DF/HCC Protocol #: 17-519

[redacted] **Protocol #:** ML39320

TITLE: A phase II study of atezolizumab in combination with stereotactic radiation for patients with triple-negative breast cancer and brain metastasis

Coordinating Center: Dana-Farber Cancer Institute

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IND Sponsor: Nancy Lin, MD

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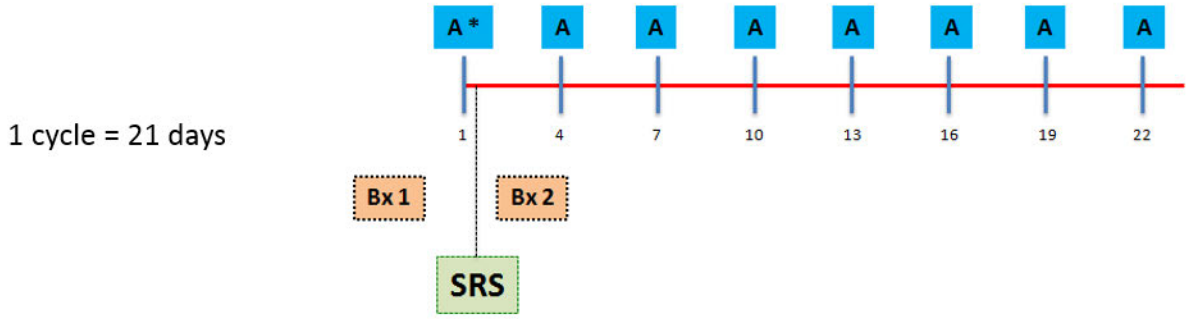
SCHEMA



TNBC – triple negative breast cancer; CNS – central nervous system; SRS – stereotactic radiation to brain metastasis;

- *D1 of Atezolizumab (A) will be administered 2-7 days before the initiation of SRS.
- Bx1 – Baseline tumor biopsy will be obtained within 28 days of starting Atezolizumab.
- Bx2 – Reassessment biopsy will be obtained between C2D1-C2D21.
- Imaging will be performed at screening within 4 weeks before starting Atezolizumab. Tumor assessments, including brain magnetic resonance imaging and computed tomography of the chest, abdomen and pelvis, will be performed every 6 weeks (± 1 week) for the first 24 weeks on treatment, and every 9 weeks (± 1 week) thereafter, with additional scans as clinically indicated.
- **Blood samples will be collected within 7 days before starting treatment (baseline), at day 1 (± 3 days) of cycle #3, #5, #9, and at progression or off protocol therapy, whichever comes first.**
- **Cerebrospinal fluid collection will be performed at 3 times points (baseline, before cycle 3, and at progression or off protocol therapy, whichever comes first).**

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SAFETY REPORTING FAX COVER SHEET

[redacted]

AE / SAE FAX No: [redacted]

[redacted]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 [redacted]

PLEASE PLACE MEDWATCH REPORT or SAFETY REPORT BEHIND THIS COVER SHEET

Version 2
Effective 14-Jan-2016

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1. OBJECTIVES

1.1 Study Design

1.1.1 This is an open-label, single arm phase II study assessing progression-free survival (PFS) in patients with triple-negative breast cancer (TNBC) and brain metastases who will receive stereotactic radiation in combination with atezolizumab. Atezolizumab will be administered at 1200mg IV every 3 weeks until disease progression in either CNS or extra-CNS site(s) according to RANO-BM and RECIST criteria, respectively. The first dose of Atezolizumab should be administered 2-7 days prior to starting radiation. Research biopsies will be performed at baseline (mandatory if there is accessible extracranial metastasis) and at Cycle 2 Day 1. An optional research biopsy will be collected at time of progression if a participant has achieved a prolonged response defined as complete or partial response or stable disease for ≥ 24 weeks).

1.2 Primary Objectives

1.2.1 To evaluate the efficacy of atezolizumab in combination with stereotactic radiation, as defined by bi-compartmental PFS according to RANO-BM criteria, in patients with TNBC and brain metastases.

1.3 Key secondary objectives

1.3.1 To evaluate the extracranial objective response rate (ORR) by RECIST 1.1 criteria among patients with TNBC and brain metastasis treated with atezolizumab in combination with stereotactic radiation [Eisenhauer *et al.*, 2009].

1.3.2 To evaluate overall survival (OS) of patients with TNBC and brain metastasis treated with atezolizumab in combination with stereotactic radiation.

1.4 Exploratory Objectives

<u>Exploratory safety objectives</u>

1.4.1 To evaluate the short term, 6-month, and 12-month safety and tolerability of atezolizumab in combination with stereotactic radiation in patients with TNBC and brain metastases.

Exploratory efficacy objectives

1.4.2 To evaluate the central nervous system (CNS) response rates according to response assessment in neuro-oncology-brain metastases (RANO-BM) criteria[Lin *et al.*, 2015] (Section 11.1.1).
1.4.3 To evaluate the CNS response rates according to response assessment in immunotherapy neuro-oncology-brain metastases (iRANO-BM) criteria[Okada *et al.*, 2015].
1.4.4 To evaluate the extracranial ORR according to immune-related response criteria (irRC)[Wolchok *et al.*, 2009].
1.4.5 To evaluate the abscopal response rate (ARR), according abscopal response definition [Golden *et al.*, 2015](Section 11.4.3).
1.4.6 To evaluate the proportion of participants with stable or responsive disease in both CNS and non-CNS at 16 and 24 weeks.
1.4.7 To evaluate PFS according to the RECIST 1.1 single-compartmental model.
1.4.8 To evaluate PFS according to the RANO-BM criteria.
1.4.9 To describe the site of first progression (CNS vs extracranial vs both)
1.4.10 To describe the type of CNS progression (new lesion(s), progression of non-target lesions, progression of target lesions, progression of stereotactic radiation-treated lesions, radiation necrosis in stereotactic radiation-treated lesions, or a combination of these)
1.4.11 To describe the type of extracranial progression (new lesion(s), progression of non-target lesions, progression of target lesions, or a combination of these)

Exploratory patient-reported outcome objectives

1.4.12 To evaluate the impact of treatment with atezolizumab and stereotactic radiation in patients with TNBC and brain metastases on PROs, as measured by the M.D. Anderson Symptom Inventory-Brain Tumor (MDASI-BT) assessment
1.4.13 To evaluate the relationship of CNS ORRs according the RANO-BM criteria with PRO endpoints, as measured by the MDASI-BT
1.4.14 To evaluate the relationship of CNS ORRs according the iRANO criteria with PRO endpoints, as measured by the MDASI-BT
1.4.15 To evaluate the impact of the study treatment, for these same patients, on general health status assessed by EQ-5D questionnaire.

Exploratory investigator-assessed neurological evaluation objectives

1.4.16 To evaluate the impact of treatment with atezolizumab and stereotactic radiation in patients with TNBC and brain metastases on investigator-assessed neurological evaluation, as measured by the Neurological Assessment in Neuro-Oncology (NANO) scale.
1.4.17 To evaluate the relationship of CNS ORRs according the RANO-BM criteria and investigator-assessed neurological evaluation, as measured by the Neurological Assessment in Neuro-Oncology (NANO) scale.
1.4.18 To evaluate the relationship of CNS ORRs according the iRANO criteria with investigator-assessed neurological evaluation, as measured by the Neurological Assessment in Neuro-Oncology (NANO) scale.

1.5 Correlative Objectives

1.5.1 To explore whether the number and/or type of mutations identified using a next generation sequencing (NGS) panel is correlated with patient outcomes (PFS, ORR, CBR, and OS).
1.5.2 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, ORR, CBR, and OS).
1.5.3 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.
1.5.4 To characterize a broad array of immune markers in metastatic TNBC (characterization will be based on histology, protein expression, and mRNA expression), and their changes with immune checkpoint blockade.
1.5.5 To explore how different immunosuppressive and/or immune-stimulating immune marker profiles at baseline correlate with patient outcomes (PFS, CNS ORR, CBR and OS).
1.5.6 To characterize changes in immune marker profiles on treatment and at time of progression
1.5.7 To characterize serial changes in immune marker profile in peripheral blood mononuclear cells (PBMCs) and in plasma over the course of the trial treatment.
1.5.8 To explore whether induction of changes in the immunosuppressive and/or immune-stimulating immune marker profile in PBMC correlates with clinical outcomes (PFS, ORR, and OS).
1.5.9 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.
1.5.10 To explore whether cfDNA load in CSF is associated with clinical outcomes (PFS, ORR, CBR, and OS).
1.5.11 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(s)

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% of these cancers are classified as triple-negative breast cancer (TNBC), comprising those with absent expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2)[Foulkes *et al.*, 2010]. Together with human epidermal growth factor receptor 2 (HER2)-positive breast cancer subtype, triple negative breast cancer (TNBC) have the highest rates of brain metastases, with studies reporting up to 50% rate of CNS involvement among those subtypes[Lin *et al.*, 2008, Niwinska *et al.*, 2010, Lin *et al.*, 2012].

Initial treatment for patients with brain metastases typically includes surgery or radiotherapy, either whole brain radiotherapy (WBRT), stereotactic radiation, or both, depending of factors such as performance status, expected prognosis as well the localization and the number of CNS metastasis[Lim *et al.*, 2014]. In patients with limited metastases and good performance status, stereotactic radiation has emerged as a preferred definitive modality because it offers excellent local control, is associated with minimal acute and long-term toxicity, and does not significantly interfere with systemic therapy schedules[Lim *et al.*, 2014].

Even with improvement in local and systemic therapies, the prognosis of patients with breast cancer BM is poor, especially in TNBC[Kennecke *et al.*, 2010, Sperduto *et al.*, 2012]. A recent large retrospective study of 865 patients with BCBMs reported the median overall survival following the diagnosis of BCBMs of 7.3 months in patients with TNBC[Sperduto *et al.*, 2013]. Therefore, there is a great necessity of developing new therapeutic strategies for TNBC BMs.

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

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

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene *Pdcd1*) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade. The mechanism by

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

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

Unlike melanoma and NSCLC, BC has not been intensively investigated for its susceptibility to immunotherapy in clinical settings. However, there 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

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. In particular, 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.

2.3.2.2 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.

2.3.2.3 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 addition, 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.

2.3.2.4 Clinical Pharmacokinetics and Immunogenicity

On the basis of 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 Stereotactic radiation

Multiple randomized controlled trials have compared stereotactic radiation with or without initial whole brain radiotherapy in patients with up to 4 brain metastases and have demonstrated equivalent overall survival outcomes (ref: Patchell JAMA 1998, JROSG 99-1 study, JAMA 2006; MDACC study Lancet Oncol 2009; EORTC 22952 study JCO 2011; NCCTG N0574 study, ASCO 2015; JCOG 0504 study ASCO 2016) whether or not WBRT is included in the initial treatment approach. Because of valid concerns about short- and long-term neurotoxicity with WBRT, stereotactic radiation has become the preferred initial approach in the treatment of patients who present with a limited number of brain metastases. In patients who develop new and/or progressive brain metastases after WBRT, stereotactic radiation can frequently be employed as salvage therapy.

However, the available data indicates that breast cancer subtype strongly influences the

outcomes after stereotactic radiation in patients with brain metastases. For example, in a retrospective study of patients treated at Dana-Farber Cancer Institute, median duration of intracranial control was significantly shorter among patients with TNBC compared to those with HER2+ breast cancer (Dyer et al, IJROBP 2012). In addition, patients with TNBC and brain metastases frequently experience extra-cranial disease progression as well; thus, control of both intracranial and extracranial compartments is critical (Lin et al, Cancer 2008).

