SUMMARY OF CHANGES – Protocol

For Protocol Amendment #6 to Amendment #7, Version #14

NCI Protocol #: 10013 (R02, PTMA #100224)
Local Protocol #: 201706104

NCI Version Date: 02/28/2023
Protocol Date: 02/28/2023

Protocol Changes#	**Section**	**Change**
1.	Throughout document	Protocol version date was updated in header.
2.	Title page	Protocol version was updated.
3.	9	Biomarker and Correlative Analysis plan and tissue sample prioritization table updated to add snRNA-seq and CODEX analyses
4.	9.2.3	Immune signature section updated to include sn-RNA-seq and CODEX analyses for the Human Tumor Atlas Network (HTAN)
5.	14	References updated throughout document to correct previous amendments and section 14

NCI Protocol #: 10013 (R02, PTMA #100224)

Local Protocol #: **201706104**

ClinicalTrials.gov Identifier: NCT02883062

TITLE: Randomized Phase 2 Study of Neoadjuvant Chemotherapy, Carboplatin and Paclitaxel, with or without Atezolizumab in Triple Negative Breast Cancer (TNBC)

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NCI-Supplied Agent(s): Atezolizumab (MPDL3280A; NSC 783608)

Other Agent(s): Paclitaxel (NSC 673089), Commercial; Carboplatin (NSC241240), Commercial

IND #: [redacted]

IND Sponsor: DCTD, NCI

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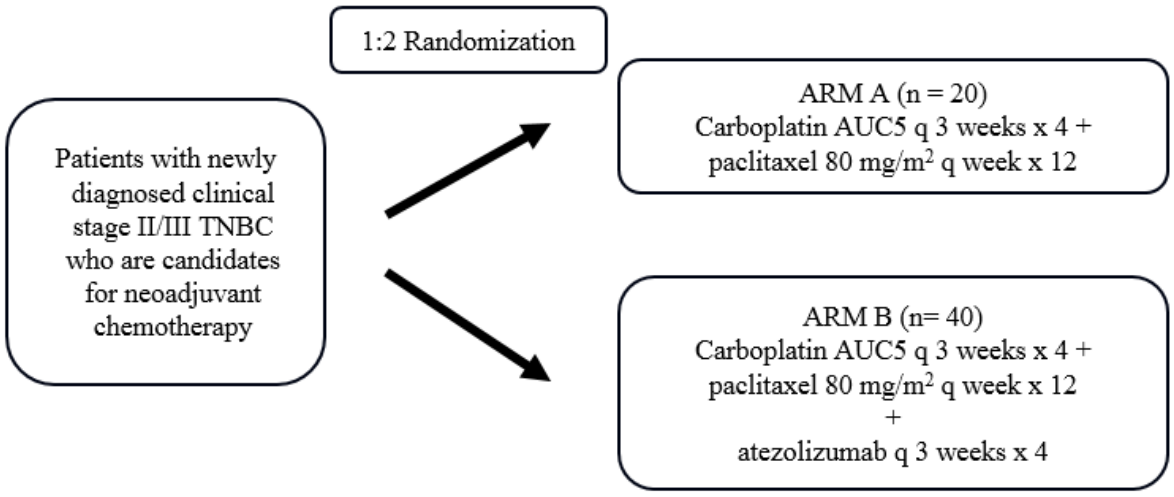
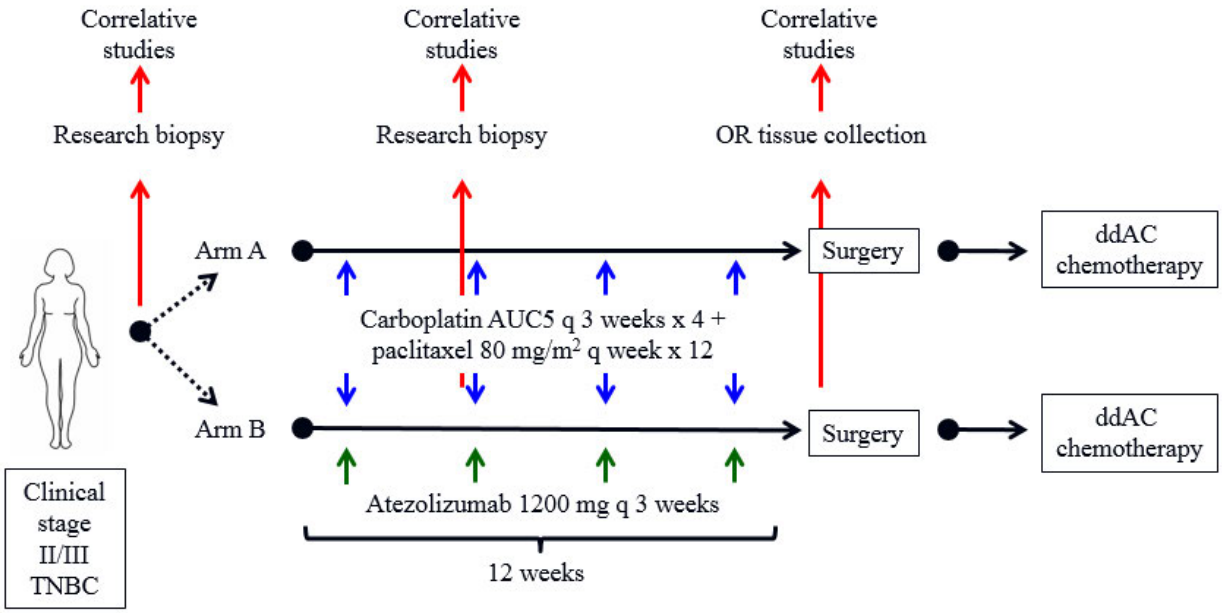
STUDY SCHEMA





This is an open label, stratified (with clinical stage as strata), randomized phase 2 clinical trial of neoadjuvant chemotherapy plus atezolizumab in patients with newly diagnosed clinical stage II/III triple neagative breast cancer. Patients will be randomized in a 1:2 ratio to carboplatin AUC5 q 3 weeks x 4 + paclitaxel 80 mg/m^2 q week x 12 (Arm A), or carboplatin AUC5 q 3 weeks x 4 + paclitaxel 80 mg/m^2 q week x 12 + atezolizumab 1200 mg q3 weeks x 4 (Arm B). Tissue and peripheral blood will be collected at baseline, after the first cycle of therapy, and at the time of surgery. We will continue to enroll patients until there are 60 evaluable patients or 72 total patients, whichever comes first.

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1. OBJECTIVES

1.1 Primary objective

Primary objective #1 is to test the hypothesis that the combination of chemotherapy and atezolizumab will increase tumor infiltrating lymphocyte (TIL) percentage compared to chemotherapy alone in patients with newly diagnosed triple negative breast cancer (TNBC) being treated with neoadjuvant chemotherapy.

TIL percentage will be measured at baseline and after initiation of therapy (day 18-22) using a well validated histopathologic assay. The TIL percentage after initiation of therapy will be compared between patients receiving neoadjuvant chemotherapy alone (Arm A) and patients receiving neoadjuvant chemotherapy in combination with atezolizumab (Arm B).

Primary objective #2 is to test the hypothesis that the combination of chemotherapy and atezolizumab will increase the pathologic complete response (pCR) rate compared to chemotherapy alone in patients with newly diagnosed TNBC being treated with neoadjuvant chemotherapy. Pathologic complete response rates will be compared between patients receiving neoadjuvant chemotherapy alone (Arm A) and patients receiving neoadjuvant chemotherapy in combination with atezolizumab (Arm B).

1.2 Secondary objective

The secondary objective is to evaluate the safety of the treatment combination of atezolizumab + carboplatin + paclitaxel. This will be measured by evaluation of toxicity as outlined in the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0.

1.3 Exploratory objectives

Exploratory objective #1 is to evaluate potential biomarkers of response to chemotherapy in combination with atezolizumab in patients with newly diagnosed TNBC. Potential biomarkers include baseline TIL percentage, baseline PD-L1 expression in the tumor and tumor infiltrating immune cells, baseline immune signature, and baseline neoantigen load. These biomarkers will be correlated with pathologic complete response.

Exploratory objective #2 is to evaluate the impact of chemotherapy in combination with atezolizumab on the immune response in patients with newly diagnosed TNBC. The immune signature and PD-L1 expression in the tumor and tumor-infiltrating immune cells will be evaluated at baseline and after initiation of therapy. The immune response will be correlated with pathologic complete response.

Exploratory objective #3 is to evaluate the impact of chemotherapy in combination with atezolizumab on neoantigen-specific T cell responses in patients with newly diagnosed TNBC.

Exploratory objective #4 is to evaluate the impact of chemotherapy in combination with atezolizumab on long-term clinical endpoints such as overall survival (OS) and disease-free survival (DFS) in patients with newly diagnosed TNBC. The correlation between long-term outcomes and baseline biomarkers will also be assessed.

2. BACKGROUND

2.1 Triple-negative breast cancer

Triple negative breast cancer (TNBC) represents approximately 10-20% of breast cancers worldwide and affects approximately 200,000 women annually [1]. It is defined by a lack of expression of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) receptor. The disease is more common in young black women and in women with a deleterious mutation in the BRCA1 gene [2, 3].

Established targeted therapies in breast cancer are directed against a nuclear or a surface receptor, such as tamoxifen or trastuzumab, respectively. However, no established targets currently exist for TNBC, so patients receive chemotherapy for systemic control of their disease [4]. Despite neoadjuvant chemotherapy trials showing that TNBC has higher pathological complete response (pCR) rates compared to hormone-receptor-positive subtypes [5], TNBC remains extremely difficult to treat in many cases, and patients have high rates of relapse, particularly due to chemotherapy resistance within the first few years [6]. TNBC patients suffer from a worse initial prognosis among all other breast cancer subtypes, and there is a dire need to develop new treatment strategies for TNBC, particularly for those with disease that is resistant to standard chemotherapy.

2.1.1 Neoadjuvant Chemotherapy in Triple Negative Breast Cancer

Neoadjuvant chemotherapy describes primary systemic therapy that is utilized prior to surgery. It was initially used for patients with locally advanced, inoperable breast cancer in an effort to improve local control and decrease distant metastases when used in combination with surgery [7]. A few years later, the National Surgical Adjuvant Breast and Bowel Project (NSABP) began a clinical trial (B-18) to evaluate the efficacy of neoadjuvant chemotherapy compared to adjuvant therapy in patients with operable breast cancer. Approximately 1500 women were randomized to receive chemotherapy either pre- or post-operatively [8]. Although there were no survival differences or differences in rates of ipsilateral tumor recurrence after lumpectomy, preoperative chemotherapy led to an improvement in rates of breast conservation. Since that time, numerous studies have confirmed the utility of neoadjuvant chemotherapy in patients with locally advanced inoperable disease as well as those with operable disease who desire breast-conserving therapy [9, 10]. In addition, the neoadjuvant platform creates an excellent model to assess pathologic tumor response and analyze tissue upon completion of systemic chemotherapy. Ultimately, this allows for the identification of molecular changes induced by treatment, and most importantly, it permits determination of predictors of chemotherapy resistance and chemotherapy sensitivity (CS) to better individualize management.

While several clinical trials involving neoadjuvant traditional chemotherapy, biologics, or different dosing schedules have recently been launched in an attempt to improve the outcome of patients with TNBC, the initial phase of neoadjuvant studies did not restrict entry criteria to only patients with TNBC. However, a large proportion (41%) of the study population in NSABP protocol B-40 was classified as having TNBC [11]. The goals of NSABP B-40 were to determine if the addition of capecitabine or gemcitabine to docetaxel followed by doxorubicin and cyclophosphamide (AC) would increase pCR rates in patients with palpable and operable HER2-negative disease and also to determine whether the addition of bevacizumab to docetaxel-based

regimens followed by AC will increase pCR rates. In the chemotherapy alone arms, the addition of capecitabine or gemcitabine to docetaxel versus docetaxel alone did not increase the pCR rates (29.7% and 31.8%, respectively, vs. 32.7%; P=0.69). Similarly, the GeparTrio study reported by Huober et al. showed a pCR rate of 39% in the TNBC patients in a trial that sought to determine the effect of switching neoadjuvant chemotherapy depending on mid-course response on pCR at time of surgery [12]. Patients received 2 cycles of docetaxel, doxorubicin, and cyclophosphamide (TAC), followed by either 4 or 6 more cycles of TAC in responders or 4 cycles of TAC versus capecitabine plus vinorelbine in non-responders. In non-responders, pCR rates were less than 10% in both groups, suggesting a lack of benefit of switching chemotherapy to these non-standard agents or even providing further chemotherapy in the adjuvant setting to that subset of patients.

2.1.2 Platinum agents for the treatment of TNBC

In contrast to the lack of data that capecitabine, vinorelbine or gemcitabine improve pCR rates, recent and increasing data indicate a potential role for platinum agents in the treatment of TNBC [13-16]. Sporadic BRCA mutations and/or homologous recombination DNA repair deficiency is known to be present in a significant fraction of TNBC patients [17-19]. This insight into the biology of TNBC provides strong rationale for the use of DNA damaging agents like platinum compounds in TNBC. Recent studies have demonstrated that the addition of carboplatin to anthracycline and taxane based neoadjuvant chemotherapy improves pCR rates in patients with TNBC, although toxicity is increased [13, 20].

For example, CALGB 40603, presented at the 2013 San Antonio Breast Cancer Symposium, evaluated the impact of the addition of carboplatin and/or bevacizumab to neoadjuvant weekly paclitaxel followed by dose-dense AC on pCR rates in TNBC [13]. This was a 2 X 2 factorial design in which patients on Arm A received weekly paclitaxel at 80 mg/m^2 for 12 weeks followed by dose dense AC for 4 cycles, patients on Arm B received weekly paclitaxel 80 mg/m^2 for 12 weeks plus bevacizumab followed by dose dense AC for 4 cycles, patients on Arm C received weekly paclitaxel 80 mg/m^2 for 12 weeks plus carboplatin followed by dose dense AC for 4 cycles, and patients on Arm D received weekly paclitaxel 80 mg/m^2 for 12 weeks plus carboplatin and bevacizumab followed by dose dense AC for 4 cycles. While there was no clear benefit from the addition of bevacizumab, pCR rates increased from 41% to 54% in patients receiving carboplatin. Similarly, a significant improvement in pCR rate (58.7% vs 37.9%, $p<0.05$) in TNBC was observed with the addition of carboplatin (AUC 1.5 weekly) to 18 weeks of weekly paclitaxel (80mg/m^2) plus non-pegylated liposomal doxorubicin (20mg/m^2) in the GeparSixto trial [20].

Kern et al. recently reported that carboplatin plus docetaxel alone is a safe and effective neoadjuvant chemotherapy regimen for TNBC. Patients were treated with six cycles of docetaxel (75mg/m^2) and carboplatin AUC 6 q3w. Grade 3/4 toxicities were rare, and 93% of patients completed six cycles of therapy. The pCR rate was 50%. After 2 and 5years, overall survival (OS) was 96.7 and 89.7% [21, 22].

Similarly, Sharma et al. recently reported on 190 patients with stage I-III TNBC treated uniformly with carboplatin and docetaxel in two independent prospective cohorts (NCT02489448, NCT01560663) [23]. In these cohorts, the pathologic complete response rate was 55%, and response rates were similar between patients with BRCA mutation-associated and

wildtype TNBC. Based on these promising results, a randomized phase 2 trial has been initiated (NCT02413320) comparing carboplatin + docetaxel alone to carboplatin + docetaxel followed by ddAC.

Of note, the combination of carboplatin and paclitaxel has proven safe in combination with atezolizumab in NSCLC as reported at ASCO 2015 (Abstract 8030).

Given the promising results of neoadjuvant carboplatin and docetaxel in several independent phase 1 clinical trials, the fact that this regimen is now being tested in a phase 2 trial, and the fact that carboplatin and paclitaxel has proven to be safe in combination with atezolizumab, we have selected carboplatin and paclitaxel as the chemotherapy backbone for this clinical trial.

2.2 Atezolizumab

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 [24]. 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 PD-L1 and its receptors, PD-1 and B7-1 (also known as CD80), both of which function as inhibitory receptors expressed on T cells.

Atezolizumab shows anti-tumor activity in both nonclinical models and cancer patients and is being investigated as a potential therapy in a wide variety of malignancies. Atezolizumab is being studies as a single agent in the advanced cancer and adjuvant therapy settings, as well as in combination with chemotherapy, targeted therapy, and cancer immunotherapy.

Atezolizumab is approved in the United States for the treatment of locally advanced or metastatic urothelial carcinoma and as well as metastatic non-small cell lung carcinoma.

2.2.1 Atezolizumab mechanism of action

PD-L1 expression is prevalent in many human tumors (*e.g.*, lung, bladder, ovarian, melanoma, colon carcinoma), and its overexpression has been associated with poor prognosis in patients with several cancers [25-28]. PD-L1 binds to two known inhibitory receptors expressed on activated T cells (PD-1 and B7.1), and receptor expression is sustained in states of chronic stimulation such as chronic infection or cancer [29, 30]. Ligation of PD-L1 with PD-1 or B7.1 inhibits T-cell proliferation, cytokine production, and cytolytic activity, leading to the functional inactivation or inhibition of T cells. Aberrant expression of PD-L1 on tumor cells has been reported to impede anti-tumor immunity, resulting in immune evasion [31]. Therefore, interruption of the PD-L1/PD-1 and PD-L1/B7.1 pathway represents an attractive strategy to reinvigorate tumor-specific T-cell immunity.

Blockade of PD-L1 or PD-1 with monoclonal antibodies has been reported to result in strong and often rapid antitumor effects in several mouse tumor models [32, 33]. These data suggest that tumor-specific T cells may be present in the tumor microenvironment in an inactive or inhibited state, and blockade of the PD-L1/PD-1 pathway can reinvigorate tumor-specific T-cell responses.

Collectively, these data establish the PD-L1/PD-1 pathway as a promising new therapeutic target in patients with advanced tumors. Immune-related adverse events (AEs) reported from the two recent studies were consistent with the role of the PD-L1/PD-1 pathway in regulating peripheral tolerance.

2.2.2 Summary of nonclinical experience

The safety, pharmacokinetics (PK), 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, PK, and toxicokinetics of atezolizumab.

Overall, the nonclinical PK and toxicokinetics observed for atezolizumab supported entry into clinical studies, including providing adequate safety factors for the proposed phase 1 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.

2.2.3 Summary of clinical experience

A summary of clinical data from company-sponsored atezolizumab trials is presented below. Details of all ongoing studies can be found in the Atezolizumab Investigator's Brochure.

2.2.3.1 Clinical PK and immunogenicity

On the basis of available preliminary PK data (0.03–20 mg/kg), atezolizumab shows linear PK at doses ≥1 mg/kg [24]. Based on an analysis of exposure, safety, and efficacy data, the following factors had no clinically relevant effect: age (21−89 years), body weight, gender, positive ATA status, albumin levels, tumor burden, region or ethnicity, renal impairment, mild hepatic impairment, level of PD-L1 expression, or ECOG status. No formal PK drug-drug interaction studies have been conducted with atezolizumab, and the interaction potential is unknown. Further details can be found in the current Investigator's Brochure.

The development of anti-therapeutic antibodies (ATAs) has been observed in patients in all dose cohorts and was associated with changes in PK for some patients in the lower dose cohorts (0.3, 1, and 3 mg/kg) [24]. Patients dosed at ≥ 10 mg/kg maintained C_{min} values well above the target serum concentration of 6 mcg/mL despite the detection of ATAs. Accordingly, the development of detectable ATAs does not appear to have a clinically significant impact on PK for doses above 10 mg/kg. To date, no relationship between the development of measurable ATAs and safety or efficacy has been observed.

2.2.3.2 Clinical safety summary

As of 10 May 2016, atezolizumab has been administered (alone or in combination with other agents) to approximately 6053 patients with solid tumor and hematologic malignancies. The first-in-human monotherapy study PCD4989g (in patients with locally advanced or metastatic

solid tumors or hematologic malignancies) provides the majority of monotherapy safety data, with 629 safety-evaluable patients as of the data extraction date. Currently, no maximum tolerated dose (MTD), no dose-limiting toxicities (DLTs), and no clear dose-related trends in the incidence of AEs have been determined.

Fatigue, decreased appetite, nausea, diarrhea, constipation, and cough were commonly reported AEs in single and combination therapy (Investigator's Brochure, 2016). AE profiles are similar across tumor types studied, including non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), triple-negative breast cancer (TNBC), and urothelial carcinoma (UC), and are consistent with the mechanism of action of atezolizumab. The overall immune-mediated AEs reported were considered moderate in severity, and the majority of patients were able to continue on atezolizumab therapy.

As of the data extraction date of December 15, 2015, there were 629 safety-evaluable patients from the first-in-human phase 1a study PCD4989g (Investigator's Brochure, 2016). The median age was 61 years. Of the 629 patients, 619 patients (98.4%) reported at least one AE of any grade or attribution to atezolizumab, and 316 patients (50.2%) experienced at least one grade 3 or 4 AE of any attribution. A total of 444 patients (70.6%) reported at least one treatment-related AE, and 86 patients (13.7%) experienced at least one treatment-related grade 3 or 4 AE. The most frequently observed AEs of any grade and attribution (occurring in ≥10% of treated patients) include fatigue, decreased appetite, nausea, pyrexia, constipation, cough, dyspnea, diarrhea, anemia, vomiting, asthenia, back pain, headache, arthralgia, pruritus, rash, abdominal pain, insomnia, peripheral edema, and dizziness.

Serious AEs (SAEs) have been reported in 261 patients (41.5%) in study PCD4989g (Investigator's Brochure, 2016). Reported SAEs were consistent with the underlying disease. Treatment-related SAEs (57 patients [9.1%]) included pyrexia, dyspnea, pneumonitis, malaise, fatigue, hypoxia, colitis, and bone pain. Pooled single-agent safety data from 1978 patients with UC, NSCLC, and other indications (including trial PCD4989g) indicate that the most frequent (>1% of patients) serious adverse drug reactions (regardless of grade) include dyspnea (3.0%), back pain (1.2%), and abdominal pain (1.1%). A list of AEs considered "expected" for atezolizumab is presented in Section 7.1.1.

Immune-Related Adverse Events

Given the mechanism of action of atezolizumab, events associated with inflammation and/or immune-related adverse events are closely monitored during the atezolizumab clinical program. To date, immune-related adverse events associated with atezolizumab include hepatitis, pneumonitis, colitis, pancreatitis, diabetes mellitus, hypothyroidism, hyperthyroidism, adrenal insufficiency, Guillain-Barré syndrome, myasthenic syndrome/myasthenia gravis, and meningoencephalitis.

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

2.2.3.3 Clinical efficacy summary

Patients with multiple tumor types were included in study PCD4989g, with the largest cohorts consisting of patients with non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), and bladder cancer [24]. Objective responses with atezolizumab monotherapy were observed in a broad range of malignancies, including NSCLC, RCC, and UC. Objective responses were

observed in a broad range of malignancies, including NSCLC, RCC, melanoma, UC, colorectal cancer, head and neck cancer, gastric cancer, breast cancer, and sarcoma. Both the preliminary and more mature efficacy data available suggest that treatment with atezolizumab as a single agent or in combination with other therapeutic agents results in anti-tumor activity across a range of tumor types and hematologic malignancies (UC, NSCLC, RCC, TNBC, melanoma, CRC, and NHL) and across lines of therapy. Clinical benefit was observed in terms of objective responses, durability of responses, and overall survival (OS). Improved efficacy of atezolizumab was observed in the unselected patient population, as well as in patients with higher PD-L1 expression on TCs or ICs (*e.g.*, NSCLC) or on ICs only (*e.g.*, mUC, RCC).[34, 35]

2.3 Study rationale

2.3.1 Rationale for checkpoint blockade therapy in TNBC

There is strong rationale for the use of checkpoint blockade therapy in patients with TNBC.

First, TNBC is a mutationally complex breast cancer subtype. Previous studies suggest that response to checkpoint blockade therapy is associated with mutational load [36]. Several recent studies have provided insight into the mutational landscape in human breast cancer, including TNBC. While the average somatic mutational frequency across almost 30 different types of cancer is approximately one mutation/megabase, the frequency among all breast cancers ranges from 0.1 to 10 [37-39]. One feature of TNBC is the increased frequency of germline and somatic TP53 and BRCA1 mutations [40]. TP53 and BRCA mutations lead to defects in DNA repair mechanisms, and consequently an increased mutational frequency. Extensive analysis of the clonal and mutational spectrum of primary TNBC suggests that TNBC is characterized by a higher mutational frequency than other subtypes of breast cancer [41-43]. The relative abundance of somatic mutations in TNBC compared to other breast cancer subtypes suggests that neoantigens that can be targeted by checkpoint blockade therapy are more likely to be present.

Second, tumor infiltrating lymphocytes (TILs) are more common in TNBC, and TILs are associated with improved outcome in TNBC following adjuvant or neoadjuvant chemotherapy [44-46]. The association between TILs and improved outcome in TNBC suggests that the adaptive immune system contributes to the response to chemotherapy.

