potentially immune-related causes, including rash, hypo-/hyperthyroidism, hepatitis/transaminitis, colitis, pneumonitis, hypophysitis, adrenal insufficiency, insulin-dependent Diabetes Mellitus, myositis, and myasthenia gravis, have been observed.

For complete details on adverse reactions, please see nivolumab investigator brochure.

7.1.2 Expected Adverse Events for Carboplatin

The following AEs related to the cytotoxic mechanism of action of carboplatin have been reported:

<u>Hematologic Toxicity</u>
Bone marrow suppression is the dose-limiting toxicity of carboplatin, including thrombocytopenia, neutropenia and anemia. Fever has also been reported in patients with neutropenia. The nadir of hematologic toxicity with single-agent carboplatin usually occurs at about day 21. Marrow suppression is usually more severe in patients with impaired kidney function. Patients with poor performance status have also experienced a higher incidence of severe leukopenia and thrombocytopenia. The incidence of anemia increases with increasing exposure to carboplatin.

<u>Gastrointestinal Toxicity</u>
Carboplatin, as a single agent or in combination, is significantly less emetogenic than cisplatin; however, patients previously treated with emetogenic agents, especially cisplatin, appear to be more prone to vomiting. Both nausea and vomiting usually cease within 24 hours of treatment and are often responsive to antiemetic measures. Other gastrointestinal effects observed frequently

were constipation and diarrhea.

Neurologic Toxicity
Peripheral neuropathies have been observed in patients receiving carboplatin, with mild paresthesias occurring most frequently. Carboplatin therapy produces significantly fewer and less severe neurologic side effects than does therapy with cisplatin. However, patients older than 65 years and/or previously treated with cisplatin appear to have an increased risk for peripheral neuropathies. Although the overall incidence of peripheral neurologic side effects induced by carboplatin is low, prolonged treatment, particularly in cisplatin pretreated patients, may result in cumulative neurotoxicity.

Nephrotoxicity
Development of abnormal renal function test results is uncommon, despite the fact that carboplatin, unlike cisplatin, has usually been administered without high-volume fluid hydration and/or forced diuresis. Creatinine clearance has proven to be the most sensitive measure of kidney function in patients receiving carboplatin, and it appears to be the most useful test for correlating drug clearance and bone marrow suppression. Patients with a baseline value of 60 mL/min or more are more likely to experience a reduction below this value during carboplatin therapy.

Hepatic Toxicity
Abnormal liver function tests, including transaminitis, have generally been mild and reversible, although the role of metastatic tumor in the liver may complicate the assessment in many patients.

Electrolyte Changes
The following serum electrolytes have been found to be anormally decreased with treatment with carboplatin: sodium, potassium, calcium and magnesium. Electrolyte supplementation is not routinely administered concomitantly with carboplatin, and these electrolyte abnormalities are rarely associated with symptoms.

Allergic Reactions
Hypersensitivity has been reported with carboplatin. These allergic reactions have been similar in nature and severity to those reported with other platinum-containing compounds, ie, rash, urticaria, erythema, pruritus, and rarely bronchospasm and hypotension. Anaphylactic reactions have been reported as part of postmarketing surveillance. These reactions have been successfully managed with standard epinephrine, corticosteroid, and antihistamine therapy.

Injection Site Reactions
Injection site reactions, including redness, swelling, and pain, have been reported during postmarketing surveillance. Necrosis associated with extravasation has also been reported.

For complete details on adverse reactions please see the carboplatin package insert.

7.1.3 Expected Adverse Events for Nab-Paclitaxel

In clinical studies, nab-paclitaxel has been associated with alopecia, myelosuppression

(primarily neutropenia), sensory neuropathy, abnormal ECG, fatigue/asthenia, myalgia/arthralgia, AST elevation, alkaline phosphatase elevation, anemia, nausea, infections, and diarrhea.

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

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

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site: 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.
  - Other AEs for the protocol that do not require expedited reporting are outlined in the next section (Expedited Adverse Event Reporting) under the sub-heading of Protocol-Specific Expedited Adverse Event Reporting Exclusions.

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

- **Expectedness** of the AE:
  Adverse events can be 'Expected' or "Unexpected'

  - Expected adverse event

  Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.

  Refer to Section 7 for a listing of expected adverse events associated with the study agent(s).

- Unexpected adverse event

For the purposes of this study, an adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk.

7.3 Expedited Adverse Event Reporting

7.3.1 Investigators **must** report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment (100 days for participants receiving nivolumab).

7.3.2 For multi-institution studies where a DF/HCC investigator is serving as the Overall Principal Investigator, 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.

7.3.3 Additionally, as mentioned in section 6.2.10, Nivolumab Infusion-Related Reactions, all Grade 3 or 4 infusion reactions should be reported within 24 hours to the study PI and reported as a SAE if criteria are met.

7.3.4 All occurrences of potential DILIs, meeting the defined criteria in Section 5.5, must be reported as SAEs.

7.4 DF/HCC Expedited Reporting Guidelines

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

Other investigative sites will report AEs to their respective IRB 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 for participants receiving 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 in reporting adverse events.

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

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

7.6 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.

7.7 Expedited Reporting to [redacted]

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

SAE Email Address: [redacted]

SAE Facsimile Number: 609-818-3804

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 [redacted] (or designee) using the same procedure used for transmitting the initial SAE report.

All SAEs should be followed to resolution or stabilization.

7.7.1 SAE Definition for Reporting to [redacted]

A Serious Adverse Event (SAE) is any untoward medical occurrence that at any dose:

- 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.

7.7.2 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 [redacted] of this event and complete one of the following forms within 24 hours of awareness of the 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, [redacted] Pregnancy Surveillance Form, or approved site SAE form. A [redacted] 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 [redacted] 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.

7.8 Routine Adverse Event Reporting

All Grade 2 or higher Adverse Events (excluding non-clinically significant laboratory values) Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must <u>also</u> be reported in routine study data submissions.**

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational agent(s) administered in this study can be found in Section 7.

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 elimination half-life ($t_{½}$) was 25 days and the typical clearance was 8.2 mL/h, which are consistent with those of full human immunoglobulin antibodies.

Table 26: Nivolumab Physical and Chemical Properties

[redacted] 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), with a maximum of 8 hours of the total 24 hours 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

Pharmaceutical Management Branch, CTEP/DCTD/NCI 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. When the dose is fixed (eg, 360 mg flat dose), nivolumab injection can be infused undiluted or diluted so as not to exceed a total infusion volume of 160 mL.

8.1.7 Administration

Nivolumab will be delivered in infusion bags with IV infusion lines over approximately 30 minutes (+/- 10 minutes) using a volumetric pump with 0.2 to 1.2 micron pore size, low-protein binding polyethersulfone membrane in-line filter.

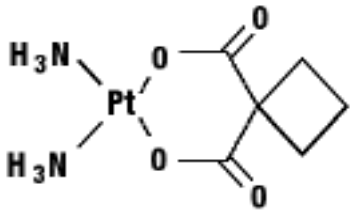
8.1.8 Ordering

Nivolumab will be provided by [redacted]. Each participating institution is responsible for completing the applicable drug supply forms to receive re-supply of Nivolumab for the duration of 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 Carboplatin

8.2.1 Description

Carboplatin is a platinum coordination compound. The chemical name for carboplatin is platinum, diammine[1,1-cyclobutanedicarboxylato(2-)- *O*,*O*']-, (*SP*-4-2), and carboplatin has the following structural formula:
Carboplatin is a crystalline powder with the molecular formula of C6H12N2O4Pt and a molecular weight of 371.25. It is soluble in water at a rate of approximately 14 mg/mL, and the pH of a 1% solution is 5 to 7. It is virtually insoluble in ethanol, acetone, and dimethylacetamide.

8.2.2 Formulation and Administration

Carboplatin will be obtained commercially and is available as a sterile, pyrogen-free, 10 mg/mL aqueous solution in 5-mL, 15-mL, 45-mL, and 60-mL vials. Carboplatin may be diluted in 0.9% sodium chloride injection, USP or 5% dextrose injection, USP. Needles or IV administration sets containing aluminum parts that may come in contact with carboplatin should not be used for the preparation or administration of the drug. Aluminum can react with carboplatin causing precipitate formation and loss of potency.

The total dose of carboplatin for each patient will be per institutional guidelines. Carboplatin should be administered after nivolumab. Carboplatin as well as premedication is to be administered and stored in accordance with local prescribing information and local institutional guidelines.

8.2.3 Storage and Stability

Carboplatin is a premixed aqueous solution of 10 mg/mL that can be further diluted to concentrations as low as 0.5 mg/mL with 5% dextrose in water (D5W) or 0.9% sodium chloride injection, USP. When prepared as directed, carboplatin aqueous solutions are stable for 8 hours at room temperature (25°C). Since no antibacterial preservative is contained in the formulation, it is recommended that carboplatin aqueous solutions be discarded 8 hours after dilution.

Unopened vials of carboplatin are stable to the date indicated on the package when stored at 25°C (77°F); excursions permitted from 15°-30°C (59°-86°F). Vials should be protected from light. Carboplatin multidose vials maintain microbial, chemical, and physical stability for up to 14 days at 25°C following multiple needle entries. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. Solutions for infusion should be discarded 8 hours after preparation.

8.2.4 Handling

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

8.2.5 Availability

Carboplatin is commercially available.

8.2.6 Preparation

Carboplatin should be prepared according to institutional guidelines.

8.2.7 Ordering

Carboplatin is commercially available. Check with the site Director of Pharmacy and/or the site research pharmacy to ensure that the agent is in stock. If the agent is not stocked, ensure that the agent can be ordered before the protocol is activated.

For complete details on drug preparation, administration, storage conditions, clinical pharmacology, pharmacokinetics, known precautions, warnings and adverse reactions please see the carboplatin package insert.

8.3 Nab-Paclitaxel

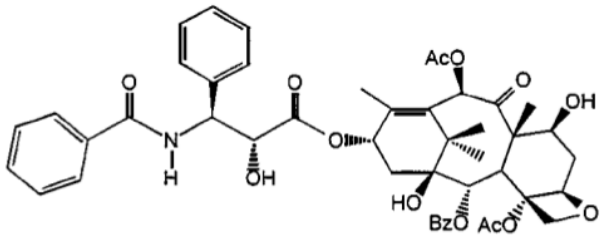
Please refer to the FDA-approved package insert for nab-paclitaxel for product information, extensive preparation instructions, and a comprehensive list of adverse events.

8.3.1 Description

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

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

Paclitaxel has the following structural formula:



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

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

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

8.3.3 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.

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

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

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

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

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

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

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

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

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

All biomarker analyses completed on blood and tissue samples for correlative data will be exploratory.

In all patients in whom tumor is safely accessible, a baseline tumor biopsy is required. We plan to use baseline biopsy tissue to perform immune profiling assays, characterization based on histology (TILs), protein expression, and mRNA expression, as detailed below. We will perform targeted panel (OncoPanel) and whole exome sequencing to determine mutational load and identify specific mutations or copy number changes in a panel of DNA repair genes and other potential biomarkers of interest. Additionally, we will bank specimens for possible future additional analyses.

We will perform single-cell RNA sequencing on the study biopsies for up to 15 first-line carboplatin-nivolumab patients, up to 15 first-line carboplatin patients and up to 15 second-line nab-paclitaxel-nivolumab patients enrolled at DFCI. These single-cell RNA studies will distinguish immune cell populations associated with response to carboplatin and nivolumab. Additionally, the studies will evaluate subclonal intratumor heterogeneity and characterize tumor subpopulations associated with resistance to therapy.

Serial blood draws for correlative science are required on this trial; blood draws will be obtained every two cycles (every 6 weeks during the Treatment Phase, every 8 weeks during the Crossover Phase) prior to the infusion of study drugs for the first 24 weeks on study treatment and then every three cycles (every 9 weeks during the Treatment Phase, every 12 weeks during the Crossover Phase), at the end-of-treatment visit in patients who go off 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 any reason other than progressive disease. Blood will be collected for flow cytometry to characterize protein expression of immune mediators and for T-cell receptor (TCR) sequencing, as detailed below, and additional blood will be banked for future analyses.

Instructions for the collection, labeling and shipment of blood, tissue, and stool samples are outlined below and further detailed in the 17-512 Lab Manual .

Please note that some of the downstream correlative plans for blood and tissue include whole exome sequencing, RNA sequencing, tumor tissue spatial profiling, single cell sequencing, single-nucleus sequencing, and TCR sequencing plus analysis of the data generated, which may be performed at the Broad Institute of MIT, Nanostring Technologies, Inc., Adaptive Biotechnologies, and Foundation Medicine. All samples will be sent in a completely de-identified fashion. Gene expression assays (e.g. BC360 panel) and digital spatial profiling (DSP) will be performed at DFCI and/or BWH Pathology using FFPE tissue samples. De-identified gene-expression and/or DSP data will be analyzed by Nanostring and returned to the PI, following the corresponding MTA. Only designated study personnel at DFCI will have access to PHI.

Specimen Collection Table

Specimen	Screening	Cycle 1 Day 1	Cycle 2 Day 1	Cycle 3 Day 1	Subsequent Cycles Day 1	End of treatment/ Progression [d]	Shipping Condition	Ship to
Lavender Top Tube (Section 9.3)		x					Ambient	Core Blood and Tissue Bank
Streck Tube (Section 9.3)		x		x	x [a]	x [c]	Ambient	Core Blood and Tissue Bank
Green Top Tubes (Section 9.3)		x		x	x [a]	x [c]	Ambient	CIO/Marianno's Lab
Archival tumor tissue (blocks or slides) (Section 9.1)	x						Ambient	DFCI 17-512 Study Team
Required fresh tumor tissue biopsy [b] (Section 9.2)	x		x			x [d]	FFPE, RNAlater: Ambient DMEM: 4ºC (ice packs) OCT: Frozen	Core Blood and Tissue Bank: OCT, RNAlater, FFPE/formalin

					(dry ice) RPMI w/ Hepes: 4ºC (ice packs)[f]	CIO/Marianno's Lab: DMEM CCPM Team: RPMI w/ Hepes[g]
Optional fresh tumor tissue biopsy (Section 9.2)					x	FFPE, RNAlater: Ambient DMEM: 4ºC (ice packs) OCT: Frozen (dry ice)	Core Blood and Tissue Bank: OCT, RNAlater, FFPE/formalin CIO/Marianno's Lab: DMEM
Stool sample collection [e] (Section 9.4)	x		x		x [f]	Ambient	Biorepository

a. Every 6 weeks (coinciding with day 1 of that cycle) for 24 weeks, then every 9 weeks. For Arm B participants who elect to cross-over to nivolumab + nab-paclitaxel, every 8 weeks (coinciding with day 1 of that cycle) for 24 weeks, then every 12 weeks.
b. Fresh tumor biopsy of accessible metastatic lesion is mandatory before treatment begins. The biopsy may be performed at screening or on Cycle 1 Day 1 prior to dosing. **For external site patients**, biopsy should be done after registration or on C1D1 prior to treatment start. See section 9.2 below.
c. Blood samples are mandatory for patients with progressive disease. For patients who come off treatment for other reasons, blood samples are optional at end of treatment and every 6-12 weeks during follow up. For Arm B participants who elect to cross-over to nivolumab + nab-paclitaxel, the study calendar is reinitated at C1D1 for Streck and Green Top tube collections (Lavender Top tube is not required). See Section 10, Study Calendar.
d. For Arm B participants who elect to cross-over to nivolumab + nab-paclitaxel, a baseline biopsy is mandatory, if safely accessible, before treatment begins. The study calendar is reinitiated for tissue collection during the Crossover phase.
e. Dietary and physical activity questionnaires will be performed at the time points of stool sample collection.
f. Stool sample will be collected at time of progression if response was observed with treatment and for Arm B participants who elect to cross-over to nivolumab + nab-paclitaxel before treatment begins. The study calendar is reinitiated for stool sample collection during the Crossover phase.
g. Only for specific subset of patients enrolled at DFCI, See Section 9.2.3.

