DF/HCC Protocol #: 17-546

TITLE: A phase II study of atezolizumab in combination with pertuzumab plus high-dose trastuzumab for the treatment of central nervous system metastases in patients with Her2-positive breast cancer

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IND #: 137053
IND Sponsor: Nancy Lin, MD
Agent(s): Atezolizumab, Trastuzumab and Pertuzumab, *supplied by Genentech*

Protocol Type / Version # / Version Date: Amendment / Version 4 / January 21, 2020

SCHEMA

N= 33
1 cycle = 21 days

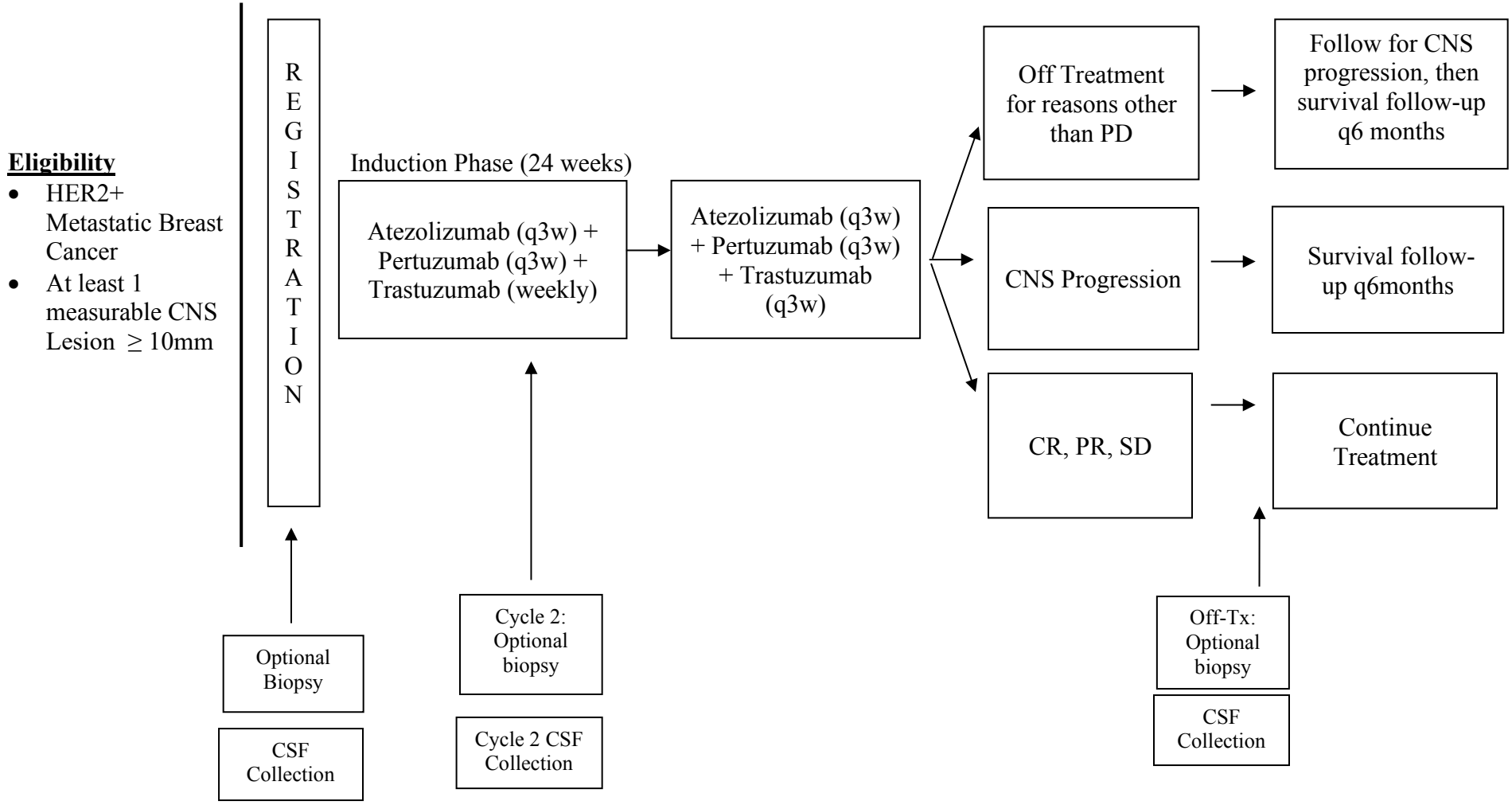


TABLE OF CONTENTS

1. OBJECTIVES

1.1 Study Design

This is an open-label, multi-center, phase II study designed to evaluate the efficacy of the combination of atezolizumab with pertuzumab plus with high-dose trastuzumab for the treatment of central nervous system (CNS) metastases in patients with HER2-positive metastatic breast cancer (MBC), as measured by objective response rate (ORR) in the CNS.

Up to 33 eligible patients will receive the following treatment: atezolizumab [1200mg intravenously (IV) every 3 weeks (q3w)], pertuzumab (loading dose of 840 mg IV, followed q3w thereafter by a dose of 420 mg IV), and high-dose trastuzumab (at a dose of 6 mg/kg weekly for the first 24 weeks, and thereafter trastuzumab 6mg/Kg IV q3w).

1.2 Primary Objective

To evaluate the efficacy of atezolizumab in combination with pertuzumab and high-dose trastuzumab for the treatment of CNS metastases in patients with HER2-positive MBC, as measured by ORR in the CNS according to response assessment in neuro-oncology-brain metastases (RANO-BM) criteria.

1.3 Secondary Objectives

1.3.1 Safety Objective

- To evaluate the safety, and tolerability of the combination of atezolizumab, pertuzumab and high-dose trastuzumab.

1.3.2 Efficacy Objectives

- To evaluate the duration of response (DOR) in the CNS
- To evaluate the efficacy of the study combination, as defined by bi-compartmental progression-free survival (PFS) according to RANO-BM criteria [Lin *et al.*, 2015] (Section 11.1.1).
- To evaluate the CNS response rates according to response assessment in immunotherapy neuro-oncology-brain metastases (iRANO-BM) criteria [Okada *et al.*, 2015].
- To evaluate the extracranial ORR according RECIST 1.1 criteria[Eisenhauer *et al.*, 2009].
- To evaluate the extracranial ORR according to immune-related response criteria (irRC)[Wolchok *et al.*, 2009].
- To evaluate clinical benefit rate at 18 and 24 weeks, defined as the proportion of participants with stable or responsive disease in both CNS and non-CNS at 18 and 24 weeks per RANO-BM criteria.
- To evaluate PFS according to the RECIST 1.1 single-compartmental model.
- To describe the site of first progression (CNS vs extracranial vs both)

- To evaluate the overall survival (OS) among patients included in this trial

1.3.3 Patient-Reported Outcome Objectives

- To evaluate the impact of the experimental treatment on PROs, as measured by the M.D. Anderson Symptom Inventory-Brain Tumor (MDASI-BT) assessment

1.3.4 Investigator-Assessed Neurological Evaluation

- To evaluate the impact of the study treatment, for these same patients, on investigator-assessed neurological evaluation, as measured by the Neurological Assessment in Neuro-Oncology (NANO) scale.

1.3.5 EQ-5D evaluation

- To evaluate the impact of the study treatment, for these same patients, on general health status assessed by EQ-5D questionnarie.

1.4 Correlative Objectives

- To explore whether the number and/or type of mutations identified using a next generation sequencing (NGS) panel is correlated with patient outcomes (PFS, CNS ORR, CBR, and OS).
- To collect blood to study cell-free DNA for quantification of tumor DNA content and copy number variation, using ultra-low pass whole genome sequencing, and to explore whether cfDNA load is associated with patient outcomes (PFS, CNS ORR, CBR, and OS).
- To collect blood to study cell-free DNA for targeted sequencing and/or whole exome sequencing To compare mutations and copy number variation between cfDNA and tumor biopsies.
- To characterize a broad array of immune markers in metastatic HER-2 positive breast cancer (characterization will be based on histology, protein expression, and mRNA expression), and their changes with immune checkpoint blockade.
- To explore how different immunosuppressive and/or immune-stimulating immune marker profiles at baseline correlate with patient outcomes (PFS, CNS ORR, CBR and OS).
- To characterize changes in immune marker profiles on treatment and at time of progression
- 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 PBMC correlates with clinical outcomes (PFS, CNS ORR, and OS).
- To collect cerebrospinal fluid (CSF) to study cell-free DNA for quantification of tumor DNA content and copy number variation, using ultra-low pass whole genome sequencing, and to compare patterns of cfDNA serially over time in CSF compared to plasma.
- To explore whether cfDNA load in CSF is associated with clinical outcomes (PFS, CNS ORR, CRR, and OS).
- To collect CSF to study cell-free DNA for targeted sequencing and/or whole exome sequencing before, on and after immunotherapy. To compare mutations and copy number variation between cfDNA in plasma versus CSF.

2. BACKGROUND

2.1 Study Disease

Breast cancer is the most frequently diagnosed cancer and the second cause of cancer death in American women[Jemal *et al.*, 2011, Siegel *et al.*, 2013]. Approximately 15%-20% overexpress human epidermal growth factor receptor 2 (HER2) and are classified as HER2 positive tumors [Slamon *et al.*, 1987, Pathmanathan *et al.*, 2012, Wolff *et al.*, 2013]. Together with triple negative breast cancer (TNBC), HER-2 positive tumors have the highest rates of brain metastases (BM), with studies reporting up to 50% rate of central nervous system (CNS) involvement among those subtypes[Lin *et al.*, 2008, Niwinska *et al.*, 2010, Lin *et al.*, 2012, Olson *et al.*, 2013, Pestalozzi *et al.*, 2013].

Initial treatment for patients with BM typically includes surgery or radiotherapy, either whole brain radiotherapy (WBRT), sterotactic radiosurgery (SRS), or both, depending on factors such as performance status, expected prognosis, as well the localization and the number of CNS metastases[Lim *et al.*, 2014]. Although median OS after a diagnosis of brain metastasis now exceeds 2 years in patients with good performance status and HER2-positive disease[Sperduto *et al.*, 2012], this outcome has resulted in patients who live long enough to have substantial morbidity from additional CNS progression post-radiation. At this time point, there are currently no systemic therapies approved for use in the treatment of these patients. Clearly, better options for the prevention and treatment of brain metastases in patients with HER2-positive breast cancer are needed.

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

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

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene *Pdcd1*) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and

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

<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 Atezolizumab (MPDL3280A)

Atezolizumab is a human immunoglobulin (Ig) G1 monoclonal antibody consisting of two heavy chains (448 amino acids) and two light chains (214 amino acids) and is produced in Chinese hamster ovary cells. Atezolizumab was engineered to eliminate Fc-effector function via a single amino acid substitution (asparagine to alanine) at position 298 on the heavy chain, which results

in a non-glycosylated antibody that has minimal binding to Fc receptors and prevents Fc-effector function at expected concentrations in humans. Atezolizumab targets human programmed death-ligand 1 (PD-L1) and inhibits its interaction with its receptor, programmed death-1 (PD-1). Atezolizumab also blocks the binding of PD-L1 to B7.1, an interaction that is reported to provide additional inhibitory signals to T cells.

Atezolizumab is being investigated as a potential therapy against solid tumors and hematologic malignancies in humans.

2.3.1 Summary of Nonclinical Experience with Atezolizumab

The nonclinical strategy of the Atezolizumab program was to demonstrate in vitro and in vivo activity, to determine in vivo pharmacokinetic (PK) behavior, to demonstrate an acceptable safety profile, and to identify a Phase I starting dose. Comprehensive pharmacology, PK, and toxicology evaluations were thus undertaken with Atezolizumab.

The safety, pharmacokinetics, and toxicokinetics of Atezolizumab were investigated in mice and cynomolgus monkeys to support intravenous (IV) administration and to aid in projecting the appropriate starting dose in humans. Given the similar binding of Atezolizumab for cynomolgus monkey and human PD-L1, the cynomolgus monkey was selected as the primary and relevant nonclinical model for understanding the safety, pharmacokinetics, and toxicokinetics of Atezolizumab.

Overall, the nonclinical pharmacokinetics and toxicokinetics observed for Atezolizumab supported entry into clinical studies, including providing adequate safety factors for the proposed Phase I starting doses. The results of the toxicology program were consistent with the anticipated pharmacologic activity of down-modulating the PD-L1/PD-1 pathway and supported entry into clinical trials in patients.

Refer to the Atezolizumab Investigator's Brochure for details on the nonclinical studies.

2.3.2 Clinical Experience with Atezolizumab

2.3.2.1 Ongoing Clinical Studies

Current studies of Atezolizumab include one ongoing Phase Ia monotherapy study, three ongoing combination studies, five Phase II studies, and one Phase III study. Details of all ongoing studies can be found in the Atezolizumab Investigator's Brochure.

Phase Ia Study PCD4989g
Study PCD4989g is a multicenter, first-in-human, open-label, dose-escalation study evaluating the safety, tolerability, immunogenicity, pharmacokinetics, exploratory pharmacodynamics, and preliminary evidence of biologic activity of Atezolizumab administered as a single agent by IV infusion every 3 weeks to patients with locally advanced or metastatic solid malignancies or hematologic malignancies. Ongoing expansion cohorts are studying the efficacy in patients with

pancreatic cancer, bladder cancer, breast cancer, esophageal cancer, prostate cancer, small-cell lung cancer, malignant lymphoma, multiple myeloma, and other less common tumor types.

Phase Ib Study GP28328

Ongoing Phase Ib Study GP28328 is evaluating the safety and pharmacology of Atezolizumab administered with bevacizumab alone (Arm A) or with bevacizumab plus leucovorin, 5-fluorouracil, and oxaliplatin (FOLFOX; Arm B) in patients with advanced solid tumors. Additional cohorts have been included to investigate Atezolizumab in combination with carboplatin plus paclitaxel, in combination with carboplatin plus pemetrexed, and in combination with carboplatin plus nab paclitaxel, pemetrexed, and cisplatin in patients with advanced or metastatic non-small cell lung cancer (NSCLC).

Phase Ib Study GP28384

Ongoing Phase Ib Study GP28384 is evaluating the safety and pharmacology of Atezolizumab administered in combination with vemurafenib in patients with previously untreated $BRAF^{V600}$-mutation–positive metastatic melanoma.

Phase Ib Study GP28363

Ongoing Phase Ib Study GP28363 is evaluating the safety and pharmacology of Atezolizumab administered in combination with cobimetinib (MEK inhibitor) in locally advanced or metastatic solid tumors.

Phase II Study GO28625 (FIR)

Ongoing, single-arm, Phase II Study GO28625 is evaluating the safety and efficacy of Atezolizumab monotherapy in PD-L1–positive patients with NSCLC. This study is evaluating whether archival or fresh tumor tissue is more predictive of response to Atezolizumab. Safety and efficacy data are not yet available for this study.

Phase II Study GO28753 (POPLAR)

Study GO28753 is a randomized, open-label, Phase II study in patients with locally advanced or metastatic NSCLC who have failed a prior platinum-containing regimen. Patients in the control arm of Study GO28753 will receive docetaxel alone. Eligible patients will be enrolled regardless of PD-L1 status and will be stratified by PD-L1 expression. The primary endpoint is overall survival (OS) for both the PD-L1–positive population and the overall study population.

Phase II Study GO28754 (BIRCH)

Ongoing, single-arm, Phase II Study GO28754 is evaluating the safety and efficacy of Atezolizumab monotherapy in PD-L1–positive patients with NSCLC. Safety and efficacy data are not yet available for this study.

Phase II Study WO29074

Ongoing Phase II Study WO29074 is evaluating the safety and efficacy of Atezolizumab monotherapy or the combination of Atezolizumab and bevacizumab versus sunitinib in treatment-naïve patients with renal cell carcinoma (RCC). Safety and efficacy data are not yet available for this study.

Phase II Study GO29293

Ongoing Study GO29293 is a single-arm, open label, Phase II study to assess the clinical benefit of Atezolizumab as a single agent in patients with locally advanced or metastatic UBC. The co-primary endpoints of this study are independent review facility (IRF)–assessed objective response rate (ORR) according to Response Evaluation Criteria in Solid Tumors, Version 1.1 (RECIST v1.1) and investigator-assessed ORR according to modified Response Evaluation Criteria in Solid Tumors (RECIST) criteria.

Phase III Study GO28915 (OAK)
Study GO28915 is a randomized, open-label, Phase III study in patients with locally advanced or metastatic NSCLC who have failed a prior platinum-containing regimen. Patients in the control arm of Study GO28915 will receive docetaxel alone. Eligible patients will be enrolled regardless of PD-L1 status and will be stratified by PD-L1 expression. The primary endpoint is OS for both the PD-L1−positive population and the overall study population.

2.3.3 Clinical Safety

The presented safety data for Atezolizumab have been derived mainly from the treatment of patients in Phase Ia Study PCD4989g. As of 10 May 2014, Atezolizumab has been administered to approximately 775 patients with solid and hematologic malignancies. No dose-limiting toxicities (DLTs) have been observed at any dose level, and no maximum tolerated dose (MTD) was established. Fatigue was the most frequently reported adverse event (AE).

Adverse Events
The following safety data are from PCD4989g, in which Atezolizumab is being used as single-agent therapy in patients with locally advanced or metastatic solid tumors or hematologic malignancies. In 412 treated patients, 97.1% reported an AE while on study. Of these AEs, 48.8% were Grade 1 or 2 in maximum severity on the basis of National Cancer Institute Common Terminology Criteria for Adverse Events, Version 4.0 (NCI CTCAE v4.0). The most frequently observed AEs (occurring in ≥ 10% of treated patients) included fatigue, nausea, decreased appetite, pyrexia, dyspnea, diarrhea, constipation, cough, headache, back pain, vomiting, anemia, arthralgia, rash, insomnia, asthenia, abdominal pain, chills, pruritus, and upper respiratory tract infection.

Grade ≥ 3 AEs were reported by 199 of 412 patients (48.3%). There were 51 patients (12.4%) who reported Grade ≥ 3 AEs that were assessed as related to study drug by the investigators. The most frequently reported related Grade ≥ 3 AEs included fatigue (5 patients [1.2%]), increased ALT and increased AST (each reported in 4 patients [1.0%]); and asthenia, autoimmune hepatitis, and hypoxia (each reported in 3 patients [0.7%]).

Immune-Related Adverse Events
Given the mechanism of action of Atezolizumab, events associated with inflammation and/or immune-mediated AEs have been closely monitored during the Atezolizumab clinical program. These include potential dermatologic, hepatic, endocrine, and respiratory events as well as events of hepatitis/elevated liver function tests (LFTs) and influenza-like illness. Expected adverse drug reactions associated with Atezolizumab include the following: hepatitis/transaminitis, hypothyroidism, infusion-related reactions (IRRs), pneumonitis, influenza-like illness, and dermatologic reactions. Potential adverse drug reactions include the following: anti-therapeutic antibodies (ATAs), colitis, endocrine disorders, hypersensitivity, neurologic disorders, and pericardial effusion.

For further details, see the Atezolizumab Investigator's Brochure.

2.3.4 Clinical Activity

As of the data cutoff of 1 January 2014, efficacy analyses were performed on 386 efficacy evaluable patients who were defined as those patients, with measurable disease at baseline, treated by 1 July 2013 in Study PCD4989g (to ensure that each patient had a minimum of 6 months follow-up). Patients with multiple tumor types were included in the study, with the largest cohorts consisting of patients with NSCLC, RCC, and bladder cancer. Objective responses with Atezolizumab monotherapy were observed in a broad range of malignancies, including NSCLC, RCC, melanoma, bladder cancer, colorectal cancer, head and neck cancer, gastric cancer, breast cancer and sarcoma. Altogether, there were 47 patients with responses with a median duration of response of 75.7 weeks (range: 11.7+ to 85.9+ weeks, where "+" denotes censored value). The majority of these responses have been durable, with 72.3% (34/47) of responses ongoing as of the clinical cutoff date.

Analyses of tumor-infiltrating immune cells for PD-L1 expression on baseline tumor tissue have been performed for Study PCD4989g. Preliminary results from Study PCD4989g suggest that PD-L1 expression in tumor-infiltrating immune cells is likely to be associated with response to Atezolizumab.

In adittion, as of the clinical cutoff date of 21 April 2014, efficacy analyses were performed on 33 immunohistochemistry (IHC) 2/3 and 36 IHC 0/1 efficacy-evaluable patients with locally advanced or metastatic urothelial bladder cancer (UBC) who were dosed by 27 January 2014 in Study PCD4989g[Powles *et al.*, 2014]. In the 33 IHC 2/3 efficacy-evaluable patients with UBC, the median follow-up was 6 months (range: 1+ to 12 months). The investigator-assessed ORR per RECIST v1.1 in this IHC 2/3 cohort was 52% (95% CI: 34%, 69%) with three complete responses. The median duration of response was not yet reached (range: 0.1+ to 42+ weeks). The median progression-free survival (PFS) was 24 weeks (range: 5 to 50+ weeks), respectively. Among 36 IHC 0/1 efficacy-evaluable patients with UBC, there were 5 patients with responses. The investigator-assessed ORR per RECIST v1.1 in this IHC 0/1 cohort was 14% (95% CI: 6%, 28%). Median duration of follow-up for these patients was 4 months (range: 1+ to 7 months). For these five responses, the median duration of response has not been reached (range: 6+ to 19+ weeks). The majority of responses have been durable, with 86.4% of responses (19 of 22) still ongoing as of the clinical cutoff date.

For further details, see the Atezolizumab Investigator's Brochure.

2.3.5 Clinical Pharmacokinetics and Immunogenicity

Based on available preliminary PK data (0.03–20 mg/kg), Atezolizumab appeared to show linear pharmacokinetics at doses ≥ 1 mg/kg. For the 1-mg/kg and 20-mg/kg dose groups, the mean clearance (CL) and the mean volume at steady state (V_{ss}) had a range of 3.20–4.43 mL/kg and 48.1–64.1 mL/kg, respectively, which is consistent with the expected profile of an IgG1 antibody in humans.

The development of ATAs has been observed in patients in all dose cohorts and was associated with changes in pharmacokinetics for some patients in the lower dose cohorts (0.3, 1, and 3 mg/kg). The development of detectable ATAs has not had a significant impact on pharmacokinetics for doses from 10–20 mg/kg. Patients dosed at the 10-, 15-, and 20-mg/kg dose levels have maintained the expected target trough levels of drug despite the detection of ATAs. To date, no clear relationship between detection of ATAs and AEs or IRRs has been observed.

2.4 Pertuzumab

Pertuzumab (Perjeta®), a humanized monoclonal antibody to the HER2 receptor, blocks ligand-dependent heterodimerization of HER2 with other HER family members. This results in the inhibition of ligand-initiated intracellular signaling. In addition, pertuzumab mediates antibody-dependent cellular cytotoxicity.

Pertuzumab has been shown in nonclinical settings to have superior anti-tumor effects when combined with trastuzumab than when used as monotherapy. Trastuzumab and pertuzumab monoclonal antibodies bind to distinct epitopes on the HER2 receptor without competing with each other, resulting in distinctive mechanisms for disrupting HER2 signaling. These mechanisms are complementary and result in augmented therapeutic efficacy when pertuzumab and trastuzumab are given in combination. Pertuzumab binds to an epitope within subdomain 2 of HER2, whereas the epitope for trastuzumab is localized to subdomain 4 (Cho et al. 2003; Franklin et al. 2004).

