Title: A phase II study of nivolumab in combination with cabozantinib for metastatic triple-negative breast cancer

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TITLE: A phase II study of nivolumab in combination with cabozantinib for metastatic triple-negative breast cancer

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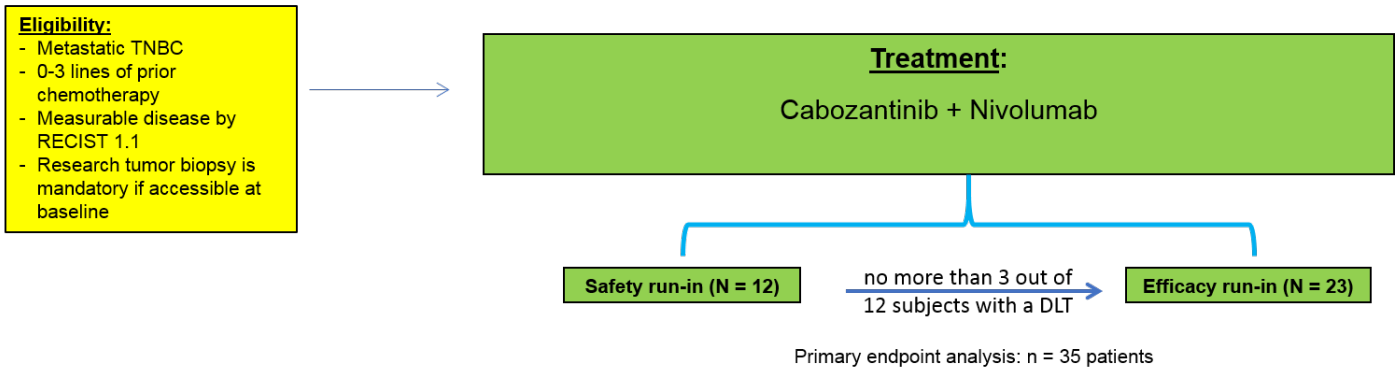
SCHEMA



There will be two research biopsies: Bx1 at baseline, and Bx2 within 14 days before starting cycle 3 day 1 (week 7).

TNBC – triple negative breast cancer.
Tumor assessment: Imaging every 8 weeks.
Biopsy 1 - Baseline tumor biopsy will be obtained within 7 days before starting cycle 1.
Biopsy 2 – Reassessment biopsy will be obtained within 14 days before starting cycle 3.
Blood samples will be collected within 14 days before starting therapy and every 4 weeks thereafter (just before the d1 of a new cycle).
1 cycle = 28 days

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1. OBJECTIVES

1.1 Study Design

1.1.1 This is an open-label, single-arm, single center, phase 2 study of cabozantinib given orally at 40mg per day in combination with nivolumab given intravenously at 480mg every four weeks in subjects with metastatic triple-negative breast cancer (TNBC) previously treated with 0 to 3 chemotherapy regimens in the metastatic setting. Mandatory research biopsies will be performed at baseline (if tissue is safely accessible) and during days 15-28 of Cycle 2 (prior to beginning Cycle 3).

1.2 Primary Objectives

1.2.1 To evaluate the efficacy of nivolumab in combination with cabozantinib, as defined by objective response rate (ORR) according to RECIST 1.1 [Eisenhauer *et al.*, 2009] in patients with metastatic TNBC previously treated with 0 to 3 lines of chemotherapy in the metastatic setting.

1.3 Secondary Objectives

Safety objectives

1.3.1 To evaluate the safety and tolerability of nivolumab in combination with cabozantinib in patients with metastatic TNBC previously treated with 0 to 3 lines of chemotherapy in the metastatic setting.

Efficacy objectives

1.3.2 To evaluate the ORR of the combination according to immune-related response criteria (irRC) [Wolchok *et al.*, 2009]. (Section 11).

1.3.3 To evaluate the Clinical Benefit Rate (CR + PR + SD ≥ 24 weeks) of the combination

1.3.4 To evaluate the progression-free survival according to the RECIST 1.1.

1.4 Correlative Objectives

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

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

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

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

1.4.5 To evaluate MET and phospho MET expression in tumor tissue at baseline by immunohistochemistry

1.4.6 To explore the effect of treatment on plasma biomarkers (sVEGFR2, sMET, IL-2, IFN-γ, TNF-α)

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

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

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

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

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

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

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

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

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

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

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

1.4.18 To explore whether the number and/or type of mutations identified using a next generation sequencing (NGS) panel is correlated with patient outcomes (PFS, ORR, and CBR). This will be done on DFCI patients only.

2. BACKGROUND

2.1 Study Disease(s)

Breast cancer (BC) is the most frequently diagnosed cancer and the second cause of cancer death in women in the Unites States[1]. Approximately, 15% of these cancers are classified as triple-negative breast cancer (TNBC), comprising those with absent expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2)[2].

Hormonal therapies are only effective for ER or PR positive breast cancer, and the monoclonal antibody trastuzumab is effective only for tumors that have amplified HER2 genes. The basal-like tumors have a poor prognosis relative to other subtypes, even with the best available chemotherapy. TNBCs have an aggressive nature with higher rates of relapse and shorter overall survival in the metastatic setting compared with other subtypes of breast cancer[3]. Little progress has been made in identifying specific molecular pathways that exist for this tumor subtype; because of this dismal prognosis, new therapies are need in TNBC setting.

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

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

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

<u>The PD-1/PD-L1 pathway in breast cancer</u>

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

PD-1 and PD-L1 differs among breast tumors subtype: HR-positive (30% PD-1; 33% PD-L1), triple-negative (70% PD-1; 59% PD-L1) and HER2-positive (60% PD-1; 20% PD-L1)[Gatalica *et al.*, 2014].

2.3 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).

2.3.1 Nivolumab nonclinical toxicology

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

2.3.2 Clinical Experience with nivolumab

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

In several ongoing clinical trials, the safety of nivolumab in combination with other therapeutics such as ipilimumab, cytotoxic chemotherapy, anti-angiogenics and targeted therapies is being explored. Most studies are ongoing and as such, the safety profile of nivolumab combinations continues to evolve. The most advanced combination under development is nivolumab and ipilimumab in subjects with MEL. Thus far, the combination of both agents results in a safety profile with similar types of AEs as either agent alone, but in some cases with greater frequency.

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

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

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

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

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

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

In addition, monotherapy or combination trials with nivolumab are on-going for subjects with SCCHN,[67] HCC,[68] CRC[69], GBM[70], and NHL.[71,72]

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

2.4 Cabozantinib

A summary of XL184 clinical and nonclinical experience is contained in the Investigator's Brochure supplied by Exelixis (ANEXO XXX). The Investigator's Brochure should be reviewed in conjunction with this study protocol.

Cabozantinib is a new chemical entity that inhibits multiple RTKs with growth-promoting and angiogenic properties. The primary targets of cabozantinib are RET, MET, VEGFR2/KDR, and KIT (see below)

CABOZANTINIB IC_{50} Values in Biochemical, Enzymatic Assays

Kinase	IC_{50} (biochemical) [nM]
RET	3.8
MET	1.8
VEGFR2/KDR	0.035
KIT	4.6

IC_{50}, concentration required for 50 % target inhibition.

Cabozantinib is an oral receptor tyrosine kinase (RTK) inhibitor, with multiple targets related to tumor growth and angiogenesis in the tumor microenvironment, including MET, and VEGFR2/KDR [Scagliotti *et al*., 2013]. Both high tumor microvessel density and elevated vascular endothelial growth factor-A (VEGF-A) expression are associated with decreased relapse-free and overall survival in early stage breast cancer, suggesting that angiogenesis is associated with a poor prognosis [Relf *et al.,* 1997, Uzzan *et al.,* 2004]. More importantly, it is well known that bevacizumab (an anti-VEGF monoclonal antibody) in combination with chemotherapy has been associated with an improvement in the rates of complete pathological response in the neoadjuvant setting and with a significant improvement in progression-free survival (PFS) in several clinical studies, although it has not prolonged OS in patients with breast cancer [Robert *et al.,* 2011]. It has been recognized that inhibition of VEGF signaling alone, although initially effective in slowing tumor growth, often culminates in the emergence of an evasive resistance

phenotype that ultimately promotes tumor invasiveness and metastasis [Sennino, 2012]. One of the potential causes of resistance to anti-angiogenic inhibitors is the overexpression of MET and of its ligand - hepatocyte grow factor (HGF); which have been associated with tumor hypoxia, increased invasiveness and metastasis, and reduced survival in metastatic breast cancer [Raghav *et al.,* 2012]. Specifically, in patients with TNBC, MET expression is elevated and associated with poorer prognosis [Ho-Yen *et al.,* 2014, Zagouri *et al.,* 2013]. In this context, cabozantinib an oral bioavailable small molecule inhibitor of multiple receptor tyrosine kinases (RTK), including VEGFR-2, was thought to be a drug to be evaluated in combination with anti-PD1 drugs in patients with TNBC. We completed a phase II trial evaluating the efficacy of cabozantinib monotherapy in heavily pre-treated patients with mTNBC [Tolaney *et al.*, 2016]. They reported a clinical benefit rate of 34%, including 9% of partial response rate.

In vivo data from pharmacodynamic experiments show that cabozantinib inhibits key RTKs that promote tumor cell proliferation, migration and/or angiogenesis (RET, MET, and VEGFR2). In xenografted tumor models, cabozantinib inhibited VEGFR2 phosphorylation in lung tissue, with an ED_{50} of 26 mg/kg. The duration of action for cabozantinib was sustained with > 50 % inhibition observed 10-24 hours post-dose at a dose level of 100 mg/kg for all targets studied.

Treatment with cabozantinib shows rapid effects on the tumor endothelium, resulting in breakdown of the vasculature beginning 24 hours after administration of cabozantinib, thus suggesting potent anti-angiogenic effects of cabozantinib. These effects translate into significant tumor growth inhibition after cabozantinib treatment in multiple tumor models including human MTC, human breast cancer, human lung carcinoma, and rat glioblastoma. Overall, the data generated *in vivo* demonstrate that the target profile of cabozantinib translates to potent anti-angiogenic activity and potent anti-tumor efficacy.

2.4.1 Cabozantinib Nonclinical Toxicology

In nonclinical toxicity studies in rodents and non-rodents, histopathological changes associated with cabozantinib administration were observed in gastrointestinal (GI) tract, bone marrow, lymphoid tissues, kidney, adrenal, and reproductive tract tissues. Histopathological changes present in bone and pancreas were considered secondary to cabozantinib administration. Cabozantinib was negative in *in vitro* bacterial, *in vitro* mammalian cell, and *in vivo* mammalian genotoxicity bioassays. In reproductive toxicity studies, cabozantinib was embryotoxic in rats, produced fetal soft tissue changes in rabbits, and decreased fertility in male and female rats.

Safety pharmacology studies of cabozantinib administration did not demonstrate adverse effects on neurobehavioral or respiratory-system function in rats; furthermore, no significant changes in electrocardiographic parameters (including corrected QT [QTc] interval) were observed by telemetry in dogs.

Cabozantinib was not an inhibitor of cytochrome P450 (CYP) 3A4 in vitro and is not predicted to have significant effects on CYP3A4 induction. Cabozantinib was shown to be an inhibitor of CYP2C8, CYP2C9*3, and CYP2C19 isozyme, *in vitro* and was also a substrate of CYP3A4-mediated metabolism. The mean plasma protein binding by cabozantinib *in vitro* was greater than 98%.

Additional toxicology information may be found in the Investigator's Brochure.

2.4.2 Clinical Experience

2.4.2.1 Clinical Safety Profile

The adverse event (AE) and serious adverse event (SAE) data summarized in the following section includes those reported and entered in the clinical database and safety database, respectively, as of 29 February 2016. The clinical studies with cabozantinib are ongoing, thus the AE data from the clinical database does not yet include all SAEs. Data from double-blinded studies are not presented. The severity of AEs was assessed using the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 or 4.0.

2.4.2.2 Adverse Events

A pooled analysis through 29 February 2016 included 2410 subjects with cancer who had been treated with single-agent cabozantinib in company-sponsored clinical trials (Section 5.4). The subjects in that dataset were predominately White (83.4%) and male (77.5%) with a median age of 64.0 years (see Table 5-3); the gender distribution reflects the inclusion of CRPC studies in the pooled analysis.

Table: Demographics and Other Baseline Characteristics for Pooled Single-Agent Studies **Subject characteristics**	**Total**
Total number of subjects exposed	**2410**
Sex	
Female	542 (22.5%)
Male	1868 (77.5%)
Age	
Mean	63.0 (11.47)
Median	64.0
Range	20 - 91
Race	
American Indian or Alaska Native	2 (0.1%)
Asian	89 (3.7%)
Black or African American	78 (3.2%)
Multiple	4 (0.2%)
Native Hawaiian or other Pacific Islander	1 (0.0%)
White	2009 (83.4%)
Other	52 (2.2%)
Not reported	175 (7.3%)

This table summarizes pooled data for cabozantinib-treated subjects with cancer in the clinical database for single-agent cabozantinib studies (XL184-001, XL184-008, XL184-201, XL184-203, XL184-205, XL184-301, XL184-306, XL184-307, XL184-308, and XL184-401).

The AE data summarized in the following sections include those reported and entered in the clinical database as of the dates presented in Table 5-1. For active clinical studies with a "Data Through" date of 29 February 2016, the AE data from the clinical database may not yet include all SAEs. A summary of pooled SAEs from company-sponsored clinical studies with single-agent cabozantinib is provided in Section 5.4.1.4.

The severity of AEs was assessed using the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 or version 4.0, and AE and SAE PTs were coded with the Medical Dictionary for Regulatory Activities (MedDRA) version 17.0 for all studies. For AEs and SAEs, multiple occurrences of the same event in any individual subject are counted once at the highest grade reported. Events that were assessed as possibly related or probably related to cabozantinib

are reported as "related," and events that were assessed as not related or unlikely related to cabozantinib are reported as "not related."

Adverse events that occurred in ≥ 10% of the 2410 subjects in the pooled single-agent studies are presented in Table below.

Table : Summary of Adverse Events Experienced by ≥ 10% of Subjects Treated with Single-Agent Cabozantinib, N = 2410

	All AEs		Related AEs	
MedDRA Preferred Term	**Subjects with AE n (%)**	**Subjects with ≥ Grade 3 AE n (%)**	**Subjects with AE n (%)**	**Subjects with ≥ Grade 3 AE n (%)**
Number of subjects with at least one event	2404 (99.8)	1979 (82.1)	2324 (96.4)	1512 (62.7)
Diarrhoea	1471 (61.0)	251 (10.4)	1300 (53.9)	226 (9.4)
Fatigue	1458 (60.5)	369 (15.3)	1281 (53.2)	312 (12.9)
Nausea	1290 (53.5)	118 (4.9)	1062 (44.1)	89 (3.7)
Decreased appetite	1283 (53.2)	136 (5.6)	1080 (44.8)	104 (4.3)
Vomiting	861 (35.7)	95 (3.9)	612 (25.4)	59 (2.4)
Weight decreased	860 (35.7)	97 (4.0)	671 (27.8)	75 (3.1)
Palmar-plantar erythrodysaesthesia syndrome	835 (34.6)	186 (7.7)	819 (34.0)	185 (7.7)
Constipation	779 (32.3)	31 (1.3)	345 (14.3)	11 (0.5)
Hypertension	708 (29.4)	330 (13.7)	603 (25.0)	284 (11.8)
Dysgeusia	637 (26.4)	2 (0.1)	605 (25.1)	2 (0.1)
Dysphonia	610 (25.3)	5 (0.2)	520 (21.6)	4 (0.2)
Asthenia	557 (23.1)	165 (6.8)	434 (18.0)	124 (5.1)
Dyspnoea	497 (20.6)	76 (3.2)	187 (7.8)	22 (0.9)
Anaemia	479 (19.9)	200 (8.3)	217 (9.0)	70 (2.9)
Stomatitis	474 (19.7)	42 (1.7)	446 (18.5)	41 (1.7)
Abdominal pain	456 (18.9)	95 (3.9)	232 (9.6)	22 (0.9)
Aspartate aminotransferase increased	446 (18.5)	69 (2.9)	382 (15.9)	46 (1.9)
Back pain	444 (18.4)	90 (3.7)	56 (2.3)	6 (0.2)
Mucosal inflammation	444 (18.4)	46 (1.9)	423 (17.6)	44 (1.8)
Pain in extremity	422 (17.5)	53 (2.2)	180 (7.5)	15 (0.6)
Headache	415 (17.2)	30 (1.2)	149 (6.2)	3 (0.1)
Alanine aminotransferase increased	389 (16.1)	74 (3.1)	343 (14.2)	58 (2.4)
Rash	367 (15.2)	15 (0.6)	300 (12.4)	13 (0.5)
Hypothyroidism	359 (14.9)	6 (0.2)	301 (12.5)	3 (0.1)
Cough	354 (14.7)	7 (0.3)	84 (3.5)	1 (0.0)
Oedema peripheral	335 (13.9)	15 (0.6)	101 (4.2)	4 (0.2)
Dizziness	307 (12.7)	12 (0.5)	154 (6.4)	3 (0.1)
Arthralgia	303 (12.6)	30 (1.2)	76 (3.2)	4 (0.2)
Dyspepsia	300 (12.4)	4 (0.2)	224 (9.3)	4 (0.2)
Hypokalaemia	300 (12.4)	90 (3.7)	136 (5.6)	42 (1.7)

Dry mouth	279 (11.6)	0	231 (9.6)	0
Urinary tract infection	269 (11.2)	35 (1.5)	31 (1.3)	3 (0.1)
Dry skin	264 (11.0)	0	219 (9.1)	0
Hypomagnesaemia	262 (10.9)	24 (1.0)	170 (7.1)	17 (0.7)
Dehydration	255 (10.6)	79 (3.3)	139 (5.8)	42 (1.7)
Muscle spasms	255 (10.6)	1 (0.0)	147 (6.1)	1 (0.0)
Hair colour changes	251 (10.4)	2 (0.1)	243 (10.1)	2 (0.1)
Pyrexia	250 (10.4)	16 (0.7)	44 (1.8)	2 (0.1)
Insomnia	244 (10.1)	0	82 (3.4)	0

AE, adverse event; MedDRA, Medical Dictionary for Regulatory Activities.
At each level of subject summarization, a subject is counted once if the subject reported one or more events.
Adverse events were coded based on MedDRA version 17.0.
Note: This table summarizes pooled data in the clinical database for single-agent cabozantinib studies (XL184-001, XL184-008, XL184-201, XL184-203, XL184-205, XL184-301 cabozantinib arm, XL184-306 cabozantinib arm, XL184-307 cabozantinib arm, XL184-308 cabozantinib arm, and XL184-401).