9.1 Archival Tissue

9.1.1 Collection

At least one formalin-fixed, paraffin-embedded (FFPE) tumor block or 10-20 (5 micron) unstained charged slides will be collected from archival tissue for future research for all participants. Tissue from time of diagnosis is preferred, but if not available, then any archival sample prior to receipt of the previous line of treatment is acceptable. Tissue needs to be located and availability must be confirmed at the time of registration, as indicated in eligibility criterion 3.1.4. Tissue may be shipped to the DFCI 17-512 study team after registration occurs.

9.1.2 Shipping of Specimen(s)

All sites will ship archival specimens at ambient temperature to:

Dana-Farber Cancer Institute
Breast Oncology
Attn: DFCI 17-512 Study Team
450 Brookline Avenue
Dana 157
Boston, MA 02215

Email the current Dana-Farber CRC with the sample information and tracking information the day before shipping specimens.

Complete a Specimen Requisition Form (17-512 Lab Manual) to be sent with the specimens; make a copy of the requisition for the participant's research chart prior to sending.

9.2 Fresh Tissue Biopsy

9.2.1 Objectives

The study will obtain paired tumor biopsies for patients enrolled (after carboplatin monotherapy and after carboplatin in combination with nivolumab). Paired biopsies will be used to evaluate changes in TILs, expression of immune markers (e.g., immune cell subsets, inhibitory and co-stimulatory pathway molecules by immunohistochemistry and/or immunofluorescence) and other relevant markers. Additionally, the biopsies will be subjected to whole exome sequencing (WES) to characterize mutational load, to capture BRCA reversion mutations (and the mutation status of most genes involved with double strange break repair), and overall mutational signature. A portion of the biopsies will be used to perform single-cell RNA sequencing to determine mRNA expression signatures in tumor and immune cells that correlate with response and resistance to treatment.

9.2.2 Collection of Specimen(s)

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

Mandatory:

- Baseline or on C1D1 prior to dosing (for external site patients, biopsy should be performed after registration or on C1D1 prior to dosing*)
- After 3-6 weeks of treatment (any time from C2D1-C3D1)

Optional:

- At time of progression

* Biopsies performed during screening prior to registration are not violations.

<u>Crossover</u>
For patients randomized to carboplatin monotherapy and who elect to crossover to nivolumab + nab-paclitaxel at time of progression, biopsies will be performed at the below timepoints:

Mandatory:

- Prior to initiation of nivolumab + nab-paclitaxel (baseline or C1D1 of crossover).
- After 3-6 weeks of crossover treatment (any time from C2D1-C3D1)

Optional:

- At time of progression on nivolumab + nab-paclitaxel

Retreatment

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

Optional:

- At time of progression, prior to restarting nivolumab
- After retreatment with nivolumab, at time of progression

Mandatory biopsies of safely accessible tumor, at baseline and prior to crossover, if applicable, should be performed within 14 days of initiating therapy.

If dosing is delayed placing the biopsy outside of the allowable window, the biopsy should be rescheduled to be within the window. If not feasible, the biopsy should be obtained as close to within the window as possible. Tissue specimens will be collected from recurrent or metastatic lesions using standard institutional procedures. The amount of tissue collected will follow the guidelines listed below. If a participant has more than one site of disease, only one site needs to be biopsied in order to go on to the study and the site is left to the discretion of the patient and their treating physician. Fine need aspirates (FNA) are not allowed. Participants who undergo a research biopsy procedure for the purpose of this protocol, and in whom inadequate tissue is obtained, are still eligible and are not required to undergo a repeat biopsy in order to enter the study.

- *Breast*: A goal of 3-6 core biopsy specimens will be obtained using standard institutional guidelines for a diagnostic core biopsy of a breast mass.
- Skin/chest wall: A goal of 1-2 5-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 will be performed 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 participant 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.
- *Pleural fluid*: A goal of 500 cc of pleural fluid will be obtained with a standard thoracentesis procedure, with or without image guidance, according to the clinical judgment of the treating physician and clinician performing the procedure. Less than the

goal amount is acceptable, and should be based upon the clinical judgment of the Investigator and the clinician performing the procedure. If more than the goal amount of fluid is obtained, then the entire specimen (with the exception of what is needed for clinical purposes, if applicable) will be stored in the tissue bank.

- *Ascites fluid*: A goal of 500 cc of ascites fluid will be obtained with a standard paracentesis procedure, with or without image guidance, according to the clinical judgment of the treating physician and clinician performing the procedure. Less than the goal amount is acceptable, and should be based upon the clinical judgment of the Investigator and the clinician performing the procedure. If more than the goal amount of fluid is obtained, then the entire specimen (with the exception of what is needed for clinical purposes, if applicable) will be stored in the tissue bank.

Please note that the above are guidelines for the amount of tissue to be obtained at the baseline biopsy, and are not meant to replace clinical judgment at the time the procedure is performed. Less than the goal quantity of tissue is accepted for each type of biopsy, and will be left to the clinical judgment of the physician performing the procedure. If ascites or pleural fluid is to be used as the investigational biopsy specimen, consideration should be given to confirming the malignant nature of the ascites or pleural fluid prior to study entry.

If a patient is undergoing resection of a lesion for clinical reasons (i.e. wedge resection of a new lung lesion for confirmation of diagnosis or re-testing of hormone receptor or HER2 status; or, resection of a chest wall lesion; or, resection of a lymph node), then the patient may opt to have a portion of that tissue stored for research at the time of the procedure (provided that the tissue is collected and processed as specified in this section and the 17-512 Lab Manual), in which case, the participant would not be required to undergo a separate research biopsy for entry into this protocol.

9.2.3 Handling and Shipping

Core biopsy specimens will be handled and processed at the time of biopsy collection. Ideally, **sufficient core biopsy samples will be obtained to allow for some to be frozen** (after embeding in OCT) and **others to be fixed in formalin** and subsequently embedded into paraffin blocks. The specific instructions for handling core biopsy material is provided in the 17-512 Lab Manual

The order of specimen collection should be:

- First core: 10% neutral buffered formalin
- Second core: RNA later
- Third core: Sterile DMEM
- Fourth core: Sterile DMEM*
- Fifth core: OCT
- Sixth core: 10% neutral buffered formalin

*If at least four cores cannot be obtained in a biopsy (allowing for 2 cores [third and fourth] to be collected in sterile DMEM), then the third core should be snap-frozen in OCT.

For up to 15 first-line carboplatin-nivolumab patients, up to 15 first-line carboplatin patients and up to 15 second-line nab-paclitaxel-nivolumab patients enrolled at DFCI, the order of specimen collection will be:

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

For **DF/HCC Collaborative Sites ONLY**, fresh tumor tissue in DMEM will not be collected at the time of the research biopsies due to shipping and processing requirements.

The order of specimen collection for DF/HCC Collaborative sites should be:

- First core: 10% neutral buffered formalin
- Second core: RNA later
- Third core: OCT
- Fourth core: 10% neutral buffered formalin
- Fifth core: 10% neutral buffered formalin
- Sixth core: 10% neutral buffered formalin

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

- All samples should be de-identified and labeled with the participant initials, study ID, date of collection, approximate time of collection, and study time point.

- Two cores in sterile DMEM should be brought as fresh tissue immediately to the lab to be processed for TILs within 1.5 hours of its collection (as described in the 17-512 Lab Manual). Sites processing DMEM cores locally will batch ship to Mariano Severgnini at the end of the study. For samples that will be processed at DFCI, the cores should be brought or shipped on ice to the lab of Mariano Severgnini at:

  Center for Immuno-Oncology
  Dana-Farber Cancer Institute
  440 Brookline Ave-Mayer 308
  Boston, MA 02215
  Phone: (617) 632-2421
  Pager: 42093

- Two cores in formalin should be made into a formalin-fixed paraffin-embedded (FFPE) block. Blocks should be brought/shipped ambient to the DF/HCC Core Blood and Tissue Bank (dfcibreastbank@partners.org). For local sites, the cores in formalin will be brought to the Brigham and Women's Specialized Histopathology Core Lab where it will be made into a block.

- One core in OCT should be brought/shipped to the DF/HCC Core Blood and Tissue Bank Please email the DF/HCC Core Blood and Tissue Bank (dfcibreastbank@partners.org) with patient name, study ID, date of collection, approximate time of collection, and study time point the day prior to collection.

- One core in RNAlater should be brought/shipped at ambient temperature to the DF/HCC Core Blood and Tissue Bank. Please email the DF/HCC Core Blood and Tissue Bank (dfcibreastbank@partners.org) with patient name, study ID, date of collection, approximate time of collection, and study time point the day prior to collection.

Ship specimens overnight Monday-Thursday only by either FedEx or UPS to::

Core Blood and Tissue Bank
Brigham and Women's Hospital
Thorn Building – Room 428
20 Shattuck Street
Boston, MA 02215
dfcibreastbank@partners.org

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

- Two cores in RPMI with Hepes: should be brought immediately to the lab at the Broad for processing. Members of the Center for Cancer Precision Medicine (CCPM) team will collect these cores to deliver them to the Broad Institute. Please email Laura Dellostritto (laura_dellostritto@dfci.harvard.edu) with study ID, study timepoint, tissue site, and collection date, location, and approximate time at least 24 hours prior to the collection.

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

Genomic sequencing as described below will be performed at the Broad Institute.

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 1-2 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(114).

- 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 and immunofluorescence assays

- 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. Subsets of different immune populations (effector/memory CD8 cells, T regulatory cells, dendritic cells, tumor associated macrophages, and Tie-2 expressing monocytes (TEM)) will be identified using immunohistochemical (IHC) and/or immunofluorescence (IF) staining on FFPE tumor slides.

- Pathologic confirmation of PD-L1 status (no PD-L1 assay restriction) is required prior to enrollment on the trial for stratification purposes. Independent central confirmation of PD-L1 status will be performed using the antibody clone SP142, as specified in Section 9.6. 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. PD-L1 IHC assays have 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)(115, 116). Other commercially available IHC PD-L1 antibodies (e.g. 28-8, SP263, 22C3, E1L3N) may be used to compare PDL1 staining.

  As specified in Section 9.6, central confirmation of PD-L1 status will be performed using the antibody clone SP142 on the baseline research biopsy; if a baseline biopsy was not performed, archival tissue will be used for central confirmation of PD-L1 status. Tumor and immune cell expression of PD-L1 will be evaluated at different cut-off points (i.e. 1%, 5%, 10%, 50%). Tumor will be considered positive if ≥1% (PD-L1)(71) of the immune cell infiltrate demonstrates unequivocal staining with SP142 on the Ventana platform. All IHC stained slides will be evaluated by a pathologist and H-scores will be reported. 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.

- FFPE tumor slides will also be assessed by IHC and/or IF assays (e.g. GeoMx DSP) for other markers, including androgen receptor (AR), as deemed appropriate and informative based on the literature at the time of correlative science performance.

Assay 3: Flow cytometry

TILs will be isolated from the biopsy specimen as described in the 17-512 Lab Manual. Surface staining followed by flow cytometry on the resultant TILs will then be performed as described in the 17-512 Lab Manual with a selection of antibodies, as deemed appropriate and informative based on the state of the immune profiling literature at the time of correlative science performance.

Assay 4: RNA analysis:

RNA analysis may be performed, and tissue for RNA analysis will be stored, in the Core Blood and Tissue Bank. 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(56), include CXCL9, CCL5, CD8ACD80, CXCL13, IGKC, CD21, IDO1, PD-1, PD-L1, PD-L2, CTLA4, and FOXP3.

Perou and others have previously shown that within TNBC, exists all of the intrinsic subtypes of breast cancer. Thus, we propose to use the PAM50 algorithm(117) and the Claudin-low predictor(118) in order to determine the intrinsic subtype of each sample. Specifically, using the RNA-seq data, a global normalization step will be performed to adjust the TNBC RNA-seq data obtained in this study relative to the TNBC subset of patients coming from the TCGA RNA-seq study. Once this normalization is complete, the PAM50 algorithm will be run as described in Parker et al. 2009, and then the Claudin-low predictor run as described in Prat et al., thus classifying samples into 1 of 6 intrinsic subtypes. The intrinsic subtype classifications will be tested for associations with disease response to treatment using univariate and multivariate testing, in models that include the standard clinical parameters and with models including other genomic predictors.

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

Assay 5: Whole Exome, Whole Genome sequencing, DNA methylation

DNA sequencing will be performed at the Broad Institute; this may include SNP-arrays for DNA copy number changes as well as exome and/or whole genome sequencing. Tumor biopsy specimens may be assessed using the most current and informative methodologies at the time that correlative science is performed. Potential assays that may be performed on frozen or FFPE metastatic samples and/or FFPE primary samples include:

- Whole Genome sequencing depth of ≥ 40X coverage, or Whole Exome sequencing depth of ≥ 500X coverage.
- AFFY SNP 6.0, or Illumina SNP arrays for genotypes and DNA copy number changes may be performed.
- DNA methylation: Illumina Infinium 450K methylation arrays.

Assay 6: T-cell receptor (TCR) sequencing

DNA will be extracted from tumor tissue samples and submitted to Adaptive Biotechnologies for survey level TCR β-chain sequencing. Tumor biopsy specimens may be assessed using the most current and informative methodologies at the time that correlative science is performed. Potential testing includes: preparation of targeted amplicon libraries by multiplex PCR targeting all TCR β-chain V and J gene segments, and then sequencing using Illumina HiSeq. Data for individual TCR sequences, including V and J gene segment identification and CDR3 sequences, can be obtained for customized analysis of T-cell repertoire diversity dynamics, as previously described(119).

Assay 7: OncoPanel testing

Patients at DF/HCC who provided consent to DFCI #11-104, #17-000 or #05-246, or who underwent clinical OncoPanel testing, will be identified. OncoPanel will be performed at CAMD using DNA extracted from archival FFPE tumor tissue or from a research tumor tissue biopsy. Results, including somatic mutations, copy number variations and structural variants, will be analyzed and associations with clinical outcomes explored. Integrated analysis of results from DNA sequencing (e.g. OncoPanel, WES, WGS, methylation), RNA sequencing (bulk tumor and single-cell/single-nuclei) and proteomics (e.g. IHC/IF) assays will also be performed, and correlated with clinical outcomes.

9.2.5 Sites Performing Correlative Studies and/or Data Analysis

BWH (Including the Immunology-Oncology Pathology Core, Scott Rodig MD, PhD; Center for Advanced Molecular Diagnostics)
Harvard Medical School (Including Peter Sorger PhD, Sandro Santagata MD, PhD)
Dana Farber Cancer Institute (DFCI Center for Immuno-Oncology, DF/HCC Core Blood and Tissue Bank, Translational Immunogenomics Lab)
The Broad Institute of MIT
Nanostring Technologies, Inc
Adaptive Biotechnologies

9.3 Blood Collection

Research blood collection is mandatory for all patients for flow cytometry and potential DNA isolation. The samples will be banked in the DF/HCC Core Blood and Tissue Bank and Center for Immuno-Oncology for these and future research purposes. These specimens will become the property of the DF/HCC.