2.5 Rationale

The recognizing that overexpression of immune checkpoint molecules in tumor microenvironment has a crucial role for antitumor immunity evasion and for cancer progression has been revolutionized the cancer treatment[Pardoll, 2012]. In particular, anti-PD-1/PD-L1 antibodies have demonstrated clinical activity in more than 15 cancer types, and have proved to increase overall survival in melanoma, non-small-cell lung carcinoma and renal cell carcinoma[Brahmer *et al.*, 2012, Topalian *et al.*, 2012, Topalian *et al.*, 2015, Rosenberg *et al.*, 2016].

Despite the fact that BC has not been considered an immunogenic neoplasm, there are accumulating preclinical and clinical evidence suggesting that the immune system is critical for disease outcome[Kroemer *et al.*, 2015]. It is now recognized that a fraction of breast tumors, especially TNBC, have substantial lymphocyte infiltration and that this pathologic feature has prognostic implications[Savas *et al.*, 2015]. Furthermore, the high rates of PD-L1 and PD-1 expression in patients with TNBCs led to clinical trials to address the role of PD-1 blockers in this population[Gatalica *et al.*, 2014]. The anti-PD-L1 atezolizumab was evaluated in patients with metastatic TNBC PD-L1 positive and showed an objective response rate (ORR) of 19% and a 6-month PFS of 27%[Emens *et al.*, 2015]. Similar results were found in a PD-L1 positive population with the anti-PD-1 inhibitor pembrolizumab (ORR of 18% and 6-month PFS of 23%)[Nanda *et al.*, 2016]. Data from a trial with avelumab has demonstrated that in a non-selected population, the use of PD-1/PD-L1 inhibitors presented an objective response rate (ORR) of 8.6%[Dirix *et al.*, 2015]. Clearly, these results show that even in the most immunogenic of breast tumors, other therapeutic strategies must be added to anti-PD-1/PD-L1 agents in order to benefit more patients. Indeed, preliminary results suggest that the combination of atezolizumab plus nab-paclitaxel is tolerable and has a very promising activity in patients with metastatic TNBC. Of note, upfront treatment showed an ORR of 66.7%[Adams *et al.*, 2016]. These results suggest that combinatorial approaches can achieve a high rate of clinical benefit.

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

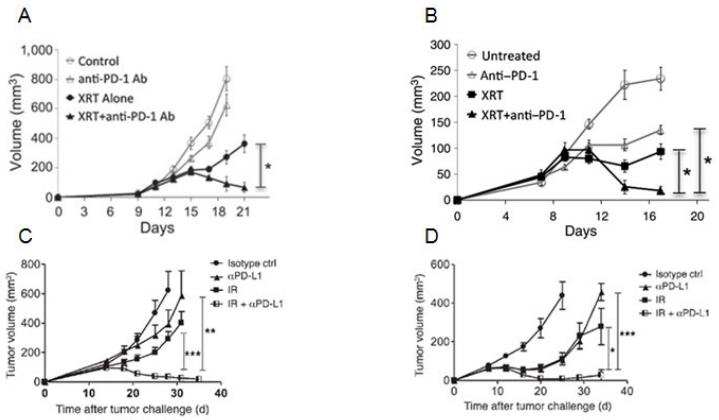


Figure 1. Radiation (XRT or IR) combined with anti– PD-1 immunotherapy significantly improves tumor control in different preclinical models of cancer - A) B16-OVA tumor cells, B) 4T1-HA tumor, C) TUBO tumor and D) MC38.

Modified from J Clin Invest. 2014;124(2):687–695 and Cancer Immunol Res. 2015 Apr;3(4):345-55.

Moreover, the effect of localized radiotherapy mediating systemic responses distant from the field of radiation (the abscopal effect), has been reported in several types of human malignancies, such as melanoma and renal cell carcinoma, after treatment combining RT and immune checkpoint inhibitors[Adamow *et al.*, 2012]. Although there is no definitive data confirming the host immune response caused the response, the tumor responses observed in these cases were accompanied by immunological changes in peripheral blood, including changes in a variety of tumor-associated antigens and a decrease in MDSCs. Additionally, the abscopal effect was recently demonstrated in mice treated with the combination of RT and anti–PD-L1 therapy, but not in groups receiving either treatment alone, suggesting that this combination may potentiate an abscopal effect on distant tumors (Figure 2)[Deng *et al.*, 2014, Dovedi *et al.*, 2015].

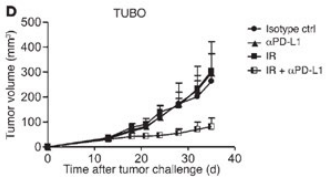


Figure 2. IR (radiation) and PD-L1 blockade synergistically amplify the antitumor effect - systemic effect (abscopal effect) of combination treatment greatly reduced the growth of secondary tumors.

Modified from J Clin Invest. 2014;124(2):687–695. doi:10.1172/JCI67313.

Given the above rationale, there is an increased interest in to investigate the benefit of combining stereotactic radiation and immune checkpoint inhibitors. Multiples series have showed that the anti-CTLA-4 ipilimumab in combination with cranial radiation is well tolerated and can result in prolonged survival[Mathew *et al.*, 2013, Kiess *et al.*, 2015, Schoenfeld *et al.*, 2015]. Recently, Ahmed et al reported good local control and minimal neurotoxicity with the combination of the anti-PD1 Nivolumab and stereotactic radiation in the management of melanoma brain metastases [Ahmed *et al.*, 2016]. Additionally, they also showed an improvement in OS and distant brain metastases control compared with a historical control. Thus, we hypothesize that RT may potentiate the efficacy of PD-L1 blockade and we propose evaluating the combination of the Atezolizumab with stereotactic radiation in patients with brain metastases due to TNBC.

Thus, we propose to evaluate the combination of atezolizumab with stereotactic radiation in patients with TNBC and brain metastases. This is an open-label, single arm phase II study. The primary endpoint is bi-compartmental PFS. Because of the strong interest in exploring a potential abscopal effect, evaluable extracranial disease is required. Patients included in this trial will be those in which a course of stereotactic radiation for CNS metastases is indicated. Patients will receive the first dose of Atezolizumab 2-7 days before the beginning of stereotactic radiation. Atezolizumab 1200mg will be administered every 3 weeks thereafter until disease progression. Research biopsies will be performed at baseline (required if accessible extra-CNS metastases) and from C2D1-C2D21.

2.6 Correlative Studies Background

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 evidence of a type I IFN transcriptional signature. This tumor phenotype is called non-T-cell-inflamed[Gajewski, 2015].

The T-cell inflamed phenotype has positive prognostic value for several types of early stage cancer, including breast cancer[Dushyanthen *et al.*, 2015, Perez *et al.*, 2015], suggesting that the attempt by the host to generate an anti-tumor immune response reflects a biologic process associated with improved patient outcomes[Gajewski, 2015]. In breast oncology, different groups have demonstrated that the amount of tumor-infiltrating lymphocytes (TILs) in a tumor specimen, commonly assessed simply by histological evaluation of a standard hematoxylin and eosin-stained slide by a trained pathologist, is a significant predictor of both response to therapy and overall disease outcomes in the neoadjuvant and adjuvant settings [Denkert *et al.*, 2010, Loi *et al.*, 2013, Adams *et al.*, 2014, Ali *et al.*, 2014, Salgado *et al.*, 2014, Denkert *et al.*, 2015,

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

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

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

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

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

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].

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. Particularly, in patients with brain metastases, research biopsies of CNS tumors are not feasible. 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

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

3.1 Eligibility Criteria

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

3.1.2 Either the primary tumor and/or metastatic tumor must be triple-negative as defined below:

- Hormone receptor status: the invasive tumor must be ER- and PR-negative, or staining present in <1% by immunohistochemistry (IHC)
- HER2 status: the invasive tumor must be Human Epidermal Growth Factor Receptor 2 Negative (HER2-negative) by the ASCO CAP guidelines

Note: In cases where both primary tumor and metastatic sample(s) have been tested for ER, PR, and HER2, the triple-negative status of the most recent sample should be used.

3.1.3 Participants must have a diagnosis of brain metastases for which stereotactic radiation is indicated, as determined by a radiation oncologist.

3.1.4 The contrast-enhancing intraparenchymal brain metastases(s) must be well circumscribed and must have a maximum diameter of ≤ 3.0 cm in any direction on the enhanced scan.

3.1.5 Participants must not have more than 5 new or progressive lesions in the brain requiring stereotactic radiation treatment (greater than 5 total brain lesions are allowed as long as no more than 5 lesions require stereotactic radiation treatment).

3.1.6 Participants must have measurable extracranial disease as defined by RECIST 1.1.

3.1.7 Participants must be willing to undergo a research biopsy at baseline and at Cycle 2 Day 1 if extracranial metastases are safely accessible. Participants for whom biopsies cannot be safely performed must be willing to submit an archival primary and/or metastatic specimen. The biopsies may be waived with prior PI approval for the first 6 participants enrolled to the safety run in phase.

3.1.8 Prior systemic therapy:

- Participants must have discontinued systemic therapy at least 14 days prior to initiating protocol therapy.
- There is no limit to the number of prior lines of systemic therapy. Participants who have not received any systemic therapy for metastatic disease are also eligible.

- Participants may initiate or continue bisphosphonate therapy on study.

3.1.9 Prior local therapy:

- Prior surgery, whole brain radiation or stereotactic radiation is allowed as long as the most recent brain progression is amenable to stereotactic radiation treatment.

3.1.10 Resolution of all chemotherapy-related or radiation-related toxicities to Grade 1 severity or lower, except for stable sensory neuropathy (≤ Grade 2 allowed) and alopecia (of any grade).

3.1.11 Participant is ≥18 years old.

3.1.12 ECOG performance status ≤2 (Karnofsky ≥60%, see Appendix A)

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

3.1.14 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 (≤2.0 in patients with known Gilberts syndrome)
– AST(SGOT)/ALT(SGPT)	≤2.5 × institutional ULN. ≤5.0 × institutional ULN for patients with documented liver metastases.
– albumin	>2.5mg/dL
– serum creatinine	≤1.5mg/dL or calculated GFR ≥60 mL/min

3.1.15 Female subjects of childbearing potential must have a negative serum or urine pregnancy test within 8 days of initiating protocol therapy.

3.1.16 For women of childbearing potential: agreement to remain abstinent (refrain from heterosexual intercourse) or use contraceptive methods that result in a failure rate of < 1% per year during the treatment period and for at least 90 days after the last dose of study treatment. A woman is considered to be of childbearing potential if she is postmenarcheal, has not reached a postmenopausal state (≥ 12 continuous months of amenorrhea with no identified cause other than menopause), and has not undergone surgical sterilization (removal of ovaries and/or uterus). Examples of contraceptive methods with a failure rate of < 1% per year include bilateral tubal ligation, male sterilization, established, proper use of hormonal contraceptives that inhibit ovulation, hormone-releasing intrauterine devices, and copper intrauterine devices. The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or postovulation methods) and withdrawal are not acceptable methods of contraception. The effects of atezolizumab on the developing human fetus are unknown and radiotherapy has known teratogenic effects so women of child-bearing potential and men must agree to use adequate contraception (barrier method of birth control; abstinence) prior to study entry and for the duration of study participation and 90 days after completion of atezolizumab administration.

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

3.2 Exclusion Criteria

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

3.2.2 Known leptomeningeal or brainstem metastases. The presence of leptomeningeal enhancement alone, without associated clinical manifestations and/or positive CSF cytology, will not be constituted as known leptomeningeal metastases.