Pertuzumab acts by blocking dimerization of HER2 with other HER family members, thereby inhibiting ligand-initiated intracellular signaling through two major signaling pathways, MAPK and PI3K. Inhibition of these pathways can result in growth arrest and apoptosis (Hanahan and Weinberg 2000). In comparison, trastuzumab binds to the juxtamembrane epitope (subdomain 4), preventing cleavage and ligand independent signal transduction. Both antibodies are also capable of activating antibody-dependent, cell-mediated cytotoxicity (Spector and Blackwell 2009). In the Phase III, pivotal study WO20698/TOC4129g (CLEOPATRA; N = 808) in patients with previously untreated HER2-positive MBC, a statistically significant and clinically meaningful improvement in PFS, based on tumor assessments by an independent review facility (IRF), was observed in patients treated with pertuzumab, trastuzumab, and docetaxel (n = 406) compared with those receiving placebo, trastuzumab, and docetaxel (n = 402). PFS was prolonged at the median by 6.1 months, and the risk of disease progression or death was reduced by 38% (*hazard* ratio [HR]: 0.62; 95% confidence interval [CI]: 0.51, 0.75; $p < 0.0001$) with an improvement in median PFS from 12.4 months to 18.5 months. Results of the investigator-assessed PFS analysis (HR: 0.65 [0.54, 0.78]; $p < 0.0001$; median 12.4 vs. 18.5 months, respectively) were consistent with those observed for IRF-assessed PFS. A second interim analysis of OS (performed one year after the primary analysis of efficacy) crossed the predefined stopping boundary for statistical significance ($p = 0.0138$), demonstrating that treatment with pertuzumab, trastuzumab, and docetaxel significantly improved OS when compared with the placebo arm (HR: 0.66; 95% CI: 0.52, 0.84; $p = 0.0008$). The updated analysis of investigator-assessed PFS demonstrated that the PFS benefit observed at the primary analysis was maintained after an additional year of follow-up. The HR of 0.69 and the increase in median PFS of 6.3 months (from 12.4 months in the placebo arm to 18.7

months in the treatment arm) were highly consistent with those from the first analysis of investigator-assessed PFS and consequently also with the primary IRF analysis (Swain et al. 2013).

Based on these data, pertuzumab was approved by the FDA for use in HER2-overexpressing MBC in combination with trastuzumab and docetaxel for first-line treatment metastatic disease. Pertuzumab is also currently approved in the early breast cancer setting. See the pertuzumab Investigator's Brochure for additional information.

2.5 Trastuzumab

Trastuzumab (Herceptin®) is a recombinant monoclonal antibody that binds specifically and with high affinity to the extracellular domain of HER2. Trastuzumab has been shown to inhibit the proliferation of human tumor cells overexpressing HER2 both in vitro and in vivo. The clinical benefit of trastuzumab in women with MBC has been demonstrated in two pivotal studies.

A Phase II trial (H0649g) assessed the activity of single-agent trastuzumab in 222 women with HER2-overexpressing MBC with progressive disease after one or more chemotherapy regimens (Cobleigh et al. 1999). An independent response evaluation committee identified 8 complete and 26 partial responses, for an objective response rate of 15% in the intent-to-treat population (95% CI: 11% to 21%). The median duration of response was 9.1 months, and the median duration of survival was 13 months. The most common adverse events, which occurred in approximately 40% of patients, were mild to moderate infusion-associated fever and/or chills. The most clinically significant event was cardiac dysfunction, which occurred in 4.7% of patients. An open-label, randomized, Phase III study (H0648g) in 469 patients with HER2-positive MBC was conducted to evaluate the efficacy of trastuzumab in combination with chemotherapy as first-line treatment. Patients who were anthracycline-naïve were randomized to receive either anthracycline plus cyclophosphamide (AC) or trastuzumab plus AC. Patients who had received prior anthracyclines in the adjuvant setting were randomized to receive either paclitaxel or trastuzumab plus paclitaxel. As determined by an independent response evaluation committee, trastuzumab prolonged median time to disease progression from 4.6 months to 7.4 months ($p < 0.001$), improved the overall response rate (complete and partial responses) from 32% to 50% ($p < 0.001$), and increased median duration of response from 6.1 to 9.1 months ($p < 0.001$). Compared to chemotherapy alone, the addition of trastuzumab significantly lowered the incidence of death at one year from 33% to 22% ($p = 0.008$) and increased median overall survival 24% from 20.3 months to 25.1 months ($p = 0.046$) (Slamon et al. 2001). The observed survival advantage remained despite crossover of 66% of patients initially randomized to chemotherapy alone who elected to receive trastuzumab upon disease progression (Tripathy et al. 2000). Fever/chills were observed with the initial trastuzumab infusion in approximately 25% of patients. Class III or IV cardiac dysfunction was observed in 16% of the trastuzumab + AC subgroup; increasing age was an associated risk factor for the development of cardiotoxicity in this treatment cohort (Slamon et al. 2001).

Based on these data, trastuzumab was approved by the U.S. Food and Drug Administration (FDA) for use in HER2-overexpressing MBC in combination with paclitaxel for first-line treatment and as a single agent for patients who have progressed on chemotherapy for metastatic disease. Subsequent randomized studies have demonstrated the value of continued trastuzumab in combination with chemotherapy or targeted therapy, even after prior progression on trastuzumab

(von Minckwitz, 2009; Blackwell, 2012). As a result, even after progression on trastuzumab, continuing the blockade of HER-2 pathway, usually with a trastuzumab containing regimen, is considered standard of care [Giordano *et al.*, 2014]. See the trastuzumab Investigator's Brochure for additional information.

2.6 Rationale

2.6.1 Rationale for choice of high dose trastuzumab and pertuzumab backbone

Unfortunately, CNS response to existing systemic anticancer therapies at standard dosages has been disappointing, in part due to the limited capacity of many of these drugs to cross the blood–brain barrier (BBB) effectively, and due the therapeutic concentration limitations resulting from active drug efflux proteins such as P-glycoprotein (Pgp), which are present in high concentrations in the luminal membranes of brain endothelium[Regina *et al.*, 2001]. Large monoclonal antibodies are not believed to cross an intact BBB. However, the BBB may be subject to increased permeability/disruption associated with radiation effects and tumor invasion. A study using positron electron tomography imaging demonstrated CNS penetration by 89Zr-trastuzumab in patients with MBC with an 18-fold higher uptake in brain tumors than in normal brain tissue[Dijkers *et al.*, 2010].

Multiple retrospective and prospective studies have demonstrated that patients treated with trastuzumab prior to the development of CNS metastases and/or after CNS metastases have improved survival outcomes. Incremental increases in uptake of trastuzumab have been observed with standard systemic dosing when the BBB is disrupted by associated radiation effects and/or tumor invasion[Dijkers *et al.*, 2010]. Subtherapeutic trastuzumab levels achieved in the CNS may be related to insufficient dosing as opposed to the inability of trastuzumab to cross the BBB. Of note, Phillips et al. (unpublished Genentech internal data) evaluated the relationship between dose-response in brain graft experiments in murine models. When evaluated in brain graft experiments, 4D5 (a murine equivalent of trastuzumab) doses up to three times the effective dose used in mammary grafts were required to achieve efficacy in brain grafts. Importantly, higher trastuzumab dosages have not been associated with increased cardiotoxicity or adverse events as observed in a Phase I study (8 patients received 500 mg intravenously every week for 8 weeks), and in two Phase II studies (Vogel et al. 2002; Leyland-Jones et al. 2010) where patients received two (N = 57) and three times (N = 47) the standard dose.

The impact of a more comprehensive blockade of cell signaling associated with the addition of pertuzumab to trastuzumab has been demonstrated in the nonclinical and clinical settings. The combination of pertuzumab and trastuzumab with docetaxel demonstrated statistically significant and clinically meaningful improvements in outcomes (PFS and OS) without an observed increase in cardiotoxicity compared with trastuzumab and docetaxel in a pivotal, Phase III, randomized, controlled trial CLEOPATRA[Swain *et al.*, 2013].

Based on the above data, the PATRICIA trial, an ongoing, Genentech-sponsored, multicenter, prospective, single-arm study is evaluating the efficacy of “high dose” trastuzumab (6 mg/kg weekly) in combination with standard dose pertuzumab in patients with HER2-positive breast

cancer and progressive brain metastases. In this study, patients are also allowed to continue concurrent systemic chemotherapy and/or endocrine therapy. Over 30 patients have been enrolled to date. Pre-planned interim analysis of the first 15 patients has demonstrated a CNS ORR of 20% (95% CI 4.3%-48.1%). At four months, 40% (95% CI 16.3%-67.7%) have had no evidence of either CNS or systemic progression. No cardiac toxicity signals of concern have been observed to date. Although preliminary, these results provide proof-of-concept that altering the trastuzumab dosing schedule may result in CNS efficacy, and provide a well-tolerated backbone upon which to add novel agents of interest.

2.6.2 Rationale for exploration of immunotherapy in HER2-positive breast cancer

There are accumulating preclinical and clinical evidence suggesting that immune system is critical for disease outcome in breast cancer, particularly in the triple-negative and HER2-positive subtypes [Kroemer *et al.*, 2015]. Different groups have been shown that immune cell infiltration differs according BC subtype: while hormonal-positive BC are poorly T cell infiltrated, a substantial proportion of HER2-positive tumors can be richly infiltrated [Loi *et al.*, 2014, Salgado *et al.*, 2014, Denkert *et al.*, 2015]. Of note, multiple concordant reports indicate that disease outcome in patients with HER2-positive breast cancer treated in the neo(adjuvant) setting with trastuzumab-based regimes improves when tumor microenvironment either have an abundant tumors lymphocyte infiltration or express immune-related signatures[Kroemer *et al.*, 2015]. Recently, in a secondary analysis of the phase III CLEOPATRA study, which evaluated the benefit of adding pertuzumab or placebo to docetaxel plus trastuzumab in the first line treatment of patients with advanced HER2-positive breast cancer, investigators showed that higher stromal tumour-infiltrating lymphocytes values are significantly associated with improved overall survival, suggesting that the effect of antitumour immunity extends to the advanced setting[Luen *et al.*, 2017]. So far, only one trial has reported data evaluating immune checkpoint inhibitors (ICI) in patients with HER2-positive breast cancer: a phase I clinical trial has evaluated the safety of the PD-L1 inhibitor avelumab in monotherapy[Dirix *et al.*, 2015]. Although the ORR was low, of note, patients did not receive concurrent anti-HER2-therapy.

Importantly, it has been demonstrated that trastuzumab induces robust tumor infiltration by lymphoid cells in patients with breast cancer[Gennari *et al.*, 2004], and preclinical data has shown that trastuzumab loses its efficacy when the gene encoding the common γ chain of activating Fcγ receptors is deleted in the host[Clynes *et al.*, 2000]. Therefore, trastuzumab might mediate anticancer effects in part via the induction of antibody-dependent cellular cytotoxicity (ADCC). In agreement with these data, there are evidence suggesting that components both of the innate and adaptive immune system participate in the clinical activity of trastuzumab. The optimal response of mouse BCs to various anti-HER2 mAbs (including trastuzumab) requires the presence not only of NK cells but also of CD8+ and CD4+ T lymphocytes[Park *et al.*, 2010, Stagg *et al.*, 2011]. Expression of PD-1, PD-L1 or both, in HER-positive breast tumor has been noted, and this may be an important mechanism of immune evasion and contribute to resistance through anti-HER2 agents. Furthermore, it has been demonstrated that anti-HER2 mAbs synergize with anti-PD-1, and can significantly improve the therapeutic activity of trastuzumab in immunocompetent mice [Stagg *et al.*, 2011].

2.6.3 Rationale for overall study design

To date, patients with active breast cancer brain metastases have been excluded from virtually all trials of immunotherapy. However, clear CNS activity has been demonstrated with immune checkpoint inhibitors in patients with advanced melanoma or non-small cell lung cancer[Weber *et al.*, 2011, Konstantinou *et al.*, 2014, Goldberg *et al.*, 2016]. Given the high prevalence of brain metastases in patients with HER2-positive breast cancer, evaluating the efficacy of checkpoint blockade in this patient population represents an opportunity for a major impact in this area of unmet medical need. Furthermore, preliminary activity from the PATRICIA trial supports the use of a high dose trastuzumab/pertuzumab backbone upon which to add checkpoint blockade.

Therefore, we hypothesize that an optimal anti-HER2 regimen, designed to better penetrate the BBB, combined with an anti-PD-L1 agent, will synergize to increase efficacy against CNS metastases in patients with HER2-positive MBC. We propose a single-arm, multi-center, phase II trial to evaluate the efficacy and safety of atezolizumab in combination with pertuzumab and high-dose trastuzumab for the treatment of CNS metastases in patients with HER2-positive MBC, as measured by ORR in the CNS according to RANO-BM criteria. Patients will receive atezolizumab [1200mg intravenously (IV) every 3 weeks (q3w)], pertuzumab (loading dose of 840 mg IV, followed q3w thereafter by a dose of 420 mg IV), and high-dose trastuzumab (at a dose of 6 mg/kg weekly for the first 24 weeks, and thereafter trastuzumab 6mg/Kg IV q3w).

2.7 Correlative Studies Background

2.7.1 Immune biomarkers

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 of 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. The lack of a significant T-cell infiltrate, and the low expression of immune checkpoint molecules, may explain the reason that non-inflamed tumor phenotype are associated with *de novo* resistance to ICI. For this group of patients, therapeutic strategies that promote a boost in innate immunity, such as a highly effective anti-HER2 therapy, will be crucial to successfully overcoming T-cell exclusion and improve the likelihood of benefit of PD-1 blockers. Therefore, the bulk of correlative science in this trial highlights our especial interest in characterize a broad array of immune markers in metastatic HER2-positive breast cancer, investigating whether those markers predict disease response to therapy.

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

These correlative projects are made possible by collaboration with Drs. 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.

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

2.7.3 Circulating free DNA

We will collaborate with Dr. Heather Parsons and investigators at the Broad Institute to study cfDNA serially over time. cfDNA provides a less invasive method by which to characterize tumor genomics. In addition, there is the potential to capture heterogeneity across multiple metastatic sites, in a more practical way than tissue biopsies of multiple sites. In patients with brain metastases, in particular, research biopsies of CNS tumors are not feasible—yet given that these tumors have often been exposed to additional therapies (for example, WBRT and/or SRS), their genetic profile may be distinct from that of other metastatic sites. Although there have been some studies describing the genomics of brain metastases, because most resections occur in the setting of a new presentation of a single brain metastasis, they do not truly reflect the patient with progressive brain metastases after local therapy. cfDNA also provides an alternate method by which to quantify tumor burden over time. Given the intended population of patients with brain metastases who will receive immune checkpoint blockade, transient increases in the size of brain metastases may be explained by: true tumor progression, radiation necrosis, or immune infiltration. There is currently no non-invasive test that has been demonstrated to match the gold standard of surgical resection, and the guidelines reflect this uncertainty[Lin *et al.*, 2013]. Developing better tests to differentiate between these entities would be a tremendous clinical advance in terms of everyday patient care. Finally, the correlation between cfDNA in plasma versus CSF is currently unknown, as is their relationship to patient outcomes in patients with brain metastases.

3. PARTICIPANT SELECTION

Participants must meet the following criteria on screening examination to be eligible to participate in the study. Screening evaluations are to be conducted within 28 days prior to start of protocol therapy unless otherwise specified. Screening laboratory assessments must be done within 8 days prior to initiating protocol therapy.

3.1 Eligibility Criteria

3.1.1 Histologically confirmed metastatic breast cancer

3.1.2 Histologically confirmed HER-2 positive by ASCO CAP 2013 guidelines by local laboratory. Central confirmation of HER-2 status is not required.

- IHC 3+ based on circumferential membrane staining that is complete, intense

  -AND/OR-

- FISH positive based on one of the three following criteria:
  - Single-probe average HER2 copy number ≥6.0 signals/cell; **OR**
  - Dual-probe HER2/CEP17 ratio ≥2.0 **OR**
  - Dual-probe HER2/CEP17 ratio <2.0 with an average HER2 copy number≥6.0 signals/cell

3.1.3 At least one measurable CNS lesion, defined as ≥ 10 mm in at least one dimension

3.1.4 Unequivocal evidence of new and/or progressive brain metastases, and **at least one** of the following scenarios:

- Treated with SRS or surgery with residual un-treated lesions remaining. Such participants are eligible for immediate enrollment on this study providing that at least one untreated lesion is measurable
- Participants who have had prior WBRT and/or SRS and then whose lesions have subsequently progressed are also eligible. In this case, lesions which have been treated with SRS may be considered as target lesions if there is unequivocal evidence, in the opinion of the treating physician, of progression following SRS.
- Participants who have not previously been treated with cranial radiation (e.g., WBRT or SRS) are eligible to enter the study, but such participants must be asymptomatic from their CNS metastases and not requiring corticosteroids for symptom control.
- Both participants who present with systemic stable/absent or progressive disease are eligible to this trial, as long as they fulfill one of the above criteria.

3.1.5 Eastern Cooperative Oncology Group (ECOG) Performance Status of 0 or 1.

3.1.6 Left ventricular ejection fraction (LVEF) ≥ 50% by echocardiogram (ECHO) or multigated acquisition (MUGA) scan

3.1.7 Stable dose of dexamethasone 2mg or less for at least 7 days prior to initiation of treatment

3.1.8 Concurrent administration of other anti-cancer therapy during the course of this study is not allowed. Note that concurrent use of supportive care medications (e.g. anti-resorptive agents, pain medications) is allowed.

3.1.9 The subject is ≥18 years old.

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

- absolute neutrophil count ≥1,000/μl
- platelets ≥75,000/μl
- hemoglobin ≥ 9 g/dL
- total bilirubin ≤ 1.5mg/dL × institutional upper limit of normal except subject with documented Gilbert's syndrome (≤5 x ULN) or liver metastasis, who must have a baseline total bilirubin ≤3.0 mg/dL;
- AST(SGOT)/ALT(SGPT) ≤ 2.5 × institutional ULN OR ≤ 5.0 × institutional ULN for patients with documented liver metastases.
- Albumin >2.5mg/dL
- serum creatinine ≤ 1.5 × ULN (or glomerular filtration rate ≥ 60 ml/min as determined by the Cockcroft-Gault equation)

3.1.11 Female subjects of childbearing potential must have a negative serum or urine pregnancy test within 8 days of initiating protocol therapy. Childbearing potential is defined as pre-menopausal women with inteact uterus and ovaries.

3.1.12 Women of child-bearing potential and men must agree to use adequate contraception (barrier method of birth control; abstinence) prior to study entry, for the duration of study participation and 4 months after completion of atezolizumab administration.

3.1.13 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.1 Visceral crisis or impending visceral crisis at time of screening.

3.2.2 CNS complications for whom urgent neurosurgical intervention is indicated (e.g., resection, shunt placement).

3.2.3 Known leptomeningeal metastases [Defined as positive CSF cytology and/or unequivocal radiological evidence of clinically significant leptomeningeal involvement. CSF sampling is not required in the absence of suggestive symptoms to exclude leptomeningeal involvement].

3.2.4 Treatment with high dose systemic corticosteroids defined as dexamethasone > 2mg/day or bioequivalent within 7 days of treatment initiation

3.2.5 Patients unable to undergo gadolinium contrast-enhanced MRI or receive IV contrast for

any reason (e.g., due to pacemaker, ferromagnetic implants, claustrophobia, extreme obesity, hypersensitity).

3.2.6 Chemotherapy or targeted therapy within 14 days prior to planned treatment start

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 ipilimumab or any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways).

3.2.8 No washout is required for endocrine therapy. If a patient has been on endocrine therapy within 28 days of study entry, that same endocrine therapy is permitted to be continued during protocol therapy, at the investigator's discretion, as is continuation of ovarian suppression in premenopausal women. Starting a new endocrine therapy during protocol therapy is not permitted

3.2.9 Current use or history of receiving a non-approved, investigational treatment within 14 days prior to planned treatment start

3.2.10 Subjects with a history of hypersensitivity to compounds of similar biologic composition to atezolizumab or any constituent of the product

3.2.11 Uncontrolled intercurrent illness, including, but not limited to, ongoing or active infection, uncontrolled hypertension, unstable angina pectoris, uncontrolled cardiac arrhythmia, congestive heart failure-New York Heart Association Class III or IV, active ischemic heart disease, myocardial infarction within the previous six months, uncontrolled diabetes mellitus, chronic liver or renal disease, or severe malnutrition.

3.2.12 Pregnant women or women who are lactating/breastfeeding due to the teratogenic potential of the study drugs

3.2.13 Active, second potentially life-threatening cancer

3.2.14 Major surgery within 21 days of planned treatment start

3.2.15 Active infection requiring iv antibiotics at treatment initiation

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

3.2.17 Symptomatic intrinsic lung disease or extensive tumor involvement of the lungs, resulting in dyspnea at rest

3.2.18 Known human immunodeficiency virus (HIV), HepBsAg, or HCV RNA. HIV-positive participants on combination antiretroviral therapy are ineligible because of the unclear effects of immune checkpoint inhibitors in this setting.

3.2.19 Live vaccines within 28 days of first dose of trial therapy and during trial treatment.

3.2.20 Known intolerance to trastuzumab or pertuzumab or atezolizumab.

3.3 Inclusion of Women and Minorities

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

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

DF/HCC 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 must be taken off-study in the CTMS (OnCore) with an appropriate date and reason entered.

4.2 Registration Process for DF/HCC Institutions

Applicable DF/HCC Policy (REGIST-101) must be followed.

4.3 General Guidelines for Other Investigative Sites

Eligible participants will be entered on study centrally at the Dana-Farber Cancer Institute by the Project Manager. All sites should email or call the Project Manager to verify slot availabilities. The required forms in Section 4.4 should be emailed or faxed to the Project Manager.

Following registration, participants should begin protocol therapy within 7 days. Issues that would cause treatment delays should be discussed with the Overall PI. If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. The Project Manager should be notified of cancellations as soon as possible.

4.4 Registration Process for Other Investigative Sites

To register a participant, the following documents should be completed by the participating site and emailed to the Project Manager at [redacted]

- Clinic visit note including medical history, physical exam, ECOG-PS, and vital signs
- Copy of required laboratory tests including: Hematology (CBC with differential), Chemistry, TSH, and pregnancy test (if applicable)
- Pathology report and documentation of ER/PR status and HER2 status

- Tumor assessments by CT or MRI
- Brain MRI
- ECHO or MUGA report
- Signed participant consent form
- HIPAA authorization form (if separate from the main consent form)
- Completed DF/HCC Eligibility Checklist

To complete the registration process, the Project Manager will

- Follow the DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) and register the participant on the protocol
- email the research nurse or data manager at the participating site with the participant study number, and registration confirmation

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

5. TREATMENT PLAN

5.1 Treatment Regimen

Patients will receive the following treatment: atezolizumab [1200mg intravenously (IV) every 3 weeks (q3w)], pertuzumab (loading dose of 840 mg IV, followed q3w thereafter by a dose of 420 mg IV), and high-dose trastuzumab (at a dose of 6 mg/kg weekly for the first 24 weeks, and thereafter trastuzumab 6mg/Kg IV q3w). Treatments will be administered on an outpatient basis.

Regimen Description					
Agent	**Premedication**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Atezolizumab	Not routinely necessary unless prior infusion reaction.	1200 mg	IV over 60 minutes (+/- 15 mins)	Day 1 (q3w)	21 days (3 weeks)
Pertuzumab	Not routinely necessary unless prior infusion reaction.	Loading dose of 840 mg followed by standard dose of 420 mg*	IV over 60 mins with 60 min obs period	Day 1 (q3w)	
Trastuzumab	Not routinely necessary unless prior infusion reaction.	6 mg/Kg	IV over 30-90 minutes**	Weekly for the first 24 weeks followed by q3w	

Atezolizumab, pertuzumab, and trastuzumab may be administered in any order.

Weekly doses of trastuzumab may be administered +4 -2 days (no less than 5 days apart).

If the initial infusion of atezolizumab is well-tolerated, subsequent infusions may be delivered over 30 minutes (+/- 10 minutes).

If the initial infusion of pertuzumab is well-tolerated, subsequent infusions may be delivered over 30 minutes, per institutional guidelines.

*No loading dose is required in the case of an interval < 6 weeks between last dose of pertuzumab and the first administration of on-study pertuzumab.

**Trastuzumab-naïve patients should receive their first dose over 90 minutes. Patients who are actively receiving trastuzumab prior to enrollment will have a dose increase to 6 mg/kg IV weekly.

5.2 Pre-Treatment Criteria

Laboratory results must be reviewed prior to dosing. If screening assessments were completed within 8 calendar days of Cycle 1 Day 1, these assessments do not need to be repeated.

5.2.1 All cycles, Day 1

- Absolute Neutrophil Count ≥1,000/mcL
- Platelets ≥50,000/mcL
- AST(SGOT)/ALT(SGPT) ≤ 2.5 × institutional ULN or ≤ 5 × institutional ULN for participants with documented liver metastases
- Creatinine ≤ 1.5 mg/dL (or glomerular filtration rate >_ 50 ml/min as determined by the Cockcroft-Gault equation)

5.2.2 All cycles, Days 8 and 15

Because trastuzumab is not expected to lead to hematologic, renal, or hepatic toxicity, there are no laboratory criteria-to-treat for day 8 or day 15 trastuzumab

5.3 Agent Administration

5.3.1 Atezolizumab Administration

The initial dose of atezolizumab will be delivered over 60 (±15) minutes. If the first infusion is tolerated without infusion-associated AEs, the second infusion may be delivered over 30 (±10) minutes. If the 30-minute infusion is well tolerated, all subsequent infusions may be delivered over 30 (±10) minutes. Premedication may be administered for Cycles≥2 at the discretion of the treating physician.

