To: CTEP Protocol and Information Office

From: Alice Chen, MD, DTC, NCI

Date: November 10, 2022

Re: Amendment to NCI Protocol 10398: A Phase 2 Study of Anti-PD-L1 Antibody (Atezolizumab) in Chondrosarcoma and Clear Cell Sarcoma

This amendment is in response to [redacted] notice regarding atezolizumab drug information updates.

Thank you for your consideration.

I. Protocol Changes:

#	Section	Comments
1.	Cover Page Header	Updated the version number and date.
2.	Cover Page	Removed the list of AIs for consistency with the current CTEP template.
3.	Cover Page	Updated the pediatric referral contact.
4.	8.1	Updated the atezolizumab drug information as requested by [redacted] of CTEP PMB.
5.	8.2	Removed instructional language that was inadvertently retained from the protocol template.

NCI Protocol #: 10398

Local Protocol #: 000081

ClinicalTrials.gov Identifier: NCT04458922

TITLE: A Phase 2 Study of Anti-PD-L1 Antibody (Atezolizumab) in Chondrosarcoma and Clear Cell Sarcoma

Corresponding Organization: LAO-NCI / National Cancer Institute LAO
National Cancer Institute
10 Center Drive, 12N226
Bethesda, MD 20892

Principal Investigator: Alice P. Chen, MD, DCTD/NCI
31 Center Drive Suite 3A44
Bethesda, MD 20892
Phone: (240) 781-3320, Fax: [redacted]
chenali@mail.nih.gov

Translational PI: [redacted], PhD

Statistician: [redacted], PhD, BRB/DCTD/NCI
9609 Medical Center Drive
Rockville, MD 20850
Phone: [redacted]

Responsible Research Nurse: [redacted], RN, DCTD/NCI
Bldg 10/[redacted]
Bethesda, MD 20892
Phone: [redacted] *Fax:* [redacted]

Responsible Research Nurse Pediatric patients: [redacted], MSN, RN, OCN, POB/CCR/NCI
Bldg 10/[redacted]
Bethesda, MD 20892
Phone: [redacted] *Fax:* [redacted]

NCI-Supplied Agent: Atezolizumab (NSC 783608)

IND #: [redacted]
IND Sponsor: DCTD, NCI
Protocol Type / Version # / Version Date: Amendment / Version A05 / November 10, 2022

Participating Organizations

LAO-11030 / University Health Network Princess Margaret Cancer Center LAO
LAO-CA043 / City of Hope Comprehensive Cancer Center LAO
LAO-CT018 / Yale University Cancer Center LAO
LAO-MA036 / Dana-Farber - Harvard Cancer Center LAO
LAO-MD017 / JHU Sidney Kimmel Comprehensive Cancer Center LAO
LAO-MN026 / Mayo Clinic Cancer Center LAO
LAO-NC010 / Duke University - Duke Cancer Institute LAO
LAO-NJ066 / Rutgers University - Cancer Institute of New Jersey LAO
LAO-OH007 / Ohio State University Comprehensive Cancer Center LAO
LAO-PA015 / University of Pittsburgh Cancer Institute LAO
LAO-TX035 / University of Texas MD Anderson Cancer Center LAO
LAO-NCI / National Cancer Institute LAO

PRÉCIS

Background:

- Clear cell sarcoma (CCS) is a rare, aggressive, deep-seated tumor that accounts for <1% of all sarcomas. CCS presents predominantly in young adults, with an overall poor prognosis due to widespread dissemination.
- Chondrosarcoma (CS) is one of the most common malignant bone tumors in adults. Conventional CS tumors have a low metastatic potential but are typically refractory to chemotherapy and radiation therapy. High-grade or dedifferentiated CS tumors have a high metastatic potential and a poor prognosis following resection alone.
- Atezolizumab is a human monoclonal antibody directed against programmed death-ligand 1 (PD-L1) with potential immune checkpoint inhibitory and antineoplastic activities. Though studies in patients with CCS and CS are limited, there is clinical evidence that immune checkpoint pathways may play a role in CCS and CS progression.

Objectives:

- Determine the objective response rates (ORR) using RECIST v 1.1 of atezolizumab in adult (≥18 years) patients with CCS and CS
- Determine duration of response (DOR) using RECIST v 1.1 and/or change in clinical symptoms (time frame: baseline until disease progression, death, loss to follow-up, initiation of another anti-cancer treatment, withdrawal of consent, or study termination)
- Measure progression-free survival (PFS) time (time frame: baseline until disease progression, death, loss to follow-up, initiation of another anti-cancer treatment, withdrawal of consent, or study termination)
- Assess the number of activated CD8+ T cells infiltrating the tumor before and after atezolizumab treatment, and correlate treatment-induced changes with clinical response
- Compare RECIST v 1.1 vs immune RECIST (iRECIST) in patients with CCS and CS on atezolizumab
- Examine changes in PD-1/PD-L1 expression in the tumor microenvironment before and after atezolizumab treatment, and correlate treatment-induced changes with clinical response
- Evaluate potential associations between atezolizumab activity and tumor genomic alterations

Eligibility:

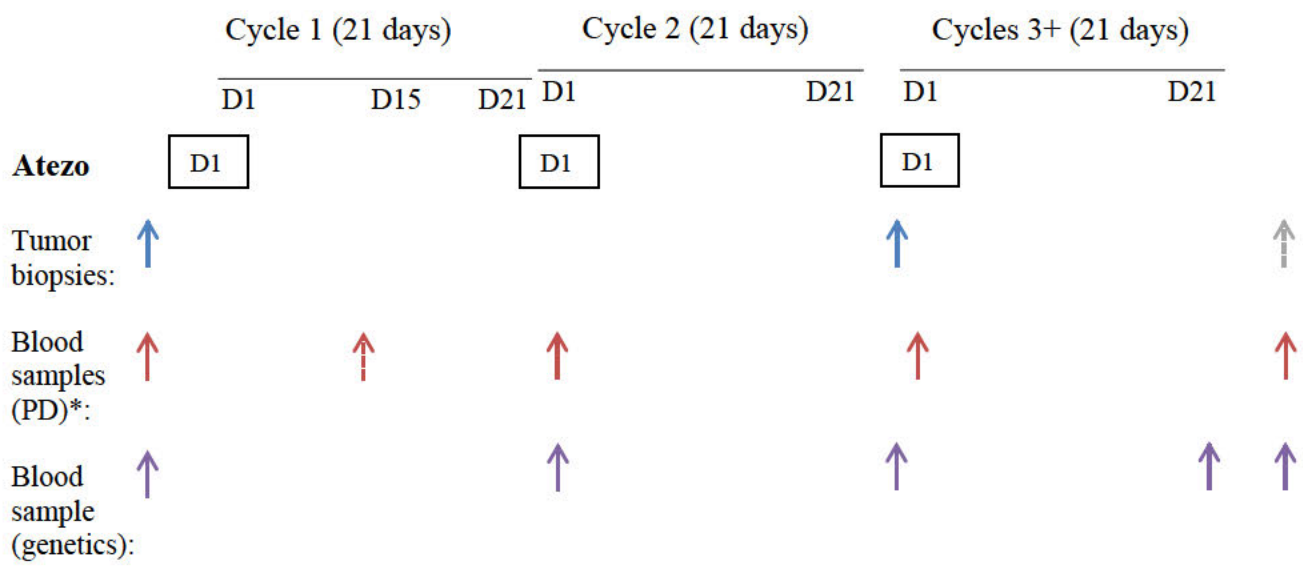
- Patients with documented *EWSR1/ATF1* or *EWSR1/CREB1* translocation or histologically confirmed CCS, documented grade 2 or 3 conventional CS, or documented dedifferentiated CS; disease must be measurable and must not be curable by surgery
- ≥2 years of age at the NCI Clinical Center (≥12 years at other participating sites)

Study Design:

- Patients will receive atezolizumab at a fixed dose of 1200 mg IV for adults and 15 mg/kg for pediatric patients once every 21 days, in 21-day cycles
- Tumor biopsies (mandatory at all sites) will be collected from adult patients (≥18 years of age) at baseline and prior to cycle 3 day 1 (±3 days; or sooner if there is clinical evidence that the patient is responding to drug) for pharmacodynamic assessment
- A cohort will be halted unless there is at least one response in the initial 9 patients accrued

- Within each histology, the trial will remain open to pediatric patients (up to a maximum of 8 patients across the three study histologies) for as long as the study is accruing adults of that histology

SCHEMA



Atezo = atezolizumab IV on Day 1 only of every 21 day-cycle (1200 mg in adults, 15 mg/kg [capped at 1200 mg] in pediatrics)

Research tumor biopsies (mandatory**) will be collected from adult patients (≥18 years of age) at baseline and prior to Cycle 3 Day 1 (±3 days; or sooner if there is clinical evidence that the patient is responding to drug) for the assessment of pharmacodynamic endpoints. One optional tumor biopsy may also be collected either on Day 1 (±2 days) of the cycle following any restaging at which a 10-19% increase in tumor volume is observed (according to RECIST v 1.1 criteria) if the patient has been on study for at least 4 cycles (the "restaging follow-up biopsy"), or at time of disease progression.

Collection of blood samples for genomic analysis is mandatory at all sites for patients ≥12 years of age at the following timepoints: baseline; C2D1, Cycle 3 Day 1 (±3 days; or sooner if there is clinical evidence that the patient is responding to drug); at the first two restaging visits (end of C3 and end of C5); every two restaging visits after that (e.g., C9, C13, etc.); and at progression.

*At NCI only, mandatory collection of blood for immunoPD from patients ≥12 years of age at baseline and before drug administration at the following timepoints: Cycle 2 Day 1, at the start of every subsequent cycle, at the time of the restaging follow-up biopsy (if applicable), and at time of disease progression. Collection on Cycle 1 Day 15 is optional.

Patients will undergo a CT scan at the end of Cycle 3 and every two cycles thereafter (every 3 cycles for patients on study for >1 year; every 4 cycles for patients on study for >2 years).

**Mandatory for patients in which biopsies are deemed safe and feasible. Patients that cannot be safely biopsied may be considered for the study upon discussion with Principal Investigator.

TABLE OF CONTENTS

1. OBJECTIVES

1.1 Primary Objectives

1.1.1 Determine the objective response rates (ORR) using RECIST v 1.1 of atezolizumab in adult (≥18 years) patients with CCS and CS

1.2 Secondary Objectives

1.2.1 Determine duration of response (DOR) using RECIST v 1.1 and/or change in clinical symptoms (time frame: baseline until disease progression, death, loss to follow-up, initiation of another anti-cancer treatment, withdrawal of consent, or study termination)

1.2.2 Measure progression-free survival (PFS) time (time frame: baseline until disease progression, death, loss to follow-up, initiation of another anti-cancer treatment, withdrawal of consent, or study termination)

1.2.3 Assess the number of activated CD8+ T cells infiltrating the tumor before and after atezolizumab treatment, and correlate treatment-induced changes with clinical response

1.3 Exploratory Objectives

1.3.1 Compare RECIST v1.1 vs immune RECIST (iRECIST) in patients with CCS and CS on atezolizumab

1.3.2 Examine changes in PD-1/PD-L1 expression in the tumor microenvironment before and after atezolizumab treatment, and correlate treatment-induced changes with clinical response

1.3.3 Evaluate potential associations between atezolizumab activity and tumor genomic alterations

2. BACKGROUND

2.1 Clear Cell Sarcoma

Clear cell sarcoma (CCS) is a rare, aggressive, deep-seated tumor that accounts for <1% of all sarcomas. The disease presents predominantly in young adults in the third decade of life as a slowly enlarging tender or painful mass in the extremities [1, 2]. The most common site of CCS is the deep soft tissues, juxtaposed to tendons, aponeuroses, or fascia of the lower extremities, particularly the area of the foot and ankle [1]. CCS is associated with local recurrence, regional lymph node metastases, and distant metastases [3]. It is usually a progressive disease with an overall poor prognosis due to widespread dissemination, with 5- and 10-year overall survival (OS) rates of 59% and 41%, respectively [4]. Molecularly, CCS is associated with a recurrent chromosomal translocation t (12;22), resulting in a fusion of the *EWSR1* gene on 22q with the

ATF1 gene on 12q and producing the chimeric protein EWS/ATF1; this translocation is present in 70-90% of cases of CCS [4]. EWSR1-CREB1 fusions have also been consistently described in CCS [5].

One of the largest CCS data sets comes from a retrospective review of 52 patients with CCS in France [4]. In this study, the majority of patients presented with localized disease; 44 patients had a local resection (5 patients received neoadjuvant chemotherapy), of which 25 had residual microscopic disease and underwent radiation treatment. Surgical treatments varied between local excision, wide local excision, radical compartment resection, and amputation. Two patients presented with metastatic disease to regional lymph nodes. Twenty-nine of the 52 patients developed a local recurrence; the time interval between initial treatment and local relapse ranged from 1 month to 20 years (median 38 months). Local recurrence was seen in 55% of the patients at 5 years and in 63% at 10 years (4). Nineteen patients developed regional lymph node metastasis (34% of them at 5 years and 41% of them at 10 years). Thirty patients developed distant metastasis (43% of them at 5 years and 62% at 10 years). The most common sites of distant metastasis were pulmonary (27 cases), bone (2 cases), and distant lymph nodes (1 case).

There are no current national guidelines for the treatment of CCS, as there is no effective treatment for this disease when metastatic. Complete surgical excision of the primary tumors, when possible, is generally accepted as the best treatment option in the non-metastatic setting. Overall, review of the literature regarding clinical experience with CCS demonstrates a lack of objective responses to standard chemotherapies and an overall poor prognosis with propensity for recurrences, early metastases, and poor overall survival.