The most common AEs (≥ 5% incidence) reported at severity of Grade 3 and above were fatigue (15.3%), hypertension (13.7%), diarrhea (10.4%), anemia (8.3%), PPES (7.7%), asthenia (6.8%), pulmonary embolism (6.1%), and decreased appetite (5.6%).

The most frequently (≥ 20% incidence) observed AEs reported as related to cabozantinib, were diarrhea (53.9%), fatigue (53.2%), decreased appetite (44.8%), nausea (44.1%), PPES (34.0%), weight decreased (27.8%), vomiting (25.4%), dysgeusia (25.1%), hypertension (25.0%), and dysphonia (21.6%).

2.4.2.3 Serious Adverse Events

The most commonly reported SAEs (≥ 1% incidence) excluding events of disease progression are shown in Table below.

Table: Summary of Serious Adverse Events Experienced by ≥ 1% of Subjects Treated with Single-Agent Cabozantinib Excluding Events of Disease Progression, N = 2410

	All SAEs		**Related SAEs**	
MedDRA Preferred Term	**Subjects with SAE n (%)**	**Subjects with ≥ Grade 3 SAE n (%)**	**Subjects with SAE n (%)**	**Subjects with ≥ Grade 3 SAE n (%)**
Subjects reporting at least one SAE	**1332 (55.3)**	**1221 (50.7)**	**602 (25.0)**	**516 (21.4)**
Pulmonary embolism	120 (5.0)	119 (4.9)	85 (3.5)	84 (3.5)
Vomiting	81 (3.4)	48 (2.0)	40 (1.7)	27 (1.1)
Nausea	72 (3.0)	44 (1.8)	47 (2.0)	31 (1.3)
General physical health deterioration	71 (2.9)	65 (2.7)	13 (0.5)	9 (0.4)
Dehydration	69 (2.9)	54 (2.2)	41 (1.7)	32 (1.3)
Pneumonia	69 (2.9)	58 (2.4)	5 (0.2)	4 (0.2)
Anaemia	59 (2.4)	49 (2.0)	17 (0.7)	13 (0.5)
Abdominal pain	53 (2.2)	43 (1.8)	13 (0.5)	9 (0.4)

Diarrhoea	52 (2.2)	36 (1.5)	42 (1.7)	31 (1.3)
Deep vein thrombosis	46 (1.9)	36 (1.5)	21 (0.9)	16 (0.7)
Fatigue	43 (1.8)	36 (1.5)	27 (1.1)	25 (1.0)
Asthenia	41 (1.7)	30 (1.2)	20 (0.8)	13 (0.5)
Back pain	41 (1.7)	36 (1.5)	1 (0.0)	1 (0.0)
Dyspnoea	39 (1.6)	26 (1.1)	7 (0.3)	5 (0.2)
Pyrexia	36 (1.5)	9 (0.4)	5 (0.2)	2 (0.1)
Urinary tract infection	35 (1.5)	25 (1.0)	4 (0.2)	3 (0.1)
Hyponatraemia	31 (1.3)	29 (1.2)	15 (0.6)	14 (0.6)
Pleural effusion	30 (1.2)	22 (0.9)	6 (0.2)	5 (0.2)
Renal failure acute	30 (1.2)	24 (1.0)	7 (0.3)	5 (0.2)
Convulsion	29 (1.2)	18 (0.7)	5 (0.2)	2 (0.1)
Decreased appetite	28 (1.2)	20 (0.8)	19 (0.8)	14 (0.6)
Bone pain	27 (1.1)	23 (1.0)	0	0
Sepsis	27 (1.1)	27 (1.1)	5 (0.2)	5 (0.2)
Metastatic pain	26 (1.1)	20 (0.8)	1 (0.0)	0
Confusional state	25 (1.0)	17 (0.7)	7 (0.3)	5 (0.2)
Constipation	25 (1.0)	9 (0.4)	9 (0.4)	4 (0.2)
Spinal cord compression	23 (1.0)	22 (0.9)	1 (0.0)	1 (0.0)

MedDRA, Medical Dictionary for Regulatory Activities; SAE, serious adverse event.
Note: Reported SAEs were coded using MedDRA version 17.0. At each level of subject summarization, a subject is counted once if the subject reported one or more events.
Note: This table summarizes pooled data from the safety database for single-agent cabozantinib studies (XL184-001, XL184-008, XL184-201, XL184-203, XL184-205, XL184-301 cabozantinib arm, XL184-306 cabozantinib arm XL184-307 cabozantinib arm, XL184-308 cabozantinib arm, and XL184-401).
Note: Disease progression is expected for subjects with advanced cancer on cabozantinib clinical trials, and as such, events of progression of underlying cancer are not included.
[a] Twenty (20) out of 29 of the observed convulsion events were reported in subjects with glioblastoma (GB) enrolled in Studies XL184-201 or XL184-205. For more information on safety observed in subjects with GB, see Section 5.4.3.1.

As of 29 February 2016, the incidence of Grade 5 AEs from the pooled single-agent studies was 10.5% (254 subjects). The Grade 5 events that occurred at the highest frequency (≥ 1% incidence) were prostate cancer (2.9%) and general physical health deterioration (1.0%). Per convention, prostate cancer was the PT for disease progression of the cancer under study for Studies XL184-203 (CRPC cohorts), XL184-306, and XL184-307. Only one event of general physical health deterioration (on Study XL184-307) was assessed as related to study treatment; no events of prostate cancer were assessed as related to study treatment.

Thirty-three (33) of the 254 subjects with Grade 5 AEs had events assessed as related to the study treatment. The only related Grade 5 AEs that occurred more than once were pulmonary embolism (n=4), death (unspecified; n=3), hemorrhage (n=2), respiratory failure (n=2), and sudden death (n=2).

Detailed information regarding the safety profile of Cabozantinib from all studies may be found in the Investigator's Brochure.

2.4.2.4 Clinical Pharmacokinetics

Pharmacokinetic (PK) analysis showed dose proportional increases in maximum plasma concentration (C_{max}) and area under the plasma concentration-vs-time curve (AUC) both for the powder-in-bottle (PIB) formulation (dose range: 0.08 to 11.52 mg/kg) and the capsule formulation (dose range: 125 mg to 175 mg). Terminal-phase half-life ($t_{1/2, z}$) values were 59.1 to 136 hours. More detailed information regarding cabozantinib PK from all studies and product metabolism in humans may be found in the Investigator's Brochure.

2.4.3 Clinical Activity

Efficacy in Phase 3 Clinical Trials

2.4.3.1 Renal Cell Carcinoma: Study XL184-308 (METEOR; N=658)

The population, enrollment, and study design of XL184-308 are summarized in Table 5-1. In order to be eligible for the study, subjects were required to have histologically or cytologically confirmed advanced RCC with a clear cell component, to have measurable disease by CT/MRI per Response Evaluation Criteria in Solid Tumors (RECIST), and to have received treatment with at least one prior VEGFR-TKI. Tumor assessments were evaluated by the blinded IRC to determine response and/or progression. Eligible subjects were randomized 1:1 to receive either cabozantinib (60 mg, tablet formulation) or everolimus (10 mg) which was the standard of care for the second-line setting at the time of the initiation of the study.

It was estimated that 375 randomized subjects would have been adequate to evaluate the primary endpoint of PFS alone. However, a much larger sample size of 650 subjects was needed to provide reasonable power for the secondary endpoint of OS. To minimize the effects of bias due to the influence of subjects with early progression and allow for longer, more robust PFS follow-up among fewer subjects, the primary analysis of PFS was planned to be conducted for the first 375 randomized subjects.

Only the first 375 subjects randomized were included in the population for the primary PFS analysis (Primary Endpoint Intent-to-Treat [PITT] population). The results of the analysis demonstrated a statistically significant improvement in PFS per IRC for subjects in the cabozantinib arm (n=187) compared with the everolimus arm (n=188): the HR adjusted for stratification factors was 0.58 (95% CI: 0.45, 0.74; stratified log-rank p-value < 0.0001). The Kaplan-Meier estimates for median duration of PFS were 7.4 months in the cabozantinib arm vs 3.8 months in the everolimus arm. Kaplan-Meier estimates of the percent of subjects event-free at 6- and 12-month landmarks after treatment randomization were higher for the cabozantinib arm than for the everolimus arm (6 months: 55% cabozantinib, 34% everolimus; 12 months: 29%, 15%). The PFS analysis was repeated in the ITT population (658 subjects), and results were similar to those obtained for the PITT population. The HR adjusted for stratification factors was 0.51 (95% CI: 0.41, 0.62).

A pre-specified interim analysis of OS was conducted for the ITT population as of the 22 May 2015 database cutoff, at the time of the primary analysis of PFS. There were a total of 202 deaths (89 cabozantinib, 113 everolimus) by this date, representing 49% (202/408) of the total required for the pre-specified primary analysis of OS. The minimum time of follow-up (from randomization of the last subject through 22 May 2015) was 5.9 months. The interim analysis demonstrated a strong trend for improvement in duration of OS for subjects in the cabozantinib arm compared with the everolimus arm: the HR, adjusted for stratification factors was 0.68 (95%

CI: 0.51, 0.90; stratified log-rank p-value = 0.006). The OS results nearly met the criteria required to reject the null hypothesis at the interim analysis; the critical p-value was ≤ 0.0019 (HR ≤ 0.645).

Following these results, in August 2015 an unplanned second interim OS analysis was specified, with a prospectively-defined cutoff date of 31 December 2015 to provide at least 12 months of follow-up from the last subject randomized. The second interim OS analysis with 13 months of follow-up demonstrated a highly statistically significant prolongation of OS for subjects in the cabozantinib arm compared with the everolimus arm: the HR, adjusted for stratification factors was 0.66 (95% CI: 0.53, 0.83; stratified log-rank p-value 0.0003). The critical value for rejecting the null hypothesis at the current analysis was p<0.0163. Kaplan-Meier estimates for median duration of OS were 21.4 months in the cabozantinib arm and 16.5 months in the everolimus arm. The proportion of subjects estimated to be alive by Kaplan-Meier analysis was greater among subjects in the cabozantinib arm compared with everolimus at each time point. The respective landmark estimates of survival were 73% and 63% at 12 months and 58% and 47% at 18 months.

The primary analysis of ORR per IRC was conducted in the ITT population at the time of the primary analysis of PFS. The same data cutoff date was used as for the PFS analysis. Tumor assessments that occurred after the individual subject PFS-censoring dates were excluded from this analysis. The ORR in the ITT population for the cabozantinib and everolimus arms, respectively, was 17% (95% CI: 13, 22) and 3% (95% CI: 2, 6) (unstratified p-value < 0.0001). All responses were PRs. Of note there was a low incidence in the cabozantinib arm of PD as best response (12% vs 27%) which indicates a low incidence of primary refractory disease with cabozantinib in this study population.

In conclusion, cabozantinib demonstrated a robust and statistically significant improvement in PFS, OS, and ORR in a Phase 3 trial for subjects with advanced RCC in the second-line setting.

2.4.3.2 Medullary Thyroid Cancer: Study XL184-301 (EXAM; N=330)

Subjects were required to have radiographically documented disease progression by modified RECIST (mRECIST) compared with a radiologic assessment performed within the previous 14 months. Tumor assessments were evaluated by the blinded IRC to determine response and/or progression.

The estimated median PFS was 11.2 months for cabozantinib versus 4.0 months for placebo (HR 0.28; 95% CI, 0.19 to 0.40; p < .001). Prolonged PFS with cabozantinib was observed across all subgroups including by age, prior TKI treatment, and RET mutation status (hereditary or sporadic). Kaplan-Meier estimates of subjects alive and progression-free at 1 year are 47.3% for cabozantinib and 7.2% for placebo.

Patient subgroups harboring any RET mutation, the RET M918T mutation, or no RET mutation all demonstrated an HR of less than 1, indicating benefit of cabozantinib treatment; however, the upper bound for the 95% CI for RET mutation negative subjects exceeded 1.

The final analysis of the secondary endpoint of OS included 218 events (217 were required) and showed a trend for increased survival in the cabozantinib arm. The estimated median OS for the cabozantinib arm was 26.6 months versus 21.1 months for the placebo arm (HR = 0.85; 95% CI 0.64, 1.12; p = 0.2409).

Because MTC is a relatively rare disease, this study was not designed to be large enough to provide high power to detect the minimum clinically meaningful difference in the secondary endpoint of OS. Instead, the study size was chosen to provide reasonable power (80%) for a large

(50%) improvement in OS (HR 0.667; improved median from 22 to 33 months). The final analysis was consistent with analyses at information fractions of 75%, 85%, and 90% which included 162, 185, and 196 of the required 217 deaths respectively. The consistency of the OS results at different information fractions supports the conclusion that although not statistically significant, the trend in increased survival with cabozantinib treatment did not likely arise by chance.

The subgroup analysis of subjects with a RET M918T mutation revealed a larger improvement in OS for the cabozantinib arm: the median OS was 44.3 months for the cabozantinib arm versus 18.9 months for the placebo arm (HR = 0.60; 95% CI 0.38, 0.94; p = 0.0255 not adjusted for multiple subgroup analyses. In the current study 126 subjects or 38% had RET M918T mutation (the majority of which were somatic mutations), representing 54% of 233 subjects whose RET M918T status was known.

The analysis of tumor response among subjects with measurable disease at baseline was conducted at the time of the primary analysis of PFS. The ORRs per IRC were 28% (95% CI: 21.9%, 34.5%) and 0% for subjects in the cabozantinib and placebo arms, respectively. All responses were PRs, no subjects in either treatment group had a confirmed CR. The p-value from the stratified Cochran-Mantel-Haenszel test was highly significant ($p < 0.0001$). The median duration of objective responses was 14.6 months (95% CI: 11.1, 17.5) for subjects in the cabozantinib arm. The median duration of the best overall response (BOR) of SD, as determined by the IRC, was 10.8 months (95% CI: 8.3, 11.2) for the cabozantinib arm compared with 5.7 months (95% CI: 5.6, 8.4) for the placebo arm.

2.4.3.3 Castration-Resistant Prostate Cancer

Study XL184-307 (COMET-1; N=1023)

The study enrolled subjects with advanced CRPC with bone metastases. The prespecified primary analysis of the primary endpoint (OS) was based on an ITT analysis of all randomized subjects. The analysis included 614 events (578 were required) and did not demonstrate a statistically significant improvement in OS for subjects in the cabozantinib arm compared with the prednisone arm: the HR, adjusted for prior cabazitaxel use, baseline Brief Pain Inventory (BPI; Item 3) score, and baseline ECOG performance status was 0.90 (95% CI: 0.76, 1.06; stratified logrank p-value 0.213). The Kaplan-Meier estimates for median duration of OS were 11.0 months in the cabozantinib arm and 9.8 months in the prednisone arm, an estimated 1.2 month difference in the medians. The secondary efficacy endpoint was the proportion of subjects with a BSR per IRC at Week 12. BSR was defined as a $\geq$ 30% decrease in total bone-scan lesion area compared with baseline without soft-tissue disease progression. The analysis demonstrated a statistically significant improvement in the cabozantinib arm compared with the prednisone arm (42% vs 3%; stratified Cochran-Mantel-Haenszel [CMH] p-value < 0.001). The median duration of BSR was 5.8 vs 1.8 months. Median PFS per Investigator was 5.6 months in the cabozantinib arm and 2.8 months in the prednisone arm. The HR adjusted for randomization stratification factors was 0.48 (95% CI 0.40, 0.57; stratified logrank p-value < 0.0001).

Study XL184-306 (COMET-2; N=119)

The study enrolled subjects with advanced CRPC with bone metastases. Enrollment was stopped before the planned study population size of 246 was reached; a total of 119 subjects were enrolled. The primary analysis for the ITT population (n=119) did not demonstrate an improvement in the proportion of subjects with a pain response at Week 6 confirmed at Week 12 in the cabozantinib arm compared with the mitoxantrone plus prednisone arm; the proportions of

subjects experiencing a confirmed pain response were 15% and 17% for the two arms, respectively. The secondary endpoints were BSR per IRC and OS. BSR was defined as a ≥ 30% decrease in total bone-scan lesion area compared with baseline without soft-tissue disease progression. The analysis of BSR per IRF at Week 12 without progression in soft tissue per mRECIST 1.1 demonstrated a greater improvement in the cabozantinib arm (31%; 95% CI: 20%, 43%) compared with the mitoxantrone plus prednisone arm (5%; 95% CI: 0%, 11%). In the analysis of OS that included 78 deaths out of the 196 pre-specified deaths, the stratified HR was 0.70 (95% CI: 0.44, 1.10). The Kaplan-Meier estimates for median duration of OS were 9.0 months in the cabozantinib arm versus 7.9 months in the mitoxantrone plus prednisone arm.

2.4.3.4 Efficacy Results in Other Clinical Trials

Cabozantinib has been studied in a broad selection of tumor types. A brief summary of the available clinical activity results for cabozantinib in these tumor types is provided in Table 5-14.

Table: Assessment of Cabozantinib Seen in Multiple Tumor Types

Tumor Type	Study	Median PFS (months)	Week 12 DCR[a] (%)
DTC	XL184-008	not reached	80[b]
RCC	XL184-008	12.9	72[b]
Ovarian Cancer	XL184-203 RDT	5.5	50
	XL184-203 NRE	4.0	NA
CRPC	XL184-203 RDT	6.8	66
	XL184-203 NRE	4.6[c]	NA
	XL184-203 NRE	6.5[d]	NA
HCC	XL184-203 RDT	5.2	66
Breast Cancer	XL184-203 RDT	4.3	47
Melanoma	XL184-203 RDT	2.8	43
NSCLC	XL184-203 RDT	4.0	38
SCLC	XL184-203 RDT	3.4	43
Pancreatic Cancer	XL184-203 RDT	2.7	35
Gastric/GEJ Cancer	XL184-203 RDT	1.4	33
GB	XL184-201	3.7[e]	NA

CRPC, castration-resistant prostate cancer; DCR, disease control rate; DTC, differentiated thyroid cancer; GB, glioblastoma; GEJ, gastroesophageal junction; HCC, hepatocellular carcinoma; PFS, progression-free survival; NA, not available; NRE, non-randomized expansion; NSCLC, non-small cell lung cancer; RCC; renal cell carcinoma; RDT, randomized discontinuation trial; SCLC, small-cell lung cancer.

Note: Data reported in this table is from the final endpoint analyses.

[a] DCR = (confirmed response [CR] + partial response [PR] + stable disease [SD])

[b] DCR at Week 17

[c] For the CRPC cohort that was assigned a dose of 40 mg qd

[d] For the CRPC cohort that was assigned a dose of 100 mg qd

[e] For Treatment Group C (100 mg qd). See Section 5.5.2.2 for further details.