The management of infusion-related reactions (IRRs) will be according to severity as follows:

- In the event that a patient experiences a mild (NCI CTCAE Grade 1) IRR, the infusion rate should be reduced to half the rate being given at the time of event onset. Once the event has

resolved, the investigator should wait for 30 minutes while delivering the infusion at the reduced rate. If tolerated, the infusion rate may then be increased to the original rate.

- In the event that a patient experiences a moderate IRR (NCI CTCAE Grade 2) or flushing, fever, or throat pain, the infusion should be immediately interrupted and the patient should receive aggressive symptomatic treatment. The infusion should be restarted only after the symptoms have adequately resolved to baseline grade. The infusion rate at restart should be half of the infusion rate that was in progress at the time of the onset of the IRR.
- For severe or life-threatening IRRs (NCI CTCAE Grade 3 or 4), the infusion should be stopped immediately, and aggressive resuscitation and supportive measures should be initiated. Patients experiencing severe or life-threatening IRRs will not receive further infusion and will be further managed as clinically indicated until the event resolves.

For anaphylaxis precautions, see APPENDIX B.

Administration of atezolizumab will be performed in a setting with emergency medical facilities and staff who are trained to monitor for and respond to medical emergencies.

5.3.2 Pertuzumab Administration

The loading dose of pertuzumab will be 840 mg administered as a 60-minute IV infusion, followed every 3 weeks thereafter by a dose of 420 mg administered over a period of 30 minutes. For patients already receiving pertuzumab as part of their ongoing systemic therapy, no loading dose is required in the case of an interval < 6 weeks between last dose of pertuzumab and the first administration of on-study pertuzumab. An observation period of 30 to 60 minutes is recommended after each pertuzumab infusion, according to each participating site's institutional guidelines.

5.3.3 Trastuzumab Administration

High-dose trastuzumab will be administered at a dose of 6 mg/kg weekly, infused intravenously over 30 -90 minutes. No loading dose is required. Trastuzumab-naïve patients should receive their first dose over 90 minutes. Patients who are actively receiving trastuzumab prior to enrollment will have a dose increase to 6 mg/kg IV weekly. The dose of trastuzumab should be based on institutional guidelines. Weekly doses may be administered +4 -2 days (no less than 5 days apart).

5.4 Definition of Dose-Limiting Toxicity

The first six participants will be assessed for dose-limiting toxicities (DLTs). After the first six patients are enrolled, accrual must pause until the DLT window passes. If there are ≥ 2 DLTs in these patients, the regimen will be declared unsafe for further study. If there are ≤ 1 DLTs observed, the study may continue with enrollment.

Dose-limiting toxicity is defined as any of the following events occurring within 21 days of Cycle 1 Day 1 treatment, if judged by the investigator to be possibly, probably, or definitely

related to study drug:

1) Death
2) ≥ Grade 3 treatment-emergent neurological toxicity
3) Asymptomatic grade 4 neutropenia or thrombocytopenia lasting ≥7 days
4) Grade 4 thrombocytopenia of any duration
5) ≥ Grade 3 Febrile neutropenia
6) ≥ Grade 3 Thrombocytopenia if associated with bleeding
7) ≥ Grade 3 elevation in AST or ALT associated with a grade 2 elevation in bilirubin that is at least possibly related to study drug (Hy's Law)
8) ≥ Grade 3 non-hematologic laboratory value if:
    a. Medical intervention is required to treat the patient, or
    b. The abnormality leads to hospitalization, or
    c. The abnormality persists >7 days
    d. Excluding:
        i. Alkaline phosphatase ≤10.0x ULN in a patient with grade 2 alkaline phosphatase elevation at baseline as a result of bone metastasis
9) ≥ Grade 3 pneumonitis of any duration
10) ≥ Grade 3 Fatigue lasting >5 days
11) New York Heart Association (NYHA) class III and IV cardiac heart failure (Appendix G)
12) An asymptomatic decline in LVEF to a value 10 percentage points below baseline or lower, and < 45%
13) ≥ Grade 3 other non-laboratory toxicity lasting >3 days despite optimal supportive care, excluding Alopecia (of any grade).

5.5 General Concomitant Medication and Supportive Care Guidelines

5.5.1 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.

5.5.2 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
- Radiation therapy
- Any systemically active oral, injected, or implanted hormonal method of contraception except for progesterone coated intrauterine devices (IUDs) that had been previously implanted.
- Estrogen replacement therapy.

- Live vaccines within 28 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids should be avoided for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology, or emergent symptoms from brain metastasis. If corticosteroids are required for this purpose, the minimum effective dose should be used.
- Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy may be required. The investigator should discuss any questions regarding this with the overall PI.

5.5.3 Supportive Care Guidelines

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.
- Anticoagulants - Anticoagulation with heparin, heparin derivatives, and/or warfarin may be given at the discretion of the treating physician. Coagulation parameters should be checked at least once monthly, or more frequently at discretion of treating physician.
- Pain medications administered per standard clinical practice are acceptable while the patient is enrolled in the study.
- Hematopoietic growth factors (e.g., granulocyte-colony stimulating factor [G-CSF], granulocyte macrophage colony stimulating factor) may be used at investigator's discretion for the management of treatment-emergent neutropenia and/or for secondary prophylaxis per NCCN guidelines or local standard practice

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

5.6 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:

- Disease progression by RANO-BM and/or RECIST 1.1 (see note below)
- Disease progression by iRANO and/or irRC criteria.
  NOTE: Please note that although the primary endpoint is Overall Response Rate in the CNS according to response assessment in neuro-oncology-brain metastases (RANO-BM) criteria, patients may remain on protocol therapy until the time of disease progression by iRANO and/or irRC criteria. The immune criteria allow treatment beyond initial radiographic worsening of disease in order to distinguish between pseudoprogression and true disease progression. For treatment beyond radiographic progression the following criteria must be met:
    - Absence of signs or symptoms of clinically significant progression;
    - No decline in ECOG performance status;
    - Absence of symptomatic rapid disease progression requiring urgent medical intervention.
- 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
- Physician Discretion. 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, Nancy Lin, MD at 617-632-2335 or nlin@partners.org.

Participants may elect to stop atezolizumab with CR after at least 24 weeks of treatment and having had at least two treatments with atezolizumab after documentation of the CR.

Subjects who stop atezolizumab with CR may be eligible for additional atezolizumab 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 atezolizumab after attaining a confirmed CR by central radiology review (TIMC) according to RECIST 1.1, was treated for at least 24 weeks with atezolizumab before discontinuing therapy, and received at least two treatments with atezolizumab beyond the date when the initial CR was declared

OR

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

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

5.7 Duration of Follow Up

An Off-Treatment visit should occur within 30 days of the last dose of study treatment.

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

Participants who are taken off protocol therapy for extracranial progression in the setting of intracranial response or stable disease will be followed for CNS progression and survival after removal from protocol therapy. It is understood that it may not always be feasible for patients to return for restaging evaluation after coming off protocol therapy, though a strong effort should be made to encourage restaging every 6-12 weeks. In this specific setting, lack of restaging scans at the interval will not constitute a protocol deviation or violation. If patients cannot return in person, local medical records, or a phone call to the participants' local provider will be requested in order to provide additional follow up information.

Participants who are removed from protocol therapy will be followed for survival every 6 months or until death. This can be a visit to the clinic, chart review/receipt of local medical records, or a phone call to the participants' local provider.

5.8 Criteria for Taking a Participant Off Study

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

- Lost to follow-up
- Withdrawal of consent
- Death
- Study closure

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.

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 are required to resume therapy within 3 weeks of the scheduled interruption. The reason for interruption must be documented in the participant's medical record.

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

No dose reductions are allowed for atezolizumab, pertuzumab or trastuzumab in this study. If any of the three drugs needs to be permanently discontinued, patients can remain on the others at descrition of their physician.

6.1 Management of toxicities attributable to atezolizumab

6.1.1 Guidelines for Dosage Modification and Treatment Interruption or Discontinuation

There will be no dose reduction for atezolizumab in this study.

Atezolizumab treatment may be temporarily suspended in patients experiencing toxicity considered to be related to study treatment. If corticosteroids are initiated for treatment of the toxicity, they must be tapered over ≥1 month to ≤ 10 mg/day oral prednisone or equivalent before atezolizumab can be resumed. If atezolizumab is withheld for >105 days, the patient will be discontinued from atezolizumab. However, atezolizumab may be withheld for >105 days to allow for patients to taper off corticosteroids prior to resuming treatment. Atezolizumab can be resumed after being withheld for >105 days if PI agrees that the patient is likely to derive clinical benefit. Atezolizumab treatment may be suspended for reasons other than toxicity (e.g., surgical procedures). The investigator will determine the acceptable length of treatment interruption.
Patients should be assessed clinically for toxicity prior to, during, and after each infusion. Any toxicity associated or possibly associated with atezolizumab treatment should be managed according to standard medical practice. Additional tests, such as autoimmune serology or biopsies, may be used to determine a possible immunogenic etiology.

Although most irAEs observed with immunomodulatory agents have been mild and self-limiting, such events should be recognized early and treated promptly to avoid potential major complications.

Discontinuation of atezolizumab may not have an immediate therapeutic effect, and there is no available antidote for atezolizumab. In severe cases, immune-related toxicities may be acutely managed with topical corticosteroids, systemic corticosteroids, mycophenolate, or TNFα inhibitors.

In general, the primary approach to Grade 1 to 2 irAEs is supportive and symptomatic care with continued treatment with atezolizumab; for higher-grade irAEs, atezolizumab should be withheld and oral and/or parenteral steroids administered. Recurrent Grade 2 irAEs may also mandate withholding atezolizumab or the use of steroids. Assessment of the risk-benefit ratio should be

made by the investigator, with consideration of the totality of information as it pertains to the nature of the toxicity and the degree of clinical benefit a given patient may be experiencing prior to further administration of atezolizumab. Atezolizumab should be permanently discontinued in patients with life-threatening irAEs.

Management of diarrhea/colitis, hepatitis/transaminitis, rash, pulmonary, and endocrine adverse events are presented in this section as they have been observed in this study and are potentially immune related.

Dose interruptions for reasons other than toxicity, such as planned surgical procedures, may be allowed, with prior PI approval. The interruption should not be greater than 84 days.

6.1.1.1 Systemic immune activation

Systemic immune activation is a rare condition characterized by an excessive immune response. Given the mechanism of action of atezolizumab, systemic immune activation is considered a potential risk when given in combination with other immunomodulating agents.

Recommendations regarding early identification and management of systemic immune activation are provided below. In the event of suspected systemic immune activation, atezolizumab should be withheld and the PI should be contacted immediately for additional guidance.

Refer to the current version of the Atezolizumab Investigator Brochure for management of immune-related events, including infusion-related reactions.

Early disease recognition is critical, and systemic immune activation should be suspected if, in the absence of an alternative etiology, the patient meets two or more of the following criteria:

- Hypotension that is refractory to aggressive IV fluid challenge
    - Vasopressor support may be required.
- Respiratory distress that requires aggressive supportive care
    - Supplemental oxygen and intubation may be required.
- Fever > 38.5°C
- Acute renal or hepatic failure
- Bleeding from coagulopathy
- Any of the following unexplained laboratory abnormalities (change from baseline): cytopenias (in two or more lineages), significant transaminitis, and coagulopathy

For patients with suspected systemic immune activation, an initial evaluation should include the following:

- CBC with peripheral smear
- PT, PTT, fibrinogen, and D-dimer
- Ferritin

- Triglycerides
- AST, ALT, and total bilirubin
- LDH
- Complete neurologic and abdominal examination (assess for hepatosplenomegaly)

Laboratory tests with normal results should be repeated frequently in patients for whom a high clinical suspicion of systemic immune activation exists.

If cytopenias are present (Grade ≥ 2 in two or more lineages) or ferritin is ≥ 3000 ng/mL, the following evaluations should also be performed:

- Bone marrow biopsy and aspirate (assess for evidence of hemophagocytosis)
- Soluble interleukin 2 (IL-2) receptor (sCD25)
- Natural killer cell activity

Cytomegalovirus, Epstein-Barr virus, and herpes-simplex virus evaluation (for reactivated or active disease).

<table>
<tr><th colspan="3">Systemic Immune Activation Diagnostic Criteria
(applicable only when alternative etiologies have been excluded)</th></tr>
<tr><th colspan="2">Major Criteria</th><th>Minor Criteria</th></tr>
<tr><td colspan="2">• Fever ≥ 38.5°C on more than one occasion
• Ferritin ≥ 3000 ng/mL
• Cytopenias (Grade ≥ 2 in two or more lineages)
• Age-adjusted soluble IL-2 receptor elevated by ≥ 2 standard deviations
• Severe dysfunction in two or more organs
• Decreased fibrinogen</td><td>• Splenomegaly
• Hemophagocytosis in bone marrow, spleen, or lymph nodes
• Elevated GGT or LFTs (AST, ALT, or total bilirubin)
• Elevated triglycerides
• Elevated LDH
• Decreased natural killer cell activity</td></tr>
<tr><th colspan="3">Diagnosis and Management of Systemic Immune Activation</th></tr>
<tr><th>Number of Criteria</th><th>Diagnosis</th><th>Action to Be Taken</th></tr>
<tr><td>≥ 4 major criteria</td><td>Consistent with systemic immune activation</td><td>• Withhold pertuzumab and trastuzumab.
• Permanently discontinue atezolizumab.
• Consider treatment with an immunosuppressive agent (i.e., tocilizumab, infliximab, cyclosporine A, or etoposide) and IV corticosteroids (i.e., methylprednisolone 1 g once daily or equivalent).
• Consider HLH-94 protocol if there is no clinical improvement.
• If event resolves within 12 weeks from last dose of atezolizumab, and if the subject was unequivocally deriving clinical benefit, the subject may be able to resume trastuzumab and pertuzumab as determined by the investigator</td></tr>
<tr><td>3 major criteria
OR
2 major plus
≥ 3 minor criteria</td><td>Probable systemic immune activation</td><td>• Withhold pertuzumab and trastuzumab.
• Depending on clinical severity, follow guidelines for “Consistent with systemic immune activation” or “Possible systemic immune activation” diagnosis.
• If event resolves within 12 weeks from last dose of atezolizumab, and if the subject was unequivocally deriving clinical benefit, the subject may be able to resume trastuzumab and pertuzumab as determined by the investigator</td></tr>
<tr><td>2 major plus
≤ 2 minor criteria
OR
1 major plus
≥ 4 minor criteria</td><td>Possible systemic immune activation</td><td>• Withhold pertuzumab, trastuzumab and atezolizumab.
• Consider treatment with IV corticosteroids.
• Follow guidelines for “Consistent with systemic immune activation” diagnosis if there is no clinical improvement or if clinical worsening occurs.
• If clinical improvement occurs, atezolizumab may be resumed following a benefit-risk assessment.
• If event resolves within 12 weeks from last dose of atezolizumab, and if the subject was unequivocally deriving clinical benefit, the subject may be able to resume trastuzumab and pertuzumab as determined by the investigator</td></tr>
</table>

GGT = γ-glutamyl transpeptidase; IL-2 = interleukin-2; IV = intravenous; LFT = liver function test.

Notes: Criteria are adapted from a Delphi Survey of 26 experts who provided helpful criteria in the positive diagnosis of hemophagocytic syndrome in adult patients (Hejblum et al. 2014).

Case reports and recommendations have been published for cytokine-release syndrome (Teachey et al. 2013; Lee et al. 2014; Maude et al. 2014), and, on the basis of etiologic similarities, these practices have been incorporated into the

Systemic Immune Activation Diagnostic Criteria (applicable only when alternative etiologies have been excluded)

above treatment recommendations.

These recommendations do not replace clinical judgment and are intended as suggested guidance.

An adverse event of systemic immune activation should be reported in the patient's medical records if it meets the criteria for "consistent with systemic immune activation" or "probable systemic immune activation" as outlined above.

6.1.1.2 Gastrointestinal Toxicity

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 atezolizumab and pertuzumab and 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 atezolizumab.

Participants experiencing intolerable Grade 2 diarrhea or Grade 3 diarrhea unable to be managed with standard antidiarrheal treatments should consult a gastrointestinal (GI) doctor for a potential endoscopy and biopsy to help distinguish between pertuzumab vs. atezolizumab mediated toxicity.

- If a GI biopsy is performed, and showed T cell infiltration indicative of atezolizumab-induced colitis, atezolizumab should be permanently discontinued. Once diarrhea returns to Grade 1, restart pertuzumab and trastuzumab.
- If a GI biopsy is performed, and showed no T cell infiltration indicative of atezolizumab-induced colitis, pertuzumab should be discontinued, and atezolizumab and trastuzumab should be resumed at the same dose once diarrhea returns to Grade 1.

Table 2 Dose Modification Guidelines for Gastrointestinal Toxicity

Toxicity	Description	Management
Diarrhea and/or Colitis	Grade 1	• Continue atezolizumab, trastuzumab and pertuzumab. • Initiate symptomatic treatment • Endoscopy is recommended if symptoms persist for > 7 days • Close monitoring.
	Grade 2	• Hold atezolizumab, and pertuzumab. • Initiate symptomatic treatment • Gastroenterology consultation recommended • For recurrent events or events that persist > 5 days: o Consider referral to gastroenterologist o Consider oral budesonide, mesalamine or 10 mg of prednisone equivalent per day o If co-existing abdominal pain, all of the above plus rule out bowel perforation and consider prednisone 60 mg per day or equivalent

Toxicity	Description	Management
		• When symptoms improve Grade ≤1, resume atezolizumab and pertuzumab o Corticosteroids must be tapered over ≥1 month to <10 mg/day oral prednisone or equivalent before resuming • Atezolizumab and pertuzumab may be resumed if the event improves to Grade ≤ 1 within 12 weeks from last dose and corticosteroids have been reduced to the equivalent of prednisone ≤ 10 mg/day. o Atezolizumab may be withheld for a longer period of time (i.e., > 12 weeks after event onset) to allow for corticosteroids (if initiated) to be reduced to ≤10 mg/day oral prednisone or equivalent. The acceptable length of the extended period of time must be determined by the PI
	Grade 3	• Hold atezolizumab and pertuzumab. – Treat with loperamide up to maximum of 8 mg daily. Other antimotilty agents may be added or substituted as clinically indicated. – If diarrhea improves to grade 2 or less within 7 days, then atezolizumab and pertuzumab may be restarted once the diarrhea resolves to </= grade 1. – If grade 3 persists > 1 week despite maximum supportive care measures, or if grade 3 lasting > 7 days recurs on re-challenge, then 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. In addition, patients should be referred for gastroenterology for consideration of biopsy to rule out atezolizumab-mediated colitis. ➢ If GI biopsy shows T cell infiltration indicative of atezolizumab-induced colitis, atezolizumab should be permanently discontinued. Once diarrhea returns to Grade 1, restart pertuzumab. ➢ If GI biopsy shows no T cell infiltration indicative of atezolizumab-induced colitis, pertuzumab should be discontinued, and atezolizumab may be resumed if the event improves to Grade ≤ 1 within 12 weeks and corticosteroids have been reduced to the equivalent of prednisone ≤ 10 mg/day. – If event does not resolve to </= grade 1 within 12 weeks of holding, permanently discontinue both atezolizumab and pertuzumab – If the subject was unequivocally deriving clinical benefit, and event resolves to </= grade 1 after more the 12 weeks, the subject may be rechallenged with atezolizumab and/or pertuzumab as determined by the PI, but the approval must be documented in writing.
	Grade 4	• Hold atezolizumab and pertuzumab and inform PI. – Treat with loperamide up to maximum of 8 mg daily. Other antimotility agents may be added or substituted as clinically indicated. • 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. • Gasteroenterology referral and confirmation biopsy. ➢ If GI biopsy shows T cell infiltration indicative of atezolizumab-induced

Toxicity	Description	Management
		colitis, atezolizumab should be permanently discontinued. Once diarrhea returns to </=Grade 1, restart pertuzumab. ➢ If GI biopsy shows no T cell infiltration indicative of atezolizumab-induced colitis, pertuzumab should be permanently discontinued. If diarrhea improved to grade 1 or better within 12 weeks from last dose of atezolizumab, ***and*** if the subject was unequivocally deriving clinical benefit, ***and*** if the GI biopsy showed no indication of T cell infiltration indicative of atezolizumab-induced colitis, the subject may be able to resume Atezolizumab as determined by the PI, but the approval must be documented in writing.

IV = intravenous; TNF-α = tumor necrosis factor alph

6.1.1.3 Hepatotoxicity

Immune-mediated hepatitis has been associated with the administration of atezolizumab.

While in this study, patients presenting with right upper-quadrant abdominal pain and/or unexplained nausea or vomiting should have LFTs performed immediately, and LFTs should be reviewed before administration of the next dose of study drug.

If LFTs increase, neoplastic, concurrent medications, viral hepatitis, and toxic etiologies should be considered and addressed, as appropriate. Imaging of the liver, gall bladder, and biliary tree should be performed to rule out neoplastic or other causes of increased LFTs. Anti-nuclear antibody, perinuclear anti-neutrophil cytoplasmic antibody, anti-liver kidney microsomal, and anti-smooth muscle antibody tests should be performed if an autoimmune etiology is considered.

Patients with LFT abnormalities should be managed according to the guidelines in Table 3. For patients with elevated LFTs at baseline (between 3-5-fold ULN) due to documented liver metastases, further elevation of LFTs may not require dose interruptions if the ALT and/or AST have risen ≤ 3-fold over baseline and if the total bilirubin elevation remains ≤ 2-fold over normal with normal PT/INR. Such cases should be also discussed with the principal investigator.

Table 3 Dose Modification Guidelines for Hepatotoxicity

Toxicity	Description	Management
LFT and/or total bilirubin abnormalities, deemed related to protocol therapy (i.e. not related to underlying liver metastases)	Grade 1	• Continue study therapy and monitor labs according to study calendar
	Grade 2	• Continue study therapy and monitor LFTs more frequently until return to baseline or Grade ≤1 • If persistent > 5 days: • hold atezolizumab; start prednisone 60 mg/day or equivalent. When LFTs improve to Grade ≤1 taper steroids over ≥1 month • Resume atezolizumab, if event resolves to Grade 1, baseline, or better within 12 weeks from the last dose of atezolizumab. • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab and contact PI

Toxicity	Description	Management
		• if the subject was unequivocally deriving clinical benefit, the subject may be able to resume Atezolizumab as determined by the PI. On second occurrence: ▪ Manage as grade 3 and 4
	Grade 3 or 4 deemed related to protocol therapy (i.e. not related to underlying liver metastases)	• Permanently discontinue atezolizumab, start prednisone 60 mg/day or equivalent and inform the PI o If the subject was unequivocally deriving clinical benefit, the subject may be able to resume Atezolizumab as determined by the PI. • 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 • Consider obtaining a hepatology consult and liver biopsy. • When LFTs improve to Grade ≤1 taper steroids over ≥1 month

IV = intravenous; LFT = liver function test; TNFα = tumor necrosis factor alpha; ULN = upper limit of normal.

6.1.1.4 Dermatologic Toxicity

Treatment-emergent rash has been associated with atezolizumab. The majority of cases of rash were mild in severity, self-limited, both with and without pruritus. 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. A dermatologist should evaluate persistent and/or severe rash or pruritus and consider biopsying the site.

Dermatologic toxicity and rash should be managed according to the guidelines in Table 4

Table 4 Dose Modification Guidelines for Dermatologic Toxicity

Toxicity	Description	Management
Dermatologic toxicity/rash (e.g., maculopapular or purpura)	Grade 1: Mild <10% BSA	• Continue study therapy. • Consider topical steroids and/or other symptomatic therapy (e.g., antihistamines).
	Grade 2: Moderate 10%–30% BSA	• Continue study therapy. • Administer topical steroids and consider higher potency topical steroids if event does not improve • Consider dermatologist referral.