While CCS typically presents in the 3rd-4th decade of life, cases occur in the pediatric age range and make up a significant percentage of patients with this disease. Gonzaga and colleagues reported patients as young as 9 years of age at diagnoses with 12% of cases presenting in patients less than 20 years of age [6]. The Italian and German Soft Tissue Sarcoma Cooperative Group reported a series of 28 pediatric patients with CCS ranging in age from 2 to 21 years [7]. In a recent analysis of the SEER database 172 cases of CCS were identified ranging in age at diagnosis from 9 to 91 years with 43 of 172 patients less than 25 years of age [8]. These reports demonstrate that CCS occurs in the pediatric age range and represents approximately 10% of cases of the disease. The prognosis for pediatric patients with CCS is not different from that adult patients and children would benefit from improved therapies for CCS thus justifying the inclusion of pediatric patients in this study.

2.2 Chondrosarcoma

Chondrosarcoma (CS) is one of the most common malignant bone tumors in adults [9]. Histologic grade, on a scale from 1-3, is considered the most important indicator of clinical behavior and prognosis [10]. Conventional CS, of which the majority of cases are low- to intermediate-grade, represents around 85% of CS overall [9, 11]. These tumors are slow growing with a low metastatic potential but are typically refractory to chemotherapy and radiation therapy. In contrast, high-grade or dedifferentiated CS (5-10% of CS overall) tumors have a high metastatic potential and a poor prognosis following resection alone [11]. In one of the largest studies of dedifferentiated CS, the 5-year survival rate was 13% [12]. Current

guidelines suggest that patients with dedifferentiated chondrosarcoma should be treated with osteosarcoma regimens (NCCN Guidelines for Bone Cancer V.1.2018).

Efforts to define the molecular characteristics of chondrosarcomas in general have noted recurrent activating mutations in isocitrate dehydrogenase (IDH) 1 and 2; the presence of these mutations was associated with shorter survival in some studies [13, 14]. More recently, multi-omics classification based on proliferation status, 14q32 locus microRNA expression, and *IDH*-related genome hypermethylation was found to be closely associated with prognosis, and pointed to a multi-step disease progression process [15]. However, a study of 89 central chondrosarcomas stratified by tumor grade found no association between *IDH* mutations and overall survival but, rather, a significant association between *IDH1/2* mutations and both relapse-free survival and metastasis-free survival in grade II and grade III chondrosarcomas[16]. Frequent point mutations in H3F3B have also been reported [17].

While significantly more common in older adults, CS is the third most common primary tumor of bone after osteosarcoma and Ewing sarcoma in pediatric patients representing approximately 5% of primary bone tumors in children. Evaluation of the SEER database of cases between 1973 and 2014 identified 247 cases of chondroma in patients ≤18 years of age, including several CS cases among patients 0-14 years of age [18]. Cases series from Finland and Norway showed an incidence of chondrosarcoma of 0.3-0.5 per million per year among pediatric patients [19, 20]. As in adult disease, there are no effective treatment options beyond a complete surgical resection for pediatric patients. In the setting of metastatic or recurrent disease, prognosis is dismal with minimal therapeutic options. Because of the occurrence of CS in pediatric patients along with the lack of effective therapeutic options for metastatic, progressive, or nonresectable disease we will include pediatric patients in this study.

2.3 Atezolizumab

Atezolizumab is a human monoclonal antibody directed against programmed death-ligand 1 (PD-L1) with potential immune checkpoint inhibitory and antineoplastic activities. Atezolizumab targets immune cells or tumor cells and prevents interaction with either programmed death-1 (PD-1) receptor or B7.1 (CD80), both of which function as inhibitory receptors expressed on T cells. Interference of the PD-L1:PD-1 and PDL1: B7.1 interactions may enhance the magnitude and quality of the tumor-specific T-cell response through increased T-cell priming, expansion, and/or effector function [21, 22]. In May 2016, atezolizumab (Tecentriq™) was approved for the treatment of patients with locally advanced or metastatic urothelial carcinoma whose disease had worsened during or following platinum-containing chemotherapy, or within 12 months of receiving platinum-containing chemotherapy. Subsequent approvals have been granted for atezolizumab (some as a monotherapy and some in combination with other agents) in non-small cell lung cancer, triple-negative breast cancer, and small cell lung cancer.

Atezolizumab was engineered to eliminate Fc-effector function via a single amino acid substitution at position 298 on the heavy chain, which results in a non-glycosylated antibody that has minimal binding to Fc receptors and, consequently, eliminates detectable Fc‑effector

function. By eliminating Fc-effector function and antibody dependent cell-mediated cytotoxicity (ADCC), antibody-mediated clearance of activated effector T cells is also eliminated.

2.3.1 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 [23-26]. PD-L1 binds to two known receptors, PD-1 and B7.1 (CD80). PD-l is expressed on activated T cells, and receptor expression is sustained in states of chronic stimulation such as chronic infection or cancer [27, 28]. Ligation of PD-L1 with PD-1 inhibits T-cell proliferation, cytokine production, and cytolytic activity, leading to the functional inactivation or inhibition of T cells (Figure 1). Similarly, while the receptor B7.1 has been best defined as a costimulatory molecule expressed by B lymphocytes and other antigen-presenting cells (APCs), B7.1 expressed on the surface of T cells may provide an additional signaling mechanism through which PD-L1 can negatively regulate T cell responses [21]. As a result, aberrant expression of PD-L1 on tumor cells and tumor-infiltrating immune cells, such as macrophages and dendritic cells, has been reported to impede anti-tumor immunity and contribute to immune evasion [29, 30].

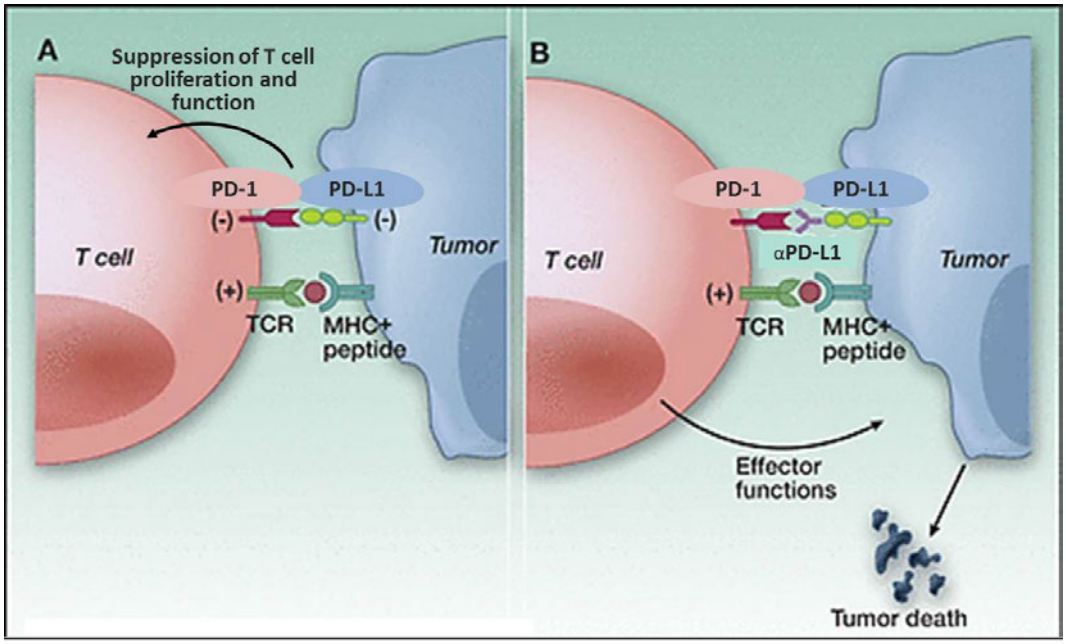


Figure 1: PD-1 and PD-L1 interaction and T cell responses. A) Binding of T-cell PD-1 by tumor PD-L1 results in the downregulation of T-cell proliferation and effector functions that destroy tumor tissue. B) Blockade of this pathway by an anti-PD-1 antibody prevents this downregulation, and allows T cells to maintain their antitumor response and ability to mediate tumor cell death (adapted from Sznol et al., 2013[31]).

Given the inhibitory effects of PD-L1 signaling on T cell proliferation and activity, targeted interruption of the PD-L1/PD-1 and PD-L1/B7.1 pathways represent an attractive strategy to reinvigorate tumor-specific T-cell immunity. The other known ligand of PD-1, programmed

death-ligand 2 (PD-L2), is primarily expressed in normal tissues such as the lung. As a result, targeting tumor-overexpressed PD-L1 is a more promising strategy than targeting PD-1, as it preserves the immune homeostatic PD-L2:PD-1 interaction in normal tissues while dually inhibiting the PD-L1:PD-1 and PD-L1:B7.1 pathways to enhance anti-tumor 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 reported from the two recent studies were consistent with the role of the PD-L1/PD-1 pathway in regulating peripheral tolerance.

2.3.2 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. Please refer to the Investigator's Brochure for details on the nonclinical studies.

2.3.3 Summary of Clinical Experience

Current clinical studies of atezolizumab include 101 company-sponsored studies with atezolizumab as a single agent or in combination with other therapies. Details of all ongoing studies can be found in the Atezolizumab Investigator's Brochure.

2.3.4 Clinical PK and Immunogenicity

2.3.4.1 Adult Patients

Atezolizumab pharmacokinetics and other data have been analyzed from a number of company-sponsored monotherapy studies (Investigator's Brochure, 2019). Exposure to atezolizumab increased dose proportionally over the dose range of 1 mg/kg to 20 mg/kg, including the fixed dose of 1200 mg administered every 3 weeks. On the basis of a population pharmacokinetic analysis that included 472 patients (Studies PCD4989g and JO28944) in the dose range of 1 mg/kg to 20 mg/kg, the typical population clearance (CL) was 0.20 L/day, the volume of

distribution at steady state (V_{ss}) was 6.9 L, and the terminal half-life ($t_{1/2}$) was 27 days. The population PK analysis suggested that steady state was obtained after 6 to 9 weeks (2 to 3 cycles) of repeated dosing. The systemic accumulation in area under the concentration-time curve (AUC), maximum concentration (C_{max}), and trough concentration (C_{min}) was 1.91, 1.46, and 2.75-fold, respectively, following intravenous administration of 1200 mg atezolizumab every 3 weeks. Atezolizumab pharmacokinetics after combination administrations with other anti-cancer agents were comparable to those following atezolizumab monotherapy administrations.

ADAs to atezolizumab have been observed at ≤ 1 mg/kg to 20 mg/kg and at 1200 mg (Investigator's Brochure, 2019). ADA status appears to have no clinically relevant impact on pharmacokinetics, safety, or efficacy.

2.3.4.2 Pediatric Patients

Clinically meaningful differences in monoclonal antibody distribution between adult and pediatric patients are not expected after taking into account differences in body size. Because infants and children are able to maintain homeostasis of immunoglobulins, it is presumed that there are no age-related developmental differences in disposition for therapeutic proteins when nonspecific elimination is the major pathway. For atezolizumab, the nonspecific antibody elimination pathway appears to be primary on the basis of the observation of linear pharmacokinetics. Furthermore, a review of the PK characteristics of 12 approved therapeutic proteins showed comparable steady-state peak and trough concentrations and terminal half-lives between pediatric and adult patients after adjustment for body size. Weight-based dosing of atezolizumab in children should therefore result in exposure similar to that in adults.

Preliminary pediatric PK data (n=48) are available from the GO29664 trial, an early-phase, multicenter, open-label, single-arm study in pediatric (<2 to 17 years of age) and young adult patients with relapsed or refractory solid tumors with known or expected PD-L1 pathway involvement. Exposure was compared between different pediatric age groups and shown to be similar to adults. Median Cmin values were 53.6 µg/mL (2 to <6 years), 54.1 µg/mL (6 to <12 years), and 62.0 µg/mL (12 to <18 years) compared with 68.0 µg/mL in adults. Cmin values in all age groups are above the concentration expected to produce maximum receptor occupancy (6 µg/mL).

15 mg/kg is the weight-based dose equivalent to the standard adult dose of 1200 mg, assuming an adult body weight of 80 kg. This dosing regimen is expected to produce exposure in children that is in a similar range to adult exposure. In the GO29664 trial, the infusion was tolerated by pediatric patients.

As of the clinical cutoff date for the 2019 Atezolizumab Investigator's Brochure, there were 87 safety-evaluable patients enrolled in Study GO29664 in 12 cohorts of patients with various tumor types including Ewing sarcoma, other soft tissue sarcomas, and osteosarcoma. Overall, atezolizumab was well-tolerated and its safety profile in pediatric patients was consistent with its known safety profile in adults. There were no new safety signals identified. The incidence of adverse events was similar across age groups and cohorts.

2.3.4.3 Clinical Safety Summary

As of May 17, 2019, atezolizumab has been administered (alone or in combination with other agents) to > 21,000 patients with solid tumors and hematologic malignancies (Investigator's Brochure, 2019). Safety findings of single-agent atezolizumab across multiple tumor types in the clinical development program are consistent with the known mechanism of action of atezolizumab and the underlying disease. Overall, treatment with atezolizumab is well tolerated with a manageable adverse event profile. Currently, no maximum tolerated dose (MTD), no dose-limiting toxicities (DLTs), and no clear dose-related trends in the incidence of adverse events (AEs) have been determined.