3.2.3 Treatment with high dose systemic corticosteroids defined as dexamethasone >2mg/day or bioequivalent within 7 days of initiating therapy.

3.2.4 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).

3.2.5 Participants who are receiving any other investigational agents.

3.2.6 Previous treatment with any anti-PD-1, PD-L1, or PD-L2 agent.

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

3.2.8 The participant has an uncontrolled intercurrent illness, including, but not limited to uncontrolled hypertension, unstable angina pectoris, uncontrolled cardiac arrhythmia, congestive heart failure-New York Heart Association Class III or IV, active ischemic heart disease, myocardial infarction within the previous six months, uncontrolled diabetes mellitus, gastric or duodenal ulceration diagnosed within the previous 6 months, severe malnutrition or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.9 Participant has a 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.

3.2.10 Has evidence of active, noninfectious pneumonitis that requires treatment with steroids.

3.2.11 Has a history of interstitial lung disease.

3.2.12 The participant is known to be positive for the human immunodeficiency virus (HIV), HepBsAg, or HCV RNA. HIV-positive participants on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with atezolizumab.

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

3.2.14 Has received a live vaccine within 28 days of planned start of study therapy.

3.2.15 The participant is pregnant or breast-feeding.

3.3 Inclusion of Women and Minorities

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

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

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

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

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

4.2 Registration Process for DF/HCC Institutions

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

5. TREATMENT PLAN

5.1 Treatment Regimen

Forty-five participants will participate in this open-label, single arm phase II study assessing PFS in patients TNBC and brain metastasis. Participants will receive stereotactic radiation in combination with Atezolizumab (1200mg IV) on day 1 of each 21-day (3 week) cycle. The first dose of Atezolizumab will be administered 2-7 days prior to beginning stereotactic radiation. Treatments will be administered on an outpatient basis.

Regimen Description

Agent	Premedication; Precautions	Dose	Route	Schedule	Cycle Length
Atezolizumab	Not routinely necessary unless prior infusion reaction.	1200 mg	IV The initial dose of atezolizumab will be delivered over 60 (± 15) minutes. If the first infusion is tolerated, 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.	Day 1, q3w	21 days (3 weeks)

5.2 Pre-Treatment Criteria

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

5.2.1 All Cycles, Day 1

– absolute neutrophil count	≥1,000/mcL
– platelets	≥75,000/mcL
– AST(SGOT)/ALT(SGPT)	≤ 2.5 × institutional ULN or ≤ 5 × institutional ULN for participants with documented liver metastases
- creatinine	≤1.5 × institutional ULN

5.2.2 Atezolizumab Administration

[redacted] will provide atezolizumab. Atezolizumab will be administered by trained medical personnel at the investigational site. Treatment compliance will be monitored through documentation of study treatment administration in the subject's medical record.

Atezolizumab will be administered in clinic on day 1 (+/- 3 days) of each cycle. Patients will receive the first dose of Atezolizumab 2-7 days before the beginning of stereotactic radiation. 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. No premedication will be allowed for the first dose of Atezolizumab. 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.

5.2.3 Other Modality(ies) or Procedures

5.2.3.1 Stereotactic radiation

5.2.4.1.1 Timing of Radiation

Radiation must begin within 14 days after brain MRI obtained with T1 post contrast sequences, with a maximum slice thickness of 1.5mm. Radiation must be delivered at Brigham and Women's Hospital or Dana-Farber Cancer Institute.

5.2.4.1.2 Dose

Lesions <2 cm in maximum diameter will be treated with stereotactic radiosurgery, generally 20 Gy in 1 fraction. Lesions between 2.0 and 2.5 cm in maximum diameter will generally be treated to 18 Gy in 1 fraction. Lesions between 2.5-3.0 cm in maximal diameter will generally be treated with stereotactic radiotherapy to 30 Gy in 5 fractions given on consecutive weekdays The volume receiving 12 Gy (V12) should be limited to <8-10cc; dose reductions are acceptable if the V12 constraint cannot be met via replanning, with a minimum dose of 16 Gy. Patients with simultaneous metastases that require 1 fraction and 5 fractions can be treated concurrently. If a metastasis that would be treated normally with stereotactic radiosurgery is adjacent to a metastasis that must receive stereotactic radiotherapy (so that a significant degree of scatter dose from the latter field will include the potential former field) it is acceptable for the treating investigator to treat both metastases with stereotactic radiotherapy.

The treating radiation oncologist may use their discretion in the best interest of the patient in the treating participants on study.

5.2.4.1.3 Physical Factors
Treatment will be delivered using megavoltage machines with 6MV photon beams using a linear accelerator equipped with stereotactic capabilities.

5.2.4.1.4 Simulation, Immobilization, and Localization

The patient will be treated in the supine position. A tightly fitting thermoplastic mask will be employed for immobilization.

5.2.4.1.5 Treatment Planning and patient setup

The gross tumor volume (GTV) will cover the metastasis as identified by contrast enhanced MRI and CT (contrast optional) obtained within 14 days of the treatment initiation date. The MRI must be obtained with a T1 post contrast sequence with a maximal slice thickness of 1.5 mm. No clinical target volume (CTV) is to be used. The planning target volume (PTV) will be 1 mm. For patients who have rapidly growing gross disease an extra 0.1-1mm of PTV margin can be added at the discretion of the treating clinician to account for growth between planning and treatment; in such cases the time between planning and treatment should be minimized to the greatest extent

possible. Treatments will be delivered through volumetric modulated arc therapy (VMAT) using a single isocenter for PTV volumes >1.5 cm in maximum diameter. For PTV volumes with a maximum dimension of 0-1.5 cm in diameter either VMAT, circular collimators, or multiple static fields will be acceptable. The prescription will generally be normalized to the 60-95% isodose line. The maximum allowed hot spot will be 170%. 100% of the GTV should be covered by the prescription dose. At least 99% (ideal) / 98% (at minimum) of the PTV should be covered by the prescription isodose line. The entire PTV should be covered by 90% (ideal) / 85% (at minimum) of the prescription dose. Daily set up with cone beam CT or stereoscopic KV imaging is mandated.

5.2.4.1.6 Dose to Critical Structures

Dose constraints for stereotactic radiation to critical structures will be as follows:

Dose constraints for stereotactic radiation

Organ at Risk	Tolerance	Dose Modification Regimen
Eyes, Optic Nerves, Optic Chiasm	Point dose ≤8 Gy	If point dose >8 Gy, undercover PTV while maintaining 100% GTV coverage. If not achievable, dose reduce GTV to highest dose which meets optic constraint, through 16 Gy (15 Gy for cavity in which gross total resection has been achieved). If 100% of GTV cannot be safely covered by 16 Gy (15 Gy for cavity in which gross total resection has been achieved), then an acceptable variation will be to convert to stereotactic radiotherapy – see Table 2
Brainstem (defined as brainstem volume minus GTV)	Dose to 0.035cc ≤12 Gy	If dose to 0.035cc >12 Gy, undercover PTV while maintaining 100% GTV coverage. If not achievable, dose reduce GTV to highest dose which meets brainstem constraint, through 16 Gy (15 Gy for cavity in which gross total resection has been achieved). If 100% of GTV cannot be safely covered by 16 Gy (15 Gy for cavity in which gross total resection has been achieved), then an acceptable variation will be to convert to stereotactic radiotherapy – see Table 2
Volume of normal brain (less the GTV) receiving >12 Gy (per individual lesion, called the V12)	≤10cc	If the V12 is >10cc, undercover PTV while maintaining 100% GTV coverage. If not achievable, dose reduce GTV to highest dose which meets constraint, through 16 Gy (15 Gy for cavity in which gross total resection has been achieved). If 100% of GTV cannot be safely covered by 16 Gy (15 Gy for cavity in which gross total resection has been

		achieved), then an acceptable variation will be to convert to stereotactic radiotherapy – see Table 2
Normal brain less the GTV (for entire brain)	Mean <5 Gy	Administer stereotactic radiation on two or more consecutive weekdays rather than a single day

Dose constraints for stereotactic radiotherapy to critical structures will be as follows:

Dose constraints for stereotactic radiotherapy (5 fractions)

Organ at Risk	Tolerance	Dose Modification Regimen
Eyes, Optic Nerves, Optic Chiasm	Point dose of 25-27 Gy	If point dose >25-27 Gy undercover PTV while maintaining 100% GTV (30 Gy in 5 fractions if gross disease; 25 Gy in 5 fractions if gross total resection has been achieved) coverage. If not achievable, dose reduce GTV to highest dose which meets constraint, through 23 Gy in 5 fractions. 100% of GTV should always receive at least 23 Gy in such a scenario.
Brainstem (defined as brainstem volume minus GTV)	Dose to 0.035cc ≤28 Gy	If dose to 0.035cc >28 Gy, undercover PTV while maintaining 100% GTV coverage (30 Gy in 5 fractions if gross disease; 25 Gy in 5 fractions if gross total resection has been achieved). If not achievable, dose reduce GTV to highest dose which meets brainstem constraint, through 23 Gy in 5 fractions. 100% of GTV should always receive at least 23 Gy in such a scenario.

5.2.4.1.7 Stereotactic Radiation Documentation Requirements

The institution will archive treatment prescription and verification images for later review by the study chair if requested. At least one port film or pretreatment alignment film/CT along with the digitally reconstructed radiographs (DRRs) from the treatment planning program or, alternatively, a simulation verification radiograph shall be acquired and kept for evaluation if requested except where geometrically impractical.

5.2.4.1.8 Stereotactic Radiation Radionecrosis Assessment

To assess participants for potential delayed radiation toxicity, a radiation follow-up visit will be performed approximately 6 months after completion of stereotactic radiation treatment. This visit should be done no earlier than 5 months and no later than 8 months post-stereotactic radiation. Another visit should occur, if possible, around 12 months after completion of radiation treatment. This visit should be performed no earlier than 11 months and no later than 14 months post-radiation. At these timepoints, brain imaging will be correlated with a clinical assessment,

performed by the radiation oncology team. All potential radiation necrosis events will be adjudicated by the PI, Nancy Lin, MD, in collaboration with the trial radiation oncologist, Ayal Aizer, MD.A diagnosis of radiation necrosis will be ascribed if:

1.Surgery on an enlarging lesion after radiation reveals a specimen showing predominantly necrosis
2. Changes consistent with necrosis on dual-phase PETCT are seen
3. Changes consistent with necrosis on serial MRI studies of the brain are seen. Changes consistent with necrosis on serial MRIs of the brain included lack of sustained growth over time in patients who did not receive central nervous system-active systemic therapy after initial enlargement, development of a non-distinct margin of enhancement on T1 post contrast sequences, development of a hypointense center on T1 post contrast images, and a large edema to enhancement ratio.

If a diagnosis of radiation necrosis is made for any patient in this cohort, we will distinguish radiographic necrosis from radiographic and symptomatic necrosis based upon the presence versus absence of associated neurologic symptoms or administration of dexamethasone / bevacizumab for neurologic symptomatology correlating with the anatomic location of the lesion in question.

Every attempt should be made for these assessments to occur in person. Due to the nature of this patient population, some participants may be on hospice or otherwise not able to travel to the study site to complete these endpoints. In these cases, the study team will check to see if any brain imaging has been completed at local facilities and will obtain those images for review. The radiation team may reach out to the patient to perform the radiation necrosis assessment over the phone. Not completing these visits in person will not constitute protocol violations.