Toxicity	Description	Management

	Grade 3: Severe >30% BSA	• Hold atezolizumab and pertuzumab, and administer oral prednisone 10 mg or equivalent.
		  • If the rash is not improved after 48–72 hours, increase dose of prednisone to 60 mg or equivalent.
		• Refer for dermatology consult
		• Restart atezolizumab and pertuzumab if rash is resolved to grade 1 or better and systemic dose is ≤ 10 mg oral prednisone equivalent per day (taper over 1 month).
		• If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab and contact PI
		  • if the subject was unequivocally deriving clinical benefit, the subject may be able to resume Atezolizumab as determined by the PI.

	Grade 4	• Permanently discontinue atezolizumab and inform the PI • If the subject was unequivocally deriving clinical benefit, the subject may be able to resume Atezolizumab as determined by the PI

BSA = body surface area; PRN = as needed.

6.1.1.5 Endocrine Toxicity

Hypothyroidism has been associated with the administration of atezolizumab.

Patients with unexplained symptoms such as fatigue, myalgias, impotence, mental status changes, or constipation should be investigated for the presence of hyponatremia, hypokaelmia and thyroid, pituitary and adrenal endocrinopathies.An endocrinologist should be consulted if an endocrinopathy is suspected.

Table 5 Dose Modification Guidelines for Endocrine Toxicity

Toxicity	Management
Asymptomatic Hypothyroidism	• Continue atezolizumab • Start treatment with thyroid replacement hormone • Monitor thyroid-stimulating hormone (TSH) weekly
Symptomatic Hypothyroidism	• Hold atezolizumab • Start thyroid replacement hormone • Monitor TSH weekly • Consider referral to an endocrinologist • Restart atezolizumab when symptoms are controlled and thyroid function is improving
Asymptomatic Hyperthyroidism	• If serum TSH < 0.5 mU/L and > 0.1 mU/L, continue Atezolizumab and monitor TSH every 4 weeks • If TSH < 0.1 mU/L, follow guidelines for symptomatic hyperthyroidism

Toxicity	Management
Symptomatic Hyperthyroidism	• Hold atezolizumab • initiate treatment with anti-thyroid drug such as methimazole as needed • Consider referral to an endocrinologist • If symptoms are controlled and thyroid function is improving, resume atezolizumab • Permanently discontinue atezolizumab for life-threatening immune-related hyperthyroidism. Inform PI.
hyperglycemia, grade 1-2	• Continue atezolizumab. • Initiate treatment if clinically indicated • Monitor for glucose control.
hyperglycemia, grade 3-4	• Hold Atezolizumab • Initiate treatment for hyperglycemia • Monitor for glucose control. • Resume atezolizumab when symptoms resolve and glucose levels are stable.
Symptomatic panhypopituitarism and any other Grade 3-4 endocrine events	• Permanently discontinue atezolizumab and treat with an initial dose of methylprednisolone 1 to 2 mg/kg per day intravenously followed by oral prednisone 1 to 2 mg/kg per day upon improvement. Inform PI • When symptoms improve to Grade ≤ 1, start steriod taper and taper over ≥ 1 month, resume atezolizumab • Consult an endocrinologist • Perform appropriate imaging • Initiate hormonre replacement therapy, if clinically indicated • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab and contact PI • if the subject was unequivocally deriving clinical benefit, the subject may be able to resume Atezolizumab as determined by the PI.

6.1.1.6 Pulmonary Toxicity

Cases of interstitial lung disease (ILD), including pneumonitis, some leading to acute respiratory distress syndrome or death, have been reported in patients receiving atezolizumab. Signs and symptoms may include dyspnea, cough, fatigue, and pulmonary infiltrates. Patients with dyspnea at rest due to complications of advanced malignancy and comorbidities may be at risk of pulmonary events.

Patients with clinically significant pulmonary symptoms will be excluded from this study (see Section 3.2).

Recommended management for pulmonary events are listed in table 6 and may include the following exams:

- 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}])

Table 6 Dose Modification Guidelines for Pulmonary Toxicity (Pneumonitis)

Toxicity	Description	Management
Pulmonary toxicity (Pneumonitis)	Grade 1	• Continue study therapy with close monitoring • Re-evaluate on serial imaging • Consider pulmonary consultation • For recurrent pneumonitis, treat as a Grade 3 or 4 event
	Grade 2	• Continue trastuzumab and pertuzumab. • Hold atezolizumab and start prednisone 60 mg/day or equivalent • Consult pulmonary and infectious disease specialists with consideration for bronchoscopy/BAL • When symptoms improve to Grade ≤ 1, taper steroids over ≥ 1 month to < 10 mg/day • First occurrence: o Atezolizumab may be resumed if the event improves to Grade ≤ 1 within 12 weeks from last dose of atezolizumab and if corticosteroids have been reduced to the equivalent of oral prednisone ≤ 10 mg/day. o Permanently discontinue atezolizumab if event does not resolve to Grade 1 or better within 12 weeks from last dose of Atezolizumab and contact the PI. If the subject was unequivocally deriving clinical benefit, the subject may be able to resume as determined by the PI • For recurrent events, o Treat as Grade 3−4 (see below)
	Grade 3-4	• Hold trastuzumab and pertuzumab. • Permanently discontinue atezolizumab start prednisone 60 mg/day or equivalent and notify PI • If symptoms are not improving after 48 hours or is worsening, add additional alternative immunosuppression (e.g., infliximab, cyclophosphamide, IVIG, or mycophenolate mofetil) to the corticosteroid regimen • Consult pulmonary and infectious diseases as bronchoscopy/BAL is recommended • When symptoms improve to Grade ≤ 1, taper steroids over ≥ 1 month • If event resolves to Grade 1 or better within 12 weeks from last dose of atezolizumab, and if the subject was unequivocally deriving clinical benefit, the subject may be able to resume as determined by the PI

6.1.1.7 Pancreatic Toxicity

Symptoms of abdominal pain associated with elevations of amylase and lipase, suggestive of pancreatitis, have been associated with administration of other immunomodulatory agents. The differential diagnosis of acute abdominal pain should include pancreatitis. Appropriate workup should include an evaluation for obstruction, as well as serum amylase and lipase tests.

Table 7 Pancreatic Toxicity

Amylase and/or lipase elevation, Grade 2	• Continue atezolizumab. • Monitor amylase and lipase weekly • For prolonged elevation (> 3 weeks) consider treatment with 10 mg/day oral prednisone or equivalent
Amylase and/or lipase elevation, Grade 3 or 4	• Withhold atezolizumab. • Refer to gastrointestinal specialist • Monitor amylase and lipase every other day • If no improvement, consider treatment with 1-2 mg/kg/day oral prednisone or equivalent • Resume atezolizumab if event resolves to Grade 1 or better within 12 weeks after event onset • Permanently discontinue atezolizumab if event does not resolve to Grade 1 or better within 12 weeks and contact PI. o if the subject was unequivocally deriving clinical benefit, the subject may be able to resume as determined by the PI • For recurrent events, permanently discontinue Atezolizumab and contact PI.
Immune-related pancreatitis, Grade 2 or 3	• Withhold atezolizumab • Refer to gastrointestinal specialist • Initiate treatment with 1-2 mg/day IV methylprednisolone or equivalent and convert to 1-2 mg/kg/day oral prednisone or equivalent • Resume atezolizumab if event resolves to Grade 1 or better within 12 weeks of event onset • Permanently discontinue atezolizumab if event does not resolve to Grade 1 or better within 12 weeks of event onset o if the subject was unequivocally deriving clinical benefit, the subject may be able to resume as determined by the PI • For recurrent events, permanently discontinue Atezolizumab and contact PI.
Immune-related pancreatitis, Grade 4	• Permanently discontinue Atezolizumab and contact PI. • Refer to gastrointestinal specialist • Initiate treatment with 1-2 mg/day IV methylprednisolone or equivalent and convert to 1-2 mg/kg/day oral prednisone or equivalent • If event does not improve within 48 hours of initiating corticosteroids, consider adding an immunosuppressive agent • Resume atezolizumab if event resolves to Grade 1 or better within 12 weeks of event onset, taper corticosteroids over $\geq$ 1 month • Permanently discontinue atezolizumab if event does not resolve to Grade 1 or better within 12 weeks of event onset if the subject was unequivocally deriving clinical benefit, the subject may be able to resume as determined by the PI

6.1.1.8 Eye Toxicity

An ophthalmologist should evaluate visual complaints. Uveitis or episcleritis may be treated with topical corticosteroid eye drops. Atezolizumab should be permanently discontinued for immune-mediated ocular disease that is unresponsive to local immunosuppressive therapy.

Ocular toxicity should be managed according to the guidelines in Table 8 and the Atezolizumab Investigator.

Table 8 Dose Modification Guidelines for Eye Toxicity

Toxicity	Description	Management
Eye toxicity (autoimmune uveitis, iritis, or episcleritis)	Symptomatic	• Hold atezolizumab. • Consult ophthalmologist and start topical corticosteroid eye drops. • Atezolizumab may be restarted following resolution of the events. • Permanently discontinue Atezolizumab for immune-mediated ocular disease that is unresponsive to local immunosuppressive therapy.
	Grade 1	• Continue atezolizumab, pertuzumab and trastuzumab. • Referral to ophthalmologist strongly recommended • Initiate treatment with topical corticosteroid eye drops/ topical immunosuppressive therapy • If symptoms persist, treat as a Grade 2 event.
	Grade 2	• Withhold atezolizumab for up to 12 weeks • Referral to ophthalmologist strongly recommended • Initiate treatment with topical corticosteroid eye drops/ topical immunosuppressive therapy • Resume atezolizumab if event resolves to Grade 1 or better within 12 weeks from last dose of atezolizumab • Permanently discontinue atezolizumab if event does not resolve to Grade 1 or better within from 12 weeks from last dose of atezolizumab. o If the subject was unequivocally deriving clinical benefit, the subject may be able to resume Atezolizumab as determined by the PI.
	Grade 3-4	• Permanently discontinue Atezolizumab and contact PI

Toxicity	Description	Management
		○ If the subject was unequivocally deriving clinical benefit, the subject may be able to resume Atezolizumab as determined by the PI.Refer to ophthalmologist • Initiate treatment with 1-2 mg/kg/day oral prednisone or equivalent • If event resolves to grade 1 or better, taper corticosteroids over ≥ 1 month.

6.1.1.9 Left Ventricular Dysfunction

Patients treated with atezolizumab are at risk of developing left ventricular dysfunction. To date, significant cardiac events, including left ventricular ejection fraction (LVEF) of <40%, have been observed infrequently in clinical trials of atezolizumab.

Patients must meet specified LVEF requirements to be included in this study (see Section 3.1).

Left ventricular function will be monitored by measurement of ejection fraction using echocardiogram (ECHO) or multiple-gated acquisition (MUGA) scans as described in Section 6.2.1.

Guidelines for management of patients who develop left ventricular dysfunction are provided in Apendix H.

6.1.1.10 Neurologic disorders

Neurologic toxicity/disorder should be managed according to the guidelines in Table 9.

Table 9 Dose Modification Guidelines for Neurologic Disorders

Neurologic disorders	
Immune-related neuropathy, Grade 2	• Withhold atezolizumab. • Resume atezolizumab if event resolves to Grade 1 or better within 12 weeks from last dose of atezolizumab • Permanently discontinue atezolizumab if event does not resolve to Grade 1 or better within from 12 weeks from last dose of atezolizumab.
Immune-related neuropathy, Grade 3 or 4	• Permanently discontinue atezolizumab.
Myasthenia gravis and Guillain-Barré, all grades	• Permanently discontinue atezolizumab.
Immune-related meningoencephalitis, all grades	• Permanently discontinue atezolizumab.

6.2 Management of toxicities attributable to pertuzumab and/or trastuzumab

Administration of trastuzumab may be delayed to assess or treat adverse events, such as changes in LVEF, as shown in Appendix J. Since pertuzumab is also associated with a risk for cardiac dysfunction, the management of cardiac safety for patients receiving both drugs in the study, as outlined in the next section, applies to both drugs.

1. Diarrhea/Colitis	Refer to section 6.1.1.2
2. Hepatotoxicity	Refer to section 6.1.1.3
3. Dermatology toxicity	Refer to section 6.1.1.4
4. Non-hematological, Grade 1 or 2 (NCI CTCAE v4.0) adverse evets, excluding cardiac[a], diarrhea/colitis, hepatotoxicity, and dermatology toxicity	Continue study treatment.
5. Non-hematological, deemed related grade 3 or 4 (NCI CTCAE v4.0) adverse events, excluding cardiac[a], diarrhea/colitis, hepatotoxicity, and dermatology toxicity	Hold study treatment (all medications in the cycle) until recovery to Grade ≤2. Toxicity resolved to Grade ≤1 within a maximum of 3 weeks calculated from **last** administration: resume study treatment. Toxicity did **not** resolve to Grade ≤2 within a maximum of 3 weeks calculated from last administration: discontinue the related study medication (pertuzumab or trastuzumab) permanently. Continue treatment as deemed suitable by the local investigator.
6. Recurrence of non-hematological, Grade 3 or 4 (NCI CTCAE v4.0; excluding cardiac[a], diarrhea/colitis, hepatotoxicity, and dermatology toxicity) toxicity upon rechallenge	Discontinue the related study medication (pertuzumab or trastuzumab) permanently. Continue treatment as deemed suitable by the local investigator.
7. Cardiac toxicity (asymptomatic drop in LVEF or symptomatic CHF)	Study treatment (all medication in the cycle) to be held, continued, or resumed according to the algorithm in Appendix F Related study medication (pertuzumab or trastuzumab) to be discontinued permanently in case of symptomatic CHF *(refer to Management of Symptomatic Cardiac Changes)*.
8. Cardiac toxicity (NCI CTCAE or other cardiac toxicities not covered by the treatment algorithm in Appendix F	Actions must follow rules 4 to 6 for non-hematological toxicities.
9. Hematological toxicity – neutropenia	Hold study treatment (all medication in the cycle) until neutrophils ≥1000/mcL

CHF = congestive heart failure; LVEF = left ventricular ejection fraction; NCI CTCAE = National Cancer Institute Common Terminology Criteria for Adverse Events; NYHA = New York Heart Association.
a Severity corresponding to NYHA classification (see Appendix G).

6.2.1 Management of Cardiac Toxicity

All patients must have a baseline evaluation of cardiac function including a measurement of LVEF by either ECHO or MUGA scan prior to study entry. Only patients with LVEF of ≥ 50% should

be entered into this study. While receiving treatment, all patients will have regular monitoring of LVEF with ECHO or MUGA (at screening, 6 weeks and 12 weeks [after Cycle1, Day 1], followed by LVEF evaluations every 3 months or as clinically indicated). During the course of therapy with pertuzumab and trastuzumab, patients should be monitored for signs and symptoms of heart failure (i.e., dyspnea, tachycardia, new unexplained cough, neck vein distention, cardiomegaly, hepatomegaly, paroxysmal nocturnal dyspnea, orthopnea, peripheral edema, and rapid unexplained weight gain). The diagnosis must be confirmed using the same method used to measure LVEF at baseline (either ECHO or MUGA).

Patients who develop signs and symptoms of heart failure NCI CTCAE v4.0 Grade 2, 3, or 4 should have atezolizumab, pertuzumab and trastuzumab held and should receive treatment for heart failure as prescribed by the Heart Failure Society of American (HFSA 2010; e.g., ACE inhibitors, angiotensin-II receptor blockers, β-blockers, diuretics, and cardiac glycosides, as needed).

Consideration should be given to obtaining a cardiac consultation. LVEF should be reassessed after 3 weeks (using the same method of measurement). If the symptoms of heart failure resolve with treatment, and cardiac function (as measured by ECHO or MUGA) improves, atezolizumab, pertuzumab and trastuzumab may be restarted after discussion with the patient concerning the risks and benefits of continued therapy. If the patient is benefiting clinically from study therapy, the benefit of continued treatment may outweigh the risk of cardiac dysfunction. If pertuzumab and trastuzumab are restarted, continued surveillance with noninvasive measures of LVEF (ECHO or MUGA) will continue per protocol.

Study treatment will be adjusted if necessary according to the algorithm described in Appendix F If an investigator is concerned that an adverse event may be related to cardiac dysfunction, an additional LVEF measurement should be performed. Trastuzumab and pertuzumab will be discontinued permanently in any patient who develops clinical signs and symptoms suggesting symptomatic CHF, with the diagnosis confirmed by a suggestive chest X-ray and a drop in LVEF by ECHO or MUGA.

CHF should be treated and monitored according to standard medical practice. At present, there are inadequate data available to assess the prognostic significance of asymptomatic drops of LVEF. Study treatment must be held in all patients for whom a drop of LVEF to <40% or 40% −45% with a 10%-point or greater drop below baseline (Appendix H). If this value is confirmed or LVEF has not recovered to > 45% or 40% − 45% and LESS than 10% below baseline with a repeat assessment within 3 weeks of the first assessment, using the same assessment method, study drug must be discontinued (see Appendix H). If the subject was unequivocally deriving clinical benefit, the subject may be able to resume atezolizumab as determined by the investigator.

Patients who resume therapy will resume pertuzumab at the study dose of 420 mg every 3 weeks (840 mg loading dose of pertuzumab required if study drug is held > 6 weeks) and trastuzumab at the study dose of 6 mg/kg every 3 weekly. Atezolizumab will resume at the study dose of 1200 mg every 3 weeks. Patients will be allowed to hold and resume therapy for a maximum of three times, after which the study drug must be discontinued.

The incidence of CHF will also be recorded throughout the study. See Appendix I for details of NYHA classification, Appendix J for LVSD according to NCI CTCAE v4.0 grading, and Appendix K for reporting conventions for LVSD/heart failure.

6.2.2 Management of Infusion Reactions

Like other monoclonal antibodies, pertuzumab and trastuzumab have been associated with infusion-related reactions (IRRs), such as chills, diarrhea, fatigue, headache, nausea, and pyrexia. The infusion rate of pertuzumab may be slowed or interrupted and appropriate medical therapies should be administered if the patient develops a significant IRR. Patients should be evaluated and carefully monitored until complete resolution of signs and symptoms.

The infusion should be discontinued immediately if the patient experiences a serious hypersensitivity reaction.

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.

All AEs and SAEs whether volunteered by the subject, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be assessed and reported, if appropriate. Each reported AE or SAE will be described by its duration (i.e., start and end dates), expectedness, regulatory seriousness criteria if applicable, suspected relationship to the atezolizumab and actions taken.

After initiation of study treatment, all adverse events will be reported until 30 days after the last dose of study treatment or until initiation of new systemic anti-cancer therapy, whichever occurs first. Serious adverse events and adverse events of special interest deemed to be reasonably related to Atezolizumab will continue to be reported until 90 days after the last dose of study treatment or until initiation of new systemic anti-cancer therapy, whichever occurs first.

7.1 Adverse Events Lists

7.1.1 Anticipated Toxicities for Atezolizumab

Atezolizumab has been associated with risks such as the following: IRRs and immune-related hepatitis, pneumonitis, colitis, pancreatitis, diabetes mellitus, hypothyroidism, hyperthyroidism, adrenal insufficiency, Guillain-Barré syndrome, myasthenic syndrome or myasthenia gravis, and meningoencephalitis. In addition, systemic immune activation (described below) is a potential risk associated with atezolizumab when given in combination with other immunomodulating agents.

Refer to the Atezolizumab Investigator's Brochure for a detailed description of anticipated safety risks for atezolizumab.

7.1.2 Anticipated Toxicities for Trastuzumab and Pertuzumab

Trastuzumab and pertuzumab have been associated with risks such as the following: cardiac dysfunction, ARRs, pulmonary AEs, neutropenia/febrile neutropenia, diarrhea, fatigue, nausea, vomiting, and decreased appetite. Please see Section 6 of the trastuzumab and pertuzumab Investigator's Brochures for a detailed description of anticipated safety risks for trastuzumab and pertuzumab.

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

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

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

  - **Expectedness:**
    Expected adverse events are those adverse events that are listed or characterized in the current adverse event list, the Package Insert, the Investigator Brochure or is included in the informed consent document as a potential risk.

    Unexpected adverse events are those not listed in the Package Insert (P.I.) or current Investigator Brochure (I.B.) or not identified. This includes adverse events for which the specificity or severity is not consistent with the description in the P.I. or I.B. For example, under this definition, hepatic necrosis would be unexpected

7.3 DF/HCC Expedited Adverse Event Reporting

Investigators must report to the Overall PI any serious adverse event (SAE) within 24 business hours of first awareness of the event (immediately if the event is fatal or life-threatening).

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

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

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

The Coordinating Center will submit SAE reports from outside institutions to the DFCI OHRS according to DFCI IRB policies and procedures in reporting adverse events.

DF/HCC Reportable AEs

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

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

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

The overall PI Investigator is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the Investigator to be possibly related.

The overall PI will also notify the FDA and all participating investigators, in a written IND

Safety Report, of any serious, unexpected AE that is considered reasonably or possibly related to the use of Atezolizumab, Trastuzumab, or Pertuzumab. An unexpected adverse event is one that is not already described in the Atezolizumab, Trastuzumab, or Pertuzumab.investigator brochure.

7.5 Expedited Reporting to Genentech

Investigators must report SAEs to Genentech within the timelines described below. The completed MedWatch/case report should be faxed (immediately upon completion to Genentech Drug Safety using the Genentech Safety Reporting Fax Cover Sheet found in Appendix N to: 650-238-6067

Serious adverse events (SAEs), pregnancy reports and AEs of special interest (AESIs), where the patient has been exposed to the Product, will be sent on a MedWatch3500A form to Roche. The Genentech-specific protocol number, ML40055, should be referenced on all submissions to Genentech., Transmission of these reports (initial and follow-up) will be either electronically or by fax and within the timelines specified below:

- **SAEs:**
  Serious AE reports that are **related** to Atezolizumab shall be transmitted to Genentech-Roche within fifteen (15) calendar days of the awareness date.
- **Other SAEs:**
  Serious AE reports that are **unrelated** to Atezolizumab shall be transmitted to Genentech-Roche within thirty (30) calendar days of the awareness date.
- **Pregnancy Reports:**
  While such reports are not serious AEs per se, any reports of pregnancy, where the fetus may have been exposed to the Product, shall be transmitted to Genentech-Roche within thirty (30) calendar days of the awareness date. Pregnancies will be followed up until the outcome of the pregnancy is known, whenever possible, based upon due diligence taken to obtain the follow-up information.
- **AESIs:**
  AESIs requiring expedited reporting shall be forwarded to Roche within fifteen (15) calendar days of the awareness date. Others shall be sent within thirty (30) calendar days.

Relevant follow-up information should be submitted to Genentech Drug Safety as soon as it becomes available and/or upon request.

7.5.1 Adverse Events of Special Interest (AESI)

The following AEs are considered events of special interest and must be reported to the Genentech Drug Safety expeditiously, irrespective of regulatory seriousness criteria:

- Cases of potential drug-induced liver injury that include an elevated ALT or AST in combination with either an elevated bilirubin or clinical jaundice, as defined by Hy's Law and based on the following observations: - Treatment-emergent ALT or AST > 3 x baseline value in combination with total bilirubin > 2 x ULN (of which ≥ 35% is direct bilirubin)
  - Treatment-emergent ALT or AST > 3 x baseline value in combination with clinical jaundice

- Suspected transmission of an infectious agent by the study treatment, as defined below:
    - Any organism, virus, or infectious particle (e.g., prion protein transmitting transmissible spongiform encephalopathy), pathogenic or non-pathogenic, is considered an infectious agent. A transmission of an infectious agent may be suspected from clinical symptoms or laboratory findings that indicate an infection in a patient exposed to a medicinal product. This term applies only when a contamination of study treatment is suspected.

- Atezolizumab AESIs:
    - Pulmonary toxicity (Pneumonitis)
    - Colitis
    - Endocrinopathies: diabetes mellitus, pancreatitis, adrenal insufficiency, hyperthyroidism, and hypophysitis
    - Hepatitis, including AST or ALT > 10xULN
    - Systemic lupus erythematosus
    - Neurological disorders: Guillain-Barré syndrome, myasthenic syndrome or myasthenia gravis, and meningoencephalitis
    - Events suggestive of hypersensitivity, infusion-related reactions, cytokine release syndrome, influenza-like illness, systemic inflammatory response syndrome, and systemic immune activation
    - Nephritis
    - Ocular toxicities (e.g., uveitis, retinitis) Myositis Myopathies, including rhabdomyolysis
    - Grade $\geq$ 2 cardiac disorders (e.g., atrial fibrillation, myocarditis, pericarditis)
    - Vasculitis
- Trastuzumab AESI:
    - Congestive heart failure
- Pertuzumab AESI:
    - Asymptomatic decline in LVEF requiring treatment or leading to discontinuation of Trastuzumab or Pertuzumab

7.6 Additional Reporting Requirements for Genentech

7.6.1 Pregnancy

If a female subject becomes pregnant while receiving the study drug or within 90 days after the last dose of study drug, a report should be completed and expeditiously submitted to Genentech, Inc. Follow-up to obtain the outcome of the pregnancy should also occur. Abortion, whether accidental, therapeutic, or spontaneous, should always be classified as serious, and expeditiously reported as an SAE. Similarly, any congenital anomaly/birth defect in a child born to a female subject exposed to the {study drug} should be reported as an SAE.