The following research blood samples are required:

Cycle 1 Day 1:

- 1 lavender top tube (10 mL) for whole blood
- 1 Streck Tube (10 mL) for whole blood
- 5 green top tubes (10 mL each) for whole blood

Every 6 weeks (coinciding with Day 1 of that cycle) for 24 weeks, then every 9 weeks:

- 1 Streck Tube (10 mL) for whole blood
- 5 green top tubes (10 mL each) for whole blood

Off Treatment (if progressive disease):

- 1 Streck Tube (10 mL) for whole blood
- 5 green top tubes (10 mL each) for whole blood

The following research blood samples are optional for patients who come off treatment for a reason other than progressive disease:

Off Treatment (for reason other than progressive disease):

- 1 Streck Tube (10 mL) for whole blood
- 5 green top tubes (10 mL each) for whole blood

Follow Up (every 6-12 weeks):

- 1 Streck Tube (10 mL) for whole blood
- 5 green top tubes (10 mL each) for whole blood

If sample collection is missed at baseline or at the time of progression then the sample should be drawn at a future appointment.

For retreatment patients, research blood samples will be drawn every 6 weeks (coinciding with Day 1 of that cycle) for 24 weeks, then every 9 weeks.

- 1 Streck Tube (10 mL) for whole blood
- 5 green top tubes (10 mL each) for whole blood

For crossover patients, research blood samples will be drawn every **8** weeks (coinciding with Day 1 of that cycle) for 24 weeks, then every **12** weeks.

- 1 Streck Tube (10 mL) for whole blood
- 5 green top tubes (10 mL each) for whole blood

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 X" [X = number of cycle] or "Progressive Disease"). If Green top tubes are unavailable, purple top or CPT tubes are acceptable substitutions.

Complete a Specimen Requisition Form (17-512 Lab Manual) to be sent with the tubes; make a copy of the requisition for the participant's research chart prior to sending.

- Green Top tubes:
  For samples obtained at local sites, these must be processed within 3-4 hrs at ambient temperature immediately after being drawn to Mariano Severgnini. For outside institutions, samples will either be processed on site for batch shipment at the end of the study or will be shipped within 24 hours of collection at ambient temperature overnight to Mariano Severgnini:

  Center for Immuno-Oncology
  Dana-Farber Cancer Institute
  440 Brookline Ave-Mayer 308Boston, MA 02215
  Phone: (617) 632-2421
  Pager: 42093

  Processing of samples will be follow the instructions included in the 17-512 Lab Manual.

- Streck tubes:

  Blood in Streck tubes should be brought/shipped at ambient temperature to the DF/HCC Core Blood and Tissue Bank for processing. Please email the DF/HCC Core Blood and Tissue Bank with patient name, study ID, date of collection, approximate time of collection, and study time point the day prior to collection.

  Core Blood and Tissue Bank
  Brigham and Women's Hospital
  Thorn Building – Room 428
  20 Shattuck Street
  Boston, MA 02215
  dfcibreastbank@partners.org

- Lavender Top tubes:

  Blood in the lavender top (EDTA) tube should be brought/shipped at ambient temperature to the DF/HCC Core Blood and Tissue Bank for processing. Please email the DF/HCC Core Blood and Tissue Bank with patient name, study ID, date of collection, approximate time of collection, and study time point the day prior to collection:

Core Blood and Tissue Bank
Brigham and Women's Hospital
Thorn Building – Room 428
20 Shattuck Street
Boston, MA 02215
dfcibreastbank@partners.org

9.3.1 Immune Markers

Blood will be collected at baseline, every 2 cycles prior to the infusion of study drugs (every 3 cycles after 24 weeks on study treatment), and at time of progression, for evaluation of immune markers. The banked samples will be used to analyze DNA, RNA and protein in future studies.

9.3.1.1 Collection

Five aliquots of 10 ml of whole blood will be collected in Green Top tubes. The blood sample will be collected and processed at baseline, every 2 cycles prior to the infusion of study drugs (every 3 cycles after 24 weeks on study treatment), and at time of progression for evaluation of immune markers.

9.3.1.2 Potential Testing

Assay 1: Flow cytometry

PBMCs will be generated as described in the 17-512 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. A selection of the following antibodies may be used, and/or additional antibodies, as deemed appropriate and informative based on the state of the immune profiling literature at the time of correlative science performance: CD8, PD-1, PD-L1, PD-L2, CD4, FOXP3, CD127.

Assay 2: TCR sequencing

PBMCs will be generated as described in the 17-512 Lab Manual, and used to assess immune cell populations. DNA will be extracted from PBMCs. Blood specimens may be assessed using the most current and informative methodologies at the time that correlative science is performed. Potential testing includes use of the immunoSeq assay for high-throughput sequencing of the CDR3 region of TCR β genes. The distribution of sequencing reads over unique TCR β rearrangements can be used to computationally estimate a lower bound on the number of unique TCR β rearrangements present in each sample, as previously described(120).

9.3.2 Cell-free DNA (cfDNA) analysis

Blood will be collected at baseline, every 6 weeks prior to infusion of study drugs (every 9 weeks after 24 weeks on study treatment) 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.

9.3.2.1 Collection of cfDNA specimen(s)

One 10 ml of whole blood will be collected in Streck Tubes. The blood sample will be collected and processed at baseline, every 6 weeks prior to infusion of study drugs (every 9 weeks after 24 weeks on study treatment) and at time of progression.

9.3.3 Germline Mutational Status

Mutations identified in target genes will be assessed using germline DNA from the baseline blood sample. We will assess germline mutational status of clinically actionable genes related to familial risks of breast, colon, ovarian, endometrial, pancreatic cancers, as well as melanoma. Genes will be analyzed both for single-base changes and large rearrangements by next-generation sequencing. Germline mutational profiling will be included as a secondary correlative analysis in this trial to explore the activity of carboplatin and nivolumab, compared to carboplatin alone, in patients with metastatic TNBC and germline *BRCA1/2* mutations.

9.3.3.1 Collection of germline specimen(s)

An additional 10 ml of whole blood will be collected in a lavender top tube at baseline. The blood sample will be collected and processed at baseline. Fill the lavender top tube completely and immediately mix by gentle inversion 8 to 10 times. Inadequate or delayed mixing may result in inaccurate results. The banked samples will be used to analyze DNA, RNA and protein in future studies.

9.3.4 Sites Performing Correlatives

DFHCC Core Blood and Tissue Bank
The Broad Institute
DFCI CIO Laboratories
Adaptive Biotechnologies
Foundation Medicine

9.4 Stool Collection

9.4.1 Collection

All stool samples will be collected by each patient at home using using a home-based, self-collection and mail method for stool that has been proven to provide nearly equivalent metagenomic and metatranscriptomic data to state-of-the-art fresh-frozen sample-collection protocol(121).

Samples will be collected at the below timepoints:

- Baseline
- After 3-6 weeks of treatment (any time from C2D1-C3D1 is allowed)
- At time of progression for those patients who achieved response (partial or complete) to

treatment
- Arm B participants who elect to cross-over to nivolumab + nab-paclitaxel.

Crossover patients will collect samples at the below timepoints:
- At time of crossover, prior to initiation of nivolumab + nab-paclitaxel
- After 4-8 weeks of crossover treatment (any time from crossover C2D1-C3D1)
- At the time of progression if response observed with nivolumab.

Retreatment patients will only collect a sample at the below timepoint:
- At the time of progression after retreatment, if response was again observed

A patient questionnaire is included in the kits. The questionnaire should be completed at the time of stool collection and mailed back along with the sample. Stool samples and questionnaires that are not collected at the protocol-specified collection time points will not be protocol violations. Crossover and retreatment stool samples are optional.

9.4.2 Handling and shipping of stool specimens

All kits will be provided to the patients at a clinic visit. Patients will also be provided with a mailer in which to return the sample. All samples will be shipped within 24 hours of collection at ambient temperature to Brigham and Women's Hospital/Harvard Cohorts Biorepository, where they will be processed and stored (see 17-512 Lab Manual) until batch shipment for analysis.

9.4.3 Analysis

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 patients with mTNBC treated with and without nivolumab (see Section 13.7.2).

9.4.4 Sites performing correlative analysis

BWH/Harvard Cohorts Biorepository
Massachusetts General Hospital (Clinical and Translational Epidemiology Unit, Andrew Chan MD, MPH)
MicroBiomeDx

9.5 Diet and Physical Activity Assessments

Dietary composition will be assessed through the Block Fat/Sugar/Fruit/Vegetable Screener (Appendix C), supplemented with 3 additional questions about fiber intake from the Block Vegetable/Fruit/Fiber Screener (Appendix D). The dietary assessment (Appendix C) contains 55 total questions and takes about 10-12 minutes to complete. Analysis produces estimates of saturated fat, trans fat, total sugars, "added sugars" (in sweetened cereals, soft drinks, and sweets), fruit and fruit juice, vegetable intake, glycemic load, glycemic index and fiber intake.

Physical activity patterns will be assessed with the Leisure Score Index of Godin Leisure-Time Exercise Questionnaire (LSI). The LSI is a short instrument which asks participants to quantify the number of minutes spent in the last week engaging in vigorous, moderate, and light/mild physical activity. The LSI has undergone extensive reliability and validity testing in a number of cancer and general populations(122). We will be using a modified form of the instrument which includes not only frequency of activity but also exercise duration. Weekly minutes of moderate and vigorous physical activity will be calculated at each time point using standard scoring algorithms.

Please refer to Appendices C and D for the Diet and Physical Activity Assessments.

Assessments will be conducted at the below timepoints (in conjunction with collection of stool samples):

- Baseline
- After 3-6 weeks of treatment (any time from C2D1-C3D1 is allowed)
- At time of progression for those patients who achieved response (partial or complete) to treatment
- Arm B participants who elect to cross-over to nivolumab + nab-paclitaxel.

Crossover patients will complete assessments at the below timepoints:

- At time of crossover, prior to initiation of nivolumab + nab-paclitaxel
- After 4-8 weeks of crossover treatment (any time from crossover C2D1-C3D1)
- At the time of progression if response observed with nivolumab.

Retreatment patients will only collect complete an assessment at the below timepoint:

- At the time of progression after retreatment, if response was again observed

The assessments should be completed at the time of stool collection and mailed back along with the sample and stool questionnaire. Diet and physical activity assessments that are not collected at the protocol-specified collection time points will not be protocol violations. Crossover and retreatment assessments are optional.

9.6 Central Confirmation of PD-L1 Status

PD-L1 status is prospectively evaluated at the time of enrollment using local institutional procedures for testing. Eligibility is determined by local test results. Central confirmation will occur retrospectively. Patients in whom a baseline biopsy was not performed need to provide archival tissue for performing this central test.

9.7 Additional analyses

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 registration unless otherwise specified. Screening assessments occurring within 3 days prior to initiating study treatment do not need to be repeated on Cycle 1 Day 1.

As detailed in the Study Calendar, a negative pregnancy test in women of child-bearing potential must be documented within 14 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 $\pm$ 4 days of the protocol-specified date, unless otherwise noted.

Dose delays of both drugs are allowed per protocol. If Day 1 dosing is delayed, all subsequent assessments will be adjusted accordingly. For patients randomized to the experimental arm, if drugs are administered on different days in the same 21-day cycle, every effort should be made to administer both drugs on the same day in subsequent cycles (all assessments being adjusted accordingly), provided that criteria are met per protocol and that 21 ± 4 days have elapsed since the administration of the previous dose of carboplatin and 21 ± 4 days since the administration of the previous dose of nivolumab.

For crossover and retreatment patients, any baseline assessments (including tumor imaging and research samples) should be completed within 14 days of Day 1 of second course treatment, unless stated otherwise.

First Course Treatment Study Calendar							
	Screening **≤ 4 weeks prior to registration**	**Cycle 1 Day 1**	**Cycle 2 Day 1**	**Cycle 3 Day 1**	**Subsequent Cycles Day 1**	**End of Treatment[j]**	**Follow-Up (every 6 months +/- 1 month)**
Demographics	X						
Medical History[a]	X						
Physical exam[b], performance status	X	X	X	X	X	X	
Concurrent medications (See Section 5.6)	X	X	X	X	X	X	
Adverse Event assessment		X	X	X	X	X	X[m]
Vital signs[c]	X	X	X	X	X	X	
Weight[d]	X	X	X	X	X	X	
Height	X						
Hematology panel[e]	X	X	X	X	X	X	
Chemistry panel[f]	X	X	X	X	X	X	
TSH/fT4	X	X	X	X	X	X	
Coagulation panel (PT/PTT)	X						
Pregnancy test[g]	X	X	X	X	X		
Single 12-lead EKG	X					X	
Tumor Assessment[h]	X			X	X[h]	X[k]	X[k]
Brain MRI[i]	X			X	X[i]	X	X
Research Blood (See Section 9.3)		X		X	X	X[n]	X[n]
Research Biopsy (See Section 9.2)	X		X			X	
Research Stool Sample + Questionnaires (See Section 9.4)	X[l]		X			X[l]	
Archival Tumor Retrieval (See Section 9.1)	X						
Survival Assessment (See Section 5.8)							X

a. Medical history includes clinically significant diseases, surgeries, and cancer history (including prior cancer therapies and procedures).
b. A complete physical examination will be performed at baseline. A limited physical exam will be completed prior to therapy on Day 1 of every cycle beginning with Cycle 1.
c. Vital signs should be collected pre-dose on dosing days and are to include measurements of heart rate, respiratory rate, systolic and diastolic blood pressure while the patient is in a seated position, and oral or tympanic temperature.

d. Weight adjustments for carboplatin will be done according to institutional standards. See Section 5.3.2
e. 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.
f. Chemistry testing includes: sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, calcium, phosphorus, magnesium, total bilirubin, total protein, albumin, ALT, AST, alkaline phosphatase, and LDH. Results must be available prior to the administration of study drug.
g. In female subjects of child-bearing potential as defined in the eligibility criteria, pregnancy test (serum or urine) must be performed within **14 days** before the first dose of study medication, and on Day 1 of each cycle.
h. Tumor assessments should consist of 1) CT and/or MRI of the chest/abdomen/pelvis, 2) any other imaging studies as clinically indicated by the treating physician. Tumor assessments will be performed at baseline, every 2 cycles/6 weeks for the first 24 weeks with a window of -7 days to + 3 days (e.g., between Cycle 2 Day 15 and Cycle 3 Day 4) and then every 3 cycles/9 weeks with a window of -7 days to + 3 days (e.g., between Cycle 11 Day 15 and Cycle 12 Day 4). Additional scans are permitted as clinically indicated. All known sites of disease documented at screening should be re-assessed at each subsequent tumor evaluation. See Section 11.1.3 Participants who have their treatment held or delayed will continue to have restaging scans that align with cycles of treatment received, rather than weeks on study.
i. Brain MRIs are not mandatory at baseline in patients without history of brain metastases and in whom there are no concerning signs or symptoms. Brain MRI should be done with and without contrast. For patients with a history of brain mets, brain MRI is required at baseline and with each restaging; if patient received radiation therapy within 6 weeks of registration, then baseline MRI is not needed. If a participant is unable to have an MRI, a CT of the brain with contrast is acceptable. If CT with contrast is contraindicated, a CT without contrast is acceptable.
j. End of treatment visit is to occur 30-45 days from final administration of study treatment. In the event a participant's condition is deteriorating or new therapy will be initiated within 30 days of the last protocol treatment, end of treatment visit may be performed early (i.e. a window of 15-45 days from final administration of study treatment).
k. For those taken off the treatment for reasons other than progressive disease, tumor measurements should continue to be repeated every 6-12 weeks.
l. Stool sample may be collected within 4 weeks of starting treatment and 4 weeks from progression, as long as no intervening therapy (otherwise, within 2 weeks).
m. An adverse event assessment is performed 100 days (-15/+30 days) after the last dose of nivolumab (Arm A and Crossover patients only). This may be conducted in person or by phone by the investigator or suitably trained and qualified delegate (e.g. MD, RN, NP, etc).
n. Blood samples are mandatory for patients with progressive disease at the end of treatment vist. Blood samples are optional for patients who come off treatment for other reasons, at the end of treatment visit and during follow up. Samples during follow up should be drawn every 6-12 weeks, alongside tumor assessments, for patients who come off treatment for reasons other than progressive disease.