5.3 General Concomitant Medication and Supportive Care Guidelines

5.3.1 Concomitant Medication Guidelines

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the overall PI.

Acceptable Concomitant Medications

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

Prohibited Concomitant Medications

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

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Investigational agents other than atezolizumab
- Radiation therapy not specified in this protocol
- 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 radiation, 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.
- Subjects may receive other medications that the investigator deems to be medically necessary.

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

5.3.2 Supportive Care Guidelines – general medications

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

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

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.

5.4 Definition of Dose-Limiting Toxicity

Dose-limiting toxicity is defined as any of the following events occurring from first dose of Atezolizumab until Cycle 3 Day 1 if judged by the investigator to be possibly, probably, or definitely related to study drug and/or radiation administration:

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

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

5.5 Criteria for Taking a Participant Off Protocol Therapy

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

- Disease progression by iRANO and/or irRC criteria.
    - Please note that although the primary endpoint is bi-compartmental PFS as

defined by standard RANO-BM (for CNS) and RECIST 1.1 (for non-CNS), 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.

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

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

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, Nancy Lin, MD at [redacted]

Participants with a documented complete response (CR) may elect to stop Atezolizumab. Subjects who stop atezolizumab with a 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 and documented CR 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.

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.

5.6 Duration of Follow Up

Participants removed from protocol therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event. Participants who are 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. Participants who are removed from protocol therapy for intracranial disease progression will be followed for survival until death.

Participants who are removed from protocol therapy before the 6-month post-radiation follow-up visit will be contacted by phone by a member of the study team to be assessed for possible latent radiation toxicities.

5.7 Criteria for Taking a Participant Off Study

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

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

6.1 Management of toxicities attributable to atezolizumab

Toxicities 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 immune-related adverse events (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, in severe cases, immune-related toxicities may require acute

management with topical corticosteroids, systemic corticosteroids, mycophenolate, or TNFα inhibitors.

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.

6.1.1 Guidelines for Dosage Modification and Treatment Interruption or Discontinuation

There will be no dose reduction for atezolizumab in this study. Patients may temporarily suspend study treatment for up to 84 days beyond the scheduled date of delayed infusion if study drug-related toxicity requiring dose suspension is experienced. If atezolizumab is held because of AEs for >84 days beyond the scheduled date of infusion, the patient will be discontinued from atezolizumab and will be followed for safety and efficacy as specified in Section 5.5.

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.

Management of hepatitis/transaminitis, colitis, rash, and hypothyroidism 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 Gastrointestinal Toxicity

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

Patients should be advised to inform the investigator if any diarrhea occurs, even if it is mild.

If the event is of significant duration or magnitude, or is associated with signs of systemic inflammation or acute phase reactants (e.g., increased CRP or platelet count or bandemia), it is recommended that sigmoidoscopy (or colonoscopy, if appropriate) with colonic biopsy with three to five specimens for standard paraffin block be performed. If possible, one or two biopsy specimens should be snap frozen and stored.

Treatment may be restarted following the resolution of colitis. In addition, if the patient is being managed with corticosteroids, treatment should not be restarted until the steroids have been tapered down to a prednisone dose ≤10 mg/day. Patients who resume treatment should be monitored closely for signs of renewed diarrhea. Table 1 provides a summary of dose modification guidelines for gastrointestinal toxicities.

Table 1 Dose Modification Guidelines for Gastrointestinal Toxicity

Toxicity	Description	Management
Diarrhea and/or Colitis	Grade 1	• Continue atezolizumab. • Initiate symptomatic treatment • Endoscopy is recommended if symptoms persist for > 7 days • Close monitoring.
	Grade 2	• Hold atezolizumab • Initiate symptomatic treatment • For recurrent events or events that persist > 5 days: start Prednisone 60 mg/day or equivalent. • When symptoms improve Grade ≤1, resume atezolizumab o Corticosteroids must be tapered over ≥1 month to <10 mg/day oral prednisone or equivalent before resuming. • 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. 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. • 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 o If event resolves to ≥ Grade 1, restart atezolizumab • If event does not resolve to > grade 1 within 12 weeks of holding, permanently discontinue o If the subject was unequivocally deriving clinical benefit, the subject may be rechallenged as

		determined by the PI. • Gastroenterology referral and confirmation biopsy
	Grade 4	• Permanently discontinue atezolizumab and inform PI o 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 Atezolizumab as determined by the PI. • Gastroenterology referral and confirmation biopsy • 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.

IV = intravenous; TNF-α = tumor necrosis factor alpha.

6.1.1.2 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 2. For patients with elevated LFTs at baseline (between 3-5 x ULN) due to documented liver metastases, further elevation of LFTs may not require dose interruptions if the ALT and/or AST have risen < 3 x baseline value and if the total bilirubin elevation remains < 2x ULN with normal PT/INR. Such cases should be also discussed with the principal investigator.

Table 2 Dose Modification Guidelines for Hepatotoxicity

Toxicity	Description	Management
LFT and/or total bilirubin abnormalities	Grade 1	• Continue Atezolizumab and monitor labs according to study calendar
	Grade 2	• Continue Atezolizumab and monitor LFTs more frequently until return to baseline or Grade ≤1 • If persistent > 5 days, hold atezolizumab, and start prednisone 60 mg/day or equivalent. When LFTs improve to Grade ≤1 taper steroids over ≥1 month • When LFTs improve to Grade ≤1 and the steroid dose is prednisone ≤ 10 mg/day or equivalent (if initiated), restart atezolizumab
	Grade 3 or 4	• 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.

6.1.1.3 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 3.

Table 3 Dose Modification Guidelines for Dermatologic Toxicity

Toxicity	Description	Management
Dermatologic toxicity/rash (e.g., maculopapular or purpura)	Grade 1: Mild <10% BSA	• Continue atezolizumab • Consider topical steroids and/or other symptomatic therapy (e.g., antihistamines).
	Grade 2: Moderate 10%–30% BSA	• Continue atezolizumab. • Administer topical steroids and consider higher potency topical steroids if events do not improve • Consider dermatologist referral.
	Grade 3: Severe >30% BSA	• Hold atezolizumab and administer oral prednisone 10 mg or equivalent. • If the rash is not improved after 48–72 hours, increase oral dose of prednisone to 60 mg or equivalent. • Refer for dermatology consult. • Restart atezolizumab 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.

6.1.1.4 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.

Hypophysitis is a rare condition, characterized by the development of hypopituitarism with multiple anterior pituitary hormone deficiencies (e.g., low levels of adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH).) MRI typically reveals a diffuse enlargement of the pituitary gland.

Cases of hypophysitis have been reported in patients receiving Atezolizumab. Among 2, 616 patients treated with single agent Atezolizumab in clinical trials, hypophysitis occurred in 2 patients. It is recommended that Atezolizumab be withheld for grade 2 or grade 3 hypophysitis

and permanently discontinued for grade 4 events. Corticosteroids and hormone replacement therapy should be administered at the discretion of the treating physician.

Table 4 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 treatment with thyroid replacement hormone • Monitor TSH weekly • Consider referral to an endocrinologist • Restart atezolizumab when symptoms are controlled
Asymptomatic Hyperthyroidism	• If serum TSH < 0.5 mU/L and >0.1 mU/L, continue atezolizumab and monitor TSH every 4 weeks. • If serum TSH < 0.1 mU/L, follow guidelines for symptomatic hyperthyroidism.
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 and CRC.
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.
Hypophysitis any grade 2 or 3 events	• Hold atezolizumab until toxicity improve to Grade ≤ 1 • Corticosteroids and hormone replacement therapy should be administered as clinically indicated. • Consult an endocrinologist • If toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks, permanently discontinue atezolizumab. Inform PI and study team. • For recurrent hypophysitis, treat as Grade 4.

Hypophysitis grade 4	• 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 steroid taper and taper over ≥ 1 month, resume atezolizumab • Consult an endocrinologist • Perform appropriate imaging • Initiate hormone 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.

6.1.1.5 Pulmonary Toxicity

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

Mild-to-moderate events of pneumonitis have been reported with atezolizumab. All pulmonary events should be thoroughly evaluated for other commonly reported etiologies such as pneumonia/infection, lymphangitic carcinomatosis, pulmonary embolism, heart failure, chronic obstructive pulmonary disease (COPD), or pulmonary hypertension. Recommended management for pulmonary events are listed in table 5 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}])

As a precaution, patients will be assessed for pulmonary signs and symptoms throughout the study.

Table 5 Dose Modification Guidelines for Pulmonary Toxicity (Pneumonitis)

Toxicity	Description	Management
Pulmonary toxicity (Pneumonitis)	Grade 1	• Continue atezolizumab with close monitoring • Re-evaluate on serial imaging • Consider pulmonary consultation
	Grade 2	• Hold and 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 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	• Permanently discontinue atezolizumab, start prednisone 60 mg/day or equivalent and notify PI and study team. • 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

6.1.1.6 Potential 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.

6.1.1.7 Potential 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 6.

Table 6 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 • 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 o If the subject was unequivocally deriving clinical benefit, the subject may be able to

Toxicity	Description	Management
		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.

6.1.1.8 Potential Cardiac Toxicity

Myocarditis refers to a diverse group of heart-specific immune processes classified by a spectrum of clinical and histopathological manifestations. It may present as mild dyspnea or chest pain, or more severe cardiogenic short or sudden death. In the absence of an infectious etiology, immune-related myocarditis is confirmed by histological evidence of inflammatory infiltrates within the myocardium, together with cardiac myocytes degenerations and necrosis of non-ischemic origin.

A cumulative analysis of the company safety database which includes data from clinical trials and post-marketing setting (data cut-off 20 February 2017), identified 2 non-fatal cases of myocarditis, including one with biopsy confirmation. Approximately 8, 000 clinical trial patients and 5, 000 post-marketing patients have been treated with Atezolizumab to date.

Atezolizumab should be permanently discontinued for all grades of myocarditis. Corticosteroids and/or additional immunosuppressive agents should be administered as clinically indicated.

Toxicity	Description	Management
Myocarditis	Symptomatic	• Permanently discontinue Atezolizumab for any grade and notify PI and study team. • Corticosteroids and/or immunosuppressive agents should be administered as clinically indicated. • Consult a cardiologist.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

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

7.1 Adverse Events Lists

7.1.1 Expected adverse events for atezolizumab

The PD-L1/PD-1 pathway is involved in peripheral tolerance; therefore, such therapy may increase the risk of immune-related AEs, specifically the induction or enhancement of

autoimmune conditions. AEs with potential immune-related causes, including rash, hypothyroidism, hepatitis/transaminitis, colitis, myositis, and myasthenia gravis, have been observed with Atezolizumab in Study PCD4989g.

Although most immune-related AEs observed with immunomodulatory agents have been mild and self-limiting, such events should be recognized early and treated promptly to avoid potential major complications[Champiat *et al.*, 2016].

A more detailed safety profile of Atezolizumab is provided in the Atezolizumab Investigator's Brochure.

7.1.2 Safety Parameters and Definitions

Specification of Safety Variables

Safety assessments will consist of monitoring and reporting adverse events (AEs) and serious adverse events (SAEs) per protocol. This includes all events of death, and any study specific issue of concern.

Adverse Events

An AE is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational medicinal product (IMP) or other protocol-imposed intervention, regardless of attribution.