Among 3178 patients treated with single-agent atezolizumab for whom pooled safety data are available, the most commonly reported AEs (≥ 10%) include fatigue, decreased appetite, nausea, cough, dyspnea, constipation, pyrexia, diarrhea, anemia, back pain, vomiting, asthenia, arthralgia, pruritus, rash, headache, urinary tract infection, and peripheral edema (Investigator's Brochure, 2019). The safety profile was generally consistent between the two highly enrolled populations (urotherlial carcinoma and non-small cell lung cancer).

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, 2017). The median age was 61 years. The median duration of treatment for this cohort is currently 12.14 weeks (range: 0.0 – 71.4), and the median number of atezolizumab cycles administered is 5.0 (range: 1 – 19 cycles). 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, 2017). 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.

2.3.4.4 Immune-Related Adverse Events

Given the mechanism of action of atezolizumab, events associated with inflammation and/or immune-mediated AEs have been closely monitored during the atezolizumab clinical program (Investigator's Brochure, 2019). These include potential dermatologic, hepatic, endocrine, gastrointestinal, and respiratory events as well as events of hepatitis/elevated liver function tests (LFTs) and influenza-like illness. 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, meningoencephalitis, myocarditis, nephritis, and myositis.

Pericardial and pleural involvement with associated effusions is common in patients with cancer and has the theoretical potential to be exacerbated by inflammation associated with antitumor immunity following PD-L1 blockade. Dyspnea, cough, fatigue, hypoxia, pneumonitis, and pulmonary infiltrates have been associated with the administration of atezolizumab and have primarily been observed in patients with underlying NSCLC.

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

2.3.4.5 Clinical Efficacy Summary

Patients with multiple tumor types were included in study PCD4989g, with the largest cohorts consisting of patients with NSCLC, RCC, and UC (Investigator's Brochure, 2017). Objective responses with atezolizumab monotherapy 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. Objective responses were recorded for 20 of 88 (22.7%) response-evaluable patients with NSCLC, including responses in squamous and non-squamous patients. The median duration of survival follow-up at the time of the data cut-off (December 2, 2014) was 22.5 months. At that timepoint, the median duration of all responders was 17.3 months, with 40% (8 of 20) of responders having an ongoing response.

Analyses of tumor cells and tumor-infiltrating immune cells for PD-L1 expression on baseline tumor tissue have been performed for study PCD4989g, including expansion cohorts of patients with NSCLC (88 efficacy-evaluable patients), UC (87 efficacy-evaluable patients), and RCC (62 efficacy-evaluable patients) [22, 34] (Investigator's Brochure, 2017). Preliminary results suggest that PD-L1 expression in tumor-infiltrating immune cells is likely to be associated with response to atezolizumab. An objective response rate (ORR) of 43% (13 of 30 patients, including 2 complete responses) was observed in UC patients with tumor-infiltrating immune cells with PD-L1 immunohistochemistry (IHC) scores of 2 or 3, whereas the ORR among IHC 0/1 UC patients was 11% (4 of 35) [34]. While the median had not been reached as of January 1, 2014, the duration of response ranged from 0.1+ to 30.3+ weeks for patients with IHC 2/3 tumor-infiltrating immune populations and from 0.1+ to 6.0+ weeks for patients with IHC 0/1. Response to atezolizumab in the UC cohort was associated with the tumor-infiltrating immune cell IHC scores (P=0.026) but not with tumor cell IHC scores (P=0.93). Similarly, a preliminary analysis of the NSCLC cohort (53 evaluable patients) and the total patient population (175 evaluable patients) of PCD4989g found that response was significantly associated with PD-L1 IHC score in tumor-infiltrating immune cells (P=0.015 in NSCLC, P=0.007 in all tumors) but not with IHC score in tumor cells (P=0.920 in NSCLC, P=0.079 in all tumors) [22]. Eighty-three percent of NSCLC patients with an IHC score of 3 (tumor-infiltrating immune cell) responded to treatment, whereas 43% of NSCLC patients with IHC 2 were limited to disease stabilization.

Extensive efficacy data are now available for patients with non-small cell lung cancer (1636 atezolizumab-treated patients) and patients with metastatic urothelial carcinoma (983 atezolizumab-treated efficacy-evaluable patients; Investigator's Brochure, 2019). The results from studies evaluating atezolizumab as monotherapy in patients with locally advanced or metastatic NSCLC demonstrated clinically meaningful overall survival (OS) improvement in the

2L/3L NSCLC intent to treat population, in comparison with standard of care, in both non-squamous and squamous histologies, and across all PD-L1 expression subgroups. Other available efficacy data suggested that treatment with atezolizumab as a single agent or in combination with other therapeutic agents resulted in anti-tumor activity across a range of other tumor types and hematologic malignancies (including pediatric-type tumors), across lines of therapy, and across PD-L1 expression subgroups.

2.4 Rationale

Wide surgical resection remains the only curative treatment for localized CCS, leaving a paucity of therapeutic options for the treatment of this often recurrent and metastatic aggressive disease [35]. Scheinberg and colleagues reported a retrospective study of young adult patients with advanced sarcomas, including CCS, with data suggesting patients with CCS may benefit from treatment with anti-PD-1 antibody pembrolizumab [36]. Complete clinical response has been reported in a case of mediastinal CCS with pembrolizumab when the agent was combined with standard fractionation radiotherapy [37]. A phase 1 study of the checkpoint inhibitor ipilimumab (anti-CTLA-4) in pediatric patients reported stable disease in one patient with CCS (n=2) [38]. Finally, one patient with advanced CCS experienced a partial response with pembrolizumab (personal communication). These results suggest that similar checkpoint inhibitor agents such as the anti-PD-L1 antibody atezolizumab may be effective in CCS, providing a possible therapeutic option for a rare disease with limited treatment possibilities. To our knowledge, treatment of patients with CCS with atezolizumab has not been reported in the literature.

Likewise, there remains an urgent need for novel CS treatments, given the recurrence rates and the chemo- and radio-resistance of the disease [39]. With the growing success and FDA approvals of immune checkpoint inhibitors in other cancer types, several studies have examined the expression of PD-1 and its ligands in bone sarcomas, including CS [40]. Dedifferentiated CS may be the subtype of CS most amenable to checkpoint inhibitor treatment, as these tumors were shown to uniquely express PD-L1 and to have tumor-infiltrating immune cells [15, 41]. More recently, however, PD-L1 expression and TILs were also reported in conventional CS specimens and were associated with tumor aggressiveness [42, 43]. Clinical reports of immune checkpoint inhibitors in CS remain scarce, since most investigations have been conducted in osteosarcoma or across wide arrays of sarcoma subtypes. Wagner and colleagues have reported a case of a near complete response to the PD-1 inhibitor nivolumab in conventional CS, following a well-documented pseudoprogression response after 4 cycles of therapy [44]. Additionally, objective responses have been reported in two patients with dedifferentiated CS with immune checkpoint inhibitors [45, 46].

This trial is designed to provide eligible patients with a promising treatment approach while also providing insight into immunotherapy mechanisms of action and potential strategies for patient selection, because despite the success that has been achieved with checkpoint inhibitor therapy in a subset of patients with melanoma, NSCLC, and some other cancers, there is no cellular marker that consistently predicts which patients will respond. Our objective endpoint will be to determine the ORRs of atezolizumab in adult and pediatric/adolescent patients with CCS and CS.

Biopsies will be used to determine immune status of the tumor microenvironment (presence and

activation of tumor-infiltrating T cells) and genomic makeup of the tumor pre- and post-treatment; these will be correlated with the response to checkpoint inhibitor therapy. We will atezolizumab investigate whether TMB correlates with response to atezolizumab; while sarcomas driven by chromosomal translocations are often low-TMB, it is thought that mutational burden increases over time as the disease progresses [47, 48]. Blood samples will be used for the analysis of immune cell subsets and measurement of T-cell activation in the periphery, allowing us to follow changes in the T-cell population longitudinally and potentially correlate increases in T-cell activation with reduction in tumor burden.

2.5 Correlative Studies Background

Although checkpoint inhibitory agents have been shown to have clinical activity, there is as yet no predictive biomarker for this class of agent. Blocking PD-L1:PD-1 and PD-L1: B7.1 interactions with atezolizumab may release the immune checkpoint blockade, affecting the activity of tumor infiltrating lymphocytes (TILs), CD4+CD25+FOXP3+ regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), T-helper 2 (Th2) CD4+T cells, M2 macrophages, and N2 neutrophils, and allowing TILs to mount an immune response [49, 50]. From the pharmacodynamic perspective, a relationship between extent of mutational load and molecular response of the T-cell receptor in CD8+ TILs could be observed following PD-L1 therapy in cases that are baseline PD-L1+ tumor/PD-1+ TILs (i.e., in cases with a high likelihood that the PD-1 pathway is the immune checkpoint). The "negative control" is that this should not occur in tumors that utilize some other immune checkpoint, as evidenced by PD-L1- tumor or PD-1- TIL phenotyping.

One of the challenges of developing assays to measure the effects of immunotherapeutic agents is that the target is an immune cell rather than a tumor. It is therefore necessary to measure primary effects on immune cells and secondary effects on the cancer cells, as well as the interactions between these two populations (Figure 2). Experimental models with an intact immune system are required for this, thereby excluding immunodeficient xenograft models and tumor cell lines.

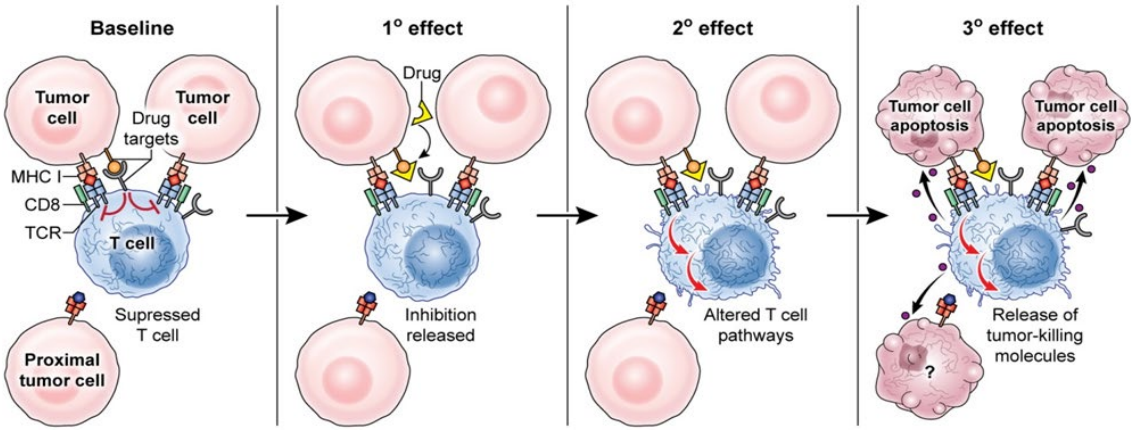


Figure 2. Immune checkpoint blockade. Assays to measure the PD effects of checkpoint blockade agents must encompass effects on the immune cell-tumor cell interface, the T cell, and adjoining tumor cells (adapted from Parchment et al. [51]).

Adult patients will undergo tumor biopsies for pharmacodynamic studies at baseline and prior to Cycle 3 Day 1 (±3 days; or sooner if there is clinical evidence that the patient is responding to drug) such as at confirmation of PR. Blood specimens will also be collected at these and other timepoints. We plan to use these research samples to evaluate the pharmacological effect of atezolizumab on immune cell components of the tumor, the tumor microenvironment, and peripheral T cell populations which will help to elucidate the mechanism of action of this agent in sarcoma and demonstrate the impact of a PD-L1 inhibitor on signaling pathways mediating the immune response. As there are differences in biological characteristics among sarcoma subtypes, pharmacodynamic comparisons will only be made within the CS and CCS sarcoma cohorts, rather than CCS and CS samples being pooled for analysis. Efforts will be made to correlate any observed treatment-induced changes with clinical response or resistance to therapy.

2.5.1 Pharmacodynamic Assessment of Drug Response

Using the multiplex quantitative immunofluorescence assay (immunoPD IFA) platform developed by PADIS (Figure 3 and Figure 4), we plan to quantify the number of activated CD8+ T cells infiltrating the tumor and characterize the activation status of the T cells around and within the tumor region. The tumor and T cell markers analyzed in the immunoPD IFA will include:

- PD-1 and PD-L1 expression
- T cell phenotypic markers CD8 and CD4
- Phosphorylation of the T-cell receptor complex (TCR-CD3 ζ) to demonstrate antigen recognition by T cells [52]
- Phosphorylated tyrosine phosphatases SHP1 (Y536) and SHP2 (Y542 and Y580) in T cells as markers of the status of PD1/PDL1 checkpoint signaling (primary PD) [53]
- Phosphorylated Zap70 kinase (Y493), an indication of further transduction of the T cell-activating TCR signal (secondary PD), that serves as a convergence point for TCR and PD1/PDL1 signaling [54]
- Tumor marker(s), such as β-catenin, to identify tumor region

	Slide no	Section thickness	IFA channel (emission wavelength)			
			340 nm	488 nm	546 nm	647 nm
	1	2 µm	H&E			
CD8/PD1 activation status panels	4	2 µm	DAPI	DNP-ZAP70-pY493	DIG-CD8	Biotin-CD3ζ-pY142
	5	2 µm	DAPI	DIG-PDL1	β-catenin-AF546	Biotin-IRF-1
	6	2 µm	DAPI	Biotin-SHP1pY536	DNP-CD8	DIG-PD1

Figure 3. PADIS is developing and validating these IF assays to report T cell activation, PD-1 and PD-1 levels, and relationship of TILs to invasive tumor margins. Test panels (such as the example shown here) include IFA multiplexes and an H&E slide for pathologist annotation and registration of adjacent slides. Additional biomarkers can be incorporated into panels as they come available. Adjacent slides may also be evaluated by HalioDx (France) using its Immunoscore® and Immunoseek® assays for independent CD8+ T-cell counts.