Third, PD-L1 expression is higher in TNBC than other breast cancer subtypes [47, 48]. PD-L1 expression has been used as a biomarker for response to checkpoint blockade therapy [35]. We have examined the expression of PD-1 and PD-L1 in the common molecular subtypes of breast cancer using a well annotated human breast cancer tissue array [48, 49]. These studies demonstrate that expression of PD-1 on tumor-infiltrating lymphocytes and/or PD-L1 on breast cancers is more common in TNBC, and expression of these proteins is associated with a poor prognosis. Other investigators have also documented increased expression of PD-L1 in TNBC [47].

Fourth, a recent phase 1 clinical trial of atezolizumab in patients with metastatic TNBC demonstrates the safety of checkpoint blockade therapy with promising response rates. In an oral presentation at the AACR Annual meeting in April, 2015 (Abstract 2859), Emens et al. reported on 54 patients with metastatic TNBC enrolled in an expansion cohort. Patients received atezolizumab at 15 mg/kg, 20 mg/kg or 1200 mg flat dose IV q3w. AEs occurred in 67% of

patients, most frequently fatigue (22%), pyrexia (15%), neutropenia (15%) and nausea (15%). Grade 3 AEs occurred in 11% of patients (adrenal insufficiency, neutropenia, nausea, vomiting, decreased WBC count). Twenty-one of the 54 patients were evaluable for efficacy, all of whom were PDL1–positive (IHC 2/3). Among the 21 evaluable patients with PD-L1-expressing tumors, the unconfirmed RECIST ORR was 24% (95% CI, 8% to 47%) which included 3 PRs and 2 CRs. Adams et al. reported the results of a phase 1b clinical trial of atezolizumab in combination with nab-paclitaxel in patients with metastatic TNBC at the 2015 SABCS Meeting (Abstract P2-11-06). 24 patients with a minimum follow-up of ≥ 3 months were evaluable for efficacy. Across all groups the ORR was 71%. Based on these results, a randomized phase 3 trial is planned (IMpassion130, NCT02425891).

2.3.2 Rationale for the combination of chemotherapy and checkpoint blockade therapy

Multiple preclinical studies and a recent phase 1 clinical trial provide strong rationale for combining chemotherapy and checkpoint blockade therapy. Chemotherapies have immune adjuvant properties. Several mechanisms have been proposed to explain how chemotherapies impact antitumor immunity, including modulation of STAT signaling, induction of immunogenic cancer cell death, and modulation of PD-L1 expression [50, 51]. Of note, several recent high impact publications using cell depletion or genetic models suggest that an intact adaptive immune system is required for the success of chemotherapy [52-54].

Of note, there is strong circumstantial evidence documenting the importance of the adaptive immune system in the response to chemotherapy in TNBC. Translational studies demonstrate that the presence of TILs in TNBC is strongly associated with response to chemotherapy. This association has been demonstrated by several independent groups using well validated immunohistochemistry assays and clinical specimens from large randomized prospective phase 3 trials (BIG 02-98, ECOG E2197, ECOG E1199) [44, 45]. This association between the presence of TILs and response to chemotherapy has been confirmed in the neoadjuvant setting (GeparSixto) [46]. Similar results have been obtained when evaluating the impact of TIL percentage after neoadjuvant chemotherapy [55-57].

Preclinical studies have specifically addressed the potential of combination chemotherapy and checkpoint blockade. Lesterhuis et al. demonstrated that CTLA-4 blockade is synergistic with gemcitabine in two non-immunogenic murine tumor models [58]. Jure-Kunkel et al. demonstrated that CTLA-4 blockade is synergistic with ixabepilone, etoposide, and gemcitabine in multiple preclinical murine tumor models, including SA1N fibrosarcoma, EMT-6 mammary carcinoma, M109 lung carcinoma, and CT-26 colon carcinoma [59]. Shalapour et al. recently published a high impact paper demonstrating that anti-PDL1 therapy enhances the efficacy of oxaliplatin chemotherapy in prostate cancer [53].

A recent phase 1 clinical trial in NSCLC confirms the potential of combination chemotherapy and atezolizumab therapy. In an oral presentation at the ASCO Annual meeting in June, 2015 (Abstract 8030), Liu et al. reported on 37 patients with locally advanced or metastatic NSCLC who were randomized to receive carboplatin-based chemotherapy regimens in combination with atezolizumab. Atezolizumab was given concurrently with chemotherapy, and no unexpected toxicities were observed. Across all arms, the ORR was 67% - significantly higher than previous experience with atezolizumab alone. The combination of carboplatin, paclitaxel and

atezolizumab has now progressed to a phase 3 trial in NSCLC (NCT02367794).

2.4 Correlative studies background

2.4.1 Tumor infiltrating lymphocyte (TIL) percentage

TIL percentage is a well-described and validated histopathologic analysis that has been correlated with clinical response to adjuvant and neoadjuvant chemotherapy in patients with TNBC [44-46]. These studies used standardized methodology for visual assessment of TIL percentage, and tissue specimens from patients enrolled in phase 3 randomized clinical trials. The studies demonstrated that TIL percentage is associated with improved overall survival. This correlation was first reported using baseline samples from the BIG 2–98 trial [45] and subsequently independently confirmed in 481 TNBC sample prospectively collected during two phase III adjuvant randomized breast cancer trials (United States Eastern Cooperative Oncology Group trials 2197 and 1199) [44].

An increase in the number of TILs has also been shown to be associated with response to checkpoint inhibition therapy in multiple independent studies. For instance, in a prospective randomized trial of ipilimumab for patients with advanced melanoma where serial tumor biopsies were obtained, Hamid et al. demonstrated that 57.1% of patients in the benefit group had a post-treatment increase in TILs, whereas only 10.0% of patients in the non-benefit group had a post-treatment increase in TILs ($p = 0.005$) [60]. Similarly, Tumeh et al. studied tumor samples from 46 patients with metastatic melanoma obtained before and after therapy with pembrolizumab. In this study, they used quantitative immunohistochemistry to evaluate the number of CD8 T cells present in the tumor and observed that an increase in the number of CD8 T cells was strongly associated with the best percent change in tumor size as measured by CT scan (n=18, Spearman r = −0.75, P = 0.0002) [61]. Finally, Herbst et al. evaluated the safety, activity and biomarkers of PD-L1 inhibition in patients treated with atezolizumab [35]. They found that after atezolizumab treatment, regressing lesions displayed a dense immune infiltratePD-L1 expression.

2.4.2 PD-L1 expression

Immune cell PD-L1 expression has recently been shown to predict response to atezolizumab therapy in multiple cancer types [35]. Subsequent studies of PD-L1 expression, including several that were performed in patients with TNBC, indicate that expression of PD-L1 should be assessed separately on tumor cells and tumor infiltrating immune cells [62, 63]. For example, Beckers et al. observed that PD-L1 was expressed by both TNBC cells and tumor infiltrating immune cells, and that cytoplasmic tumoral PD-L1 expression as well as stromal PD-L1 expression was associated with favorable patient outcome. Further study is clearly required to define the association between PD-L1 expression and clinical response to atezolizumab in patients with TNBC.

One of the exploratory objectives is to evaluate potential biomarkers of response to combination therapy with chemotherapy and atezolizumab in patients with newly diagnosed TNBC. We will correlate baseline PD-L1 expression in tumor infiltrating immune cells and/or tumor cells with pathologic complete response in patients being treated with combination therapy.

2.4.3 Immune signature

One of the exploratory objectives is to evaluate potential biomarkers of response to combination therapy with chemotherapy and atezolizumab in patients with newly diagnosed TNBC. Immune signature analysis will be performed using the NanoString platform. We will correlate immune signature at baseline with pathologic complete response in patients being treated with combination therapy. In addition, we will assess the change in immune signature following therapy. Immune signature analyses will be performed at baseline, after initiation of therapy (day 18-22), and at time of surgery.

Denkert et al. defined an immune signature correlated with pathologic complete response following neoadjuvant chemotherapy in the GeparSixto clinical trial. Twelve immunologically relevant mRNA markers were selected for detailed evaluation in breast cancer tissue, including T-cell markers, B-cell markers, chemokines, and immune checkpoint parameters (CXCL9, CCL5, CD8A, CD80, CXCL13, IGKC, CD21, IDO1, PD-1, PD-L1, CTLA4, FOXP3). Three different immune subtypes of tumors with different expression of immunologic genes were defined.

Likewise, gene expression analyses have been performed in an attempt to define biomarkers of response to atezolizumab in NSCLC and bladder cancer [64, 65]. In NSCLC, B- and NK-cell signatures were associated with clinical benefit [64].

In bladder cancer, baseline immune signature associated with an effector T cell signature (including *CD8A*, *GZMA*, *IFNG*) and/or NK gene signature (*NKG2* family members) were associated with clinical benefit [65].

2.4.4 Neoantigen Load

Neoantigen load has been correlated with response to checkpoint blockade therapy in preclinical models [66] and human clinical trials [36].

To estimate neoantigen load we will use recently developed sequencing and epitope prediction pipelines [66].

One of the exploratory objectives is to evaluate potential biomarkers of response to combination therapy with chemotherapy and atezolizumab in patients with newly diagnosed TNBC. We will correlate baseline neoantigen load with pathologic complete response in patients being treated with combination therapy.

2.4.5 Neoantigen-specific T cell response

We recently described a strategy to identify and prioritize neoantigens in murine sarcomas [66]. We used this strategy to demonstrate that checkpoint blockade therapy targets neoantigens [66].

To evaluate the T cell response to multiple different candidate neoantigens, a high throughput assay system is required, and we have collaborated with Dr. [redacted] and [redacted] at The Netherlands Cancer Institute in Amsterdam to develop bar-coded MHC class I tetramers by UV-induced epitope exchange capable of recognizing multiple different neoantigen-specific T cells simultaneously. We will then identify candidate neoantigens using the optimized sequencing and epitope prediction algorithms that we have established. Once we have identified and prioritized neoantigens, we will generate MHC class I tetramers by UV-

induced epitope exchange, and perform multiparameter flow cytometry, or time-of-flight mass spectrometry (CyTOF) to determine if neoantigen-specific T cells are present/functional in the peripheral blood or primary breast cancers.

3. PATIENT SELECTION

3.1 Eligibility criteria

1. Patients must have histologically confirmed new diagnosis of breast cancer.
2. ER and PR < Allred score of 3 or ≤ 5% positive staining cells in the invasive component of the tumor (provided the patient is being treated as triple negative breast cancer).
3. HER2 negative by FISH or IHC staining 0 or 1+ according to NCCN guidelines.
4. Clinical stage T2-T4c, any N, M0 primary tumor by AJCC 7th edition clinical staging.
5. Eligible for neoadjuvant chemotherapy.
6. No prior therapy for this disease.
7. Age ≥ 18 years.
8. ECOG performance status ≤ 2 (Karnofsky ≥ 60%, see **Appendix A**).
9. Patients must have normal organ and marrow function as defined below:

– leukocytes	≥ 2,500/mcL
– absolute neutrophil count	≥ 1,500/mcL
– platelets	≥ 150,000/mcL
– hemoglobin	≥ 10 g/dL
– total bilirubin	≤ 1.5 × institutional upper limit of normal (ULN) (however, patients with known Gilbert disease who have serum bilirubin level ≤3 × ULN may be enrolled)
– AST(SGOT)/ALT(SGPT)	≤ 3 × ULN
– alkaline phosphatase	≤ 2.5 × ULN
– creatinine clearance	≥ 30 mL/min/1.73 m^2 by Cockcroft-Gault: $\frac{(140 - age) \times (weight\ in\ kg) \times (0.85\ if\ female)}{72 \times (serum\ creatinine\ in\ mg/dL)}$
– INR and aPTT	≤ 1.5 × ULN (This applies only to patients who **do not** receive therapeutic anticoagulation; patients receiving therapeutic anticoagulation, such as low-molecular-weight heparin or warfarin, should be on a stable dose.

10. Administration of atezolizumab may have an adverse effect on pregnancy and poses a risk to the human fetus, including embryo-lethality. Women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation, and for 180 days after the last dose of paclitaxel. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.
11. Ability to understand and the willingness to sign an IRB-approved written informed consent document.

3.2 Exclusion criteria

1. Known metastatic disease.
2. Invasive cancer in the contralateral breast.
3. Patients with a previous history of non-breast malignancy are eligible only if they meet the following criteria for a cancer survivor: (1), and (2)
    - Has undergone potentially curative therapy for all prior malignancies.
    - Has been considered disease-free for at least 1 year (with the exception of basal cell or squamous cell carcinoma of the skin or carcinoma-in-situ of the cervix.
4. Patients with prior allogeneic bone marrow transplantation or prior solid organ transplantation.
5. Treatment with any other investigational agent within 4 weeks prior to Cycle 1, Day 1.
6. Treatment with systemic immunosuppressive medications (including, but not limited to, prednisone, cyclophosphamide, azathioprine, methotrexate, thalidomide, and anti-tumor necrosis factor [anti-TNF] agents) within 2 weeks prior to Cycle 1, Day 1.
    - Patients who have received acute, low dose, systemic immunosuppressant medications (*e.g.*, a one-time dose of dexamethasone for nausea) may be enrolled.
    - The use of inhaled corticosteroids and mineralocorticoids (*e.g.*, fludrocortisone) for patients with orthostatic hypotension or adrenocortical insufficiency is allowed.
7. Patients taking bisphosphonate therapy for symptomatic hypercalcemia. Use of bisphosphonate therapy for other reasons (*e.g.*, osteoporosis) is allowed.
8. Known hypersensitivity to Chinese hamster ovary cell products or other recombinant human antibodies.
9. History of severe allergic, anaphylactic, or other hypersensitivity reactions to chimeric or humanized antibodies or fusion proteins
10. History of allergic reactions attributed to compounds of similar chemical or biologic composition to other agents used in study.
11. Known clinically significant liver disease, including active viral, alcoholic, or other hepatitis; cirrhosis; fatty liver; and inherited liver disease.
    - Patients with past or resolved hepatitis B infection (defined as having a negative hepatitis B surface antigen [HBsAg] test and a positive anti-HBc [antibody to hepatitis B core antigen] antibody test) are eligible.
    - Patients positive for hepatitis C virus (HCV) antibody are eligible only if polymerase chain reaction (PCR) is negative for HCV RNA.
12. History or risk of autoimmune disease, including, but not limited to, systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, vascular thrombosis associated with antiphospholipid syndrome, Wegener's granulomatosis, Sjögren's syndrome, Bell's palsy, Guillain-Barré syndrome, multiple sclerosis, autoimmune thyroid disease, vasculitis, or glomerulonephritis.

- Patients with a history of autoimmune hypothyroidism on a stable dose of thyroid replacement hormone may be eligible.
- Patients with controlled Type 1 diabetes mellitus on a stable insulin regimen may be eligible.
- Patients with eczema, psoriasis, lichen simplex chronicus of vitiligo with dermatologic manifestations only (*e.g.*, patients with psoriatic arthritis would be excluded) are permitted provided that they meet the following conditions:
    - Patients with psoriasis must have a baseline ophthalmologic exam to rule out ocular manifestations
    - Rash must cover less than 10% of body surface area (BSA)
    - Disease is well controlled at baseline and only requiring low potency topical steroids (*e.g.*, hydrocortisone 2.5%, hydrocortisone butyrate 0.1%, flucinolone 0.01%, desonide 0.05%, aclometasone dipropionate 0.05%)
    - No acute exacerbations of underlying condition within the last 12 months (not requiring psoralen plus ultraviolet A radiation [PUVA], methotrexate, retinoids, biologic agents, oral calcineurin inhibitors; high potency or oral steroids)

13. History of idiopathic pulmonary fibrosis, pneumonitis (including drug induced), organizing pneumonia (*i.e.*, bronchiolitis obliterans, cryptogenic organizing pneumonia, etc.), or evidence of active pneumonitis on screening chest computed tomography (CT) scan. History of radiation pneumonitis in the radiation field (fibrosis) is permitted.
14. Patients with active tuberculosis (TB) are excluded.
15. Severe infections within 4 weeks prior to Cycle 1, Day 1, including, but not limited to, hospitalization for complications of infection, bacteremia, or severe pneumonia.
16. Signs or symptoms of infection within 2 weeks prior to Cycle 1, Day 1.
17. Received oral or intravenous (IV) antibiotics within 2 weeks prior to Cycle 1, Day 1. Patients receiving prophylactic antibiotics (*e.g.*, for prevention of a urinary tract infection or chronic obstructive pulmonary disease) are eligible.
18. Major surgical procedure within 28 days prior to Cycle 1, Day 1 or anticipation of need for a major surgical procedure during the course of the study with the exception of the planned breast cancer surgery that is part of the trial design.
19. Administration of a live, attenuated vaccine within 4 weeks before Cycle 1, Day 1 or anticipation that such a live, attenuated vaccine will be required during the study and up to 5 months after the last dose of atezolizumab.
    - Influenza vaccination should be given during influenza season only (approximately October to March). Patients must not receive live, attenuated influenza vaccine within 4 weeks prior to Cycle 1, Day 1 or at any time during the study.
20. Patients requiring treatment with a RANKL inhibitor (e.g. denosumab) who cannot discontinue it before treatment with atezolizumab.
21. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection,

symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia (including symptomatic sinus bradycardia), or psychiatric illness/social situations that would limit compliance with study requirements.

22. Patients positive for human immunodeficiency virus (HIV) are NOT excluded from this study, but HIV-positive patients must have:
    - A stable regimen of highly active anti-retroviral therapy (HAART)
    - No requirement for concurrent antibiotics or antifungal agents for the prevention of opportunistic infections
    - A CD4 count above 250 cells/mcL and an undetectable HIV viral load on standard PCR-based tests

23. Pregnant women are excluded from this study because atezolizumab is an agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with atezolizumab, breastfeeding should be discontinued if the mother is treated with atezolizumab. These potential risks may also apply to other agents used in this study.

3.3 Inclusion of women and minorities

This trial is open to women and members of all minority groups. Men will not be eligible as breast cancer occurs primarily in women.

NIH policy requires that women and members of minority groups and their subpopulations be included in all NIH-supported biomedical and behavioral research projects involving NIH-defined clinical research unless a clear and compelling rationale and justification establishes to the satisfaction of the funding Institute & Center (IC) Director that inclusion is inappropriate with respect to the health of the subjects or the purpose of the research. Exclusion under other circumstances must be designated by the Director, NIH, upon the recommendation of an IC Director based on a compelling rationale and justification. Cost is not an acceptable reason for exclusion except when the study would duplicate data from other sources. Women of childbearing potential should not be routinely excluded from participation in clinical research. Please see http://grants.nih.gov/grants/funding/phs398/phs398.pdf.

4. REGISTRATION PROCEDURES

4.1 Investigator and research associate registration with CTEP

4.1.1 CTEP registration procedures

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all investigators participating in any NCI-sponsored clinical trial to register and to renew their registration annually. Registration requires the submission of:

- a completed ***Statement of Investigator Form*** (FDA Form 1572) with an original signature
- a current Curriculum Vitae (CV)
- a completed and signed ***Supplemental Investigator Data Form*** (IDF)
- a completed ***Financial Disclosure Form*** (FDF) with an original signature

Fillable PDF forms and additional information can be found on the CTEP website at http://ctep.cancer.gov/investigatorResources/investigator_registration.htm.

For questions about Investigator Registration, please contact the ***CTEP Investigator Registration Help Desk*** by email at pmbregpend@ctep.nci.nih.gov.

4.1.2 CTEP associate registration procedures/CTEP-IAM account

The Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) application is a web-based application intended for use by both Investigators (*i.e.*, all physicians involved in the conduct of NCI-sponsored clinical trials) and Associates (*i.e.*, all staff involved in the conduct of NCI-sponsored clinical trials).

Associates will use the CTEP-IAM application to register (both initial registration and annual re-registration) with CTEP and to obtain a user account.

Investigators will use the CTEP-IAM application to obtain a user account only. (See CTEP Investigator Registration Procedures above for information on registering with CTEP as an Investigator, which must be completed before a CTEP-IAM account can be requested.)

An active CTEP-IAM user account is required to access all CTEP applications and, if applicable (*e.g.*, all Network trials), all Cancer Trials Support Unit (CTSU) applications and websites.

Additional information can be found on the CTEP website at http://ctep.cancer.gov/branches/pmb/associate_registration.htm.

For questions about Associate Registration or CTEP-IAM Account Creation, please contact the ***CTEP Associate Registration Help Desk*** by email at ctepreghelp@ctep.nci.nih.gov.

4.2 Site registration

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

Each investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can be approved to enroll patients. Assignment of site registration status in the CTSU

Regulatory Support System (RSS) uses extensive data to make a determination of whether a site has fulfilled all regulatory criteria including but not limited to: an active Federal Wide Assurance (FWA) number, an active roster affiliation with the Lead Network or a participating organization, a valid IRB approval, and compliance with all protocol specific requirements.

Sites participating on the NCI CIRB initiative that are approved by the CIRB for this study are not required to submit separate IRB approval documentation to the CTSU Regulatory Office. For sites using the CIRB, IRB approval information is received from the CIRB and applied to RSS in an automated process. Signatory Institutions must submit a Study Specific Worksheet for Local Context (SSW) to the CIRB via IRBManager to indicate their intention to open the study locally. The CIRB's approval of the SSW is then communicated to the CTSU Regulatory Office. In order for the SSW approval to be processed, the Signatory Institution must inform the CTSU which CIRB-approved institutions aligned with the Signatory Institution are participating in the study.

4.2.1 Downloading regulatory documents

Site registration forms may be downloaded from the NCI Protocol #10013 protocol page located on the CTSU Web site. Permission to view and download this protocol is restricted and is based on person and site roster data housed in the CTSU RSS. To participate, Investigators and Associates must be associated with the Corresponding or Participating protocol organization in the RSS.

- Go to https://www.ctsu.org and log in using your CTEP-IAM username and password.
- Click on the Protocols tab in the upper left of your screen.
- Either enter the protocol # in the search field at the top of the protocol tree, or
- Click on the By Lead Organization folder to expand, then select LAO-NC010 and protocol #10013.
- Click on LPO Documents, select the Site Registration documents link, and download and complete the forms provided. (Note: For sites under the CIRB initiative, IRB data will load to RSS as described above.)

4.2.2 Requirements For NCI Protocol 10013 site registration:

- CTSU Transmittal Sheet (optional)
- IRB approval (for sites not participating via the NCI CIRB; local IRB documentation, an IRB-signed CTSU IRB Certification Form, Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form, or combination is accepted)
- Study Chair Approval

4.2.3 Submitting regulatory documents

Submit required forms and documents to the CTSU Regulatory Office, where they will be entered and tracked in the CTSU RSS.

Regulatory Submission Portal: www.ctsu.org (members' area) → Regulatory Tab →

Regulatory Submission

When applicable, original documents should be mailed to:

CTSU Regulatory Office
1818 Market Street, Suite 1100
Philadelphia, PA 19103

Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support.

4.2.4 Checking site registration status

You can verify your site registration status on the members' section of the CTSU website.

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password.
- Click on the Regulatory tab at the top of your screen.
- Click on the Site Registration subtab.
- Enter your 5-character CTEP Institution Code and click on Go.

Note: The status given only reflects compliance with IRB documentation and institutional compliance with protocol-specific requirements as outlined by the Lead Network. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with the NCI or their affiliated networks.

4.3 Patient registration

4.3.1 OPEN/IWRS

Patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). OPEN is a web-based registration system available to users on a 24/7 basis. It is integrated with the CTSU Enterprise System for regulatory and roster data interchange and with the Theradex Interactive Web Response System (IWRS) for retrieval of patient registration/randomization assignment. Patient enrollment data entered by Registrars in OPEN / IWRS will automatically transfer to the NCI's clinical data management system, Medidata Rave.

The OPEN system will provide the site with a printable confirmation of registration and treatment information. Please print this confirmation for your records.

4.3.2 OPEN/IWRS user requirements

OPEN/IWRS users must meet the following requirements:

- Have a valid CTEP-IAM account (*i.e.*, CTEP username and password).
- To enroll patients: Be on an ETCTN Corresponding or Participating Organization roster with the role of Registrar.
- Have regulatory approval for the conduct of the study at their site.

Prior to accessing OPEN/IWRS, site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes.
- If applicable, all patients have signed an appropriate consent form and HIPAA authorization form.

4.3.3 Patient Enrollment Instructions

N/A

4.3.4 OPEN/IWRS Questions?

Further instructional information on OPEN is provided on the OPEN tab of the CTSU website at https://www.ctsu.org or at https://open.ctsu.org. For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

4.4 Patient Randomization

Patients will be randomized in a 1:2 ratio between Arm A (chemotherapy alone, n = 20) and Arm B (chemotherapy plus atezolizumab, n = 40) and will be stratified by clinical stage (stage II vs. stage III). The randomization table will be generated using the SAS program PROC PLAN. Enrollment will continue until 60 evaluable or 72 total patients are enrolled, whichever comes first. Enrollment of up to 72 patients is to account for up to 20% of patients being non-evaluable for primary endpoint(s). Randomization will occur with OPEN registration.

4.5 General guidelines

Following registration, patients should begin protocol treatment within 14 days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

5. TREATMENT PLAN

5.1 Agent administration

Reported adverse events and potential risks are described in **Section 7**. Appropriate dose modifications are described in **Section 6**. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Patients randomized to Arm A will be treated with neoadjuvant carboplatin + paclitaxel (paclitaxel IV (80 mg/m^2) qw and then carboplatin IV q3w to achieve an initial target area under the curve (AUC) of 5 milligrams per milliliters per minute (mg/mL/min)). The capped dose of carboplatin is 750 mg. Carboplatin will be administered first, followed by paclitaxel or in the order as per institutional standards (i.e., paclitaxel prior to carboplatin). The premedications for paclitaxel should be administered prior to carboplatin or per institutional standards. There is a +/- 1 day window associated with all treatment days.