This includes the following:

- AEs not previously observed in the patient that emerge during the protocol-specified AE reporting period, including signs or symptoms that were not present prior to the AE reporting period
- Complications that occur as a result of protocol-mandated interventions (e.g., invasive procedures such as cardiac catheterizations).
- If applicable, AEs that occur prior to assignment of study treatment associated with medication washout, no treatment run-in, or other protocol-mandated intervention.
- Preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period.
    -

Serious Adverse Events

An AE should be classified as an SAE if the following criteria are met:

- It results in death (i.e., the AE causes or leads to death).
- It is life threatening (i.e., the AE, in the view of the investigator, places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.).
- It requires or prolongs inpatient hospitalization.
- It results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the subject's ability to conduct normal life functions).
- It results in a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to the IMP.

- It is considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the subject or may require medical/surgical intervention to prevent one of the outcomes listed above).

7.1.3 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.

7.1.3.1 Adverse Events of Special Interest

The following AEs are considered of special interest and must be reported to the [redacted] 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.
- 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 ≥ 2 cardiac disorders (e.g., atrial fibrillation, myocarditis, pericarditis)

7.2 Methods and Timing for Assessing AND Recording Safety variables

The investigator is responsible for ensuring that all AEs and SAEs that are observed or reported during the study, are collected and reported to the FDA, appropriate IRB(s), and [redacted] in accordance with CFR 312.32 (IND Safety Reports).

7.2.1 Adverse Event Reporting Period

The study period during which all AEs and SAEs must be reported begins after informed consent is obtained- and initiation of study treatment and ends 30 days following the last administration of study treatment or study discontinuation/termination, whichever is earlier. After this period, investigators should only report SAEs that are attributed to prior study treatment.

7.2.2 Assessment of Adverse Events

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 reported appropriately. Each reported AE or SAE will be described by its duration (i.e., start and end dates), regulatory seriousness criteria if applicable, suspected relationship to the atezolizumab (see following guidance), and actions taken.

To ensure consistency of AE and SAE causality assessments, investigators should apply the following general guideline:

Yes
There is a plausible temporal relationship between the onset of the AE and administration of atezolizumab, and the AE cannot be readily explained by the subject's clinical state, intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response to atezolizumab; and/or the AE abates or resolves upon discontinuation of atezolizumab or dose reduction and, if applicable, reappears upon re-challenge.

No
Evidence exists that the AE has an etiology other than atezolizumab (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to atezolizumab administration (e.g., cancer diagnosed 2 days after first dose of study drug).

Expected adverse events are those adverse events that are listed or characterized in the Package Insert (P.I) or current Investigator Brochure (I.B).

Unexpected adverse events are those not listed in the P.I or current 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 if the P.I. or I.B. only referred to elevated hepatic enzymes or hepatitis.

7.3 Procedures for Eliciting, Recording, and Reporting Adverse Events

7.3.1 Eliciting Adverse Events

A consistent methodology for eliciting AEs at all subject evaluation time points should be adopted. Examples of non-directive questions include:

- "How have you felt since your last clinical visit?"
- "Have you had any new or changed health problems since you were last here?"

7.3.2 Specific Instructions for Recording Adverse Events

Investigators should use correct medical terminology/concepts when reporting AEs or SAEs. Avoid colloquialisms and abbreviations.

7.3.2.1 Diagnosis vs. Signs and Symptoms

If known at the time of reporting, a diagnosis should be reported rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, it is acceptable to report the information that is currently available. If a diagnosis is subsequently established, it should be reported as follow-up information.

7.3.2.2 Deaths

All deaths that occur during the protocol-specified AE reporting period (see Section 7.2.1), regardless of attribution, will be reported to the appropriate parties. When recording a death, the event or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report "Unexplained Death".

7.3.2.3 Preexisting Medical Conditions

A preexisting medical condition is one that is present at the start of the study. Such conditions should be reported as medical and surgical history. A preexisting medical condition should be re-assessed throughout the trial and reported as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important

to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

7.3.2.4 Hospitalizations for Medical or Surgical Procedures

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE. If a subject is hospitalized to undergo a medical or surgical procedure as a result of an AE, the event responsible for the procedure, not the procedure itself, should be reported as the SAE. For example, if a subject is hospitalized to undergo coronary bypass surgery, record the heart condition that necessitated the bypass as the SAE.

Hospitalizations for the following reasons do not require reporting:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for preexisting conditions
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study or
- Hospitalization or prolonged hospitalization for scheduled therapy of the target disease of the study.

7.3.2.5 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 [redacted]. 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.

7.3.2.6 Post-Study Adverse Events

The investigator should expeditiously report any SAE occurring after a subject has completed or discontinued study participation if attributed to prior study drug 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.

7.3.2.7 Reconciliation

The Sponsor agrees to conduct reconciliation for the product. [redacted] 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 [redacted] 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. The sponsor shall receive reconciliation guidance documents within the 'Activation Package'.

7.3.2.8 AEs of Special Interest (AESIs)

AEs of Special Interest are defined as a potential safety problem, identified as a result of safety monitoring of the Product.

The atezolizumab Events of Special Interest are listed in section 7.1.3.1

7.4 Reporting to Principal investigator

7.4.1 Investigators **must** report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of study treatment on the local institutional SAE form within 1 business day of awareness. Report serious adverse events by telephone, email, or facsimile to:

Nancy Lin, MD
[redacted]

7.5 Expedited Reporting to [redacted]

Investigators must report all SAEs to [redacted] within the timelines described below. The completed MedWatch 3500A form should be faxed to [redacted] Drug Safety using the [redacted] fax coversheet found in APPENDIX I at:

[redacted]

Relevant follow-up information should be submitted to [redacted] Drug Safety as soon as it becomes available.

Serious AE reports and AEs of Special Interest, whether related or unrelated to atezolizumab, will be transmitted to [redacted] within one (1) business day of the awareness date.

Additional reporting requirements to [redacted] include the following:

- Any reports of pregnancy following the start of administration with atezolizumab and within the follow-up period (for female patients within 4 months after the last dose of atezolizumab or the partner of a male patient within 4 months of completing therapy) will be transmitted to [redacted] within one (1) business day 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.

In addition to SAEs, pregnancy reports and AESIs, the following Special Situations Reports should be collected and transmitted to [redacted] even in the absence of an Adverse

Event within thirty (30) calendar days:

- Data related to product usage during pregnancy or breastfeeding
- Data related to overdose, abuse, misuse, inadvertent/erroneous administration, medication error or occupational exposure, with or without association with an AE/SAE unless otherwise specified in the protocol
- Data related to a suspected transmission of an infectious agent via a medicinal product (STIAMP)

7.5.1.1 MedWatch 3500A Reporting Guidelines

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (section 5) of the MedWatch 3500A form:

- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome if known
- Supportive laboratory results and diagnostics
- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

Follow-up Information

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500A report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form
- Summarizing new information and faxing it with a cover letter including patient identifiers (i.e. D.O.B. initial, patient number), protocol description and number, if assigned, brief adverse event description, and notation that additional or follow-up information is being submitted (The patient identifiers are important so that the new information is added to the correct initial report)

Occasionally [redacted] may contact the reporter for additional information, clarification, or current status of the patient for whom an adverse event was reported. For questions regarding SAE reporting, you may contact the [redacted] Drug Safety representative noted above or the MSL assigned to the study. Relevant follow-up information should be submitted to [redacted] Drug Safety as soon as it becomes available and/or upon request.

MedWatch 3500A (Mandatory Reporting) form is available at
http://www.fda.gov/medwatch/getforms.html

For questions related to safety reporting, please contact [redacted] Drug Safety:
[redacted]

7.6 Reporting to the Institutional Review Board (IRB)

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

7.7 Expedited Reporting to the Food and Drug Administration (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 to the use of atezolizumab. An unexpected adverse event is one that is not already described in the atezolizumab Investigator Brochure. Such reports are to be telephoned or faxed to the FDA and [redacted] within 7 calendar days of first learning of the event.

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. An unexpected adverse event is one that is not already described in the atezolizumab investigator brochure.

7.8 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. Immediate Reporting of Adverse Events and Events of Clinical Interest to [redacted]

7.9 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. **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 [redacted]

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. 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 [redacted]

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.

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.

In all patients in whom a tumor outside the field of radiation is accessible, a baseline tumor biopsy is required. We plan to use baseline biopsy tissue to perform a number of immune profiling assays, detailed below. On baseline tumor biopsies, we will perform characterization

based on histology (TILs), protein expression, and mRNA expression. Additionally, we will bank specimens for possible future DNA analysis, and other further testing.

Serial blood draws for correlative science are required on this trial; blood draws will be obtained according to the schedule in Table 9-1. We will perform flow cytometry on each blood draw to characterize protein expression of immune mediators, as 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	1-10 mL Streck Tube 5- 10mL green top tubes*
	Start day of stereotactic radiation	1-10 mL Streck Tube 5- 10mL green top tubes*
	Cycle 3 Day 1	1-10 mL Streck Tube 5- 10mL green top tubes*
	Cycle 5 Day 1	1-10 mL Streck Tube 5- 10mL green top tubes*
	Cycle 9 Day 1	1-10 mL Streck Tube 5- 10mL green top tubes*
	At progression or off protocol therapy	1-10 mL Streck Tube 5- 10mL green top tubes*
Fresh Tissue	Pre-treatment (2-7 days prior to stereotactic radiation)	5-7 cores
	Cycle 2 Day 1 to day 21	5-7 cores
	Optional 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 sterile collection tubes
	Cycle 2 Day 15 to day 21	3-10 mL in sterile collection tubes
	At progression or off protocol therapy	3-10 mL in sterile collection tubes

* EDTA (purple top) tubes or CPT tubes may be used interchangeably with green top tubes.

9.1 Archival Tissue Collection

1 block or 15, 4-micron unstained, charged slides will be collected for future research.

9.2 Fresh Tissue Collection

9.2.1 Collection

Biopsies are required at baseline (pre-treatment) and C2D1 (performed as close to C2D1 as possible but between C2D1 and C2D21). An optional biopsy may be collected at the time of progression for patients who achieved an objective response and/or a prolonged stable disease (≥ 24 weeks). The biopsies may be waived on the first 6 participants enrolled to the safety run in phase.

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 [redacted] 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 RNA Later 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 C.

9.2.2 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.

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

  [redacted]

  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 [redacted] for later use for RNA sequencing. Please notify the lab of [redacted]

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

- Cores in RNAlater should be brought to the DF/HCC Clinical Trial Core Laboratory at the address provided here:

  Dana-Farber Cancer Institute
  [redacted]

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

9.3 Blood Collection

9.3.1 Collection

Research blood collection is mandatory for all patients for flow cytometry and potential DNA

isolation. The samples will be banked in the DFCI breast tissue repository for these and future research purposes. These specimens will become the property of the DF/HCC.

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

The following research blood samples are required:

Cycle 1 Day 1:

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

Day 1 of Stereotactic Radiation:

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

Cycles 3, 5, and 9 Day 1:

- 1-10 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):

- 1-10 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:

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

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

9.3.2 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 3-4hrs of its being drawn at ambient temperature immediately after being drawn to [redacted] at:

- 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.

  Blood in Streck tubes should be brought to the Clinical Trial Core Laboratory [redacted] on Smith 9 for processing.

9.4 Cerebrospinal fluid (CSF)

9.4.1 Collection

While CSF collection is optional, it will be encouraged. We plan to collect CSF for at least 10 patients 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 optional*:

Screening (baseline):
• 3-10 mL in sterile collection tubes

Cycle 2 Day 15 to day 21:
• 3-10 mL in sterile collection tubes

Off Treatment (at progression or off protocol therapy, whichever comes first):
• 3-10 mL in sterile collection tubes

*Streck Cell-Free DNA BCT tubes may be used in place of sterile collection tubes. After collection, gently invert tube 10 times to ensure proper mixing of stabilization agent and store at room temperature until processing.