PADIS has demonstrated the use of this IFA in identifying and enumerating CD8+ T cells within tumor tissue that has been segmented using the tumor marker β-catenin. The number of activated CD8+ T cells present inside and outside of the tumor marker-positive area will be reported for biopsies collected at baseline and on Cycle 3 Day 1, as modulation of the tumor immune microenvironment is expected to require an extended response time frame. T-cell activation status will be determined through detection of pZAP70 and pCD3ζ phosphorylation. This endpoint will be correlated with patient response to immunotherapy. Figure 4 shows the clinical feasibility of the immunoassay demonstrated in a tissue microarray core specimen.

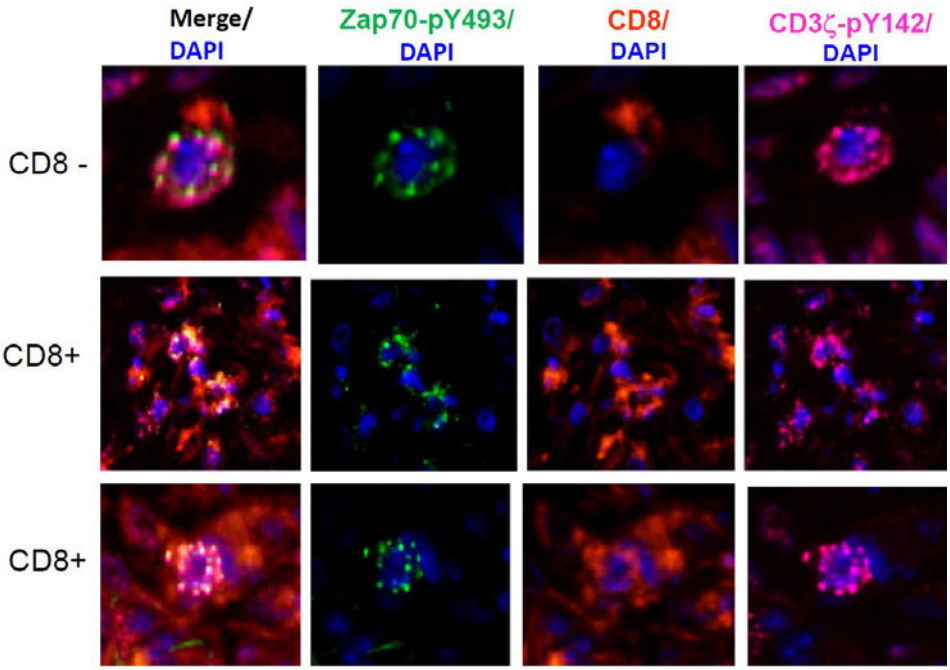


Figure 4. Performance of the immunoPD IFA on T cells captured in a colorectal cancer tumor microarray (collected with an ischemia time of 5 minutes or less), demonstrating our ability to identify activated T cells in tissue specimens collected from patients under Developmental Therapeutics Clinic SOPs.

While there is evidence to suggest that PD-L1 expression is associated with response to anti-PD-1/PD-L1 checkpoint inhibitors, there are not enough data available to definitively establish PD-L1 as a predictive biomarker for atezolizumab response in sarcoma. PADIS immunoPD IFA panels will be used in an exploratory fashion to measure baseline expression of PD-L1 in responders and non-responders, as well as the effects of atezolizumab on PD-L1 expression in tumor cells and PD-1 expression in T cells. Multiplex IFA panels measuring these markers in conjunction with CD3, CD8, and β-catenin are in development.

Intracellular staining and flow cytometry assays evaluating the immune signaling and activation in peripheral T cells will also be performed on blood samples collected at select sites. Peripheral T-cell subsets will be identified using phenotypic surface markers CD3, CD4, and CD8. Activation status will be determined based on expression of intracellular phosphomarkers (pZAP70, pSHP1/SHP2, and pCD3ζ). PADIS has demonstrated clinical validation of this flow cytometry assay using blood specimens collected at DTC clinic (a manuscript is in progress). These studies will allow us to follow changes in the T-cell population longitudinally and potentially correlate increases in T-cell activation with reduction in tumor burden. The data obtained from this continuous analysis may also inform the adjustment of biopsy timepoints to capture drug-induced immune changes in tumor tissue.

2.5.2 Immunopharmacodynamic Assay Validation Summary

As suitable immunocompetent animal models were not available for methods development and biomarker validation, an in vitro system was established to activate T cells from healthy donors recruited through the Research Donor Program at FNLCR. A manuscript describing this process is in preparation. Testing for activation biomarkers and immune signaling was performed using multicolor flow cytometry. After identifying biomarker/antibody pairs, control FFPE blocks were prepared using activated donor T cells and control tissue (liver); these provide an ongoing and consistent control set of known reactivity that can be used over the time course of months to years until exhausted. Each of the monoclonal antibody lots that will be used in this clinical trial were validated by time course of marker activation and dilution of the reporter antibody conjugate.

The multicolor flow cytometry data obtained by PADIS demonstrate the ability to evaluate T-cell activation in blood specimens; activation of known internal signaling pathways in CD4+ and CD8+ T cells is a multiday event in vitro, and there is a variable time to activation for each of the identified biomarkers of interest.

Antibodies to PD-1 and PD-L1 were also screened and analytically validated (Figure 5). PD-1 could be identified in control human thymus and PD-L1 was identified in human xenograft tissues and in tissue microarray. Both colorimetric IHC and immunofluorescence were used in evaluation of the FFPE slides.

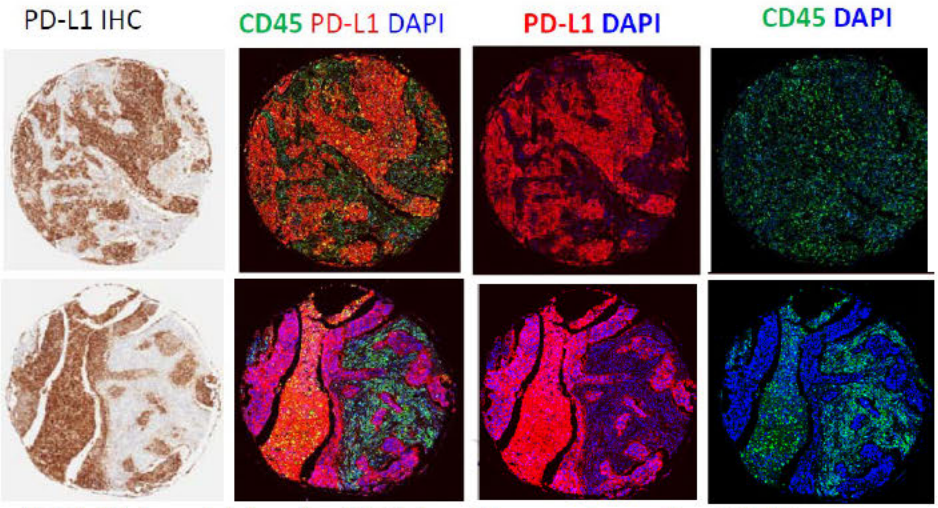


Figure 5. Multiplex staining for PD-L1 and leucocyte marker CD45 on a non-small cell lung cancer tumor microarray sample showing PD-L1-positive tumor cells and some infiltrating immune cells.

Analytical validation of assay performance used competition with free peptide to block antibody binding; Western blots demonstrating single band reactivity were performed for each marker.

To demonstrate the ability of the multiplex assays to identify co-labeled cells, analytical performance on FFPE-prepared stimulated T cells was performed—this is important in establishing a "bridge" between the multi-color flow cytometry assays used in methods development on the stimulated donor T cells and identification of the relevant biomarkers in the cells passed through the formaldehyde fixation and paraffin embedding process that would be encountered in processing patient biopsies for analysis T cell activation.

Finally, we established that quantitation of activated CD8+ T cells can be accomplished using the standard Definiens image analysis tools used by PADIS.

2.5.3 Genetic Analysis

Collecting tumor biopsy tissue on this trial will also make it possible to investigate the genomic differences between responders and non-responders through analysis of whole exome and RNA sequencing data. We will assess whether changes in the genomic makeup of the tumor are associated with treatment response and/or progression, and whether TMB in pre-treatment tissue correlates with patient outcome. This will be done retrospectively; if adequate tissue is available for a given patient, whole-exome sequencing will be performed on biopsies collected throughout the study. The data will not be available until personally identifying information is removed from the sample; sequencing data will not be used to prescreen patients.

Whole blood samples will be collected at screening to obtain plasma and mononuclear cells. All sequencing analysis from the plasma and mononuclear cells will be performed as retrospective research sequencing. The nucleic acid extracted from the patient's mononuclear cells will be

sequenced to distinguish germline from tumor-specific alterations and thus to call somatic mutations in the whole exome sequencing results from the tumor tissue specimens and, possibly, from the cell-free DNA.

Blood for circulating tumor DNA (ctDNA) analysis will be collected before treatment, throughout the trial, and at the time of disease progression. Once a patient has come off study, ctDNA from blood samples correlating to clinically informative time points will be analyzed by MoCha with the TSO500 targeted sequencing assay to quantitatively assess longitudinal changes in ctDNA levels (as a percentage of total circulating, cell-free DNA) and to identify any mutations that may be associated with atezolizumab sensitivity or resistance.

If possible, we will explore changes to mutational load over the therapy duration as there is some support for the hypothesis that mutations accumulate over time [55, 56]. These findings may be relevant for whether "better" responses are observed in later versus earlier line patients. In addition to evaluating changes in tumor mutations over time (post-treatment), extensive molecular characterization of pre-treatment tumor biopsy tissue from each patient enrolled on this trial will enable identification of valuable potential biomarkers to predict which patients are more or less likely to respond to treatment.

For patients who choose to undergo optional "restaging follow-up" or progression biopsies, the first 2 flash-frozen biopsy cores will be reserved for analysis by the CLIA-certified Oncomine version 3 (OCAv3) targeted sequencing assay to identify mutations in patient tumor tissue. Results from the OCAv3 assay **will be** returned to patients, in the form of an Oncomine report, for use in selecting future treatment options.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have documented *EWSR1/ATF1* or *EWSR1/CREB1* translocation or histologically confirmed clear cell sarcoma, documented grade 2 or 3 conventional chondrosarcoma, or documented dedifferentiated chondrosarcoma. The disease must not be curable by surgery.

3.1.2 Patients must have measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded for non-nodal lesions and short axis for nodal lesions) as ≥20 mm (≥2 cm) by chest x-ray or as ≥10 mm (≥1 cm) with CT scan, MRI, or calipers by clinical exam. See Section 11 (Measurement of Effect) for the evaluation of measurable disease.

3.1.3 Patients with newly diagnosed, unresectable, metastatic and measurable clear cell sarcoma, *EWSR1/ATF1* or *EWSR1/CREB1* translocation, grade 2 or 3 conventional chondrosarcoma, or dedifferentiated chondrosarcoma will also be eligible if they show clinical evidence of disease progression (including history and increasing physical symptoms). On-study documentation will include a physician's rationale that supports

evidence of clinical disease progression (i.e., increasing tumor pain).

3.1.4 Age ≥2 years at the NCI Clinical Center (≥12 years at other participating sites)

3.1.5 ECOG performance status ≤2 (Karnofsky or Lansky ≥70%, see Appendix A).

3.1.6 Life expectancy of greater than 3 months.

3.1.7 Patients must have adequate organ and marrow function as defined below:

– absolute neutrophil count	≥1,000/mcL
– platelets	≥100,000/mcL
– hemoglobin	≥8 g/dL
– total bilirubin	≤ 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 × institutional ULN (AST and/or ALT ≤5 × ULN for patients with liver involvement)
– alkaline phosphatase	≤2.5 × ULN (≤5 × ULN for patients with documented liver involvement or bone metastases)
– creatinine	For adult patients (≥18 years of age): ≥30 mL/min/1.73 m^2 by Cockroft-Gault

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

For pediatric patients (<18 years of age), a serum creatinine based on age and gender as follows:

Serum Creatinine for Age/Gender		
Age	**Maximum Serum Creatinine (mg/dL)**	
	Male	**Female**
2 to <6 years	0.8	0.8
6 to <10 years	1	1
10 to <13 years	1.2	1.2
13 to <16 years	1.5	1.4
16 to <18 years	1.7	1.4
The threshold creatinine values in this table were derived from the Schwartz formula for estimating GFR [57], utilizing child length and stature data published by the CDC.		

3.1.8 Patients with **treated brain metastases** are eligible if follow-up brain imaging after central nervous system (CNS)-directed therapy shows no evidence of progression for ≥1

month after treatment of the brain metastases.

3.1.9 Patients with **new or progressive brain metastases** (active brain metastases) or **leptomeningeal disease** are eligible if the treating physician determines that immediate CNS-specific treatment is not required and is unlikely to be required during the first 2 cycles of therapy.

3.1.10 Patients with a prior malignancy whose natural history or treatment does not have the potential to interfere with the safety or efficacy assessment of the investigational regimen are eligible for this trial.