Patients randomized to Arm B will be treated with neoadjuvant carboplatin + paclitaxel as described above, but will also receive concurrent atezolizumab at 1200 mg every 3 weeks. Atezolizumab will be administered first, followed by carboplatin, followed by paclitaxel or in the order as per institutional standards (i.e., paclitaxel prior to carboplatin). The premedications for paclitaxel should be administered prior to carboplatin or per institutional standards. There is a +/- 1 day window associated with all treatment days.

Agent	Premedications; Precautions	Dose	Route	Schedule
Atezolizumab (Arm B only)	N/A	1200 mg	IV over 60 min (+/- 15 min) for first infusion; IV over 30 min (+/- 10 min) for second infusion. If tolerated, stay with 30 min rate thereafter.	q3 weeks x 4 cycles
Paclitaxel	In order to avoid the occurrence of severe hypersensitivity reactions, premedicate with dexamethasone 20 mg PO (12 and 6 hours prior), diphenhydramine or equivalent 50 mg IV 30-60 min prior, and famotidine 20 mg IV 30-60 min prior or per institutional standards.	80 mg/m^2	IV over 1 hour (+/- 15 min)	q week x 12 (4 cycles)
Carboplatin	Premedicate with palonosetron 0.25 mg IV 30-60 min prior or other 5HT3 antagonist or per institutional standards.	AUC5	IV over 30 min (+/- 10 min)	q3 weeks x 4 cycles

5.1.1 Atezolizumab

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. For the first infusion, the patient's vital signs (heart rate, respiratory rate, blood pressure, and temperature) should be determined within 60 minutes before, during (every 15 [± 5] minutes), and 30 (± 10) minutes after the infusion. For subsequent infusions, vital signs will be collected within 60 minutes before and within 30 minutes after the infusion. Vital signs should be collected during the infusion only if clinically indicated. Patients will be informed about the possibility of delayed post-infusion symptoms and instructed to contact their study physician if they develop such symptoms.

Premedication is not permitted for the first dose of atezolizumab. Premedication with antihistamines or antipyretics/analgesics (e.g. acetaminophen) may be administered for subsequent infusions at the discretion of the treating physician. The management of infusion-related reactions will be according to severity as follows:

- In the event that a patient experiences a grade 1 infusion-related reaction during Cycle 1, 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 grade 2 infusion-related reaction, 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 infusion-related reaction. For subsequent infusions, administer oral premedication with antihistamine and anti-pyretic and monitor closely for infusion-related reactions.
- For grade 3 or 4 infusion-related reactions, the infusion should be stopped immediately, and aggressive resuscitation and supportive measures should be initiated (e.g., oral or IV antihistamine, anti-pyretic, glucocorticoids, epinephrine, bronchodilators, oxygen). Atezolizumab should be permanently discontinued. Resumption of atezolizumab may be considered in patients who are deriving benefit and have fully recovered from the immune-related event; retreatment requires consultation with, and consent of, the trial Principal Investigator (PI).

For anaphylaxis precautions, use the following procedure:

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 atezolizumab 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.

5.1.2 Paclitaxel

Note: Contact of the undiluted concentrate with plasticized PVC equipment of devices used to prepare solutions for infusion is not recommended. In order to minimize patient exposure to the plasticizer DEHP [di-(2-ethylhexyl)phthalate], which may be leached from PVC infusion bags or sets, diluted paclitaxel solutions should be stored in bottles (glass, polypropylene) or plastic bags (polypropylene, polyolefin) and administered with polyethylene-lined administration sets.

All patients should be premedicated prior to paclitaxel administration in order to prevent severe hypersensitivity reactions. Such premedication should consist of dexamethasone 20 mg PO administered approximately 12 and 6 hours before paclitaxel, diphenhydramine (or equivalent) 50 mg IV 30-60 minutes prior to paclitaxel, and famotidine 20 mg IV 30-60 minutes prior to paclitaxel.

Paclitaxel must be diluted prior to infusion. Paclitaxel should be diluted in 0.9% Sodium Chloride Injection, USP, 5% Dextrose Injection, USP, 5% Dextrose and 0.9% Sodium Chloride Injection, USP, or 5% Dextrose in Ringer's Injection to a final concentration of 0.3 to 1.2 mg/mL.

Administer paclitaxel IV over one hour through an in-line filter with a microporous membrane not greater than 0.22 microns. Use of filter devices such as IVEX-2 filters which incorporate short inlet and outlet PVC-coating tubing has not resulted in significant leaching of DEHP. The Chemo Dispensing Pin device or similar devices with spikes should not be used with vials of paclitaxel since they can cause the stopper to collapse resulting in loss of sterile integrity of the paclitaxel solution.

5.1.3 Carboplatin

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

All patients should be premedicated prior to carboplatin administration in order to prevent emesis. Such premedication should consist of palonosetron 0.25 mg IV 30-60 minutes prior to carboplatin (or other 5HT3 antagonist).

Carboplatin can be diluted to concentrations as low as 0.5 mg/mL with 5% Dextrose in Water (D5W) or 0.9% Sodium Chloride Injection, USP.

5.2 General concomitant medication and supportive care guidelines

Because there is a potential for interaction of atezolizumab, paclitaxel, and carboplatin with other concomitantly administered drugs, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential for drug interactions. The study team should check a frequently-updated medical reference for a list of drugs to avoid or minimize use of.

5.2.1 Atezolizumab general concomitant medication guidelines

Concomitant therapy includes any prescription medications or over the counter preparations used by a patient between the 7 days preceding the screening evaluation and the treatment discontinuation visit.

Patients who experience infusion-associated symptoms may be treated symptomatically with acetaminophen, ibuprofen, diphenhydramine, and/or cimetidine or another H2 receptor antagonist, as per standard practice (for sites outside the United States, equivalent medications may be substituted per local practice). Serious infusion associated events manifested by dyspnea, hypotension, wheezing, bronchospasm, tachycardia, reduced oxygen saturation, or respiratory distress should be managed with supportive therapies as clinically indicated (*e.g.*, supplemental oxygen and β_2-adrenergic agonists; see **Section 5.1.1**).

Systemic corticosteroids and TNFα inhibitors may attenuate potential beneficial immunologic effects of treatment with atezolizumab but may be administered at the discretion of the treating physician. If feasible, alternatives to corticosteroids should be considered. Premedication may be administered for Cycles ≥2 at the discretion of the treating physician. The use of inhaled corticosteroids and mineralocorticoids (*e.g.*, fludrocortisone) for patients with orthostatic hypotension or adrenocortical insufficiency is allowed. Megestrol administered as appetite stimulant is acceptable while the patient is enrolled in the study.

Patients who use oral contraceptives, hormone-replacement therapy, prophylactic or therapeutic anticoagulation therapy (such as low-molecular-weight heparin or warfarin at a stable dose level), or other allowed maintenance therapy (see **Section 3.2**) should continue their use. Females of reproductive potential should use highly effective means of contraception.

5.2.2 Atezolizumab excluded therapies

Any concomitant therapy intended for the treatment of cancer, whether health authority-approved or experimental, is prohibited. This includes but is not limited to the following:

- Chemotherapy, hormonal therapy, immunotherapy, radiotherapy, investigational agents, or herbal therapy (except for maintenance therapies outlined in **Section 3.2** and **Section 5.2.1**).
    - After Cycle 1, certain forms of radiotherapy may be considered for pain palliation if patients are deriving benefit (*e.g.*, treatment of known bony metastases); atezolizumab administration may be suspended during radiotherapy.

It is strongly recommended that:

- Traditional herbal medicines not be administered because the ingredients of many herbal medicines are not fully studied and their use may result in unanticipated drug-drug interactions that may cause, or confound assessment of, toxicity.
- The use of a RANKL inhibitor (denosumab) be discontinued during the study; this agent could potentially alter the activity and the safety of atezolizumab.

Patients are not allowed to receive immunostimulatory agents, including, but not limited to, IFN-α, IFN-γ, or IL-2, during the entire study. These agents, in combination with atezolizumab, could potentially increase the risk for autoimmune conditions.

Patients should also not be receiving immunosuppressive medications, including, but not limited to, cyclophosphamide, azathioprine, methotrexate, and thalidomide. These agents could potentially alter the activity and the safety of atezolizumab. Systemic corticosteroids and anti-TNFα agents may attenuate potential beneficial immunologic effects of treatment with atezolizumab but may be administered at the discretion of the treating physician. If feasible, alternatives to these agents should be considered.

In addition, all patients (including those who discontinue the study early) should not receive other immunostimulatory agents for 10 weeks after the last dose of atezolizumab.

5.2.3 Paclitaxel general concomitant medication guidelines

Patients should receive premedications as described in **Section 5.1.2**.

The metabolism of paclitaxel is catalyzed by cytochrome P450 enzymes CYP2C8 and CYP3A4. Caution should be exercised when administering paclitaxel concomitantly with known substrates or inhibitors of the cytochrome P450 isoenzymes CYP2C8 and CYP3A4. Caution should be exercised when paclitaxel is concomitantly administered with known substrates (e.g., midazolam, buspirone, felodipine, lovastatin, eletriptan, sildenafil, simvastatin, and triazolam), inhibitors (e.g., atazanavir, clarithromycin, indinavir, itraconazole, ketoconazole, nefazodone, nelfinavir, ritonavir, saquinavir, and telithromycin), and inducers (e.g., rifampin and carbamazepine) of CYP3A4.

Caution should also be exercised when paclitaxel is concomitantly administered with known substrates (e.g., repaglinide and rosiglitazone), inhibitors (e.g., gemfibrozil), and inducers (e.g., rifampin) of CYP2C8.

5.2.4 Carboplatin general concomitant medication guidelines

Carboplatin can induce emesis. The incidence and intensity of emesis have been reduced by using premedication with antiemetics. Patients will be premedicated with an antiemetic prior to paclitaxel as described in **Section 5.1.2**, so no additional guidance is provided.

The renal effects of nephrotoxic compounds may be potentiated by carboplatin.

5.3 Surgery

Standard treatment for curative intent in stages II-III TNBC includes AC. If a patient experiences significant toxicity from neoadjuvant chemotherapy, that patient will proceed to surgery and

should complete standard chemotherapy if able postoperatively. Surgery should be scheduled to take place 3-6 weeks following the last dose of neoadjuvant chemotherapy. A surgical delay beyond the timeframe described above requires a discussion with the principal investigator.

The type of breast surgery (either mastectomy or lumpectomy) is determined by the treating surgeon according to the institutional standard, with the goal of completely excising the tumor. A negative margin is required unless further excision is not possible.

The actual surgical/therapeutic approached taken will be recorded as:

- Partial mastectomy at first attempt (removal of the cancer as well as some of the breast tissue around the tumor and the fascia over the chest muscles below the tumor, if indicated)
- Re-excision after partial mastectomy as first attempt
- Total mastectomy after partial mastectomy as first attempt
- Modified radical mastectomy (record preservation or removal of pectoralis minor)
- Radical mastectomy (does not include resection of less than 25% of pectoralis major)
- Remained inoperable
- Disease progressed
- Other

The type of lymph node surgery will be determined by the treating surgeon according to the institutional standard.

5.4 Adjuvant Treatment

Dose-dense AC (ddAC) will be given in the adjuvant setting as per routine care. ddAC consists of doxorubicin at 60 mg/m^2 on Day 1 of each 2-week cycle plus cyclophosphamide at 600 mg/m^2 on Day 1 of each 2-week cycle for a total of 4 cycles.

5.5 Specimen collection for correlative studies

The study design presents unique opportunities for tissue acquisition and correlative science in the context of neoadjuvant chemotherapy. We will obtain tumor tissue at baseline, following initiation of therapy, and at the time of definitive surgery.

All patients will have research core biopsies of the tumor performed at baseline and after the first cycle of therapy (Day 18-22). Tumor tissue will be obtained from all patients at the time of surgery.

Specialized tissue specimen collection/shipment kits will be used for this study. Kits can be requested by emailing the Tissue Procurement Core at WUMC at tbank@wustl.edu, referencing "Kit request" in the subject line. Provide first and last name, phone number, email, and direct shipping address. Reference protocol short title "Neoadj Chemo and Atezolizumab in TNBC" and number of kits needed. Limit of 3 kits per request. If more than 3 kits are needed, please provide justification. Kits will be distributed within 10 days of request receipt. Each kit contains the necessary materials that are needed for the biospecimen (tissue and blood) collection at one time point on this trial.

The required biopsies include two core biopsies in 10% buffered formalin and two core biopsies

frozen immediately at bedside in separate OCT blocks to preserve the RNA and DNA of the tumor. The first core should be placed in 10% buffered formalin, the second in OCT, the third in formalin, and the fourth in OCT.

5.5.1 Required pre-treatment core biopsy for biomarker and correlative studies

- A research biopsy is mandatory for all patients prior to initiating therapy.
- It is recommended to use the specimen collection/shipping kits provided.
- The needle used to obtain the core biopsies should be 14 G.
- It is strongly suggested that core biopsies be image-guided.
- Tissue may be obtained concurrent with another procedure (clip placement, axillary node FNA, port placement) or as a separate procedure.
- The required biopsies include two core biopsies in 10% buffered formalin and two core biopsies frozen immediately at bedside in separate OCT blocks to preserve the RNA and DNA of the tumor.
- It is also permissible to submit samples that were taken prior to study enrollment, such as during the diagnostic biopsy procedure as long as: a) the additional (un-sectioned) frozen biopsies were prepared in cryomolds and OCT at the bedside as described in the kit and held with an accession number in a pathology department at -70C or lower until release to the CSB and b): the two formalin-fixed biopsies were placed in 10% buffered formalin overnight and processed into two separate paraffin blocks.

5.5.2 Required on-treatment core biopsy for correlative studies

- A research biopsy is mandatory for all patients after one cycle of therapy and should be obtained on day 18-22.
- It is recommended to use the specimen collection/shipping kits provided.
- The needle used to obtain the core biopsies should be 14 G.
- It is strongly suggested that core biopsies be image-guided.
- The required biopsies include two core biopsies in 10% buffered formalin and two core biopsies frozen immediately at bedside in separate OCT blocks to preserve the RNA and DNA of the tumor.

5.5.3 Required tissue collection at time of surgery for all patients after the completion of neoadjuvant chemotherapy for correlative studies

- Following completion of neoadjuvant chemotherapy, an intra-operative core biopsy of residual tumor by the surgeon prior to its resection is required. If no gross residual tumor can be identified, then a core biopsy of the tumor bed should be done prior to its resection.
- The required biopsies include: Two core biopsies in 10% buffered formalin and two core biopsies frozen immediately at bedside in separate OCT blocks to preserve the RNA and DNA of the tumor.
- Alternatively, but not preferred, the site pathologist can remove the tumor samples during the dissection of the surgical specimen (using a 5-mm skin punch biopsy

device) as long as the samples are frozen or fixed within 30 minutes of removal from the patient.

5.5.4 Required peripheral blood collection for PBMC isolation

- Peripheral blood will be collected from all patients at the time points noted above (pre-treatment, on-treatment after one cycle of therapy (Day 18-22), after 3 cycles of therapy, and post-treatment at the time of surgery).
- At each time point, collect six 10mL green-top (sodium heparin) tubes (BD Vacutainer, Catalog # 366480).

Details about specimen processing and shipping are included below in **Section 9**.

5.6 Duration of therapy

In the absence of treatment delays due to adverse event(s), treatment may continue for 4 cycles of paclitaxel + carboplatin (with or without atezolizumab) or until one of the following criteria applies:

- Disease progression; note that subjects can be treated beyond progression if pseudoprogression is strongly suspected or confirmed
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator

5.7 Duration of follow up

Patients will be followed for one year after removal from study or until death, whichever occurs first. Follow-up will be at 6 months and one year post-surgery. Patients who come off study prior to surgery will be followed for 30 days. Patients removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

5.8 Criteria for removal from study

Patients will be removed from study when any of the criteria listed in **Section 5.6** applies. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

6. DOSING DELAYS/DOSE MODIFICATIONS

6.1 Atezolizumab

6.1.1 General AE management and dose modification guidelines

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

Patients may temporarily suspend study treatment for up to 42 days (6 weeks) beyond the scheduled date of delayed infusion if study drug-related toxicity requiring dose suspension is experienced. Treatment with paclitaxel and carboplatin may continue if atezolizumab is temporarily suspended. If atezolizumab is held because of AEs for >42 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 this protocol. If the AE resolves within 42 days and the patient is receiving corticosteroid therapy for the event, atezolizumab may be held for longer than 42 days (up to 4 weeks) in order to allow tapering of the steroid dose to ≤ 10 mg oral prednisone or equivalent. Treatment with paclitaxel and carboplatin may continue if atezolizumab is permanently discontinued.

Dose interruptions for reasons other than toxicity, such as surgical procedures, may be allowed. The acceptable length of interruption will be at the discretion of the study PI in consultation with CTEP.

Atezolizumab must be **permanently discontinued** if the patient experiences any of the following events, regardless of benefit:

- Grade 4 pneumonitis
- AST or ALT >5×ULN or total bilirubin >3×ULN
- Grade 4 diarrhea or colitis
- Grade 4 hypophysitis
- Any grade myasthenic syndrome/myasthenia gravis, Guillain-Barré or meningoencephalitis
- Grade 4 ocular inflammatory toxicity
- Grade 4 pancreatitis or any grade of recurrent pancreatitis
- Grade 4 rash
- Any grade myocarditis

Treatment may, under limited and compelling circumstances, be resumed in patients who have recovered from the following events, but only after consultation with the trial Principal Investigator:

- Grade 3 pneumonitis
- Grade 3 ocular inflammatory toxicity
- Grade 3 or 4 infusion-related reactions

Any 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 [67]. 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, or other immunosuppressive agents. The investigator should consider the benefit-risk balance prior to further administration of atezolizumab.

For detailed information regarding management of adverse events associated with atezolizumab, please refer to the most current version of the Atezolizumab Investigator's Brochure and the FDA product label.

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 benefit-risk balance 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. In all cases, treatment with paclitaxel and carboplatin may continue if atezolizumab is temporarily suspended or permanently discontinued as long as the treating physician deems the patient a candidate for chemotherapy.

Patients should be assessed clinically (including review of laboratory values) for toxicity prior to, during, and after each infusion. If unmanageable toxicity due to atezolizumab occurs at any time during the study, treatment with atezolizumab should be discontinued.

6.1.2 Management of specific AEs

Management of certain AEs of concern, including immune-related pneumonitis, hepatitis, colitis, endocrinopathies, pancreatitis, neuropathies, meningoencephalitis, and potential ocular toxicities are presented in the Atezolizumab Investigator's Brochure. See **Section 5.1.1** for guidelines for the management of infusion-related reactions and anaphylaxis.

Atezolizumab has been associated with risks such as the following: IRRs and immune–related hepatitis, pneumonitis, colitis, pancreatitis, diabetes mellitus, hypothyroidism, hyperthyroidism, adrenal insufficiency, hypophysitis, Guillain-Barré syndrome, myasthenic syndrome or myasthenia gravis, meningoencephalitis, myocarditis, nephritis, myositis, and severe cutaneous adverse reactions. Immune-mediated reactions may involve any organ system and may lead to hemophagocytic lymphohistiocytosis and macrophage activation syndrome.

Pulmonary events

Dyspnea, cough, fatigue, hypoxia, pneumonitis, and pulmonary infiltrates have been associated with the administration of atezolizumab. Patients will be assessed for pulmonary signs and symptoms throughout the study.

All pulmonary events should be thoroughly evaluated for other commonly reported etiologies such as pneumonia or other infection, lymphangitic carcinomatosis, pulmonary embolism, heart failure, chronic obstructive pulmonary disease, or pulmonary hypertension. Management guidelines for pulmonary events are provided in the table below.

Event	Management
Pulmonary event, Grade 1	● Continue atezolizumab and monitor closely. ● Re-evaluate on serial imaging. ● Consider patient referral to pulmonary specialist.
Pulmonary event, Grade 2	● Withhold atezolizumab for up to 12 weeks after event onset. [a] ● Refer patient to pulmonary and infectious disease specialists and consider bronchoscopy or BAL. ● Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day oral prednisone. ● If event resolves to Grade 1 or better, resume atezolizumab. [b] ● If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab. ● For recurrent events, treat as a Grade 3 or 4 event.
Pulmonary event, Grade 3 or 4	● Permanently discontinue atezolizumab. ● Bronchoscopy or BAL is recommended. ● Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day oral prednisone. ● If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. ● If event resolves to Grade 1 or better, taper corticosteroids over ≥1 month.

BAL = bronchoscopic alveolar lavage

[a] 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 the equivalent of ≤10 mg/day oral prednisone. The acceptable length of the extended period of time will be determined by the investigator and the Medical Monitor.

[b] If corticosteroids have been initiated, they must be tapered over ≥1 month to the equivalent of ≤10 mg/day oral prednisone before atezolizumab can be resumed.

Hepatic events

Immune-mediated hepatitis has been associated with the administration of atezolizumab. Eligible patients must have adequate liver function, as manifested by measurements of total bilirubin and hepatic transaminases, and liver function will be monitored throughout study treatment. Management guidelines for hepatic events are provided in the table below.

Patients with right upper-quadrant abdominal pain and/or unexplained nausea or vomiting should have liver function tests (LFTs) performed immediately and reviewed before administration of the next dose of study drug.

For patients with elevated LFTs, concurrent medication, viral hepatitis, and toxic or neoplastic etiologies should be considered and addresses, as appropriate.

Event	Management
Hepatic event, Grade 1	• Continue atezolizumab. • Monitor LFTs until values resolve to within normal limits or to baseline values.
Hepatic event, Grade 2	**All events:** • Monitor LFTs more frequently until return to baseline values. **Events of >5 days' duration:** • Withhold atezolizumab for up to 12 weeks after event onset. [a] • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day oral prednisone. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab.
Hepatic event, Grade 3 or 4	• Permanently discontinue atezolizumab. • Consider patient referral to gastrointestinal specialist for evaluation and liver biopsy to establish etiology of hepatic injury. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day oral prednisone. • If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, taper corticosteroids over ≥1 month.

LFT = liver function test.

[a] 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 the equivalent of ≤10 mg/day oral prednisone. The acceptable length of the extended period of time will be determined by the investigator.

[b] If corticosteroids have been initiated, they must be tapered over ≥1 month to the equivalent of ≤10 mg/day oral prednisone before atezolizumab can be resumed.

Gastrointestinal events

Immune-mediated colitis has been associated with the administration of atezolizumab. Management guidelines for diarrhea or colitis are provided in the table below.

All events of diarrhea or colitis should be thoroughly evaluated for other more common etiologies. For events of significant duration or magnitude or associated with signs of systemic inflammation or acute-phase reactants (e.g., increased C-reactive protein, platelet count, or bandemia): Perform sigmoidoscopy (or colonoscopy, if appropriate) with colonic biopsy, with three to five specimens for standard paraffin block to check for inflammation and lymphocytic infiltrates to confirm colitis diagnosis.

Event	Management
Diarrhea or colitis, Grade 1	• Continue atezolizumab. • Initiate symptomatic treatment. • Endoscopy is recommended if symptoms persist for >7 days. • Monitor closely.

Event	Management
Diarrhea or colitis, Grade 2	• Withhold atezolizumab for up to 12 weeks after event onset. [a] • Initiate symptomatic treatment. • Patient referral to GI specialist is recommended. • For recurrent events or events that persist >5 days, initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day oral prednisone. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab.
Diarrhea or colitis, Grade 3	• Withhold atezolizumab for up to 12 weeks after event onset. [a] • Refer patient to GI specialist for evaluation and confirmatory biopsy. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab.
Diarrhea or colitis, Grade 4	• Permanently discontinue atezolizumab. • Refer patient to GI specialist for evaluation and confirmation biopsy. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, taper corticosteroids over ≥1 month.

GI = gastrointestinal.

[a] 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 the equivalent of ≤10 mg/day oral prednisone. The acceptable length of the extended period of time must be determined by the investigator.

[b] If corticosteroids have been initiated, they must be tapered over ≥1 month to the equivalent of ≤10 mg/day oral prednisone before atezolizumab can be resumed.

Endocrine disorders

Thyroid disorders, adrenal insufficiency, diabetes mellitus, and pituitary disorders have been associated with the administration of atezolizumab. Management guidelines for endocrine events are provided in the table below.

Patients experiencing one or more unexplained AEs possibly indicative of endocrine dysfunction (including headache, fatigue, myalgias, impotence, mental status changes, and constipation) should be investigated for the presence of thyroid, pituitary, or adrenal endocrinopathies. The patient should be referred to an endocrinologist if an endocrinopathy is suspected. Thyroid-stimulating hormone (TSH) and free T3 and T4 levels should be obtained to determine whether thyroid abnormalities are present. Pituitary hormone levels and function tests [*e.g.*, TSH, growth hormone, luteinizing hormone, follicle stimulating hormone, testosterone, prolactin,

adrenocorticotropic hormone (ACTH) levels, and ACTH stimulation test] and MRI of the brain (with detailed pituitary sections) may help to differentiate primary pituitary insufficiency from primary adrenal insufficiency. The table below describes dose management guidelines for hyperthyroidism, hypothyroidism, symptomatic adrenal insufficiency, and hyperglycemia.