9.4.2 Handling and Shipping of CSF specimens

CSF tubes should be brought or shipped to the DF/HCC Clinical Trial Core Laboratory [redacted] provided here:

Please email the DF/HCC Clinical Trials Core Laboratory [redacted]) 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 -80ºC 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 according to intensity (0=no staining, 1=weak staining, 2=moderate staining, 3=strong staining), staining patter (M=predominantly cell membrane; C=predominantly cell cytoplasm), and the percentage of cells showing positive staining (0-100%). The 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.

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 initiating protocol therapy unless otherwise specified. Screening assessments occurring within 1 week prior to initiating study treatment do not need to be repeated on Cycle 1 Day 1.

Screening laboratory assessments must be done within 8 days prior to initiating protocol therapy. For women of childbearing potential, as defined in the eligibility criteria, a pregnancy test must be completed within 8 days prior to initiating protocol therapy. If a urine pregnancy test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

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

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

	Screening	Cycle 1			Cycle 2		Cycle 3	Cycle 4+		Cycle 5	Cycle 6		Off-Treatment[s]	Follow-Up
Day	Day -28 to day -1	Day 1	RT Day 1	10 days after RT	Day 1	Day 15-21	Day 1	Day 1	Day 15-21	Day 1	Day 1	Day 15-21		
Informed consent	X													
SRS Planning Appointment[a]	X													
Medical history[b]	X													
Physical exam[c]	X	X			X		X	X		X	X		X	
Radiation Oncologist Visit[d]			X[d]	X[d]										
Adverse event evaluation	X	X			X		X	X		X	X		X	
Vital signs[e]	X	X			X		X	X		X	X		X	
Weight	X	X			X		X	X		X	X			
Concurrent medications[f]	X	X			X		X						X	
ECOG Performance status	X	X			X		X	X		X	X		X	
Neurological Assessment	X	X			X		X						X	
Brain MRI[g]	X					X			X			X	X	X
CAP CT and/or MRI[h]	X					X			X			X	X	X
Hematology panel[i]	X	X			X		X						X	
Chemistry panel[j]	X	X			X		X						X	
TSH [k]	X	X			X		X	X		X	X			
Pregnancy test[l]	X													
Research Blood - cfDNA[m]		X	X				X	X[m]		X[m]	X[m]		X	X

Research Blood - Immune Markers[n]	X	X	X				X	X[n]		X[n]	X[n]		X	
Research Biopsy[o]	X				X								X	
Cerebrospinal Fluid Collection[p]	X					X							X	
Questionnaire[q]		X					X	X		X	X		X	X
General Impression Worksheet[t]	X				X		X	X		X	X			
Archival Tissue Collection	X													
Atezolizumab		X			X		X	X		X	X			
Post RT Follow-up Visit[u]											X[u]			
Survival Status[r]														X

Atezolizumab: 1200mg given IV q3 weeks on Day 1 of each cycle + Stereotactic Radiation: radiation will start 2-7 days after the first dose of atezolizumab

a. Stereotactic radiation planning appointment must occur at least 7 days prior to initiating radiation
b. Medical history includes clinically significant disease, surgeries, and cancer history (including prior cancer therapies and procedures).
c. 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.
d. Participants will be seen by a radiation oncologist on day 1 of stereotactic radiation and 10 days after radiation completion (+/- 4 days).
e. Vital signs to include: heart rate, systolic and diastolic blood pressures while the patient is in a seated position, respiratory rate, and temperature.
f. Selected medications of interest received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment, including dosage, frequency, route, and dates of administration will be documented
g. T-1 weighted perfusion MRI will be used. Screening MRI must be done within 14 days of the radiation initiation. Subsequent assessments should be done during the last 15-21 days of a cycle, q6weeks for the first 24 weeks (i.e. C2, 4, 6, 8) and then can be reduced to q9 weeks (i.e. C11, 14, 17, etc). If progression is suspected, an unscheduled assessment is permitted. An objective response (CR, PR should be confirmed by repeat assessments 4-6 weeks after initial finding. Patients with CNS PD are allowed to continue on study for 4-6 weeks to confirm PD on MRI. 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 beginning a new cancer therapy regimen. 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 CNS restaging after a patient has been taken off protocol therapy will not constitute a protocol violation.
h. CT and/or MRI should be of the chest, abdomen and pelvis. Additional imaging studies (CT neck, plain films, etc.) are permitted as clinically indicated. The same radiographic procedures and technique must be used throughout the study for each patient. Assessments should be done during the last 15-21 days of a cycle, q6weeks for the first 24 weeks (i.e. C2, 4, 6, 8) and then can be reduced to q9 weeks (i.e. C11, 14, 17, etc.) until progression. For those taken off-study for reasons other than progressive disease, tumor measurements should continue to be repeated every 6-12 weeks until progression or beginning a new cancer therapy regimen. Failure to complete restaging after a patient has been taken off protocol therapy will not constitute a protocol violation.
i. Hematology: hemoglobin, hematocrit, platelet count, RBC count, WBC count, percent and absolute differential count.
j. Chemistry: sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, calcium, total bilirubin, total protein, albumin, ALT, AST, alkaline phosphatase, and LDH.
k. Beginning with cycle 4, TSH will be done every other cycle on Day 1 (i.e. cycles 4, 6, 8, etc.).
l. In female subjects of child-bearing potential as defined in the eligibility criteria, serum or urine pregnancy test must be performed within **8 days** prior to initiating protocol therapy
m. cfDNA is required to be drawn on C1D1, Day 1 of RT, C3D1, C5D1, C9D1, at each restaging visit, and at disease progression or discontinuation of protocol therapy, whichever comes first.
n. Immune markers will be drawn on C1D1, Day 1 of RT, C3D1, C5D1, C9D1, and at disease progression or discontinuation of protocol treatment, whichever comes first.
o. Baseline tumor biopsy should be obtained within 28 days of the first dose of Atezolizumab. The C2D1 biopsy should be performed as close to C2D1 as possible but may be collected between C2D1 and C2D21. Both the baseline and C2 biopsy are required for participants with accessible disease outside of the field of radiotherapy. A biopsy at the time of disease progression is optional for subjects who have accessible disease and have achieved an objective response (CR or PR) and/or experienced prolonged stable disease for ≥ 24 weeks.
p. CSF collection will be performed at baseline, C2 day 15 to day 21, and at disease progression or discontinuation of protocol therapy, whichever comes first (±3 days) in at least 10 participants.
q. NANO Scale, MDASI0BT and EQ-5D to be administered at baseline, C3D1, C5D1, C7D1, C9D1, 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 beginning a new cancer therapy regimen when possible. Failure to complete questionnaires after a patient has been taken off protocol therapy will not constitute a protocol violation.
r. Participants will be followed for overall survival every 6 months or until death. This can be a visit to the clinic or a phone call to the participant or the participant's local provider.
s. Off-Treatment visit should occur within 30 days of the last dose of study treatment. Tumor assessments (including brain MRI and CAP CT/MRI do not need to be repeated if done within 28 days of the off-treatment visit).
t. General Impression Worksheet to be completed at baseline and the end of each 3-week cycle of treatment.
u. To assess participants for potential delayed radiation toxicity, a stereotactic radiation follow-up visit will be performed approximately 6 months and 12 months after completion of stereotactic radiation treatment. This visit may occur any time post 6 months (as early as 5 months/as late as 8 months) or post 12 months (as early as 11 months/as late as 14 months) after completion of stereotactic radiation treatment. Please see section5.2.4.1.8 for more information surrounding these visit requirements.

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 achieve a sustainable response after 1 year on protocol therapy, re-evaluation can be performed every 12 weeks.

11.1 Antitumor Effect – CNS disease

Tumor response and progression for CNS disease will be assessed centrally by the DF/HCC Tumor Imaging Metrics Core according to RANO-BM criteria and according to iRANO criteria. Please refer to the full publications for additional details.

11.1.1 Response Assessment in Neuro-Oncology-Brain Metastases(RANO-BM) Criteria:

11.1.1.1 Definitions

- Definition of 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).
- Definition of 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.

11.1.1.2 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'.

11.1.1.3 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.

11.1.1.4 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.

11.1.1.5 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 after SRS or 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 (in the case of SRS/SRT) are strongly encouraged. (2) Surgical pathology obtained via biopsy or resection. (3) For SRS/SRT treated lesions, an advanced imaging modality such as perfusion MR imaging, MR spectroscopy, or ^{18}FLT or ^{18}FDG positron emission tomography (PET) may be used as additional evidence of tumor progression or treatment effect/radionecrosis. Upon review of the literature and extensive discussions by the Working Group, we were not able to conclude that any one modality or approach can be recommended across all patients to distinguish between radiation necrosis versus true progression, as the literature is not sufficiently robust, and recommend clinical judgment and involvement of a multidisciplinary team. We recognize this is less than satisfactory and agree that developing more sensitive and specific methods for distinguishing between treatment effect and tumor progression are needed. 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.

Table 1

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.

11.2.3.1 Evaluation of Target Lesions

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

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

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

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

11.2.3.2 Evaluation of Non-Target Lesions

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

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

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

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

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

11.2.3.3 Evaluation of New Lesions

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

11.2.3.4 Evaluation of Best Overall Response

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

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

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

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

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

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

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

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

11.2.4 Duration of Response

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

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

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

11.2.5 Clinical Benefit rate

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

11.3 Antitumor Effect – Hematologic Tumors

N/A

11.4 Other Response Parameters

11.4.1 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.

11.4.1.1 Impact of New Lesions on irRC

New lesions in and of themselves do not qualify as progressive disease. However, their contribution to total tumor burden is included in the SPD which in turn feeds into the

irRC criteria for tumor response. Therefore, new non-measurable lesions will not discontinue any subject from the study.

11.4.1.2 Definition of Target Lesions Response Using irRC

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

11.4.1.3 Definition of Non-Target Lesions Response Using irRC

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

11.4.1.4 Definition of Overall Response Using irRC

Overall response using irRC will be based on these criteria:

- **Immune-Related Complete Response (irCR):** Complete disappearance of all tumor lesions (target and 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