3.1.11 Patients with known history or current symptoms of cardiac disease, or history of treatment with cardiotoxic agents, should have a clinical risk assessment of cardiac function using the New York Heart Association Functional Classification. To be eligible for this trial, patients should be class 2B or better.

3.1.12 Willingness to provide biopsy samples for research purposes (patients ≥18 years of age only).

3.1.13 Administration of atezolizumab may have an adverse effect on pregnancy and poses a risk to the human fetus, including embryo-lethality. Female patients of child-bearing potential and male patients 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 5 months (150 days) after the last dose of study agent. 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.

3.1.14 Ability to understand and the willingness to sign a written informed consent document or a parent/guardian able to do the same.

3.2 Exclusion Criteria

3.2.1 Any prior therapy must have been completed ≥4 weeks or, if known, ≥5 half-lives of the prior agent (whichever is shorter) prior to enrollment on protocol (minimum of 1 week between prior therapy and study enrollment), and the participant must have recovered to eligibility levels from prior toxicity. Patients should be at least 6 weeks out from nitrosoureas and mitomycin C. Prior definitive radiation should have been completed ≥4 weeks or palliative radiation should have been completed ≥2 weeks prior to study enrollment and all associated toxicities resolved to eligibility levels (patients on study may be eligible for palliative radiotherapy to non-targeted lesions after 2 cycles of therapy at the PI's discretion). Patients who have had prior monoclonal antibody therapy must have completed that therapy ≥6 weeks (or 3 half-lives of the antibody, whichever is shorter) prior to enrollment on protocol (minimum of 1 week between prior therapy and study enrollment). A patient who has received a cumulative dose of >350 mg/m^2 of anthracycline (regardless of cardioprotectant) may only be enrolled if their

ejection fraction measured by an echocardiogram is within normal institutional limits.

3.2.2 Prior treatment with anti-PD-1, or anti-PD-L1 therapeutic antibody or pathway-targeting agents.

- Patients who have received prior treatment with anti-CTLA-4 may be enrolled, provided the following requirements are met:
  - Minimum of 12 weeks from the first dose of anti-CTLA-4 and >6 weeks from the last dose
  - No history of severe immune-related adverse effects from anti-CTLA-4 (NCI CTCAE Grade 3 and 4)

3.2.3 Treatment with any other investigational agent within 4 weeks (or within five half-lives of the investigational product, whichever is shorter) prior to Cycle 1, Day 1 (minimum of 1 week between prior therapy and study enrollment). Patients must be ≥ 2 weeks since any investigational agent administered as part of a Phase 0 study (also referred to as an "early Phase I study" or "pre-Phase I study" where a sub-therapeutic dose of drug is administered) at the Coordinating Center PI's discretion, and should have recovered to eligibility levels from any toxicities.

3.2.4 Treatment with systemic immunostimulatory agents (including, but not limited to, interferon-α or interleukin-2 [aldesleukin]) within 6 weeks prior to Cycle 1, Day 1.

3.2.5 Treatment with systemic immunosuppressive medications (including, but not limited to, prednisone [>10 mg/day], 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 systemic mineralocorticoids (e.g., fludrocortisone) for patients with orthostatic hypotension or adrenocortical insufficiency is allowed.

3.2.6 Patients taking bisphosphonate therapy for symptomatic hypercalcemia. Use of bisphosphonate therapy for other reasons (e.g., bone metastasis or osteoporosis) is allowed.

3.2.7 Known hypersensitivity to Chinese hamster ovary cell products or other recombinant human antibodies.

3.2.8 History of severe allergic, anaphylactic, or other hypersensitivity reactions to chimeric or humanized antibodies (i.e., antibodies with generic names ending in "ximab" or "zumab", respectively) or fusion proteins

3.2.9 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac

arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.10 Pregnant women are excluded from this study because atezolizumab is an investigational agent with the unknown 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.

3.2.11 Patients with a history of HIV-positive on antiretroviral therapy are eligible with an undetectable viral load. For these patients, an HIV viral load test must be completed within 28 days prior to enrollment.

3.2.12 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. For these patients, HBsAg and anti-HBc tests must be done within 28 days prior to enrollment.
- Patients positive for hepatitis C virus (HCV) antibody are eligible only if polymerase chain reaction (PCR) is negative for HCV RNA. For these patients, an HCV RNA test must be done within 28 days prior to enrollment.

3.2.13 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 autoimmune hyperthyroid disease not requiring immunosuppressive treatment 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, or 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)

3.2.14 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.

3.2.15 Patients with active tuberculosis (TB) are excluded.

3.2.16 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.

3.2.17 Signs or symptoms of infection within 2 weeks prior to Cycle 1, Day 1.

3.2.18 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.

3.2.19 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.

3.2.20 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.

3.3 Screening Evaluation

Patient eligibility screening tests will be performed as described below. Eligibility history, physical examination, laboratory evaluations, urinalysis, and EKG are to be conducted within 8 days prior to enrollment. As noted in Section 5, if protocol therapy is started within 8 days of these eligibility screening evaluations, values from the screening evaluations may be used as baseline measurements; if >8 days have passed since the screening evaluations, the medical history, physical examination, laboratory evaluations, urinalysis, and EKG must be repeated prior to starting protocol therapy. ECHO must be done within 28 days prior to enrollment. ECHO and baseline imaging scans must be done within 28 days prior to the start of protocol therapy; eligibility screening results (ECHO and radiographs) can be used if done ≤28 days prior to start of protocol therapy and the site investigator determines that they are of acceptable quality.

3.3.1 Histologic confirmation: For patients enrolling on the basis of CCS diagnosis, in the absence of documented *EWSR1* translocation, histological confirmation of CCS will be required at the enrollment site prior to enrollment. Reports from outside institutions will

be accepted for documentation of *EWSR1/ATF1* or *EWSR1/CREB1* translocation, grade 2 or 3 conventional chondrosarcoma, or dedifferentiated chondrosarcoma.

3.3.2 History and Physical Examination - complete history and physical examination (including height, weight, vital signs, and performance score [ECOG, Karnofsky, or Lansky, see Appendix A]) will be conducted within 8 days prior to both enrollment and the start of protocol therapy.

3.3.3 Imaging Studies - every participant should have an evaluation of known sites of disease, conducted via a CT scan of the chest/abdomen/pelvis. This scan may be done at the enrolling institution; alternatively, images or reports from scans conducted at an outside institution may be used. Either way, the scan, image, or report used to determine eligibility must be *at least* as recent as the time of disease progression determination on the patient's last treatment. Scans for baseline tumor measurements must be done within 28 days prior to the start of protocol therapy; radiographs used to determine eligibility can be used for baseline measurements if the scans were conducted ≤28 days prior to start of protocol therapy and the site investigator determines that they are of acceptable quality. At the discretion of the PI and as clinically indicated, the following imaging studies may also be performed: MRI or CT scan with contrast of the brain, MRI liver, or MRI for other disease sites.

3.3.4 Laboratory Evaluation - laboratory data are to be obtained within 8 days prior to both enrollment and start of protocol therapy:

- Hematological Profile: CBC with differential, hemoglobin, platelets
- Biochemical Profile: ACTH, albumin, alkaline phosphatase (ALP), AM cortisol, bicarbonate, total bilirubin, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium, TSH, free T4, amylase, lipase
- Urinalysis
- Serum or urine pregnancy test for female participants of childbearing potential
- PT and PTT only for patients undergoing research biopsies

3.3.5 Cardiac Evaluation

- EKG - to be performed within 8 days prior to both enrollment and start of protocol therapy
- ECHO - to be performed within 28 days prior to both enrollment and start of protocol therapy

4. REGISTRATION PROCEDURES

4.1 Investigator and Research Associate Registration with CTEP

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account at https://ctepcore.nci.nih.gov/iam. In addition, persons with a registration type of Investigator

(IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) (*i.e.*, clinical site staff requiring write access to Oncology Patient Enrollment Network (OPEN), Rave, or acting as a primary site contact) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) at https://ctepcore.nci.nih.gov/rcr.

RCR utilizes five person registration types.

- IVR: MD, DO, or international equivalent,
- NPIVR: advanced practice providers (*e.g.*, NP or PA) or graduate level researchers (*e.g.*, PhD),
- AP: clinical site staff (*e.g.*, RN or CRA) with data entry access to CTSU applications (*e.g.*, Roster Update Management System [RUMS], OPEN, Rave,),
- Associate (A): other clinical site staff involved in the conduct of NCI-sponsored trials, and
- Associate Basic (AB): individuals (*e.g.*, pharmaceutical company employees) with limited access to NCI-supported systems.

RCR requires the following registration documents:

Documentation Required	IVR	NPIVR	AP	A	AB
FDA Form 1572	✓	✓			
Financial Disclosure Form	✓	✓	✓		
NCI Biosketch (education, training, employment, license, and certification)	✓	✓	✓		
GCP training	✓	✓	✓		
Agent Shipment Form (if applicable)	✓				
CV (optional)	✓	✓	✓		

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and Cancer Trials Support Unit (CTSU) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and Institutional Review Boards (IRBs) covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Addition to a site roster,
- Assign the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN,
- Act as the site-protocol Principal Investigator (PI) on the IRB approval, and
- Assign the Clinical Investigator (CI) role on the Delegation of Tasks Log (DTL).

In addition, all investigators act as the Site-Protocol PI, consenting/treating/drug shipment, or as the CI on the DTL must be rostered at the enrolling site with a participating organization (*i.e.*, Alliance).

Additional information is located on the CTEP website at

https://ctep.cancer.gov/investigatorResources/default.htm. For questions, please contact the **RCR Help Desk** by email at RCRHelpDesk@nih.gov.

4.2 Site Registration

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

IRB Approval

Sites participating with the NCI Central Institutional Review Board (NCI CIRB) must submit the Study Specific Worksheet for Local Context (SSW) to the CIRB using IRBManager to indicate their intent to open the study locally. The NCI CIRB's approval of the SSW is automatically communicated to the CTSU Regulatory Office, but sites are required to contact the CTSU Regulatory Office at CTSURegPref@ctsu.coccg.org to establish site preferences for applying NCI CIRB approvals across their Signatory Network. Site preferences can be set at the network or protocol level. Questions about establishing site preferences can be addressed to the CTSU Regulatory Office by emailing the email address above or calling 1-888-651-CTSU (2878).

Sites using their local IRB or REB must submit their approval to the CTSU Regulatory Office using the Regulatory Submission Portal located in the Regulatory section of the CTSU website. Acceptable documentation of local IRB/REB approval includes:

- Local IRB documentation,
- IRB-signed CTSU IRB Certification Form, and/or
- Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form.

In addition, the Site-Protocol PI (*i.e.*, the investigator on the IRB/REB approval) must meet the following five criteria to complete processing of the IRB/REB approval record:

- Holds an Active CTEP status,
- Rostered at the site on the IRB/REB approval (*applies to US and Canadian sites only*) and on at least one participating roster,
- If using NCI CIRB, rostered on the NCI CIRB Signatory record,
- Includes the IRB number of the IRB providing approval in the Form FDA 1572 in the RCR profile, and
- Holds the appropriate CTEP registration type for the protocol.

Additional Requirements

Additional requirements to obtain an approved site registration status include:

- An active Federalwide Assurance (FWA) number,
- An active roster affiliation with the Lead Protocol Organization (LPO) or a Participating Organization, and
- Compliance with all protocol-specific requirements (PSRs).

4.2.1 Downloading Regulatory Documents

Download the site registration forms from the protocol-specific page located on the CTSU members' website. Permission to view and download this protocol and its supporting documents is restricted based on person and site roster assignment. To participate, the institution and its associated investigators and staff must be associated with the LPO or a Participating Organization on the protocol.

- Log on to the CTSU members' website (https://www.ctsu.org) using your CTEP-IAM username and password,
- Click on *Protocols* in the upper left of your screen
    - Enter the protocol number in the search field at the top of the protocol tree, or
    - Click on the By Lead Organization folder to expand, then select *LAO-NCI*, and protocol number *10398*,
- Click on *Documents*, select *Site Registration*, and download and complete the forms provided. (Note: For sites under the CIRB initiative, IRB data will load automatically to the CTSU as described above.)

4.2.2 Protocol Specific Requirements For P10398 Site Registration

Upon site registration approval in RSS, the enrolling site may access OPEN to complete enrollments. The enrolling site will select their credentialed provider treating the subject in the OPEN credentialing screen, and may need to answer additional questions related to treatment in the eligibility checklist.

- A teleconference training led by the Coordinating Center is required for each participating site prior to the site's initial activation in RSS.

4.2.3 Submitting Regulatory Documents

Submit required forms and documents to the CTSU Regulatory Office via the Regulatory Submission Portal on the CTSU website.

To access the Regulatory Submission Portal, log on to the CTSU members' website → Regulatory → Regulatory Submission.

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

Delegation of Tasks Log (DTL)

Each site must complete a protocol-specific DTL using the DTL application in the Delegation Log section on the CTSU members' website. The Clinical Investigator (CI) is required to review and electronically sign the DTL prior to the site receiving an Approved site registration status

and enrolling patients to the study. To maintain an approved site registration status the CI must re-sign the DTL at least annually and when a new version of the DTL is released; and activate new task assignments requiring CI sign-off. Any individual at the enrolling site on a participating roster may initiate the site DTL. Once the DTL is submitted for CI approval, only the designated DTL Administrators or the CI may update the DTL. Instructions on completing the DTL are available in the Help Topics button in the DTL application and include a Master Task List, which describes DTL task assignments, CI signature, and CTEP registration requirements.