2.5 Rationale

Recognizing that overexpression of immune checkpoint molecules in the tumor microenvironment has a critical role for antitumor immunity evasion and for cancer progression has revolutionized cancer treatment[4]. In particular, anti-PD-1/PD-L1 antibodies have demonstrated clinical activity in more than 15 cancer types and two PD-1 inhibitors (nivolumab

and pembrolizumab) are FDA approved for treatment of advanced melanoma, non-small-cell lung carcinoma and renal cell carcinoma[5-7]. More than 1,000 clinical trials of checkpoint blockers are currently ongoing.

Immune checkpoint inhibitors (ICI), have revolutionized cancer therapy for patients with advanced solid tumors [Leach *et al.,* 1996, Pardoll, 2012, Freeman *et al.,* 2000]. A large body of preclinical and clinical evidence suggesting that the immune system can recognize and fight against breast cancer [Kroemer *et al.,* 2015, Savas *et al.,* 2015]. It is now recognized that a fraction of breast tumors, especially TNBC, have substantial lymphocyte infiltration and that this pathologic feature has prognostic implications [Savas *et al.*, 2015]. Furthermore, the high rates of PD-L1 and PD-1 expression in patients with TNBCs led to clinical trials to address the role of PD-1 blockers in this population [Gatalica *et al.,* 2014].

The recently presented KEYNOTE 086 trial, the largest study evaluating the efficacy of PD-1 inhibitors (Pembrolizumab given in monotherapy; flat dose of 200mg every 3 weeks) in patients with mTNBC showed different outcomes based on the number of previous lines of therapy: cohort A included 170 patients who had received ≥1 prior chemotherapy for metastatic disease, and showed an objective response rate of 4.7% (2% - 9%) [Adams *et al.,* 2015] ; Perhaps, more important is the duration of response with the PD-1 inhibitors in patients with mTNBC: 6.3 months; with more than 5 (63%) responders without progression of disease at study data cut off; cohort B included 52 patients no prior systemic anticancer therapy for metastatic disease, and showed an objective response rate of 23% (14% - 36%) [Adams *et al.,* 2017]. While checkpoint blockade shows activity in a subset of mTNBC, these results suggest that there must be additional immunosuppressive factors causing resistance to immunotherapy in this population, and other agents must be added to anti-PD-1/PD-L1 treatment in order to enhance the benefit of immunotherapy in TNBC.

Despite BC not being considered an immunogenic neoplasia, an increasing body of preclinical and clinical evidence suggests that the interaction with the immune system is critical for disease outcome in this disease as well[8]. It is now recognized that a fraction of breast tumors, especially TNBC, have substantial lymphocyte infiltration and that this pathologic feature has prognostic implications[9]. Furthermore, the high rates of PD-L1 and PD-1 expression in patients with TNBCs led to clinical trials to address the role of PD-1 blockers in this population[10]. The anti-PD-L1 atezolizumab was evaluated in patients with metastatic TNBC PD-L1 positive and showed an objective response rate (ORR) of 19% and a 6-month PFS of 27%[11]. Similar results were found in a PD-L1 positive population with the anti-PD-1 inhibitor pembrolizuamb (ORR of 18% and 6-month PFS of 23%)[12]. Data from a trial with avalumab has demonstrated that in a non-selected population, the use of PD-1/PD-L1 inhibitors presented an objective response rate (ORR) of 8.6%[13].

Clearly, these results show that, while checkpoint blockade may show activity in TNBC, other agents must be added to anti-PD-1/PD-L1 treatment in order to enhance the benefit. In addition, the fact that the majority of patients with advanced cancer, including TNBC, still do not derive clinical benefit from these drugs suggests that additional immunosuppressive mechanisms within the tumor microenvironment have a role in de novo resistance to these therapies. Some of these mechanisms have been identified, and now provide targets for combination therapy[14]. In this context, vascular endothelial growth factor-A (VEGF-A), the key molecule in tumor angiogenesis, has been recognized as a critical mediator of tumor immune suppression[15]. In addition to its role in angiogenesis, VEGF modulates anti-tumor immunity on multiple levels, including promotion and expansion of inhibitory immune cell subsets, such as T regulatory cells

(Tregs) and myeloid-derived suppressor cells (MDSCs), inhibition of dendritic cell (DC) maturation, mitigation of effector T cell responses, and alteration of lymphocyte development and trafficking[16]. Consequently, targeting angiogenesis may be an effective strategy to increase the efficacy of primarily T cell-directed immunotherapy, such as immune checkpoint blockade [17].

Recently, Voron at al. showed in a mouse model that that VEGF-A produced in the tumor microenvironment enhances expression of PD-1 and other inhibitory checkpoints mediating CD8+ T cell exhaustion, which could be reverted by different anti-angiogenic agents (anti-VEGF-A antibody or tyrosine kinase inhibitors (TKI), such as sunitinib, which target VEGF receptor family member (VEGFR). The administration of masatinib, a TKI that does not target VEGFR, was not able to revert the PD-L1 overexpression VEGF-induced (Figure 1). Importantly, the authors demonstrated in vitro that the expression of PD-1 on CD8+ T cells is induced by VEGF-A in a VEGFR-2-dependent manner, but is not VEGFR-1-dependent. Because anti–VEGF-A treatment decreased PD-1 expression on intratumoral CD8+ T cells but does not fully restore the steady-state level of PD-1 expression (Figure 2a), the authors hypothesized that VEGF-A blockade could help to sensitize T cells to anti–PD-1 antibody treatment in some tumors. Interestingly, anti– PD-1 antibody induced a significant antitumor effect only in VEGF-deleted tumor-bearing mice and not in wild-type tumor-bearing mice (Fig. 2b), suggesting that VEGF produced by tumor cells limits anti–PD-1–induced antitumor effects. Next, the authors combined anti-VEGF-A antibody with anti–PD-1 antibody in a CT26 mouse model of colorectal cancer expressing high levels of VEGF-A. Anti–VEGF-A treatment reduced tumor growth, and anti–PD-1 antibody alone had no significant effect, but the anti–VEGF-A and anti–PD-1 combination induced a strong antitumor effect as compared with anti–VEGF-A alone or anti-PD-1 alone (Fig. 2c). This result suggests that high VEGF-A levels may be involved in resistance to anti–PD-1 treatments and that antiangiogenic therapies targeting VEGF-A–VEGFR-2 could synergize with anti–PD-1 treatment strategies and enhance anti–PD-1–dependent antitumor effects.

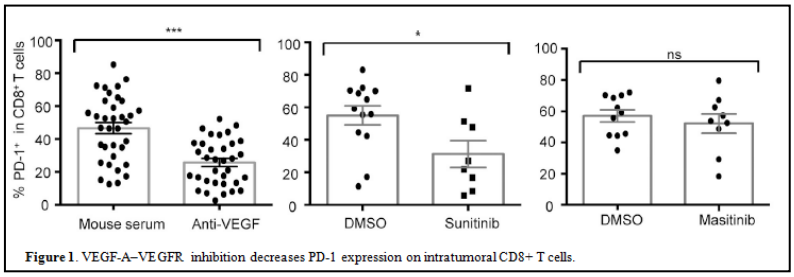


Figure 1. VEGF-A–VEGFR inhibition decreases PD-1 expression on intratumoral CD8+ T cells.

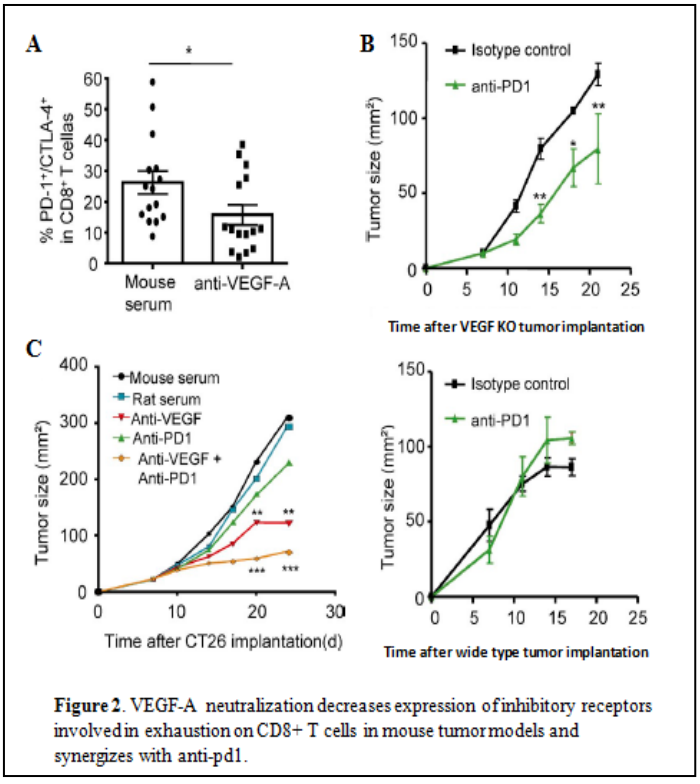


Figure 2. VEGF-A neutralization decreases expression of inhibitory receptors involved in exhaustion on CD8+ T cells in mouse tumor models and synergizes with anti-pd1.

In the clinical setting, a phase I study using bevacizumab (anti-VEGF antibody) plus the immune checkpoint ipilimumab (anti-CTLA4) conducted in patients with advanced melanoma showed an objective partial response rate of 17% and a disease control rate

of 67%[18]. Currently, several clinical trials are evaluating the combination of immune checkpoint blockade and angiogenesis inhibition in cancer patients.

Both high tumor microvessel density and elevated VEGF expression are associated with decreased relapse-free and OS in early stage BC, suggesting that angiogenesis is associated with a poor prognosis[19, 20]. Moreover, it is well known that bevacizumab in combination with chemotherapy has been associated with improvement in complete pathological response in neoadjuvant setting and with significantly improvement in progression-free survival (PFS) in several studies, although it has not prolonged OS in BC [21]. It has been recognized that inhibition of VEGF signaling alone, although initially effective in slowing tumor growth, often culminates in emergence of an evasive resistance phenotype that ultimately promotes tumor invasiveness and metastasis [22]. In this context, cabozantinib an oral bioavailable small molecule inhibitor of multiple receptor tyrosine kinases (RTK), including VEGFR-2, seems to be a very interesting drug to evaluate in combination with anti-PD1 drugs in patients with TNBC. Another important property of cabozantinib is that it inhibits MET, a RTK that promotes cell proliferation, invasion, and survival when activated by its ligand, hepatocyte growth factor (HGF, also known as the "scatter factor")[23]. *In vivo,* besides its antiangiogenic effects, cabozantinib rapidly induces tumor cell apoptosis, resulting in tumor regression in various xenograft models. In addition, increased expression of MET has been implicated in the development of resistance to antiangiogenic drugs in preclinical models of several cancers [24]. More recently, Balan et al reported that the MET-induced signaling promotes PD-L1 overexpression in renal cancer cells, establishing a link between MET pathway and antitumor immunity evasion [25].

Of note, MET and HGF overexpression have been associated with tumor hypoxia, increased invasiveness and metastasis, and reduced survival in metastatic breast cancer [26]. Specifically, in patients with TNBC, MET expression is elevated and associated with poorer prognosis [27, 28] . Preclinical data showed that cabozantinib synergizes with immunotherapy in different tumor mice models [Kwilas *et al.,* 2014, Lu *et al.,* 2017]. Recently, in a phase II trial, cabozantinib monotherapy demonstrated a clinical benefit rate of 34%, including 9% of partial response rate, in heavily pre-treated patients with mTNBC[29]. Furthermore, correlative studies performed in this trial showed: 1) high baseline plasma concentration of sMET was associated with longer PFS and tended to correlate with clinical benefit; 2) the concentration of plasma HGF was lower in patients with clinical benefit versus those without, though this association did not reach statistical significance; 3) cabozantinib treatment was associated with changes in biomarker concentrations that are consistent with anti-vascular effects and increases in tissue hypoxia, i.e., increases in plasma CAIX, PlGF, VEGF and SDF1α; 4) Flow cytometric analyses showed a significant and persistent increase in the fraction of circulating CD3+ T cells after cabozantinib therapy, which appeared to be driven by an increase in the CD4–/CD8+ cytotoxic T lymphocyte (CTL) population, and a significant decrease in the CD14+ monocytic population, which is considered as an immunosuppressive myeloid population. These findings highlight the possibility that combinations of cabozantinib with immunotherapy will improve the outcomes in this population. In the clinical setting, other groups including investigators at DF/Harvard Cancer Center have been explored the immunological effects of the combination of antiangiogenic drugs plus ICI. Two early phase trials showed an increase in the tumor-infiltrating lymphocytes rates in patients with melanoma and kidney cancer [Hodi *et al.,* 2014, Wallin *et al.,* 2016]. Altogether, these data highlight the possibility that combinations of cabozantinib with immunotherapy will improve the outcomes in this population.

Based on the above rationale, we hypothesize that cabozantinib (by increasing the number of CTLs) will synergize with nivolumab (which will activate the CTLs), resulting in improvement of efficacy of immunotherapy. We propose to test this hypothesis by evaluating the combination of cabozantinib plus nivolumab (anti-PD-1 monoclonal antibody) in patients with metastatic TNBC in an open-label, single arm phase II study. The primary endpoint will be to evaluate the efficacy of cabozantinib in combination with nivolumab, as defined by objective response rate (ORR) according to RECIST 1.1 in patients with metastatic TNBC previously treated with 0 to 3 lines of chemotherapy in the metastatic setting. Additionally, we will perform correlative studies in order to explore the effect of treatment on serum markers relevant to VEGF-VEGFR2 and HGF/MET pathways, as well on immunologic biomarkers (plasma sVEGFR2, IL-2, IFN-γ, TNF-α, sMET) in peripheral blood. In addition, research biopsies will be performed at baseline (mandatory if the lesion is accessible) and within 7-14 days before starting cycle 3 of treatment.

2.6 Correlative Studies Background

2.6.1 Blood and Tissue Analysis

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

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

The T-cell inflamed phenotype has positive prognostic value for several types of early stage cancer, including breast cancer[Dushyanthen *et al.*, 2015, Perez *et al.*, 2015], suggesting that the attempt by the host to generate an anti-tumor immune response reflects a biologic process associated with improved patient outcomes[Gajewski, 2015]. In breast oncology, different groups have demonstrated that the amount of tumor-infiltrating lymphocytes (TILs) in a tumor specimen, commonly assessed simply by histological evaluation of a standard hematoxylin and eosin-stained

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

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

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

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

These correlative projects are made possible by collaboration with Drs. Scott Rodig and Evisa Gjini, 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.

2.6.2 Microbiome Analysis

Breast Cancer (BC) is the most frequently diagnosed cancer and the second cause of cancer death in American women [DeSantis *et al.,* 2015]. In the advanced setting, despite multiple

available systemic therapies, virtually all patients will die from their disease. Thus, the exploration of new treatments, such as immune checkpoint inhibitors (ICI), including nivolumab, is imperative.

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

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

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

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

2.6.3 Tumor Genomic Profile

In addition to the immune microenvironment, intrinsic tumor factors may be associated with response to immune checkpoint inhibitors. Although some of the mechanisms related to de novo or acquired resistance to ICI have been recently described, including loss of function in beta-2- microglobulin or defects in the interferon signaling pathway[Gao *et al.*, 2016, Zaretsky *et al.*, 2016], the knowledge of immune resistance remains largely unknown. Several gene/pathways have been described as possible candidates of having an immunosuppressive role in different

advanced solid tumor, including MYC amplification[Casey *et al.*, 2016], activation in WNT-β-catenin pathway[Spranger *et al.*, 2015], activation in MAPK pathway, loss of PTEN[Li *et al.*, 2016, Peng *et al.*, 2016, George *et al.*, 2017]. On the other hand, few possible biomarkers of response to ICI have emerged, including mutational load[Snyder *et al.*, 2014, Rizvi *et al.*, 2015], tumor aneuploidy[Davoli *et al.*, 2017], mismatch repair defects[Le *et al.*, 2015], and BRCA2 mutation[Hugo *et al.*, 2016]. Notably, there is no data on genomic mechanisms of de novo resistance to anti-PD-1 therapy in patients with breast cancer.

Therefore, as a correlative study to this trial, we will to explore whether the number and/or type of mutations identified using a next generation sequencing (NGS) panel – OncoPanel - is correlated with patient outcomes (PFS, ORR, and CBR). This tool is a cancer genomic assay to detect somatic mutations, copy number variations and structural variants in tumor DNA extracted from fresh, frozen or formalin-fixed paraffin-embedded samples. The OncoPanel assay surveys exonic DNA sequences of 447 cancer genes and 191 regions across 60 genes for rearrangement detection. DNA is isolated from tissue containing at least 20% tumor nuclei and analyzed by massively parallel sequencing using a solution-phase Agilent SureSelect hybrid capture kit and an Illumina HiSeq 2500 sequencer. The targeted NGS assay (OncoPanel) will be performed at the Center for Advanced Molecular Diagnostics (Department of Pathology, Brigham and Women's Hospital). This assay has been extensively validated and is used as a CLIA-approved clinical molecular test in our institution without any additional sequencing assays to validate the findings [Wagle *et al.*, 2012].

3. PARTICIPANT SELECTION

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

3.1 Eligibility Criteria

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

3.1.2 Estrogen-receptor and progesterone-receptor expression both <10% by immunohistochemistry (IHC) and HER2 negative by IHC or non-amplified as determined by the current ASCO-CAP criteria. If patient has more than one histological result, the most recent one has to be considered for inclusion.

3.1.3 Participants must have measurable disease by RECIST version 1.1

3.1.4 Participants must agree to undergo a research biopsy, if tumor is safely accessible, at baseline and within14 days prior to C3D1. Participants for whom newly-obtained samples cannot be provided (e.g. inaccessible or participant safety concern) may submit an archived specimen.

Note: After the first 6 participants undergo biopsy on cabozantinib, we will review their adverse event profiles to ensure no more than a 20% rate of grade 3 or higher bleeding or wound healing complications occur. If more than 2 patients (>20%) have safety concerns, we will reassess the safety of collecting the research biopsies. Full review of all grade (including grade 1 and 2) may also prompt changes and will be reviewed by the study team. Exelixis may be consulted if necessary.

3.1.5 Prior chemotherapy: Participants may have received 0-3 prior chemotherapeutic regimens for metastatic breast cancer and must have been off treatment with chemotherapy for at least 14 days prior to registration. Participants should also be adequately recovered from acute toxicities of prior treatment.

3.1.6 Prior biologic therapy: Patients must have discontinued all biologic therapy at least 14 days prior to registration.

3.1.7 Prior radiation therapy: Patients may have received prior radiation therapy in either the metastatic or early-stage setting. Radiation therapy must be completed per the following timelines:

i) Radiotherapy to the thoracic cavity or abdomen within 4 weeks prior to registration.

ii) Radiotherapy to bone lesions within 2 weeks prior to registration.

iii) Radiotherapy to any other site within 4 weeks prior to registration.