Event	Management
Asymptomatic hypothyroidism	• Continue atezolizumab. • Initiate treatment with thyroid replacement hormone. • Monitor TSH weekly.
Symptomatic hypothyroidism	• Withhold atezolizumab. • Initiate treatment with thyroid replacement hormone. • Monitor TSH weekly. • Consider patient referral to endocrinologist. • Resume atezolizumab when symptoms are controlled and thyroid function is improving.
Asymptomatic hyperthyroidism	**TSH ≥0.1 mU/L and <0.5 mU/L:** • Continue atezolizumab. • Monitor TSH every 4 weeks. **TSH <0.1 mU/L:** • Follow guidelines for symptomatic hyperthyroidism.
Symptomatic hyperthyroidism	• Withhold atezolizumab. • Initiate treatment with anti-thyroid drug such as methimazole or carbimazole as needed. • Consider patient referral to endocrinologist. • Resume atezolizumab when symptoms are controlled and thyroid function is improving. • Permanently discontinue atezolizumab.
Symptomatic adrenal insufficiency, Grade 2–4	• Withhold atezolizumab for up to 12 weeks after event onset. [a] • Refer patient to endocrinologist. • Perform appropriate imaging. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event resolves to Grade 1 or better and patient is stable on replacement therapy, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better or patient is not stable on replacement therapy while withholding atezolizumab, permanently discontinue atezolizumab.
Hyperglycemia, Grade 1 or 2	• Continue atezolizumab. • Investigate for diabetes. If patient has Type 1 diabetes, treat as a Grade 3 event. If patient does not have Type 1 diabetes, treat as per institutional guidelines. • Monitor for glucose control.

Event	Management
Hyperglycemia, Grade 3 or 4	• Withhold atezolizumab. • Initiate treatment with insulin. • Monitor for glucose control. • Resume atezolizumab when symptoms resolve and glucose levels are stable.
Hypophysitis (pan-hypopituitarism), Grade 2 or 3	• Withhold atezolizumab for up to 12 weeks after event onset. [a] • Refer patient to endocrinologist. • Perform brain MRI (pituitary protocol). • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • Initiate hormone replacement if clinically indicated. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab. • For recurrent hypophysitis, treat as a Grade 4 event.
Hypophysitis (pan-hypopituitarism), Grade 4	• Permanently discontinue atezolizumab. • Refer patient to endocrinologist. • Perform brain MRI (pituitary protocol). • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • Initiate hormone replacement if clinically indicated.

MRI = magnetic resonance imaging; TSH = thyroid-stimulating hormone.

[a] 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 the equivalent of ≤10 mg/day oral prednisone. The acceptable length of the extended period of time must be determined by the investigator.

[b] If corticosteroids have been initiated, they must be tapered over ≥1 month to the equivalent of ≤10 mg/day oral prednisone before atezolizumab can be resumed.

Ocular events

An ophthalmologist should evaluate visual complaints (e.g., uveitis, retinal events).

Management guidelines for ocular events are provided in the table below.

Event	Management
Ocular event, Grade 1	• Continue atezolizumab. • Patient referral to ophthalmologist is strongly recommended. • Initiate treatment with topical corticosteroid eye drops and topical immunosuppressive therapy. • If symptoms persist, treat as a Grade 2 event.

Event	Management
Ocular event, Grade 2	• Withhold atezolizumab for up to 12 weeks after event onset. [a] • Patient referral to ophthalmologist is strongly recommended. • Initiate treatment with topical corticosteroid eye drops and topical immunosuppressive therapy. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab.
Ocular event, Grade 3 or 4	• Permanently discontinue atezolizumab. • Refer patient to ophthalmologist. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day oral prednisone. • If event resolves to Grade 1 or better, taper corticosteroids over ≥1 month.

[a] 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 the equivalent of ≤10 mg/day oral prednisone. The acceptable length of the extended period of time must be determined by the investigator.

[b] If corticosteroids have been initiated, they must be tapered over ≥1 month to the equivalent of ≤10 mg/day oral prednisone before atezolizumab can be resumed.

Immune-mediated- Myocarditis

Immune-mediated myocarditis has been associated with the administration of atezolizumab. Immune-mediated myocarditis should be suspected in any patient presenting with signs or symptoms suggestive of myocarditis, including, but not limited to, laboratory (e.g., B-NP [B-Natriuretic Peptide]) or cardiac imaging abnormalities, dyspnea, chest pain, palpitations, fatigue, decreased exercise tolerance, or syncope.

Immune-mediated myocarditis needs to be distinguished from myocarditis resulting from infection (commonly viral, e.g., in a patient who reports a recent history of gastrointestinal illness), ischemic events, underlying arrhythmias, exacerbation of preexisting cardiac conditions, or progression of malignancy. All patients with possible myocarditis should be urgently evaluated by performing cardiac enzyme assessment, an electrocardiogram (ECG), a chest X-ray, an echocardiogram, and a cardiac MRI as appropriate per institutional guidelines. A cardiologist should be consulted. An endomyocardial biopsy may be considered to enable a definitive diagnosis and appropriate treatment, if clinically indicated.

Patients with signs and symptoms of myocarditis, in the absence of an identified alternate etiology, should be treated according to the guidelines in the table below.

Event	Management
Immune-related myocarditis, Grade 2	• Withhold atezolizumab for up to 12 weeks after event onset [a]. • Refer patient to cardiologist. • Initiate treatment as per institutional guidelines and consider antiarrhythmic drugs, temporary pacemaker, ECMO, or VAD as appropriate.

Event	Management
	• Consider treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab.
Immune-related myocarditis, Grade 3-4	• Permanently discontinue atezolizumab. • Refer patient to cardiologist. • Initiate treatment as per institutional guidelines and consider antiarrhythmic drugs, temporary pacemaker, ECMO, or VAD as appropriate. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, taper corticosteroids over ≥1 month.

ECMO = extracorporeal membrane oxygenation; VAD = ventricular assist device.

[a] 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 the equivalent of ≤10 mg/day oral prednisone. The acceptable length of the extended period of time must be documented by the investigator.

[b] If corticosteroids have been initiated, they must be tapered over ≥1 month to the equivalent of ≤10 mg/day oral prednisone before atezolizumab can be resumed.

Infusion-Related Reactions and Cytokine-Release Syndrome

No premedication is indicated for the administration of Cycle 1 of atezolizumab. However, patients who experience an infusion-related reaction (IRR) or cytokine-release syndrome (CRS) with atezolizumab may receive premedication with antihistamines, antipyretics, and/or analgesics (e.g., acetaminophen) for subsequent infusions. Metamizole (dipyrone) is prohibited in treating atezolizumab-associated IRRs because of its potential for causing agranulocytosis.

IRRs are known to occur with the administration of monoclonal antibodies and have been reported with atezolizumab. These reactions, which are thought to be due to release of cytokines and/or other chemical mediators, occur within 24 hours of atezolizumab administration and are generally mild to moderate in severity.

CRS is defined as a supraphysiologic response following administration of any immune therapy that results in activation or engagement of endogenous or infused T cells and/or other immune effector cells. Symptoms can be progressive, always include fever at the onset, and may include hypotension, capillary leak (hypoxia), and end-organ dysfunction (Lee et al., 2019). CRS has been well documented with chimeric antigen receptor T-cell therapies and bispecific T-cell engager antibody therapies but has also been reported with immunotherapies that target PD-1 or PD-L1 (Rotz et al., 2017; Adashek and Feldman 2019) including atezolizumab.

There may be significant overlap in signs and symptoms of IRRs and CRS, and in recognition of

the challenges in clinically distinguishing between the two, consolidated guidelines for medical management of IRRs and CRS are provided in the table below.

Management Guidelines for Infusion-Related Reactions and Cytokine-Release Syndrome

Event	Management
Grade 1[a] Fever[b] with or without constitutional symptoms	• Immediately interrupt infusion. • Upon symptom resolution, wait 30 minutes and then restart infusion at half the rate being given at the time of event onset. • If the infusion is tolerated at the reduced rate for 30 minutes, the infusion rate may be increased to the original rate. • If symptoms recur, discontinue infusion of this dose. • Administer symptomatic treatment,[c] including maintenance of IV fluids for hydration. • In case of rapid decline or prolonged CRS (> 2 days) or in patients with significant symptoms and/or comorbidities, consider managing as per Grade 2. • For subsequent infusions, consider administration of oral premedication with antihistamines, anti-pyretics, and/or analgesics, and monitor closely for IRRs and/or CRS.
Grade 2[a] Fever[b] with hypotension not requiring vasopressors **and/or** Hypoxia requiring low-flow oxygen[d] by nasal cannula or blow-by	• Immediately interrupt atezolizumab infusion. • Upon symptom resolution, wait for 30 minutes and then restart infusion at half the rate being given at the time of event onset. • If symptoms recur, discontinue infusion of this dose. • Administer symptomatic treatment.[c] • For hypotension, administer IV fluid bolus as needed. • Monitor cardiopulmonary and other organ function closely (in the ICU, if appropriate). Administer IV fluids as clinically indicated and manage constitutional symptoms and organ toxicities as per institutional practice. • Rule out other inflammatory conditions that can mimic CRS (*e.g.*, sepsis). If no improvement within 24 hours, initiate workup and assess for signs and symptoms of HLH or MAS. • Consider IV corticosteroids (*e.g.*, methylprednisolone 2 mg/kg/day or dexamethasone 10 mg every 6 hours). • Consider anti-cytokine therapy.[e] • Consider hospitalization until complete resolution of symptoms. If no improvement within 24 hours, manage as per Grade 3, that is, hospitalize patient (monitoring in the ICU is recommended), permanently discontinue atezolizumab. • If symptoms resolve to Grade 1 or better for 3 consecutive days, the next dose of atezolizumab may be administered. For subsequent infusions, consider administration of oral premedication with antihistamines, anti-pyretics, and/or analgesics and monitor closely for IRRs and/or CRS. • If symptoms do not resolve to Grade 1 or better for 3 consecutive days, contact the sponsor-investigator.
Grade 3[a] Fever[b] with hypotension requiring a vasopressor (with or without	• Permanently discontinue atezolizumab.[f] • Administer symptomatic treatment.[c] • For hypotension, administer IV fluid bolus and vasopressor as needed. • Monitor cardiopulmonary and other organ function closely; monitoring in the ICU is recommended. Administer IV fluids as clinically indicated and manage constitutional symptoms and organ toxicities as per institutional practice. • Rule out other inflammatory conditions that can mimic CRS (*e.g.*, sepsis). If no

vasopressin) **and/or** Hypoxia requiring high-flow oxygen[d] by nasal cannula, face mask, non-rebreather mask, or venturi mask	improvement within 24 hours, initiate workup and assess for signs and symptoms of HLH or MAS. ● Administer IV corticosteroids (*e.g.*, methylprednisolone 2 mg/kg/day or dexamethasone 10 mg every 6 hours). ● Consider anti-cytokine therapy.[e] ● Hospitalize patient until complete resolution of symptoms. If no improvement within 24 hours, manage as per Grade 4, that is, admit patient to ICU and initiate hemodynamic monitoring, mechanical ventilation, and/or IV fluids and vasopressors as needed; for patients who are refractory to anti-cytokine therapy, experimental treatments may be considered at the discretion of the investigator.
Grade 4[a] Fever[b] with hypotension requiring multiple vasopressors (excluding vasopressin) and/or Hypoxia requiring oxygen by positive pressure (e.g., CPAP, BiPAP, intubation and mechanical ventilation)	● Permanently discontinue atezolizumab.[f] ● Administer symptomatic treatment.[c] ● Admit patient to ICU and initiate hemodynamic monitoring, mechanical ventilation, and/or IV fluids and vasopressors as needed. Monitor other organ function closely. Manage constitutional symptoms and organ toxicities as per institutional practice. ● Rule out other inflammatory conditions that can mimic CRS (*e.g.*, sepsis). If no improvement within 24 hours, initiate workup and assess for signs and symptoms of HLH or MAS. ● Administer IV corticosteroids (*e.g.*, methylprednisolone 2 mg/kg/day or dexamethasone 10 mg every 6 hours). ● Consider anti-cytokine therapy.[e] For patients who are refractory to anti-cytokine therapy, experimental treatments[g] may be considered at the discretion of the investigator. ● Hospitalize patient until complete resolution of symptoms.

ASTCT= American Society for Transplantation and Cellular Therapy; BiPAP= bi-level positive airway pressure; CAR= chimeric antigen receptor; CPAP= continuous positive airway pressure; CRS= cytokine-release syndrome; HLH= hemophagocytic lymphohistiocytosis; IRR = infusion-related reaction; MAS= macrophage activation syndrome.

Note: The management guidelines have been adapted from NCCN guidelines for management of CAR T-cell-related toxicities (Version 2.2019).

a. Grading system for management guidelines is based on ASTCT consensus grading for CRS. NCI CTCAE (version as specified in the protocol) should be used when reporting severity of IRRs, CRS, or organ toxicities associated with CRS on the Adverse Event eCRF. Organ toxicities associated with CRS should not influence overall CRS grading.
b. Fever is defined as temperature ≥38°C not attributable to any other cause. In patients who develop CRS and then receive anti-pyretic, anti-cytokine, or corticosteroid therapy, fever is no longer required when subsequently determining event severity (grade). In this case, the grade is driven by the presence of hypotension and/or hypoxia.
c. Symptomatic treatment may include oral or IV antihistamines, anti-pyretics, analgesics, bronchodilators, and/or oxygen. For bronchospasm, urticaria, or dyspnea, additional treatment may be administered as per institutional practice.
d. Low flow is defined as oxygen delivered at ≤6 L/min, and high flow is defined as oxygen delivered at >6 L/min.
e. There are case reports where anti-cytokine therapy has been used for treatment of CRS with immune checkpoint inhibitors (Rotz *et al*. 2017; Adashek and Feldman 2019), but data are limited, and the role of such treatment in the setting of antibody-associated CRS has not been established.
f. Resumption of atezolizumab may be considered in patients who are deriving benefit and have fully recovered from the immune-related event. Patients can be re-challenged with atezolizumab according to institutional guidelines and the above table. For subsequent infusions, administer oral

premedication with antihistamines, anti-pyretics, and/or analgesics, and monitor closely for IRRs and/or CRS. Premedication with corticosteroids and extending the infusion time may also be considered after considering the benefit-risk ratio.

g. Refer to Riegler *et al*. for information on experimental treatments for CRS.

Pancreatic events

Symptoms of abdominal pain associated with elevations of amylase and lipase, suggestive of pancreatitis, have been associated with the administration of atezolizumab. The differential diagnosis of acute abdominal pain should include pancreatitis. Appropriate workup should include an evaluation for ductal obstruction, as well as serum amylase and lipase tests. Management guidelines for pancreatic events, including pancreatitis, are provided in the table below.

Event	Management
Amylase and/or lipase elevation, Grade 2	**Amylase and/or lipase >1.5–2.0 × ULN:** • Continue atezolizumab. • Monitor amylase and lipase weekly. • For prolonged elevation (e.g., >3 weeks), consider treatment with corticosteroids equivalent to 10 mg/day oral prednisone. **Asymptomatic with amylase and/or lipase >2.0–5.0 × ULN:** • Treat as a Grade 3 event.
Amylase and/or lipase elevation, Grade 3 or 4	• Withhold atezolizumab for up to 12 weeks after event onset. [a] • Refer patient to GI specialist. • Monitor amylase and lipase every other day. • If no improvement, consider treatment with corticosteroids equivalent to 1–2 mg/kg/day oral prednisone. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab. [c] • For recurrent events, permanently discontinue atezolizumab.
Immune-related pancreatitis, Grade 2 or 3	• Withhold atezolizumab for up to 12 weeks after event onset. [a] • Refer patient to GI specialist. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab. • For recurrent events, permanently discontinue atezolizumab.
Immune-related pancreatitis, Grade 4	• Permanently discontinue atezolizumab. • Refer patient to GI specialist.

Event	Management
	• Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, taper corticosteroids over ≥1 month.

GI = gastrointestinal.

[a] 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 the equivalent of ≤10 mg/day oral prednisone. The acceptable length of the extended period of time must be determined by the investigator.

[b] If corticosteroids have been initiated, they must be tapered over ≥1 month to the equivalent of ≤10 mg/day oral prednisone before atezolizumab can be resumed.

Dermatologic events

Treatment-emergent rash has been associated with atezolizumab. The majority of cases of rash were mild in severity and self-limited, with or without pruritus. A dermatologist should evaluate persistent and/or severe rash or pruritus. A biopsy should be considered unless contraindicated. Management guidelines for dermatologic events are provided in the table below.

Event	Management
Dermatologic event, Grade 1	• Continue atezolizumab. • Consider treatment with topical corticosteroids and/or other symptomatic therapy (*e.g.*, antihistamines).
Dermatologic event, Grade 2	• Continue atezolizumab. • Consider patient referral to dermatologist. • Initiate treatment with topical corticosteroids. • Consider treatment with higher-potency topical corticosteroids if event does not improve.
Dermatologic event, Grade 3	• Withhold atezolizumab for up to 12 weeks after event onset. [a] • Refer patient to dermatologist. • Initiate treatment with corticosteroids equivalent to 10 mg/day oral prednisone, increasing dose to 1-2 mg/kg/day if event does not improve within 48-72 hours. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab.
Dermatologic event, Grade 4	• Permanently discontinue atezolizumab.

Event	Management
Stevens-Johnson syndrome or toxic epidermal necrolysis (any grade)	**Additional guidance for Stevens-Johnson syndrome or toxic epidermal necrolysis:** • Withhold atezolizumab for suspected Stevens-Johnson syndrome or toxic epidermal necrolysis. • Confirm diagnosis by referring patient to a specialist (dermatologist, ophthalmologist, or urologist as relevant) for evaluation and, if indicated, biopsy. • Follow the applicable treatment and management guidelines above. • If Stevens-Johnson syndrome or toxic epidermal necrolysis is confirmed, permanently discontinue atezolizumab.

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 the equivalent of ≤10 mg/day oral prednisone. The acceptable length of the extended period of time must be determined by the investigator.

[b] If corticosteroids have been initiated, they must be tapered over ≥1 month to the equivalent of ≤10 mg/day oral prednisone before atezolizumab can be resumed.

Neurologic disorders

Myasthenia gravis and Guillain-Barré syndrome have been observed with single agent atezolizumab. Patients may present with signs and symptoms of sensory and/or motor neuropathy. Diagnostic work-up is essential for an accurate characterization to differentiate between alternative etiologies.

Event	Management
Immune-related neuropathy, Grade 1	• Continue atezolizumab. • Investigate etiology.
Immune-related neuropathy, Grade 2	• Withhold atezolizumab for up to 12 weeks after event onset. [a] • Investigate etiology. • Initiate treatment as per institutional guidelines. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab.
Immune-related neuropathy, Grade 3 or 4	• Permanently discontinue atezolizumab. • Initiate treatment as per institutional guidelines.
Myasthenia gravis and Guillain-Barré syndrome (any grade)	• Permanently discontinue atezolizumab. • Refer patient to neurologist. • Initiate treatment as per institutional guidelines. • Consider initiation of corticosteroids equivalent to 1–2 mg/kg/day oral or IV prednisone.

[a] 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 the equivalent of ≤10 mg/day oral prednisone. The acceptable length of the extended period of time must be determined by the investigator.

[b] If corticosteroids have been initiated, they must be tapered over ≥1 month to the equivalent of ≤10 mg/day oral prednisone before atezolizumab can be resumed.

Immune-Mediated Meningoencephalitis

Immune-related meningoencephalitis is an identified risk associated with the administration of atezolizumab. Immune-related meningoencephalitis should be suspected in any patient presenting with signs or symptoms suggestive of meningitis or encephalitis, including, but not limited to, headache, neck pain, confusion, seizure, motor or sensory dysfunction, and altered or depressed level of consciousness. Encephalopathy from metabolic or electrolyte imbalances needs to be distinguished from potential meningoencephalitis resulting from infection (bacterial, viral, or fungal) or progression of malignancy, or secondary to a paraneoplastic process.

All patients being considered for meningoencephalitis should be urgently evaluated with a CT scan and/or MRI scan of the brain to evaluate for metastasis, inflammation, or edema. If deemed safe by the treating physician, a lumbar puncture should be performed and a neurologist should be consulted.

Patients with signs and symptoms of meningoencephalitis, in the absence of an identified alternate etiology, should be treated according to the guidelines.

Event	Management
Immune-related meningoencephalitis, all grades	• Permanently discontinue atezolizumab. • Refer patient to neurologist. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, taper corticosteroids over ≥1 month.

Renal events

Immune-mediated nephritis has been associated with the administration of atezolizumab. Eligible patients must have adequate renal function. Renal function, including serum creatinine, should be monitored throughout study treatment. Patients with abnormal renal function should be evaluated and treated for other more common etiologies (including prerenal and postrenal causes, and concomitant medications such as non-steroidal anti-inflammatory drugs). Refer the patient to a renal specialist if clinically indicated. A renal biopsy may be required to enable a definitive diagnosis and appropriate treatment.

Patients with signs and symptoms of nephritis, in the absence of an identified alternate etiology, should be treated according to the guidelines in the table below.

Event	Management
Renal event, Grade 1	• Continue atezolizumab. • Monitor kidney function, including creatinine, closely until values resolve to within normal limits or to baseline values.
Renal event, Grade 2	• Withhold atezolizumab for up to 12 weeks after event onset. [a] • Refer patient to renal specialist. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day oral prednisone. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab.
Renal event, Grade 3 or 4	• Permanently discontinue atezolizumab. • Refer patient to renal specialist and consider renal biopsy. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day oral prednisone. • If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, taper corticosteroids over ≥1 month.

[a] 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 the equivalent of ≤10 mg/day oral prednisone. The acceptable length of the extended period of time must be determined by the investigator.

[b] If corticosteroids have been initiated, they must be tapered over ≥1 month to the equivalent of ≤10 mg/day oral prednisone before atezolizumab can be resumed.

Immune-Mediated Myositis

Immune-mediated myositis has been associated with the administration of atezolizumab. Myositis or inflammatory myopathies are a group of disorders sharing the common feature of inflammatory muscle injury; dermatomyositis and polymyositis are among the most common disorders. Initial diagnosis is based on clinical (muscle weakness, muscle pain, skin rash in dermatomyositis), biochemical (serum creatine kinase increase), and imaging (electromyography/MRI) features, and is confirmed with a muscle biopsy.

Patients with signs and symptoms of myositis, in the absence of an identified alternate etiology, should be treated according to the guidelines in table below.

Event	Management
Immune-mediated myositis, Grade 1	• Continue atezolizumab. • Refer patient to rheumatologist or neurologist. • Initiate treatment as per institutional guidelines.
Immune-mediated myositis, Grade 2	• Withhold atezolizumab for up to 12 weeks after event onset [a] and contact Medical Monitor. • Refer patient to rheumatologist or neurologist. • Initiate treatment as per institutional guidelines.

Event	Management
	• Consider treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If corticosteroids are initiated and event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab and contact Medical Monitor.
Immune-mediated myositis, Grade 3	• Withhold atezolizumab for up to 12 weeks after event onset [a] and contact Medical Monitor. • Refer patient to rheumatologist or neurologist. • Initiate treatment as per institutional guidelines. • Respiratory support may be required in more severe cases. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone, or higher-dose bolus if patient is severely compromised (*e.g.*, cardiac or respiratory symptoms, dysphagia, or weakness that severely limits mobility); convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, resume atezolizumab. [b] • If event does not resolve to Grade 1 or better while withholding atezolizumab, permanently discontinue atezolizumab and contact Medical Monitor. • For recurrent events, treat as a Grade 4 event.
Immune-mediated myositis, Grade 4	• Permanently discontinue atezolizumab and contact Medical Monitor. • Refer patient to rheumatologist or neurologist. • Initiate treatment as per institutional guidelines. • Respiratory support may be required in more severe cases. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone, or higher-dose bolus if patient is severely compromised (*e.g.*, cardiac or respiratory symptoms, dysphagia, or weakness that severely limits mobility); convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, taper corticosteroids over ≥1 month.

[a] 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 the equivalent of ≤10 mg/day oral prednisone. The acceptable length of the extended period of time must be agreed upon by the investigator and the Medical Monitor.

[b] If corticosteroids have been initiated, they must be tapered over ≥1 month to the equivalent of ≤10 mg/day oral prednisone before atezolizumab can be resumed.

Hemophagocytic Lymphohistiocytosis and Macrophage Activation Syndrome

Immune-mediated reactions may involve any organ system and may lead to hemophagocytic lymphohistiocytosis (HLH) and macrophage activation syndrome (MAS).