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

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

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

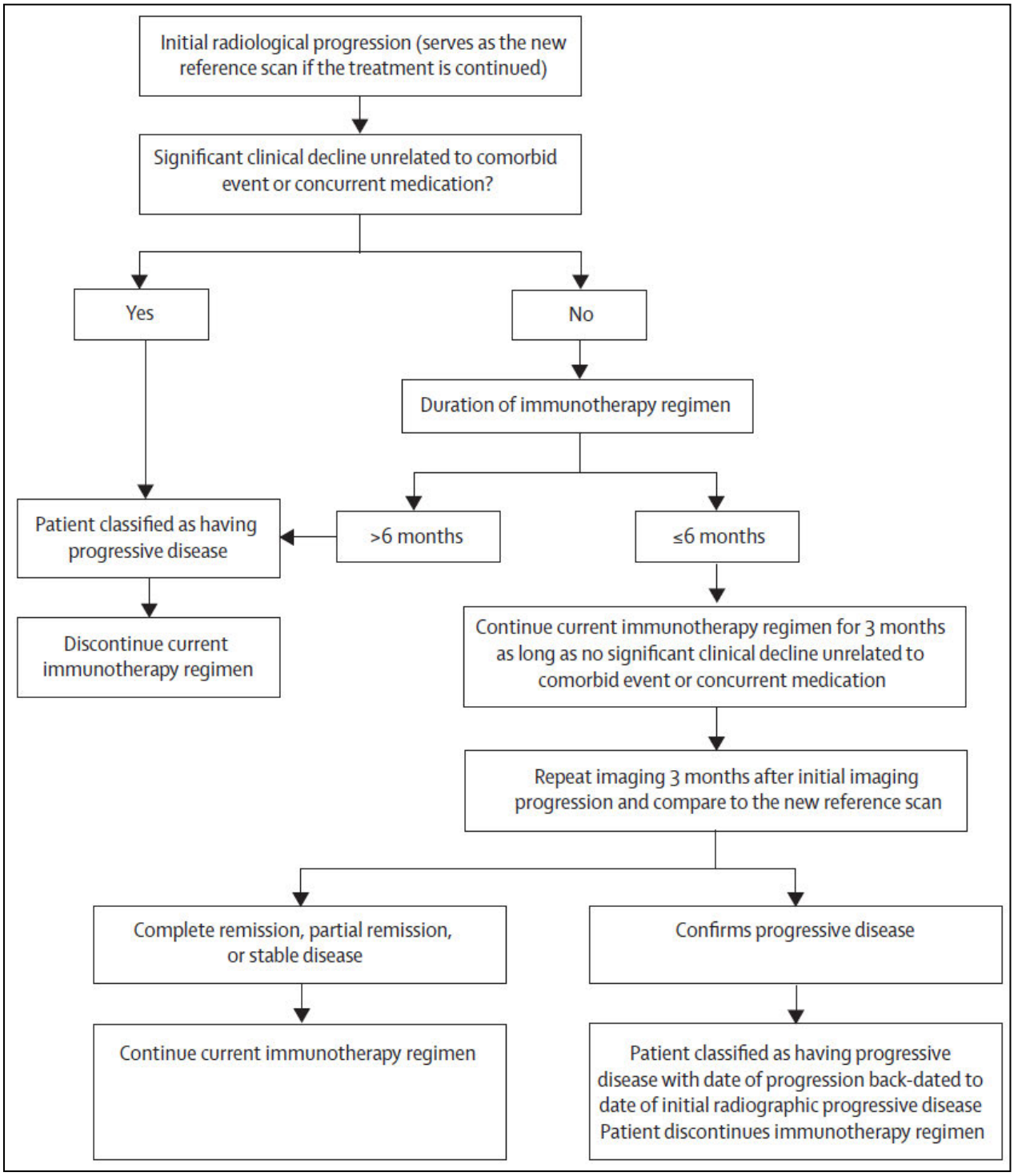
11.4.2 Definition of immunotherapy Response Assessment in Neuro-Oncology (iRANO)

Standard RANO criteria differ according to whether the target population is high-grade

glioma, low-grade brain tumors, or solid tumor brain metastases[Lin *et al.*, 2015] [Wen *et al.*, 2010, 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.

Figure 1: iRANO treatment algorithm for the assessment of progressive imaging findings 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.

11.4.3Progression-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.

11.4.3 Definition of Abscopal response

As described previously, abscopal response is defined as a decrease in the longest diameter of at least 30% in any measurable (≥1 cm) non-irradiated lesion from baseline[Golden *et al.*, 2015]. In patients with more than three lesions, the non-irradiated lesions will be measured individually for response to treatment. The best abscopal responding lesion will be reported.

11.4.3.1 Definition of overall response according abscopal response definition

- Complete abscopal response is defined as the complete disappearance of a measurable non-irradiated lesion.
- Partial abscopal response is defined as at least a 30% decrease in the longest diameter.
- Progressive disease **is** defined as at least a 20% increase in the longest diameter of the best measurable non-irradiated lesion.
- Stable disease is defined as insufficient shrinkage or growth to qualify for a partial abscopal or complete abscopal response or progressive disease.

11.4.4 Patient-Reported Outcome Measure

11.4.4.1 The PRO outcome measure for this study is as follows: Scores from the MDASI-BT assessment

11.4.5 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[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.

11.4.6 EQ-5D evaluation

In order to evaluate the impact of the study treatment, on general health status we will have participants complete the EQ-5D questionnaire (APPENDICES E or F).

12. DATA REPORTING / REGULATORY REQUIREMENTS

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

12.1 Data Reporting

12.1.1 Method

ODQ will collect, manage, and perform quality checks on the data for this study.

12.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC are responsible for submitting data and/or data forms to the ODQ according to the schedule set by ODQ.

12.1.3 Study close-out

Any study report submitted to the FDA by the overall PI will be copied to [redacted] This includes all IND annual reports and the Clinical Study Report (final study report). Additionally, any literature articles that are a result of the study should be sent to [redacted]

All IND annual reports submitted to the FDA by the Sponsor-Investigator should be copied to [redacted] Copies of such reports should be faxed to [redacted] Drug Safety: [redacted]

For Investigator-Initiated IND Studies, some additional reporting requirements for the FDA apply in accordance with the guidance set forth in 21 CFR § 600.80.

12.2 Data Safety Monitoring

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

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

12.3 Multicenter Guidelines

N/A

12.4 Collaborative Agreements Language

N/A

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

This is an open-label, single arm phase II study to assess PFS in patients with TNBC and brain metastasis who will receive stereotactic radiation in combination with atezolizumab. The target enrollment is 45 participants.

Primary endpoint
The primary endpoint is PFS defined as time from first dose of atezolizumab (day 1 cycle 1) to progression or death due to any cause. Progression will be defined according to the bi-compartmental model proposed in the RANO-BM publication and is defined as the first detection of radiologic progression of intracranial (per RANO-BM criteria; Section 11.1), extracranial (per RECIST 1.1 criteria; Section 11.2), or both or unequivocal progression of non-measurable disease in the opinion of the treating physician (an explanation must be provided); with each compartment (CNS and non-CNS) assessed separately.

Key secondary endpoints
Secondary endpoints include extracranial objective response rate (ORR), defined as an extracranial complete (CR) or partial (PR) response according to RECIST 1.1, and OS, defined as the time from the first dose of atezolizumab to death due to any cause.

13.2 Sample Size, Accrual Rate and Study Duration

The primary endpoint is bi-compartmental PFS according to RANO-BM criteria. A sample size of 45 was chosen to achieve 80 % power to detect a difference between the null hypothesis median PFS of 2 months and alternative of 3.5 months at a one-sided type I error of 0.05.

The null hypothesis was derived from two sources: First, a prospective trial testing the combination of irinotecan and iniparib in patients with metastatic triple-negative breast cancer and new/progressive brain metastases reported a median time to progression (which included both intracranial and extracranial progression) of 2.14 months[Anders *et al.*, 2014]. Next, in a retrospective study describing outcomes of breast cancer patients after initial stereotactic radiation, time to intracranial progression was significantly shorter in patients with triple-negative breast cancer, relative to the luminal A subtype (HR 28.0 in multivariable model, p=0.003[Dyer *et al.*, 2012]. Although the numbers are small, the point estimate for the time to intracranial progression in the triple-negative subset was 2.5 months [Dyer *et al.*, 2012].

Anticipated accrual of approximately 2 patients per month, it will require approximately 24 months of patient accession to reach 45 patients. The primary analysis of PFS and OS will be

time-driven and performed after 6 months of additional follow-up.

13.3 Stratification Factors

N/A

13.4 Interim Monitoring Plan

A safety run-in analysis will be performed after the first 6 patients are enrolled and have completed the assessment period for dose-limiting toxicity (DLT). The DLT assessment period encompasses the period from first dose of atezolizumab until Cycle 3 Day 1, to allow sufficient from stereotactic radiation treatment to assess for DLT. If there are 3 or more dose-limiting toxicity (DLTs) in the first 6 patients included, the study will be closed to further enrollment. If this occurs, then any plans to re-open the study with a modified treatment plan will need to be formally reviewed and approved by the sponsor and by the DF/HCC SRC/IRB prior to activation. If there are less than 3 DLT within the first 6 patients enrolled, then the study will proceed to full accrual. All patients who receive at least one dose of study drug will be included in the efficacy analysis.

Table: Probability of continuing enrollment with ≤2 DLTs out of 6 subjects.

	Safety run-in using 6 subjects						
True DLT rate	0.1	0.2	0.3	0.4	0.5	0.6	0.7
Probability of continuing enrollment	98%	90%	74%	54%	34%	18%	7%

To assess for potential delayed radiation toxicities, a 6-month safety visit will be performed. Patients who stop protocol therapy before the 6 months post stereotactic radiation treatment visit will be evaluated by phone by a member of the study team. If at any point during the trial the risk of delayed radiation toxicity is deemed to be excessive, the trial will be terminated.

In addition to the safety run-in in the first 6 patients treated, a continual assessment of symptomatic necrosis at 6 months and at 1 year will be conducted, using the method of Ivanova, et al (25) that applies Pocock-style boundaries for the maximum-tolerated rates of toxicity. Specifically, rates of necrosis at 6 months that are 25% or greater or at 1 year that are 50% or greater are considered unacceptable. For each toxicity rule, the upper limit of the probability of early stopping, φ, is set at 0.1 and the proportion with maximum-tolerated toxicity, θ, is defined as half the unacceptable rate. The following gives the sequential stopping rules tabulated according to the number of events. For instance, if 3 events of symptomatic necrosis at 6 months are observed in the first 6 patients enrolled, or if 8 events are observed in the first 30 patients, the study would be terminated for excessive toxicity.

Boundary for necrosis at 6 months	3	4	5	6	7	8	9	10	11
Maximum number enrolled	≤6	≤10	≤14	≤19	≤25	≤30	≤36	≤41	≤47
Boundary for necrosis at 1 year	5	6	7	8	9	10	11	12	13
Maximum number enrolled	≤8	≤10	≤13	≤16	≤19	≤21	≤24	≤27	≤30
Boundary for necrosis at 1 year	14	15	16	17	18	19			
Maximum number enrolled	≤34	≤37	≤40	≤43	≤46	≤47			

The probabilities of stopping the trial early for vary true rates of each classification of toxicity are presented below:

True rate of necrosis at 6 months	**Prob of stopping early, φ**	**E[N]**		**True rate of necrosis at 1 year**	**Prob of stopping early, φ**	**E[N]**
0.125	0.0994	43.91		0.25	0.1000	43.93
0.20	0.4708	34.48		0.30	0.2529	40.06
0.30	0.9169	18.94		0.40	0.7254	27.13
0.40	0.9966	10.40		0.50	0.9692	15.43
0.50	1.0000	6.64		0.60	0.9993	9.32

13.5 Analysis of Primary Endpoint

The primary endpoint is bi-compartmental PFS defined as time from first dose of atezolizumab (day 1 cycle 1) to progression or death due to any cause. Progression is defined as the first detection of radiologic progression of intracranial (per RANO-BM criteria; Section 11.1), extracranial (per RECIST 1.1 criteria; Section 11.2), or both or unequivocal progression of non-measurable disease in the opinion of the treating physician (an explanation must be provided). The PFS will be analyzed using Kaplan–Meier product-limit estimates and will be plotted with two-sided 90% confidence bands using Greenwood's formula for the variance. For evaluating median survival, a Brookmeyer-Crowley like test will be conducted using the one-sided alpha = 0.05.

13.6 Analysis of Key Secondary Endpoints

13.6.1 Extracranial Objective Response:

The design of this study, which involves radiation to CNS metastases in the setting of evaluable extracranial disease, provides a unique opportunity to test whether there is a signal consistent with an abscopal effect with the addition of stereotactic radiation to atezolizumab. Extracranial response will be assessed according to RECIST 1.1, and will be evaluated as a key secondary objective using a one-sample exact binomial test (one-sided type I error ≤ 0.05).