Additional information on any Trastuzumab or Pertuzumab-exposed pregnancy and infant will be requested by Roche Drug Safety at specific time points (i.e. after having received the initial

report, at the end of the second trimester, 2 weeks after the expected date of delivery, and at 3, 6, and 12 months of the infant's life).

7.6.2 Post-Study Adverse Events

The investigator should expeditiously report any SAE occurring after a subject has completed or discontinued study participation if attributed to Atezolizumab exposure. If the investigator should become aware of the development of cancer or a congenital anomaly in a subsequently conceived offspring of a female subject who participated in the study, this should be reported as an SAE.

7.6.3 Reconciliation

The Sponsor agrees to conduct reconciliation for the product. Genentech and the Sponsor will agree to the reconciliation periodicity and format, but agree at minimum to exchange quarterly line listings of cases received by the other party.

If discrepancies are identified, the Sponsor and Genentech will cooperate in resolving the discrepancies. The responsible individuals for each party shall handle the matter on a case-by-case basis until satisfactory resolution.

7.6.4 Product Complaints

A product complaint is any written or oral information received from a complainant that alleges deficiencies related to identity, quality, safety, strength, purity, reliability, durability, effectiveness or performance of a product after it has been released and distributed to the commercial market or clinical trial.

How to file a complaint:
For all Investigator Initiated Studies (interventional and non-interventional):
Product Complaints **with** an AE (adverse event) should be reported via email/fax to:
Usds_aereporting-d@gene.com OR 650-238-6067

Product Complaints **without** an AE (adverse event) should be reported via email to:

- For Interventional Investigator Initiated Studies: kaiseraugst.global_impcomplaint_management@roche.com
- For Non-Interventional Investigator Initiated Studies: us-acmo-d@gene.com

All complaints must be filed within 1 business day for pre-approved products and 15 calendar days for approved products. Complaints can be reported using a Medwatch, CIOMS or any Genentech-approved reporting form (same as SAEs, AESI etc.).

7.7 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.

7.8 Routine Adverse Event Reporting

All Grade 2 or higher Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. Abnormal laboratory results deemed to be not-clinically significant (NCS) by a treating investigator do not need to be entered as an adverse event in the 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 and other agents administered in this study can be found in Section 7.1.

8.1 Atezolizumab

8.1.1 Description

Atezolizumab is a human immunoglobulin (Ig) G1 monoclonal antibody consisting of two heavy chains (448 amino acids) and two light chains (214 amino acids) and is produced in Chinese hamster ovary cells. Other name: MPDL3280A. Atezolizumab targets human programmed death-ligand 1 (PD-L1) and inhibits its interaction with its receptor, programmed death-1 (PD-1). Atezolizumab also blocks the binding of PD-L1 to B7.1, an interaction that is reported to provide additional inhibitory signals to T cells.

8.1.2 Form

The atezolizumab drug product is provided in a single-use, 20-cc USP/Ph. Eur. Type 1 glass vial as a colorless-to-slightly-yellow, sterile, preservative-free clear liquid solution intended for IV administration.

The vial is designed to deliver 20 mL (1200 mg) of Atezolizumab solution but may contain more than the stated volume to enable delivery of the entire 20 mL volume. The Atezolizumab drug product is formulated as 60 mg/mL atezolizumab in 20 mM histidine acetate, 120 mM sucrose, 0.04% polysorbate 20, pH 5.8.

8.1.3 Storage and Stability

Atezolizumab must be refrigerated at 2°C – 8°C (36°F – 46°F) upon receipt until use. Atezolizumab vials should not be used beyond the expiration date provided by the manufacturer. No preservative is used in the atezolizumab drug product; therefore, each vial

is intended for single use only. Discard any unused portion of drug left in a vial. Vial contents should not be frozen or shaken and should be protected from direct sunlight.

For further details, see the atezolizumab Investigator's Brochure.

8.1.4 Compatibility

Atezolizumab will be delivered in infusion bags with IV infusion lines that have product contacting surfaces of polyvinyl chloride (PVC) or polyolefin and 0.2 μm in-line filters (filter membrane of polyethersulfone [PES]). No incompatibilities have been observed between Atezolizumab and PVC or polyolefin infusion materials (bags or infusion lines).

8.1.5 Handling

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

8.1.6 Availability

Atezolizumab is an investigational agent and will be supplied free of charge from Genentech.

8.1.7 Preparation

Atezolizumab can be diluted to concentrations between 2.4 mg/mL and 9.6 mg/mL in IV bags containing 0.9% NaCl. The prepared solution for infusion should be used immediately to limit microbial growth in case of potential accidental contamination. If not used immediately, in-use storage time and conditions prior to use are the responsibility of the user. In 250-mL IV infusion bags, the dose solution may be stored at 2°C–8°C (36°F-46°F) for 24 hours or at ambient temperature ≤ 25°C (77°F) for 8 hours.

Atezolizumab vials should not be used beyond the expiration date provided by the manufacturer. No preservative is used in the atezolizumab drug product; therefore, each vial is intended for single use only. Discard any unused portion of drug left in a vial. Vial contents should not be frozen or shaken and should be protected from direct sunlight.

8.1.8 Administration

Atezolizumab will be delivered in infusion bags with IV infusion lines that have product contacting surfaces of polyvinyl chloride (PVC) or polyolefin and 0.2 μm in-line filters (filter membrane of polyethersulfone [PES]). No incompatibilities have been observed between atezolizumab and PVC or polyolefin infusion materials (bags or infusion lines).

Administration of atezolizumab will be performed in a setting with emergency medical facilities and staff who are trained to monitor for and respond to medical emergencies.

The initial dose of atezolizumab will be delivered over 60 (± 15) minutes. If the first infusion is tolerated without infusion-associated AEs, the second infusion may be delivered over 30 (± 10) minutes. If the 30-minute infusion is well tolerated, all subsequent infusions may be delivered over 30 (± 10) minutes Patients will be informed about the possibility of delayed post-infusion symptoms and instructed to contact their study physician if they develop such symptoms.

8.1.9 Ordering

Atezolizumab will be obtained directly from Genentech.

8.1.10 Accountability

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

8.1.11 Destruction and Return

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

8.2 Pertuzumab

8.2.1 Description

Pertuzumab (Perjeta®) is a humanized monoclonal antibody to the HER2 receptor that blocks ligand-dependent heterodimerization of HER2 with other HER family members. This results in the inhibition of ligand-initiated intracellular signaling. In addition, pertuzumab mediates antibody-dependent cellular cytotoxicity.

8.2.2 Form, storage and stability

Pertuzumab is provided as a single-use formulation containing 30 mg/mL pertuzumab in 20 mM L-histidine acetate (pH 6.0), 120 mM sucrose, and 0.02% polysorbate 20. Each 20-mL vial contains 420 mg of pertuzumab (14.0 mL/vial). Pertuzumab does not contain any antimicrobial preservative. Therefore, care must be taken to ensure the sterility of the prepared solution for infusion and should be prepared by a healthcare professional. The appropriate dose of pertuzumab should be withdrawn from the vial using aseptic techniques and added to 250 mL 0.9% sodium chloride for injection for subsequent patient administration. For information on the formulation, packaging, and handling of pertuzumab, see the pertuzumab Investigator's Brochure.

8.2.3 Compatibility

No incompatibilities between pertuzumab and polyvinylchloride, polyethylene, or non-PVC polyolefin bags have been observed. Dextrose (5%) in water (D5W) solution should not be used to dilute pertuzumab since it has been shown to be chemically and physically unstable in such solutions (dilute formulations of pertuzumab liquid formulations in D5W IV bags did not maintain stable pH after storage at room temperature [27°C − 33°C] for 24 hours followed by 24 hours at refrigerator temperature [2°C − 8°C]).

8.2.4 Handling

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

8.2.5 Availability

Pertuzumab is an investigational agent and will be supplied free of charge from Genentech.

8.2.6 Preparation

Pertuzumab can be diluted into a 250 mL 0.9% sodium chloride PVC or non-PVC polyolefin infusion bag. The dose solution should be used immediately. If not used immediately, it can be stored at 2°C–8°C for up to 24 hours.

Pertuzumab vials should not be used beyond the expiration date provided by the manufacturer. No preservative is used in the pertuzumab drug product; therefore, each vial is intended for single use only. Discard any unused portion of drug left in a vial. Vial contents should not be frozen or shaken and should be protected from direct sunlight.

8.2.7 Administration

The loading dose of pertuzumab will be 840 mg administered as a 60-minute IV infusion, followed every 3 weeks thereafter by a dose of 420 mg administered over a period of 30 to 60 minutes. For patients already receiving pertuzumab as part of their ongoing systemic therapy, no loading dose is required in the case of an interval < 6 weeks between last dose of pertuzumab and the first administration of on-study pertuzumab. An observation period of 30 to 60 minutes is recommended after each pertuzumab infusion, according to each participating site's institutional guidelines.

8.2.8 Ordering

Pertuzumab will be obtained directly from Genentech.

8.2.9 Accountability

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

8.2.10 Destruction and Return

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

8.3 Trastuzumab

8.3.1 Description

Trastuzumab (Herceptin®) is a recombinant monoclonal antibody that binds specifically and with high affinity to the extracellular domain of HER2. Trastuzumab has been shown to inhibit the proliferation of human tumor cells overexpressing HER2 both in vitro and in vivo.

8.3.2 Form, storage and stability

This study will use trastuzumab from investigational supply. Trastuzumab is a sterile, white to pale yellow, preservative free lyophilized powder for intravenous (IV) administration, supplied as a 150 mg vial. Each single-dose vial of trastuzumab delivers 150 mg trastuzumab, 136.2 mg α,α-trehalose dihydrate, 3.4 mg L-histidine HCl monohydrate, 2.2 mg L-histidine, and 0.6 mg polysorbate 20.

Use appropriate aseptic technique. Reconstitute each 150 mg vial of single-dose Trastuzumab with 7.4 mL of sterile water for injection (SWFI) to yield a solution containing 21mg/mL trastuzumab that delivers 7.15 mL (150 mg trastuzumab), at a pH of approximately 6.

Use of other reconstitution diluents should be avoided. Determine the dose of trastuzumab needed, based on a loading dose of 8 mg trastuzumab/kg body weight for q3wk dosing schedules or a maintenance dose of 6 mg/kg trastuzumab/kg body weight for q3w dosing schedules. Calculate the correct dose using 21 mg/mL trastuzumab solution. Withdraw this amount from the vial and add it to an infusion bag containing 250 mL of 0.9% sodium chloride, USP. **DEXTROSE (5%) SOLUTION SHOULD NOT BE USED**. Gently invert the bag to mix the solution. The reconstituted preparation results in a colorless to pale yellow transparent solution. Parenteral drug products should be inspected visually for particulates and discoloration prior to administration.

Trastuzumab should not be mixed or diluted with other drugs. Trastuzumab should not be filtered during administration.
Trastuzumab vials must be used within 24 hours after dilution when stored at 2°C to 8°C.

For information on the formulation, packaging, and handling of trastuzumab, see the pharmacy manual and the trastuzumab Investigator's Brochure.

8.3.3 Compatibility

No incompatibilities between trastuzumab and polyvinylchloride, polyolefin, or polypropylene bags have been observed. Dextrose 5% solution should not be used because it causes aggregation of the protein. Trastuzumab should not be mixed or diluted with other drugs.

8.3.4 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.

8.3.5 Availability

Trastuzumab is an investigational agent and will be supplied free of charge from Genentech.

8.3.6 Preparation

Trastuzumab vials should not be used beyond the expiration date provided by the manufacturer. No preservative is used in the trastuzumab drug product; therefore, each vial is intended for single use only. Discard any unused portion of drug left in a vial. Vial contents should not be frozen or shaken and should be protected from direct sunlight.

8.3.7 Dosage and administration

High-dose trastuzumab will be administered at a dose of 6 mg/kg weekly, infused intravenously over 30 -90 minutes. No loading dose is required. Trastuzumab-naïve patients should receive their first dose over 90 minutes. Patients who are actively receiving trastuzumab prior to enrollment will have a dose increase to 6 mg/kg IV weekly. The dose of trastuzumab should be based on institutional guidelines. Weekly doses may be administered +4 -2 days apart.

8.3.8 Ordering

Trastuzumab will be obtained directly from Genentech

8.3.9 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.)

8.3.10 Destruction and Return

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

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

All patients will be asked to provide archival tumor tissue (both primary and metastatic tissue will be requested if available; either paraffin blocks or 15 unstained slides, ideally 4-micron thickness). However, if archival tissue is not available or not evaluable, that will not be a basis to exclude the patient from any portion of the trial or the planned analysis.

Patients with an accessible tumor outside the field of radiation will be asked to undergo an optional baseline tumor biopsy. We plan to use baseline biopsy tissue to perform several 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 further testing.

Serial blood draws for correlative science are required on this trial; blood draws will be obtained per the schedule in Table 9-1. 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.

Table 9-1 Summary of Research Tissue and Blood Specimen Collection

Research Sampling	**Time point**	**Contents**
Blood	Cycle 1 Day 1	1-9 mL Streck Tube 5- 10mL green top tubes[a,b]
	Cycle 3 Day 1	1-9 mL Streck Tube 5- 10mL green top tubes[a,b]
	Cycle 5 Day 1	1-9 mL Streck Tube 5- 10mL green top tubes[a,b]
	Cycle 9 Day 1	1-9 mL Streck Tube 5- 10mL green top tubes[a,b]
	At progression or off protocol therapy	1-9 mL Streck Tube 5- 10mL green top tubes[a,b]
Fresh Tissue Biopsy (optional)	Pre-treatment	5-7 cores
	Cycle 2 Day 1 to 21	5-7 cores

Research Sampling	Time point	Contents
	At progression for patients who achieved an objective response and/or a prolonged stable disease (≥ 24 weeks)	5-7 cores
Archival Tissue	Anytime	1 block or 15, 4 micron thick unstained slides
CSF	Screening	3-10 mL in Streck Tubes
	Anytime before cycle 3	3-10 mL in sterile collection tubes
	At progression or off protocol therapy	3-10 mL in sterile collection tubes

a. EDTA (purple top) tubes or CPT tubes may be used interchangeably with green top tubes.
b. This collection will only be performed at DFCI due to the time sensitive nature of processing.

9.1 Archival Tissue Collection

1 block or 15, 4 microns thick unstained, charged slides will be collected for future research. Archival tissue sample does not need to be collected prior to registration.

9.2 Fresh Tissue Collection

9.2.1 Collection at Dana-Farber Cancer Institute

Biopsies are optional at all timepoints [baseline (pre-treatment), Cycle 2 Day 1, and at the time of progression for patients who achieved an objective response and/or a prolonged stable disease (≥ 24 weeks)]. The Cycle 2 Day 1 biopsy should be performed as close to Cycle 2 Day 1 as possible but may be collected anytime between Cycle 2 Day 1 and Cycle 2 Day 21.

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 [redacted] The specimens in RNALater and formalin may be stored over the weekend and shipped on Monday. Specimens in RNAlater and formalin should be stored at room temperature until shipment.

Ideally, five core biopsies will be obtained:

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

The order of specimen collection should be:

- First core: 10% neutral buffered formalin

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

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

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

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

9.2.2 Collection at Northwestern University Medical Centter

Biopsies are optional at all timepoints [baseline (pre-treatment), Cycle 2 Day 1, and at the time of progression for patients who achieved an objective response and/or a prolonged stable disease (≥ 24 weeks)]. The Cycle 2 Day 1 biopsy should be performed as close to Cycle 2 Day 1 as possible but may be collected anytime between Cycle 2 Day 1 and Cycle 2 Day 21.

The specimens in RNALater and formalin may be stored over the weekend and shipped on Monday. Specimens in RNAlater and formalin should be stored at room temperature until shipment.

Ideally, five core biopsies will be obtained:

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

The order of specimen collection should be:

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

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

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

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.

- Complete the requisition form (Appendix G) for the sample

- Cores 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** ideally, though and additional 2-hour window is allowed. In addition, a small piece of one core 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 [redacted]

- Cores in formalin collected at DFCI 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 formalin collected at Northwestern should either be processed into an FFPE block per local policies and shipped to the current DFCI CRC or can be shipped overnight to the DF/HCC Clinical Trial Laboratory, who can process the sample into an FFPE block.

If FFPE Block:
Dana-Farber Cancer Institute
Attn: 17-546 Study Team
450 Brookline Ave., DA157
Boston, MA 02215

If in Formalin:
Brigham and Women's Hospital
Attn: Breast Tissue/Blood Bank
Thorn Building – Room 428
20 Shattuck Street
Boston, MA 02115

If mailing to the the DF/HCC Clinical Trials Core Laboratory, please email dfcibreastbank@partners.org with participant name, study ID, date of collection, approximate time of collection, and study time point the day prior to collection. Any tissue remaining after study-specific protocol testing occurs will be banked in the DF/HCC Clinical Trial Core

Laboratory and may be used for additional or future analyses as needed.

- Cores in RNAlater should be brought or shipped overnight to the DF/HCC Clinical Trial Core Laboratory with the sample requisition (Appendix G):

Brigham and Women's Hospital
Attn: Breast Tissue/Blood Bank
Thorn Building – Room 428
20 Shattuck Street
Boston, MA 02115
dfcibreastbank@partners.org

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

- Cores in OCT collected at Northwestern may be stored locally at -80°C and batch shipped overnight on dry ice to OR shipped overnight on dry ice on the day of collection to the DF/HCC Clinical Trial Core Laboratory with the sample requisition (Appendix G) to:

Brigham and Women's Hospital
Attn: Breast Tissue/Blood Bank
Thorn Building – Room 428
20 Shattuck Street
Boston, MA 02115
dfcibreastbank@partners.org

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

9.3 Blood Collection

9.3.1 Collection at Dana-Farber Cancer Institute

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

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

The following research blood samples are required:

Cycle 1 Day 1:

- 1-9 mL Streck Tube for whole blood
- 5- 10mL green top tubes for whole blood

Cycles 3, 5, and 9 Day 1:

- 1-9 mL Streck Tube for whole blood
- 5- 10mL green top tubes for whole blood

Off Treatment (at progression or off protocol therapy, whichever comes first):

- 1-9 mL Streck Tube for whole blood
- 5- 10mL green top tubes for whole blood

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

- 1-9 mL Streck Tube for whole blood
- 5- 10mL green top tubes for whole blood

If green top tubes are unavailable, CPT tubes or purple tops may be substituted.

9.3.2 Collection at Northwestern University Medical Center

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.

The following research blood samples are required:

Cycle 1 Day 1:

- 1-9 mL Streck Tube for whole blood

Cycles 3, 5, and 9 Day 1:

- 1-9 mL Streck Tube for whole blood

Off Treatment (at progression or off protocol therapy, whichever comes first):

- 1-9 mL Streck Tube for whole blood

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

- 1-9 mL Streck Tube for whole blood

9.3.3 Handling and Shipping

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

- Green Top tubes:
  Must be processed within 3-4 hours of being drawn at ambient temperature immediately after being drawn to Mariano Severgnini at:

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

- Streck tubes:

  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.

  Streck tubes and sample requisition (Appendix G) should be shipped to:

  Brigham and Women's Hospital
  Attn: Breast Tissue/Blood Bank
  Thorn Building – Room 428
  20 Shattuck Street
  Boston, MA 02115
  dfcibreastbank@partners.org

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

9.4 Cerebrospinal fluid (CSF)

9.4.1 Collection

CSF collection is mandatory, except in patients in whom the treating investigator believes CSF collection would be associated with excessive risk (e.g. risk of herniation, significant midline shift, need for therapeutic anticoagulation) . We plan to collect CSF at baseline for cytology and potential DNA isolation, before cycle 3 day 1, and at progression or off protocol therapy, whichever comes first. 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.

The following CSF blood samples are mandatory:

Screening (baseline):

- 2-10 mL in sterile collection tubes (Northwestern should use Streck Tubes)
  - Only 5cc of CSF is required per tube

Between C2D1 and C3D1:

- 2-10 mL in sterile collection tubes (Northwestern should use Streck Tubes)
  - Only 5cc of CSF is required per tube

Off Treatment (at progression or off protocol therapy, whichever comes first):

- 2-10 mL in sterile collection tubes (Northwestern should use Streck Tubes)
  - Only 5cc of CSF is required per tube

The first (baseline) CSF collection is billed to the patient's insurance. It is recommended that the following clinical tests be ordered at the baseline CSF collection, in addition to the research collection: Glucose, Cytology, and Total Protein.

9.4.2 Handling and Shipping

CSF tubes should be brought or shipped overnight on the day of collection at ambient temperature to the DF/HCC Clinical Trial Core Laboratoryat the following address:

Brigham and Women's Hospital
Attn: Breast Tissue/Blood Bank
Thorn Building – Room 428
20 Shattuck Street

Boston, MA 02115
dfcibreastbank@partners.org

Please email the DF/HCC Clinical Trials Core Laboratory (dfcibreastbank@partners.org) with participant name, study ID, date of collection, approximate time of collection, and study time point the day prior to collection.

Note: All liquid transfers should be performed in a sterile laminar flow hood.

1. Process samples within 2 hours of collection. Those collected with Streck preservative can be processed within 24 hours.
2. Transfer CSF to a 15mL Falcon tube
3. Spin 15mL tubes containing CSF at 1900g for 10 minutes at room temperature with the brake reduced to 6
    a. A small pellet may be visible after the spin
    b. If also using sample for single cell analysis, reduce speed to 400-700g to pellet cells
4. Carefully remove tubes from centrifuge and transfer 6 mL CSF to a barcoded FluidX 10mL tube
    a. Transfer any additional CSF to a separate FluidX 10mL tube
    b. Note: If FluidX tubes are unavailable, store samples in well-labeled cryotubes
    c. If also using sample for single cell analysis, lyse red blood cells in pellet (after collecting supernatant) using 1X BD Pharm Lyse per the manufacturer's protocol and resuspend in RPMI
5. Store tube(s) at -80^{o}C until analysis

9.5 Planned Assays for Correlative Objectives

All of the below-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.

9.5.1 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. 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 specimen may be categorized as lymphocyte predominant breast cancer (LPBC), defined as a tumor that contains >60% stromal lymphocytes, or non-LPBC.

9.5.2 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

Chen et al[Chen *et al.*, 2013] describe a semi-quantitative scoring method, which is in accordance with typical biomarker scoring in anatomic and surgical pathology. Briefly, staining is scored per intensity (0=no staining, 1=weak staining, 2=moderate staining, 3=strong staining), staining patter (M=predominantly cell membrane; C=predominantly cell cytoplasm), and the percentage of cells showing positive staining (0-100%). The semi-quantitative scoring is performed for: 1) the neoplastic tumor cells and 2) the non-neoplastic infiltrating immune cells. Significant discordant results have been rare during case evaluations.[Chen *et al.*, 2013]

It should be noted that the above staining protocols are based on standard methods used at the time of protocol writing. It is possible that at the time protein expression assays are conducted, novel and improved methods for staining will exist. In this case, we plan to use the best available, best validated experimental method available at the time.

9.5.3 Flow cytometry, genomic analysis of biopsy tissue

TILs will be isolated from the biopsy specimen and assessed by surface staining as described in the lab manual for this protocol.

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

neoantigen burden, may also be performed.

9.5.4 Analysis of PBMCs

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

9.5.5 Analysis of cell-free DNA

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

9.6 Additional analysis

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

10. STUDY CALENDAR

Screening evaluations are to be conducted within 28 days prior to start of protocol therapy unless otherwise specified. Screening laboratory assessments must be done within 8 calendar days prior to initiating protocol therapy.