Patients with suspected HLH should be diagnosed according to published criteria by McClain and Eckstein (2017). A patient should be classified as having HLH if five of the following eight criteria are met:

- Fever ≥ 38.5°C
- Splenomegaly
- Peripheral blood cytopenia consisting of at least two of the following:
  - Hemoglobin < 90 g/L (9 g/dL) (<100 g/L [10 g/dL] for infants < 4 weeks old)
  - Platelet count < 100×10^9/L (100,000/mcL)
  - ANC < 1.0×10^9/L (1000/mcL)
- Fasting triglycerides >2.992 mmol/L (265 mg/dL) and/or fibrinogen <1.5 g/L (150 mg/dL)
- Hemophagocytosis in bone marrow, spleen, lymph node, or liver
- Low or absent natural killer cell activity
- Ferritin >500 mg/L (500 ng/mL)
- Soluble interleukin 2 (IL-2) receptor (soluble CD25) elevated ≥ 2 standard deviations above age-adjusted laboratory-specific norms

Patients with suspected MAS should be diagnosed according to published criteria for systemic juvenile idiopathic arthritis by Ravelli et al. (2016). A febrile patient should be classified as having MAS if the following criteria are met:

- Ferritin >684 mg/L (684 ng/mL)
- At least two of the following:
  - Platelet count ≤ 181×10^9/L (181,000/mcL)
  - AST ≥48 U/L
  - Triglycerides >1.761 mmol/L (156 mg/dL)
  - Fibrinogen ≤3.6 g/L (360 mg/dL)

Patients with suspected HLH or MAS should be treated according to the guidelines in below.

Event	Management
Suspected HLH or MAS	• Permanently discontinue atezolizumab and contact Medical Monitor. • Consider patient referral to hematologist. • Initiate supportive care, including intensive care monitoring if indicated per institutional guidelines. • Consider initiation of IV corticosteroids and/or an immunosuppressive agent. • If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, taper corticosteroids over ≥1 month.

Event	Management

HLH= hemophagocytic lymphohistiocytosis; MAS= macrophage activation syndrome.

6.2 Systemic Chemotherapy

Atezolizumab will be held when paclitaxel and/or carboplatin are held. If there is a need to discontinue chemotherapy (such as severe hypersensitivity reaction), patients should continue to surgery.

Paclitaxel Dose Levels

Level	Dose
Starting Dose	80 mg/m2
Dose Level -1	65 mg/m2
Dose Level -2	50 mg/m2
Dose Level -3	Discontinue

Carboplatin Dose Levels

Level	Dose
Starting Dose	AUC 5
Dose Level -1	AUC 4
Dose Level -2	AUC 3
Dose Level -3	Discontinue

6.2.1 General Treatment management for paclitaxel and carboplatin

Dose modifications must be based on AEs that occurred between treatments AND AEs present on the scheduled treatment day. Dose modifications must be based on the AE requiring the greatest modification. Chemotherapy doses that have been reduced may not be escalated. Chemotherapy should be held for at least 1 week until any AE requiring dose modification returns to ≤ grade 1 unless indicated otherwise below. If recovery to ≤ grade 1 (or to other level specified) has not occurred after 3 weeks of delay, therapy must be discontinued.

CTCAE v 5.0 grade	**Modifications for AEs that occurred between treatments but did NOT require a delay in treatment (for paclitaxel) or resolve prior to the next treatment cycle (for carboplatin); treatment may NOT proceed until clinically significant AEs are ≤ grade 1 (except neutrophils, which must be ≥ 1000/mm3 and bilirubin, which must be ≤ the baseline grade)**	**Modifications for AEs that require a delay in the next treatment; hold and check weekly, and resume treatment when toxicity is ≤ grade 1 (with the exception of neutrophils and bilirubin); if toxicity has not resolved to ≤ grade 1 after 3 weeks of delay, DISCONTINUE PACLITAXEL AND CARBOPLATIN**
Neutrophil count decreased		
Grade 2	Maintain doses	Maintain dose of paclitaxel.
Grades 3, 4		Hold paclitaxel until ANC ≥ 1000/mm3. Maintain dose of paclitaxel and add G-CSF (if already receiving G-CSF, reduce paclitaxel by one dose level). Hold carboplatin until ANC ≥ 1000/mm3. If recovery takes 1 week, maintain dose of carboplatin and add G-CSF. If recovery takes 2 weeks, reduce carboplatin by one dose level and add G-CSF. If recovery takes 3 weeks, discontinue carboplatin. If already receiving G-CSF and recovery takes 1 week, reduce carboplatin by one dose level. If already receiving G-CSF and recovery takes > 1 week, discontinue carboplatin.
Platelet count decreased		
Grades 2, 3	Maintain doses	Hold both until platelets ≥ 75,000/mm3. If recovery takes 1 week, maintain

		dose of paclitaxel and reduce carboplatin by one dose level. If recovery takes > 1 week, reduce paclitaxel by one dose level and reduce carboplatin by two dose levels or discontinue.
Grade 4	Reduce both by one dose level	Hold until platelets ≥ 75,000/mm3, then reduce paclitaxel by one dose level and reduce carboplatin by two dose levels or discontinue.
Anemia		
Grade 3, 4	Chemotherapy should not proceed. Transfusion is acceptable for improving the hemoglobin value to allow therapy to continue without delay. The patient should be assessed to rule out other causes of anemia. Use of erythropoiesis-stimulating agents is prohibited.	
Diarrhea (if related to chemotherapy)		
Grade 2	Maintain doses	Maintain paclitaxel dose or reduce by one dose level. Reduce carboplatin by one dose level.
Grade 3	Reduce both by one dose level	
Grade 4	Reduce paclitaxel by one dose level or discontinue. Reduce carboplatin by two dose levels or discontinue.	
Mucositis – oral (if related to chemotherapy)		
Grade 2	Maintain doses	Maintain paclitaxel dose or reduce by one dose level. Reduce carboplatin by one dose level.
Grade 3	Reduce both by one dose level	
Grade 4	Reduce paclitaxel by one dose level or discontinue. Reduce carboplatin by two dose levels or discontinue.	
Vomiting (if related to chemotherapy and despite antiemetics)		
Grade 2	Maintain doses or reduce both by one dose level	
Grades 3, 4	Reduce both by one dose level or discontinue	Reduce paclitaxel by one dose level or discontinue. Reduce carboplatin by two dose levels or discontinue.
Bilirubin, AST, alkaline phosphatase		
Grade 2	Maintain doses or reduce both by one dose level.	Hold until bilirubin returns to the baseline grade and AST and alk phos have returned to ≤ grade 1, then

		reduce both by one dose level
Grade 3	Reduce paclitaxel by one dose level or discontinue. Reduce carboplatin by one dose level or discontinue.	Hold until bilirubin returns to the baseline grade and AST and alk phos have returned to ≤ grade 1, then reduce paclitaxel by one dose level or discontinue and reduce carboplatin by two dose levels or discontinue.
Grade 4	Discontinue both.	
Infection or febrile neutropenia		
Grade 2	Maintain doses and add G-CSF prophylaxis for subsequent chemotherapy cycles if neutropenia was present. (If grade 2 criteria for infection include topical antibiotics or other local treatment, use of G-CSF is at the investigator's discretion.)	
Grade 3	Maintain doses and add G-CSF prophylaxis for subsequent chemotherapy cycles. If receiving prophylactic G-CSF, reduce both by one dose level.	
Grade 4	Maintain doses or reduce both by one dose level and add G-CSF prophylaxis for subsequent chemotherapy cycles. If receiving prophylactic G-CSF, reduce both by one dose level or discontinue.	
Creatinine increased		
Grades 2, 3	Maintain paclitaxel. Hold carboplatin until serum creatinine is ≤ grade 1 AND measured or calculated creatinine clearance is ≥ 30 mL/min. If creatinine clearance is > 50 mL/min, maintain dose of carboplatin. If creatinine clearance is 30-50 mL/min, reduce carboplatin by one dose level. If measured or calculated creatinine clearance is < 30 mL/min but all other non-renal function AEs have resolved to ≤ grade 1 on the scheduled Day 1, carboplatin must be held. If measured or calculated creatinine clearance subsequently improves to ≥ 30 mL/min, carboplatin may be resumed. The missed carboplatin dose will not be made up.	
Grade 4	Maintain paclitaxel. Discontinue carboplatin.	
Other clinically significant AEs (at the discretion of the investigator)		
Grade 2	Maintain doses or reduce both by one dose level	
Grade 3	Reduce both by one dose level	
Grade 4	Reduce paclitaxel by one dose level or discontinue. Reduce carboplatin by two dose levels or discontinue.	

6.2.2 Treatment management for paclitaxel-related neuropathy

Nervous System	**1-7 days duration**	**Persistent for > 7 days OR caused the**

Disorders **(Paresthesias, peripheral sensory neuropathy)**		**next treatment to be delayed**
Grade 1	Maintain dose	
Grade 2	Maintain dose (must be resolved to ≤ grade 1 on the next treatment day)	Reduce by one dose level (hold paclitaxel and carboplatin for persistent grade 2 neuropathy; when ≤ grade 1, resume treatment with dose modification for paclitaxel but no dose modification for carboplatin. If grade 2 toxicity persists after 3 weeks of delay, discontinue all chemotherapy)
Grade 3	First episode: Reduce by one dose level (must be resolved to ≤ grade 1 on the next treatment day) Second episode: Discontinue paclitaxel	Discontinue paclitaxel
Grade 4	Discontinue paclitaxel	

6.2.3 Treatment management for paclitaxel-related musculoskeletal pain

Note: these instructions only apply to patients with musculoskeletal pain not controlled by analgesics. Use of narcotics and NSAIDs is encouraged to maintain the paclitaxel dose if possible.

Musculoskeletal and Connective Tissue Disorders **(Arthralgia, myalgia)**	**1-7 days duration**	**Persistent for > 7 days OR caused the next treatment to be delayed**
Grade 1	Maintain dose	
Grade 2	Maintain dose	Maintain dose or reduce by one dose level (hold paclitaxel and carboplatin for persistent grade 2 musculoskeletal pain; when ≤ grade 1, resume treatment with dose modification for paclitaxel but no dose modification for carboplatin. If grade 2 toxicity persists after 3

		weeks of delay, discontinue all chemotherapy)
Grade 3	First episode: Reduce by one dose level Second episode: Discontinue paclitaxel	First episode Reduce by one dose level or discontinue (hold paclitaxel and carboplatin for persistent grade 3 musculoskeletal pain; when ≤ grade 1, resume treatment with dose modification for paclitaxel but no dose modification for carboplatin. If grade 3 toxicity persists after 3 weeks of delay, discontinue all chemotherapy) Second episode Discontinue paclitaxel

6.2.4 Other treatment management instructions

If a paclitaxel-related hypersensitivity reaction occurs despite premedication, treatment as medically indicated will be instituted. For ≤ grade 3 allergic reaction or grade 3 anaphylaxis, continuation of paclitaxel is at the investigator's discretion. Following a grade 4 allergic reaction or grade 4 anaphylaxis, paclitaxel must be permanently discontinued.

Carboplatin must be permanently discontinued if a grade 3 or 4 hypersensitivity reaction occurs that the investigator attributes to carboplatin.

Unless otherwise specified, paclitaxel or carboplatin that is held due to toxicity will not resume until the toxicity has resolved to ≤ grade 1.

If carboplatin is held due to toxicity, paclitaxel should be held. If therapy is held for > 2 weeks due to persistent carboplatin toxicity, discontinue carboplatin.

If carboplatin is discontinued due to toxicity, the remaining paclitaxel doses should be administered.

Paclitaxel and carboplatin must be completed within 18 weeks. Any of the 12 paclitaxel doses or 4 carboplatin doses remaining after 18 weeks following the first paclitaxel and carboplatin doses should not be administered.

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 AEs (**Section 7.1**) and the characteristics of an observed AE (**Section 7.2**) will determine whether the event requires expedited reporting via the CTEP Adverse Event Reporting System (CTEP-AERS) **in addition** to routine reporting.

7.1 Comprehensive adverse events and potential risks list (CAEPR)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset of AEs, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and

is identified with ***bold*** and ***italicized*** text. The SPEER is a list of events that are protocol-specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/adverse_effects.htm for further clarification.

NOTE: The highest grade currently reported is noted in parentheses next to the AE in the SPEER. Report **ONLY** AEs higher than this grade expeditiously. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

7.1.1 CAEPR for atezolizumab

Version 2.3, March 11, 2021[1]

Adverse Events with Possible Relationship to Atezolizumab (MPDL3280A) (CTCAE 5.0 Term) [n= 3097]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		
CARDIAC DISORDERS			
		Heart failure[2]	
		Myocarditis[2]	
		Pericardial effusion[2]	
		Pericardial tamponade[2]	
		Pericarditis[2]	
ENDOCRINE DISORDERS			
		Adrenal insufficiency[2]	
		Endocrine disorders - Other (diabetes)[2]	
	Hyperthyroidism[2]		
		Hypophysitis[2]	
	Hypothyroidism[2]		
EYE DISORDERS			
		Eye disorders - Other (ocular inflammatory toxicity)[2]	
		Uveitis[2]	
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 2)***
		Colitis[2]	
	Diarrhea Dysphagia		***Diarrhea (Gr 2)***
	Nausea		***Nausea (Gr 2)***
		Pancreatitis[2]	
	Vomiting		***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr 2)***
	Fever[3] Flu like symptoms[3]		
HEPATOBILIARY DISORDERS			
		Hepatic failure[2]	

Adverse Events with Possible Relationship to Atezolizumab (MPDL3280A) (CTCAE 5.0 Term) [n= 3097]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
		Hepatobiliary disorders - Other (hepatitis)[2]	
IMMUNE SYSTEM DISORDERS			
	Allergic reaction[3]		
		Anaphylaxis[3]	
		Cytokine release syndrome[3]	
		Immune system disorders - Other (systemic immune activation)[2]	
INFECTIONS AND INFESTATIONS			
Infection[4]			
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Infusion related reaction[3]		
INVESTIGATIONS			
	Alanine aminotransferase increased[2]		
	Alkaline phosphatase increased[2]		
	Aspartate aminotransferase increased[2]		
	Blood bilirubin increased[2]		
		Creatinine increased	
	GGT increased[2]		
	Lipase increased*		
		Platelet count decreased	
	Serum amylase increased*		
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 2)***
		Hyperglycemia[2]	
	Hypokalemia		
	Hyponatremia		
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia2		
	Back pain		
		Generalized muscle weakness	
	Myalgia		
		Myositis[2]	
NERVOUS SYSTEM DISORDERS			
		Ataxia[2]	
		Encephalopathy[2]	
		Nervous system disorders - Other (encephalitis non-infective)[2]	
		Guillain-Barre syndrome[2]	
		Nervous system disorders - Other (meningitis non-infective)[2]	
		Myasthenia gravis[2]	
		Paresthesia[2]	
		Peripheral motor neuropathy[2]	
		Peripheral sensory neuropathy[2]	
RENAL AND URINARY DISORDERS			

Adverse Events with Possible Relationship to Atezolizumab (MPDL3280A) (CTCAE 5.0 Term) [n= 3097]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
		Acute kidney injury	
		Renal and urinary disorders - Other (nephritis)[2]	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		***Cough (Gr 2)***
	Dyspnea		
	Hypoxia		
	Nasal congestion		***Nasal congestion (Gr 2)***
		Pleural effusion[2]	
		Pneumonitis[2]	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
		Bullous dermatitis[2]	
		Erythema multiforme[2]	
	Pruritus		
	Rash acneiform		
	Rash maculo-papular		
		Skin and subcutaneous tissue disorders – Other (drug reaction with eosinophilia and systemic symptoms [DRESS])[2]	
	Skin and subcutaneous tissue disorders - Other (lichen planus)[2]		
		Skin and subcutaneous tissue disorders – Other (exanthematous pustulosis)[2]	
		Stevens-Johnson syndrome[2]	
		Toxic epidermal necrolysis[2]	

*Denotes adverse events that are <3%.

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Atezolizumab, being a member of a class of agents involved in the inhibition of "immune checkpoints," may result in severe and possibly fatal immune-mediated adverse events probably due to T-cell activation and proliferation. Immune-mediated adverse reactions have been reported in patients receiving atezolizumab. Adverse events potentially related to atezolizumab may be manifestations of immune-mediated adverse events. In clinical trials, most immune-mediated adverse reactions were reversible and managed with interruptions of atezolizumab, administration of corticosteroids and supportive care.

[3]Infusion reactions, including high-grade hypersensitivity reactions, anaphylaxis, and cytokine release syndrome, which have been observed following administration of atezolizumab, may manifest as fever, chills, shakes, itching, rash, hypertension or hypotension, or difficulty breathing during and immediately after administration of atezolizumab.

[4]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

Adverse events reported on atezolizumab (MPDL3280A) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that atezolizumab (MPDL3280A) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (pancytopenia); Febrile neutropenia

CARDIAC DISORDERS - Cardiac arrest; Ventricular tachycardia

GASTROINTESTINAL DISORDERS - Constipation; Dry mouth; Ileus

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Edema limbs; Malaise; Multi-organ failure

HEPATOBILIARY DISORDERS - Portal vein thrombosis

INVESTIGATIONS - Lymphocyte count decreased; Neutrophil count decreased; Weight loss; White blood cell decreased

METABOLISM AND NUTRITION DISORDERS - Hypophosphatemia; Tumor lysis syndrome

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Bone pain; Muscle cramp: Pain in extremity

NERVOUS SYSTEM DISORDERS - Headache

PSYCHIATRIC DISORDERS - Confusion; Insomnia; Suicide attempt

REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Breast pain

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Bronchopulmonary hemorrhage; Pulmonary hypertension; Respiratory failure

SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin[2]; Hyperhidrosis

VASCULAR DISORDERS - Hypertension; Hypotension; Thromboembolic event

Note: Atezolizumab (MPDL3280A) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.1.2 Adverse event list for paclitaxel

Common adverse reactions are neutropenia, leukopenia, thrombocytopenia, anemia, infections, bleeding, hypersensitivity reactions, abnormal ECGs, peripheral neuropathy, myalgia/arthralgia, nausea, vomiting, diarrhea, alopecia, elevated liver enzymes, and injection site reaction.

Please refer to the package insert for the comprehensive list of adverse events.

7.2 Adverse event characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized until March 31, 2018 for AE reporting. CTCAE version 5.0 will be utilized for AE reporting beginning April 1, 2018. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent that are ***bold and italicized*** in the CAEPR (*i.e.*, those listed in the SPEER column, **Section 7.1.1**) should be reported through CTEP-

AERS only if the grade is above the grade provided in the SPEER.
- Other AEs for the protocol that do not require expedited reporting are outlined in **Section 7.3.4**.

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

7.3 Expedited adverse event reporting

7.3.1 CTEP-AERS

Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP Web site (https://eapps-ctep.nci.nih.gov/ctepaers). The reporting procedures to be followed are presented in the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" which can be downloaded from the CTEP Web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm). These requirements are briefly outlined in the tables below (**Section 7.3.3**).

In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.

7.3.2 Distribution of adverse event reports

CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Principal Investigator and Adverse Event Coordinator(s) (if applicable) of the Corresponding Organization or Lead Organization, the local treating physician, and the Reporter and Submitter. CTEP-AERS provides a copy feature for other e-mail recipients.

7.3.3 Expedited reporting guidelines

Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

Note: A death on study requires both routine and expedited reporting, regardless of causality. Attribution to treatment or other cause must be provided.

Death due to progressive disease should be reported as **Grade 5 "Disease progression"** under the system organ class (SOC) "General disorders and administration site conditions." Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Phase 1 and Early Phase 2 Studies: Expedited Reporting Requirements for Adverse Events

that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria MUST be immediately reported to the NCI via electronic submission within the timeframes detailed in the table below.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days	24-Hour 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR.

Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" - The AE must initially be submitted electronically within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" - A complete expedited report on the AE must be submitted electronically within 10 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 3, 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

[2]For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

7.3.4 Adverse events of special interest in atezolizumab studies

The following AEs are considered of special interest in patients receiving atezolizumab and must be reported expeditiously through CTEP-AERS, irrespective of regulatory seriousness criteria:

- Pneumonitis
- Colitis
- Endocrinopathies: diabetes mellitus, pancreatitis, adrenal insufficiency, hyperthyroidism, hypophysitis, and adrenal insufficiency
- Hepatitis, including AST or ALT > 10 x ULN
- Systemic lupus erythematosus
- Neurological disorders: Guillain-Barré syndrome, myasthenic syndrome or myasthenia gravis, and meningoencephalitis
- Events suggestive of hypersensitivity, cytokine release, influenza-like illness, systemic inflammatory response syndrome, systemic immune activation, or infusion-related reactions
- Nephritis
- Ocular toxicities (e.g. uveitis, retinitis)
- Myositis
- Myopathies, including rhabdomyolysis
- Grade ≥2 cardiac disorders (e.g. atrial fibrillation, myocarditis, pericarditis)
- Vasculitis

7.3.5 Special situations reports

- 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
- Data related to a suspected transmission of an infectious agent via a medicinal product (STIAMP)
- Lack of therapeutic efficacy

7.4 Routine adverse event reporting

All Adverse Events **must** be reported in routine study data submissions. **AEs reported expeditiously through CTEP-AERS must <u>also</u> be reported in routine study data submissions.**

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. AEs are reported in a routine manner at scheduled times during the trial using Medidata Rave. For this trial the Adverse Event CRF is used for routine AE reporting in Rave.

7.5 Secondary malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary

malignancy is not considered a metastasis of the initial neoplasm.

CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported expeditiously via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (*e.g.*, acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol. For this trial, use the Adverse Event CRF in Rave.

7.6 Second malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine AE reporting unless otherwise specified.

8. PHARMACEUTICAL INFORMATION

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

8.1 CTEP IND agent: Atezolizumab

Other Names: Tecentriq™, MPDL3280A

Classification: monoclonal antibody

M.W.: 150 KD

Mode of Action: anti-PD-L1

8.1.1 Description

Atezolizumab is a humanized IgG1 monoclonal antibody consisting of two heavy chains (448 amino acids) and two light chains (214 amino acids). Atezolizumab targets human PD-L1 and inhibits its interaction with its receptor 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 [68].

8.1.2 How Supplied

Atezolizumab is provided by Genentech/F.Hoffmann-La Roche LTD and distributed by the Pharmaceutical Management Branch, CTEP, NCI. The agent is supplied in a single-use, 20-mL glass vial as a colorless-to-slightly-yellow, sterile, preservative-free clear liquid solution intended for IV administration. Atezolizumab is formulated as 60 mg/mL atezolizumab in 20 mM histidine acetate, 120 mM sucrose, 0.04% polysorbate 20, at a pH of 5.8. 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.

8.1.3 Preparation

The prescribed dose of atezolizumab should be diluted in 250 mL 0.9% NaCl and infused through a 0.2 micrometer in-line filter. The IV bag may be constructed of PVC or PO; the IV infusion line may be constructed of PVC or PE; and the 0.2 micrometer in-line filter may be constructed of PES. The prepared solution may be stored at 2°C-8°C or room temperature for up to 8 hours.

8.1.4 Storage

2°C-8°C (36°F-46°F) Vial contents should not be frozen or shaken and should be protected from direct sunlight.

If a storage temperature excursion is identified, promptly return atezolizumab to 2°C-8°C (36°F-46°F) and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to pmbafterhours@mail.nih.gov for determination of suitability.

8.1.5 Stability

Stability studies are ongoing.

CAUTION: No preservative is used in atezolizumab; therefore, the vial is intended for single use only. Discard any unused portion of drug remaining in a vial.

8.1.6 Route of Administration

IV infusion.

8.1.7 Method of Administration

Atezolizumab is administered as an intravenous infusion over 60 minutes. If the first infusion is tolerated, all subsequent infusions may be delivered over 30 minutes. Do not administer atezolizumab as an intravenous push or bolus. No premedication is indicated for administration of Cycle 1 of atezolizumab. Patients who experience an infusion related reaction with Cycle 1 of atezolizumab may receive premedication with antihistamines or antipyretics/analgesics (e.g. acetaminophen) for subsequent infusions.

8.1.8 Potential Drug Interactions

Cytochrome P450 enzymes as well as conjugation/glucuronidation reactions are not involved in the metabolism of atezolizumab. No drug interaction studies for atezolizumab have been conducted or are planned. There are no known interactions with other medicinal products or other form of interactions.

8.1.9 Patient Care Implication

Female patients of childbearing potential should utilize contraception and take active measures to avoid pregnancy while undergoing atezolizumab treatment and for at least 5 months (150 days) after the last dose of atezolizumab.

8.1.10 Availability

Atezolizumab is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

8.1.11 Atezolizumab ordering and agent accountability

NCI-supplied agents may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained). The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), Biosketch/Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one

institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

In general, sites may order initial agent supplies when a subject is being screened for enrollment onto the study.

Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees can submit agent requests through the PMB Online Agent Order Processing (OAOP) application. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account and the maintenance of an "active" account status and a "current" password. For questions about drug orders, transfers, returns, or accountability, call or email PMB any time. Refer to the PMB's website for specific policies and guidelines related to agent management.

8.1.12 Atezolizumab inventory records

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, dispensing and final disposition of all agents received from the PMB using the appropriate NCI Investigational Agent (Drug) Accountability Record (DARF) available on the CTEP forms page. Store and maintain separate NCI Investigational Agent Accountability Records for each agent, strength, formulation and ordering investigator on this protocol.

8.1.13 Investigator Brochure Availability

The current versions of the IBs for the agents will be accessible to site investigators and research staff through the PMB OAOP application. Access to OAOP requires the establishment of a CTEP IAM account and the maintenance of an "active" account status and a "current" password. Questions about IB access may be directed to the PMB IB Coordinator via email.