In prior studies evaluating immune checkpoint inhibitors in metastatic triple negative breast cancer (which excluded patients with active CNS metastases), the extracranial response rate has ranged from 8.6% in an unselected population[Dirix *et al.*, 2015] (Dirix et al, 2015), to 18-19% in patients selected for tumor PD-L1 expression[Emens *et al.*, 2015, Nanda *et al.*, 2016]. Based on these prior studies of single agent PD1/PDL1 blockade in unselected triple negative breast cancer, the null hypothesis is that the extra-CNS response rate is 10% or lower.

With a sample size of 45 defined by the primary endpoint of PFS, there will be 82% power to reject the null hypothesis if the true extra-CNS response rate is 25% with stereotactic radiation given in combination with atezolizumab.

The observed extra-CNS response rate will be reported with a two-sided 90% confidence level which given the sample size will have a maximum width of 26%. In addition, we intend to evaluate extracranial objective according abscopal response as by Golden et al [Golden *et al.*, 2015].

13.6.2 Overall survival

Overall survival is defined as the time from first dose of atezolizumab (day 1 cycle 1) to death from any cause. Patients who are alive at the end of the study will be censored at the date of last known alive. OS will be analyzed using Kaplan–Meier product-limit estimates and will be plotted with two-sided 90% confidence bands using Greenwood's formula for the variance. For evaluating median survival, a Brookmeyer-Crowley like test will be conducted using the one-sided alpha = 0.05. Based on prior studies[Eichler *et al.*, 2008, Lin *et al.*, 2008, Nam *et al.*, 2008, Niwinska *et al.*, 2010, Anders *et al.*, 2011], the null hypothesis is that median overall survival is 5 months or less.

With a sample size of 45 defined by the primary endpoint of PFS, there will be 90% power to reject the null hypothesis if the true median OS is 10 months when patients are given stereotactic radiation in combination with atezolizumab. The Approximate Upper Critical Value to the hypothesis test is a median OS of 7.4 months, under the assumption of nonparametric exponential family of density function for the distribution of failure times. The following table shows the level of power if smaller levels of improvement in survival are true which may be clinically relevant.

Alternative Hypothesis: Median OS (HR versus null)	**Power**
10 months (HR = 0.5)	90%
8.33 months (HR = 0.6)	75%
8 months (HR = 0.625)	70%
7.5 months (HR = 0.667)	61%

13.7 Analysis of Exploratory and Correlative Endpoints

Efficacy Endpoints

Intracranial Objective Response:

There is debate in the field as to the best criteria to use for assessment of response and progression in patients with brain metastases and in patients receiving immunotherapy, and this study provides an opportunity to evaluate efficacy using multiple published criteria in the setting of stereotactic radiation and to compare and contrast these.

All patients who initiate protocol therapy will be evaluated for CNS response according RECIST 1.1, RANO-BM and iRANO-BM criteria.

Lesions treated with stereotactic radiation as part of this protocol will be specifically designated in the case report forms. Based on prior literature, the response rate for stereotactic radiation-treated lesions should be high[Dyer *et al.*, 2012].

In this study, stereotactic radiation-treated lesions can be designated as target lesions, as long as they are reproducibly measurable, and at least 1 cm in longest dimension. It is anticipated that some patients may enter the study with previously treated and stable CNS lesions that are not re-treated as part of the study. These lesions should generally be designated as non-target lesions for the purpose of this study.

Based on a high CNS response rate previously reported with stereotactic radiation alone, we do not necessarily anticipate a detectable increase in the response rate when stereotactic radiation is given in combination with immune checkpoint therapy.

Clinical benefit:

The proportion of patients with stable or responsive disease in both CNS and non-CNS (each compartment assessed separately; CNS by RANO-BM and non-CNS by RECIST 1.1) at 16 and 24 weeks will be calculated.

Progression free survival

Historically, PFS has been assessed using RECIST 1.1, which assumes a “single-compartment” summation model. Using RECIST 1.1, up to 2 target lesions may be chosen from each organ site,

with up to 5 total lesions overall. Although the brain can be included as an organ site in RECIST 1.1, frequently, investigators have designated brain metastases as non-target lesions. If brain metastases are instead designated as target lesions, then using RECIST 1.1 as currently written would require that up to 2 lesions in the brain are summed with other target lesions outside of the brain, and progression only deemed when the sum of all longest dimensions exceeds 20% of the sum of nadir on study, and there is an absolute increase of at least 5 mm in at least one lesion.

RANO-BM proposes a bi-compartmental PFS model, such that the CNS and extracranial compartments are evaluated separately, and in this study, bi-compartmental PFS is the primary endpoint. However, there are little to no data comparing the RECIST 1.1 single-compartment model and RANO-BM bi-compartmental model.

In addition, the use of immunotherapy further complicates the assessment of progression. Both irRC and iRANO allow patients to be treated beyond the first demonstration of radiologic progression in order to distinguish between true progression and pseudoprogression. In addition, new lesions do not automatically constitute progressive disease. If true progression is subsequently documented, the date of progression is backdated to the original demonstration of radiographic worsening.

This study will provide a unique opportunity to compare and contrast:

-PFS according to RECIST 1.1, single compartment model, with first progression event deemed an event
-PFS according to RANO-BM, two-compartment model, with first progression event deemed an event
-PFS according to irRC, single compartment model
-PFS according to iRANO, two-compartment model

Safety and tolerability

All patients will be evaluable for toxicity from the time of their first treatment with any study agent. Toxicity will be graded according to NCI CTCAE, Version 4.0. Toxicities will be summarized by maximum grade.

Radiation Necrosis

Brain metastases treated with stereotactic radiation which enlarge post treatment will be evaluated for radiation necrosis by the treating radiation oncologist, via neuroradiology assessment, and/or through the use of dual-phase PET-CT studies (Horky *et al*., 2011*)*, as clinically indicated, according to standard of care. The cumulative incidence of necrosis will be evaluated as the time from first dose of atezolizumab (day 1 cycle 1) to detected necrosis. Patients without detected necrosis will be censored at the end of the study, whether due to death or other reason. Cumulative incidence will be analyzed using Kaplan–Meier product-limit estimates and will be plotted with two-sided 90% confidence bands using Greenwood's formula for the variance.

Patient-reported outcomes

The PRO measure for this study will be the scores from the MDASI-BT assessment.

Neurological examination

In order to standardize the evaluation of key neurological exam components, this study will use the Neurological Assessment in Neuro-Oncology (NANO) scale.

Correlative endpoints

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

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

With a sample size of 45 and assuming that there will be patients without accessible lesions, the table below indicates the power available to detect 20%, 30%, or 40% increases of PD-L1 positivity rate. The power calculation is based on McNemar's test with 1-sided alpha of 0.05 and assuming 2% of patients unexpectedly show PD-L1 positivity only in the baseline assessment,

Increase of PD-L1 positivity rate at C3D1	# of paired biopsies (baseline and at cycle 3)	Power
20%	20	64%
	30	79%
	40	88%
30%	20	85%
	30	95%
	40	98%
40%	20	96%
	30	99%
	40	99%

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

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 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.

For paired assessments of WES of tumoral DNA and CSF at baseline and at time of progression, 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	# of paired samples (tumor and CSF)	True Cohen's kappa
20%	20	0.63
	30	0.53
	40	0.46
30%	20	0.61
	30	0.51
	40	0.44
40%	20	0.60
	30	0.50
	40	0.43

The association of baseline CSF 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.

13.8 Reporting and Exclusions

13.8.1 Evaluation of Efficacy

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

Subanalyses may then be performed on the basis of a subset of participants, excluding

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

13.8.2 Evaluation of Safety

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

14. PUBLICATION PLAN

The results should be made public within 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

- Tourniquet
- Oxygen
- Epinephrine for subcutaneous, intravenous, and/or endotracheal use in accordance with standard practice
- Antihistamines
- Corticosteroids
- Intravenous infusion solutions, tubing, catheters, and tape

PROCEDURES

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

1. Stop the study drug infusion.
2. Apply a tourniquet proximal to the injection site to slow systemic absorption of study drug. Do not obstruct arterial flow in the limb.
3. Maintain an adequate airway.
4. Administer antihistamines, epinephrine, or other medications as required by patient status and directed by the physician in charge.
5. Continue to observe the patient and document observation.

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 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 ENGLIGH QUESTIONARIE

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.

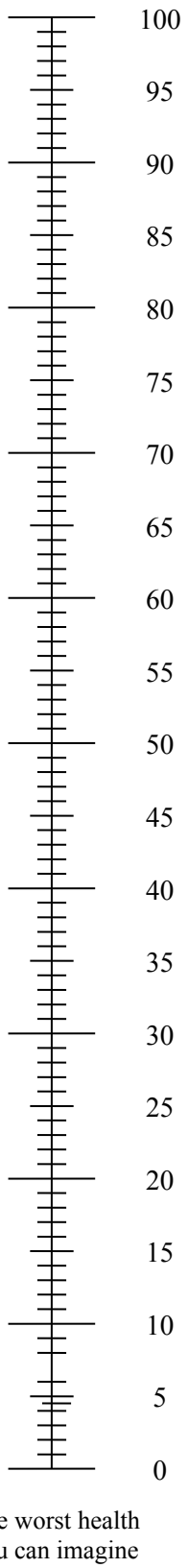
This scale is numbered from 0 to 100.

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.

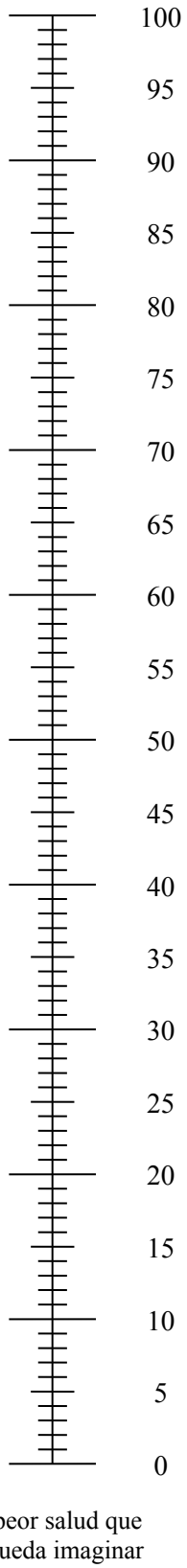
La escala está numerada de 0 a 100.

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: 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 ECOG or Karnofsky Performance Status (see Appendix A for definitions): _____

APPENDIX H GUIDELINES FOR COLLECTING RESEARCH BIOPSY TISSUE

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

Skin/chest wall: Because the yield of malignant tissue from skin/chest wall biopsies tends to be relatively low, if a patient has another accessible site of disease (i.e. lymph node, liver), that site should be biopsied preferentially. If skin/chest wall is the only biopsy-accessible site, then a goal of 2 5-mm punch biopsies will be obtained.

Lymph node: A goal of 5-7 core biopsy specimens will be obtained using an 18-gauge needle.

Liver: A goal of 5-7 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. lymph node, liver), that site should be biopsied preferentially. If bone is the only biopsy-accessible site, then a goal of 5-7 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 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 I: [redacted] SAFETY REPORTING FAX COVERSHEET

[redacted]

SAFETY REPORTING FAX COVER SHEET

[redacted]

AE / SAE FAX No: [redacted]

[redacted]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 [redacted]

PLEASE PLACE MEDWATCH REPORT or SAFETY REPORT BEHIND THIS COVER SHEET

Version 2
Effective 14-Jan-2016