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

3.1.8 The subject is ≥18 years old.

3.1.9 ECOG performance status ≤1(Karnofsky ≥60%, see Appendix A)

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

- absolute neutrophil count ≥1,000/mcL
- platelets ≥100,000/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 ≤ 3 × institutional ULN for participants with documented liver metastases
- creatinine >1.5 ×within normal institutional ULN OR creatinine clearance ≥40 mL/min (using Cockcroft-Gault formula) for participants with creatinine levels above institutional ULN.
- Urine protein/creatinine ratio (UPCR) ≤1

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

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

3.1.12 Female and male participants of childbearing potential 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. The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or postovulation methods) and withdrawal are not acceptable methods of contraception.

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

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

3.2 Exclusion Criteria

3.2.1 Major surgery within 12 weeks before the first dose of study treatment. Complete wound healing from major surgery must have occurred 1 month before the first dose of study treatment. Minor surgery (including uncomplicated tooth extractions) within 28 days before the first dose of study treatment with complete wound healing at least 10 days before the first dose of study treatment. Allclinically relevant ongoing complications from prior surgery should be resolved before the first dose of study treatment.

3.2.2 The participant has tumor in contact with, invading, or encasing major blood vessels or radiographic evidence of significant cavitary pulmonary lesions.

3.2.3 The subject has pathologic evidence of tumor invading the GI tract (esophagus, stomach, small or large bowel, rectum or anus), or any evidence of endotracheal or endobronchial tumor within 28 days before the first dose of cabozantinib;

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

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

3.2.6 The participant has received a prior c-Met inhibitor

3.2.7 Has received 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)

3.2.8 Known brain metastases that are untreated, symptomatic, or require therapy to control symptoms. Participants with a history of treated central nervous system (CNS) metastases are eligible. Treated brain metastases are defined as those having no evidence of progression for ≥ 1 month after treatment, and no ongoing requirement for corticosteroids, as ascertained by clinical examination and brain imaging (magnetic resonance imaging or CT scan) completed during screening. Any corticosteroid use for brain metastases must have been discontinued without the subsequent appearance of symptoms for ≥2 weeks prior to registration. Treatment for brain metastases may include whole brain radiotherapy, radiosurgery, or a combination as deemed appropriate by the treating physician. Participants with CNS metastases treated by neurosurgical resection or brain biopsy performed within 2 months before day 1 will be excluded.

3.2.9 The subject has uncontrolled, significant intercurrent or recent illness including, but not limited to, the following conditions:

a. Cardiovascular disorders including:

i. Congestive heart failure (CHF): New York Heart Association (NYHA) Class III (moderate) or Class IV (severe) at the time of screening;
ii. Concurrent uncontrolled hypertension defined as sustained blood pressure (BP) > 150 mm Hg systolic or > 100 mm Hg diastolic despite optimal antihypertensive treatment within 7 days of the first dose of study treatment;
iii. Any history of congenital long QT syndrome;
iv. Any of the following within 6 months before the first dose of study treatment:
- unstable angina pectoris;
- clinically-significant cardiac arrhythmias;
- stroke (including transient ischemic attack (TIA), or other ischemic event);
- myocardial infarction;

b. GI disorders particularly those associated with a high risk of perforation or fistula formation including:
i. Tumors invading the GI tract, active peptic ulcer disease, active inflammatory bowel disease (eg, Crohn's disease), active diverticulitis, cholecystitis, symptomatic cholangitis or appendicitis, acute pancreatitis or acute obstruction of the pancreatic duct or common bile duct, or gastric outlet obstruction
ii. Abdominal fistula, GI perforation, bowel obstruction, intra-abdominal abscess within 6 months before randomization,
Note: Complete healing of an intra-abdominal abscess must be confirmed prior to randomization

c. Other clinically significant disorders that would preclude safe study participation;

3.2.10 QTcF interval >500 msec

Note: Three ECGs must be performed for eligibility determination. If the average of these three consecutive results for QTcF is ⩽ 500 msec, the subject meets eligibility in this regard.

3.2.11 Thromboembolic events requiring therapeutic anticoagulation. Concomitant anticoagulation with oral anticoagulants (eg, warfarin, direct thrombin and Factor Xa inhibitors), platelet inhibitors (eg, clopidogrel) are prohibited.

Note: Low-dose aspirin for cardioprotection (per local applicable guidelines) and low-dose LMWH are permitted (in subjects who are on a stable dose of LMWH for at least 6 weeks before the first dose of study treatment, and who have had no clinically significant hemorrhagic complications from the anticoagulation regimen or the tumor).

Note: Subjects with a venous filter (eg vena cava filter) are not eligible for this study).

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

3.2.13 Participant has an active infection requiring IV antibiotics

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

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

3.2.16 Known hypersensitivity to any of the components of cabozantinib or nivolumab.

3.2.17 The participant has received a live vaccine within 28 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine. The use of the inactivated seasonal influenza vaccine (Fluzone®) is allowed.

3.2.18 The subject has experienced any of the following:

- Clinically significant gastrointestinal bleeding within 6 months before the first dose of study treatment
- Clinically significant hemoptysis within 3 months of the first dose of study treatment
- Any other signs indicative of pulmonary hemorrhage within 3 months before the start of study treatment

3.2.19 The participant is unable to swallow oral dosage forms.

3.2.20 The participant is pregnant or breastfeeding.

Inclusion of Women and Minorities

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

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

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

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

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

4.2 Registration Process for DF/HCC Institutions

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

5. TREATMENT PLAN

5.1 Treatment Regimen

This is an open-label, single-arm, phase 2 study of nivolumab given every 28 days intravenously in combination with cabozantinib given orally once daily for 28 days. Thirty-five participants will be enrolled to the study to assess the efficacy of the combination as defined by objective response rate (ORR) according to RECIST 1.1, in patients with metastatic TNBC.

No investigational or commercial agents of therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Table 1: Regimen Description

Agent	Premedication ; Precautions	Dose	Route	Schedule	Cycle Length
Nivolumab (BMS-936558-01)	Not routinely necessary unless prior infusion reaction.	480 mg	IV	Every 28 days (4 weeks)	28 days (4 weeks)
Cabozantinib	Not necessary	40mg	Oral	Continuous, once daily	28 days (4 weeks)

5.2 Pre-Treatment Criteria

5.2.1 Cycle 1, Day 1

Participants do not need to re-meet eligibility criteria on Cycle 1, Day1if screening laboratory evaluations are completed within 3 days of Cycle 1 Day 1.

- absolute neutrophil count ≥1,000/mcL
- platelets ≥100,000/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 ≤ 3 × institutional ULN for participants with documented liver metastases

- creatinine >1.5 ×within normal institutional ULN OR creatinine clearance ≥40 mL/min (using Cockcroft-Gault formula) for participants with creatinine levels above institutional ULN.
- urine protein/creatinine ratio (UPCR) ≤ 1.

5.2.2 Subsequent Cycles, Day 1

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

5.3 Agent administration

On days when Nivolumab and Cabozantinib are administered on the same day (i.e. Day 1 of each cycle) the order of agent administration does not matter..

5.3.1 Nivolumab Administration

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 (USP to concentrations no less than 0.35 mg/mL) or 5% Dextrose.

Nivolumab will be administered every 28 days at a dose of 480mg given intravenously over 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.

5.3.2 Cabozantinib administration

Cabozantinib will be administered orally, once daily for 28 days at a dose of 40 mg. Cabozantinib should be taken 2 hours after a meal (water is permitted) with a full glass of water (8oz/240mL). Participants should continue to fast for 1 hour after taking cabozantinib. Participants will be provided with dosing instructions, a drug diary and a sufficient supply of cabozantinib for continuous daily dosing. Cabozantinib should be dosed on an outpatient basis even on days where Nivolumab and Cabozantinib are dose together (i.e. Day 1 of each cycle). While taking Cabozantinib, participants should avoid consumption of grapefruit and Seville oranges.

5.4 Definition of Dose-Limiting Toxicity (DLT)

We will perform a safety run-in analysis on the first 6 participants enrolled to the trial. If there are 2 or more dose-limiting toxicity (DLTs) in the first 6 participants, the trial will be closed for further enrollment.

After the first safety run-in of 6 patients, which ended on 4/3/2018, 2 DLTs had been identified. Given that new safety data provided by Exelixis using the same dosages of Nivolumab and Cabozantinib proved to be tolerable, this study will enroll an additional 6 participants in a second safety run-in. If there are 2 or more DLTs in the second safety run-in, the trial will be closed to further enrollment. If less than or equal to 1, the trial will continue to enroll to the efficacy run-in.

Dose-limiting toxicity is defined as any of the following occurring during the first cycle (28 days) of therapy, if judged by the investigator to be possibly, probably, or definitely related to study drug administration:

- Any grade 5 toxicity
- Grade 3 Thrombocytopenia if associated with bleeding
- Grade 4 Thrombocytopenia of any duration
- Grade 3 or higher Febrile neutropenia
- Any other grade 4 hematologic toxicity lasting ≥14 days, unless deemed by the investigator to be clinically insignificant
- ≥ Grade 3 elevation in AST or ALT
- Grade 2 bilirubin elevation
- Cases of Hy's Law defined as:
    - AST or ALT 3X ULN and bilirubin 2X ULN, not cholestatic and probably caused by study drug.
- Any ≥ Grade 3 non-hematologic laboratory value <u>if:</u>
    - Medical intervention is required to treat the patient, or
    - The abnormality leads to hospitalization, or
    - The abnormality persists >7 days

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

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

5.5 General Concomitant Medication and Supportive Care Guidelines

5.5.1 Concomitant Medication Guidelines

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during

the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the overall PI.

Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care and documented in the medical record. Selected medications of interest received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment, including dosage, frequency, route, and dates of administration will be recorded.

Concomitant medications that are known to prolong the QTc interval and should be used with caution in subjects who receive cabozantinib until they have permanently discontinued cabozantinib treatment (refer to http://www.qtdrugs.org for a list of drugs which have the potential to prolong the QTc interval).

Every effort to avoid radiation therapy while participating on this protocol should be made; however, if it is of clinical necessity for the patient, the circumstances should be discussed with the PI and it may be permitted.

Prohibited Concomitant Medications

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

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Investigational agents other than nivolumab and cabozantinib
- Any systemically active oral, injected, or implanted hormonal method of contraception except for progesterone coated intrauterine devices (IUDs) that had been previously implanted.
- Estrogen replacement therapy.
- Live vaccines within 28 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine. The use of the inactivated seasonal influenza vaccine (Fluzones, BCG, and ty
- Systemic glucocorticoids should be avoided for any purpose other than to modulate symptoms from radiation or an event of clinical interest of suspected immunologic etiology. If corticosteroids are required for this purpose, the minimum effective dose should be used. The use of physiologic doses of corticosteroids (10 mg prednisone daily or equivalent) can be used without principal investigator (PI) authorization.

- Concomitant anticoagulation with oral anticoagulants (eg, warfarin, direct thrombin and Factor Xa inhibitors), platelet inhibitors (eg, clopidogrel) are prohibited. Note: Low-dose aspirin for cardioprotection (per local applicable guidelines) and low-dose LMWH are permitted. Anticoagulation with therapeutic doses of LMWH is allowed.

- Subjects may receive other medications that the investigator deems to be medically necessary.

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

5.5.2 Supportive Care Guidelines – general medications

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

- Standard therapies for pre-existing medical conditions unless listed as prohibited therapy. Any medication intended solely for supportive care (e.g., analgesics, anti-diarrheal, anti-depressants) may be used at the investigator's discretion. Antiemetics and anti-diarrheal medications should not be administered prophylactically before initial treatment with study drugs. At the discretion of the investigator, prophylactic antiemetic and anti-diarrheal medication(s) may be used as per standard clinical practice before subsequent doses of study drugs or before, during or after radiation treatment.
- Bisphosphonate or denosumab therapy to be used in accordance with the approved labeled indication and/or nationally recognized treatment guidelines. Participants already receiving bisphosphonate/denosumab at the time of study entry can continue the treatment. Participants may initiate treatment with bisphosphonate/denosumab after study entry with physician discretion.
- Concomitant anticoagulation with oral anticoagulants (eg, warfarin, direct thrombin and Factor Xa inhibitors), platelet inhibitors (eg, clopidogrel) are prohibited. Note: Low-dose aspirin for cardioprotection (per local applicable guidelines) and low-dose LMWH are permitted. Anticoagulation with therapeutic doses of LMWH is allowed.
- 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:

Cytochrome P450: Preliminary data from a clinical drug interaction study (Study XL184-008) show that clinically relevant steady-state concentrations of cabozantinib appear to have no

marked effect on the AUC of co-administered rosiglitazone, a CYP2C8 substrate. Therefore, cabozantinib is not anticipated to markedly inhibit CYP2C8 in the clinic, and by inference, is not anticipated to markedly inhibit other CYP450 isozymes that have lower [I]/Ki values compared to CYP2C8 (*i.e.*, CYP2C9, CYP2C19, CYP2D6, CYP1A2, and CYP3A4). In vitro data indicate that cabozantinib is unlikely to induce cytochrome P450 enzymes, except for possible induction of CYP1A1 at high cabozantinib concentrations (30 μM).

Cabozantinib is a CYP3A4 substrate (but not a CYP2C9 or CYP2D6 substrate), based on data from in vitro studies using CYP-isozyme specific neutralizing antibodies.

Preliminary results from a clinical pharmacology study, XL184-006, showed that concurrent administration of cabozantinib with the strong CYP3A4 inducer, rifampin, resulted in an approximately 80% reduction in cabozantinib exposure (AUC values) after a single dose of cabozantinib in healthy volunteers. Co-administration of cabozantinib with strong inducers of the CYP3A4 family (*e.g.*, dexamethasone, phenytoin, carbamazepine, rifampin, rifabutin, rifapentin, phenobarbital, and St. John's Wort) may significantly decrease cabozantinib concentrations. The chronic use of strong CYP3A4 inducers should be avoided. Other drugs that induce CYP3A4 should be used with caution because these drugs have the potential to decrease exposure (AUC) to cabozantinib (*e.g.*, chronic use of modafinil) should be avoided because of its potential to reduce cabozantinib exposure. Selection of alternate concomitant medications with no or minimal CYP3A4 enzyme induction potential is recommended. In addition, caution must be used when discontinuing treatment with a strong CYP3A4 inducer in a subject who has been concurrently receiving a stable dose of cabozantinib, as this could significantly increase the exposure to cabozantinib.

Preliminary results from a clinical pharmacology study, XL184-007, showed that concurrent administration of cabozantinib with the strong CYP3A4 inhibitor, ketoconazole, resulted in a 33-39% increase in the cabozantinib exposure (AUC values) after a single dose of cabozantinib in healthy volunteers. Co-administration of cabozantinib with strong inhibitors of the CYP3A4 family (*e.g.*, ketoconazole, itraconazole, clarithromycin, indinavir, nefazodone, nelfinavir, and ritonavir) may increase cabozantinib concentrations. Grapefruit / grapefruit juice and Seville oranges may also increase plasma concentrations of cabozantinib. Strong CYP3A4 inhibitors and other drugs that inhibit CYP3A4 should be used with caution because these drugs have the potential to increase exposure (AUC) to cabozantinib. Selection of alternate concomitant medications with no or minimal CYP3A4 enzyme inhibition potential is recommended.

Because in vitro studies only assessed the metabolizing capacity of the CYP3A4, CYP2C9, and CYP2D6 pathways, the potential for drugs that inhibit/induce other CYP450 pathways (*e.g.*, CYP2C8, CYP2C19, CYP2B6, CYP1A2) to alter cabozantinib exposure is not known. Therefore, these drugs should be used with caution when given with cabozantinib.

Please refer to the Flockhart drug interaction tables for lists of substrates, inducers, and inhibitors of selected CYP450 isozyme pathways (Flockhart 2007; *http://medicine.iupui.edu/clinpharm/ddis/table.aspx*).

Protein Binding: Cabozantinib is highly bound (≥ 99.7%) to human plasma proteins. Therefore, highly protein bound drugs should be used with caution with cabozantinib because there is a potential displacement interaction that could increase free concentrations of cabozantinib and/or a co-administered highly protein-bound drug (and a corresponding increase in pharmacologic effect).

In vitro data suggest that cabozantinib is unlikely to be a substrate for P glycoprotein (P-gp), but it does appear to have the potential to inhibit the P-gp transport activity.

Additional details related to these overall conclusions are provided in the Investigators Brochure.

5.6 Criteria for Taking a Participant Off Protocol Therapy

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

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

3. Participants who have attained a confirmed complete response (CR) that have been treated for at least 24 weeks on protocol therapy and had at least two treatments with nivolumab beyond the date when the initial CR was declared. Participants who stop nivolumab with CR may be eligible for additional nivolumab therapy if they progress after stopping study treatment. This retreatment is termed the Second Course Phase Therapy. See additional details below.

- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Participant demonstrates an inability or unwillingness to comply with the medication regimen and/or documentation requirements
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator

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

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, Sara Tolaney, phone: 617-632-2335, or email: stolaney@partners.org.

Second Course Phase (Retreatment Period)

Participants may elect to stop nivolumab and cabozantinib 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 cabozantinib after attaining an investigator-determined confirmed CR according to RECIST 1.1, was treated for at least 24 weeks with nivolumab before discontinuing therapy, and received at least two treatments with nivolumab beyond the date when the initial CR was declared

AND

- experienced an investigator-determined confirmed radiographic disease progression after stopping their initial treatment with nivolumab
- did not receive any anti-cancer treatment other than cabozantinib since the last dose of nivolumab
- meets all other study inclusion/exclusion criteria, as per Section 3.

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.

5.7 Duration of Follow Up

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

Participants who are alive and free of disease progression at the time of removal from protocol therapy will be followed for first disease progression event after removal from protocol therapy.

Tumor assessments should continue to be performed every 6-12 weeks on these participants.

5.8 Criteria for Taking a Participant Off Study

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

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

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

6. DOSING DELAYS/DOSE MODIFICATIONS

Dose delays 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/Modficiation

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.

Subjects may hold each drug independently of one another and if one must permanently be discontinued, they may continue on the other.

Guidelines for the management of AEs (ie, dose interruptions and dose reductions) are presented in the next sections. Cabozantinib and nivolumab have class-specific safety profiles based on their mechanism of action but may also cause AEs that overlap. For management of AEs

which can be clearly attributed to Cabozantinib 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.

Cabozantinib may only be reduced by one dose level (see Table 1 below). If study treatment of cabozantinib is restarted after being withheld or interrupted, the subject should be instructed not to make up the missed doses of cabozantinib.

Starting Dose	First Level dose Reduction
40 mg cabozantinib	Reduce to 20 mg cabozantinib

For toxicities that were considered unrelated to cabozantinib: If the subject recovers from his or her toxicities to CTCAE v.4.0 Grade ≤ 1 or to the baseline value (or lower), then treatment with cabozantinib may be restarted with no change in dose.