8.1.14 CTEP useful links and contacts

- CTEP Forms, Templates, Documents: http://ctep.cancer.gov/forms/
- NCI CTEP Investigator Registration: pmbregpend@ctep.nci.nih.gov
- PMB policies and guidelines: http://ctep.cancer.gov/branches/pmb/agent_management.htm
- PMB Online Agent Order Processing (OAOP) application: https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx
- CTEP Identity and Access Management (IAM) account: https://eapps-ctep.nci.nih.gov/iam/
- CTEP Associate Registration and IAM account help: ctepreghelp@ctep.nci.nih.gov
- PMB email: pmbafterhours@mail.nih.gov
- PMB phone and hours of service: (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET)

8.2 Commercial agent: carboplatin

8.2.1 Product Description

Carboplatin is supplied as a sterile, pyrogen-free, 10 mg/mL aqueous solution of carboplatin. Carboplatin is a platinum coordination compound. The chemical name for carboplatin is platinum, diamine [1,1-cyclobutane-dicarboxylato(2-)-0,0']-,(SP-4-2). It has a molecular formula of $C_6H_{12}N_2O_4Pt$ and a molecular weight of 371.25. It is soluble in water at a rate of approximately 14 mg/mL, and the pH of a 1% solution is 5 to 7. It is virtually insoluble in ethanol, acetone, and dimethylacetamide.

8.2.2 Solution Preparation

Please refer to the package insert for standard preparation instructions.

8.2.3 Route of Administration

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

Carboplatin will be administered over 30 minutes.

8.2.4 Agent Ordering

Carboplatin is commercially available.

8.2.5 Prescribing Information

Please refer to the package insert for full prescribing information on carboplatin.

8.3 Commercial agent: paclitaxel

8.3.1 Product Description

Paclitaxel is obtained via a semi-synthetic process from Taxus baccata. The chemical name for paclitaxel is 5β,20-Epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2R,3S)-N-benzoyl-3-phenylisoserine. Paclitaxel Injection, USP is a clear, colorless to slightly yellow viscous solution. It is supplied as a nonaqueous solution intended for dilution with a suitable parenteral fluid prior to intravenous infusion. Paclitaxel Injection, USP is available in 30 mg (5 mL), 100 mg (16.7 mL), and 300 mg (50 mL) multidose vials. Each mL of sterile nonpyrogenic solution contains 6 mg paclitaxel, USP, 527 mg of purified polyoxyl 35 castor oil, and 49.7% (v/v) dehydrated alcohol, USP.

8.3.2 Solution Preparation

Please refer to the package insert for standard preparation instructions.

8.3.3 Route of administration

Contact of the undiluted concentrate with plasticized PVC equipment or devices used to prepare solutions for infusion is not recommended. In order to minimize patient exposure to the plasticizer DEHP [di-(2-ethylhexyl)phthalate], which may be leached from PVC infusion bags or

sets, diluted paclitaxel solutions should be stored in bottles (glass, polypropylene) or plastic bags (polypropylene, polyolefin) and administered through polyethylene-lined administration sets.

Administer paclitaxel as an IV infusion over 1 hour.

8.3.4 Agent ordering

Paclitaxel is commercially available.

8.3.5 Prescribing Information

Please refer to the package insert for full prescribing information on paclitaxel.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

The study design provides unique opportunities for tissue collection in the context of neoadjuvant chemotherapy therapy for triple negative breast cancer. Research core biopsies will be obtained at baseline, and after one cycle of therapy. Additional tumor tissue will be obtained at the time of surgery. PBMC will be obtained at baseline, after one and three cycles of therapy, and at the time of surgery.

A summary of the correlative analyses and prioritization of sample use is provided below. Many of the correlative analyses planned will be conducted in collaboration with the Cancer Immune Monitoring and Analysis Center at Dana-Farber Cancer Institute (DFCI-CIMAC) and at Washington University as part of the Human Tumor Analysis Network (WU-HTAN).

Sample Prioritization for Correlative Studies							
Priority	**Biomarker Name**	**Assay (CLIA: Y/N)**	**Use in protocol / Purpose**	**Sample types and Time points of sample collection**		**Mandatory or optional?**	**Assay Lab**
1-tissue	TIL percentage by H&E	H&E (CLIA: N)	Integral: Measure antitumor immune response following combination chemotherapy and atezolizumab (co-primary endpoint). Correlate baseline TIL percentage with pathologic complete response (exploratory objective).	Tumor FFPE	Baseline, D18-22, surgical resection	M	DFCI-CIMAC
2-tissue	TIL cell density (cells/mm2) by multiplex IF (CD3, CD8, CD20, CD4, FoxP3, PDL1, as tumor marker)	IF (CLIA: N)	Exploratory: Measure antitumor immune response following combination chemotherapy and atezolizumab (co-primary endpoint). Correlate baseline immune cell percentage with pathologic complete response (exploratory objective).	Tumor FFPE	Baseline, D18-22, surgical resection	M	DFCI-CIMAC
3-tissue	PD-L1 expression (VENTANA PD-L1 (SP142) Assay)	IHC (CLIA: N)	Exploratory: Correlate baseline PD-L1 expression with pathologic complete response Measure antitumor immune response following combination chemotherapy and atezolizumab therapy	Tumor FFPE	Baseline, D18-22, surgical resection	M	Histo-GeneX

Sample Prioritization for Correlative Studies							
Priority	**Biomarker Name**	**Assay (CLIA: Y/N)**	**Use in protocol / Purpose**	**Sample types and Time points of sample collection**		**Mandatory or optional?**	**Assay Lab**
4-tissue	Immune signature (RNA-Seq)	RNA-Seq (CLIA: N)	Exploratory: Correlate baseline immune signature with pathologic complete response Measure antitumor immune response following combination chemotherapy and atezolizumab therapy (	Tumor Frozen core	Baseline, D18-22, Surgical resection	M	DFCI-CIMAC
5-tissue	Immune signature (NanoString PanCancer Immune panel)	Nanostring (Only if RNAseq is not performed)	Exploratory: Correlate baseline immune signature with pathologic complete response Measure antitumor immune response following combination chemotherapy and atezolizumab therapy	Tumor FFPE	Baseline, D18-22, Surgical resection	M	DFCI-CIMAC
6-tissue	Neoantigen load	Tumor tissue and normal blood whole exome sequencing (CLIA: N)	Exploratory: Correlate baseline neoantigen load with pathologic complete response	Fresh tissue or FFPE	Baseline, D18-22, Surgical resection	O	DFCI-CIMAC
7-tissue	T cell receptor (TcR) repertoire analysis	T cell receptor (TcR) sequencing	Exploratory: Measure antitumor immune response based on T cell clonal changes following combination chemotherapy and atezolizumab therapy	Fresh tissue or FFPE	Baseline, D18-22, Surgical resection	O	DFCI-CIMAC
8-tissue	Immune signature	sn-RNA-Seq whole transcriptome	Exploratory: Correlate baseline immune signature with pathologic complete response Measure antitumor immune response following combination chemotherapy and atezolizumab therapy	Tumor Frozen core	Baseline, D18-22, Surgical resection	O	WU-HTAN (Gillanders, Ding)

Sample Prioritization for Correlative Studies							
Priority	**Biomarker Name**	**Assay (CLIA: Y/N)**	**Use in protocol / Purpose**	**Sample types and Time points of sample collection**		**Mandatory or optional?**	**Assay Lab**
9-tissue	Immune signature	CODEX	Exploratory: Correlate baseline immune signature with pathologic complete response Measure antitumor immune response following combination chemotherapy and atezolizumab therapy	Tumor Frozen core	Baseline, D18-22, Surgical resection	O	WU-HTAN (Gillanders, Ding)
1-blood	Neoantigen load	Tumor tissue and normal blood whole exome sequencing (CLIA: N)	Exploratory: Correlate baseline neoantigen load with pathologic complete response	blood	Baseline or any other timepoint	O	DFCI-CIMAC
2-blood	Neoantigen-specific T cell response	CyTOF or multiparameter flow cytometry with neoantigen-specific tetramers or sn-RNA-Seq	Exploratory: Measure neoantigen-specific antitumor immune response following combination chemotherapy and atezolizumab therapy	blood	Baseline, D18-22, Surgical resection	O	Schreiber /Gillanders, Immune Monitoring Laboratory WUSM
3-blood	Peripheral blood immune reconstitution	CyTOF or multiparameter flow cytometry	Exploratory: Assess peripheral blood immune reconstitution following combination chemotherapy and atezolizumab therapy	blood	Baseline, D18-22, Surgical resection	O	DFCI-CIMAC
4-blood	T cell receptor (TcR) repertoire analysis	T cell receptor (TcR) sequencing	Exploratory: Measure antitumor immune response based on T cell clonal changes following combination chemotherapy and atezolizumab therapy	blood	Baseline, D18-22, Surgical resection	O	DFCI-CIMAC

9.1 Integral correlative study

9.1.1 TIL percentage by H&E

Rationale: TIL percentage is a well-described and validated histopathologic analysis that has been correlated with clinical response to adjuvant and neoadjuvant chemotherapy in patients with TNBC [44-46]. These studies used standardized methodology for visual assessment of TIL

percentage, and tissue specimens from patients in enrolled in phase 3 randomized clinical trials. The studies demonstrated that TIL percentage is associated with improved overall survival. This correlation was first reported using baseline samples from the BIG 2–98 trial [45] and subsequently independently confirmed in 481 TNBC sample prospectively collected during two phase III adjuvant randomized breast cancer trials (United States Eastern Cooperative Oncology Group trials 2197 and 1199) [44].

An increase in the number of TILs has been shown to be associated with response to checkpoint inhibition therapy in multiple independent studies. For instance, in a prospective randomized trial of ipilimumab for patients with advanced melanoma where serial tumor biopsies were obtained, Hamid et al. demonstrated that 57.1% of patients in the benefit group had a post-treatment increase in TILs, whereas only 10.0% of patients in the non-benefit group had a post-treatment increase in TILs ($p = 0.005$) [60]. Similarly, Tumeh et al. studied tumor samples from 46 patients with metastatic melanoma obtained before and after therapy with pembrolizumab. In this study, they used quantitative immunohistochemistry to evaluate the number of CD8 T cells present in the tumor and observed that an increase in the number of CD8 T cells was strongly associated with the best percent change in tumor size as measured by CT scan (n=18, Spearman r = −0.75, P = 0.0002) [61]. Finally, Herbst et al. evaluated the safety, activity and biomarkers of PD-L1 inhibition in patients treated with atezolizumab [35]. They found that after atezolizumab treatment, regressing lesions displayed a dense immune infiltrate.

The primary objective of the clinical trial is to test the hypothesis that the combination of chemotherapy and atezolizumab will increase TIL percentage compared to chemotherapy alone in patients with newly diagnosed TNBC being treated with neoadjuvant chemotherapy.

TIL percentage will be measured at baseline, and after initiation of therapy using a well validated histopathologic assay. The TIL percentage after initiation of therapy will be compared between patients receiving neoadjuvant chemotherapy alone (Arm A), and patients receiving neoadjuvant chemotherapy in combination with atezolizumab (Arm B).

Tissue will be obtained at three time points (baseline, after initiation of therapy (day 18-22), and at the time of surgery), providing three opportunities to measure TIL percentage. We expect that TIL percentage will be readily measured at baseline and at day 22, but that TIL percentage may be difficult to measure at the time of surgery. We anticipate that the pCR may be 50%, or greater depending on the arm.

As this is a randomized clinical trial, we anticipate that the baseline TIL percentage will be similar between the two arms. As such, for the primary endpoint, we will compare the TIL percentage between arms at day 22. We hypothesize that the TIL percentage will be increased in Arm B (chemotherapy plus atezolizumab).

At each time point, two cores will be placed into FFPE. Both cores will be assessed by H&E for the presence of invasive breast cancer and for TIL percentage. If no invasive cancer is present in either of the two FFPE cores at day 22, the sample will be considered inevaluable. If invasive cancer is present in only one of the two FFPE cores, the sample will be considered evaluable, and TIL percentage will be determined based on the FFPE core containing invasive breast cancer. If invasive cancer is present in both cores, TIL percentage will be assessed independently in the two cores and the result will be averaged between the two cores in order to minimize sampling bias as a potential confounder. Please note if the day 22 sample is inevaluable, the patient is inevaluable for the primary endpoint and will be replaced so that at least 60 patients

who are evaluable for the primary endpoint are enrolled. These patients will continue on protocol and will be evaluated for the secondary and exploratory endpoints.

Hypothesis: The combination of chemotherapy and atezolizumab will increase TIL percentage, and pathologic complete response rate compared to chemotherapy alone in patients with newly diagnosed TNBC being treated with neoadjuvant chemotherapy.

Assay methodology: TIL percentage will be evaluated as described by Wimberly et al. [69] and a recent International Working Group [70].

TIL percentage will be assessed in collaboration with Drs. [redacted] who [redacted] the DFCI Tissue Biomarker Laboratory a member of the NCI Cancer [redacted]onitoring and Analysis Centers (CIMACs) - Cancer Immunologic Data Commons (CIDC) Network. Hematoxylin and eosin (H&E) stains are routinely performed for quality control for IHC staining. H&E staining will allow the assessments for: Viable Tissue (% nucleated cells), Viable Tumor cells (% nucleated cells), Lymphocyte Influx (0- to 3+), Necrosis (% area), Fibrosis (% nucleated cells). TIL percentage can also be calculated as detailed below:

Protocol for assessing tumor-infiltrating lymphocytes (TILs) in breast cancer

To assess tumor-infiltrating lymphocytes (TILs) in breast cancer, we will follow the protocol published by Salgado et al, 2015 [70]. More specifically, to report the percentage of TILs for the stromal compartment we will follow these steps:

Step 1. We will define the area for TILs evaluation

The TILs will be evaluated within the borders of the invasive tumor including the invasive edge and a single value reported. TILs outside of the tumor borders, tumor zones with crush artifacts, necrosis and hyalinization will be excluded.

Step 2. We will scan tumor at low magnification, focusing on the tumor stroma and stromal TILs.

We plan to analyze only one section per patient, with the parameters of the section being 4-5µM, and we will use a x200-400 magnification to assess the average TILs in the stromal area by the pathologist who will report his score in 10% increments (0-100%) in as much detail as possible. For assessment of percentage values, the dissociated growth pattern of lymphocytes will be taken into account.

Step 3. We will determine the type of inflammatory infiltrate

We are only going to score only mononuclear cells (lymphocytes and plasma cells). This analysis will not include granulocytic infiltrates in necrotic areas.

Step 4. We will include the tumor in one of three groups based on low magnification and we will assess % stromal TILs.

Group A: tumor with no/minimal immune cells (0-10% stromal TILs).

Group B: tumor with intermediate/heterogeneous infiltrate (10-40% stromal TILs).

Group C: Tumor with high immune infiltrate (40-90% stromal TILs).

Step 5. We will report the percentage of stromal lymphocytes

We will report the average of the stromal area and will not focus on hotspots.

For intermediate groups, we will evaluate different areas at higher magnification, taking into consideration that lymphocytes do not form aggregates, therefore even with 90-100% stroma TILs there will still be some space between the individual lymphocytes.

Of note, measurement of TIL percentage will be performed by two pathologists in 10% increments. The SOP has been reviewed by the CTEP Biomarker Review Committee and harmonized with investigators at Dana Farber Cancer Institute (Rodig) and at Yale University (Schalper).

To evaluate inter-laboratory reproducibility of TIL assessment in the H&E biopsy specimens, we will perform the following analyses:

The precision of each biomarker in each lab will be measured in terms of standard deviation. The repeatability and between-lab reproducibility for each biomarker will also be assessed using the following linear mixed model,

$y = \mu + B + e$

μ : general expectation (population mean)

B: laboratory component of variation

e: random error

Expectation and variance of B are assumed to be 0 and $V(B)$ respectively. Expectation of e is assumed to be 0 and its variance, $V(e)$, is assumed to be equal in all laboratories. The standard deviation of repeatability, $\sqrt{V(e)}$, represents precision under the hypothetical condition where independent measures are obtained with the same method on the identical tests in the same laboratory by the same technician using the same equipment within short intervals of time. While the reproducibility standard deviation, $\sqrt{V(B)+V(e)}$, measures the precision under conditions where measures are obtained with the same method on identical tests in different laboratories with different technicians using different equipment.

The overall agreement of TIL percentage between two cores will also be assessed using intraclass correlation of coefficients.

TIL percentage will also be measured by multiplexed quantitative fluorescence (QIF) (see **Section 9.2.1**). There is a concern that measurement of TIL percentage by H&E is only semiquantitative and subject to interobserver variation. Measurement of TIL percentage by two protocols, and in collaboration with two groups will address this issue.

Investigator's Experience and Competence with the Assay: TIL percentage will be assessed in collaboration with Drs. [redacted] at Dana Farber Cancer Institute, and Dr. [redacted] at Yale University Cancer Center.

Drs. [redacted] and [redacted] are experienced clinical pathologists with experience evaluating breast specimens. The assay will be performed in a CLIA-certified laboratory.

Justification for Number of Patients and Specimens: Based on a two-sample t-test, the designed sample size (20 patients in Arm A and 40 patients in Arm B) provides 80% power at 1-sided alpha = 0.1 to detect a minimum of ~15% difference between the treatment arms. We expect this will be a clinically meaningful difference. Data from several phase III trials, have demonstrated that for every 10% increase in TIL percentage at baseline, there is a 15% to 20% increase in DFS

and OS in TNBC patients [44-46].

9.1.1.1 Collection of specimens

Four research core biopsies will be collected prior to treatment and after one cycle of therapy. At each time point, two cores will be placed in 10% buffered formalin and these will provide material for this assay. Please see **Section 5.5** for additional details on specimen collection.

9.1.1.2 Handling of specimens

The research core biopsies will be kept in 10% buffered formalin and shipped same-day to the Tissue Procurement Core. If they cannot be shipped on the day of collection, the research core biopsies may be kept in 10% buffered formalin overnight for fixation of tissue, followed by dehydration and paraffin infiltration using standard procedures. Each core is prepared into an individual paraffin block. Each block should be labeled with

- Study number
- Subject ID number
- Date of specimen acquisition

Blocks will be shipped in batch to the Tissue Procurement Core Facility at WUSM as detailed below.

9.1.1.3 Shipping of specimen(s)

Specimens should be shipped to the Washington University Tissue Procurement Facility at the address below:

425 S. Euclid Ave., Room 5120
St. Louis, MO 63110-1005
Phone: 314-454-7615
Email: tbank@wudosis.wustl.edu

All samples should be labeled with institutional study number, subject ID number, date of specimen acquisition, sample collection time and patient initials. They should be accompanied by a specimen submission form.

Specimens may be sent to the Tissue Procurement Facility on Monday through Thursday for next day deliver. **The Tissue Procurement Facility cannot receive specimens on Saturdays, Sundays, or holidays. Do not send specimens on Friday, Saturday, or the day before a holiday.**

Arrange for Federal Express pick-up through your usual institutional procedures. On the day that specimens are sent, please email the Tissue Procurement Facility at tbank@wustl.edu as described in the specimen shipping instructions, including the tracking number of the shipment and number of kits shipped. Ship blocks at room temperature to the Tissue Procurement Facility at Washington University and attach a copy of the Block Shipment Log.

9.2 Exploratory correlative studies

9.2.1 TIL Percentage by multiplexed quantitative fluorescence (QIF)

Rationale: TIL percentage is a well-described and validated histopathologic analysis that has been correlated with clinical response to adjuvant and neoadjuvant chemotherapy in patients with TNBC [44-46]. However, there is a concern that measurement of TIL percentage by H&E is only semiquantitative and subject to interobserver variation. We will also measure TIL percentage by multiplexed quantitative fluorescence (QIF).

Hypothesis: The combination of chemotherapy and atezolizumab will increase TIL percentage, and pathologic complete response rate compared to chemotherapy alone in patients with newly diagnosed TNBC being treated with neoadjuvant chemotherapy.

Assay methodology: TIL percentage by multiplexed quantitative fluorescence (QIF) will be evaluated in the DFCI Tissue Biomarker Laboratory led by Dr. [redacted] The DFCI Tissue Biomarker Laboratory will conduct multiplex immunofluorescence biomarker imaging for multi-label analysis of formalin- FFPE tissue biopsy samples. The PerkinElmer's multiplex biomarker imaging systems will be used to precisely measure and quantify multiple fluorescent molecular markers simultaneously, even when co-localized within a single tissue section. The data for each marker is spectrally unmixed, generating clear and accurate images for each of the individual labels in a multi-labeled tissue section with no crosstalk. Specifically, we will use the Opal chemistry and multispectral microscopy Vectra™ 3.0 and Polaris™ systems (Perkin-Elmer), which includes the Nuance software; analysis will be performed using the inForm software. The proposed panels for the trial samples include: CD3, CD8, CD20, CD4, FoxP3, PDL1. T-cell subpopulations are defined by expression of specific markers (CD3, CD4, CD8, FoxP3), B cells by CD20, and all immune populations are evaluated as cell density per tumor tissue (number of positive cells by mm square of tumor area. The staining procedure will be performed by specialized technicians under pathologist supervision, and the scanning and analysis is done by senior scientists and a pathologist trained in the use of Vectra and InForm. Finally, the data is consolidated using Inform Image Analysis Software (PerkinElmer) for the final report.

Investigator's Experience and Competence with the Assay: TIL percentage by multiplexed quantitative fluorescence (QIF) will be assessed in collaboration with Drs. [redacted] at Dana Farber Cancer Institute, a Cancer Immune Monitoring and Analysis Centers (CIMACS) laboratory that provides state-of-the-art analyses for genomic, phenotypic and functional characterization of responses of patients in early phase clinical trials using analytically-validated assays.

Justification for Number of Patients and Specimens: Based on a two-sample t-test, the designed sample size (20 patients in Arm A and 40 patients in Arm B) provides 80% power at 1-sided alpha = 0.1 to detect a minimum of ~15% difference between the treatment arms. Note that for TIL percentage and neoantigen-specific T cell response, targeting the differences between two treatment arms, the power justification was based on an alpha = 0.1 (see **Section 13.1** for the rationale for this alpha level). For the PD-L1 expression, immune signature, and neoantigen load, the power justification was based on the more conventional alpha = 0.05.

9.2.1.1 Collection of specimens

Four research core biopsies will be collected prior to treatment and after one cycle of therapy. At each time point, two cores will be placed in 10% buffered formalin and these will provide

material for this assay. Please see **Section 5.5** for additional details on specimen collection.

9.2.1.2 Handling of specimens

The research core biopsies will be kept in 10% buffered formalin and shipped same-day to the Tissue Procurement Core. If they cannot be shipped on the day of collection, the research core biopsies may be kept in 10% buffered formalin overnight for fixation of tissue, followed by dehydration and paraffin infiltration using standard procedures. Each core is prepared into an individual paraffin block. Each block should be labeled with

- Study number
- Subject ID number
- Date of specimen acquisition

Blocks will be shipped in batch to the Tissue Procurement Core Facility at WUSM as detailed below.

9.2.1.3 Shipping of specimen(s)

Please see **Section 9.1.1.3** for detailed instructions on shipping of samples to the Tissue Procurement Core Facility at WUSM.

9.2.2 PDL1 Expression

Rationale: PD-L1 expression is higher in TNBC than other breast cancer subtypes [47, 48]. We have examined the expression of PD-1 and PD-L1 in the common molecular subtypes of breast cancer using a well annotated human breast cancer tissue array [48, 49]. These studies demonstrate that expression of PD-1 on tumor-infiltrating lymphocytes and/or PD-L1 on breast cancers is more common in TNBC, and expression of these proteins is associated with a poor prognosis. Other investigators have also documented increased expression of PD-L1 in TNBC [47].

PD-L1 expression has also been demonstrated to be a biomarker for response to atezolizumab therapy [35]. Through serial on-treatment tumor biopsy analysis, a decrease in tumor burden was associated with increased PD-L1 expression at baseline, and an increase in PD-L1 expression on tumor-infiltrating immune cells following initiation of therapy [35]. Combined, these studies provide strong rationale for assessing PD-L1 levels at baseline and after one cycle of therapy.

Hypothesis: (1) PD-L1 expression on tumor-infiltrating immune cells at baseline predicts pathologic complete response. (2) Increased PD-L1 expression on tumor-infiltrating immune cells after one cycle of therapy predicts pathologic complete response.

Assay methodology: PD-L1 expression on tumor-infiltrating immune cells and on tumor cells will be performed at HistoGeneX, using the VENTANA PD-L1 (SP142) Assay that is FDA approved for PD-L1 expression.

Investigator's Experience and Competence with the Assay: HistoGeneX Laboratories is a CAP and CLIA accredited global laboratory system that serves pharma and biotech drug development sponsors and functions as a diagnostic laboratory for oncology offices worldwide. They have validated the VENTANA PD-L1 (SP142) immunohistochemistry assay for clinical use.

Justification for Number of Patients and Specimens: PD-L1 scoring will include cut-point analyses of ≥ 1% and ≥ 5%, as these thresholds have been used in previous studies. We will also test other thresholds using a ROC analysis as detailed below.