4.2.4 Checking Site Registration Status

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

- Log on to the CTSU members' website
- Click on *Regulatory* at the top of your screen
- Click on *Site Registration*
- Enter your 5-character CTEP Institution Code and click on Go

Note: The status shown only reflects institutional compliance with site registration requirements as outlined above. 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

The Oncology Patient Enrollment Network (OPEN) is a web-based registration system available on a 24/7 basis. OPEN is integrated with CTSU regulatory and roster data and with the Lead Protocol Organization (LPOs) registration/randomization systems or Theradex Interactive Web Response System (IWRS) for retrieval of patient registration/randomization assignment. OPEN will populate the patient enrollment data in NCI's clinical data management system, Medidata Rave.

Requirements for OPEN access:

- A valid CTEP-IAM account.
- To perform enrollments or request slot reservations: Be on an LPO roster, ETCTN Corresponding roster, or Participating Organization roster with the role of Registrar. Registrars must hold a minimum of an AP registration type.
- If a DTL is required for the study, the registrar(s) must hold the OPEN Registrar task on the DTL for the site.
- Have an approved site registration for a protocol prior to patient enrollment.

To assign an Investigator (IVR) or Non-Physician Investigator (NPIVR) as the treating, crediting, consenting, drug shipment (IVR only), or receiving investigator for a patient transfer in

OPEN, the IVR or NPIVR must list the IRB number used on the site's IRB approval on their Form FDA 1572 in RCR. If a DTL is required for the study, the IVR or NPIVR must be assigned the appropriate OPEN-related tasks on the DTL.

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

- Patient has met all eligibility criteria within the protocol stated timeframes, and
- All patients have signed an appropriate consent form and HIPAA authorization form (if applicable).

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

Access OPEN at https://open.ctsu.org or from the OPEN link on the CTSU members' website. Further instructional information is in the OPEN section of the CTSU website at https://www.ctsu.org or https://open.ctsu.org. For any additional questions, contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

Patient enrollment for this study will be facilitated using the Slot Reservation System in conjunction with the registration system in OPEN. Prior to discussing protocol entry with the patient, all site staff must use the IWRS Slot Reservation System to ensure that a slot on the protocol is available to the patient. Once a slot reservation confirmation is obtained, site staff may then proceed to enroll the patient to this study. Slots can be reserved for a maximum of 14 calendar days.

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

Following registration, patients should begin protocol treatment within 8 days. Issues that would cause treatment delays should be discussed with the Principal Investigator.

4.3.2 OPEN/IWRS Questions?

Further instructional information on OPEN is provided on the OPEN link 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.

Theradex has developed a Slot Reservations and Cohort Management User Guide, which is available on the Theradex website: http://www.theradex.com/clinicalTechnologies/?National-Cancer-Institute-NCI-11. This link to the Theradex website is also on the CTSU website OPEN tab. For questions about the use of IWRS for slot reservations, contact the Theradex Helpdesk at 609-619-7862 or Theradex main number 609-799-7580; CTMSSupport@theradex.com.

4.4 General Guidelines

Following registration, patients should begin protocol treatment within 8 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

This is a multicenter open-label phase 2 trial evaluating atezolizumab in adult subjects ≥18 years of age and in pediatric/adolescent subjects ≥2 years of age with advanced CCS or CS. Patients will be divided into cohorts based on histology upon enrollment. There will be three cohorts: histologically confirmed CCS (or EWSR1 translocation), conventional CS (grade 2 or 3), and dedifferentiated CS.

Patient evaluations will be performed throughout the study as described below. Baseline history, physical examination, laboratory evaluations, urinalysis, and EKG are to be conducted within 8 days prior to the start of protocol therapy. If protocol therapy is started within 8 days of these eligibility screening evaluations (see Section 3.3), values from the screening evaluations may be used as baseline measurements; if >8 days have passed since the screening evaluations, the medical history, physical examination, laboratory evaluations, urinalysis, and EKG must be repeated prior to starting protocol therapy. Baseline imaging scans and ECHO must be done within 28 days prior to the start of protocol therapy.

History and physical examination will be done within 8 days of Day 1 of all cycles. Labs (serum chemistries and CBC with differential) will be performed at baseline as described above, on Cycle 1 Day 15 [± 1 day], and then up to 3 days before the start of each subsequent cycle (LDH and protein do not need to be checked again after baseline). TSH will be checked at baseline (within 8 days of Cycle 1 Day 1) and then up to 3 days before the start of each subsequent cycle. EKG will be repeated as clinically indicated after the baseline measurement described above.

Patients will have a CT scan at baseline (within 28 days prior to start of protocol therapy). Patients will undergo a CT scan and be re-evaluated for response at the end of Cycle 3 and every two cycles thereafter (every 3 cycles for patients on study for >1 year; every 4 cycles for patients on study for >2 years). Given the concern of pseudoprogression ("tumor flare"), patients that are clinically well may continue on therapy following RECIST progression if they meet the criteria outlined in Section 11.4.

Research biopsies (mandatory for adult patients at all sites) and blood samples for PD studies (mandatory for patients ≥12 years of age) will be collected as described in Section 9.2 and Section 9.3.

5.1 Agent Administration

Treatment will be administered on an outpatient basis according to the regimen table below. Reported adverse events and potential risks are described in Section 7. Administration of atezolizumab will be performed in a setting with emergency management capabilities and staff

who are trained to monitor for and respond to medical emergencies.

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

Regimen Description				
Atezolizumab	***Dose***	***Route***	***Schedule***	***Cycle Length***
Adults ≥18 yrs	1200 mg	IV	Day 1, week 1	21 days
Pediatrics ≥2 yrs	15 mg/kg (1200 mg max)	IV	Day 1, week 1	21 days

For anaphylaxis precautions, see the management guidelines. Atezolizumab infusions will be administered per the instructions outlined in the table below:

Administration of First and Subsequent Atezolizumab Infusions

First Infusion	Subsequent Infusions
• No premedication is permitted prior to the atezolizumab infusion. • Vital signs (pulse rate, respiratory rate, blood pressure, and temperature) should be measured within 60 minutes prior to the infusion. • Atezolizumab should be infused over 60 (± 15) minutes. • If clinically indicated, vital signs should be measured every 15 (± 5) minutes during the infusion and at 30 (± 10) minutes after the infusion. • Patients should be informed about the possibility of delayed post-infusion symptoms and instructed to contact their study physician if they develop such symptoms.	• If the patient experienced an infusion-related reaction with any previous infusion, premedication with antihistamines, antipyretics, and/or analgesics may be administered for subsequent doses at the discretion of the investigator. • Vital signs should be measured within 60 minutes prior to the infusion. • Atezolizumab should be infused over 30 (± 10) minutes if the previous infusion was tolerated without an infusion-related reaction, or 60 (± 15) minutes if the patient experienced an infusion-related reaction with the previous infusion. • If the patient experienced an infusion-related reaction with the previous infusion or if clinically indicated, vital signs should be measured during the infusion and at 30 (± 10) minutes after the infusion.

For anaphylaxis precautions, use the following procedure:

Equipment Needed

- Monitoring devices: ECG monitor, blood pressure monitor, oxygen saturation monitor, and thermometer
- Oxygen

- Epinephrine for subcutaneous, intravenous, and/or endotracheal use in accordance with standard practice
- Antihistamines
- Corticosteroids
- IV 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. Call for additional medical assistance.
3. Ensure that appropriate monitoring is in place, with continuous ECG and pulse oximetry monitoring, if possible.
4. Administer antihistamines, epinephrine, or other medications as required by patient status and directed by the physician in charge.
5. Continue to observe the patient and document observation.
6. Draw serum/plasma samples for immunogenicity testing.
7. Ask participant to return for washout immunogenicity sample if appropriate.

Atezolizumab Dose Preparation

The prescribed dose of atezolizumab should be diluted in 0.9% NaCl to a concentration between 3.2 mg/mL and 16.8 mg/mL and infused through a 0.2 or 0.22 micrometer in-line filter. The IV bag may be constructed of polyvinyl chloride (PVC), polyolefin (PO), polyethylene (PE), or polypropylene (PP); the IV infusion line may be constructed of PVC, PE, or polybutadiene (PB); and the 0.2 or 0.22 micrometer in-line filter may be constructed of polyethersulfone (PES) or polysulfone (PSU). The prepared solution may be stored at 2°C–8°C for up to 24 hours or at ambient < 25°C (77°F) for 8 hours. If the dose solution is stored at 2°C–8°C (36°F–46°F), it should be removed from refrigeration and allowed to reach room temperature prior to administration. This time includes storage and time for administration for infusion. Do not shake or freeze infusion bags containing the dose solution.

5.2 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of atezolizumab 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. Appendix B (Patient Drug Information Handout and Wallet Card) is available.

Palliative radiotherapy to non-targeted lesions may be permitted for patients receiving atezolizumab after completing 2 cycles of therapy and achieving stable or responding disease, at the Principal Investigator's discretion.

Drugs to be avoided:

- Traditional herbal or homeopathic or natural medicines should be limited and used at the discretion of the investigator
- 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.
- Immunostimulatory agents, including but not limited to interferon (IFN)-α, IFN-γ, anti-TNF-α, or IL-2 (aldesleukin) (prohibited prior to and during the study and for 10 weeks after the last dose of atezolizumab; see Section 3.2.4). These agents, in combination with atezolizumab, could potentially increase the risk for autoimmune conditions.
- Immunosuppressive medications, including but not limited to cyclophosphamide, azathioprine, methotrexate, and thalidomide. These agents could potentially alter the activity and the safety of atezolizumab.
- Live vaccines and live, attenuated vaccines (prohibited during the study and for 100 days after the last dose of study drug).
- Initiation of granulocyte colony-stimulating factors (e.g., filgrastim and biosimilar products, sargramostim, and/or pegfilgrastim) should be discussed with the Medical Monitor.

5.3 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- Disease progression
  - With the caveat that patients who show evidence of clinical benefit may be permitted to continue atezolizumab treatment after disease progression, if they meet the criteria outlined in Section 11.4.
- 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
- Patient non-compliance
- Pregnancy
  - All women of childbearing potential should be instructed to contact the investigator immediately if they suspect they might be pregnant (*e.g.*, missed or late menstrual period) at any time during study participation.
  - The investigator must immediately notify CTEP in the event of a confirmed pregnancy in a patient participating in the study.
- Termination of the study by sponsor
- The drug manufacturer can no longer provide the study agent

The reason(s) for protocol therapy discontinuation, the reason(s) for study removal, and the corresponding dates must be documented in the Case Report Form (CRF).

5.4 Duration of Follow-Up

Patients will be followed for 90 days after the last dose of atezolizumab is administered or until the patient enrolls on another protocol or death, whichever comes first. Follow-up will consist of three telephone calls from the Study Team: one call between Day 27 and Day 30 after the last dose of atezolizumab, one call between Day 57 and Day 60 after the last dose, and one call between Day 87 and Day 90 after the last dose. Patients removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

5.5 Criteria for Removal from Treatment and/or Study

Patients will be removed from study for one of the following reasons: completed 30-day follow-up period, toxicities are unresolved but stabilized, patient enrolls on another protocol, patient receives other type of treatment-intent therapy, pregnancy, or death. The reason for study removal and the date the patient was removed must be recorded on the case report form.

6. DOSING DELAYS/DOSE MODIFICATIONS

6.1 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 84 days (12 weeks) beyond the scheduled date of delayed infusion if study drug-related toxicity requiring dose suspension is experienced. If atezolizumab is held because of AEs for >84 days beyond the scheduled date of infusion, the patient will be discontinued from atezolizumab and will be followed for safety and efficacy as specified in this protocol. If the AE resolves within 84 days and the patient is receiving corticosteroid therapy for the event, atezolizumab may be held for longer than 84 days (up to 4 weeks) in order to allow tapering of the steroid dose to ≤10 mg oral prednisone or equivalent.

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.

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.

6.2 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 **the Agent Administration Guidelines** in this document, including the "**Administration of First and Subsequent Atezolizumab Infusions**" table 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.

[*Use the following paragraph in studies enrolling patients with lung cancer. Studies not enrolling lung cancer patients may delete.*] Pleural and pericardial effusion

Patients experiencing dyspnea, chest pain, or unexplained tachycardia should be evaluated for the presence of a pericardial effusion. Patients with pre-existing pericardial effusion should be followed closely for pericardial fluid volume measurements and impact on cardiac function. When intervention is required for pericardial or pleural effusions, atezolizumab should be held, and appropriate workup includes cytology, lactate dehydrogenase (LDH), glucose, cholesterol, protein concentrations (with pleural effusions), and cell count.

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 and will have computed tomography (CT) scans of the chest performed at every tumor assessment.

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]

Event	Management
	• 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 must 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 addressed, as appropriate.

Event	Management
Hepatic event, Grade 1	• Continue atezolizumab.

Event	Management
	• 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 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.

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.

Event	Management
	• Monitor closely.
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 measured 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.