Subjects unable to tolerate a daily dose of 20 mg should discontinue study treatment.

For dose reductions not triggered by myelosuppression or by Grade 4 AEs affecting major organs (eg, central nervous system, cardiac, hepatic, renal) dose re-escalation to the participants previous dose of cabozantinib (not higher than 40 mg/day) may be allowed after discussion with the PI.

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. Nivolumab doses may be delayed up to 3 days from scheduled dose.

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 toxicities:

- Any Grade 3 skin, drug-related adverse event
- Grade 2 diarrhea or colitis
- Grade 2 pneumonitis
- Grade 2 hypophysitis
- Grade 2 adrenal insufficiency
- Serum creatinine more than 1.5 and up to 6 times the upper limit of normal
- Grade 1 or 2 new-onset moderate or severe neurologic signs or

symptoms

- Any grade 3 adverse event
    - First occurrence: omit dose
    - Recurrence of same grade 3 AE: discontinue nivolumab permanently
- AST or ALT > 3xULN and up to >5xULN
- Total bilirubin >3xULN

After omitting a dose, the following criteria must be met to resume treatment with Nivolumab:

- AE has resolved to Grade ≤ 1 or baseline value, 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.

Participants should restart treatment at the next scheduled time point per protocol.

If treatment is withheld > 9 weeks from the last dose of either cabozantinib or nivolumab, the patient should discontinue study therapy.

Additionally, Nivolumab treatment should be permanently discontinued for the following:

- 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 the re-treatment period OR requires systemic treatment
- Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding
- Creatinine greater than 6 times ULN
- Grade 4 diarrhea or colitis
- Grade 3 or 4 pneumonitis
- Grade 4 hypophysitis
- Grade 3 or 4 adrenal insufficiency
- Grade 4 hyperglycemia (related to study drug)
- Grade 4 skin related AE
- Immune-mediated encephalitis
- Recurrent Grade 3 event (unless otherwise specified in tables below)

- Any AE that requires 10mg or more of prednisone or equivalent for >9 weeks

- 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:

- Grade 4 amylase or lipase abnormalities (refer to below table)

- Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset

- 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 Cabozantinib and Nivolumab

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 both cabozantinib and 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.

Immune-mediated colitis has been associated with the administration of nivolumab.

Participants experiencing interolable Grade 2 diarrhea 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 distinguish between cabozantinib vs. nivolumab mediated toxicity. If a biopsy is performed and:

- there is T cell infiltration indicative of nivolumab-induced colitis, nivolumab should be permanently discontinued. Once diarrhea returns to Grade 1, restart cabozantinib at a

reduced dose.

- there is no T cell infiltration indicative of nivolumab-induced colitis, when diarrhea returns to Grade 1 nivolumab should be resumed at the same dose at the discretion of the investigator. Cabozantinib should also be resumed at a reduced dose.

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 ≤ 10 mg/day. Patients who resume treatment should be monitored closely for signs of renewed diarrhea.

Table 2: Managment of Diarrhea/Colitis

Grade	Management
Grade 1	• Continue nivolumab and cabozantinib. • Start Anti-diarrheal agent (e.g., Imodium®) – up to 3 agents are permitted • Close monitoring.
Grade 2	• Hold nivolumab and cabozantinib • Administer anti-diarrheal agent (e.g., Imodium®). • If symptoms persist > 5 days or recur, initiate therapy with Prednisone 60 mg/day or equivalent • If improved to Grade ≤1 within 9 weeks: o Taper steriods over ≥ 1 month o Restart Nivolumab when corticosteroids have been reduced to the equivalent of prednisone ≤ 10 mg/day. o Restart Cabozantinib at a reduced dose when corticosteroids have been reduced to the equivalent of prednisone ≤ 10 mg/day.
Grade 3	• Hold nivolumab and cabozantinib. • Treat with IV steroids (1-2 mg/kg/day methylprednisolone or equivalent) and convert to oral steroids (prednisone 60 mg/day or equivalent) after improvement • If improved to Grade ≤1 within 9 weeks: o Taper steriods over ≥ 1 month o Restart Nivolumab when corticosteroids have been reduced to the equivalent of prednisone ≤ 10 mg/day. o Restart Cabozantinib at a reduced dose when corticosteroids have been reduced to the equivalent of prednisone ≤ 10 mg/day.
Grade 4	• Permanently discontinue nivolumab and hold cabozantinib. • Treat with IV steroids (1−2 mg/kg/day methylprednisolone or equivalent) and convert to oral steroids (prednisone 60 mg/day or equivalent) after improvement • When symptoms improve to Grade ≤ 1, taper steroids over ≥ 1 month • If symptoms are not improving after 48 hours of initiating steroids or are worsening, addition of an alternative immunosuppressive agent (e.g., mycophenolate or TNF-α antagonist) may be considered. • If the subject was unequivocally deriving clinical benefit, the subject may be able to resume cabozantinib at 20mg daily as determined by the investigator.

6.2.2 Hepatobilary Disorders

Elevations of ALT, AST, and bilirubin have been observed during treatment with cabozantinib. 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. Participants on this study may enter with increased ALT/AST serum levels up to 3 × ULN to the guidelines in Table 3 should be used for dose modifications.

Table 3. Management of Elevation of transaminases

Transaminase elevation CTCAE v4.0	**Intervention**
Grade 1 **AST or ALT > ULN to 3.0 x ULN and/or Total bilirubin > ULN – 1.5 x ULN**	Continue study treatment Monitor liver function tests (LFTs) weekly for at least 4 weeks.
Grade 2 **AST or ALT > 3.0 to ≤ 5.0 x ULN and/or Total bilirubin > ULN – 1.5 x ULN to ≤3 x ULN**	Hold cabozantinib and nivolumab. Monitor LFTs at least twice weekly until return to baseline or Grade ≤1 Consider referral to a hepatologist. If persistent > 5 days, start prednisone 60 mg/day or equivalent. When LFTs resolve to baseline or Grade ≤1 and steroid dose is prenisone ≤ 10 mg/day or equivalent, restart Nivolumab and Cabozantinib at a reduced dose.
Grade 3 **AST or ALT > 5.0 to ≤ 20.0 x ULN and/or Total bilirubin > ULN – 3.0 x ULN to ≤10.0 x ULN**	Hold cabozantinib and nivolumab. Monitor LFTs every 48−72 hours until LFTs return to baseline or Grade ≤1 Consider referral to hepatologist and liver biopsy to establish etiology of hepatic injury Start prednisone 60 mg/day or equivalent With first occurrence: • If LFTs do not resolved to Grade < 3 within 7 days, permanently discontinue nivolumab and continue holding cabozantinib. • When LFTs resolve to baseline or Grade ≤ 1, taper steroids

	over ≥1 month. When steroid dose is prenisone ≤ 10 mg/day or equivalent, restart Nivolumab and Cabozantinib at a reduced dose. Subjects receiving cabozantinib at a daily dose of 20 mg may be restarted at the same dose if deemed safe at the discretion of the principal investigator. If recurs: permanently discontinue nivolumab . If the subject was unequivocally deriving clinical benefit, the subject may be able to resume cabozantinib at 20mg daily as determined by the investigator.
Grade 4 **AST or ALT > 200 x ULN and/or Total bilirubin > ULN 10.0 x ULN**	Permanently discontinue nivolumab and hold cabozantinib and inform the principle investigator. Obtain hepatology consult and liver biopsy to establish etiology of hepatic injury. Start prednisone 60 mg/day or equivalent. If LFT results do not decrease within 48 hours after initiation of systemic steroids, consider addition of an alternative immunosuppressive agent (e.g., mycophenolate) to the corticosteroid regimen. If LFTs resolve to baseline or Grade ≤ 1, taper steroids over ≥1 month. If the subject was unequivocally deriving clinical benefit, the subject may be able to resume cabozantinib at 20mg daily as determined by the investigator.

6.2.3 Dermatologic Disorders

Palmar-plantar erythrodysesthesia syndrome (also known as hand-foot syndrome), skin rash (including blisters, erythematous rash, macular rash, skin exfoliation, dermatitis acneiform, and papular rash), pruritus, dry skin, and erythema have been reported in cabozantinib-treated participants.

All participants on study should be advised on prophylactic skin care. This includes the use of hypoallergenic moisturizing creams, ointment for dry skin, and sunscreen with sun protection factor $\geq$ 30, avoidance of exposure of hands and feet to hot water, protection of pressure sensitive areas of hands and feet, and use of thick cotton gloves and socks to prevent injury and to keep the palms and soles dry. Participants with pre-existing skin disorders should be carefully monitored for signs of infection (e.g., abscess, cellulitis, or impetigo).

Early signs of hand-foot syndrome could be tingling, numbness, and slight redness or mild hyperkeratosis. Early manifestations include painful, symmetrical red and swollen areas on the palms and soles. The lateral sides of the fingers or periungual zones may also be affected.

Adequate interventions are required to prevent worsening of skin symptoms such as blisters, desquamations, ulcerations, or necrosis of affected areas.

Aggressive management of symptoms is recommended, including early dermatology referral. In the case of study treatment-related skin changes (eg, rash, hand-foot syndrome), the investigator may request that additional assessments be conducted with the subject's consent. These assessments may include digital photographs of the skin changes and/or a biopsy of the affected skin and may be repeated until the skin changes resolve.

Table 4: Management of Treatment-emergent PPE Syndrome

CTCAE v.4.0 Grade	Intervention
1	Continue Cabozantinib and Nivolumab treatment may be continued at the current dose if PPE is clinically insignificant and tolerable. Start urea 20% cream twice daily and clobetasol 0.05% cream once daily. Reassess at least weekly; if PPE worsens at any time or does not improve after 2 weeks, proceed to the intervention guidelines for Grade 2.
2	Cabozantinib and Nivolumab treatment may be continued if PPE is tolerated. Cabozantinib may be dose reduced in intolerable. Start urea 20% cream twice daily and clobetasol 0.05% cream once daily and add analgesics (eg, NSAIDs/GABA agonists) for pain control if needed. Reassess at least weekly; if PPE does not improve within 2 weeks or worsens or affects self-care, proceed to the intervention guidelines for Grade 3.

3	Hold Cabozantinib and Nivolumab treatment Continue treatment of skin reaction with clobetasol 0.05% cream twice daily and analgesics. If PPE resolves to Grade ≤2, resume Nivolumab (at same dose) and Cabozantinib (at reduced dose). If recurs and is intolerable or does not improve within 6 weeks, permanently discontinue cabozantinib and hold Nivolumab until resolves to Grade ≤2.

CTCAE, Common Terminology Criteria for Adverse Events; GABA, gamma-amino butyric acid; NSAID, non-steroidal anti-inflammatory drug; PPE, palmar-plantar erythrodysesthesia.

Treatment-emergent rash has also 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. A biopsy should be performed unless contraindicated. Low-grade rash and pruritus irAEs have been treated with symptomatic therapy (e.g., antihistamines). Topical or parenteral corticosteroids may be required for more severe symptoms.

Table 5: Management of Skin Rash and Oral Lesions

Grade	**Management**
≤ 1	Continue protocol therapy
2	Continue protocol therapy and monitor.
3	Omit dose of nivolumab and cabozantinib until ≤ Grade 1
4	Discontinue nivolumab and hold cabozantinib. If the subject is unequivocally deriving clinical benefit, the subject may be able to resume cabozantinib at 20mg daily once resolved to ≤ Grade .1
• Patients with bullous lesions must be evaluated for vasculitis, Steven-Johnson syndrome, TEN, and autoimmune bullous disease including oral lesions of bullous pemphigus/pemphigoid. • Pruritus may occur with or without skin rash and should be treated symptomatically. • Note skin rash typically occurs early and may be followed by additional events particularly during steroid taper.	

6.2.4 Thyroid Function Disorders

Changes in thyroid function tests (TFTs) and hypothyroidism have been reported with nivolumab, cabozantinib and other tyrosine kinase inhibitor treatment as a result of altered

thyroid hormone regulation by mechanisms that seem to be specific for each agent (Torino et al. 2009). Preliminary data from ongoing studies indicate that treatment-emergent elevation of thyroid stimulating hormone (TSH) by cabozantinib may be dose-dependent in fashion.

- Cabozantinib and nivolumab do not need to be held for asymptomatic participants.

Management of thyroid dysfunction (eg, symptomatic hypothyroidism) should follow accepted clinical practice guidelines.

6.3 Management of toxicities attributable to Cabozantinib

Table 6: General Dose Modifications of Cabozantinib for Treatment Related AEs

CTCAE v.4.0 Grade	Recommended Guidelines for Management[1]
Grade 1 AEs	Continue cabozantinib treatment at the current dose level if AE is manageable and tolerable. Add supportive care as indicated.
Grade 2 AEs which are tolerable and are easily managed	Continue cabozantinib treatment at the current dose level with supportive care or appropriate intervention. At the discretion of the investigator, cabozantinib should be dose reduced or interrupted. Note: It is recommended that dose holds be as brief as possible.
Grade 2 AEs which are intolerable and cannot be adequately managed	Cabozantinib should be held unless the toxicity can be easily managed with a dose reduction and optimal medical care. If held, cabozantinib can either restarted at same dose or dose reduced per investigator discretion. If same toxicity recurs at grade ≥2, the dose should be held and reduced when resolves to baseline. Note: It is recommended that dose holds be as brief as possible.
Grade 3 AEs (excludging clinically insignificant laboratory abnormalities)	Hold Cabozantinib unless the toxicity can be easily managed with a dose reduction and optimal medical care. Note: It is recommended that dose holds be as brief as possible.
Grade 4 AEs (excludging clinically	Hold cabozantinib. Discontinue cabozantinib unless the following criteria are

insignificant laboratory abnormalities)	met: • Subject is deriving clear clinical benefit as determined by treating investigator and PI. • Toxicity can be managed with a dose reduction following recovery to Grade 1 (or baseline) and optimal medical care

*AE: adverse event

[1] Study treatment dose adjustment is only needed if the toxicity was deemed related to cabozantinib treatment or had an unclear relationship to cabozantinib treatment.

6.3.1 Gastrointestinal Disorders

The most common GI AEs reported in clinical studies with cabozantinib are diarrhea, oral pain, dyspepsia, stomatitis, and dysphagia.

Nausea and Vomiting

Antiemetic agents are recommended as clinically appropriate at the first sign of nausea and vomiting or as prophylaxis to prevent emesis, along with supportive care according to clinical practice guidelines.

- The 5-HT3 receptor antagonists are the preferred antiemetic
- The following antiemtics should be avoided as they impact CYP3A4 which could impact cabozantinib exposure:
  - NK-1 receptor antagonists and dexamethasone
  - Glucocorticoids
  - Nabilone (weak inhibitor)

When therapy with antiemetic agents does not control the nausea or vomiting to tolerable levels, study treatment should be temporarily interrupted or dose reduced.

Stomatitis and Mucositis

Preventive measures may include a comprehensive dental examination to identify any potential complications before study treatment is initiated. Removal of local factors should be instituted as indicated, such as modification of ill-fitting dentures and appropriate care of gingivitis. During treatment with cabozantinib good oral hygiene and standard local treatments such as nontraumatic cleansing and oral rinses (e.g., with a weak solution of salt and baking soda) should be maintained. The oral cavity should be rinsed and wiped after meals, and dentures should be cleaned and brushed often to remove plaque. Local treatment should be instituted at the earliest onset of symptoms. Obtain bacterial/viral culture if oral infection is suspected and treat infection as indicated by local guidelines. When stomatitis interferes with adequate nutrition and local therapy is not adequately effective, dose reduction or temporary withholding of cabozantinib should be considered.

6.3.2 Hematological Disorders

Hematological toxicities (ie, neutropenia and thrombocytopenia) and associated complications have been observed after administration of cabozantinib and may be managed with dose interruptions 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. Participantss with hematologic 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 such as red blood cell transfusions may be managed according to institutional guidelines.

6.3.3 Fatigue, Anorexia and Weightloss Disorders

Fatigue has been reported during treatment with cabozantinib and nivolumab, the dose modifications are recommended for both drugs. Common causes of fatigue such as anemia, deconditioning, emotional distress (depression and/or anxiety), nutrition, sleep disturbance, and hypothyroidism should be ruled out and/or these causes treated according to standard of care. Individual non-pharmacological and/or pharmacologic interventions directed to the contributing and treatable factors should be given. Pharmacological management with psychostimulants such as methylphenidate should be considered after disease specific morbidities have been excluded.

Dose reduction of study treatment should be considered when general or pharmacological measures have not been successful in reducing symptoms. Dose interruption may be considered for Grade ≥ 3 fatigue despite optimal management, at the investigator's discretion.

Anorexia and weight loss should be managed according to local standard of care including nutritional support. Pharmacologic therapy such as megestrol acetate should be considered for appetite enhancement. Should these interventions prove ineffective, dose holds and reductions may be considered for Grade ≥ 3 anorexia or weight loss. If anorexia and/or weight loss do not recur after a dose reduction, dose of cabozantinib may be re-escalated to the previous dose.

6.3.4 Wound Healing

VEGF inhibitors can cause wound healing complications and wound dehiscence which may occur even long after a wound has been considered healed. Therefore, surgical and traumatic wounds must have completely healed prior to starting cabozantinib treatment and be monitored for wound dehiscence or wound infection while the subject is being treated with cabozantinib.

6.3.5 GI perforation/fistula and non-GI fistula formation

GI perforation/fistula and non-GI fistula formation have been reported with cabozantinib and other approved drugs that inhibit VEGF pathways.

After starting cabozantinib, subjects should be monitored for early signs of GI perforation such as abdominal pain, nausea, emesis, constipation, and fever especially if known risk factors for developing GI perforation or fistula are present.

- Discontinue cabozantinib treatment in participants diagnosed with GI or non-GI perforation/fistula.

6.3.6 Hypertension

Hypertension is a common class effect of drugs that inhibit VEGF pathways and has been reported in subjects treated with cabozantinib. Blood pressure should be monitored in a constant position at each visit (either sitting or supine). In general, subjects with known hypertension should be optimally managed prior to study entry.

Decisions to decrease or hold the dose of study treatment must be based on blood pressure readings taken by a medical professional and must be confirmed with a second measurement at least 5 minutes following the first measurement. Other than for hypertension requiring immediate therapy, the presence of new or worsened hypertension should be confirmed at a second visit before taking therapeutic action. It is recommended that this second visit occurs within 1 week.