All assessments must be performed prior to administration of any study agent unless otherwise specified. The following windows apply:

- Screening assessments performed within 8 days prior to Day 1 of Cycle 1 do not have to be repeated for Day 1 of Cycle 1.
- Day 1 study assessments for Cycle 2 and beyond must be performed within +/- 3 days of the protocol-specified date.
- Weekly trastuzumab (first 24 weeks) must be given within +4/- 2 days of the protocol-specified date.
- Restaging scans are performed within the last 15-21 of the previous cycle to ensure scans are reviewed in advance.
- LVEF assessments may be performed up to 7 days prior to scheduled evaluation

Deviations within +/- 2 days of the protocol-specified timepoints for treatment of study assessments are permitted and should be recorded on the minor deviation log.

If a participant's condition is deteriorating, laboratory evaluations should be repeated within 48

hours prior to initiation of the next cycle of therapy.

It is understood that it may not always be feasible for patients to return for restaging evaluation after coming off protocol therapy; however, it strongly encouraged. Failure to complete restaging assessments or questionnaires after a patient has been taken off protocol therapy will not constitute a protocol violation.

	Pre-Study	Cycle 1	Cycle 2	Cycle 3	Cycle 5	Subsequent Cycles	Follow-up	
	Day 28 to Day -1	D1	D1 ± 3 days	D1 ± 3 days	D1 ± 3 days	D1 of each cycle ± 3 days	Off-Treatment within 30 days	Follow-up
Physical exam, ECOG PS	X	X	X	X	X	X	X	
Medical History [a]	X							
Vital signs, weight [b]	X	X	X	X	X	X	X	
CBC w/differential [c]	X	X	X	X	X	X	X	
Chemistry [d]	X	X	X	X	X	X	X	
TSH [e]	X	X	X	X		X[e] (even cycles)		
Pregnancy Test [f]	X							
Echocardiogram or MUGA [g]	X			X	X	X[g]		
Neurological Assessment	X	X	X	X	X	X	X	
Tumor Measurements [h]	X			X	X	X[h]	X	X[h]
Brain MRI [i]	X			X	X	X[i]	X	X[i]
Research Blood [j]		X		X		X	X	
Research Biopsy [j, k]	X		X[k]				X	
CSF Collection [l]	X		X				X	
Archival Tissue Collection [j]	X							
NANO Scale, MDASI-BT, and EQ-5D questionnaires [m]	X			X	X	X[m]	X	X[m]
General Impression Worksheet [n]	X			X	X	X[n]	X[n]	
AE Assessment [o]	X	X	X	X	X	X	X[o]	
Safety Follow-Up [p]/ Survival status [q]								X[q]

a. A complete physical examination, including neurological examination, will be performed at screening. A limited physical exam, to include a neurological exam, will be performed at subsequent Day 1 visits.
b. Vital signs to include: heart rate, systolic and diastolic blood pressures while the patient is in a seated position. Vital signs and weight will be assessed before treatment on Day 1 of every 3-week cycle
c. Hematology: hemoglobin, hematocrit, platelet count, RBC count, WBC count, neutrophil percent and absolute differential count.
d. Chemistry: sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, calcium, total bilirubin, total protein, albumin, ALT, AST, alkaline phosphatase, and LDH.
e. Beginning with cycle 4, TSH is collected every other cycle on Day 1 (i.e. cycles 4, 6, 8, etc).
f. In female subjects of child-bearing potential as defined in the eligibility criteria, serum or urine pregnancy test must be performed within 8 days of C1D1. If a urine pregnancy test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
g. LVEF evaluations will be assessed at screening, on cycle 3 day 1 (- 7 days) and cycle 5 day 1 (- 7 days), followed by LVEF evaluation every 3 months (- 7 days) during the treatment period. Measurements will be done by either ECHO or MUGA scan (with ECHO as the preferred method).
h. Clinical and radiological tumor assessments will be performed by CT and/or MRI of the chest, abdomen and pelvis at baseline and repeated on the last 15-21 days of a cycle every 6 weeks for the first 24 weeks and then reduced to every 9 weeks until progression. The same radiographic procedures and technique must be used throughout the study for each patient. For participants who have not progressed after 1 year on protocol therapy, re-evaluation can be performed every 12 weeks. For those taken off-treatment for reasons other than progressive disease, tumor measurements should continue to be repeated every 6-12 weeks until progression or initiation of a new anticancer regimen; failure to adhere to this schedule will not be considered a protocol violation.
i. Screening MRI must be done within 28 days of C1D1. Subsequent assessments should be done during the last 15-21 days of a cycle, q6weeks for the first 24 weeks and then can be reduced to q9 weeks. If progression is suspected, an unscheduled assessment is permitted. For those taken off-study for reasons other than progressive disease in the CNS, assessments should continue to be repeated every 6-12 weeks until progression or initiation of a new anticancer regimen; failure to adhere to this schedule will not be considered a protocol violation.
j. See **Section 9** for further information regarding research blood, biopsy, and archival tissue collection
k. Research biopsies are optional at all 3 timepoints (baseline, at Cycle 2 Day 1, and at the time of progression). The Cycle 2 Day 1 biopsy should be performed as close to Cycle 2 Day 1 as possible but may be collected between Cycle 2 Day 1 and Cycle 2 Day 21.
l. CSF collection will be performed at 3 time-points (baseline, before cycle 3, and at progression or off treatment (whichever comes first). It is recommended that the following clinical tests be ordered at the baseline CSF collection, in addition to the research collection: Glucose, Cytology, and Total Protein. CSF collection is required except in patients in whom the treating investigator believes CSF collection would be associated with excessive risk (e.g. risk of herniation, significant midline shift, need for therapeutic anticoagulation)
m. MDASI-BT, NANO Scale, and EQ-5D questionnaires (Appendices C, D, E and F) will be completed at baseline (which can occur during screening or on cycle 1 day 1), and day 1 of Cycles 3, 5, 9 and Off-Treatment. For those taken off-treatment for reasons other than progressive disease, questionnaires should continue to be repeated every 6-12 weeks at the time of tumor assessments until progression or initiation of a new anticancer regimen.
n. General Impression Worksheet (Appendix L) to be completed by the treating physician at baseline (which can occur during screening or on cycle 1 day 1) and at the end of each 3-week cycle until and at progression or off treatment (whichever comes first)
o. Adverse events will be collected until 30 days after removal from study therapy.
p. Off-Treatment visit should occur within 30 days (+ 7 days) of the date the decision is made to remove the participant from protocol therapy. Tumor assessments (including brain MRI and CAP CT/MRI do not need to be repeated if done within 28 days of off-treatment visit).
q. Survival status collected every 6 months or until death either via clinic visit or telephone call to the patient or patient's local provider

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. For participants who have not progressed after 1 year on protocol therapy, re-evaluation can be performed every 12 weeks.

Central review by TIMC will take place for all participants to determine response and progression in the CNS and in non-CNS disease sites. For clinical decision-making, central radiology results will be used at DF/HCC sites. Outside sites may use local review of restaging scans to determine disease response for clinical decision-making, if central radiology results are not available in real-time. Participating sites will submit imaging scans (either hard copy or through secure electronic interface) to the coordinating center to be reviewed by TIMC for central review. The TIMC review will be entered in the Case Report Forms and used for analysis of antitumor effect.

11.1 Antitumor Effect – CNS disease

Tumor response and progression for CNS disease will be assessed using Neuro-Oncology-Brain Metastases (RANO-BM) Criteria.

11.1.1 Definitions

- Measurable Disease: Measurable disease is defined as a contrast enhancing lesion that can be accurately measured in at least one dimension with a minimum size of 10 mm, visible on two or more axial slices that are preferably ≤ 5 mm apart with 0-mm skip (and ideally ≤ 1.5 mm apart with 0-mm skip). In addition, although the longest diameter in the plane of measurement is to be recorded, the diameter perpendicular to the longest diameter in the plane of measurement should be at least 5 mm for the lesion to be considered measurable. In the event the MRI is performed with thicker slices, the size of the measurable lesion at baseline should be at least two times the slice thickness. If there are interslice gaps, this also needs to be considered in determining the minimum size of measurable lesions at baseline. Measurement of tumor around a cyst or surgical cavity represents a particularly difficult challenge. In general, such lesions should be considered non-measurable unless there is a nodular component measuring ≥ 10 mm in longest diameter and ≥ 5 mm in the perpendicular plane. The cystic or surgical cavity should not be measured in determining response (Figure 1 in the original publication).
- Non-measurable Disease: All other lesions, including lesions with longest dimension < 10 mm, lesions with borders that cannot be reproducibly measured, dural metastases, bony skull metastases, cystic-only lesions, and leptomeningeal disease.

11.1.2 Specifications of Methods of Measurement

- Method of Assessment: 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. It is important to use imaging techniques that are consistent across all imaging time points in order to ensure that the assessment of interval appearance or disappearance of lesions or of

change in size is not affected by scan parameters such as slice thickness. Use of thin section imaging (for example, Appendix A of the original publication) is particularly important when evaluating lesions < 10 mm in LD and/or small changes in lesion size.

- Imaging Modality: Gadolinium-enhanced MRI is the best currently available, sensitive, and reproducible method to measure CNS lesions selected for response assessment. Suggested brain MRI specifications are detailed in Appendix A of the original publication. A sum of the diameters for all target lesions will be calculated and reported as the baseline sum of longest diameters (sum LD). All other CNS lesions should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as 'present', 'absent', or 'unequivocal progression'.

11.1.3 Definition of Best Overall CNS Response

- Best overall CNS response represents a composite of radiographic CNS target and non-target response (see definitions above), corticosteroid use, and clinical status. In non-randomized trials where CNS response is the primary endpoint, confirmation of PR or CR at least 4 weeks later is required to deem either one the best overall response. At each protocol-specified time point, a response assessment should occur and CNS assessments should be coincident with extra-CNS assessment. Table 1 shows the additional corticosteroid and clinical status requirements to deem a PR or CR.

11.1.4 Evaluation of Target Lesions

- **Complete response (CR):** Disappearance of all CNS target lesions sustained for at least 4 weeks; no new lesions; no corticosteroids; stable or improved clinically.
- **Partial response (PR):** At least a 30% decrease in the sum LD of CNS target lesions, taking as reference the baseline sum LD sustained for at least 4 weeks; no new lesions; stable to decreased corticosteroid dose; stable or improved clinically.
- **Progressive disease (PD):** At least a 20% increase in the sum LD of CNS 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 elative increase of 20%, at least one lesion must increase by an absolute value of ≥ 5 mm to be considered progression.
- **Stable disease (SD):** Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD while on study.

11.1.5 Evaluation of Non-Target Lesions

Non-target lesions should be assessed qualitatively at each of the time points specified in the protocol.

- **CR:** Requires all of the following: disappearance of all enhancing CNS non-target lesions, no new CNS lesions.
- **Non-CR/Non-PD:** Persistence of one or more non-target CNS lesion(s).
- **PD:** Any of the following: unequivocal progression of existing enhancing non-target CNS lesions, new lesion(s) (except while on immunotherapy-based treatment), or unequivocal progression of existing tumor-related non-enhancing (T2/FLAIR) CNS lesions. In the case of immunotherapy-based treatment, new lesions alone may not constitute progressive disease (see "Guidance in the case of new lesion(s) while on immunotherapy" below).

Special Notes on the Assessment of Target and Non-Target CNS Lesions:

a) *Target lesions that become too small to measure:* While on study, all CNS target lesions should have their actual measurement recorded, even when very small (e.g., 2 mm). If the lesion disappears, the value should be recorded as 0 mm. However, if the lesion is sufficiently small (but still present) that the radiologist does not feel comfortable assigning an exact measure, a default value of 5 mm should be recorded on the case report form.
b) *Lesions that coalesce on treatment*: As lesions coalesce, a plane between them may be maintained that would aid in obtaining maximum LD of each individual lesion. If the lesions have truly coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximum LD for the 'coalesced' lesion.
c) *Definition of new lesion(s):* The finding of a new CNS lesion should be unequivocal and not due to technique or slice variation. A new lesion is one that was not present on prior scans. If the MRI is obtained with ≤ 1.5 mm slice thickness, then the new lesion should also be visible in axial, coronal, and sagittal reconstructions of ≤ 1.5 mm projections. If a new lesion is equivocal, for example because of its small size (i.e., ≤ 5 mm), continued therapy may be considered, and follow up evaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan showing the new lesion. In the case of immunotherapy, new lesions alone may not constitute progressive disease (see "Guidance in the case of new lesion(s) while on immunotherapy" below).
d) *Definition of Unequivocal Progression of Non-Target Lesion(s):* When the patient also has measurable disease, to achieve 'unequivocal progression' on the basis of non-target disease alone, there must be an overall level of substantial worsening in non-target disease such that, even in the presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. When the patient has only non-measurable disease, there must be an overall level of substantial worsening to merit discontinuation of therapy.
e) *Guidance in the Case of Uncertain Attribution of Radiographic Findings and/or Equivocal Cases:* The RANO-BM group acknowledges that in the case of patients followed during immunotherapy-based approaches, there may be radiographic evidence of enlargement of target and non-target lesions which may not necessarily represent tumor progression. If there is evidence of radiographic progression but there is clinical evidence supporting the possibility that the radiological changes are due to treatment effect (and not to progression of cancer), additional evidence is required to distinguish true progression versus treatment effect as standard MRI alone is not sufficient. The methods used to distinguish between the two entities should be specified prospectively in the clinical protocol. one or more of the following options: (1) Repeat the scan at the next protocol scheduled evaluation or sooner, and generally within ~6 weeks. An investigator may choose a shorter time interval in the case of progressive symptoms or other clinically concerning findings. If there is continued increase in enhancement concerning for tumor growth, then this may be consistent with radiographic progression and the patient should be taken off study (Figure 2 in the original publication). If the lesion is stable or decreased in size, then this may be consistent with treatment effect and the patient may remain on study (Figure 3 in the original publication). For patients with equivocal results even on the next restaging scan, the scan may be repeated

again at a subsequent protocol scheduled evaluation or sooner although surgery and/or use of an advanced imaging modality are strongly encouraged. (2) Surgical pathology obtained via biopsy or resection. We should also note that these advanced imaging modalities have not been extensively studied with regards to immunotherapy-based approaches and therefore are cannot be recommended for distinguishing tumor progression versus immune-related changes at this time. Regardless of the additional testing obtained, if subsequent testing demonstrates that progression has occurred, the date of progression should be recorded as the date of the scan at which this issue was first raised. Patients may also have an equivocal finding on a scan (for example, a small lesion that is not clearly new). It is permissible to continue treatment until the next protocol scheduled evaluation. If the subsequent evaluation demonstrates that progression has indeed occurred, the date of progression should be recorded as the date of the initial scan where progression was suspected.

Notes Regarding Corticosteroid Use and Clinical Deterioration:

a) An increase in corticosteroid dose alone, in the absence of clinical deterioration related to tumor, will not be used as a sole determinant of progression. Patients with stable imaging studies whose corticosteroid dose was increased for reasons other than clinical deterioration related to tumor do not qualify for stable disease or progression. They should be observed closely. If their corticosteroid dose can be reduced back to baseline, they will be considered as having stable disease; if further clinical deterioration related to tumor becomes apparent, they will be considered to have progression.
b) The definition of clinical deterioration is left to the discretion of the treating physician, but it is recommended that a decline in the KPS from 100 or 90 to 70 or less, a decline in KPS of at least 20 points from 80 or less, or a decline in KPS from any baseline to 50 or less, for at least 7 days, be considered neurologic deterioration unless attributable to comorbid events, treatment-related toxicity, or changes in corticosteroid dose.

Summary of the Proposed RANO Response Criteria for CNS Metastases

Criterion	CR	PR	SD	PD
Target lesions	None	≥30% decrease in sum LD relative to baseline	<30% decrease relative to baseline but<20% increase in sum LD relative to nadir	≥20% increase in sum LD relative to nadir*
Non-target lesions	None	Stable or improved	Stable or improved	Unequivocal PD*
New lesion(s)**	None	None	None	Present*
Corticosteroids	None	Stable or decreased	Stable or decreased	NA+
Clinical status	Stable or improved	Stable or improved	Stable or improved	Worse*
Requirement for response	All	All	All	Any+

Abbreviations: CNS = central nervous system; CR = complete response; LD= longest dimension; NA = not applicable; PD = progressive disease; PR= partial response; RANO= Response Assessment in Neuro-Oncology; SD = stable disease.
*Progression occurs when this criterion is met.
**New lesion = new lesion not present on prior scans and visible in at least 2 projections. If a new lesion is equivocal, for example because of its small size, continued therapy may be considered, and follow up evaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan showing the new lesion. For immunotherapy based approaches, new lesions alone to do not define progression (See "Guidance in the Case of New Lesion(s) while on Immunotherapy").
+Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration.

11.2 Antitumor Effect – non-CNS disease

Response and progression in extracranial 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.

11.2.1 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.

11.2.2 Disease Parameters

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

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

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

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

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

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

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions**

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

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

11.2.3 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.

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.

11.2.4 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.

11.2.5 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).

11.2.6 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.

11.2.7 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	

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

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.
Note: Participants with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

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

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

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

11.2.8 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.

11.2.9 Clinical Benefit rate

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

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.

11.3.2 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.

11.3.3 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.

11.3.4 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).

11.3.5 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

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

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

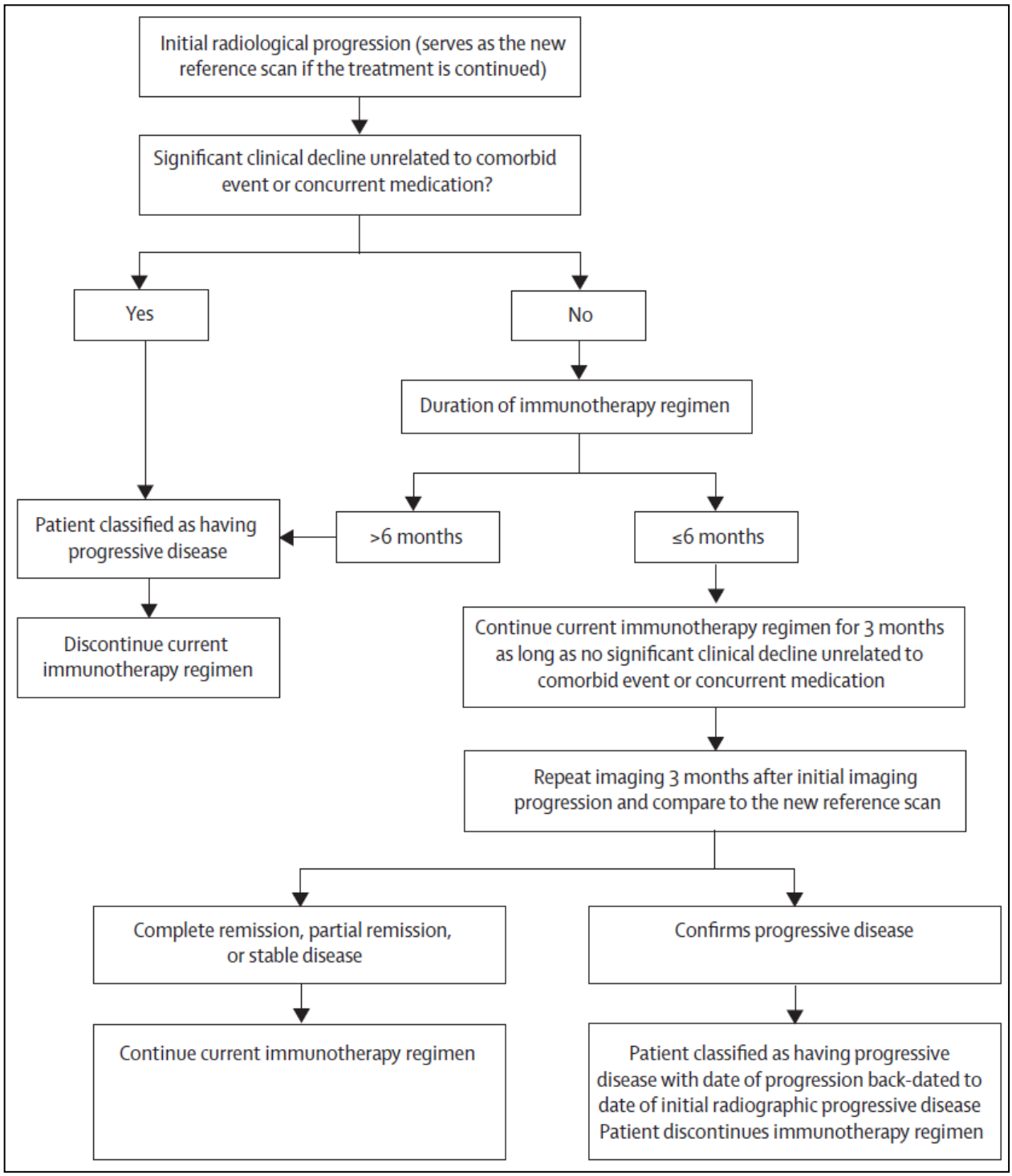
irCR or irPR determinations included in the irBOR assessment must be confirmed by a second (confirmatory) evaluation meeting the criteria for response and performed no less than 4 weeks after the criteria for response are first met.

11.3.7 Definition of immunotherapy Response Assessment in Neuro-Oncology (iRANO)

Standard RANO criteria differ according to whether the target population is high-grade glioma, low-grade brain tumors, or solid tumor brain metastases [Wen et al., 2010{Lin, 2015 #1185, Van Den Bent et al., 2011]}. Given the clinical success of modern immunothery trials, iRANO was designed to integrate seamlessly into these different "backbone" RANO criteria (Okada et al, 2015). iRANO takes an algorithmic approach as to whether a patient may remain on protocol therapy following the first "disease progression" event after beginning protocol threapy, and provides the ability for patients to remain on protocol therapy through this initial event. The key component of the iRANO criteria is specific additional guidance for the determination of progressive disease in patients with neuro-oncological malignancies undergoing immunotherapy (Figure 1). Specifically, the iRANO criteria advocate for the confirmation of radiographic progression in appropriate patients defined by clinical status and time from initiation of immunotherapy.

If disease progression is confirmed on subsequent scan(s), then the date of progression is backdated to the original date of radiographic worsening.

iRANO treatment algorithm for the assessment of progressive imaging fi ndings in patients with neuro-oncological malignancies undergoing immunotherapy [Okada *et al.*, 2015].



iRANO=immunotherapy Response Assessment in Neuro-Oncology.

In patients who have imaging findings that meet RANO criteria for progressive disease within 6 months of starting immunotherapy including the development of new lesions, confirmation of radiographic progression on follow-up imaging before defining the patient as nonresponsive to treatment might be needed provided that the patient does not have new or substantially worse neurological deficits. Such patients might be allowed a window of up 3 months before confirming disease progression with the scan that first showed initial progressive changes as the new reference scan for comparison with subsequent imaging studies.

If RANO criteria for progressive disease are met on the follow-up scan 3 months later, non-responsiveness to treatment should be assumed, and the date of progressive disease should be back-dated to the initial date when it was first identified (table 1). Patients who develop substantial new or worsened neurological deficits not due to comorbid events or a change in co-administered medication at any time within the 3-month follow-up window should be designated as non-responsive to treatment and should discontinue immunotherapy. For these patients, the date of actual tumor progression should also be back-dated to the date when radiographic progressive disease was initially identified.

If radiographic findings at the 3-month follow-up meet RANO criteria for stable disease, partial response, or complete response compared with the original scan meeting criteria for progression, and no new or worsened neurological deficits are identified, such patients should be deemed as deriving clinical benefit from therapy and allowed to continue treatment. Patients who develop worsening radiographic findings compared with the pretreatment baseline scan more than 6 months from starting immunotherapy are expected to have a low likelihood of ultimately deriving clinical benefit and should be regarded as non-responsive to treatment with a recommendation to discontinue therapy.

11.3.8 Progression-free Survival

RANO-BM proposes evaluating of progression-free survival according to a bi-compartmental model, i.e. each compartment (CNS and non-CNS) is evaluated separately, CNS according to RANO-BM and non-CNS according to RECIST 1.1. Progression in either compartment is deemed an overall progression event and site of first progression (CNS or non-CNS) is captured as a unique data element in the CRFs.

RECIST 1.1 uses instead a summation approach. With RECIST 1.1, up to 2 target lesions per organ may be assessed and the longest dimension of all target lesions (i.e. CNS and non-CNS) are summed for evaluation of response and progression. As with RANO-BM, unequivocal worsening of target lesions in either CNS or non-CNS compartments also constitutes a progression event. Unlike RANO-BM, RECIST 1.1 relies primarily on radiographic findings and does not include neurological status or corticosteroid use.