Crossover Therapy Study Calendar: Nivolumab + nab-Paclitaxel															
	Crossover Screening	**Cycle 1 Crossover**			**Cycle 2 Crossover**			**Cycle 3 Crossover**			**Subsequent Cycles Crossover**			**End of Crossover Treatment[j]**	**Follow-Up (every 6 months +/- 1 month)**
	≤ 14 days prior to crossover D1[a]	**Day 1**	**Day 8**	**Day 15**	**Day 1**	**Day 8**	**Day 15**	**Day 1**	**Day 8**	**Day 15**	**Day 1**	**Day 8**	**Day 15**		
Physical exam[b], performance status	X	X		X	X		X	X		X	X		X	X	
Concurrent medications (See Section 5.6)	X	X		X	X		X	X			X			X	
Adverse Event assessment		X		X	X		X	X			X			X	X[m]
Vital signs[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Hematology panel[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Chemistry panel[e]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
TSH/fT4	X	X			X			X			X			X	
Pregnancy test[f]	X	X			X			X			X				
Single 12-lead EKG	X[g]													X	
Tumor Assessment[h]	X							X			X[h]			X[k]	X[k]
Brain MRI[i]	X							X			X[i]			X	X
Research Blood (See Section 9.3)	X[n]							X			X			X[n]	X[n]
Mandatory Research Biopsy (See Section 9.2)	X				X									X	
Research Stool Sample + Questionnaires (See Section 9.4)	X[l]				X									X[l]	
Survival Assessment (See Section 5.8)															X

a. Crossover screening assessments may overlap with the most recent first course treatment visit (i.e end of treatment/progression visit). Note: labs should occur on C1D1 of crossover therapy (See Section 5.7, Crossover Phase, Criteria to Treat)
b. A complete physical examination will be performed at baseline. A limited physical exam will be completed prior to therapy on Days 1 and 15 of every cycle beginning with Cycle 1.

c. Vital signs should be collected pre-dose on dosing days and are to include measurements of heart rate, respiratory rate, systolic and diastolic blood pressure while the patient is in a seated position, and oral or tympanic temperature.
d. 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.
e. Chemistry testing includes: sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, calcium, phosphorus, magnesium, total bilirubin, total protein, albumin, ALT, AST, alkaline phosphatase, and LDH. Results must be available prior to the administration of study drug.
f. In female subjects of child-bearing potential as defined in the eligibility criteria, pregnancy test (serum or urine) must be performed within **7 days** before the first dose of crossover treatment, and on Day 1 of each cycle.
g. If EKG performed at the end of treatment visit on First Course Treatment and within 14 days of crossover C1D1, the EKG does not need to be repeated.
h. Tumor assessments should consist of 1) CT and/or MRI of the chest/abdomen/pelvis, 2) any other imaging studies as clinically indicated by the treating physician. Tumor assessments will be performed at baseline, every 2 cycles/8 weeks for the first 24 weeks with a window of -7 days to + 3 days (e.g., between Cycle 2 Day 15 and Cycle 3 Day 4) and then every 3 cycles/12 weeks with a window of -7 days to + 3 days (e.g., between Cycle 11 Day 15 and Cycle 12 Day 4). Additional scans are permitted as clinically indicated. If tumor assessments performed at the end of treatment visit on First Course Treatment and within 14 days of crossover C1D1, tumor assessments do not need to be repeated during crossover screening. All known sites of disease documented at screening should be re-assessed at each subsequent tumor evaluation. See Section 11.1.3. Participants who have their treatment held or delayed will continue to have restaging scans that align with cycles of treatment received, rather than weeks on study.
i. Brain MRIs are not mandatory during crossover screening in patients without history of brain metastases and in whom there are no concerning signs or symptoms. Brain MRI should be done with and without contrast. For patients with a history of brain mets, brain MRI is required at baseline for crossover and with each restaging. If a participant is unable to have an MRI, a CT of the brain with contrast is acceptable. If CT with contrast is contraindicated, a CT without contrast is acceptable.
o. End of treatment visit is to occur 30-45 days from final administration of crossover study treatment. In the event a participant's condition is deteriorating or new therapy will be initiated within 30 days of the last protocol treatment, end of treatment visit may be performed early (i.e. a window of 15-45 days from final administration of crossover study treatment).
j. For those taken off crossover treatment for reasons other than progressive disease, tumor measurements should continue to be repeated every 6-12 weeks.
k. Stool sample may be collected within 4 weeks of starting treatment and 4 weeks from progression, as long as no intervening therapy (otherwise, within 2 weeks).
l. 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 (e.g. MD, RN, NP, etc).
m. Blood samples are mandatory for crossover patients prior to C1D1 of crossover therapy and for patients with progressive disease at the end of crossover treatment visit. For patients who come off crossover treatment for other reasons, blood samples are optional at the end of treatment visit and during follow up. Samples during follow up should be drawn every 6-12 weeks, alongside tumor assessments, for patients who come off crossover treatment for reasons other than progressive disease.

11. MEASUREMENT OF EFFECT

For the purposes of this study, participants should be re-evaluated for response every 2 cycles for the first 24 weeks and then every 3 cycles thereafter.

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(1) and immune related response criteria(2). 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 present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for target disease response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

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

11.1.2 Disease Parameters

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

Note: Tumor lesions that are situated in a previously irradiated area might be considered measurable if the investigator thinks it appropriate to include them.

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

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

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

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

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

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

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

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

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

11.1.3.1 Evaluation of Target Lesions

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

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

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

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

11.1.3.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization

of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

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

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

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

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

11.1.3.3 Evaluation of New Lesions

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

11.1.3.4 Evaluation of Best Overall Response

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

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

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥ 4 wks Confirmation**
CR	Non-CR/Non-PD	No	PR	≥ 4 wks Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-	No	PR	

	PD/not evaluated			
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥ 4 wks from baseline**
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 Time to Response

Time to objective response: The time to objective response is defined as the time from randomization to the date of the first documented CR or PR (whichever is first recorded).

11.1.6 Clinical Benefit Rate

Clinical Benefit Rate: Clinical Benefit Rate (CBR) is defined as CR, PR and stable disease (SD) for ≥ 24 weeks.

11.1.7 Survival

Overall Survival: Overall Survival (OS) is defined as the time from randomization (or registration) to death due to any cause, or censored at date last known alive.

Progression-Free Survival: Progression-Free Survival (PFS) is defined as the time from randomization (or registration) to the earlier of progression or death due to any cause. Participants alive without disease progression are censored at date of last disease evaluation.

Time to Progression: Time to Progression (TTP) is defined as the time from randomization (or registration) to progression, or censored at date of last disease evaluation for those without progression reported.

11.1.8 Response Review

Central Review will be conducted by the DF/HCC Tumor Imaging Metric Core for DF/HCC Institutions.

11.2 Other Response Parameters

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

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

11.2.1.1 Impact of New Lesions on irRC

New lesions 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.

11.2.1.2 Definition of Target Lesions Response Using irRC

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

11.2.1.3 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).

11.2.1.4 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

11.2.1.5 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.

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 Board (DSMB) will review and monitor study progress, toxicity, safety and other data from this study. The board is chaired by a medical oncologist from outside of DF/HCC and has external and internal representation. Information that raises any questions about participant safety or protocol performance will be addressed by the Overall PI, statistician and study team. Should any major concerns arise, the DSMB will offer recommendations regarding whether or not to suspend the study.

The DSMB will meet twice a year to review accrual, toxicity, response and reporting information. Information to be provided to the DSMB may include: participant accrual; treatment regimen information; adverse events and serious adverse events reported by category; summary of any deaths on study; 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 B.

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

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

This is an open-label, multi-institutional, randomized phase 2 study of carboplatin at AUC 6 intravenously every 3 weeks, with or without nivolumab 360 mg intravenously every 3 weeks, in subjects with metastatic triple-negative breast cancer previously treated with 0 prior lines of therapy in the metastatic setting. The target enrollment is 132 first-line patients; 66 per arm of treatment.

The primary endpoint is to compare in the intent-to-treat study population the progression-free survival (PFS) of carboplatin in combination with nivolumab versus carboplatin alone, in patients with previously untreated metastatic TNBC. PFS is defined according to RECIST 1.1 criteria (Section 11.1).

<u>Secondary endpoints include:</u>

- Overall response rate (ORR) according to RECIST 1.1 (Section 11.1) and immune-related response criteria (irRC) (Section 11.2)
- Overall survival (OS)
- Clinical benefit rate (CBR) according to RECIST 1.1, defined as CR, PR or stable disease for $\geq$ 24 weeks
- Duration of response (DOR) and time to objective response (TTOR)
- Toxicity graded according to NCI CTCAE, Version 4.0

The above endpoints will be assessed in the following study populations:
-Intent-to-treat
-PD-L1-positive
-Germline *BRCA1* or *BRCA2* mutation carriers
-Crossover to nab-paclitaxel plus nivolumab after progression on carboplatin alone

13.2 Sample Size, Accrual Rate and Study Duration

Based on data of recent trials in a similar population of patients with TNBC treated with carboplatin, a median PFS of 3 months would be expected with carboplatin in monotherapy(30, 31). We anticipate that achieving a PFS of at least 5 months for combination therapy (Arm A) in the present study would make the regimen worthy of further investigation. Considering a one-sided 0.05 type-I error and 85% power to detect a 2-month absolute difference in median PFS at the time of final analysis (hazard ratio = 0.6), 132 first-line patients would need to be enrolled on the trial.

The final analysis will occur when 115 PFS events are observed, or 9 months after the last patient is randomized, whichever occurs first. An interim analysis for futility will occur when 50% information is obtained (57 PFS events). The study would stop early if the observed HR from a Cox model >1.0 (Arm A: Arm B). It is estimated that the interim analysis will occur approximately 21 months after the first patient is randomized.

The expected accrual rate is 5-6 patients per month and, considering an estimated enrollment of 40 first-line patients prior to activation of the study amendment to first-line metastatic setting only, accrual of 132 first-line patients is anticipated to be completed at 18 months from activation of the crossover amendment. The expected study duration to primary analysis of PFS is 40 months from study activation.

13.3 Stratification Factors for Secondary Analyses

Considering the differences in clinical activity with carboplatin reported in the TNT trial for patients who carry a germline *BRCA1* or *BRCA2* mutation(31), compared to those without a known *BRCA* mutation, germline *BRCA* status (mutation vs. wild-type/unknown) will be a stratification factor.

Platinum therapy in the neo-/adjuvant setting may preclude resistance to carboplatin upon rechallenge or inability to maintain dose and/or duration of carboplatin due to toxicity. Thus, prior exposure to platinum will be a stratification factor.

In order to avoid a potential selection bias based on PD-L1 status, PD-L1-negative status is restricted to a maximum of 60% of patients randomized on the trial. PD-L1 status will be a stratification factor. In IMpassion130, 40% of patients were found to be PDL1-positive. When restricting PDL1-negative status to 60% of patients enrolled on DFCI 17-512, assuming 40% of PDL1-positive patients (n=48/120) and similar distribution between treatment arms (n=24 per arm), there would be 52% power to demonstrate an improvement in median PFS corresponding to HR 0.60 in the PDL1-positive group.

13.4 Interim Monitoring Plan

We will perform a safety run-in analysis in the first 12 patients randomized to the experimental arm. If there are 4 or more dose-limiting toxicity (DLTs) in the first 12 patients included, enrollment will be halted to discuss whether the study will be amended, with re-evaluation of the appropriate dosing schedule and study design, or closed. Section 5.4 describes the DLT definitions used in this current study, which will be assessed in the first cycle (21 days of cycle 1). In the case that enrollment continues, these first 12 patients will be included in the efficacy analysis.

Additionally, we will perform a safety run-in analysis in the first 12 participants who crossover to nivolumab + nab-paclitaxel. If there are 4 or more dose-limiting toxicity (DLTs) in the first 12 participants treated with nivolumab + nab-paclitaxel, crossover will be halted to discuss whether the study will be amended, with re-evaluation of the appropriate crossover dosing schedule and design, or the option to crossover closed. Section 5.4 describes the DLT definitions used in this current study, which will be assessed in the first cycle of crossover treatment (28 days of cycle 1 crossover). In the case that crossover treatment with nivolumab + nab-paclitaxel continues, these first 12 patients will be included in the efficacy analysis.

An additional interim analysis for futility will occur when 50% information is obtained (after 57 PFS events have occurred). The study would stop early if the Z-statistic from a stratified logrank test is less than 0 (corresponding to an observed HR > 1.0 under a stratified Cox model). It is estimated that the interim analysis will occur at approximately 21 months from study activation.

13.5 Analysis of Primary Endpoint

The primary objective is to compare the median PFS achieved with the experimental combination of carboplatin plus nivolumab versus carboplatin alone, and this will be assessed among all first-line patients who initiated protocol therapy according to the randomized treatment assignment.

PFS is defined as the time from study randomization to disease progression according to 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. Definitive analysis of the efficacy data will be performed when 115 PFS events are recorded and will be based on the intent-to-treat principle where all patients will be analyzed as randomized.

The primary comparison in PFS between the two treatment arms will be performed by the stratified log-rank test with prior exposure to platinum (yes or no), germline *BRCA* status (mutation or wild-type/unknown), and PD-L1 status (positive or negative). Factor(s) will be dropped from the stratified logrank test if an insufficient number of patients/events allow the model to converge. The test will be one-sided with a significance level at 0.05.

The Kaplan-Meier estimates will also be calculated separately for patients allocated to each treatment regimen. Multivariable Cox proportional hazards models will be used to estimate treatment efficacy in PFS with adjusting for potential confounders such as age at study entry, stage

(locally advanced or metastatic), *BRCA* mutation status (positive, negative or unknown), prior exposure to platinum (yes or no) and PD-L1 status (positive or negative). Potential interactions between treatment and clinical factors and biomarkers, including *BRCA* mutation, will be explored in the Cox models. Model diagnostics will be performed to check whether the proportionality assumption is valid in those models. Should it fail, appropriate analysis, such as models with time-dependent effects, will be explored. An exploratory subset analysis in the eligible-only population will also be performed, and results of this analysis will be compared with those from the intent-to-treat population in the primary analysis. Any substantial difference will be carefully examined.