Table 7: Management of Treatment-emergent Hypertension

Criteria for Dose Modification	Intervention
> 140 mm Hg (systolic) and < 160 mm Hg OR > 90 mm Hg (diastolic) and < 110 mm Hg	• Maintain dose of cabozantinib and nivolumab • Optimize antihypertensive treatment (i.e., increase dose of existing medications and/or add new antihypertensive medications). Reevaluate in one week • If after optimal antihypertensive therapy (usually to include 3 agents) BP returns (< 150 mm Hg systolic or <100 mm Hg diastolic maintain dose of cabozantinib and nivolumab. • If after optimal antihypertensive therapy BP is not < 150 mm Hg systolic or < 100 mm Hg diastolic, reduce cabozantinib treatment by one dose level and continue nivolumab. Reevaluate in one week. • If optimized antihypertensive therapy (usually to include 3 agents) and cabozantinib dose reduction does not result in BP < 150 mm Hg systolic or < 100 mmHg diastolic, cabozantinib treatment should be interrupted; Continue patient on nivolumab.
≥ 160 mm Hg (systolic) OR ≥ 110 mm Hg (diastolic)	• Reduce cabozantinib treatment by one dose level and continue nivolumab. Optimize antihypertensive treatment (i.e., increase dose of existing medications and/or add new antihypertensive medications). Monitor participant closely for hypotension. • Reevaluate in one week. • If after optimal antihypertensive therapy (usually to include 3 agents) upper limits of BP (< 150 mm Hg systolic or <100 mm Hg diastolic) keep cabozantinib on the reduced dose and keep nivolumab. • Interrupt cabozantinib treatment if upper limits of BP (≥ 150 mm Hg systolic or ≥ 100 mm Hg diastolic) are sustained manageable or if subject is symptomatic. • Restart cabozantinib treatment at the most tolerable dose and reescalate cabozantinib dose only if BP falls to and is sustained at < 140 mm Hg systolic and < 90 mm Hg diastolic.
>180 mm Hg systolic or > 120 mm Hg diastolic or if subject is symptomatic.	• Hold cabozantinib treatment and continue nivolumab • Optimize antihypertensive treatment (i.e., increase dose of existing medications and/or add new antihypertensive medications). Reevaluate in one week

	• Restart cabozantinib at reduced dose and re-escalate cabozantinib dose only if BP falls to and is sustained at < 140 mm Hg systolic and < 90 mm Hg diastolic. • Permanently interrupt cabozantinib treatment if upper limits of BP (≥ 150 mm Hg systolic or ≥ 100 mm Hg diastolic) are sustained and not adequately manageable or if subject is symptomatic.
Hypertensive crisis or hypertensive encephalopathy	• Discontinue cabozantinib treatment. • Hold nivolumab until resolution.

BP, blood pressure.

6.3.7 Thromboembolic Events

Thromboembolic complications are frequent in cancer patients due to procoagulant changes induced by the malignancy or anti-cancer therapy including inhibitors of VEGF pathways. Deep vein thrombosis and PE have been observed in clinical studies with cabozantinib; including fatal events.

- Subjects who develop a PE or DVT should have cabozantinib treatment held until therapeutic anticoagulation with heparins (e.g., LMWH) is established.
- Cabozantinib treatment may be resumed at the same dose in subjects who are stable and have uncomplicated PE or DVT and are deriving clinical benefit from cabozantinib treatment.
- During anticoagulation treatment, subjects need to be monitored on an ongoing basis for bleeding risk and signs of bleeding which may require additional or more frequent laboratory tests according to institutional guidelines.
- If there are any signs of clinically relevant bleeding, cabozantinib treatment should be stopped immediately and the PI contacted to discuss further study participation.
- Subjects with life-threatening PE or DVT should have study treatment discontinued unless toxicity can be managed and subject is deriving clear clinical benefit as determined by the investigator and agreed by the PI.

Arterial thrombotic events (e.g., transient ischemic attack, myocardial infarction) have been observed rarely in studies with cabozantinib. Subjects should be evaluated for preexisting risk factors for arterial thrombotic events such as diabetes mellitus, hyperlipidemia, hypertension, coronary artery disease, history of tobacco use, and cardiac or thromboembolic events that occurred prior to initiation of study treatment.

- Cabozantinib treatment should be discontinued in patients who develop an acute myocardial infarction or any other clinically relevant arterial thromboembolic complication.

6.3.8 Hemorrhagic Events

Hemorrhagic events have been reported with cabozantinib and other approved drugs that inhibit VEGF pathways.

- Discontinue cabozantinib treatment in participants who have been diagnosed with a severe bleeding complication (Grade 3 or 4) suspected to be related to drug.

6.3.9 Proteinuria

Proteinuria is an expected AE with the inhibition of VEGF pathways and has been observed in cabozantinib clinical studies. Nephrotic syndrome has been reported with cabozantinib and other inhibitors of VEGF pathways.

Table 8 Management of Treatment-emergent Proteinuria

Severity of Proteinuria (UPCR)	**Intervention**
≤ 1 (≤ 113.1 mg/mmol)	• Continue therapy with cabozantinib and nivolumab
> 1 and < 3.5 (> 113.1 and < 395.9 mg/mmol)	• Continue therapy with cabozantinib and nivolumab • Consider confirming with a 24-hour protein assessment within 7 days o If UPCR ≤ 2 or urine protein ≤ 2 g/24 hours on 24-hour urine collection, cabozantinib continue at the same dose. o If UPCR > 2 on repeat UPCR testing or urine protein > 2 g/24 hours on 24-hour urine collection, hold cabozantinib and repeat testing in 7 days. ▪ If UPCR decreases to < 2, restart cabozantinib at a reduced dose. ▪ If UPCR remains > 2 mg/mg continue holding cabozantinib and repeat testing every 7 days until decreases to <2 or max hold is reached. When <2 restart cabozantinib at a reduced dose. •
≥ 3.5 (≥ 395.9 mg/mmol)	• Hold cabozantinib and nivolumab • Repeat UPCR and perform 24-hour protein assessment within 7 days • If ≥ 3.5 on repeat UPCR, continue to hold cabozantinib treatment and check UPCR once per week. o If UPCR decreases to < 2, restart cabozantinib treatment at a reduced dose and monitoring of urine protein/creatinine should continue weekly until the UPCR decreases to < 1.

	• Restart nivolumab once UPCR is ≤ 1
Nephrotic syndrome	• Discontinue cabozantinib and nivolumab.

UPCR, urine protein to creatinine ratio.

6.3.10 Hypophosphatemia

Hypophosphatemia has been reported during treatment with cabozantinib.

Mild hypophosphatemia is usually asymptomatic or symptoms can be non-specific such as weakness, bone pain, rhabdomyolysis, or altered mental status. Other causes of hypophosphatemia such as poor nutrition, chronic alcoholism, malabsorption, excessive antacid use, glucocorticoids use, kidney dysfunction, respiratory alkalosis, vitamin D deficiency should be ruled out and/or these causes treated according to standard of care.

- Mild to moderate hypophosphatemia should be managed by oral replacement including food that are high in phosphate (diary items, meats, beans) and/or oral phosphate supplements according to standard clinical practice guidelines.

6.3.11 Osteonecrosis of the Jaw

Osteonecrosis of the jaw (ONJ) has been reported with cabozantinib, the use of anti-angiogenic drugs, bisphosphonates and denosumab in cancer patients. Additional risk factors for ONJ have been identified such as use of corticosteroids, chemotherapy, local radiotherapy, poor oral hygiene, smoking, dental or orofacial surgery procedures, and cancer disease itself.

- As a preventive measure, invasive dental procedures should be avoided if possible in subjects who have previously been treated with or concomitantly receive bisphosphonates or denosumab.
- In cases where dental procedures are unavoidable, the risks and benefits of a dental procedure, the extent of the procedure, the risk of developing osteonecrosis of the jaw should be carefully considered when deciding on the duration of a temporary treatment interruption of cabozantinib. If clinically possible, treatment with cabozantinib should be held for approximately 4 weeks prior to a dental procedure and resumed after complete healing has occurred.
- Participants experiencing osteonecrosis should have study treatment with cabozantinib held and be managed according to clinical guidelines. . Participants may be able to restart cabozantinib if in their best interest after discussion with the PI.

6.3.12 QTc Prolongation

The effect of orally administered cabozantinib 140 mg qd on QTc interval was evaluated in a placebo-controlled study in subjects with MTC. A mean increase in QTcF of 10-15 ms was observed after 4 weeks after initiating cabozantinib treatment. A concentration-QTc

relationship could not be definitively established. Changes in cardiac wave form morphology or new rhythms were not observed. No cabozantinib-treated subjects in this study had a QTcF > 500 ms.

Unless otherwise specified, only subjects with a baseline QTcF ⩽ 500 msec are eligible for this study. Cabozantinib should be used with caution in subjects with QT prolongation risk, a history of QT interval prolongation, or who are taking antiarrhythmics or drugs known to prolong the QT interval. Concomitant treatment with strong CYP3A4 inhibitors, which may increase cabozantinib plasma concentrations, should be avoided.

If at any time on study there is an increase in QTcF to an absolute value > 500 ms or an increase of > 60 ms above baseline, two additional ECGs must be performed with intervals not less than 3 min apart within 30 min after the initial ECG.

If the average QTcF from the three ECGs is > 500 ms or increased by > 60 ms above baseline, the following actions must be taken:

- Withhold study treatment
- Immediately notify the Overall PI
- Hospitalize symptomatic subjects (eg, with palpitations, dizziness, syncope, orthostatic hypotension, a significant ventricular arrhythmia on ECG) for a thorough cardiology evaluation and management
- Consider cardiology consultation for asymptomatic subjects for evaluation and management
- Check electrolytes, especially magnesium, potassium and calcium; correct abnormalities as clinically indicated
- Check concomitant medications for any medication that may have contributed to QT prolongation, and if possible, discontinue these medications (http://www.qtdrugs.org)
- Repeat ECG triplicates hourly until the average QTcF is ≤ 500 msec, or otherwise
- Subjects with QTc prolongation and symptoms must be monitored closely until the QTc elevation and symptoms have resolved. Study treatment may be restarted at a reduced dose level if all of the following conditions are met:
- Symptoms are determined to be unrelated to the QT interval prolongation
- The QTcF value > 500 ms or increase of > 60 ms above baseline is not confirmed according to protocol procedures
- Study treatment has been interrupted through a minimum of 1 week following the return of the QTcF to ≤ 500 msec or return to ≤ 60 ms above baseline.
- QT prolongation can be unequivocally associated with an event other than cabozantinib administration and is treatable/has been resolved
- Overall PI has reviewed all available information and has agreed to the continuation of study treatment

Following reinitiation of study treatment, ECGs must be repeated weekly for 2 weeks, then every 2 weeks for 1 month, then according to the protocol-defined time points.

All study treatment must be permanently discontinued if either of the following applies:

- Cardiac evaluation confirms that symptoms are the consequence of QT interval prolongation
- Recurrence of QTcF prolongation (confirmed by central ECG lab) after reinitiation of study treatment at a reduced dose

6.4 Management of toxicities attributable to Nivolumab

6.4.1 Steroid and immunosuppresive agents use on treatment with 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 < 10 mg/day prednisone or equivalent prior to re-initiation of study therapy.

6.4.2 Elevated Amylase and/or Lipase

Patients may develop symptomatic and/or radiographic evidence of pancreatitis, DM, or pancreatic dysfunction. Amylase and lipase should be checked if there is clinical suspicion for pancreatitis. Asymptomatic elevations can be self-limited and treatment continued per guidelines above. Corticosteroids do not seem to alter the natural history of lipase/amylase elevations. Laboratory values tend to fluctuate on a day-to-day basis and eventually return to baseline or low grade over the course of weeks, whether or not subjects receive corticosteroids. Asymptomatic elevations should be monitored approximately on a weekly basis

Patients with symptomatic or radiographic pancreatitis should discontinue nivolumab and consider consulting a gastroenterologist for management.

Table 9: Elevated Amylase and/or Lipase

Grade	Management/Next Dose for Nivolumab
≤ 3	Continue protocol therapy if asymptomatic. Ensure that participants have associated symptoms consistent with pancreatitis, such as abdominal pain, or hyperglycemia or radiographic pancreatic inflammation.
4	Hold nivolumab and cabozantinib. Ensure that participants have associated symptoms consistent with pancreatitis, such as abdominal pain, or hyperglycemia or radiographic pancreatic inflammation. If patient remains asymptomatic, resume treatment when ≤ Grade 1.

6.4.3 Pulmonary Toxicity

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 11.1.3).

Table 10: Management of Pneumonitis

Grade	Management
≤ 1	Continue protocol therapy if radiologic findings only; dose may be held for further evaluation per investigator discretion
2	Hold nivolumab pending evaluation.

Grade	Management
	Resume protocol therapy after pulmonary and/or ID consultation if lymphocytic pneumonitis is excluded. Patient should discontinue nivolumab if prolonged steroids are required unless an alternative etiology of findings is identified. Withhold cabozantinib until resolved to ≤ Grade 1.
3	Discontinue nivolumab. Withhold cabozantinib until resolved to ≤ Grade 1.
4	Discontinue nivolumab. Withhold cabozantinib until resolve to ≤ Grade 1.
Distinguishing inflammatory pneumonitis is often a diagnosis of exclusion for patients who do not respond to antibiotics and have no causal organism identified including influenza. Most patients with respiratory failure or hypoxia will be treated with steroids. Bronchoscopy may be required and analysis of lavage fluid for lymphocytic predominance may be helpful. Patients with new lung nodules should be evaluated for sarcoid like granuloma. Please consider recommending seasonal influenza inactivated vaccine for all patients.	

6.4.4 Fatigue

Table 11: Management of Fatigue

Grade	Management
≤ 2	Continue protocol therapy
3	Hold nivolumab and cabozantinib. When ≤ Grade 2, resume treatment with nivolumab at the same dose and cabozantinib at a reduced dose. If it recurs, discontinue cabozantinib.
4	Hold nivolumab and discontinue cabozantinib. When ≤ Grade 2, resume treatment with nivolumab at the same dose.
Fatigue is the most common adverse event associated with immune checkpoint therapy. Grade 2 or greater fatigue should be evaluated for associated or underlying organ involvement including pituitary, thyroid, hepatic, or muscle (CPK) inflammation.	

6.4.5 Neurologic Events

The principal treatments for neurologic toxicity are dose delay, corticosteroids, and

IV immunoglobulin as outlined in the safety algorithm. Patients with any CNS events including aseptic meningitis, encephalitis, symptomatic hypophysitis, myopathy, peripheral demyelinating neuropathy, cranial neuropathy (other than peripheral n. VII), Guillain-Barre syndrome, and myasthenia gravis should discontinue nivolumab.

Table 12: Management of Neurologic Events

Grade	**Management**
≤ 1	Continue protocol therapy
2	Hold nivolumab If treatment with steriods is required, permanently discontinue nivolumab. If no treatment with steroids is required, and it has resolved to ≤ Grade 1, resume nivolumab at same dose. Participants are permitted to resume treatment with nivolumab for peripheral isolated CN VII (Bell's palsy) when stable.
3	Discontinue nivolumab. Hold cabozantinib. If the subject was unequivocally deriving clinical benefit, the subject may be able to resume cabozantinib at a lower dose as determined by the investigator.
4	Discontinue nivolumab. Hold cabozantinib. If the subject was unequivocally deriving clinical benefit, the subject may be able to resume cabozantinib at a lower dose as determined by the investigator.

6.4.6 Endocrinopathy including hypophysitis and adrenal insufficiency

Note all patients with symptomatic pituitary enlargement, exclusive of hormone deficiency, but 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 13: Management of Endocrinopathy including hypophysitis and adrenal insufficiency

Grade	Management
≤ 2	Asymptomatic abnormalities do not require dose delay*. If symptomatic, hold until on a stable replacement hormone regimen. If treated with steroids patients must be stable off of steroids for two weeks with the exception of adrenal replacement therapy.
3	Hypophysitis: Hold nivolumab and cabozantinib* until ≤ Grade 1, then resume at same dose. Other endocrinopathies: Hold nivolumab and cabozantinib* until ≤ Grade 1 and stable on replacement hormones, then resume at same dose.
4	Discontinue nivolumab. Discontinue cabozantinib. If the subject was unequivocally deriving clinical benefit, the subject may be able to resume cabozantinib at a lower dose as determined by the investigator.
*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	

6.4.7 Creatinine Levels/Renal Insufficiencies

Table 14: Management of Creatinine Levels/Renal Insufficiencies

Grade	Management
≤ 1	Continue protocol therapy. Monitor weekly until return to baseline.
2-3 (>1.5 baseline to ≤ 6 3.0 x ULN)	Hold nivolumab and cabozantinib until ≤ Grade 1. Monitor creatinine every 2-3 days. Start 0.5 to 1.0 mg/Kg/day methylprednisolone IV or oral equivalent. Consider consulting nephrology and obtaining a renal biopsy.
4 (>6.0 x ULN)	Discontinue nivolumab and hold cabozantinib. Monitor creatinine daily. Start 1.0 – 2.0 mg/Kg/day methylprednisolone IV or oral equivalent.

Grade	Management
	Consult nephrologist and consider obtaining a renal biopsy. If the subject was unequivocally deriving clinical benefit, the subject may be able to resume cabozantinib at a lower dose as determined by the investigator.

6.4.8 Infusion Reactions

Since nivolumab contains only human immunoglobulin protein sequences, 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 an 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 15: Management of Infusion reaction

Grade	Management
≤ 1	Continue nivolumab infusion, remain at bedside. Continue cabozantinib. Institute prophylactic medications with subsequent infusions.
2	Hold nivolumab until resolution to ≤ Grade 1. Refer to management and rechallenging instructions below. Continue cabozantinib.
3	Discontinue nivolumab. Continue cabozantinib.
4	Discontinue nivolumab. Continue cabozantinib.

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 [ie, not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae [eg, 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)

6.4.9 Fever

Patients with fever should be evaluated as clinically appropriate. Patients may experience isolated fever during infusion reactions or up to several days after infusion. Evaluation over the course of 1-2 weeks should be done for other autoimmune events that may present as fever.

Grade	Management
≤ 1	Continue nivolumab and cabozantinib and monitor.
2	Hold nivolumab and cabozantinib until ≤ Grade 1
3	Hold nivolumab and cabozantinib until ≤ Grade 1
4	Hold nivolumab and cabozantinib until ≤ Grade 1

6.4.10 All other non-laboratory adverse events

Table 16: General Guidance for All other non-laboratory adverse events

Grade	Management/Next Dose for Nivolumab
≤ 1	Continue protocol therapy.
2	Hold nivolumab until ≤ Grade 1 OR baseline (exceptions as noted below)
3	Omit dose of nivolumab if not recurrent (exceptions as noted below)
4	Discontinue nivolumab therapy
Recommended management: Low-grade events that are considered drug related and any high-grade events of unclear etiology should be fully evaluated and treated with systemic corticosteroids if an alternative etiology is not identified.	

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

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

7.1 Adverse Events Lists

7.1.1 Expected adverse event for nivolumab

The PD-L1/PD-1 pathway is involved in peripheral tolerance; therefore, such therapy may increase the risk of immune-related AEs, specifically the induction or enhancement of autoimmune conditions. AEs with potentially immune-related causes, including rash, hypothyroidism, hepatitis/transaminitis, colitis, myositis, and myasthenia gravis, have been observed.