It is unknown what the correlation between RANO-BM and RECIST 1.1 is with respect to PFS and with respect to any relationships between PFS and OS. In this study, data will be collected prospectively to allow calculation of PFS according to both methods.

11.3.9 Patient-Reported Outcome Measure

The PRO outcome measure for this study is as follows: Scores from the MDASI-BT assessment (APPENDIX C)

11.3.10 Investigator-Assessed Neurological Evaluation

In order to standardize the evaluation of key neurological exam components, this study will use the Neurological Assessment in Neuro-Oncology (NANO) scale (APPENDIX D)[Nayak *et al.*, 2014]. The scale was developed by an international group of neuro-oncologists convened bi-weekly between June 2012 and July 2013 as an objective and quantifiable metric of neurologic function evaluable during a routine office examination that will integrate into the existing RANO criteria[Lin *et al.*, 2015]. The NANO scale is intended to serve as a quick, oncology-friendly, quantifiable, evaluation of eight relevant neurologic domains based on direct examination by clinicians during routine office visits. The scale defines criteria for domain-specific and overall scores of response, progression and stable disease. In addition, a given domain is scored non-assessed if the clinician neglects to examine the domain or non-evaluable if the domain cannot be accurately assessed due to pre-existing conditions, co-morbid events, and/or concurrent medications.

11.3.11 EQ-5D evaluation

In order to evaluate the impact of the study treatment, general health status will be assessed by EQ-5D questionarie (APPENDIX E).

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

Each investigative site is responsible for submitting data and/or data forms to the ODQ according to the schedule set by the ODQ.

12.2 Data Safety Monitoring

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

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

The Data and Safety Monitoring Plan (DSMP) for this study is located in Appendix O.

12.3 Multicenter Guidelines

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

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

12.4 Collaborative Research and Future Use of Samples

Tissue, blood, and CSF fluid 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. Collectively, this research may help doctors to better understand:

- Breast cancer, including causes of breast cancer, and reasons why past, current, and future treatments are effective or not
- The effects of the study treatment
- Which patients may benefit most, and least, from the study treatment
- The human microbiome and its role in disease onset and progression
- Other diseases

These samples will be stored in a laboratory at Dana-Farber/Brigham & Women's Hospital. The specimens will be retained indefinitely and will only be accessible by designated researchers.

These samples may be used for future research studies and may be provided to collaborating investigators both within and outside of DF/HCC. Samples and data may be shared with outside non-profit academic investigators as well as with for-profit pharmaceutical investigators or commercial entities, with whom we collaborate. When samples are sent to investigators, and when any research is performed on samples, the samples will be identified with a code, but will not contain patient identification information such as name, birthdate, or medical record numbers.

In order to allow the greatest amount of research to be performed on the study specimens, researchers for this study may share results of genetic sequencing with other scientists. De-identified specimens or genetic data may be placed into one or 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 phase II study to evaluate the efficacy of the combination of atezolizumab with pertuzumab plus high-dose trastuzumab for the treatment of CNS metastases in patients with HER2-positive metastatic breast cancer, as measured by ORR in the CNS. The target enrollment is 33 patients.

Primary Endpoint
The primary endpoint is the confirmed ORR in the CNS according to response assessment in neuro-oncology-brain metastases (RANO-BM) criteria (Section 11).

Secondary endpoints include:
Secondary endpoints include PFS according to RANO-BM bi-compartiment model, as well according to RECIST 1.1 single compartment model, objective extra-CNS response rates, according to RECIST 1.1, irRC, RANO-BM, and iRANO-BM criteria (as defined in Section 11); DOR, clinical benefit rate, OS, safety, tolerability, patient-reported outcome, and investigator-assessed neurological evaluation, and EQ-5D evaluation.

Correlative science objectives include:
- To explore whether the number and/or type of mutations identified using a next generation sequencing (NGS) panel is correlated with patient outcomes (PFS, CNS ORR, CBR, and OS).
- To collect blood to study cell-free DNA for quantification of tumor DNA content and copy number variation, using ultra-low pass whole genome sequencing, and to explore whether cfDNA load is associated with patient outcomes (PFS, CNS ORR, CBR, and OS).

- To collect blood to study cell-free DNA for targeted sequencing and/or whole exome sequencing
- To compare mutations and copy number variation between cfDNA and tumor biopsies.
- To characterize a broad array of immune markers in metastatic HER-2 positive breast cancer (characterization will be based on histology, protein expression, and mRNA expression), and their changes with immune checkpoint blockade.
- To explore how different immunosuppressive and/or immune-stimulating immune marker profiles at baseline correlate with patient outcomes (PFS, CNS ORR, CBR and OS).
- To characterize changes in immune marker profiles on treatment and at time of progression
- 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 PBMC correlates with clinical outcomes (PFS, CNS ORR, and OS).
- To collect cerebrospinal fluid (CSF) to study cell-free DNA for quantification of tumor DNA content and copy number variation, using ultra-low pass whole genome sequencing, and to compare patterns of cfDNA serially over time in CSF compared to plasma.
- To explore whether cfDNA load in CSF is associated with clinical outcomes (PFS, CNS ORR, CRR, and OS).
- To collect CSF to study cell-free DNA for targeted sequencing and/or whole exome sequencing before, on and after immunotherapy. To compare mutations and copy number variation between cfDNA in plasma versus CSF.

13.2 Sample Size, Accrual Rate and Study Duration

This study uses a Simon 'optimal' two-stage design with a one-sided type I error of 0.1 and type II error of 0.1 (90% power) to detect the difference between null (15%) and alternative (35%) CNS response rates. In the first stage, 19 patients will be enrolled. If less than 4 patients have a confirmed CNS response, the study will be discontinued after stage 1. If 4 or more patients have CNS response, the study will continue to stage 2 with additional 14 patients enrolled. If there are 8 or more responses among the 33 patients, the regimen will be declared worthy for further study. If the true response rate is 15%, the chance that the regimen is declared ineffective after stage 1 is 68.4% and the chance the regimen is declared ineffective after stage 2 is 90.4% (exact type I error=0.096). If the true response rate is 35%, the chance that the regimen is falsely declared ineffective is 9.6% (exact power = 90.4%).

H0	H1	Total # of patients	# of patients in stage 1	Maximal # of responses to claim regiment inactive in stage 1	Maximal total # of responses to claim regiment inactive in stage 2	Prob. of discontinuation after stage 1 if H0 is true
15%	35%	33	19	3	7	68.4%

13.3 Interim Monitoring Plan

An initial safety run-in of 6 patients who have received at least 1 dose of protocol therapy wil be included. After up to 6 patients are enrolled, a safety pause in accrual will be implemented. If 2 or more patients develop a DLT within the DLT reporting period (within 21 days of C1D1 treatment), the regimen will be deemed overly toxic and the study will close to further enrollment. Patients already enrolled and who are receiving clinical benefit will be allowed to continue on protocol therapy. However, no new patients will be enrolled. If 1 or fewer of the first 6 patients develop a DLT within the DLT reporting period, the study will re-open to accrual.

In addition, an interim analysis for futility is planned to minimize the likelihood of exposing study patients to an inactive regimen. The interim analysis will be performed after 19 patients who have received at least 1 dose of protocol therapy have been enrolled in the study and have been evaluated. If less than 4 patients have CNS response, the study will be discontinued after stage 1. If 4 or more patients have CNS response, the study will continue to stage 2 with an additional 14 patients enrolled. The design was done using a Simon 'optimal' two-stage design with a one-sided type I error of 0.1 and type II error of 0.1 (90% power). If the true response rate is 15%, the chance that the regimen is declared ineffective after stage 1 is 68.4%.

13.4 Analysis of Primary Endpoint

The primary endpoint is ORR in the CNS, which will be assessed among all patients who initiated protocol therapy. CNS response will be assessed using RANO-BM criteria as defined in section 11.

Patients who initiate protocol therapy will be in included in the efficacy analysis population. In the efficacy analysis population, any patient without sufficient data to determine response (e.g., non-evaluable patients) will be classified as a non-responder. The estimate of the ORR with 90% Clopper-Pearson exact CI will be presented.

13.5 Analysis of Secondary Endpoints

Safety Endpoints

The safety population consists of all patients who took at least one dose of any protocol treatment and who have at least one post-baseline safety assessment. Toxicity will be graded according to NCI CTCAE, Version 4.0. Toxicities will be summarized by maximum grade. Incidence rate of each toxicity will be reported.

Efficacy Endpoints

Duration of response (DOR) will be evaluated among patients who had CR or PR. DOR is defined as the time from CR or PR achieved until renewed disease progression is detected in the CNS. DOR will be calculated per RANO-BM criteria, and descriptive statistics will be used to summarize the intervals observed.

Bi-compartmental PFS per RANO-BM criteria, single-compartmental PFS per RECIST 1.1 criteria, and OS will be also analyzed using Kaplan–Meier product-limit estimatesand 90%

confidence bands. PFS is defined as the time from first dose of atezolizumab (day 1 cycle 1) to disease progression or death due to any cause, whichever occurred first. Patients alive without disease progression are censored at the date of last disease evaluation. OS is defined as the time from first dose of atezolizumab (day 1 cycle 1) to death due to any cause. If death was not observed, patients will be censored at the date they were last known alive.

CNS ORR per iRANO-BM criteria, extracrainial ORR per RECIST 1.1, and extracrainial ORR per irRC will be reported with 90% exact confidence intervals. Clinical benefit rate at 18 and 24 weeks is defined as CR, PR, SD, respectively, ≥ 18 and 24 weeks. Clinical benefit will be calculated using RANO-BM criteria. Clinical benefit rate will be reported respectively with 90% exact confidence intervals.

The sites of first progression (CNS vs. extracraniavs. both) will be tabulated respectively.

The M.D. Anderson Symptom Inventory-Brain Tumor (MDASI-BT) will be used to assess patient-reported outcomes. The MDASI-BT will assess 13 symptom items and 6 interference items from the core MDAST, as well as 9 symptoms specific to brain tumors. Scores of each item will be calculated following the MDASI-BT scoring guideline.

Patients' neurological function will be assessed using the Neurological Assessment in Neuro-Oncology (NANO) scale. Scoring of the NANO scale will follow the NANO scale socring guideline.

General heath status of patients enrolled in this study will be assessed thorugh EQ-5D questionarie.

Correlative endpoints

Analyses of correlative scientific endpoints are exploratory and hypothesis-generating. Any promising findings will be tested in future studies.

Among the patients who agree to undergo optional extracranial biopsies, we will describe the landscape of somatic mutations, copy number alterations, and characterize the immune microenvironment. We will explore whether the mutation burden (as assessedd in fresh tumor specimens, plasma cfDNA, or CSF cfDNA) is correlated with patient outcomes (PFS, CNS ORR, CBR, and OS).

We aim to assess cfDNA in the CSF for several purposes; 1) to evaluate the tumor fraction (TFx) using ultra low-pass whole genome sequencing (UL-WGS) and to explore whether baseline or on-study TFx correlates with clinical outcomes; 2) for those patients with CSF TFx ≥ 10%, to perform whole exome sequencing (WES) and to compare this with WES results from plasma cfDNA in the same patients; 3) to describe the trajectory of TFx at baseline, on-study, and time of progression; 4) to describe copy number, mutations, and mutational load at baseline and time of progression in the cfDNA derived from CSF.

To explore the relationship between correlative endpoints obtained from cerebrospinal fluid (CSF) to genetic alterations detected in the tumor and plasma, patient and disease characteristics, and clinical outcomes, the following analyses are planned: cell-free DNA (cfDNA) from serial

CSF samples and plasma will be quantified using ultra-low pass whole genome sequencing, evaluated as both a continuous factor, and using the pre-defined threshold of TFx > 10% as a dichotomous variable; whole exome sequencing (WES) will be performed to determine copy number and mutation calls. We will describe and compare mutations, copy number variation, and tumor mutational burden between cfDNA in blood and CSF both at baseline, as well as in baseline versus time-of-progression samples.

For paired assessments of genomic alterations comparing between cfDNA in plasma and CSF, concordance will be assessed as the proportion of overall agreement using bootstrapped standard error estimates and confidence intervals, and kappa statistics to assess non-zero agreement. The following table shows the true Cohen's Kappa statistic there will be 80% power to detect given the prevalence of the phenotype, number of paired samples, and using a two-sided alpha = 0.05

Prevalence of phenotype	# of paired samples (plasma cfDNA and CSF cfDNA)	True Cohen's kappa
20%	20	0.63
	30	0.53
30%	20	0.61
	30	0.51
40%	20	0.60
	30	0.50

The association of baseline CSF and blood assessments to PFS and OS will be explored using Kaplan-Meier estimation and Cox proportional hazard models, and the association to ORR and CBR will be assessed using logistic models. Serial assessments will be characterized using descriptive statistics, and the association to clinical outcome will be modeled as post-baseline time-varying covariates (PFS and OS) and longitudinal mixed effects models (ORR and CBR). All analyses will be exploratory and hypothesis generating and point estimates will be reported with 95% confidence intervals.

13.6 Reporting and Exclusions

13.6.1 Evaluation of Efficacy

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

13.6.2 Evaluation of Safety

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

safety data of any kind would be excluded.

14. PUBLICATION PLAN

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

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APPENDIX A PERFORMANCE STATUS CRITERIA

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

APPENDIX B ANAPHYLAXIS PRECAUTIONS

EQUIPMENT NEEDED

- Monitoring devices: ECG monitor, blood pressure monitor, oxygen saturation monitor, and thermometer
- Oxygen
- Epinephrine for intravenous, intramuscular, and endotracheal administration in accordance with institutional guidelines
- Antihistamines
- Corticosteroids
- Intravenous infusion solutions, tubing, catheters, and tape

PROCEDURES

In the event of a suspected anaphylactic reaction during study treatment infusion, the following procedures should be performed:

1. Stop the study treatment infusion.
2. Call for additional medical assistance.
3. Ensure that appropriate monitoring is in place, with continuous ECG and pulse oximetry monitoring, if possible.
4. Administer antihistamines, epinephrine, or other medications as required by participant status and as directed by the physician in charge.
5. Continue to observe the participant and document observations.
6. Draw serum/plasma samples for immunogenicity testing.

Ask participant to return for washout immunogenicity sample if appropriate.

APPENDIX C M.D. ANDERSON SYMPTOM INVENTORY-BRAIN TUMOR (MDASI-BT)

The MDASI-BT consists of 28 items and is a multi-symptom measure of cancer-related symptoms that are sensitive to disease and treatment changes. The MDASI-BT is composed of the symptom severity scale and the symptom interference scale. In the symptom severity scale, patients rate the severity of their symptoms in the last 24 hours on 0 − 10 numeric scales, ranging from "not present" to "as bad as you can imagine." In the symptom interference scale, patients rate interference with daily activities caused by their symptoms on 0 − 10 numeric scales ranging from "did not interfere" to "interfered completely." This instrument is brief, takes less than five minutes to complete, is easily understood and validated in the cancer population[Armstrong *et al.*, 2006].

The English and Spanish versions of the MDASI-BT are below.

Date: _____ **Institution:** _____

Participant Initials: _____ **Hospital Chart #:** _____

Participant Number: _____

MD Anderson Symptom Inventory - Brain Tumor (MDASI - BT)

Part I. How **severe** are your symptoms?

People with cancer frequently have symptoms that are caused by their disease or by their treatment. We ask you to rate how severe the following symptoms have been ***in the last 24 hours.*** Please select a number from 0 (symptom has not been present) to 10 (the symptom was as bad as you can imagine it could be) for each item.

	Not Present 0	1	2	3	4	5	6	7	8	9	As Bad As You Can Imagine 10
1. Your **pain** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
2. Your **fatigue (tiredness)** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
3. Your **nausea** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
4. Your **disturbed sleep** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
5. Your feelings of being **distressed (upset)** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
6. Your **shortness of breath** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
7. Your problem with **remembering things** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
8. Your problem with **lack of appetite** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
9. Your feeling **drowsy (sleepy)** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
10. Your having a **dry mouth** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
11. Your feeling **sad** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
12. Your **vomiting** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
13. Your **numbness or tingling** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
14. Your **weakness** on one side of the body at its WORST?	○	○	○	○	○	○	○	○	○	○	○
15. Your difficulty **understanding** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
16. Your difficulty **speaking (finding the words)** at its WORST?	○	○	○	○	○	○	○	○	○	○	○

Date: _____ **Institution:** _____

Participant Initials: _____ **Hospital Chart #:** _____

Participant Number: _____

	Not Present 0	1	2	3	4	5	6	7	8	9	As Bad As You Can Imagine 10
17. Your **seizures** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
18. Your difficulty **concentrating** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
19. Your **vision** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
20. Your change in **appearance** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
21. Your change in **bowel pattern (diarrhea or constipation)** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
22. Your **irritability** at its WORST?	○	○	○	○	○	○	○	○	○	○	○

Part II. How have your symptoms **interfered** with your life?

Symptoms frequently interfere with how we feel and function. How much have your symptoms interfered with the following items ***in the last 24 hours***? Please select a number from 0 (symptoms have not interfered) to 10 (symptoms interfered completely) for each item.

	Did Not Interfere 0	1	2	3	4	5	6	7	8	9	Interfered Completely 10
23. **General activity?**	○	○	○	○	○	○	○	○	○	○	○
24. **Mood?**	○	○	○	○	○	○	○	○	○	○	○
25. **Work (including work around the house)?**	○	○	○	○	○	○	○	○	○	○	○
26. **Relations with other people?**	○	○	○	○	○	○	○	○	○	○	○
27. **Walking?**	○	○	○	○	○	○	○	○	○	○	○
28. **Enjoyment of life?**	○	○	○	○	○	○	○	○	○	○	○

Fecha: _____ **Institución:** _____

Iniciales del participante: _____ **Planilla del hospital N.º:** _____

Número del participante: _____

Cuestionario básico de síntomas M D Anderson (Tumor Cerebral) (MDASI-BT)

Parte I: ¿Qué tan **severos (graves)** son sus síntomas?

Las personas con cáncer frecuentemente tienen síntomas causados por la enfermedad o el tratamiento. Le pedimos que califique qué tan severos han sido los siguientes síntomas ***durante las últimas 24 horas***. Para cada pregunta, por favor, llene el círculo que represente qué tan severo fue el síntoma, teniendo en cuenta que 0 representa que el síntoma no estuvo presente y 10 significa que el síntoma fue el peor que pueda imaginar (marque un solo círculo).

	No Estuvo Presente										**El Peor Que Pueda Imaginar**
	0	1	2	3	4	5	6	7	8	9	10
1. ¿Su PEOR **dolor**?	○	○	○	○	○	○	○	○	○	○	○
2. ¿Su PEOR **fatiga (cansancio)**?	○	○	○	○	○	○	○	○	○	○	○
3. ¿Su PEOR **náusea**?	○	○	○	○	○	○	○	○	○	○	○
4. ¿Su PEOR **desvelo**?	○	○	○	○	○	○	○	○	○	○	○
5. ¿Su PEOR **sufrimiento emocional**?	○	○	○	○	○	○	○	○	○	○	○
6. ¿Su PEOR **falta de aire**?	○	○	○	○	○	○	○	○	○	○	○
7. ¿Su PEOR **dificultad para recordar** las cosas?	○	○	○	○	○	○	○	○	○	○	○
8. ¿Su PEOR **falta de apetito**?	○	○	○	○	○	○	○	○	○	○	○
9. ¿Su PEOR **somnolencia (adormilado)**?	○	○	○	○	○	○	○	○	○	○	○
10. ¿Su PEOR **sequedad bucal**?	○	○	○	○	○	○	○	○	○	○	○
11. ¿Su PEOR **tristeza**?	○	○	○	○	○	○	○	○	○	○	○
12. ¿Su peor **vómito**?	○	○	○	○	○	○	○	○	○	○	○
13. ¿Su PEOR **adormecimiento, entumecimiento,** u **hormigueo**?	○	○	○	○	○	○	○	○	○	○	○
14. ¿Su PEOR **debilidad** en un lado del cuerpo?	○	○	○	○	○	○	○	○	○	○	○
15. ¿Su PEOR **dificultad para comprender**?	○	○	○	○	○	○	○	○	○	○	○
16. ¿Su PEOR **dificultad para hablar (encontrar las palabras adecuadas)**?	○	○	○	○	○	○	○	○	○	○	○

Fecha: _____ **Institución:** _____

Iniciales del participante: _____ **Planilla del hospital N.º:** _____

Número del participante: _____

	No Estuvo Presente 0	1	2	3	4	5	6	7	8	9	El Peor Que Pueda Imaginar 10
17. ¿Sus PEORES **ataques o espasmos en su cuerpo**?	○	○	○	○	○	○	○	○	○	○	○
18. ¿Su PEOR **dificultad para concentrarse**?	○	○	○	○	○	○	○	○	○	○	○
19. ¿Su PEOR **dificultad para ver**?	○	○	○	○	○	○	○	○	○	○	○
20. ¿Su PEOR alteración en su **aspecto físico**?	○	○	○	○	○	○	○	○	○	○	○
21. ¿Su PEOR **(diarrea o estreñimiento)**?	○	○	○	○	○	○	○	○	○	○	○
22. ¿Su PEOR **irritabilidad**?	○	○	○	○	○	○	○	○	○	○	○

Parte II. ¿Cómo han **interferido (afectado)** sus síntomas con su estilo de vida?

Los síntomas frecuentemente interfieren con lo que sentimos y con lo que hacemos. ***En las últimas 24 horas***, ¿qué tanto han interferido sus síntomas con lo siguiente? Para cada pregunta, por favor seleccione un número del 0 (sus síntomas no han interferido) al 10 (sus síntomas han interferido completamente).

	No Han Interferido 0	1	2	3	4	5	6	7	8	9	Interfirieron Totalmente 10
23. **¿Actividad en general?**	○	○	○	○	○	○	○	○	○	○	○
24. **¿Estado de ánimo?**	○	○	○	○	○	○	○	○	○	○	○
25. **¿Trabajo normal (incluyendo los que haceres del hogar)?**	○	○	○	○	○	○	○	○	○	○	○
26. **¿Relaciones con otras personas?**	○	○	○	○	○	○	○	○	○	○	○
27. **¿Capacidad para caminar?**	○	○	○	○	○	○	○	○	○	○	○
28. **¿El poder disfrutar de la vida?**	○	○	○	○	○	○	○	○	○	○	○

APPENDIX D NEUROLOGICAL ASSESSMENT IN NEURO-ONCOLOGY (NANO) SCALE

Neurologic Assessment in Neuro-Oncology (NANO) Scale

Scoring assessment is based on direct observation and testing performed during clinical evaluation and is not based on historical information or reported symptoms. Please check 1 answer per domain. Please check "Not assessed" if testing for that domain is not done. Please check "Not evaluable" if a given domain cannot be scored accurately due to pre-existing conditions, co-morbid events and/or concurrent medications.