The predictive ability of PD-L1 expression on pathologic complete response (pCR) will be described as the area under ROC curves (AUC). The designed sample size of 60 patients (~30 pCr and ~30 non-pCR) provides 80% power at 1-sided alpha=0.05 to detect a minimum AUC of 0.68 (against AUC = 0.50, or no discriminating information). This was computed using the SAS macro developed by [redacted] and assuming the PD-L1 expression measured at an ordinal scale [http://www.bio.ri.ccf.org/html/rocanalysis.html].

9.2.2.1 Collection of specimens

Four research core biopsies will be collected prior to treatment and after one cycle of therapy. At each time point, two cores will be placed in 10% buffered formalin and these will provide material for this assay. Please see **Section 5.5** for additional details on specimen collection.

9.2.2.2 Handling of specimens

The research core biopsies will be kept in 10% buffered formalin and shipped same-day to the Tissue Procurement Core. If they cannot be shipped on the day of collection, the research core biopsies may be kept in 10% buffered formalin overnight for fixation of tissue, followed by dehydration and paraffin infiltration using standard procedures. Each core is prepared into an individual paraffin block. Each block should be labeled with

- Study number
- Subject ID number
- Date of specimen acquisition

Blocks will be shipped in batch to the Tissue Procurement Core Facility at WUSM as detailed below. The Tissue Procurement Core at WUSM will ship specimens to HistoGeneX following the directions in the HistoGeneX analysis plan.

9.2.2.3 Shipping of specimen(s)

Please see **Section 9.1.1.3** for shipping of samples.

9.2.3 Immune signature

Rationale: Tumor infiltrating lymphocytes (TILs) are more common in TNBC than in other breast cancer subtypes, and TILs are associated with improved outcome in TNBC following adjuvant, or neoadjuvant chemotherapy [44-46]. The association between TILs and improved outcome in TNBC suggests that the adaptive immune system contributes to the response to chemotherapy.

One of the underlying hypotheses of this clinical trial is that PD-1/PD-L1 signaling restrains immune responses in TNBC, and atezolizumab therapy will enhance immune responses in the context of neoadjuvant chemotherapy.

Denkert et al. recently defined a mRNA immune signature that correlated with pathologic

complete response in the GeparSixto breast cancer clinical trial. Twelve immunologically relevant mRNA markers were selected for detailed evaluation in breast cancer tissue, including T-cell markers, B-cell markers, chemokines, and immune checkpoint parameters [46].

Gene expression analysis of tumor biopsies during anti-PD-L1 treatment showed a dramatic increase in CD8 and Th1-related cytokines and activation markers that corresponded to reduced tumor burden [35].

In NSCLC, B- and NK-cell signatures were associated with clinical benefit [64].

In bladder cancer, baseline immune signature associated with an effector T cell signature (including *CD8A*, *GZMA*, *IFNG*) and/or NK gene signature (*NKG2* family members) were associated with clinical benefit [65].

Research core biopsies will be subjected to RNA extraction and analysis to assess expression of immune-related genes. The NanoString technology is a variation on the DNA microarray, and permits analysis of up to 800 transcripts in a single reaction [71]. This platform has been validated for application to both RNA from frozen tissue and RNA extracted from FFPE samples [72-75]. The nCounter®PanCancer Immune Profile Panel developed by NanoString, Inc. allows analysis of 770 genes for 24 different immune cell types and populations, 30 common cancer antigens and functional markers including checkpoint blockade genes (http://www.nanostring.com/).

Hypotheses: (1) Immune signature at baseline predicts pathologic complete response. (2) A change in the immune signature after one cycle of therapy predicts pathologic complete response.

Assay methodology: Several immune signatures will be assessed, including the immune signatures cited above, and immune signatures developed by Genentech using RNA-Seq from biopsy specimens collected in OCT blocks. RNA will be extracted from OCT blocks at the Tissue Procurement Facility using the RNeasy® kit (Qiagen). RNA will be analyzed at the DFCI CIMAC Translational Immunogenomics Laboratory led by Dr. [redacted] in collaboration with the Broad Institute using Tru-Seq Strand Specific Large Insert RNA Sequencing. The Tru-Seq platform includes plating, poly-A selection and strand specific cDNA synthesis, library preparation (450-550bp insert size), and sequencing (101bp paired reads); sequence coverage is up to 50 million paired reads or 50 million total reads.

Whole transcriptome analyses for sn-RNA-seq will also be performed using the 10x Genomics platform as previously reported by Drs. Ding.and Gillanders at the Washington University Human Tumor Atlas Research Center for HTAN (WU-HTAN)[76].

Alternatively, if specimens from OCT blocks are limiting, the Nanostring PanCancer Immune panel may be completed from 100ng of total RNA isolated from frozen tissue or FFPE tissue. RNA will be analyzed using the nCounter analysis platform per the manufacturer's instructions at the DFCI Tissue Biomarker Laboratory led by Dr. [redacted] DFCI technicians will follow NanoString™ nCounter® protocols in accordance with the manufacturer's instructions. The data will be analyzed using Nanostring nSolver Analysis Software for the final report.

Investigator's Experience and Competence with the Assay: DFCI CIMAC uses the PanCancer Immune Profiling Panel, an nCounter panel from NanoString™ optimized to develop profiles of the human immune response in all cancer types. This 770-gene panel includes markers for 24 different immune cell types and populations, 30 common cancer antigens, key checkpoint

blockade genes, and genes representing all categories of immune responses.

The WU HTAN Co-PIs Gillanders and Ding have established sn-RNA-Seq and CODEX assays optimized for core biopsy sized specimens that have been used to evaluate immune signatures in pancreas and breast cancer tissues. The Ding lab has extensive experience generating high quality snRNA-seq data across several tissue types using 10x Genomics platform. In total we have generated data for over 500 samples with over 70 specifically relevant for breast cancer. Instrumentation, methods, and bioinformatic tools are in-place for all data to be generated. The Ding lab also performed successful snRNA-seq experiments on core biopsies of breast cancer and pancreatic cancer retrieving from 5 to 10 thousand cells with ~2,000-4,000 median genes per cell indicative of high data quality. CODEX (CO-Detection by indexing) is a relatively new multiplex imaging system that utilizes antibodies conjugated to oligonucleotide barcodes, which are then visualized via fluorescently labeled reporters [77, 78]]. Unlike traditional multiplex IF, all antibodies can be stained simultaneously, which minimizes antigen decay. The Ding lab has developed a panel of DNA-barcoded antibodies for common cell type markers, proliferation markers, breast cancer subtype markers, ductal carcinoma in-situ markers and immune cell state markers.

Justification for Number of Patients and Specimens: The predictive ability of immune signature on pathologic complete response (pCR) will also be described as the area under ROC curves (AUC). Sample size justification will be similar to that of PD-L1 expression.

9.2.3.1 Collection of specimens

Four research core biopsies will be collected prior to treatment and after one cycle of therapy. At each time point, two cores will be immediately frozen in separate OCT blocks and these will provide material for this assay. Please see **Section 5.5** for additional details on specimen collection.

9.2.3.2 Handling of specimens

The research core biopsies will be stored at -70ºC on site. Each block should be labeled with

- Study number
- Subject ID number
- Date of specimen acquisition

Blocks will be shipped in batch to the Tissue Procurement Core Facility at WUSM as detailed below.

9.2.3.3 Shipping of specimen(s)

Please see **Section 9.1.1.3** for shipping of samples.

9.2.4 Neoantigen load

Rationale: Neoantigen load has been correlated with response to checkpoint blockade therapy in preclinical models [66] and human clinical trials [36]. We have developed robust next-generation sequencing strategies for the identification of neoantigens [79].

Hypothesis: Neoantigen load predicts pathologic complete response.

Assay methodology: Neoantigen load will be measured as previously described [79].

The DFCI CIMAC Translational Immunogenomics Laboratory led by Dr. [redacted] in collaboration with the Broad Institute will conduct the WES, which is ideal for unbiased detection of somatic mutations and copy number changes in tumor samples. Analysis will be performed on genomic DNA, fresh frozen tissue, FFPE scrolls etc.as necessitated by specimen availability and yields. The DFCI CIMAC/Broad's process includes hybrid capture (Illumina Rapid Capture Enrichment – 37Mb target), sequencing (Illumina, 76bp paired reads), and a sample identification quality control check, as well as data storage (5 years). Our hybrid selection libraries for deep somatic exomes typically meet or exceed 85% of targets at 50X, and for standard exomes, meet or exceed 80% at 20x. The DFCI/Broad CIMAC has the ability to process up to 188 samples per week with no minimum batch size. Processing times vary and depend on current demand.

We have developed and optimized an epitope prediction algorithm for the identification and prioritization of neoantigens. This optimized epitope prediction algorithm is described below and in our previous publications [66, 79]. We have established this algorithm in collaboration with Dr. [redacted] an internationally-known expert in tumor immunology [80-88]. [redacted] and [redacted] were one of the first to use next generation sequencing technologies to identify neoantigens, demonstrating that these antigens are important tumor rejection antigens [80]. [redacted] and Gillanders have now optimized the epitope prediction algorithm and have demonstrated that cancer vaccines targeting neoantigens are associated with antitumor immunity [66].

The goal of the optimized epitope prediction algorithm is to identify neoantigens and quantify the neoantigen load. The algorithm uses a combination of binding algorithms and processing algorithms.

Mutations that are expressed in the breast cancer will be identified using the sequencing data analysis pipeline in collaboration with Li Ding, Ph. D., an expert in genomic analysis at Washington University. The predicted amino acid sequences corresponding to the expressed mutations will be pipelined through three class I MHC epitope-binding algorithms provided by the Immune Epitope Database and Analysis Resource (http://www.immuneepitope.org): (i) Stabilized Matrix Method (SMM) [89], (ii) Artificial Neural Network (ANN) [90], and (iii) NetMHCpan [91].

A prioritized list of binding epitopes (i.e. $IC_{50} < 500$ nM) will be generated after calculating the median binding affinity value for each mutant sequence (affinity value expressed as $1/IC_{50} \times 100$).

Investigator's Experience and Competence with the Assay: The DFCI CIMAC/Broad Institute employs state of the art sequencing platforms and is a work leader in genomic analysis. Drs. [redacted] and Gillanders have considerable expertise in identifying neoantigens using sequencing technologies [66, 79, 80]. Dr. Ding's team at Washington University has developed a collection of widely-used computational tools for genomics at McDonnell Genome Institute, including VarScan, SomaticSniper, SciClone, BreakDancer, BreakFusion, MSIsensor, Pindel-C, GenomeVIP, HotSpot3D, and MuSiC. Dr. Ding has also played significant roles in The Cancer Genome Atlas (TCGA), the International Cancer Genome Consortium (ICGC), and the Clinical

Proteomic Tumor Analysis Consortium (CPTAC), PDX (patient-derived xenografts) Development and Trial Centers Research Network (PDXNet).

Dr. Ding and Dr. Gillanders are co-PIs on the Human Tumor Atlas Research Center grant, an NCI Cancer Moonshot initiative. Dr. Ding will work across the Cancer Moonshot network with Dr. [redacted] at DFCI CIMAC to complete the interpretation of the genomic correlative data for this study.

Justification for Number of Patients and Specimens: The predictive ability of neoantigen load on pathologic complete response (pCR) will also be described as the area under ROC curves (AUC). Sample size justification will be similar to that of PD-L1 expression.

9.2.4.1 Collection of specimens

Peripheral blood specimens will be collected pretreatment, after cycles 1, and 3 of therapy, and at the time of surgery as detailed in Section 5.4.4. The risks of venipuncture are minimal. For each subject six 10mL green-top (sodium heparin) tubes (BD Vacutainer, Catalog # 366480) will be collected at each time point.

Four research core biopsies will be collected prior to treatment. Two cores will be immediately frozen in separate OCT blocks and these, along with the PBMCs from any timepoint will provide material for this assay. Two core biopsies will also be collected in 10% buffered formalin and will provide an alternate source of material if there is insufficient yield from OCT blocks. Please see **Section 5.5** for additional details on specimen collection.

9.2.4.2 Handling of specimens

The research core biopsies will be stored at -70ºC on site. Each block should be labeled with:

- Study number
- Subject ID number
- Date of specimen acquisition

Blocks will be shipped in batch to the Tissue Procurement Core Facility at WUSM as detailed below.

9.2.4.3 Shipping of specimen(s)

Please see **Section 9.1.1.3** for shipping of samples.

9.2.5 T cell receptor (TcR) sequencing repertoire analysis

Rationale: The T cell receptor (TCR) repertoire is an important component of the adaptive immune response and recognition of antigens. Successful activation of the anti-tumoral response is dependent on the binding of the TCR to the neoantigen-major histocompatibility complex; thus a more diverse TCR repertoire has a greater potential for recognizing neoantigens. Recent studies examining the TCR repertoire in the context of cancer immunotherapy suggest that the TCR clonal repertoire may be modulated by immunotherapy and correlated with patient response in breast cancer[92] and other cancers [93-95]. We will conduct an exploratory analysis to evaluate the clonal alteration and immune response by profiling the TCRs circulating in the

peripheral blood, and within the tumor, at baseline and following one cycle of therapy, in collaboration with the CIMAC DFCI Translational Immunogenomics Laboratory.

Hypothesis: We hypothesize that the TCR repertoire in patients will be altered after one cycle of therapy.

Assay methodogy: The DFCI CIMAC Translational Immunogenomics Laboratory led by Drs. [redacted] and [redacted] offers a method for amplification of TCR CDR3 segments from bulk RNA derived from PBMC and fresh frozen specimens. This RNA-based method amplifies TCR alpha and beta CDR3 segments, adding sample-specific barcodes, and preparing libraries suitable for loading on Illumina sequencers to detect all productive T-cell receptor alpha variable region (TRAV) and T-cell receptor beta variable region (TRBV) T-cell subfamilies. In addition, the NCI and CIMACs (including DFCI CIMAC) are also considering TCR sequencing from bulk DNA derived from PBMC and FFPE specimens. This DNA-based method utilizes validated kits from Adaptive Technologies (Seattle, WA, ImmunoSeq Survey or Deep Sequencing) to identify human TCR beta clonotypes.

For CTEP 10013, the biobank will perform dual RNA/DNA extraction from blood/tissue and send nucleic acid material to the DFCI CIMAC. The DFCI CIMAC will evaluate specimen quality/quantity and decide upon the most suitable assay (RNA-based TCRseq vs. DNA-based TCRseq) to be analyzed on-site at the DFCI CIMAC in consultation with the trial team, NCI, and the CIMAC-CIDC network.

9.2.5.1 Collection of specimens

Peripheral blood specimens will be collected pretreatment, after one cycles 1, and 3 of therapy, and at the time of surgery as detailed in **Section 5.4.4**. The risks of venipuncture are minimal. For each subject six 10mL green-top (sodium heparin) tubes (BD Vacutainer, Catalog # 366480) will be collected at each time point.

Four research core biopsies will be collected prior to treatment. Two cores will be immediately frozen in separate OCT blocks and these, along with PBMCs from baseline and post-cycle 1 will provide material for this assay. Please see **Section 5.5** for additional details on specimen collection.

9.2.5.2 Handling of specimens

The research core biopsies will be stored at -70°C on site. Each block should be labeled with:

- Study number
- Subject ID number
- Date of specimen acquisition

9.2.5.3 Shipping of specimen(s)

Please see **Section 9.1.1.3** for shipping of samples.

9.2.6 Neoantigen-specific T cell response

Rationale: Once we have identified and prioritized neoantigens (**Section 9.2.3**), we will generate MHC class I tetramers by UV-induced epitope exchange, and perform multiparameter flow cytometry, or time-of-flight mass spectrometry (CyTOF) to determine the frequency and function of neoantigen-specific T cells in the peripheral blood of patients with TNBC, or if frequency and function of neoantigen-specific T cells changes during treatment. We developed a high throughput assay system in collaboration with Dr. [redacted] and [redacted] at The Netherlands Cancer Institute in Amsterdam to develop bar-coded MHC class I tetramers by UV-induced epitope exchange capable of recognizing multiple different neoantigen-specific T cells simultaneously.

Hypothesis: Atezolizumab therapy is associated with an increase in the frequency and function of neoantigen-specific T cells in the peripheral blood of patients.

Assay methodology: We will use peptide-MHC tetramers refolded with conditional ligands that can be exchanged for any desired peptide upon exposure to ultraviolet light [96]. Briefly, in order to generate a desired MHC class I allele, recombinant human MHC class I heavy chain and β2 microglobulin light chains are first produced in *Escherichia coli*, isolated as inclusion bodies, and refolded with photocleavable allele-specific conditional ligands as described [96]. Peptide-MHC monomers are then desalted, biotinylated and purified by gel filtration FPLC. For ultraviolet-induced ligand exchange and combinatorial encoding, peptide-MHC monomers are subjected to long-wave UV light for one hour in the presence of conventional peptides of desired sequence prior to tetramerization by the addition of titrated amounts of streptavidin-fluorochrome conjugates [97]. Peripheral blood mononuclear cells are then stained in a combinatorial fashion such that each tetramer is labeled with two separate fluorochromes, allowing for the staining of up to 28 tetramers in one individual sample in two-color codes by utilizing eight separate streptavidin-fluorochrome conjugates.

Peptide-MHC tetramers will be constructed by microscale UV-mediated peptide exchange for each neoantigen identified for which a suitable MHC class I allele is available and used to identify neoantigen-specific CD8 T cells. For these studies, we will make use of conventional flow cytometry as well as the CyTOF2 instrument in the Center for Human Immunology and Immunotherapy Programs (CHIIPs) at WUSM. This technique allows the monitoring of expression of up to 49 parameters on a single cell using isotopically tagged probes, which will allow the simultaneous measurement not only of the presence of tumor-specific CTL, but also unprecedented analysis of their function, such as expression of the surface molecules LAG-3 and TIM-3 that correlate with CD8 T-cell dysfunction, measurement of intracellular cytokine production, and assessment of surface molecules to establish whether neoantigen-specific CD8 T cells adopt a naïve or antigen-experienced phenotype.

Investigator's Experience and Competence with the Assay: Drs. [redacted] and Gillanders have experience studying the frequency and function of neoantigen-specific T cells in preclinical models [66].

Justification for Number of Patients and Specimens: Preliminary data from our phase I trial for mammaglobin-A DNA vaccine, though on patients with stable metastatic breast cancer, showed that the frequency of antigen-specific T cells can be measured with ~20% coefficient of variability (COV=SD/Mean). If assuming similar variability, the designed sample size (20 patients in Arm A and 40 patients in Arm B) provides 80% power at 1-sided alpha = 0.2 to detect a minimum of 10% difference between the two treatment arms.

9.2.6.1 Collection of specimens

Peripheral blood specimens will be collected pretreatment, after cycles 1, and 3 of therapy, and at the time of surgery as detailed in **Section 5.4.4**. The risks of venipuncture are minimal.

For each subject six 10mL green-top (sodium heparin) tubes (BD Vacutainer, Catalog # 366480) will be collected at each time point.

9.2.6.2 Handling of specimens

If collected Monday through Thursday, ship tubes on day of collection at room temperature.

If not shipped on day of collection, isolate and store PBMCs using the PBMC Isolation Procedure (see protocol below).

Label each sample (tube or cryovial) with:

- Study Number
- Subject ID Number
- Collection Date
- Collection Time
- PBMC volume in mL (for cryovials only)

PBMC Isolation Procedure

Collection

1. Collect blood in specified heparin tube(s).

Blood Separation

1. Fractionate the whole blood by centrifuging at 400 X g for 25 min at room temperature on Ficoll with the brake off. This will separate the blood into an upper plasma layer, a lower red blood cell (RBC) layer, and a thin interface containing the peripheral blood mononuclear cells (PBMC) / buffy coat. Fractionate the blood as soon as possible after collection.
2. Gently recover the buffy coat (PBMC) with a fresh disposable pipet, Pasteur pipet, or 1000 ul micropipettor with a sterile tip. Try not to uptake the RBC layer below the buffy coat or plasma on top of the buffy coat.
3. Place the recovered buffy coat into a 50mL centrifuge tube and fill up to 45mL using sterile Phosphate Buffered Saline (PBS), and centrifuge the PBMC at 400 X g.
4. Resuspend the PBMC in PBS and count the cells.
5. Proceed to section below, "Freezing collected cells."

Freezing PBMC

1. Wash the PBMC a second time. Resuspend the cell pellet at 10^7 cells/mL in freezing medium.
2. Label cryovials (1.8mL) and record the total number of PBMC and number of vials.
3. Transfer one mL cell suspension into each vial and leftover volume in the final vial.
4. Transfer the cryovials into a control-rate freezing container and immediately store the container at -80°C. Allow samples to freeze at -80°C for a minimum of 8 hours

before transferring.

5. Use the long forceps to take out the cryovial(s).

9.2.6.3 Shipping of specimen(s)

Please see **Section 9.1.1.3** for general shipping information. PBMC samples will either be shipped at room temperature or with OCT blocks on dry ice.

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 1 week prior to start of protocol therapy. Scans and x-rays must be done ≤ 4 weeks prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy. Patients receiving atezolizumab will be assessed for pulmonary signs and symptoms throughout the study. There is a +/- 1 day window for all assessments detailed below.

	Pre-Study	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12	Surgery[10]	Off Study[5]	Follow-up[8]
Atezolizumab[1]		A			A			A			A					
Paclitaxel		B	B	B	B	B	B	B	B	B	B	B	B			
Carboplatin		C			C			C			C					
Informed consent	X															
Demographics	X															
Medical history	X															
Concurrent meds	X	X----------X														
Physical exam	X	X			X			X			X				X	
Vital signs	X	X			X			X			X				X	
Height	X															
Weight	X	X		X		X		X		X		X			X	
Performance status	X	X			X			X			X				X	
CBC w/diff, plts	X	X	X	X	X	X	X	X	X	X	X	X	X		X	
Serum chemistry[2]	X	X			X			X			X				X	
Coagulation panel[7]	X															
EKG (as indicated)	X															
Randomization	X[3]															
Adverse event evaluation		AE assessment will take place with every physician visit (Q3W).													X	
Tumor measurements	X	Tumor measurements are repeated every 3-6 weeks. Documentation (radiologic) must be provided for patients removed from study for progressive disease.													X	
Radiologic evaluation	X	Radiologic measurements should be performed at baseline and prior to surgery.													X	
B-HCG	X[4]															
FFPE and fresh frozen tissue for TILs, PDL1 exp., immune sig., and neoantigen load[9]	X			X[6]										X		
PBMCs for T cell response[9]	X			X[6]						X				X		
Record review for response and survival																X

A: Atezolizumab: Dose as assigned; Q3W x 4 doses; to be administered first (if randomized to Arm B)
B: Paclitaxel: Dose as assigned; QW x 12 doses; to be administered last (for all groups)
C Carboplatin: Dose as assigned; Q3W x 4 doses; to be administered first (if randomized to Arm A) or second (if randomized to Arm B)
1. ONLY if randomized to Arm B.
2: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
3: Will take place after eligibility is confirmed.
4: Serum pregnancy test (women of childbearing potential).
5: Off-study evaluation for patients who come off treatment prior to surgery.
6: Day 18-22.

7: INR, aPTT
8: Follow up at 6 months and one year post-surgery will consist of routine monitoring and collection of data on response and survival.
9: Refer to Section 9.0 for further information on correlative studies.
10: 3-6 weeks after last dose of neoadjuvant chemotherapy.

11. MEASUREMENT OF EFFECT

11.1 Clinical response

Physical examination: Within 14 days of registration and every 3 to 6 weeks after the start of neoadjuvant treatment (that is, at the end of either every cycle or every even-numbered cycle) the longest axis and the perpendicular axis of the measurable lesion should be measured and recorded in metric notation by tape, ruler or caliper technique on the case report forms.

WHO criteria will be used to assess clinical response.

Complete Response (CR) is defined as the disappearance of all known disease based on a comparison between the pre-treatment measurements and the measurements taken at the completion of neoadjuvant therapy. In addition there is no appearance of new lesions.

Partial Response (PR) is defined as a 50% or greater decrease in the product of the bi-dimensional measurements of the lesion (total tumor size) between the pre-treatment measurements and the measurements taken at the completion of neoadjuvant therapy. In addition there can be no appearance of new lesions or progression of any lesion.

No Change (NC) occurs when a 50% decrease in total tumor size cannot be established nor has a 25% increase in the size of the lesion been demonstrated.

Progressive Disease (PD) is defined as a 25% or greater increase in the total tumor size of the lesion from its pretreatment measurements or the appearance of new lesions.

Treatment Resistance. A patient is said to have resistant disease if progressive disease is documented any time during neoadjuvant therapy.

11.2 Pathologic response

A **pathologic complete response (pCR)** is defined as no histology evidence of invasive tumor cells in the surgical breast specimen and sentinel or axillary lymph nodes.

All eligible women who have been treatment with combination therapy are included in the analysis of pCR. A patient is considered to not to have a pCR if any of the following are true:

- There is histologic evidence of invasive tumor cells in the surgical breast specimen or the axillary lymph nodes.
- The patient has discontinued neo-adjuvant treatment early due to refusal, toxicity, or radiographic or clinical evidence of progression and then goes straight to surgery where there is histologic evidence of invasive tumor cells in the surgical breast specimen and the axillary lymph nodes.
- The patient has discontinued neo-adjuvant treatment early due to refusal, toxicity or radiographic or clinical evidence of progression and then receives alternative treatment.
- The patient refuses surgery or is unable to undergo surgery due to a co-morbid condition.