13.6 Analysis of Secondary Endpoints

Efficacy Endpoints
All patients who initiated protocol therapy will also be evaluated for ORR (according to RECIST 1.1 and irRC), for CBR, DOR, TTOR and OS.

Radiographic response will be assessed using RECIST 1.1 criteria and irRC, as defined in section 11. Objective response will require confirmatory scans as indicated. The ORR (CR + PR), according to RECIST 1.1 and irRC, will be reported with 95% exact confidence intervals. Clinical benefit is defined as CR, PR or SD ≥ 24 weeks according to RECIST 1.1. CBR will be reported with 95% exact confidence intervals. Median DOR and TTOR will be reported with ranges. Overall survival will be reported with Kaplan Meier estimates.

For OS, the stratified log-rank test will be one-sided with significance level 0.05. Treatment efficacy in these secondary endpoints will be assessed by Cox models adjusting for clinical factors such as age at study entry, stage (locally advanced or metastatic), *BRCA* mutation status (positive, negative or unknown), prior exposure to platinum (yes or no) and PD-L1 status (positive or negative) Specifically, treatment efficacy, including PFS and these secondary endpoints, will be assessed in patients with known germline *BRCA* mutation. Dichotomous endpoints of response will be compared using Cochran-Maentel-Haenszel chi-squared tests with significance level 0.05.

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

13.7 Correlative endpoints

13.7.1 Tumor biopsies and TIL assessment

Previous studies have demonstrated that, in addition to direct cytotoxic effects, chemotherapy-induced cell death can be immunogenic, causing T cell tumor infiltration and sensitizing tumors to immune checkpoint blockade(123). Cytotoxic CD8+ T cell infiltration has been reported to increase after treatment with carboplatin across different tumor types(124-126). 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(99). Also, modifications in molecular signature of tumor microenvironment also correlated with response rate to this drug.

Tumor-infiltrating lymphocytes (TILs) by histological assessment will be quantitative and evaluated as a percentage of cells stained (0-100%). Data from Loi and colleagues suggest that the mean number of TILs in first-line metastatic TNBC is likely similar to that observed in first-line metastatic HER2-positive samples from the CLEOPATRA study(127). Thus, the median TIL value was 10%, with a mean number of TILs of 17% (standard deviation as a continuous variable = 21%). TILs will be analyzed as a continuous variable using descriptive statistics to summarize the distribution observed among study participants.

A) To assess the relationship between baseline TILs and progression-free survival, we plan to perform a mandatory research biopsy of a safely accesible tumor lesion at baseline. The association of TILs and PFS will be evaluated in a proportional hazards regression model using non-linear cubic spline functions knotted at evenly spaced quintiles and covariates for the treatment effect and study stratification factors(128). Linear and non-linear components of the model will be assessed using Wald-type tests. If a non-linear association is detected, the point estimate and 95% prediction bands will be reported for the log relative hazard over the entire distribution of TILs. If no significant non-linear effects are observed, the step-down model will evaluate the the linear multivariate proportional hazards regression model and report adjust hazard ratio and 95% confidence interval for a linear increase in TILs. In addition, modification of a treatment effect will be explored as an interaction test, and if non-significant (two-sided alpha = 0.05) the step-down model with main effects will be reported. Assuming a 10% assay failure rate (n = 60 evaluable per arm) and variability in TILs as report by Loi and colleagues, there will be 92% power to detect a linear main effect in the nivolumab-containing arm which corresponds to a hazard ratio of 0.8 for every 10% difference in TILs(129). Power calculations were performed using the powerSurvEpi package in R v3.1.1.

B) To assess the absolute change in the percentage of TILs before and after starting treatment, a mandatory biopsy at 3-6 weeks post-treatment (any time between C2D1 and C3D1) is required, if tumor is safely accessible. This on-treatment biopsy will be performed in, at least, the first 36 patients with accessible disease in each arm. Assuming the drop-out/non-evaluable rate will be 10%, 32 paired samples will be available. Descriptive statistics will be used to summarize the distribution of TILs observed at baseline and after 3-6 weeks of therapy (e.g. mean, standard deviation, median, and inter-quartile range). The evaluation of change in TILs within each arm will be based on a Wilcoxon signed rank test (absolute difference) using a one-sided alpha = 0.1 for each arm. Based on variability of TILs reported by Loi and colleagues in paired specimens before and after treatment with chemotherapy for TNBC, with 32 evaluable paired specimens anticipated per arm, there will be an 80% power to detect an increase in TILs from 22.3% to 30.9%, under an assumption of an additive shift model with a Gaussian common density function.

Table of Target Effects with Different Sample Sizes per Arm

N required biopsy (per arm)	**Drop-out/non-evaluable rate**	**N evaluable (per arm)**	**Δ TILs**
32	10%	28	9.2

34	10%	30	8.9
36	10%	32	8.6

If paired biopsies at baseline and at 3-6 weeks after treatment are obtained in 59 paired samples in each arm (assuming a drop-out/non-evaluable rate of 10% in the entire study population), there will be statistical power to detect the following change in TILs between both study arms. Assuming that the change in TILs from pre-treatment to 3-6 weeks after starting protocol therapy is 38% (Δ TILs = 8.6) in Arm A (Carboplatin+Nivolumab) and 20% (Δ TILs = 4.5) in Arm B (Carboplatin alone), with a standard deviation of 8, using a Wilcoxon-Mann-Whitney test with one-sided alpha of 0.1, the study has 91% power to detect the difference of change in TILs between Arm A (Carboplatin+Nivolumab) and Arm B (Carboplatin alone).

C) For secondary correlative endpoints to characterize the expression of tumor markers by immunohistochemistry (IHC) and/or immunofluorescence (IF), descriptive statistics and agglomerative hierarchical clustering techniques will be used to summarize the distribution and patterns of profiles observed in baseline samples. Tests of association with clinical outcomes (PFS, OS, and response) will be exploratory using Cox proportional hazard and logistic regression models, respective, and inferences will use Benjaminin-Hochberg step-up tests to control the false discovery rate with multiplex assays, and any positive findings will require validation in future studies with independent samples for unbiased estimates of the degress of association with treatment effects and clinical outcomes.

Optional repeat biopsies will be performed at time of progression, to explore changes in TILs and other tissue markers. Descriptive statistics will be used to summarize the change in TILs, and inferences on treatment-effects will use non-parametric tests (e.g. Wilcoxon rank sum) with two-sided alpha 0.05. The association of changes in TILs with clinical outcomes of PFS and OS will be exploratory and hypothesis generating in the subset of patients with evaluable repeat biopsies. Analysis plans will consider using Cox models with time-varying covariates to account for the post-baseline assessments.

D) Single-cell/single-nuclei RNA sequencing will be performed on biopsies at baseline, on treatment, and, when available, at progression for a subset of first-line therapy patients, and a subset of patients who elect to crossover to nab-paclitaxel plus nivolumab, enrolled at DFCI to assess the relationship of timepoint-specific tumor and immune cell subpopulations with clinical outcomes in exploratory analyses. In addition, changes in single-cell/single-nuclei RNA expression signatures from baseline will be correlated with response. Standard preprocessing and quality control algorithms will be performed to filter out low quality cells and to normalize the expression data. Differentially expressed genes will be identified using the Benjamini-Hochberg procedure to control the false discovery rate for multiple comparisons. Descriptive statistics and clustering techniques, including principal component analysis and t-distributed stochastic neighbor embedding, will be employed to summarize the distributions of the expression data and identify tumor and immune cell subpopulations. Changes in cell subpopulations between baseline, on-treatment, and at progression biopsies will be assessed with Wilcoxon signed-rank tests, while differences in cell subpopulations between responders and non-responders will be compared with Mann-Whitney tests. Preliminary associations of tumor and immune cell

subpopulations with progression-free and overall survival will be explored using Cox proportional hazard models.

13.7.2 Analysis of intestinal microbiome

Overall, we plan to describe the landscape of gut microbiota in patients with mTNBC, and the changes in their gut microbiota after two cycles of treatment and, if available, at time of progression. 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 Wilcoxon 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. Diversity of gut microbiota will be estimated by Shannon index. In order to explore the association of response (objective response according RECIST 1.1 and progression-free survival) to baseline microbiota diversity, and changes from baseline in microbiota, inference will be based on Wilcoxon rank sum tests and estimates of predictive value along the continuous scales will be visualized using receiver operating characteristic (ROC) curves and reported with c-index and confidence intervals derived from variance estimates of Somers rank correlation.

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 patients with mTNBC, treated with and without nivolumab. 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.

To assess the relationships between α-diversity and physical activity, dietary components, or BMI, we will divide the diversity measures into tertiles or at the median and compare the clinical characteristics according to the resulting diversity classes.

13.8 Reporting and Exclusions

13.8.1 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.

For the primary endpoint of this trial, patients with previously untreated metastatic TNBC who initiate protocol therapy (as first-line therapy in the metastatic setting), enrolled prior or post-crossover amendment, will be included. An exploratory efficacy evaluation will be conducted in all second-line patients who initiate protocol therapy (second-line carboplatin plus nivolumab prior to crossover amendment and second-line nab-paclitaxel plus

nivolumab after crossover amendment).

13.8.2 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.

15. REFERENCES

1. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). European journal of cancer (Oxford, England : 1990). 2009;45(2):228-47.
2. Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbe C, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res. 2009;15(23):7412-20.
3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA: a cancer journal for clinicians. 2016;66(1):7-30.
4. Perou CM. Molecular Stratification of Triple-Negative Breast Cancers. The Oncologist. 2010;15(Supplement 5):39-48.
5. O'Brien KM, Cole SR, Tse CK, Perou CM, Carey LA, Foulkes WD, et al. Intrinsic breast tumor subtypes, race, and long-term survival in the Carolina Breast Cancer Study. Clinical cancer research : an official journal of the American Association for Cancer Research. 2010;16(24):6100-10.
6. Liedtke C, Mazouni C, Hess KR, Andre F, Tordai A, Mejia JA, et al. Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. J Clin Oncol. 2008;26(8):1275-81.
7. Ono M, Tsuda H, Shimizu C, Yamamoto S, Shibata T, Yamamoto H, et al. Tumor-infiltrating lymphocytes are correlated with response to neoadjuvant chemotherapy in triple-negative breast cancer. Breast Cancer Res Treat. 2012;132(3):793-805.
8. Liu S, Lachapelle J, Leung S, Gao D, Foulkes WD, Nielsen TO. CD8+ lymphocyte infiltration is an independent favorable prognostic indicator in basal-like breast cancer. Breast Cancer Res. 2012;14(2):R48.
9. Andre F, Zielinski CC. Optimal strategies for the treatment of metastatic triple-negative breast cancer with currently approved agents. Annals of Oncology. 2012;23(suppl 6):vi46-vi51.
10. Sparano JA, Makhson AN, Semiglazov VF, Tjulandin SA, Balashova OI, Bondarenko IN, et al. Pegylated Liposomal Doxorubicin Plus Docetaxel Significantly Improves Time to Progression Without Additive Cardiotoxicity Compared With Docetaxel Monotherapy in Patients With Advanced Breast Cancer Previously Treated With Neoadjuvant-Adjuvant Anthracycline Therapy: Results From a Randomized Phase III Study. Journal of Clinical Oncology. 2009;27(27):4522-9.
11. Dear RF, McGeechan K, Jenkins MC, Barratt A, Tattersall MH, Wilcken N. Combination versus sequential single agent chemotherapy for metastatic breast cancer. Cochrane Database Syst Rev. 2013(12):CD008792.
12. Sledge GW, Neuberg D, Bernardo P, Ingle JN, Martino S, Rowinsky EK, et al. Phase III trial of doxorubicin, paclitaxel, and the combination of doxorubicin and paclitaxel as front-line chemotherapy for metastatic breast cancer: an intergroup trial (E1193). J Clin Oncol. 2003;21(4):588-92.
13. Gennari A, Stockler M, Puntoni M, Sormani M, Nanni O, Amadori D, et al. Duration

of chemotherapy for metastatic breast cancer: a systematic review and meta-analysis of randomized clinical trials. J Clin Oncol. 2011;29(16):2144-9.
14. Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science. 2018;359(6371):97-103.
15. Kaufman PA, Awada A, Twelves C, Yelle L, Perez EA, Velikova G, et al. Phase III open-label randomized study of eribulin mesylate versus capecitabine in patients with locally advanced or metastatic breast cancer previously treated with an anthracycline and a taxane. J Clin Oncol. 2015;33(6):594-601.
16. Robert NJ, Dieras V, Glaspy J, Brufsky AM, Bondarenko I, Lipatov ON, et al. RIBBON-1: randomized, double-blind, placebo-controlled, phase III trial of chemotherapy with or without bevacizumab for first-line treatment of human epidermal growth factor receptor 2-negative, locally recurrent or metastatic breast cancer. J Clin Oncol. 2011;29(10):1252-60.
17. Gray R, Bhattacharya S, Bowden C, Miller K, Comis RL. Independent review of E2100: a phase III trial of bevacizumab plus paclitaxel versus paclitaxel in women with metastatic breast cancer. J Clin Oncol. 2009;27(30):4966-72.
18. Burstein HJ, Elias AD, Rugo HS, Cobleigh MA, Wolff AC, Eisenberg PD, et al. Phase II study of sunitinib malate, an oral multitargeted tyrosine kinase inhibitor, in patients with metastatic breast cancer previously treated with an anthracycline and a taxane. J Clin Oncol. 2008;26(11):1810-6.
19. Baselga J, Gomez P, Greil R, Braga S, Climent MA, Wardley AM, et al. Randomized phase II study of the anti-epidermal growth factor receptor monoclonal antibody cetuximab with cisplatin versus cisplatin alone in patients with metastatic triple-negative breast cancer. J Clin Oncol. 2013;31(20):2586-92.
20. Stemmler HJ, diGioia D, Freier W, Tessen HW, Gitsch G, Jonat W, et al. Randomised phase II trial of gemcitabine plus vinorelbine vs gemcitabine plus cisplatin vs gemcitabine plus capecitabine in patients with pretreated metastatic breast cancer. British Journal of Cancer. 2011;104(7):1071-8.
21. Fountzilas G, Athanassiadis A, Kalogera-Fountzila A, Aravantinos G, Bafaloukos D, Briasoulis E, et al. Paclitaxel by 3-h infusion and carboplatin in anthracycline-resistant advanced breast cancer. A phase II study conducted by the Hellenic Cooperative Oncology Group. Eur J Cancer. 1997;33(11):1893-5.
22. Perez EA, Hillman DW, Stella PJ, Krook JE, Hartmann LC, Fitch TR, et al. A phase II study of paclitaxel plus carboplatin as first-line chemotherapy for women with metastatic breast carcinoma. Cancer. 2000;88(1):124-31.
23. Loesch D, Robert N, Asmar L, Gregurich MA, O'Rourke M, Dakhil S, et al. Phase II multicenter trial of a weekly paclitaxel and carboplatin regimen in patients with advanced breast cancer. J Clin Oncol. 2002;20(18):3857-64.
24. Fountzilas G, Kalofonos HP, Dafni U, Papadimitriou C, Bafaloukos D, Papakostas P, et al. Paclitaxel and epirubicin versus paclitaxel and carboplatin as first-line chemotherapy in patients with advanced breast cancer: a phase III study conducted by the Hellenic Cooperative Oncology Group. Ann Oncol. 2004;15(10):1517-26.
25. Fountzilas G, Dafni U, Dimopoulos MA, Koutras A, Skarlos D, Papakostas P, et al. A randomized phase III study comparing three anthracycline-free taxane-based regimens, as first line chemotherapy, in metastatic breast cancer: a Hellenic Cooperative Oncology Group study. Breast Cancer Res Treat. 2009;115(1):87-99.