Date Assessment Performed (day/month/year): _____
Study time point (i.e. baseline, cycle 1, day 1, etc):_____
Assessment performed by (please print name): _____

Domains	**Key Considerations**
Gait 0 ☐ Normal 1 ☐ Abnormal but walks without assistance 2 ☐ Abnormal and requires assistance (companion, cane, walker, etc.) 3 ☐ Unable to walk ☐ Not assessed ☐ Not evaluable	• Walking is ideally assessed by at least 10 steps
Strength 0 ☐ Normal 1 ☐ Movement present but decreased against resistance 2 ☐ Movement present but none against resistance 3 ☐ No movement ☐ Not assessed ☐ Not evaluable	• Test each limb separately • Recommend assess proximal (above knee or elbow) and distal (below knee or elbow) major muscle groups • Score should reflect worst performing area • Patients with baseline level 3 function in one major muscle group/limb can be scored based on assessment of other major muscle groups/limb
Ataxia (upper extremity) 0 ☐ Able to finger to nose touch without difficulty 1 ☐ Able to finger to nose touch but difficult 2 ☐ Unable to finger to nose touch ☐ Not assessed ☐ Not evaluable	• Non-evaluable if strength is compromised • Trunk/lower extremities assessed by gait domain • Particularly important for patients with brainstem and cerebellar tumors • Score based on best response of at least 3 attempts
Sensation 0 ☐ Normal 1 ☐ Decreased but aware of sensory modality 2 ☐ Unaware of sensory modality ☐ Not assessed ☐ Not evaluable	• Recommend evaluating major body areas separately (face, limbs and trunk) • Score should reflect worst performing area • Sensory modality includes but not limited to light touch, pinprick, temperature and proprioception • Patients with baseline level 2 function in one major body area can be scored based on assessment of other major body areas

Visual Fields

- 0 ☐ Normal
- 1 ☐ Inconsistent or equivocal partial hemianopsia (≥quadrantopsia)
- 2 ☐ Consistent or unequivocal partial hemianopsia (≥quadrantopsia)
- 3 ☐ Complete hemianopsia
- ☐ Not assessed
- ☐ Not evaluable

- Patients who require corrective lenses should be evaluated while wearing corrective lenses
- Each eye should be evaluated and score should reflect the worst performing eye

Facial Strength

- 0 ☐ Normal
- 1 ☐ Mild/moderate weakness
- 2 ☐ Severe facial weakness
- ☐ Not assessed
- ☐ Not evaluable

- Particularly important for brainstem tumors
- Weakness includes nasolabial fold flattening, asymmetric smile and difficulty elevating eyebrows

Language

- 0 ☐ Normal
- 1 ☐ Abnormal but easily conveys meaning to examiner
- 2 ☐ Abnormal and difficulty conveying meaning to examiner
- 3 ☐ Abnormal. If verbal, unable to convey meaning to examiner. OR non-verbal (mute/global aphasia)
- ☐ Not assessed
- ☐ Not evaluable

- Assess based on spoken speech. Non-verbal cues or writing should not be included.
- **Level 1**: Includes word finding difficulty; few paraphasic errors/neologisms/word substitutions; but able to form sentences (full/broken)
- **Level 2**: Includes inability to form sentences (<4 words per phrase/sentence); limited word output; fluent but "empty" speech.

Level of Consciousness

- 0 ☐ Normal
- 1 ☐ Drowsy (easily arousable)
- 2 ☐ Somnolent (difficult to arouse)
- 3 ☐ Unarousable/coma
- ☐ Not assessed
- ☐ Not evaluable

- None

Behavior

- 0 ☐ Normal
- 1 ☐ Mild/moderate alteration
- 2 ☐ Severe alteration
- ☐ Not assessed
- ☐ Not evaluable

- Particularly important for frontal lobe tumors
- Alteration includes but is not limited to apathy, disinhibition and confusion
- Consider subclinical seizures for significant alteration

NANO response criteria:

Definition of Neurologic Response: An overall NANO score will be determined following assessment of each domain and will include one of five possible outcomes: neurologic response; neurologic progression; neurologic stability; not assessed; and non-evaluable.

Neurologic response: ≥ 2 level improvement in at least 1 domain without worsening in other domains from baseline or best level of function.

Neurologic progression: 1) ≥ 2 level worsening from baseline or best level of function within ≥ 1 domain; or 2) worsening to the highest score within ≥ 1 domain.

Neurologic stability: a score of neurologic function that does not meet criteria for neurologic response, neurologic progression, non-evaluable or not assessed.

Non-evaluable (NE): if it is more likely than not that a factor other than underlying tumor activity contributed to an observed change in neurologic function. Such factors may include changes in concurrent medications or a co-morbid event.

Not assessed (NA): if the clinician omits evaluation of that particular domain during their examination. If a particular domain is marked NA at baseline, then that domain cannot be considered for progression or response.

In general, the assessment and scoring of all domains is encouraged.

APPENDIX E EQ-5D ENGLISH QUESTIONNARIE

Health Questionnaire

English version for the USA

Under each heading, please check the ONE box that best describes your health TODAY.

MOBILITY

I have no problems walking ❑
I have slight problems walking ❑
I have moderate problems walking ❑
I have severe problems walking ❑
I am unable to walk ❑

SELF-CARE

I have no problems washing or dressing myself ❑
I have slight problems washing or dressing myself ❑
I have moderate problems washing or dressing myself ❑
I have severe problems washing or dressing myself ❑
I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑
I have slight problems doing my usual activities ❑
I have moderate problems doing my usual activities ❑
I have severe problems doing my usual activities ❑
I am unable to do my usual activities ❑

PAIN / DISCOMFORT

I have no pain or discomfort ❑
I have slight pain or discomfort ❑
I have moderate pain or discomfort ❑
I have severe pain or discomfort ❑
I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

I am not anxious or depressed	❑
I am slightly anxious or depressed	❑
I am moderately anxious or depressed	❑
I am severely anxious or depressed	❑
I am extremely anxious or depressed	❑

We would like to know how good or bad your health is TODAY.

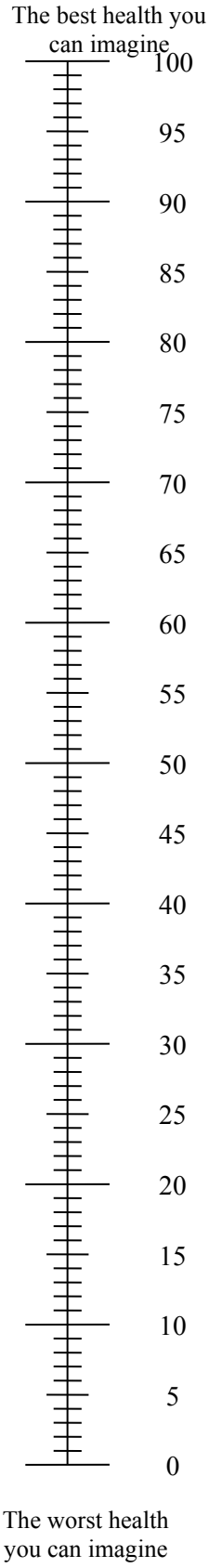
This scale is numbered from 0 to 100.

100 means the best health you can imagine.
0 means the worst health you can imagine.

Mark an X on the scale to indicate how your health is TODAY.

Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



APPENDIX F EQ-5D SPANISH QUESTIONNAIRE

Cuestionario de Salud

Versión en español para los EE. UU.

(Spanish version for the USA)

Debajo de cada encabezamiento, marque UNA casilla, la que mejor describe su salud HOY.

MOVILIDAD

No tengo problemas para caminar ❑
Tengo problemas leves para caminar ❑
Tengo problemas moderados para caminar ❑
Tengo problemas graves para caminar ❑
No puedo caminar ❑

CUIDADO PERSONAL

No tengo problemas para lavarme o vestirme solo/a ❑
Tengo problemas leves para lavarme o vestirme solo/a ❑
Tengo problemas moderados para lavarme o vestirme solo/a ❑
Tengo problemas graves para lavarme o vestirme solo/a ❑
No puedo lavarme o vestirme solo/a ❑

ACTIVIDADES DE TODOS LOS DÍAS *(Ej.: trabajar, estudiar, hacer las tareas domésticas, actividades familiares o actividades de ocio)*

No tengo problemas para realizar mis actividades de todos los días ❑
Tengo problemas leves para realizar mis actividades de todos los días ❑
Tengo problemas moderados para realizar mis actividades de todos los días ❑
Tengo problemas graves para realizar mis actividades de todos los días ❑
No puedo realizar mis actividades de todos los días ❑

DOLOR / MALESTAR

No tengo dolor ni malestar ❑
Tengo dolor o malestar leve ❑
Tengo dolor o malestar moderado ❑
Tengo dolor o malestar intenso ❑
Tengo dolor o malestar extremo ❑

ANSIEDAD / DEPRESIÓN

No estoy ansioso/a ni deprimido/a	❑
Estoy levemente ansioso/a o deprimido/a	❑
Estoy moderadamente ansioso/a o deprimido/a	❑
Estoy muy ansioso/a o deprimido/a	❑
Estoy extremadamente ansioso/a o deprimido/a	❑

Nos gustaría saber lo buena o mala que es su salud HOY.

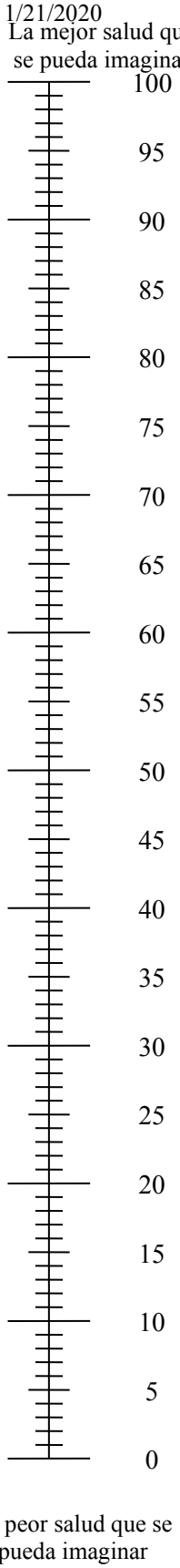
La escala está numerada de 0 a 100.

100 representa la mejor salud que se pueda imaginar.
0 representa la peor salud que se pueda imaginar.

Por favor haga una X en la escala para indicar cuál es su estado de salud HOY.

Ahora, por favor escriba en la casilla que encontrará a continuación el número que ha marcado en la escala.

SU SALUD HOY = ☐



APPENDIX G SPECIMEN REQUISITION FORM

Complete this form and include with the specimen shipment. Label ALL materials with participant initials, DFCI participant study ID, and the date the specimen was obtained. **Include a pathology report with any archival tissue specimens being submitted.**

Ship specimen(s) to: Brigham and Women's Hospital, Attn: Breast Tissue/Blood Bank, Thorn Building – Room 428, 20 Shattuck Street, Boston, MA 02115

Subject Information

Participant Initials (FML): _____ Participant Study ID: _____ DFCI Assigned ONC ID: _____

Date of specimen shipment: _____

Specimen Type *(indicate inclusion in shipment by checking box)*	**Quantity submitted** (#tubes or cores)	**Date sample collected**	**Timepoint**	**Comments**
☐ Five 10 ml green top tubes whole blood			☐ Baseline ☐ Cycle _____ ☐ Progression	
☐ One 9mL Streck Tube whole blood				
Fresh Tissue ☐ Biopsy core in RNA Later ☐ Biopsy cores in formalin ☐ Biopsy cores in DMEM (frozen in OCT for Northwestern)			☐ Baseline ☐ Cycle 2 ☐ Progression	
Archival Tissue ☐ FFPE Block (archival tissue) **OR** ☐ 15 4-micron unstained slides **AND** ☐ Pathology Report				Primary or Metastatic sample? (circle one)
Cerebrospinal Fluid (CSF) ☐ Two 10 mL sterile collection tubes (Streck Tubes for Northwestern)			☐ Baseline ☐ Before Cycle 3 ☐ Progression	

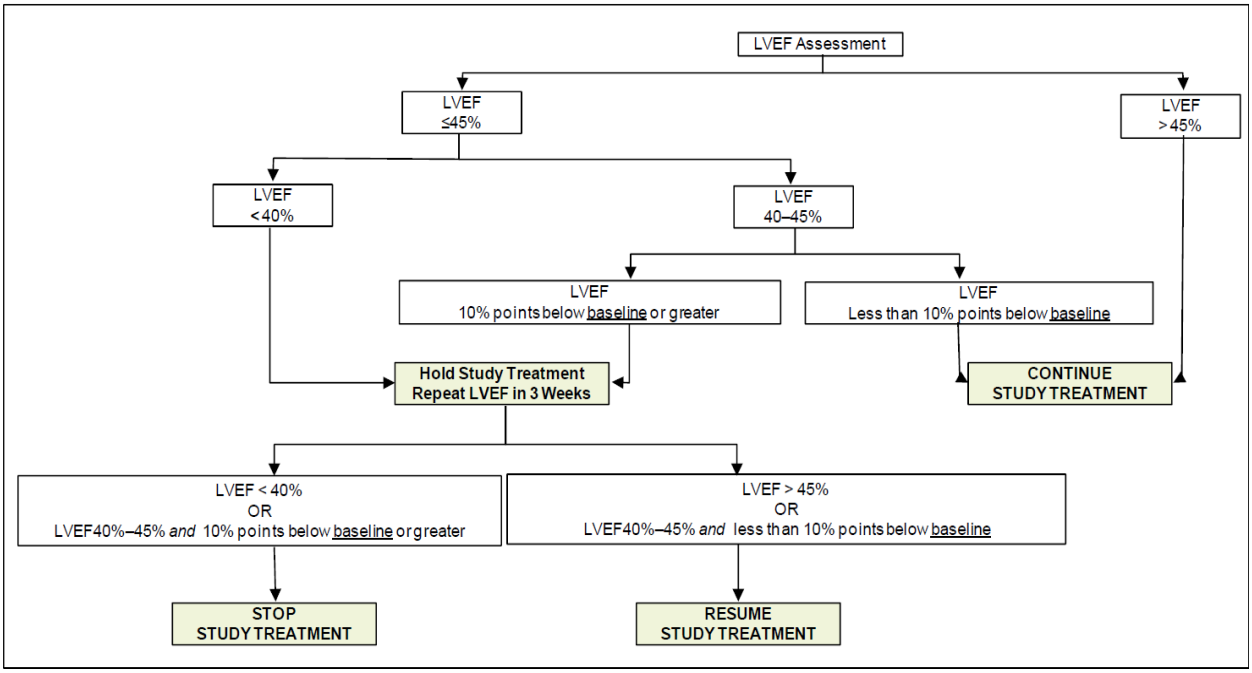
Site Responsible Contact:_____

Email:_____

Phone Number: _____

APPENDIX H ASYMPTOMATIC DECLINE IN LVEF

Algorithm for Continuation and Discontinuation of Atezolizumab, Pertuzumab and Trastuzumab Based on LVEF Assessments



APPENDIX I NEW YORK HEART ASSOCIATION CLASSIFICATION OF FUNCTIONAL CARDIAC CAPACITY

Class	
I	No limitation: Ordinary physical activity does not cause undue fatigue, dyspnea, or palpitation.
II	Slight limitation of physical activity: Such patients are comfortable at rest. Ordinary physical activity results in fatigue, palpitations, dyspnea, or angina.
III	Marked limitation of physical activity: Although patients are comfortable at rest, less than ordinary physical activity will lead to symptoms.
IV	Inability to carry on physical activity without discomfort: Symptoms of congestive heart failure are present even at rest. With any physical activity, increased discomfort is experienced.

From: Criteria Committee, New York Heart Association, Inc. Diseases of the heart and blood vessels. Nomenclature and criteria for diagnosis. 6th ed. Boston, Little, Brown and Co, 1964:114.

APPENDIX J LEFT VENTRICULAR SYSTOLIC DYSFUNCTION GRADING

NCI CTCAE Grade	Left Ventricular Systolic Dysfunction Severity
1 –	
2 –	
3	Symptomatic due to drop in ejection fraction responsive to intervention
4	Refractory or poorly controlled heart failure due to drop in ejection fraction; intervention such as ventricular assist device, intravenous vasopressor support, or heart transplant indicated
5	Death

NCI CTCAE = National Cancer Institute Common Terminology Criteria for Adverse Events.
Note: Based on the most recent version of NCI CTCAE (v 4.0), which can be found at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm
LVSD Definition: A disorder characterized by failure of the left ventricle to produce adequate output despite an increase in distending pressure and in end-diastolic volume. Clinical manifestations may include dyspnea, orthopnea, and other signs and symptoms of pulmonary congestion and edema.

APPENDIX K REPORTING CONVENTIONS FOR LEFT VENTICULAR SYSTOLIC DYSFUNCTION/HEART FAILURE

Observation	How to Report	Term to be Reported	Grading
Asymptomatic decline in LVEF $<$10% points from baseline or tan an LVEF $\geq$45%	No additional reporting required, LVEF results to be reported on eCRF	N/A	N/A
Asymptomatic decline in LVEF $\geq$10% points from baseline or tan an LVEF $<$45%	AE (eCRF)	"Ejection fraction decreased" [a]	NCI CTCAE for "ejection fraction decreased"
Asymptomatic decline in LVEF requiring treatment or leading to discontinuation of pertuzumab and trastuzumab	AE (eCRF) and on-serious AESI (reported on an SAE form)	"Ejection fraction decreased" [a]	NCI CTCAE for "ejection fraction decreased"
Heart failure/CHF (symptomatic LVSD)	AE (eCRF) and SAE (SAE form)	"Heart failure"	NCI CTCAE for "heart failure" and NYHA Class

AE = adverse event; CHF = congestive heart failure; eCRF = electronic Case Report Form; LVEF = left ventricular ejection fraction; LVSD = left ventricular systolic dysfunction; N/A = not applicable; NCI CTCAE = National Cancer Institute Common Terminology Criteria for Adverse Events; NYHA = New York Heart Association; SAE = serious adverse event.
Note: Any symptomatic LVSD event must be reported as "heart failure."
[a] Report the status "asymptomatic" and the LVEF value in the comments field as appropriate.

APPENDIX L: GENERAL IMPRESSION WORKSHEET
(to be completed at baseline and at the end of each 3-week cycle)

Patient_____Examiner_____Date_____

In the opinion of the treating physician, overall, has the patient had clinical deterioration since baseline?

( ) YES

( ) NO

In the opinion of the treating physician, overall, has the patient had clinical deterioration since his/her last visit

( ) YES

( ) NO

Is the patient currently taking corticosteroids?

( ) YES

( ) NO

If yes, please list name of medication and dose (e.g. decadron, 4 mg QD):
_____

Please indicate the patient's Karnofsky Perfomance Status (see Appendix A for definitions): _____

APPENDIX M GUIDELINES FOR COLLECTIING 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 after the procedure, or according to standard institutional guidelines. After liver biopsies, patients will be observed a minimum of 4 hours (range 4-6 hours) after the procedure, or according to standard institutional guidelines. Less than the goal quantity of tissue is accepted for each type of biopsy, and will be left to the clinical judgment of the physician performing the procedure.

Risks of Anesthesia

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

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

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

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

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

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

For Effusions and Ascites

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

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

For Fine Needle Aspiration Samples

A goal of 3 passes:

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

APPENDIX N GENENTECH SAFETY REPORTING FAX COVERSHEET

Genentech
A Member of the Roche Group

SAFETY REPORTING FAX COVER SHEET

Genentech Supported Research

AE / SAE FAX No: (650) 238-6067

Genentech Study Number	
Principal Investigator	
Site Name	
Reporter name	
Reporter Telephone #	
Reporter Fax #	

Initial Report Date	[DD] / [MON] / [YY]
Follow-up Report Date	[DD] / [MON] / [YY]

Subject Initials (Enter a dash if patient has no middle name)	[ ] - [ ] - [ ]

SAE or Safety Reporting questions, contact Genentech Drug Safety: (888) 835-2555

PLEASE PLACE MEDWATCH REPORT or SAFETY REPORT BEHIND THIS COVER SHEET

Version 2
Effective 14-Jan-2016

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

DFCI IRB Protocol #: 17-546

TABLE OF CONTENTS

15. INTRODUCTION

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

15.1 Purpose

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

15.2 Multi-Center Data and Safety Monitoring Plan Definitions

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

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

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

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

Coordinating Center: The entity (i.e. Lead Institution, Medical Monitor, Contract Research Organization (CRO), etc) that provides administrative support to the DF/HCC

Sponsor in order that he/she may fulfill the responsibilities outlined in the protocol document and DSMP, and as specified in applicable regulatory guidelines (i.e. CTEP Multi-Center Guidelines). In general, the Lead Institution is the Coordinating Center for the DF/HCC Multi-Center Protocol.

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

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

16. GENERAL ROLES AND RESPONSIBILITIES

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

16.1 DF/HCC Sponsor

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

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

- Identify and qualify Participating Institutions and obtain accrual commitments prior to extending the protocol to that site.
- Monitor accrual and address Participating Institutions that are not meeting their accrual requirements.

16.2 Coordinating Center

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

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

16.3 Participating Institution

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

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

- Document the delegation of research specific activities to study personnel.
- Commit to the accrual of participants to the protocol.
- Submit protocol and/or amendments to their local IRB of record.
- Maintain regulatory files as per sponsor requirements.

- Provide the Coordinating Center with regulatory documents or source documents as requested.
- Participate in protocol training prior to enrolling participants and throughout the trial as required (i.e. teleconferences).
- Update Coordinating Center with research staff changes on a timely basis.
- Register participants through the Coordinating Center prior to beginning research related activities.
- Submit Serious Adverse Event (SAE) reports to local IRB per institutional requirements and to the Coordinating Center, in accordance with DF/HCC requirements.
- Submit protocol deviations and violations to local IRB per institutional requirements and to the DF/HCC Sponsor in accordance with DF/HCC requirements.
- Order, store and dispense investigational agents and/or other protocol mandated drugs per federal guidelines and protocol requirements.
- Have office space, office equipment, and internet access that meet HIPAA standards.
- Participate in any quality assurance activities and meet with monitors or auditors at the conclusion of a visit to review findings.
- Promptly provide follow-up and/or corrective action plans for any monitoring queries or audit findings.

17. DF/HCC REQUIREMENTS FOR MULTI-CENTER PROTOCOLS

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

17.1 Protocol Distribution

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

17.2 Protocol Revisions and Closures

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

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

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

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

17.3 Informed Consent Requirements

The DF/HCC approved informed consent document will serve as a template for the informed consent for Participating Institutions. The Participating Institution consent form must follow the consent template as closely as possible and should adhere to specifications outlined in the DF/HCC Guidance Document on Model Consent Language for Investigator-Sponsored Multi-Center Trials. This document will be provided separately to each Participating Institution upon request.

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

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

17.4 IRB Documentation

The following must be on file with the Coordinating Center:

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

17.5 IRB Re-Approval

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

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

17.6 Participant Confidentiality and Authorization Statement

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

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

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

17.6.1 DF/HCC Multi-Center Protocol Confidentiality

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

17.7 DF/HCC Multi-Center Protocol Registration Policy

17.7.1 Participant Registration and Randomization

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

The Coordinating Center will review the submitted documents in order to verify eligibility and consent. To complete the registration process, the Coordinating Center will:

- Register the participant on the study with the DF/HCC Clinical Trial Management System (CTMS).
- Upon receiving confirmation of registration, the Coordinating Center will inform the Participating Institution and provide the study specific participant case number, and, if applicable, assigned treatment and/or dose level.

Treatment or other protocol-specific interventions may not begin without confirmation from the Coordinating Center that the participant has been registered.

17.7.2 Initiation of Therapy

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

17.7.3 Eligibility Exceptions

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

17.8 DF/HCC Protocol Case Number

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

17.8.1 Protocol Deviations, Exceptions and Violations

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

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

17.8.2 Definitions

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

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

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

17.8.3 Reporting Procedures

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

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

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

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

17.9 Safety Assessments and Toxicity Monitoring

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

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

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

17.9.1 Guidelines for Reporting Serious Adverse Events

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

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

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

17.9.2 Guidelines for Processing IND Safety Reports

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

17.10 Data Management

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

17.10.1 Data Forms Review

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

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

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

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

18. REQUISITIONING INVESTIGATIONAL DRUG

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

Participating Institutions should order their own agent regardless of the supplier. (i.e., NCI or a pharmaceutical company.)

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

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

19. MONITORING: QUALITY CONTROL

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

19.1 Ongoing Monitoring of Protocol Compliance

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

The Coordinating Center will implement ongoing monitoring activities to ensure that Participating Institutions are complying with regulatory and protocol requirements, data quality, and participant safety. Monitoring practices may include but are not limited to source data verification, and review and analysis of eligibility requirements, informed consent procedures, adverse events and all associated documentation, review of study drug administration/treatment, regulatory files, protocol departures reporting, pharmacy records, response assessments, and data management.

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

On-Site Monitoring: On-site monitoring will occur on an as-needed basis. Participating Institutions will be required to provide access to participants’ complete medical record and source documents for source documentation verification during the visit. In addition, Participating Institutions should provide access to regulatory documents, pharmacy records,

local policies related to the conduct of research, and any other trial-related documentation maintained by the Participating Site. On-site monitoring visits can be substituted with remote (virtual) monitoring visits at the discretion of the Principal Investigator.

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

19.2 Monitoring Reports

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

19.3 Accrual Monitoring

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

20. AUDITING: QUALITY ASSURANCE

Auditing is a method of Quality Assurance and involves the systematic and independent examination of all trial related activities and documents. Audits determine if evaluated activities were appropriately conducted and whether data was generated, recorded and analyzed, and accurately reported per the protocol, applicable Policies, and the Code of Federal Regulations (CFR).

20.1 DF/HCC Internal Audits

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

20.2 Audit Notifications

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

20.3 Audit Reports

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

20.4 Participating Institution Performance

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

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