7.1.2 Expected adverse event for cabozantinib

The general adverse event profile of cabozantinib includes GI symptoms (such as nausea, vomiting, and diarrhea), fatigue, anorexia, palmar-plantar erythrodysesthesia (PPE) syndrome, skin rash, elevated ALT and AST, increased pancreatic enzymes with rare cases of pancreatitis, as well as side effects associated with inhibition of VEGF signaling such as thrombotic events (eg, pulmonary embolism [PE] and deep vein thrombosis [DVT]), hypertension, proteinuria,

hemorrhagic events, and rare cases of gastrointestinal [GI] perforation and rectal/perirectal abscess. Additionally, events of arterial thromboembolism (transient ischemic attack [TIA], myocardial infarction [MI]) have been reported. For a full listing of safety events please refer to the Cabozantinib Investigational Brochure.

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.

7.3 Expedited Adverse Event Reporting

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.

Investigators **must** report to the Overall PI any serious adverse event (SAE) suspected to be related to study drug or protocol-specified procedure within 30 days of the last dose of treatment.

7.3.1 Reporting to the Institutional Review Board (IRB)

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

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

The Overall PI, as study sponsor, will be responsible for all communications with the FDA.

Any event that is both serious and unexpected must be reported to the Food and Drug Administration (FDA) as soon as possible and no later than 7 days (for a death or life-threatening event) or 15 days (for all other SAEs) after the investigator's or institution's initial receipt of the information.

SAEs should be reported on MedWatch Form 3500A, which can be accessed at: http://www.accessdata.fda.gov/scripts/medwatch/.

MedWatch SAE forms should be sent to the FDA at:

Phone: 1-800-FDA-1800 (1-800-332-1088)

Fax: 1-800-FDA-0178 (1-800-332-0178)

http://www.accessdata.fda.gov/scripts/medwatch/

7.3.3 Expedited Reporting to Hospital Risk Management

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

7.3.4 Expedited Reporting to BMS

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

SAE Email Address: Worldwide.Safety@BMS.com

SAE Facsimile Number: 609-818-3804

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

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

All SAEs should be followed to resolution or stabilization.

7.3.5 Expedited Reporting to Exelexis

As soon as an investigator becomes aware of an AE that meets the definition of 'serious,' this must be documented on an SAE Report Form or in an electronic database and include the following: (i) all SAEs that occur after starting cabozantinib and through 30 days after the decision to discontinue study treatment and (ii) any SAEs assessed as related to study treatment or study procedures, from the time of informed consent, even if the SAE occurs more than 30 days after the decision to discontinue study treatment.

All SAEs that are assessed by the PI as **related** to drug or study procedure and all pregnancy/lactation reports regardless of outcome must be sent to Exelixis within one (1) business day of the PI's knowledge of the event. The reports, on a MedWatch 3500A Form, must be sent to drugsafety@exelixis.com or fax 650-837-7392.

- The PI will perform adequate due diligence with regard to obtaining follow-up information on incomplete reports. All follow-up information must be sent to Exelixis within one (1) business day of the PI's receipt of the new information. Upon Exelixis request, the PI will query for follow-up information.

7.4 Routine Adverse Event Reporting

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

8. PHARMACEUTICAL INFORMATION

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

8.1 Nivolumab

8.1.1 Description

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

Table 8.1.1: Nivolumab Physical and Chemical Properties

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

8.1.2 Storage and Stability

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

The administration of undiluted and diluted solutions of Nivolumab must be completed within 24 hours of preparation. If not used immediately, the infusion solution may be stored up to 24 hours in a refrigerator at 2°-8°C (36°-46°F) and a maximum of 4 hours of the total 24 hours can be at room temperature (20°-25°C, 68°-77°F) and room light. The maximum 4-hour period under room temperature and room light conditions includes the product administration period.

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

8.1.3 Compatibility

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

8.1.4 Handling

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

8.1.5 Availability

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

8.1.6 Preparation

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

8.1.7 Administration

Nivolumab will be delivered in infusion bags with IV infusion lines over 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 BMS.

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.)

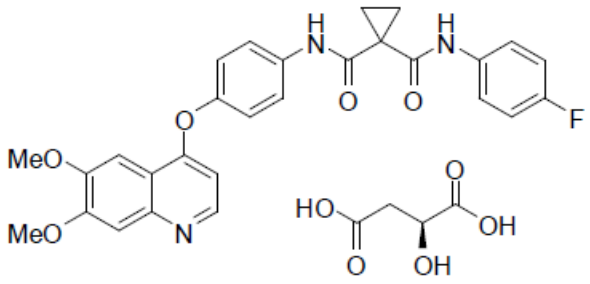
8.1.10 Destruction and Return

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 Cabozantinib

8.2.1 Description

Cabozantinib is the (*S*)-malate salt of cabozantinib, a kinase inhibitor. Cabozantinib (*S*)-malate is described chemically as *N*-(4-(6,7-dimethoxyquinolin-4-yloxy)phenyl)-*N'*-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide, (2*S*)-hydroxybutanedioate. The molecular formula is $C_{28}H_{24}FN_3O_5 \cdot C_4H_6O_5$ and the molecular weight is 635.6 Daltons as malate salt. The chemical structure of cabozantinib (*S*)-malate salt is:



In vitro biochemical and/or cellular assays have shown that cabozantinib inhibits the tyrosine kinase activity of MET, VEGFR-1, -2 and -3, AXL, RET, ROS1, TYRO3, MER, KIT, TRKB, FLT-3, and TIE-2. These receptor tyrosine kinases are involved in both normal cellular function and pathologic processes such as oncogenesis, metastasis, tumor angiogenesis, drug resistance, and maintenance of the tumor microenvironment.

8.2.2 Form

Cabozantinib/XL184 Tablets

The Sponsor will provide each investigator with adequate supplies of cabozantinib, which will be supplied as 20-mg yellow film-coated tablets. The 20-mg tablets are round. The components of the tablets are listed in Table 10.

Table 10: Cabozantinib Tablet Components and Composition

Ingredient	Function	% w/w
Cabozantinib Drug Substance (25% drug load as free base)	Active Ingredient	31.7
Microcrystalline Cellulose (Avicel PH-102)	Filler	38.9
Lactose Anhydrous (60M)	Filler	19.4
Hydroxypropyl Cellulose (EXF)	Binder	3.0
Croscarmellose Sodium (Ac-Di-Sol)	Disenegrant	6.0
Colloidal Silicon Dioxide,	Glidant	0.3
Magnesium Stearate	Lubricant	0.75
Opadry Yellow Film Coating which includes: - HPMC 2910 / Hypromellose 6 cp - Titanium dioxide - Triacetin - Iron Oxide Yellow	Film Coating	4.00

8.2.3 Storage and Stability

Store cabozantinib at 20°C to 25°C (68°F to 77°F); excursions are permitted from 15°C to 30°C (59°F to 86°F) [see USP Controlled Room Temperature].

8.2.4 Compatibility

Not applicable

8.2.5 Availability

Cabozantinib is an investigational agent and will be supplied free-of-charge from Exelixis.

8.2.6 Preparation

Not applicable

8.2.7 Administration

Cabozantinib is administered once daily as an oral tablet. Participants will be provided with a sufficient supply of study treatment and instructions for taking the study treatment on days without scheduled clinic visits. After fasting (with exception of water) for 2 hours, participants will take study treatment daily each morning with a full glass of water (minimum of 8 oz/ 240 mL) and continue to fast for 1 hour after each dose of study treatment. If doses are withheld, the original schedule of assessments should be maintained when cabozantinib is restarted. The participant should be instructed to not make up the missed doses and to maintain the planned dosing schedule. Participants must be instructed to not make up missed doses that are vomited.

8.2.8 Ordering

Cabozantinib will be provided by Exelixis.

8.2.9 Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of cabozantinib using the NCI Drug Accountability Record or another comparable drug accountability form.

8.2.10 Destruction and Return

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

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

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

Serial blood draws for correlative science are required on this trial; blood draws will be obtained every 4 weeks prior to the infusion of study drugs, at the end-of-treatment visit in patients who go off study for progressive disease, and all efforts will be made to obtain an additional blood draw at the time of progressive disease, in patients who went off study for anything other than progressive disease. On each blood draw, we will perform flow cytometry to characterize protein expression of immune mediators, detailed below, and additional blood will be banked for future testing.

All patients will additionally be asked to provide a stool sample at three separate timepoints: prior to treatment, during treatment, and at the time of disease progression. A fourth collection may be requested from patients who experience grade ≥ 2 diarrhea after discussion with the PI.

This collection is not required, but is strongly encouraged. These samples will be analyzed for microbiota content. Please refer to the separate collection information sheet for additional correlative details including collection, processing, and shipping instructions.

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

Summary of Specimens

Research Sampling	Timepoint	Contents	Destination
Archival Tissue	Anytime on study	1 FFPE Block or 10-20 5 micron unstained slides	Current DFCI CRC
Fresh Tissue	Baseline	5-7 cores	1 DMEM Core – JF406 / Mariano Severgnini
	Prior to C3D1	5-7 cores	
			RNAlater Cores – Breast Bank
			Formalin Cores – Current DFCI CRC
			1-2 DMEM Cores – DA520 / Wu Lab
Blood	Baseline, Restaging Visits, Time of Progression	1 – 10mL Streck Tube (cfDNA)	Breast Bank
		5 – 10mL green tops (PBMCs)*	JF406 / Mariano Severignini
		1 – 10mL EDTA purple tops (Plasma Biomarker)	MGH Steele Lab / Dan Duda
	C2D1	1 – 10mL EDTA purple tops (Plasma Biomarker)	MGH Steele Lab / Dan Duda
		5 – 10mL green tops (PBMCs)*	JF406 / Mariano Severignini

	Every Cycle, Day 1	1 – 10mL EDTA purple tops (Plasma Biomarker)	MGH Steele Lab / Dan Duda
Stool	Baseline	Home Collection Kit (DNA Genotek)	BWH/Harvard Cohorts Biorepository
	Prior to C3D1		
	End of treatment		
	Optional collection at the time of grade ≥ 2 diarrhea		

* Green tops may be substituted with 10mL purple EDTA tubes or 10mL CPT tubes as availability allows

9.1 Archival Tissue

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

9.2 Fresh Tissue Biopsy

9.2.1 Objectives:

- Characterizing immune markers in metastatic TNBC.
- To evaluate MET and phospho MET expression in tumor tissue at baseline by immunohistochemistry

9.2.2 Collection

Biopsies are required at screening and C3D1 (Any time from C2D14-C2D28 is allowed).

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

Ideally five core biopsies will be obtained:

- One core should be placed in 10% neutral buffered formalin tube supplied by the study.
- One core should be placed in RNAlater
- Two core should be placed in sterile DMEM

The order of specimen collection should be:

- First core: 10% neutral buffered formalin
- Second core: Sterile DMEM

- Third core: RNAlater
- Fourth core: Sterile DMEM
- Fifth core: Sterile DMEM

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

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

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

9.2.3 Handling and Shipping

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

- All samples should be de-identified and labeled with the Participant initials, Participant Study ID number and date of procedure.
- Core #5 in sterile DMEM should be brought as fresh tissue immediately to the lab of Mariano Severgnini at:

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

Cores must arrive to the lab to be processed for TILs (as described below) within 1.5 hours of its collection, though an additional 2 hour window is allowed In addition, a small piece of onecore will be immediately frozen in liquid nitrogen upon arrival to Mariano Severgnini, for later use for RNA sequencing. Please notify the lab of expected specimen collection approximately one week in advance of specimen drop-off (contacts: Tara Patel, tara_patel@dfci.harvard.edu; or Amy Cunningham, amy_cunningham@dfci.harvard.edu; or Martha Holland, marthak_holland@dfci.harvard.edu).

- Cores in formalin should be brought to the Brigham and Women's SHL lab (with appropriate work order submitted and printed) on the 6th floor of the Thorn building, where a block will be made. An email will be sent to the CRC within 2-3 days to confirm that the block has been made. The block should then be picked up from the SHL lab and brought to Dr. Scott Rodig on the 6th floor of the Thorn building.

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

- Core #2 and Core #4 in Sterile DMEM should be brought immediately to the Translational Immunogenomis Laboratory (TIGL) located on Dana 520. As soon as the

biopsy procedure is scheduled, please notify Derin Keskin derin_keskin@dfci.harvard.edu of the date and time. Sterile DMEM in conical tubes will be provided by the TIGL can can be picked up from Dana 520.

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

9.2.4 Potential Testing

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

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

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

Assay 2: Immunohistochemistry

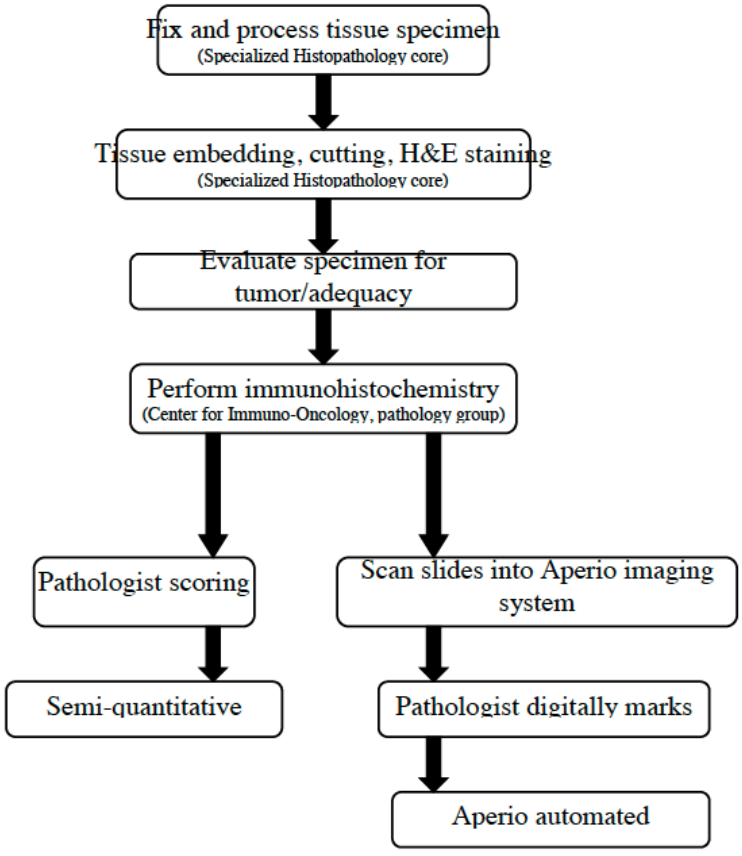
Tissue will be collected and fixed by 10% neutral buffered formalin overnight, dehydrated, and paraffin embedded. Four micrometer-thick sections will be cut. The paraffin blocks and unstained slides will be stored at room temperature. All immunohistochemical staining will be performed in the Center for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

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

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

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

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



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

subset of slides will be reviewed by a second pathologist to ensure concordance of interpretation.

The semi-quantitative scoring for this study is in accordance with those published previously and, as described above, will include scores for both the neoplastic and non-neoplastic cells within the tumor microenvironment. Data derived from pathologist visual scoring (semi-quantitative, but with increased specificity for delineating neoplastic and non-neoplastic cells) and pathologist-assisted, automated scoring (quantitative, but without accurately delineating neoplastic and non-neoplastic cells) for each marker of interest will be assessed for its clinical value. As necessary, the data from combinations of markers will also be considered (i.e. combined scores from PD-L1 and PD-L2 expression). All data will be analyzed in conjunction with the biostatistics group.

Assay 3: Flow cytometry

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

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

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

Assay 4: RNA analysis

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

Messenger RNA (mRNA) expression within tumor biopsy specimens may be assessed using the most current and informative methodologies at the time that correlative science is performed on all specimens. NanoString signatures and comprehensive RNA sequencing may be used. Potential genes of interest, based

on prior immune profiling of breast tumors,[Denkert *et al.*, 2015] include CXCL9, CCL5, CD8ACD80, CXCL13, IGKC, CD21, IDO1, PD-1, PD-L1, PD-L2, CTLA4, and FOXP3.

Assay 5: Evaluate c-Met and phospho-c-Met expression in tumor tissue

Tumor tissue sections will be deparaffinized prior to antigen retrieval, blocking, and incubation with primary antibodies. Patients will be dichotomized as to whether or not their tumor expresses c-Met and the association between expression and response to therapy will be evaluated

Assay 6: Single cell sequencing

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

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

9.2.5 Sites Performing Correlatives

BWH
DFCI CIO
DFCI Clinical Trials Core Lab
Translation Immunogenomics Laboratory (TIGL)
The Broad Institute of MIT

9.3 Blood Collection

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

Please note that if green top tubes are unavailable, they may be substituted with either purple top tubes or CPT tubes.

The following research blood samples are required:

Baseline / Cycle 1 Day 1:

- 1-10 mL Streck Tube for cfDNA
- 5-10mL green top tubes for PBMCs
- 1-10mL purple top EDTA tube for plasma biomarkers

Cycle 2 Day 1:

- 5-10mL green top tubes for PBMCs
- 1-10mL purple top EDTA tube for plasma biomarkers

All other cycles, Day 1:

- 1 - 10mL purple top EDTA tube for plasma biomarkers

Every Restaging Visit:

- 1- 10mL Streck Tube for whole blood
- 5-10mL green top tubes for PBMCs
- 1-10mL purple top EDTA tube for plasma biomarkers

Off Treatment (if for progressive disease):

- 1-10 mL Streck Tube for whole blood
- 5- 10mL green top tubes for whole blood
- 1-10mL purple top EDTA tube for plasma biomarkers

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

- 1-10 mL Streck Tube for whole blood
- 5-10mL green top tubes for whole blood
- 1-10mL purple top EDTA tube for plasma biomarkers

9.3.1.1 Handling and Shipping

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

- PBMCs (Green/Purple/CPT):

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

  Must be processed within 3-4 hours of being drawn. Will be hand carried at ambient temperature to Mariano Severgnini. Please contact the lab approximately one week in advance to notify of upcoming specimen drop off (contacts: Tara Patel, tara_patel@dfci.harvard.edu; or Amy Cunningham, Amy_Cunningham@dfci.harvard.edu; or Martha Holland, marthak_holland@dfci.harvard.edu). Please deliver to:

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

- cfDNA (Streck tube):

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

  Tube precautions:
  - DO NOT FREEZE OR REFRIGERATE TUBES as this could result in cfDNA breakage. Blood collected in the Streck tube can be stored for 14 days between 6-37 degrees Celsius.
  - Do not use tubes after expiration date.
  - Fill the tube completely; overfilling or underfilling of tubes will result in an incorrect blood-to-additive ratio and may lead to incorrect analytical results.