Event	Management
	• 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.
Hyperglycemia, Grade 3 or 4	• Withhold atezolizumab. • Initiate treatment with insulin. • Monitor for glucose control. • Consider referral to endocrinologist, particularly if patient is deemed to have atezolizumab-induced diabetes; if so, obtain C-peptide level paired with glucose, autoantibody levels (e.g. GAD65, islet cell autoantibodies), and hemoglobin A1C level. • If patient is found to have diabetic ketoacidosis or hyperglycemic hyperosmolar syndrome, treat as per institutional guidelines with appropriate management and laboratory values (*e.g.* anion gap, ketones, blood pH, *etc*.) reported. • 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.
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. • 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 symptoms resolve to below Grade 2 (*i.e.* patient is completely asymptomatic), resume atezolizumab. [b] • If symptoms do not resolve to below Grade 2 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 symptoms resolve to below Grade 2, 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 Principal Investigator.
Grade 3[a] Fever[b] with hypotension requiring a vasopressor (with or without vasopressin) **and/or** Hypoxia requiring high-flow oxygen[d] by nasal cannula, face mask, non-rebreather mask, or venturi mask	• Permanently discontinue atezolizumab. • 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 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,	• Permanently discontinue atezolizumab. • 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[f] may be considered at the discretion of the investigator.

BiPAP, intubation and mechanical ventilation)	• 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. 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:**

Event	Management
	• 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. • 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. Although uncommon, cases of severe cutaneous adverse reactions such as Stevens-Johnson syndrome and toxic epidermal necrolysis have been reported with atezolizumab. 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 for evaluation and if indicated, biopsy. • 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 for evaluation and if indicated, biopsy. • 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.
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.

[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.

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. Management guidelines for neurologic disorders are provided in the table below.

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 symptoms resolve to below Grade 2, resume atezolizumab. [b] • If symptoms do not resolve to below Grade 2 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-mediated meningoencephalitis is an identified risk associated with the administration of atezolizumab. Immune-mediated 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 in the table below.

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.

Event	Management
	• Initiate treatment as per institutional guidelines. • 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.

Event	Management

[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.

HLH= hemophagocytic lymphohistiocytosis; MAS= macrophage activation syndrome.

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 (Sections 7.2 and 7.3) 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)

Comprehensive Adverse Events and Potential Risks list (CAEPR) for Atezolizumab (MPDL3280A, NSC 783608)

The Comprehensive Adverse Events 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, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (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/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 3097 patients.* Below is the CAEPR for Atezolizumab (MPDL3280A).

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. 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.

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	
		Pericardial effusion (2)	
		Pericardial tamponade (2)	
		Pericarditis (2)	
ENDOCRINE DISORDERS			
		Adrenal insufficiency	
		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)***
	Diarrhea		***Diarrhea (Gr 2)***
	Dysphagia		
	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)	
		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]			

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%)**	
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			
	Arthralgia (2)		
	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	
		Peripheral sensory neuropathy (2)	
RENAL AND URINARY DISORDERS			
		Acute kidney injury	

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%)**	
		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)		
		Skin and subcutaneous tissue disorders - Other (exanthematous pustulosis)	
		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.

7.2 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 5.0 will be utilized for AE reporting. 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 website 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) 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

The Cancer Therapy Evaluation Program Adverse Event Reporting System (CTEP-AERS) integration enables evaluation of post-baseline AEs entered in Rave to determine whether they require expedited reporting, and facilitates entry in CTEP-AERS for those AEs requiring expedited reporting.

All AEs that occur after baseline are collected in Medidata Rave using the Adverse Event form, which is available for entry at each treatment or reporting period, and used to collect AEs that start during the period or persist from the previous reporting period. The Clinical Research Associate (CRA) will enter AEs that occur prior to the start of treatment on a baseline form that is not included in the Rave-CTEP-AERS integration. AEs that occur prior to enrollment must begin and end on the baseline Adverse Event form and should not be included on the standard Adverse Events form that is available at treatment unless there has been an increase in grade.

Prior to sending AEs through the rules evaluation process, site staff should verify the following on the Adverse Event form in Rave:

- The reporting period (course/cycle) is correct, and
- AEs are recorded and complete (no missing fields) and the form is query-free (fields added to the form during study build do not need to be query-free for the integration call with CTEP-AERS to be a success).

The CRA reports AEs in Rave at the time the Investigator learns of the event. If the CRA modifies an AE, it must be re-submitted for rules evaluation.

Upon completion of AE entry in Medidata Rave, the CRA submits the AE for rules evaluation by completing the Expedited Reporting Evaluation form. Both NCI and protocol-specific reporting rules evaluate the AEs submitted for expedited reporting. A report is initiated in CTEP-AERS using information entered in Medidata Rave for AEs that meet reporting requirements. The CRA completes the report by accessing CTEP-AERS via a direct link on the Medidata Rave Expedited Reporting Evaluation form.

In the rare occurrence that 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 that was phoned in must be entered immediately into CTEP-AERS using the deep link from Medidata Rave.

Additional information about the CTEP-AERS integration is available on the CTSU

website:

- Study specific documents: Protocols > Documents > Education and Promotion, and
- Expedited Safety Reporting Rules Evaluation user guide: Resources > CTSU Operations Information > User Guides.

NCI requirements for SAE reporting are available on the CTEP website:

- NCI Guidelines for Investigators: Adverse Event Reporting Requirements is available at https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf.

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 as long as the death occurred within 30 days after the last administration of the investigational agent. Attribution to treatment or other cause must be provided.

Death due to progressive disease should be reported as **Grade 5 "Disease progression"** in 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 Additional Protocol-Specific Expedited Adverse Event Reporting Exclusions

For this protocol only, the AEs/grades listed below do not require expedited reporting via CTEP-AERS. However, they still must be reported through the routine reporting mechanism (Section 7.4):

CTCAE SOC	Adverse Event	Grade	≥24h Hospitalization[a]
Investigations	Lymphocyte count decreased	Any	Regardless
Skin and subcutaneous tissue disorders	Alopecia	Any	Regardless
Blood and lymphatic system disorders	Anemia	2	Regardless
Metabolism and nutrient disorders	Hypoalbuminemia; hyperglycemia; hyperuricemia; hypernatremia; hyponatremia; hypokalemia; hypophosphatemia; hypomagnesemia	2	Regardless
Investigations	INR increased; activated partial thromboplastin time prolonged	2	Regardless

[a] Indicates that an adverse event required hospitalization for ≥24 hours or prolongation of hospitalization by ≥24 hours of a patient.

7.3.5 Adverse Events of Special Interest in Atezolizumab Studies

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

Drug-Specific AESIs

- Systemic lupus erythematosus
- Nephritis
- Ocular toxicities (*e.g.*, uveitis, retinitis)
- Grade ≥2 cardiac disorders (*e.g.*, atrial fibrillation, myocarditis, pericarditis)
- Events suggestive of hypersensitivity, infusion-related reactions, cytokine release syndrome, macrophage activating syndrome and hemophagocytic lymphohistiocytosis.
- Vasculitis
- Autoimmune hemolytic anemia
- Severe cutaneous reactions (e.g., Stevens-Johnson syndrome, dermatitis bullous, toxic epidermal necrolysis)

Non Drug-Specific AESIs

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

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

7.4 Routine Adverse Event Reporting

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

Although not an adverse event in and of itself, pregnancy as well as its outcome must be documented via **CTEP-AERS**. In addition, the ***Pregnancy Information Form*** included within the NCI Guidelines for Adverse Event Reporting Requirements must be completed and submitted to CTEP. Any pregnancy occurring in a patient or patient's partner from the time of consent to 150 days after the last dose of study drug must be reported and then followed for outcome. Newborn infants should be followed until 30 days old. Please see the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" (at http://ctep.cancer.gov/protocolDevelopment/adverse_effects.htm) for more details on how to report pregnancy and its outcome to CTEP.

7.6 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.

7.7 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 agent administered in this study can be found in Section 7.1.

8.1 Atezolizumab (NSC #783608)

Other Names: Tecentriq™, MPDL3280A

Classification: monoclonal antibody

M.W.: 150 KD

Mode of Action: anti-PD-L1

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 (Butte et al. 2007).

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. Each 20 mL vial contains 1200 mg of atezolizumab and is formulated in glacial acetic acid (16.5 mg), L-histidine (62 mg), polysorbate 20 (8 mg), and sucrose (821.6 mg), with 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.

Preparation:

The prescribed dose of atezolizumab should be diluted in 0.9% NaCl to a concentration between 3.2 mg/mL and 16.8 mg/mL and infused with or without a low-protein binding 0.2

or 0.22 micrometer in-line filter. The IV bag may be constructed of polyvinyl chloride (PVC), polyolefin (PO), or polyethylene (PE). The prepared solution may be stored at 2°C-8°C for up to 24 hours or at ambient ≤ 25°C (77°F) for 6 hours from the time of preparation. If the dose solution is stored at 2□C–8□C (36□F–46°F), it should be removed from refrigeration and allowed to reach room temperature prior to administration. These times include the storage and administration times for the infusion. Do not shake or freeze infusion bags containing the dose solution.

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.

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.

Route of Administration: IV infusion

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 premedications with subsequent infusions.

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.

Patient Care Implications:

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

09/22 MJB

8.2 Availability

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

Atezolizumab is provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and the DCTD, NCI (see Section 12.5).

8.2.1 Agent Ordering and Agent Accountability

8.2.1.1 NCI-supplied agents may be requested by eligible participating Investigators (or their authorized designee) at each participating institution. The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The eligible participating investigators at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), NCI Biosketch, Agent Shipment Form, 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 participating investigator at that institution.

Atezolizumab may be ordered once a patient has been registered to the trial.

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, a "current" password, and active person registration status. 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.

8.2.1.2 Agent 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.

8.2.2 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, a "current" password and active person registration status. Questions about IB access may be directed to the PMB IB Coordinator via email.

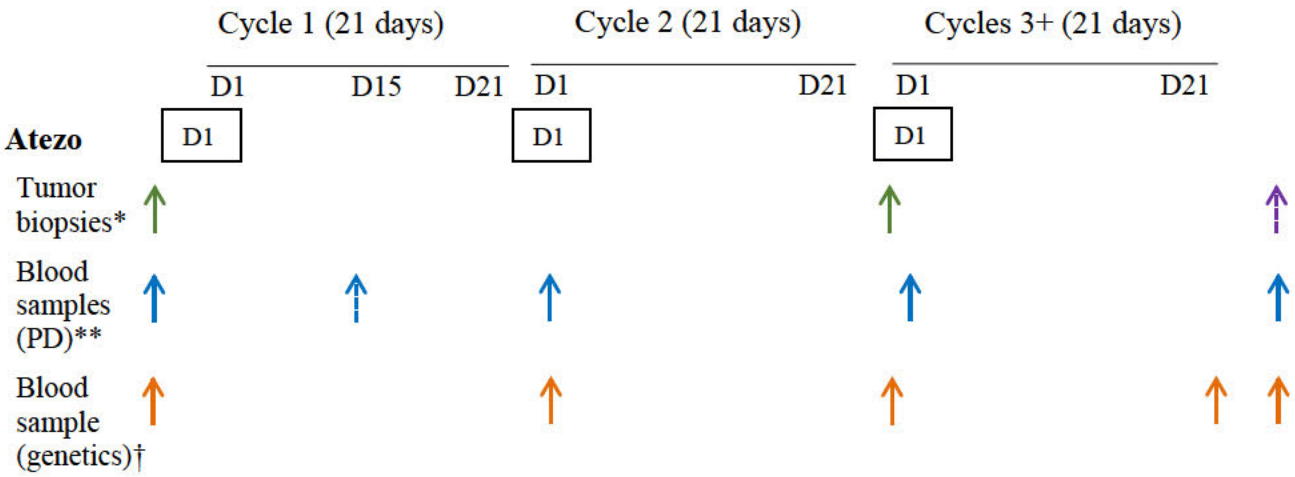
8.3 Useful Links and Contacts

- CTEP Forms, Templates, Documents: http://ctep.cancer.gov/forms/

- NCI CTEP Registration: RCRHelpDesk@nih.gov
- PMB policies and guidelines: http://ctep.cancer.gov/branches/pmb/agent_management.htm
- PMB Online Agent Order Processing (OAOP) application: https://ctepcore.nci.nih.gov/OAOP
- CTEP Identity and Access Management (IAM) account: https://ctepcore.nci.nih.gov/iam/
- CTEP IAM account help: ctepreghelp@ctep.nci.nih.gov
- IB Coordinator: IBCoordinator@mail.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)

9. CORRELATIVE STUDIES

We plan to evaluate the effect of the study drug on immune cell components of the tumor, tumor microenvironment, and peripheral blood. The correlative studies that will be carried out on the paired (pre- and post-treatment) biopsies and blood samples will help elucidate the mechanism of action of atezolizumab on signaling pathways mediating the immune response, focusing on subsets of effector cells. Additionally, the paired biopsy samples will be used to evaluate the tumor microenvironment to see if any changes or lack thereof are associated with either response or resistance to therapy. Blood samples will be collected and the nucleic acid from the mononuclear cells will be sequenced to serve as a germline control for calling somatic alterations in the whole exome sequencing results from the tumor specimens and, possibly, cell-free DNA.



*At all sites (patients ≥18 years of age). **At NCI only (patients ≥12 years of age). †All patients ≥12 years of age.

	Baseline and C3D1 Biopsies	Progression or Restaging Follow-up Biopsy (optional)	Blood Samples for ImmunoPD Analysis	Blood Sample for Genetic Analysis
Collection Information	All cores flash frozen	All cores flash frozen	6-mL NaHep tube	Two 10-mL Streck tubes at every time point
First Priority Assay(s)	PD assays (first 2 cores)	Oncomine (first core)	PD assays (1 tube)	Germline WES (1 tube at baseline)
Second Priority Assay(s)	WES/RNASeq (remaining cores)	PD assays (second and third cores) WES/RNASeq (remaining cores)		ctDNA sequencing by TSO500
Reporting to Patients	None	Oncomine report	None	None

Figure 6. Summary of specimen collection and prioritization for correlative studies.