26. Loesch D, Asmar L, McIntyre K, Doane L, Monticelli M, Paul D, et al. Phase II trial of gemcitabine/carboplatin (plus trastuzumab in HER2-positive disease) in patients with metastatic breast cancer. Clin Breast Cancer. 2008;8(2):178-86.
27. Maisano R, Zavettieri M, Azzarello D, Raffaele M, Maisano M, Bottari M, et al. Carboplatin and gemcitabine combination in metastatic triple-negative anthracycline- and taxane-pretreated breast cancer patients: a phase II study. J Chemother. 2011;23(1):40-3.
28. O'Shaughnessy J, Osborne C, Pippen JE, Yoffe M, Patt D, Rocha C, et al. Iniparib plus chemotherapy in metastatic triple-negative breast cancer. N Engl J Med. 2011;364(3):205-14.
29. Byrski T, Huzarski T, Dent R, Gronwald J, Zuziak D, Cybulski C, et al. Response to neoadjuvant therapy with cisplatin in BRCA1-positive breast cancer patients. Breast Cancer Res Treat. 2009;115(2):359-63.
30. Isakoff SJ, Mayer EL, He L, Traina TA, Carey LA, Krag KJ, et al. TBCRC009: A Multicenter Phase II Clinical Trial of Platinum Monotherapy With Biomarker Assessment in Metastatic Triple-Negative Breast Cancer. J Clin Oncol. 2015;33(17):1902-9.
31. Tutt A, Ellis P, Kilburn L, Gilett C, Pinder S, Abraham J, et al. Abstract S3-01: The TNT trial: A randomized phase III trial of carboplatin (C) compared with docetaxel (D) for patients with metastatic or recurrent locally advanced triple negative or BRCA1/2 breast cancer (CRUK/07/012). Cancer Research. 2015;75(9 Supplement):S3-01-S3-.
32. Schreiber RD, Old LJ, Smyth MJ. Cancer Immunoediting : Integrating Suppression and Promotion. (i).
33. Schreiber RD. Cancer Immunoediting : Integrating Suppression and Promotion. 2012;1565(2011).
34. Mlecnik B, Bindea G, Angell HK, Berger A, Lagorce C, Lugli A, et al. Towards the introduction of the ‘ Immunoscore ’. 2014:199-209.
35. Tosolini M, Camus M, Berger A, Wind P, Lagorce-page C. References and Notes 1. 2006;313(September):1960-5.
36. Denkert C, von Minckwitz G, Brase JC, Sinn BV, Gade S, Kronenwett R, et al. Tumor-Infiltrating Lymphocytes and Response to Neoadjuvant Chemotherapy With or Without Carboplatin in Human Epidermal Growth Factor Receptor 2-Positive and Triple-Negative Primary Breast Cancers. Journal of Clinical Oncology. 2015;33(9):983-91.
37. Adams S, Gray RJ, Demaria S, Goldstein L, Perez Ea, Shulman LN, et al. Prognostic Value of Tumor-Infiltrating Lymphocytes in Triple-Negative Breast Cancers From Two Phase III Randomized Adjuvant Breast Cancer Trials: ECOG 2197 and ECOG 1199. Journal of Clinical Oncology. 2014;32(27):JCO.2013.55.0491--JCO.2013.55.0491-.
38. Pedoeem A, Azoulay-Alfaguter I, Strazza M, Silverman GJ, Mor A. Programmed death-1 pathway in cancer and autoimmunity. Clin Immunol. 2014;153(1):145-52.
39. Zhang X, Schwartz JC, Guo X, Bhatia S, Cao E, Lorenz M, et al. Structural and functional analysis of the costimulatory receptor programmed death-1. Immunity. 2004;20(3):337-47.
40. Lazar-Molnar E, Yan Q, Cao E, Ramagopal U, Nathenson SG, Almo SC. Crystal structure of the complex between programmed death-1 (PD-1) and its ligand PD-L2. Proc Natl Acad Sci U S A. 2008;105(30):10483-8.
41. Lin DY, Tanaka Y, Iwasaki M, Gittis AG, Su HP, Mikami B, et al. The PD-1/PD-L1 complex resembles the antigen-binding Fv domains of antibodies and T cell receptors. Proc Natl Acad Sci U S A. 2008;105(8):3011-6.

42. Cheng X, Veverka V, Radhakrishnan A, Waters LC, Muskett FW, Morgan SH, et al. Structure and interactions of the human programmed cell death 1 receptor. J Biol Chem. 2013;288(17):11771-85.
43. Sheppard KA, Fitz LJ, Lee JM, Benander C, George JA, Wooters J, et al. PD-1 inhibits T-cell receptor induced phosphorylation of the ZAP70/CD3zeta signalosome and downstream signaling to PKCtheta. FEBS Lett. 2004;574(1-3):37-41.
44. Ott PA, Hodi FS, Robert C. CTLA-4 and PD-1/PD-L1 blockade: new immunotherapeutic modalities with durable clinical benefit in melanoma patients. Clin Cancer Res. 2013;19(19):5300-9.
45. Yao S, Chen L. PD-1 as an immune modulatory receptor. Cancer J. 2014;20(4):262-4.
46. Nishimura H, Agata Y, Kawasaki A, Sato M, Imamura S, Minato N, et al. Developmentally regulated expression of the PD-1 protein on the surface of double-negative (CD4-CD8-) thymocytes. Int Immunol. 1996;8(5):773-80.
47. Huang X, Venet F, Wang YL, Lepape A, Yuan Z, Chen Y, et al. PD-1 expression by macrophages plays a pathologic role in altering microbial clearance and the innate inflammatory response to sepsis. Proc Natl Acad Sci U S A. 2009;106(15):6303-8.
48. Pena-Cruz V, McDonough SM, Diaz-Griffero F, Crum CP, Carrasco RD, Freeman GJ. PD-1 on immature and PD-1 ligands on migratory human Langerhans cells regulate antigen-presenting cell activity. J Invest Dermatol. 2010;130(9):2222-30.
49. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol. 2008;26:677-704.
50. Karim R, Jordanova ES, Piersma SJ, Kenter GG, Chen L, Boer JM, et al. Tumor-expressed B7-H1 and B7-DC in relation to PD-1+ T-cell infiltration and survival of patients with cervical carcinoma. Clin Cancer Res. 2009;15(20):6341-7.
51. Taube JM, Anders RA, Young GD, Xu H, Sharma R, McMiller TL, et al. Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Sci Transl Med. 2012;4(127):127ra37.
52. Sanmamed MF, Chen L. Inducible expression of B7-H1 (PD-L1) and its selective role in tumor site immune modulation. Cancer J. 2014;20(4):256-61.
53. Topalian SL, Drake CG, Pardoll DM. Targeting the PD-1/B7-H1(PD-L1) pathway to activate anti-tumor immunity. Curr Opin Immunol. 2012;24(2):207-12.
54. Kroemer G, Senovilla L, Galluzzi L, André F, Zitvogel L. review Natural and therapy-induced immunosurveillance in breast cancer. Nature Publishing Group. 2015;21(10):1128-38.
55. Denkert C, Loibl S, Noske A, Roller M, Muller BM, Komor M, et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol. 2010;28(1):105-13.
56. Denkert C, von Minckwitz G, Brase JC, Sinn BV, Gade S, Kronenwett R, et al. Tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2-positive and triple-negative primary breast cancers. J Clin Oncol. 2015;33(9):983-91.
57. Loi S, Sirtaine N, Piette F, Salgado R, Viale G, Van Eenoo F, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin

with doxorubicin-based chemotherapy: BIG 02-98. Journal of Clinical Oncology. 2013;31(7):860-7.
58. Dushyanthen S, Beavis PA, Savas P, Teo ZL, Zhou C, Mansour M, et al. Relevance of tumor-infiltrating lymphocytes in breast cancer. BMC Medicine. 2015;1:1-13.
59. Gatalica Z, Snyder C, Maney T, Ghazalpour A, Holterman DA, Xiao N, et al. Programmed cell death 1 (PD-1) and its ligand (PD-L1) in common cancers and their correlation with molecular cancer type. Cancer Epidemiol Biomarkers Prev. 2014;23(12):2965-70.
60. Emens L, Adams S, Loi S, Schmid P, Schneeweiss A, Rugo H, et al. Abstract OT1-01-06: A phase III randomized trial of atezolizumab in combination with nab-paclitaxel as first line therapy for patients with metastatic triple-negative breast cancer (mTNBC). Cancer Research. 2016;76(4 Supplement):OT1-01-6.
61. Emens LA, Braiteh FS, Cassier P, DeLord J-P, Eder JP, Shen X, et al. Abstract PD1-6: Inhibition of PD-L1 by MPDL3280A leads to clinical activity in patients with metastatic triple-negative breast cancer. Cancer Research. 2015;75(9 Supplement):PD1-6.
62. Nanda R, Chow LQM, Dees EC, Berger R, Gupta S, Geva R, et al. Pembrolizumab in Patients With Advanced Triple-Negative Breast Cancer: Phase Ib KEYNOTE-012 Study. Journal of Clinical Oncology. 2016;34(21):2460-7.
63. Dirix L, Takacs I, Nikolinakos P, Jerusalem G, Arkenau H-T, Hamilton E, et al. Abstract S1-04: Avelumab (MSB0010718C), an anti-PD-L1 antibody, in patients with locally advanced or metastatic breast cancer: A phase Ib JAVELIN solid tumor trial. Cancer Research. 2016;76(4 Supplement):S1-04-S1-.
64. Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al. Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer. N Engl J Med. 2018;379(22):2108-21.
65. Administration FaD. Nivolumab (Opdivo): Highlights of Prescribing Information http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/125527s000lbl.pdf2015 [
66. Nishino M, Giobbie-Hurder A, Hatabu H, Ramaiya NH, Hodi FS. Incidence of Programmed Cell Death 1 Inhibitor-Related Pneumonitis in Patients With Advanced Cancer: A Systematic Review and Meta-analysis. JAMA Oncol. 2016;2(12):1607-16.
67. Kok M, Voorwerk L, Horlings H, Sikorska K, van der Vijver K, Slagter M, et al. Adaptive phase II randomized trial of nivolumab after induction treatment in triple negative breast cancer (TONIC trial): Final response data stage I and first translational data. Journal of Clinical Oncology. 2018;36(15_suppl):1012-.
68. Kelland L. The resurgence of platinum-based cancer chemotherapy. Nat Rev Cancer. 2007;7(8):573-84.
69. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nature reviews Cancer. 2012;12(4):252-64.
70. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366(26):2455-65.
71. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366(26):2443-54.
72. Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer Cell. 2015;27(4):450-61.
73. Savas P, Salgado R, Denkert C, Sotiriou C, Darcy PK, Smyth MJ, et al. Clinical

relevance of host immunity in breast cancer: from TILs to the clinic. Nature reviews Clinical oncology. 2015.
74. Carbognin L, Pilotto S, Nortilli R, Brunelli M, Nottegar A, Sperduti I, et al. Predictive and Prognostic Role of Tumor-Infiltrating Lymphocytes for Early Breast Cancer According to Disease Subtypes: Sensitivity Analysis of Randomized Trials in Adjuvant and Neoadjuvant Setting. Oncologist. 2016;21(3):283-91.
75. Dieci MV, Criscitiello C, Goubar A, Viale G, Conte P, Guarneri V, et al. Prognostic value of tumor-infiltrating lymphocytes on residual disease after primary chemotherapy for triple-negative breast cancer: a retrospective multicenter study. Ann Oncol. 2015;26(7):1518.
76. Gatalica Z, Snyder C, Maney T, Ghazalpour a, Holterman Da, Xiao N, et al. Programmed Cell Death 1 (PD-1) and Its Ligand (PD-L1) in Common Cancers and Their Correlation with Molecular Cancer Type. Cancer Epidemiology Biomarkers & Prevention. 2014;23(12):2965-70.
77. Chen DS, Mellman I. Oncology meets immunology: The cancer-immunity cycle. Immunity. 2013;39(1):1-10.
78. Adams S, Diamond JR, Hamilton EP, Pohlmann PR, Tolaney SM, Molinero L, et al., editors. Phase Ib trial of atezolizumab in combination with nab-paclitaxel in patients with metastatic triple-negative breast cancer (mTNBC). 2016 American Society of Clinical Oncology (ASCO) Annual Meeting; 2016; Chicago, IL (USA): J Clin Oncol 34, 2016 (suppl; abstr 1009).
79. Bracci L, Schiavoni G, Sistigu A, Belardelli F. Immune-based mechanisms of cytotoxic chemotherapy: implications for the design of novel and rationale-based combined treatments against cancer. Cell death and differentiation. 2014;21(1):15-25.
80. Hato SV, Khong A, de Vries IJ, Lesterhuis WJ. Molecular pathways: the immunogenic effects of platinum-based chemotherapeutics. Clinical cancer research : an official journal of the American Association for Cancer Research. 2014;20(11):2831-7.
81. Tutt A, Ellis P, Kilburn L, Gilett C, Pinder S, Abraham J, et al. TNT: A randomized phase III trial of carboplatin (C) compared with docetaxel (D) for patients with metastatic or recurrent locally advanced triple negative or BRCA1/2 breast cancer. San Antonio Breast Cancer Symposium. 2014:Abstr S3-01.
82. Bhattacharyya A, Ear US, Koller BH, Weichselbaum RR, Bishop DK. The breast cancer susceptibility gene BRCA1 is required for subnuclear assembly of Rad51 and survival following treatment with the DNA cross-linking agent cisplatin. The Journal of biological chemistry. 2000;275(31):23899-903.
83. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. The New England journal of medicine. 2015;372(26):2509-20.
84. Rizvi NA, Hellmann MD, Brahmer JR, Juergens RA, Borghaei H, Gettinger S, et al. Nivolumab in Combination With Platinum-Based Doublet Chemotherapy for First-Line Treatment of Advanced Non-Small-Cell Lung Cancer. J Clin Oncol. 2016;34(25):2969-79.
85. Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol. 2010;28(19):3167-75.
86. Kanda S, Goto K, Shiraishi H, Kubo E, Tanaka A, Utsumi H, et al. Safety and efficacy of nivolumab and standard chemotherapy drug combination in patients with advanced non-small-cell lung cancer: a four arms phase Ib study. Ann Oncol. 2016;27(12):2242-50.