  Blood in Streck tubes should be brought to the Clinical Trial Core Laboratory (Deborah Dillon, MD) on Smith 9 for processing.

  In small batches or at the end of the trial, samples will be shipped to the Broad Institute under the care of Nikhil Wagle, MD for genomic sequencing.

- Plasma biomarkers (10mL purple tube):

  This tube should be shipped the same day, on wet ice, ideally within 2 hours of collection to:

  Dr. Dan G. Duda
  Steele Laboratories for Tumor Biology
  Attn: Mrs. Anna Khachatryan / Mrs. Julia Kahn
  MGH, Cox-734
  100 Blossom St.
  Boston, MA 02114, USA
  Phone: (617) 726-4088 or (617) 726-8143 or (617) 724-1353
  Fax: (617) 724-5841
  Pager: 14082
  Email: annak@steele.mgh.harvard.edu, julia@steele.mgh.harvard.edu

  Please email Anna Khachatryan and Julia Kahn ahead of the shipment so the lab can expect it. Samples may be couriered via Skycom.

9.3.1.2 Potential Testing

Assay 1: Flow cytometry

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

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

Assay 2: Evaluate potential plasma biomarkers of cabozantinib

Exploratory analyses of potential biomarkers of cabozantinib activity will be performed by measuring proteins in the plasma and circulating cells at baseline, and on days 1 of each cycle of therapy, and, if available, at the time of progression. 8cc of blood will be collected in purple top (plasma EDTA) vacutainers, with a minimum of 5cc required. Each sample will be shipped on wet ice to the CLIA certified clinical correlative studies core of the Steele Laboratories. Blood will be separated by centrifugation into plasma and a cellular phase by standard methods. The plasma will be prepared in standard fashion and stored at –78 degrees Celsius until analysis. Plasma analysis will be carried out for a panel of circulating angiogenic and inflammatory molecules previously identified as potential biomarkers of response to anti-VEGF therapy in TNBC patients. They include VEGF, placental-derived growth factor (PlGF), VEGF-C, VEGF-D, sVEGFR1, basic

fibroblast growth factor (bFGF), and sTie-2 (using a 7-plex Growth Factor array) and granulocyte-macrophage colony stimulating factor (GM-CSF), interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and interleukin (IL)-1β, IL-2, IL-6, IL-8, IL-10, and IL-12 heterodimer p70 (using a 9-plex Inflammatory Factor array; both Meso-Scale Discovery, Gaithersburg, MD), and stromal-derived factor 1alpha (SDF1α) using ELISA from R&D Systems (Minneapolis, MN). In addition, we will measure the plasma concentration of biomarkers that are related to cabozantinib activity: HGF, s-MET, s-c-KIT and sVEGFR2 using ELISA from R&D Systems (Minneapolis, MN). Finally, we will evaluate biomarkers of tumor hypoxia, by measuring plasma carbonic anhydrase IX **(**CAIX) levels as well as biomarkers of osteoclast and osteoblast activity (plasma C-telopeptide and total alkaline phosphatase) using ELISA from R&D Systems (Minneapolis, MN). The number of circulating cells will be evaluated by using cell counters at routine visits and by flow cytometry in fresh blood samples collected at the same time-points as for plasma measurements. The fraction of stem/progenitor cell, lymphocyte, and myeloid populations of total circulating mononuclear cells were counted by flow cytometry using a LSR-II cytometer and FACSDiva software in fresh blood samples using the following markers: CD3, CD4, CD8, CD14, CD25, CD34, CD45, CD56, CD127, and CD133 (Becton Dickinson), as described{Tolaney, 2016 #1433}.

All biomarker analyses will be done in the CLIA-certified Core of the Steele Laboratories at MGH, under the supervision of Dr. Dan G. Duda.

9.3.2 Cell-free DNA (cfDNA) analysis

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

9.3.2.1 Collection of cfDNA specimen(s)

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

9.3.2.2 Handling and shipping of cfDNA specimens

One 10 ml Streck tube will be collected and processed at baseline, restaging visits and at time of progression for evaluation of cfDNA. Fill the Streck tube completely and immediately mix by gentle inversion 8 to 10 times. Inadequate or delayed mixing may result in accurate results. Ship within 24 hours of collection at ambient temperature overnight to:

Dana-Farber Cancer Institute
Attn: Lynda Chichester

Smith 9th Floor, Rm 948
450 Brookline Avenue
Boston, MA 02215
dfcibreastbank@partners.org

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

Tube precautions:

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

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

At a future date, these banked cfDNA samples will be shipped in batches to The Broad Institute under to care of Nikhil Wagle, MD, where they will be sequenced on their Genomics Platform.

9.3.2.3 Sites Performing Correlatives

DFCI Clinical Trials Core Lab
The Broad Institute
Steele Laoratories of MGH
DFCI CIO Laboratories

9.4 Stool Collection

9.4.1.1 Handling and shipping

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

- Baseline
- After two cycles of therapy

- At the end of treatment
- Optional collection at the time of grade ≥ 2 diarrhea

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

Please refer to the separate stool information sheet for collection and processing instructions.

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

9.4.2 Analysis of DNA extraction from stool samples

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

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

9.4.3 Shotgun sequencing and metabolic pathway reconstruction of stool samples

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

9.4.4 Sites performing correlative analysis

BWH/Harvard Cohorts Biorepository
Microbiome Dx

9.5 Additional analysis

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

10. STUDY CALENDAR

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

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

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

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

	Screening[a]	Cycle 1		C2			Cycle 3	Cycle 4 +		End of Treatment[p]	Follow-Up
Day		Day 1	Day 15	Day 1	Day 15	Day 22	Day 1	Day 1	Day 22		
Informed consent	X										
Medical History[b]	X										
Physical exam[c]	X	X	X	X	X		X	X		X	
Concurrent medications[d]	X										
ECOG	X	X		X			X	X		X	
Adverse event evaluation		X	X	X	X		X	X		X	
Vital signs[e]	X	X	X	X	X		X	X		X	
Hematology panel[f]	X	X	X	X			X	X		X	
Chemistry panel[g]	X	X	X	X			X	X		X	
Urine Protein Creatinine Ratio	X			X			X	X		X	
TSH/fT4	X	X		X			X	X[h]		X	
LDH[s]	X						X				
Coagulation panel (PT/PTT)	X										
Pregnancy test[i]	X										
Triplicate 12- lead EKG	X										
Tumor Assessment[j]	X					X			X	X[k]	X[k]
Brain MRI[l]	X										
Research Blood[m]		X		X			X	X		X	X
Research Biopsy[n]	X						X				
Archival Tumor Retrieval[o]	X										
Research Stool Collection[q]	X						X			X	
Stool Questionnaire[r]	X						X			X	

Cabozantinib: 40mg PO days 1-28 of a 28 day cycle
Nivolumab: 480mg IV on day 1 of a 28 day cycle.

a. Screening assessments are to be conducted within 28 days prior to start of protocol therapy unless otherwise specified. If these screening assessments occur within 3 days before start of study treatment, then they may serve as the baseline Cycle 1 Day 1 values.
b. Medical history includes clinically significant diseases, surgeries, and cancer history (including prior cancer therapies and procedures).
c. A complete physical examination will be performed at baseline. A limited physical exam will be completed prior to therapy on Days 1 and 15 for Cycles 1 and 2 and on Day 1 of every cycle beginning with Cycle 3.
d. Selected medications of interest taken from the time informed consent is signed through 30 days after the last dose of study therapy will be recorded. This includes: name, indication, dosage, frequency, route, and dates of administration.
e. Vital sign assessments include measurements of heart rate, systolic and diastolic blood pressures, respiratory rate, temperature and weight.
f. Hematology includes: hemoglobin, hematocrit, platelet count, RBC count, WBC count, and percent and absolute differential count. Results must be available prior to the administration of study drug.
g. Chemistry testing includes: sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, calcium, total bilirubin, total protein, albumin, ALT, AST, alkaline phosphatase, and LDH. Results must be available prior to the administration of study drug.
h. TSH and free T4 will be performed during screening, on Day 1 of Cycles 1-4 and then every other cycle on Day 1.
i. In female subjects of child-bearing potential as defined in the eligibility criteria, pregnancy test must be performed within **7 days** before the first dose of study medication.
j. Tumor assessments should consist of 1) CT and/or MRI of the chest/abdomen/pelvis, 3) any other imaging studies (CT neck, plain films, etc.) as clinically indicated by the treating physician. The same radiographic procedures and technique must be used throughout the study for each patient (e.g., if the patient had CT chest/abdomen/pelvis performed during screening, then she should subsequently undergo CT performed using the same radiologic protocol throughout the remainder of the study). Tumor assessments will be performed at baseline, between days 22-28 of Cycles 2, 4, and 6 and then between days 22-28 of every 3 cycles (C9, C12, C15, etc.). Additional scans are permitted as clinically indicated. All known sites of disease documented at screening should be re-assessed at each subsequent tumor evaluation.
k. For those taken off the study for reasons other than progressive disease, tumor measurements should continue to be repeated every 6-12 weeks.
l. Brain MRI should be done with and without contrast. 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.
m. Collected at screening, every D1, end of treatment (if removed for progressive disease) and optionally at time of progression. See section 9.0 for description of collections at each visit.
n. Baseline tumor biopsy should be obtained within 28 days of initiating protocol therapy. The C3D1 tumor biopsy should be performed as close to C3D1 as possible but may be collected up to 14 days prior.
o. Archival tumor sample should be collected (block or if not possible, 10-20 5mincron, unstained positively charged slides).
p. End of treatment visit is to occur within 30 days of final administration of study treatment. End of treatment assessments do not have to be repeated if the same assessments were performed within 7 days (28 days for tumor assessments) prior to the visit.
q. Baseline stool collection should be obtained within 28 days before starting protocol therapy. The C3D1 stool collection should be performed as close to C3D1 as possible, but may be collected up to 14 days prior. A sample will additionally be collection at the time of disease progression. An optional stool sample may be collected at the time of grade ≥ 2 diarrhea after discussion with the PI. As these collections are for exploratory correlative purposes, failure to provide a sample at these timepoints will not constitute a protocol violation. See section 9 and/or lab manual for stool collection and processing instructions.

r. Each stool collection kit will contain a questionnaire for the patients to complete regarding the conditions surrounding their collection. These will be a part of the kit and are not to be administered in clinic. Failure to complete these questionnaires at the required or optional timepoints will not constitute a protocol violation
s. LDH to be collected with screening labs and at C3D1.

11. MEASUREMENT OF EFFECT

In this study, response and progression in the CNS and in non-CNS sites will be evaluated and recorded separately in this trial. For the purposes of this study, participants should be re-evaluated for response every 6 weeks for the first 24 weeks and then every 9 weeks 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 [Eisenhauer *et al.*, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

11.1.1 RECIST 1.1 Definitions

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

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

11.1.2 Disease Parameters

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

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

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

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

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

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

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

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

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

11.1.3 Methods for Evaluation of Disease

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

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

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm in diameter as

assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

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

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

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

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

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

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

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

Note: A ‘positive’ FDG-PET scan lesion means one which is FDG avid with an uptake

greater than twice that of the surrounding tissue on the attenuation corrected image.

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

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

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

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

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

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

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

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

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

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

Tumor markers. Tumor markers alone cannot be used to assess response. 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	
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

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

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

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

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

11.1.4 Duration of Response

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

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

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

11.1.5 Clinical Benefit rate

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

11.2 Antitumor Effect – Hematologic Tumors

N/A

11.3 Other Response Parameters

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

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

11.3.1.1 Impact of New Lesions on irRC

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

11.3.1.2 Definition of Target Lesions Response Using irRC

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

(i.e. taking SPD of all target lesions and any new lesions) when compared to SPD at nadir.

11.3.1.3 Definition of Non-Target Lesions Response Using irRC

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

11.3.1.4 Definition of Overall Response Using irRC

Overall response using irRC will be based on these criteria:

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

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

Immune-Related Response Criteria Definitions

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

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

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

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

12. DATA REPORTING / REGULATORY REQUIREMENTS

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

12.1 Data Reporting

12.1.1 Method

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

12.1.2 Responsibility for Data Submission

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

12.2 Data Safety Monitoring

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

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

12.3 Multicenter Guidelines

N/A

12.4 Collaborative Research and Future Use of Data and Biospecimens

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

These samples and any data generated as a part of these clinical trials may be used for future research studies and may be provided to collaborating investigators both within and outside of the DF/HCC for either correlative endpoints or secondary use. Samples and data may be shared with outside non-profit academic investigators, as well as with for-profit pharmaceutical investigators or commercial 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, single-arm,single-center, phase 2 study of cabozantinib (40mg per oral daily) in combination with nivolumab (480mg intravenously on day 1, every 28 days) in subjects with metastatic triple-negative breast cancer previously treated with 0 to 3 chemotherapy regimens in the metastatic setting. The target enrollment is 35 patients.

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

Secondary endpoints include:
Secondary endpoints include the ORR of the combination according to immune-related response criteria (irRC) [Wolchok *et al.*, 2009] (Section 11), the clinical benefit rate, and PFS according to RECIST 1.1.

Blood and Tissue correlative science objectives include:

- To characterize a broad array of immune markers in metastatic TNBC (characterization will be based on histology, protein expression, and mRNA expression).
- To explore how different immunosuppressive and/or immune-stimulating immune marker profiles at baseline correlate with disease response to therapy (PFS, objective response assessed by RECIST 1.1 and immune-related response criteria).
- To characterize changes in tumor-infiltrating lymphocytes, PD-L1 expression and immune gene signatures in the tissue microenvironment (TME) from baseline to after 2 cycles of the experimental combination.
- To explore whether induction of changes in the immunosuppressive and/or immune-stimulating immune marker profile in TME correlates with disease response to therapy (response assessed by RECIST 1.1 and immune-related response criteria).
- To evaluate MET and phospho MET expression in tumor tissue at baseline by immunohistochemistry
- To explore the effect of treatment on plasma biomarkers (sVEGFR2, sMET, IL-2, IFN-γ, TNF-α)
- To characterize serial changes in immune marker profile in peripheral blood mononuclear cells (PBMCs) and in plasma over the course of the trial treatment.
- To explore whether induction of changes in the immunosuppressive and/or immune-stimulating immune marker profile in PBMCs correlates with disease response to therapy (response assessed by RECIST 1.1 and immune-related response criteria).

- To investigate whether there is an immune marker in circulating PBMCs that corresponds to tumor infiltrating lymphocyte (TIL) percentage in baseline tumor.
- To collect blood to study cell-free DNA for comparison to tumor specimens before and after immunotherapy

Stool and microbiome correlative science endpoints:

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

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

13.2 Sample Size, Accrual Rate and Study Duration

Based on data of recent trials with a similar population treated with anti-PD-1/PD-1 agents or cabozantinib in monotherapy{Tolaney, 2016 #1433}{Dirix, 2015 #1075}{Nanda, 2016 #1389}, and considering that our population will not be previously selected by PD-L1 expression status, a true ORR of 10% or less would not be of clinical interest, and is the null hypothesis to the Simon optimal two-stage design. A true rate of 30% would be considered a clinically meaningful level of response, so the sample size was chosen to have higher power (90%) to declare the combination effective at this rate, while controlling the one-sided Type I error at no more than 5% under the null.

Using Simon's optimal two-stage design, in the first stage, 18 patients will be enrolled. Of note, in this first stage we will perform a safety run-in analysis. If there are 2 or more dose-limiting toxicity (DLTs) in the first 6 patients included, the trial will be closed for further enrollment. Dose-

limiting toxicity (DLT) will be assessed in the first cycle. In the case of enrollment will continued, this first 6 patients will be included in the efficacy analysis. Thus, according to Simon's optimal two-stage design, if in this first stage there are at least 3 patients with ORR, accrual will continue to the second stage where an additional 17 patients will be enrolled. If there are at least 7 patients with ORR among the 35 patients, the regimen will be considered worthy of further study. If the true response rate is 10%, the chance the regimen is declared worthy of further study is less than 5% (exact alpha = 0.047). If the true response rate is 30%, the chance that the regimen is declared worthy of further study is 90%

Table below shows the probability of continuing enrollment with ≤1 DLTs out of the first 6 patients.

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

The expected accrual rate is 3-4 patients per month, and the accrual is expected to complete within 12 months.

13.3 Stratification Factors

NA

13.4 Interim Monitoring Plan

In the first stage we will perform a safety run-in analysis. If there are 2 or more dose-limiting toxicity (DLTs) in the first 6 patients included, the trial will be closed for further enrollment. Section 5.3 describes the DLT definitions used in this current study, which will be assessed in the first cycle (28 days of cycle 1). As of April 2018, 2 DLTs were identified during the first safety run-in.

The study will perform a safety run-in comprising another 6 patients, given that the toxicities seen were not consistent with other safety data available. Similar to the first safety run-in, if there are 2 or more DLTs in these patients, the trial will be closed to further enrollment.

If enrollment will continue, these first 12 patients will be included in the efficacy analysis. An additional interim analysis will happen after 18 patients enroll in the first stage. If there is at least 3 responses, accrual will continue to the second stage where up to 17 additional patients will be enrolled.

13.5 Analysis of Primary Endpoint

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

criteria; Section 11).

13.6 Analysis of Secondary Endpoints

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

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

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

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

With a sample size of 35 and assuming that there will be patients without accessible lesions, the table below indicates the power available to detect 20%, 30%, 40%, or 50% increases of PD-L1 positivity rate. The power calculation is based on McNemar's test with 1-sided alpha of 0.05 and assuming 2% of patients unexpectedly show PD-L1 positivity only in the baseline assessment. The calculation was done using East v6.3 (Cytel Inc).

Increase of PD-L1 positivity rate post treatment	# of paired biopsies available	Power
20%	15	54%
	20	64%
	25	72%
	30	79%
30%	15	75%
	20	85%
	25	91%
	30	95%
40%	15	90%
	20	96%
	25	98%
	30	> 99%
50%	15	97%
	20	> 99%
	25	> 99%
	30	> 99%

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

13.7 Reporting and Exclusions

13.7.1 Evaluation of Efficacy

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

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

13.7.2 Evaluation of Safety

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

14. PUBLICATION PLAN

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

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APPENDIX A PERFORMANCE STATUS CRITERIA

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

APPENDIX B GUIDELINES FOR COLLECTING RESEARCH BIOPSY TISSUE

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

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

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

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

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

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

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

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

Risks of Research Biopsy and Procedures for Minimizing Risk

Potential risks according to site are:
Skin/chest wall (punch biopsy):

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

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

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

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

Breast (core biopsy):

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

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

Pleural fluid (thoracentesis):

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

Ascites fluid (paracentesis):

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

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

Risks of Anesthesia

Local Anesthesia

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

Intravenous Conscious Sedation

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

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

General Anesthesia

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

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

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

For Effusions and Ascites

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

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

For Fine Needle Aspiration Samples

A goal of 3 passes:

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