11.3 Diagnosis of breast cancer recurrence and other cancer events

11.3.1 Local Recurrence

Local recurrence is defined as histologic evidence of ductal carcinoma in situ or invasive breast

cancer in the ipsilateral breast or chest wall.

11.3.2 Regional recurrence

Regional recurrence is defined as the cytologic or histologic evidence of disease in the ipsilateral internal mammary, ipsilateral supraclavicular, ipsilateral infraclavicular and/or ipsilateral axillary nodes or soft tissue of the ipsilateral axilla.

11.3.3 Distant recurrence

Distant recurrence is defined as the cytologic, histologic, and/or radiographic evidence of disease in the skin, subcutaneous tissue, lymph nodes (other than local or regional metastasis), lung, bone narrow, central nervous system or histologic and/or radiographic evidence of skeletal or liver metastasis.

11.3.4 Second primary breast cancer

Second primary breast cancer is defined histologic evidence of ductal carcinoma in situ or invasive breast cancer in the contralateral breast or chest wall.

11.3.5 Second primary cancer (non-breast)

Any non-breast second primary cancer other than squamous or basal cell carcinoma of the skin, melanoma in situ, or carcinoma in situ of the cervix is to be reported and should be confirmed histologically whenever possible.

11.3.6 Death

Underlying cause of death is to be reported.

12. STUDY OVERSIGHT AND 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 Study oversight

This protocol is monitored at several levels, as described in this section. The Protocol Principal Investigator is responsible for monitoring the conduct and progress of the clinical trial, including the ongoing review of accrual, patient-specific clinical and laboratory data, and routine and serious adverse events; reporting of expedited adverse events; and accumulation of reported adverse events from other trials testing the same drug(s). The Protocol Principal Investigator and statistician have access to the data at all times through the CTMS web-based reporting portal.

The Protocol Principal Investigator will have, at a minimum, quarterly conference calls with the Study Investigators to review accrual, progress, and pharmacovigilance.

All Study Investigators at participating sites who register/enroll patients on a given protocol are responsible for timely submission of data via Medidata Rave and timely reporting of adverse events for that particular study. This includes timely review of data collected on the electronic CRFs submitted via Medidata Rave.

All studies are also reviewed in accordance with the enrolling institution's data safety monitoring plan.

12.2 Data reporting

Data collection for this study will be done exclusively through Medidata Rave. Access to the trial in Rave is granted through the iMedidata application to all persons with the appropriate roles assigned in the Regulatory Support System (RSS). To access Rave via iMedidata, the site user must have an active CTEP IAM account (https://eapps-ctep.nci.nih.gov/iam) and the appropriate Rave role (Rave CRA, Read-Only, or Site Investigator) on either the Corresponding Organization or Participating Organization roster at the enrolling site.

Upon initial site registration approval for the study in RSS, all persons with Rave roles assigned on the appropriate roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site users must log into the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM user name and password, and click on the "accept" link in the upper right-corner of the iMedidata page. Please note, site users will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen.

Users that have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in RSS will also receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website, Rave tab under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website under the Rave tab or by contacting the CTSU Help Desk at 1-888-823-5923 or by e-

mail at ctsucontact@westat.com.

12.2.1 Method

This study will be monitored by the Clinical Trials Monitoring Service (CTMS). Data will be submitted to CTMS at least once every two weeks via Medidata Rave (or other modality if approved by CTEP). Information on CTMS reporting is available at: http://www.theradex.com/clinicalTechnologies/?National-Cancer-Institute-NCI-11. On-site audits will be conducted on an 18-36 month basis as part of routine cancer center site visits. More frequent audits may be conducted if warranted by accrual or due to concerns regarding data quality or timely submission. For CTMS monitored studies, after users have activated their accounts, please contact the Theradex Help Desk at (609) 799-7580 or by email at CTMSSupport@theradex.com for additional support with Rave and completion of CRFs.

12.2.2 Responsibility for data submission

For ETCTN trials, it is the responsibility of the PI(s) at the site to ensure that all investigators at the ETCTN Sites understand the procedures for data submission for each ETCTN protocol and that protocol specified data are submitted accurately and in a timely manner to the CTMS via the electronic data capture system, Medidata Rave.

Data are to be submitted via Medidata Rave to CTMS on a real-time basis, but no less than once every 2 weeks. The timeliness of data submissions and timeliness in resolving data queries will be tracked by CTMS. Metrics for timeliness will be followed and assessed on a quarterly basis. For the purpose of Institutional Performance Monitoring, data will be considered delinquent if it is greater than 4 weeks past due.

Data from Medidata Rave and CTEP-AERS is reviewed by the CTMS on an ongoing basis as data is received. Queries will be issued by CTMS directly within Rave. The queries will appear on the Task Summary Tab within Rave for the CRA at the ETCTN to resolve. Monthly web-based reports are posted for review by the Drug Monitors in the IDB, CTEP. Onsite audits will be conducted by the CTMS to ensure compliance with regulatory requirements, GCP, and NCI policies and procedures with the overarching goal of ensuring the integrity of data generated from NCI-sponsored clinical trials, as described in the ETCTN Program Guidelines, which may be found on the CTEP (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm) and CTSU websites.

An End of Study CRF is to be completed by the PI, and is to include a summary of study endpoints not otherwise captured in the database, such as (for phase 1 trials) the recommended phase 2 dose (RP2D), and a description of any dose-limiting toxicities (DLTs). CTMS will utilize a core set of eCRFs that are Cancer Data Standards Registry and Repository (caDSR) compliant (http://cbiit.nci.nih.gov/ncip/biomedical-informatics-resources/interoperability-and-semantics/metadata-and-models). Customized eCRFs will be included when appropriate to meet unique study requirements. The PI is encouraged to review the eCRFs, working closely with CTMS to ensure prospectively that all required items are appropriately captured in the eCRFs prior to study activation. CTMS will prepare the eCRFs with built-in edit checks to the extent possible to promote data integrity.

CDUS data submissions for ETCTN trials activated after March 1, 2014, will be carried out by the CTMS contractor, Theradex. CDUS submissions are performed by Theradex on a monthly basis. The trial's lead institution is responsible for timely submission to CTMS via Rave, as above.

Further information on data submission procedures can be found in the ETCTN Program Guidelines (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm).

12.3 CTEP multicenter guidelines

N/A

12.4 Collaborative agreements language

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator"

(http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.

2. For a clinical protocol where there is an investigational Agent used in combination with (an)other Agent(s), each the subject of different Collaborative Agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data"):

   a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NCI, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

   b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own Agent.

   c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement

will be made available to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm). Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to: Email: ncicteppubs@mail.nih.gov.

The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

12.5 Genomic data sharing plan

Data will be submitted no more than 6 months after the analytical dataset is finalized. Relevant phenotypic data, exposure data, and descriptive information will be shared, along with metadata necessary to reproduce published analyses. Specimen acquisition, experimental procedures, and data processing and analysis methods will be sent when data is submitted. Data will be available in dbGaP through both its unrestricted and controlled-access databases. Data will be made available for general research use.

This genomic data sharing plan will be IRB-approved as part of this project.

13. STATISTICAL CONSIDERATIONS

13.1 Study design/primary objective

TIL percentage and pathologic complete response (pCR) will be defined as the co-primary endpoints for this study. The primary objectives are to test the hypothesis that the combination of chemotherapy and atezolizumab will increase TIL percentage and pCR compared to chemotherapy alone in patients with newly diagnosed TNBC being treated with neoadjuvant chemotherapy. TIL percentage will be measured at baseline, and after initiation of therapy using a well validated histopathologic assay. The TIL percentage after initiation of therapy and pCR will be compared between patients receiving neoadjuvant chemotherapy alone (Arm A), and patients receiving neoadjuvant chemotherapy in combination with atezolizumab (Arm B).

Patients will be randomized in a 1:2 ratio between Arm A (20 patients), chemotherapy alone, and Arm B chemotherapy plus atezolizumab (40 patients). Enrollment will continue until 60 evaluable patients in both primary endpoints are enrolled or until 72 total patients are enrolled, whichever comes first. Up to 72 patients will be enrolled to account for up to 20% of patients being non-evalubalbe for the primary endpoint(s).

Data analysis will be performed following the modified intent-to-treat (ITT) principle, where patients who are randomized but are not evaluable for the primary endpoint will be excluded from the primary analysis. pCR-evaluable population includes all eligible women who have been randomized and received at least one dose of combination therapy, while TIL-evaluable population includes all eligible women who have evaluable TIL percentage after one cycle of therapy (day 18-22). The balance of demographic and baseline clinical characteristics between two arms will be compared using t-test, Mann-Whitney rank-sum test, or Fisher's exact as appropriate. The baseline and post-treatment TIL percentages between the two arms will be summarized using descriptive statistics at each time point.

13.1.1 Co-primary endpoint of TIL

In the primary analysis, a mixed model will be used to compare TIL percentages after initiation of therapy (day 18-22) while adjusting for the potential effect of baseline TILs.

$$Y_{ijk} = \beta_0 + \beta_1 * \mathrm{T}_j + \beta_2 * \mathrm{G}_k + \beta_3 * \mathrm{T}_j * \mathrm{G}_k + U_i + \delta_{ijk},$$

where Y_{ijk} is the TIL for ith subject measured at time j (j=1, 2 for baseline and 18-22 days post-treatment, respectively) in the treatment group k (k=1, 2 for Arm A and Arm B, respectively). The parameter β_0 denotes the overall intercept, while $\beta_1, \beta_2, and\ \beta_3$ are the fixed effects for timing (T), treatment (G) and their interaction, respectively. U_i and δ_{ijk}, represent the random intercept and measurement error respectively, both assuming to follow normal distributions and independent of each other, $U_i \sim N(0, \sigma_I^2)$ and $\delta_{ijk} \sim N(0, \sigma_E^2)$.

The mixed model can account for the potential correlation among TILs measured in the same individual and can borrow information across different time points. The mixed model could therefore achieve more statistical power than a two-sample t-test. Data transformation will be performed if necessary to better satisfy the assumption of normality distribution. Based on two-

sample t-test, n = 60 patients provides 80% power at 1-sided alpha = 0.1 to detect a minimum difference of 0.6*SD, where SD represents the measurement error of TIL. Based on an analysis of 314 TNBC, Denkert et al. found that the mean TIL percentage is 34.8% with a standard deviation of 24.7% (personal communication). If assuming similar variability in our study, the designed sample size allows 80% power to detect ~15% difference between arms. We expect this will be a clinically meaningful difference. Data from several phase III trials, have demonstrated that for every 10% increase in TIL percentage at baseline, there is a 15% to 20% increase in DFS and OS for TNBC patients [44-46]. Similar results have been obtained when evaluating the impact of TIL percentage after neoadjuvant chemotherapy [55-57]. Please note that we believe that an alpha = 0.10 is appropriate for these calculations, as in the context of a randomized phase 2 trial, the study is designed to ensure that an inferior treatment would have low probability of being selected, rather than to ensure that the best treatment is definitely selected. Therefore, a large false positive error (alpha) is acceptable [98, 99].

Although the primary analysis will be performed following the ITT principle, those patients with missing TIL measurements after initiation of therapy (day 18-22) will be excluded. Every effort will be made to minimize the number of missing TIL measurements (see **Section 9.1.1**), and we expect that very few patients will have missing TIL measurements. However, we will cap the proportion of patients with missing TIL measurements that can be replaced at 20%, with a corresponding cap on the total accrual. To prevent missing TIL measurements from affecting study integrity, we will prospectively track missing data.

Specifically, baseline demographic and clinical characteristics of randomized patients with and without missing TILs will be compared for evidence of selection bias. Missing data for each arm will be tabulated. If more than 5% of the randomized patients have missing TILs after initiation of therapy (day 18-22), a sensitivity analysis will also be performed using multiple imputation methods to fill in missing values and compare the difference between treatment arms.

13.1.2 Co-primary endpoint of pCR

The co-primary objective is to test the hypothesis that the combination of chemotherapy and atezolizumab will increase the pathologic complete response rate compared to chemotherapy alone in patients with newly diagnosed TNBC being treated with neoadjuvant chemotherapy. We acknowledge that the study is powered for a correlative endpoint.

Pathologic complete response rates will be summarized using contingency tables and compared using Chi-square test or Fisher's exact test between patients receiving neoadjuvant chemotherapy alone (Arm A), and patients receiving neoadjuvant chemotherapy in combination with atezolizumab (Arm B). Pathologic complete response is defined as the absence of residual invasive or noninvasive tumor cells in breast and lymph nodes (ypT0 ypN0), consistent with the recommendations of the Cortazar meta-analysis [100], and the FDA Guidance for Industry document.

The combination of carboplatin + docetaxel is actively being explored in the neoadjuvant setting for TNBC. Two early phase clinical trials have been reported [21-23]. The results of these studies have been very promising.

We assume that a pCR rate of 40% or below will not warrant further investigation (null hypothesis). A sample size of 60 patients (20 patients in Arm A, and 40 patients in Arm B) provides 80% power at 1-sided alpha = 0.10 to detect a minimum 29% improvement in

pathologic complete response rates (69% vs. 40%).

13.2 Sample size/accrual rate

The planned sample size is 60 evaluable patients (20 in Arm A receiving neoadjuvant therapy alone and 40 in Arm B receiving neoadjuvant chemotherapy in combination with atezolizumab) or 72 total patients, whichever comes first. It is anticipated that accrual will be completed over the course of 24 months across all participating sites.

Patients will be considered inevaluable for TIL if no invasive cancer is present in either of the two FFPE cores at day 22. Otherwise, patients who have received at least one study-mandated treatment and have one or more evaluable cores on day 22 will be considered evaluable. Please note if a patient is inevaluable for TIL, she will be replaced so that at least 60 TIL-evaluable patients are enrolled. These patients who are inevaluable for TIL will continue on protocol and will be evaluated for pCR as well as the secondary and exploratory endpoints. Although the sample size of 60 evaluable patients will be maintained, we will not modify the randomization process once it is implemented. The exact ratio at the end of the study may be slightly off the 20:40 target.

13.3 Planned enrollment report

Racial Categories	**Ethnic Categories**				**Total**
	Not Hispanic or Latino		**Hispanic or Latino**		
	Female	**Male**	**Female**	**Male**	
American Indian/ Alaska Native	0	0	0	0	**0**
Asian	2	0	0	0	**2**
Native Hawaiian or Other Pacific Islander	1	0	0	0	**1**
Black or African American	15	0	0	0	**15**
White	51	0	2	0	**53**
More Than One Race	1	0	0	0	**1**
Total	**70**	**0**	**2**	**0**	**72**

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13.4 Analysis of secondary endpoint

13.4.1 Safety

The incidence of toxicities by grade in each arm will be listed and 95% confidence intervals will also be calculated. The designed sample size allows us a reasonable precision to estimate the toxicity rate. Taking grade 4/5 toxicity as an example, if the "true" rate is 5% or less, there is <10% chance to observed 3 or more toxicities out of 20 patients. Conversely, if the true rate is 15% or higher, then there is >95% chance to observe at least 1 toxicities out of 20 patients.

13.4.2 Futility monitoring plan

An interim analysis will be performed after the first 30 patients are evaluated for the primary endpoints. The conditional power will be calculated for futility analysis based on the accumulated data. Conditional power is defined as the projected power to reject the null hypothesis at the end of the study given the data accrued up to the interim analysis. Therefore, a small value of conditional power indicates a highly likely, if not inevitable, conclusion of negative finding given the current data. If conditional power of 0.3 or less is obtained at the interim analysis for both TIL and pCR, enrollment will be suspended and the study investigators will consult with CTEP staff. A review of all available data will be performed, and a decision will be made with the input of CTEP staff as to whether to stop the trial for futility. The untransformed data will be provided to CTEP and assessed to ensure that the stated primary endpoint will be measurable. The raw TILs data will be submitted for review of both the baseline distribution of the data and the assay performance and for potentially planning a post hoc analysis.

13.4.3 Safety stopping rule

All grade 2 and 3 immunologic adverse events in addition to all grade 4 and 5 adverse events will be reviewed at each regularly scheduled study conference call. In the event of excessive adverse events thought to be possibly, probably, or definitely related to atezolizumab rather than standard of care chemotherapy (with excessive defined as grade 3 immunologic adverse events and any grade 4 or 5 events occurring in 3 of the first 20 patients), enrollment will be temporarily discontinued and all available data on adverse events will be assessed by a review committee in order to make a recommendation of continuation of accrual, suspension followed by formal amendment and reactivation, or study closure.

13.5 Analysis of exploratory endpoints

Exploratory objective #1 is to evaluate potential biomarkers of response (as measured by pCR) to chemotherapy in combination with atezolizumab in patients with newly diagnosed TNBC. Potential biomarkers include baseline TIL percentage, baseline PD-L1 expression in the tumor and tumor infiltrating immune cells, baseline immune signature, and baseline neoantigen load. Univariate and multivariate logistic regression models will be used to evaluate predictive ability of these baseline biomarkers on the response to carboplatin-based chemotherapy in combination with atezolizumab therapy. Receiver operating characteristic (ROC) curves will be used to explore the optimal cut-off points for each biomarker to differentiated patients who achieve pCR versus those who do not. In the analysis of PD-L1 expression, the performance of existing cut-off points (e.g., ≥ 1% and ≥ 5%) will also be assessed. To take full advantage of such a

randomized design, each model will also include the interaction term between treatment assignment and the baseline biomarker. This allows not only to answer the question whether the biomarker is predictive of pCR, but also whether the predictive ability is the same across two arms. We acknowledge that the study may not have adequate power to detect such interaction terms. The predictive ability will also be summarized using the area under ROC curves (AUC), and the 95% confidence intervals of AUC will be obtained using bootstrap resampling methods.

Exploratory objective #2 is to evaluate the impact of chemotherapy in combination with atezolizumab on the immune response in patients with newly diagnosed TNBC. The immune signature and PD-L1 expression in the tumor and tumor-infiltrating immune cells will be evaluated at baseline, after initiation of therapy (day 18-22), and at the time of surgery. The temporal changes of immune signature and PD-L1 expression in each arm will be summarized using contingency tables or descriptive statistics (means, standard deviations, medians, etc.) as appropriate. The differences of temporal change between treatment groups (Arm A versus Arm B) or their association with clinical outcome (pCR vs. non-pCR) will also be assessed musing mixed model (PD-L1 expression) or generalized linear mixed model (immune signatures).

Exploratory objective #3 is to evaluate the impact of chemotherapy in combination with atezolizumab on neoantigen-specific T cell responses in patients with newly diagnosed TNBC. The frequency and function of neoantigen-specific T cell across the two arms in the peripheral blood at each time point will be summarized using tools for high throughput analysis [98, 99]. The differences of temporal changes in neoantigen-specific T cell between treatment groups (Arm A versus Arm B) or their association with clinical outcome (pCR vs. non-pCR) will also be assessed using mixed models, followed by ad-hoc adjustment for multiple comparisons.

Exploratory objective #4 is to evaluate the impact of chemotherapy in combination with atezolizumab on long-term clinical endpoints such as overall survival (OS) and disease-free survival (DFS) in patients with newly diagnosed TNBC. OS is defined as time from randomization to death due to any causes and these patients alive will be censored at the last clinical contact. DFS is defined as time from randomization to disease recurrence or death due to any causes, and patients alive and free of disease will be censored at the last clinical contact. The distribution of OS and DFS in the two treatment arms will be described using Kaplan-Meier product limit methods and compared by log-rank tests. Proportional hazard Cox models will be used to evaluate predictive ability of baseline biomarkers (as listed in the Exploratory objective #1) on OS and DFS. To take full advantage of such a randomized design, each model will also include the interaction term between treatment assignment and the baseline biomarker. We acknowledge that the study may not have adequate power to detect such interaction terms. The predictive ability will also be summarized using concordance statistics (C-index) whose interpretation is analogous to the area under ROC curves (AUC), and the 95% confidence intervals of C-index will be obtained using bootstrap resampling methods.

13.6 Reporting and exclusions

13.6.1 Evaluation of toxicity

All patients will be evaluable for toxicity from the time of their first treatment with neoadjuvant chemotherapy. The incidences of toxicities across the two arms will be summarized using contingency tables and compared by 2-sample Chi-square test or Fisher's exact test.

13.6.2 Evaluation of response

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) no change, 4) progressive disease, 5) treatment resistance, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.] In addition, pathologic response will be determined as described in **Section 11.2**.

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

All conclusions should be based on all eligible patients. Subanalyses may then be performed on the basis of a subset of patients, 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 patients from the analysis should be clearly reported. The 95% confidence intervals should also be provided.

13.6.3 Evaluation of biomarkers

Biomarker evaluable population includes all eligible women who have evaluable biomarkers after one cycle of therapy (day 18-22). Four research core biopsies will be collected prior to treatment and after one cycle of therapy (day 18-22). At each time point, two cores will be placed in 10% buffered formalin and these will provide material for biomarkers. If no invasive cancer is present in either of the two FFPE cores at day 22, the sample will be considered inevaluable for biomarkers. Tumor tissues will also be obtained at the time of surgery, providing the third time point to measure TIL percentage. We expect that TIL percentage will be readily measured at baseline and at day 22, but evaluable TIL percentage may be difficult to measure at the time of surgery. We anticipate that the pCR may be 50%, or greater depending on the arm.

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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 PATIENT DRUG INFORMATION HANDOUT AND WALLET CARD

Information for Patients, Their Caregivers, and Non-Study Healthcare Team on Possible Interactions with Other Drugs and Herbal Supplements

The patient _____ is enrolled on a clinical trial using the experimental study drug, **atezolizumab** in combination with standard of care paclitaxel and carboplatin. This clinical trial is sponsored by the National Cancer Institute. This form is addressed to the patient, but includes important information for others who care for this patient.

These are the things that you as a healthcare provider need to know:

Immunostimulatory agents, including but not limited to IFN-α, IFN-γ, anti-TNF-α, or IL-2 are prohibited during the study and for 10 weeks after the last dose of atezolizumab due to potential for increased risk of autoimmune conditions. Also to be avoided are immunosuppressive medications, including but not limited to cyclophosphamide, azathioprine, methotrexate, and thalidomide, which could potentially alter the activity and the safety of atezolizumab. Cytochrome P450 enzymes as well as conjugation/glucuronidation reactions are not involved in the metabolism of atezolizumab. No drug interaction studies for atezolizumab have been conducted. There are no known interactions with other medicinal products or other forms of interactions. However, ingredients for such medicines have not been fully studied, and their use may result in unanticipated drug-drug interactions that may cause or confound assessment of toxicity.

Initiation or increased dose of granulocyte colony stimulating factors (e.g., granulocyte colony stimulating factor, granulocyte/macrophage colony stimulating factor, and/or pegfilgrastim) should be discussed with the study's principle investigator.

Caution should also be exercised when paclitaxel is concomitantly administered with known substrates (e.g., repaglinide and rosiglitazone), inhibitors (e.g., gemfibrozil), and inducers (e.g., rifampin) of CYP2C8.

Patients should also be advised to avoid traditional herbal or homeopathic or natural medicines

To the patient: Take this paper with you to your medical appointments and keep the attached information card in your wallet.

Atezolizumab may interact with other drugs which can cause side effects. For this reason, it is very important to tell your study doctors of any medicines you are taking before you enroll onto this clinical trial. It is also very important to tell your doctors if you stop taking any regular medicines, or if you start taking a new medicine while you take part in this study. When you talk about your current medications with your doctors, include medicine you buy without a prescription (over-the-counter remedy), or any herbal supplements such as St. John's Wort. It is helpful to bring your medication bottles or an updated medication list with you.
Many health care providers can write prescriptions. You must tell all of your health care

providers (doctors, physician assistants, nurse practitioners, pharmacists) you are taking part in a clinical trial.

These are the things that you (the patient) and your prescribers need to know:

Before you enroll onto the clinical trial, your study doctor will work with your regular health care providers to review any medicines and herbal supplements that might interact with the study drug.

- Please be very careful! Over-the-counter drugs (including herbal supplements) may contain ingredients that could interact with your study drug. Speak to your doctors or pharmacist to determine if there could be any side effects.
- Your regular health care provider should check a frequently updated medical reference or call your study doctor before prescribing any new medicine or discontinuing any medicine. Your study doctor's name is _____ and he or she can be contacted at _____.

STUDY DRUG INFORMATION WALLET CARD You are enrolled on a clinical trial using the experimental study drug Atezolizumab. This clinical trial is sponsored by the NCI. Atezolizumab may interact with other drugs that you are taking. Because of this, it is very important to: ➢Tell your doctors if you stop taking any medicines or if you start taking any new medicines. ➢Tell all of your health care providers (doctors, physician assistants, nurse practitioners, or pharmacists) that you are taking part in a clinical trial. ➢Check with your doctor or pharmacist whenever you need to use an over the counter medicine or herbal supplement.	➢Before you enroll onto the clinical trial, your study doctor will work with your regular health care providers to review any medicines and herbal supplements that may interact with atezolizumab. ➢Before prescribing new medicines, your regular health care providers should go to a frequently-updated medical reference for a list of drugs to avoid, or contact your study doctor. ➢Your study doctor's name is _____ and he/she can be contacted at _____