87. Long GV, Tykodi SS, Schneider JG, Garbe C, Gravis G, Rashford M, et al. Assessment of nivolumab exposure and clinical safety of 480 mg every 4 weeks flat-dosing schedule in patients with cancer. Ann Oncol. 2018;29(11):2208-13.
88. Wainberg ZA, Hochster HS, Kim EJ-H, George B, Kalyan A, Chiorean EG, et al. Phase I study of nivolumab (Nivo) + nab-paclitaxel (nab-P) + gemcitabine (Gem) in advanced pancreatic cancer (APC). Journal of Clinical Oncology. 2019;37(4_suppl):298-.
89. Goldman JW, Waterhouse D, George B, O'Dwyer P, Chen T, Trunova N, et al. P1.03-026 Interim Results of a Phase I Study of Nivolumab plus Nab-Paclitaxel/Carboplatin in Patients with NSCLC. Journal of Thoracic Oncology. 2017;12(11):S1960-S1.
90. Sharma P, Allison JP. The future of immune checkpoint therapy. 2015;348(6230).
91. Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, et al. new england journal. 2012:2443-54.
92. Hwu W-j, Ph D, Topalian SL, Hwu P, Chen S, Ph D, et al. Safety and Activity of Anti–PD-L1 Antibody in Patients with Advanced Cancer. 2012:2455-65.
93. Gajewski TF. The Next Hurdle in Cancer Immunotherapy_ Overcoming the Non–T-Cell–Inflamed Tumor Microenvironment. Seminars in Oncology. 2015;42(4):663-71.
94. Ribas A. Adaptive Immune Resistance : How Cancer Protects from Immune Attack. 2015(September):915-20.
95. Perez EA, Thompson EA, Ballman KV, Anderson SK, Asmann YW. Genomic Analysis Reveals That Immune Function Genes Are Strongly Linked to Clinical Outcome in the North Central Cancer Treatment Group N9831 Adjuvant Trastuzumab Trial. 2015;33(7).
96. Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Annals of Oncology. 2014;26(2):259-71.
97. Ali HR, Provenzano E, Dawson S, Blows FM, Liu B, Shah M, et al. Association between CD8 + T-cell in fi ltration and breast cancer survival in 12 439 patients. 2014(June):1536-43.
98. Denkert C, Loibl S, Noske A, Roller M, Mu BM, Komor M, et al. J OURNAL OF C LINICAL O NCOLOGY Tumor-Associated Lymphocytes As an Independent Predictor of Response to Neoadjuvant Chemotherapy in Breast Cancer. 2015;28(1):105-14.
99. Herbst RS, Soria J-c, Kowanetz M, Fine GD, Hamid O, Gordon MS, et al. antibody MPDL3280A in cancer patients. Nature. 2014;515(7528):563-7.
100. Trinchieri G. Cancer Immunity: Lessons From Infectious Diseases. J Infect Dis. 2015;212 Suppl 1:S67-73.
101. Human Microbiome Project C. Structure, function and diversity of the healthy human microbiome. Nature. 2012;486(7402):207-14.
102. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010;464(7285):59-65.
103. Roy S, Trinchieri G. Microbiota: a key orchestrator of cancer therapy. Nat Rev Cancer. 2017.
104. Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Science. 2015;350(6264):1084-9.
105. Vetizou M, Pitt JM, Daillere R, Lepage P, Waldschmitt N, Flament C, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science.

2015;350(6264):1079-84.
106. Goedert JJ, Jones G, Hua X, Xu X, Yu G, Flores R, et al. Investigation of the association between the fecal microbiota and breast cancer in postmenopausal women: a population-based case-control pilot study. J Natl Cancer Inst. 2015;107(8).
107. Liu K, Lu C. Gut microbes modulate host response to immune checkpoint inhibitor cancer immunotherapy. Transl Cancer Res. 2018;7(Suppl 5):S608-S10.
108. Matson V, Fessler J, Bao R, Chongsuwat T, Zha Y, Alegre ML, et al. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. Science. 2018;359(6371):104-8.
109. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillere R, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science. 2018;359(6371):91-7.
110. Alvarez-Mercado AI, Navarro-Oliveros M, Robles-Sanchez C, Plaza-Diaz J, Saez-Lara MJ, Munoz-Quezada S, et al. Microbial Population Changes and Their Relationship with Human Health and Disease. Microorganisms. 2019;7(3).
111. Fayfman M, Flint K, Srinivasan S. Obesity, Motility, Diet, and Intestinal Microbiota-Connecting the Dots. Curr Gastroenterol Rep. 2019;21(4):15.
112. Ticinesi A, Lauretani F, Tana C, Nouvenne A, Ridolo E, Meschi T. Exercise and immune system as modulators of intestinal microbiome: implications for the gut-muscle axis hypothesis. Exerc Immunol Rev. 2019;25:84-95.
113. Gopalakrishnan V, C S, J M, al e, editors. The gut microbiome of metastatic melanoma patients initiating systemic therapy is influenced by host factors including diet, probiotic and antiobotic use. Society for Immunotherapy of Cancer; 2018; Washington, DC.
114. Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol. 2015;26(2):259-71.
115. Chen BJ, Chapuy B, Ouyang J, Sun HH, Roemer MG, Xu ML, et al. PD-L1 expression is characteristic of a subset of aggressive B-cell lymphomas and virus-associated malignancies. Clinical cancer research : an official journal of the American Association for Cancer Research. 2013;19(13):3462-73.
116. Shi M, Roemer MG, Chapuy B, Liao X, Sun H, Pinkus GS, et al. Expression of programmed cell death 1 ligand 2 (PD-L2) is a distinguishing feature of primary mediastinal (thymic) large B-cell lymphoma and associated with PDCD1LG2 copy gain. The American journal of surgical pathology. 2014;38(12):1715-23.
117. Parker JS, Mullins M, Cheang MC, Leung S, Voduc D, Vickery T, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol. 2009;27(8):1160-7.
118. Prat A, Parker JS, Karginova O, Fan C, Livasy C, Herschkowitz JI, et al. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Res. 2010;12(5):R68.
119. Riaz N, Havel JJ, Makarov V, Desrichard A, Urba WJ, Sims JS, et al. Tumor and Microenvironment Evolution during Immunotherapy with Nivolumab. Cell. 2017;171(4):934-49 e16.
120. Robins HS, Campregher PV, Srivastava SK, Wacher A, Turtle CJ, Kahsai O, et al. Comprehensive assessment of T-cell receptor beta-chain diversity in alphabeta T cells. Blood. 2009;114(19):4099-107.
121. Franzosa EA, Morgan XC, Segata N, Waldron L, Reyes J, Earl AM, et al. Relating

the metatranscriptome and metagenome of the human gut. Proceedings of the National Academy of Sciences of the United States of America. 2014;111(22):E2329-38.
122. Jacobs D, Ainsworth B, Hartman T, Leon A. A simultaneous evaluation of 10 commonly used physical activity questionnaires. Medicine & Science in Sports & Exercise. 1993;25:81-91.
123. Pfirschke C, Engblom C, Rickelt S, Cortez-Retamozo V, Garris C, Pucci F, et al. Immunogenic Chemotherapy Sensitizes Tumors to Checkpoint Blockade Therapy. Immunity. 2016;44(2):343-54.
124. Hodge JW, Garnett CT, Farsaci B, Palena C, Tsang KY, Ferrone S, et al. Chemotherapy-induced immunogenic modulation of tumor cells enhances killing by cytotoxic T lymphocytes and is distinct from immunogenic cell death. Int J Cancer. 2013;133(3):624-36.
125. Giaccone G, Camidge DR, Liu SV, Powderly JD, Hodi FS, Gettinger S, et al., editors. Safety, activity and biomarkers of atezolizumab (MPDL3280A) with platinum-based chemotherapy (chemo) in non-small cell lung cancer (NSCLC): A Phase Ib study. European Cancer Congress 2015; 2015 Sept 25-29, 2015; Vienna, Austria: Abstr 513.
126. Peng J, Hamanishi J, Matsumura N, Abiko K, Murat K, Baba T, et al. Chemotherapy Induces Programmed Cell Death-Ligand 1 Overexpression via the Nuclear Factor-kappaB to Foster an Immunosuppressive Tumor Microenvironment in Ovarian Cancer. Cancer Res. 2015;75(23):5034-45.
127. Luen SJ, Salgado R, Fox S, Savas P, Eng-Wong J, Clark E, et al. Tumour-infiltrating lymphocytes in advanced HER2-positive breast cancer treated with pertuzumab or placebo in addition to trastuzumab and docetaxel: a retrospective analysis of the CLEOPATRA study. Lancet Oncol. 2017;18(1):52-62.
128. Harrell FE. Regression modeling strategies: R package version 3.6-3 http://CRAN.R-project.org/package=rms2013 [
129. Hsieh FY, Lavori PW. Sample-size calculations for the Cox proportional hazards regression model with nonbinary covariates. Control Clin Trials. 2000;21(6):552-60.

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 MULTI-CENTER DATA AND SAFETY MONITORING PLAN

Dana-Farber/Harvard Cancer Center
Multi-Center Data and Safety Monitoring Plan

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 should serve as a reference for any sites external to DF/HCC that will be participating in the research protocol.

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: Dana-Farber Cancer Institute (DFCI) is 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 (Food and Drug Administration (FDA) 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. Within DF/HCC, this person is the Overall Principal Investigator 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, etc.). 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 or Project Manager) 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. 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 for 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 Clinical Trials Research Informatics Office (CTRIO): 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, [redacted], 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).
- 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 correspondence, as applicable.
- Review registration materials for eligibility and register participants from Participating Institutionsin 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.
- 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 local requirements and to the Coordinating Center, in accordance with DF/HCC requirements.
- Submit protocol deviations and violations to local IRB per local 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.

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

Refer to protocol Section 4.3 and 4.4 of the protocol for the participant registration process.

3.7.2 Initiation of Therapy

Participants must be registered with the DF/HCC CTMS before receiving treatment. Treatment 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. This number is unique to the participant on this trial and must be used for CRF/eCRF completion and correspondence. 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 deviation 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.

All protocol violations must be sent to the Coordinating Center in a timely manner.

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 treatment 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 7.0.

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 and submit to their IRB according to their institutional policies and procedures.

3.10 Data Management

The DF/HCC CTRIO develops case report forms (CRF/eCRFs), for use with the protocol. These forms are designed to collect data for each study. The DF/HCC CTRIO 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

Nivolumab will be ordered from [redacted] The ordering of investigational agent is specified in the protocol Section 8.1.8.

Carboplatin 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.

5. MONITORING: QUALITY CONTROL

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

5.1 Ongoing Monitoring of Protocol Compliance

The Participating Institutions will be required to submit participant source documents to the DF/HCC Lead Institution or designee for monitoring. Participating Institutions may also be subject to on-site monitoring conducted by the DF/HCC Lead Institution.

The DF/HCC Lead Institution will implement on-site and virtual monitoring activities to ensure that Participating Institutions are complying with regulatory and protocol requirements, data quality, and subject safety. At minimum, the DF/HCC Lead Institution, or designee, will monitor each participating site twice a year while patients are receiving study treatment. Should a Participating Institution be monitored once and then not accrue any additional participants or participant visits, then a second monitoring visit may not be necessary.

Monitoring practices may include but are not limited to: source verification, review and analysis of the following: eligibility requirements of all participants, informed consent procedures, adverse events and all associated documentation, study drug administration / treatment, regulatory records and site trial master files, protocol deviations, pharmacy records, response assessments, and data management. Additionally, ongoing communication will occur through regularly scheduled teleconferences and by email. Source documents from Participating Institutions, will be collected at specific timepoints to support the primary and/or secondary endpoints.

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 verification during on-site visits. Upon request, 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. If there are protocol compliance concerns, issues that impact subject safety or the integrity of the study, or trends identified based on areas of need, additional monitoring visits may occur.

Virtual Monitoring: On-site monitoring visits will be supplemented with virtual monitoring visits. The DF/HCC Lead Institution will request source documentation from Participating Institutions as needed to complete virtual monitoring activities. Participating Institutions will be asked to forward de-identified copies of participants' medical record and source documents to the DF/HCC Lead Institution to aid in the source documentation verification process.

In addition to monitoring performed by the Coordinating Center, the DF/HCC ODQ may monitor data for timeliness of submission, completeness, and adherence to protocol requirements. The DF/HCC Lead Institution or designee and, if applicable, the ODQ Data Analysts will perform ongoing protocol data compliance monitoring with the support of the Participating Institution's Coordinators, the Principal Investigators, and the Protocol Chair.

5.2 Monitoring Reports

The DF/HCC Sponsor will review all monitoring reports for on-site and remote monitoring of Participating Institutions 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. Participating Institutions may also be subject to an audit as determined by the DF/HCC Sponsor.

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 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. Its main focus is to measure whether standards and procedures were followed. Auditing is 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, Standard Operating Procedures (SOPs), and the Code of Federal Regulations (CFR).

6.1 Audit Plan: DF/HCC Sponsored Trials

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 Notification

It is the Participating Institution's responsibility to notify the Coordinating Center of all scheduled audit dates (internal or external) and re-audit dates (if applicable), which 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 these reports to the DF/HCC ODQ per DF/HCC policy for review by the DF/HCC Audit Committee. Based upon the audit assessments the DF/HCC Audit Committee could accept or conditionally accept the audit rating and final report. Conditional approval could require the DF/HCC Sponsor to implement recommendations or require further follow-up. 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

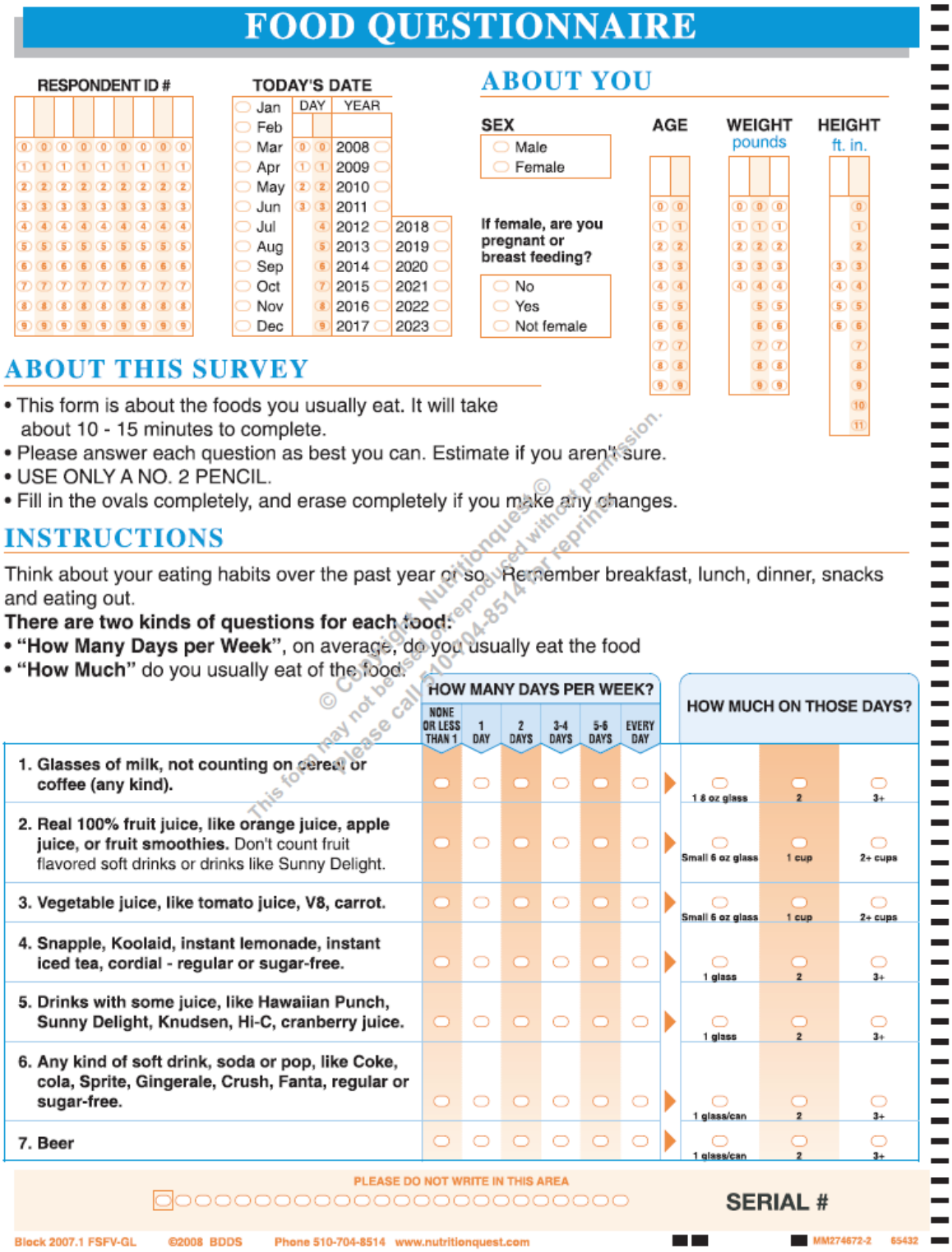
The DF/HCC Sponsor and the DFCI IRB are charged with considering the totality of an institution's performance in considering institutional participation in the protocol.

6.4.1 Corrective Actions

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.

APPENDIX C DIET AND PHYSICAL ACTIVITY ASSESSMENTS

Please note: the below is a copy of the **Block Fat/Sugar/Fruit/Vegetable Screener** for reference. *Please use the official Scantron version of the Screener rather than the appendix.*



FOOD QUESTIONNAIRE

RESPONDENT ID #

TODAY'S DATE

Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec

DAY

YEAR 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019 2020 2021 2022 2023

ABOUT YOU

SEX: Male, Female

If female, are you pregnant or breast feeding? No, Yes, Not female

AGE

WEIGHT pounds

HEIGHT ft. in.

ABOUT THIS SURVEY

- This form is about the foods you usually eat. It will take about 10 - 15 minutes to complete.
- Please answer each question as best you can. Estimate if you aren't sure.
- USE ONLY A NO. 2 PENCIL.
- Fill in the ovals completely, and erase completely if you make any changes.

INSTRUCTIONS

Think about your eating habits over the past year or so. Remember breakfast, lunch, dinner, snacks and eating out.

There are two kinds of questions for each food:

- **"How Many Days per Week"**, on average, do you usually eat the food
- **"How Much"** do you usually eat of the food.

	HOW MANY DAYS PER WEEK? NONE OR LESS THAN 1	1 DAY	2 DAYS	3-4 DAYS	5-6 DAYS	EVERY DAY	HOW MUCH ON THOSE DAYS?		
1. Glasses of milk, not counting on cereal or coffee (any kind).							1 8 oz glass	2	3+
2. Real 100% fruit juice, like orange juice, apple juice, or fruit smoothies. Don't count fruit flavored soft drinks or drinks like Sunny Delight.							Small 6 oz glass	1 cup	2+ cups
3. Vegetable juice, like tomato juice, V8, carrot.							Small 6 oz glass	1 cup	2+ cups
4. Snapple, Koolaid, instant lemonade, instant iced tea, cordial - regular or sugar-free.							1 glass	2	3+
5. Drinks with some juice, like Hawaiian Punch, Sunny Delight, Knudsen, Hi-C, cranberry juice.							1 glass	2	3+
6. Any kind of soft drink, soda or pop, like Coke, cola, Sprite, Gingerale, Crush, Fanta, regular or sugar-free.							1 glass/can	2	3+
7. Beer							1 glass/can	2	3+

PLEASE DO NOT WRITE IN THIS AREA

SERIAL #

Block 2007.1 FSFV-GL ©2008 BDDS Phone 510-704-8514 www.nutritionquest.com MM274672-2 65432

	HOW MANY DAYS PER WEEK?						HOW MUCH ON THOSE DAYS?		
	NONE OR LESS THAN 1	1 DAY	2 DAYS	3-4 DAYS	5-6 DAYS	EVERY DAY			
8. Eggs, or breakfast sandwiches with eggs, like Egg McMuffins (McDonalds).	○	○	○	○	○	○	○ 1 egg	○ 2	○ 3+
9. Cold cereal, any kind.	○	○	○	○	○	○	○ 1 small bowl	○ 1 medium bowl	○ 1 large bowl
10. Hot Cereal, cooked cereal like oatmeal or porridge, grits, or cream of wheat.	○	○	○	○	○	○	○ 1 small bowl	○ 1 medium bowl	○ 1 large bowl
11. Real sugar or honey in coffee or tea or on cereal.	○	○	○	○	○	○	○ 1 tsp	○ 2	○ 3+
12. Cheese, sliced cheese or cheese spread, including on sandwiches.	○	○	○	○	○	○	○ 1 slice	○ 2	○ 3+
13. Lunch meats like bologna, salami, sliced ham, turkey lunch meat, or any other cold meat cuts.	○	○	○	○	○	○	○ 1 slice	○ 2	○ 3+
14. Hamburgers, cheeseburgers, meat balls or meat loaf.	○	○	○	○	○	○	○ 1 small/3 oz	○ 1 large	○ 2 large
15. Hot dogs, or sausage like Polish, Italian or chorizo.	○	○	○	○	○	○	○ 1 hotdog	○ 2	○ 3+
16. Other beef or pork, such as steak, roast beef, ribs, or in sandwiches, tacos, burritos.	○	○	○	○	○	○	○ 3 oz small	○ 4-6 oz medium	○ 7+ oz large
17. Fried chicken, including chicken nuggets, wings, chicken patty.	○	○	○	○	○	○	○ 1 medium piece	○ 2 medium pieces or 6 nuggets	○ 3 medium pieces
18. Fish, any kind.	○	○	○	○	○	○	○ 2 oz	○ 4 oz	○ 6 oz
19. Pizza.	○	○	○	○	○	○	○ 1 slice	○ 2	○ 3+
20. Spaghetti, lasagna, other pasta, or noodles.	○	○	○	○	○	○	○ 1 cup	○ 2	○ 3+
21. Rice, or dishes made with rice.	○	○	○	○	○	○	○ 1 cup rice	○ 2	○ 3+
22. Green salad and vegetables you put in green salad.	○	○	○	○	○	○	○ 1 cup	○ 2	○ 3+
23. Any kind of fruit, fresh or canned (not counting juice).	○	○	○	○	○	○	○ 1 fruit or 1/2 cup	○ 2 fruits or 1 cup	○ 3 fruits or 2 cups
24. French fries, home fries, hash browns.	○	○	○	○	○	○	○ small (McD)	○ medium	○ large
25. Potatoes not fried, like baked, mashed.	○	○	○	○	○	○	○ 1/2 cup or 1/2 potato	○ 1 cup	○ 2+ cups
26. Vegetable soup, or stew with vegetables.	○	○	○	○	○	○	○ 1 cup	○ 1 1/2 cups	○ 2+ cups

	HOW MANY DAYS PER WEEK?						HOW MUCH ON THOSE DAYS?		
	NONE OR LESS THAN 1	1 DAY	2 DAYS	3-4 DAYS	5-6 DAYS	EVERY DAY			
27. ALL other vegetables you eat, as a side dish or in any kind of dish, not counting salad or potatoes.	○	○	○	○	○	○	○ 1/2 cup altogether	○ 1 cup	○ 2 cups
28. Bread, rolls, bagels.	○	○	○	○	○	○	○ 1 slice	○ 2	○ 3+
29. Biscuits, muffins, croissants.	○	○	○	○	○	○	○ 1	○ 2	○ 3+
30. Snack chips like potato chips, tortilla, corn chips, Fritos, Doritos, popcorn (not pretzels).	○	○	○	○	○	○	○ 1 small handful	○ 1 oz bag 1 cup	○ Big bag 2 cups
31. Crackers, like Ritz, soda-crackers, Cheez-Its, or any other snack cracker.	○	○	○	○	○	○	○ 3-4 small crackers	○ 5-10 crackers	○ a lot
32. Ice cream, ice cream bars.	○	○	○	○	○	○	○ 1/2 cup	○ 1 cup	○ 2+ cups
33. Doughnuts.	○	○	○	○	○	○	○ 1	○ 2	○ 3+
34. Cake, cookies, or snack cakes like cupcakes, Twinkies or any other pastry.	○	○	○	○	○	○	○ 1 small piece	○ 1 medium	○ 2+
35. Pie including fast food pies or snack pies.	○	○	○	○	○	○	○ 1 small piece	○ 1 medium	○ 2+
36. Chocolate candy like chocolate bars, M&Ms, Mars Bars, Reeses.	○	○	○	○	○	○	○ 1 mini	○ 1 medium	○ 1 large
37. Any other candy (not chocolate) like hard candy, Lifesavers, Skittles, Starburst.	○	○	○	○	○	○	○ 1-2 pieces	○ 1/2 package	○ 1 package
38. Margarine (not butter) on bread or on vegetables.	○	○	○	○	○	○	○ 1 teaspoon	○ 2 teaspoons	○ 3 teaspoons
39. Butter (not margarine) on bread or on vegetables.	○	○	○	○	○	○	○ 1 teaspoon	○ 2 teaspoons	○ 3 teaspoons
40. Fat or oil in cooking.	○	○	○	○	○	○			

For each of the questions below, please fill in the oval that best describes your usual eating habits.

41. What kind of milk do you usually drink?	○ Whole milk ○ Reduced-fat 2% milk ○ Low-fat 1% milk	○ Skim milk ○ Soy milk ○ Rice milk	○ I don't drink milk or soy milk
42. If you drink soft drinks or pop, is it usually:	○ Diet or sugar free soft drinks	○ Regular ○ I don't drink soft drinks	
43. If you drink Snapple, KoolAid, instant iced tea, or instant lemonade, is it usually:	○ Sugar-free ○ Regular	○ I don't drink these	
44. If you eat hot dogs, are they usually:	○ Low Fat or turkey hot dogs	○ Regular hot dogs ○ I don't eat hot dogs	
45. If you eat lunch meats, are they usually:	○ Low Fat or turkey	○ Regular	○ I don't eat lunch meats
46. If you eat snacks like chips, are they usually:	○ Trans-fat free	○ Regular	○ I don't know ○ I don't eat them

For each of the questions below, please fill in the oval that best describes your usual eating habits.

47. If you eat crackers, are they usually:
- Trans-fat free
- Triscuits, Graham crackers, Ry-Vita
- Saltines or other snack crackers
- I don't eat them

48. If you eat ice cream, is it usually:
- Low carb, low sugar
- Low fat or ice milk
- Regular
- Premium
- I don't eat it

49. If you eat cake, snack cakes, cookies and other pastries, are they usually:
- Low carb, low sugar
- Low fat
- Regular
- I don't eat it

50. If you eat chocolate candy, is it usually:
- Low carb, low sugar
- Low fat
- Regular
- I don't eat it

51. If you eat other candy, not chocolate, is it usually:
- Sugar-free
- Regular
- I don't eat it

52. When you use margarine, is it usually:
- Stick margarine
- Soft tub margarine
- Low-fat margarine
- Butter-margarine blend
- Non-hydrogenated and trans-fat free
- I don't eat it

53. What kind of fat or oil do you usually use in cooking? MARK ONLY 1 or 2.
- Spray oil (i.e. Pam), or no oil
- Butter
- Butter/margarine blend
- Stick margarine
- Soft tub margarine
- Low-fat margarine
- Corn oil, vegetable oil
- Olive oil, canola oil
- Lard, fatback, bacon fat
- Crisco
- Trans-fat free brand
- I don't know, or don't cook

54. What kind of cold cereal do you usually eat?
Choose 1 or 2 that you eat most often.
(If you usually eat just one kind, mark one.)
- Low-carb cereals like Atkins, Low-Carb Special K
- Cheerios (plain), Shredded Wheat, Wheat Chex, Wheaties
- Sweetened cereals like Frosted Flakes, Honey Nut Cheerios, Fruit Loops, Cap'n Crunch, granola, instant sweetened oatmeal
- Other cold cereals, like Corn Flakes, Rice Krispies, Bran Flakes
- I don't eat cereal.

55. What kind of bread do you usually eat?
- Italian, French or local bakery
- Regular sliced white bread
- Dark bread like rye, cracked wheat
- 100% whole wheat
- I don't know or I don't eat bread

SOME LAST QUESTIONS ABOUT YOU

Are you
- Hispanic or Latino
- Not Hispanic or Latino

What race do you consider yourself to be? (MARK ALL THAT APPLY)
- White
- Black or African American
- Asian
- American Indian or Alaska Native
- Native Hawaiian or Other Pacific Islander
- Do not wish to provide this information

Thank you very much for filling out this questionnaire.
Please take a minute to go back and fill in anything you may have skipped.

PLEASE DO NOT WRITE IN THIS AREA

SERIAL #

APPENDIX D DIET AND PHYSICAL ACTIVITY ASSESSMENTS

Below is a copy of the **Gordin Leisure-Time Exercise Questionnaire (LSI)** with three additional questions about fiber intake from the **Block Vegetable/Fruit/Fiber Screener**.

Godin Leisure-Time Questionnaire

When answering these questions please:

- only count exercise sessions that lasted 10 minutes or longer in duration.
- only count exercise that was done during free time (i.e., not occupation or housework).
- note that the main difference between the first three categories is the intensity of the endurance (aerobic) exercise.
- please write the average frequency on the first line and the average duration on the second.
- if you did not do any exercise in one of the categories, please write in "0".

Considering a typical week (7 days) how many times on the average did you do the following kinds of exercise in the last month?

	Times Per Week	Average Duration (min.)
a. VIGOROUS/STRENUOUS EXERCISE (HEART BEATS RAPIDLY, SWEATING) (e.g., running, aerobics classes, cross country skiing, vigorous swimming, vigorous bicycling).	_____	_____
b. MODERATE EXERCISE (NOT EXHAUSTING, LIGHT PERSPIRATION) (e.g., fast walking, tennis, easy bicycling, pilates, easy swimming, popular and folk dancing).	_____	_____
c. LIGHT/MILD EXERCISE (MINIMAL EFFORT, NO PERSPIRATION) (e.g., easy walking, yoga, golfing with a cart, and bowling).	_____	_____
d. STRENGTH TRAINING (eg. Dumbbells or Nautilus machines)	_____	_____

In addition to the above, please complete the below questions from the Dietary Fruit-Vegetable-Fiber Screener ©

Think about your eating habits over the past month or so. About how often do you eat each of the following foods? Remember breakfast, lunch, dinner, snacks and eating out. Mark one box for each food.

Fiber	Less than 1/Week	Once a Week	2-3 times a Week	4-6 times a Week	Once a Day	2+ a Day
Fiber cereals like Raisin Bran, Shredded Wheat or Fruit-n-Fiber	☐	☐	☐	☐	☐	☐
Beans such as baked beans, pinto, kidney, or lentils (not green beans)	☐	☐	☐	☐	☐	☐
Dark bread such as whole wheat or rye	☐	☐	☐	☐	☐	☐

BLOCK DIETARY DATA SYSTEMS
www.nutritionquest.com
(510) 704-8514