For NIH Clinical Center specimens only: At least 24 hours prior to tumor biopsy or blood sample collection, the research nurse will contact the NCI Phase I/II PK/PD Support Group in NIH Building 10: E-mail (preferred): NCIPK-PDsupportgroup@mail.nih.gov, Pager (preferred): 102-12798, Phone: 240-858-3963, Fax: 301-480-5871. For biopsies, tubes pre-labeled with the information specified in Section 9.4, biopsy date, and site of tissue biopsy will be provided. Initial processing and shipping of the samples will be completed as described below.

9.1 Biomarker Plan

Research tumor biopsies will be collected from adult patients (≥18 years of age) for the assessment of pharmacodynamic endpoints. These biopsies are mandatory for adult patients (≥18 years of age) for whom biopsies are deemed safe and feasible. Patients that cannot be safely biopsied may be considered for the study upon discussion with Principal Investigator.

At all sites, collection of whole blood for genetic analysis is mandatory for patients ≥12 years of age. The nucleic acid extracted from the patient's mononuclear cells will be sequenced to

distinguish germline from tumor-specific alterations and thus enable the calling of somatic mutations in whole exome sequencing results. The nucleic acid extracted from plasma will be used to evaluate DNA alterations found in cell-free, circulating tumor DNA and their potential correlation with response to atezolizumab.

At NCI only, mandatory peripheral blood collections from patients ≥12 years of age will also be used for assessment of pharmacodynamic endpoints. See Section 9.3.2.

Specimen collection time points and assay priorities are specified in the table below.

List of Biomarker Assays in Order of Priority

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
Tissue-based Biomarkers							
1	CD8+ T-cell infiltration into tumor (CD8, tumor marker [e.g., β-catenin])	PADIS immunoPD multiplex IFA CLIA: N	Integrated To examine the effects of atezolizumab on the antitumor immune response	Tumor biopsy tissue	Baseline, C3D1, restaging follow-up/ progression	M; ≥18 y/o (O; ≥18 y/o at restaging follow-up/ progression	PADIS, FNLCR Lab PI: [redacted]
2	CD8+ T-cell signaling and integration of stimulatory & suppressive receptor pathways (pZAP70, pCD3ζ, pSHP1, pSHP2)	PADIS immunoPD multiplex IFA CLIA: N	Integrated To examine the effects of atezolizumab on tumor-infiltrating T cell activation	Tumor biopsy tissue	Baseline, C3D1, restaging follow-up/ progression	M; ≥18 y/o (O; ≥18 y/o at restaging follow-up/ progression	PADIS, FNLCR Lab PI: [redacted]
3	PD-L1/PD-1	PADIS immunoPD multiplex IFA CLIA: N	Exploratory To examine the effects of atezolizumab on tumor PD-L1 expression and T-cell PD-1 expression	Tumor biopsy tissue	Baseline, C3D1, restaging follow-up/ progression	M; ≥18 y/o (O; ≥18 y/o at restaging follow-up/ progression	PADIS, FNLCR Lab PI: [redacted]

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
4	Gene and mRNA variants and transcript levels; tumor mutational burden	Whole-exome sequencing (WES) and RNASeq CLIA: N	Exploratory To uncover mutations in key pathways (immune response, neoantigen presentation) that may be correlated with patient response to atezolizumab; to assess whether tumor mutational burden correlates with response	Tumor biopsy tissue	Baseline, C3D1, restaging follow-up/ progression	M; ≥18 y/o (O; ≥18 y/o at restaging follow-up/ progression	MoCha, FNLCR Lab PI: [redacted]
5	Gene variants in the Oncomine version 3 panel	Oncomine OCAv3 CLIA: Y	Exploratory To uncover mutations in key pathways (immune response, neoantigen presentation) that may be correlated with patient response to atezolizumab; a clinical report may be returned to patients	Tumor biopsy tissue	Restaging follow-up/ progression	O; ≥18 y/o	MoCha, FNLCR Lab PI: [redacted]
Blood-based Biomarkers							
1	T-cell activation (pZAP70, pCD3ζ, pSHP1, and pSHP2)	PADIS immunoPD phospho-flow cytometry assay CLIA: N	Integrated To examine the effects of atezolizumab on the antitumor immune response	Peripheral whole blood	Baseline, C1D15, C2D1; first day of each subsequent cycle; restaging follow-up and/or progression	M; ≥12 y/o (O; ≥12 y/o on C1D15)	PADIS, FNLCR Lab PI: [redacted]

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
2	Germline control for WES	NGS CLIA: N	Exploratory To compare germline genomic profile to tumor specimen	Peripheral whole blood	Baseline	M; ≥12 y/o	MoCha, FNLCR Lab PI: [redacted]
3	Circulating tumor DNA levels and gene variants found in cfDNA	Illumina TSO500 CLIA: N	Exploratory Hypothesis generation (to assess longitudinal changes in ctDNA levels that occur with atezolizumab treatment and to uncover molecular alterations in ctDNA associated with atezolizumab response or resistance)	Peripheral whole blood	Baseline, C2D1, C3D1 (±3 days, or sooner if there is clinical evidence that the patient is responding to drug), at the first two restagings (end of C3 and end of C5), every two restaging visits after that (i.e., C9, C13, etc.)	M; ≥12 y/o	MoCha, FNLCR Lab PI: [redacted]

9.2 Correlative Assays in Tumor Tissue

Tumor biopsies (mandatory when deemed safe and feasible) will be collected for research from adult patients (≥18 years of age).

Pharmacodynamic Analysis (ImmunoPD)

Pharmacodynamic assays will be performed on these tumor specimens to evaluate the effect of atezolizumab on immune cell components of the tumor and microenvironment. The results of these assays will help to elucidate the mechanism of action of atezolizumab in sarcoma and demonstrate the impact of a PD-L1 inhibitor on signaling pathways mediating the immune response. Efforts will be made to correlate any observed treatment-induced changes with clinical response or resistance to therapy.

Activated CD8+ T cells will be defined by the expression of TCR activation (Zeta chain phosphorylation) or phosphorylated Zap70 (pY493); CD8+ cells present within the tumor section that are positive for these markers will be quantified. Other targets of PD assays will include PD-L1 and PD-1 levels in cells present in the biopsy, suppressive immune checkpoint signaling (SHP1/SHP2 phosphorylation), CD4, and a tumor marker (e.g., β-catenin) to identify tumor region.

These biomarkers will be measured through multiplex immunofluorescence assays designed and validated by PADIS (See Section 2.5). Measurements on the Nikon A1/Definiens platform will include cell enumeration and proximity of tumor cells (by pathology analysis and tumor marker IFA) to T-cells. Monoclonal antibodies to all markers have been validated by the standard PADIS approach, and PADIS has previously validated and reported on the tumor cell marker set.

Genetic Analysis

We will also perform exploratory genomic sequencing (WES and RNA-Seq) on biopsy tissue, with the goal of identifying potential correlations between atezolizumab activity and tumor genomic alterations (such as those involved in relevant immune pathways, neoantigen presentation, *etc*.). MSI status and mutational signatures may also be assessed. We will assess whether changes in the genomic makeup of the tumor is associated with treatment response and/or progression, and whether TMB correlates with patient outcome. Germline DNA obtained from PBMCs will be used as a point of comparison to identify somatic mutations. In addition to evaluating changes in tumor mutations over time (post-treatment), extensive molecular characterization of pre-treatment tumor biopsy tissue from each patient enrolled on this trial will enable identification of valuable potential biomarkers to predict which patients are more or less likely to respond to treatment.

At progression (or pre-progression, following any restaging at which a 10-19% increase in tumor volume is observed), tumor tissue from the optional biopsy will also be analyzed by the CLIA-certified OCAv3 assay, and an Oncomine report will be returned to patients to inform future treatment.

9.2.1 Timing of Tumor Biopsies

Biopsies will be collected from adult patients at:

- Baseline
- Prior to Cycle 3 Day 1 (±3 days). If, at any point before Cycle 3 Day 1, there is clinical evidence that the patient is responding to drug, the biopsy will be conducted then instead of at the Cycle 3 Day 1 (±3 days) timepoint.
- *Optional*: Day 1 (±2 days) of the cycle following any restaging at which a 10-19% increase in tumor volume is observed (according to RECIST v1.1 criteria) if the patient has been on study for at least 4 cycles (the "restaging follow-up biopsy"), or at time of disease progression

9.2.2 Tumor Biopsy Procedure

Serial tumor biopsies will be obtained by the Interventional Radiology team by a cutaneous approach, a dermatologist for skin lesions, or an ENT for lesions that are easily biopsiable through ENT exam. The investigators will meet regularly with the Interventional Radiology team to review patient scans and discuss patients' medical history. If a site is deemed appropriate for biopsy with minimal risk to the participant by agreement between the investigators and the biopsy team, an attempt for biopsy will be made. Because approximately 20% of tumor biopsies collected on research trials are not usable due to the presence of stroma or normal and/or necrotic tissue and paired biopsies are necessary for analysis, up to 5 core biopsies ≥18-gauge in diameter and ≥1 cm in length, or equivalent, will be obtained during each procedure to try and ensure adequate tumor content and quality. If possible, the lesion from which each biopsy is taken, and whether the specimen came from the center or periphery of the lesion, will be documented.

Acceptable biopsy procedures are:

- Percutaneous biopsy with local anesthetic.
- Excisional cutaneous biopsy with local anesthetic
- Other biopsy with local anesthetic and/or sedation that has been shown to have a risk of severe complications < 2%. *Note:* This risk determination is made by the interventional radiologist after determining the complication rate for the patient through discussion of the patient's scans and medical history with the investigators.

The use of imaging to facilitate biopsies will be decided by members of the biopsy team and may include ultrasound, CT scan, or MRI. Should a CT scan be needed for biopsy, the number of scans for each procedure will be limited to the minimum number needed to safely obtain a biopsy. Tumor biopsies and local anesthesia will be administered only if they are considered to be of low risk to the participant, as determined by the investigators and the biopsy team. Cores from different areas of the tumor are preferred when feasible. The clinical, radiologic, and pharmacodynamic members of the research team will meet monthly to review the adequacy of the biopsy specimens for analysis.

Baseline biopsies will be performed following patient enrolling on study. If an initial attempt at a baseline biopsy is unsuccessful, the patient will be given an option to proceed with a repeated

attempt. A separate consent form must be signed for each biopsy procedure, so patients may choose not to undergo subsequent biopsies. If the baseline biopsy is unsuccessful or the patient refuses to undergo subsequent biopsies, no further biopsies will be performed but the patient will remain on study, receive study medication, and other correlative studies will be performed.

9.2.3 Tumor Specimen Processing

Biopsy cores will be processed as described in NCI DCTD SOP340507. All external sites are required to follow NCI DCTD SOP340507 for biopsy collection, processing, and shipment; the SOP can be found by following the link below.
http://dctd.cancer.gov/ResearchResources/biomarkers/docs/par/SOP340507_Biopsy_Frozen.pdf

A webinar training session with PADIS staff will be held with each participating site prior to their first biopsy collection to review proper procedure. The sites should contact NCI_PD_Support@mail.nih.gov to initiate training and clarify shipping procedures.

Dermatologic and ENT biopsies should be collected per guidance from FNLCR PD Central Receiving (NCI_PD_Support@mail.nih.gov, 301-846-1951, or 301-846-6747).

Briefly, the biopsy cores (up to 5 per time point) or equivalent tissue, will be individually transferred into 1.5-mL pre-chilled cryovials and then flash frozen in liquid nitrogen. In accordance with SOP340507, biopsy specimens are collected into pre-chilled 1.5-mL Sarstedt, O-ring screw cap tubes (VWR, Cat#: 83009-010) and then flash-frozen in liquid nitrogen or a dry ice/ethanol bath. It is ***imperative*** that biopsies are flash frozen *within 2 minutes of collection* in order to preserve key pharmacodynamic biomarkers.

9.2.4 Baseline and C3D1 Biopsy Specimens

The frozen **baseline** and **C3D1** biopsy specimens are transferred to PADIS in pairs on dry ice, where they are stored at -80°C or colder, and subsequently processed within 7-10 days for analysis or as directed by the Principal Investigator. Biopsy samples will be analyzed as described above; any additional samples will be kept for future analysis in the Frederick National Laboratories CR Biorepository in liquid nitrogen freezers. Additional studies, if performed, will be conducted following an amendment to the current protocol.

Biopsy specimens should be labeled with:

- Sample type (e.g., Biopsy)
- Time point (e.g., C01D01 pre dose)
- Collection date and time
- Sample ID, containing:
    - CTEP protocol number
    - Unique patient ID (Do NOT include patient name, medical record number, or initials)
    - 500-series sample collection number (e.g., 500, 501, 502, etc.)
    - Pass/Core identifier (e.g., A, B, C, etc.)

For example, the sample ID for the first core of a baseline sample collected for NCI DTC

patient # [redacted] should be: 10398_NCIDTC_[redacted]_500A.

9.2.4.1 Shipping Baseline and C3D1 Biopsy Specimens from Non-NIH Sites

Shipping Address:

Biopsies for PD analysis should be shipped on dry ice via FedEx (using PADIS account number) to:

Attn: [redacted]
NCI-F/FNLCR
1073 Beasley Street, Building 1073
Fort Detrick
Frederick, MD 21701
Phone: [redacted]

Shipping Method:

Baseline and post-treatment biopsy samples should be held at -80ºC or colder and shipped to PADIS together upon completion of the second biopsy. Participating sites are required to create a FedEx shipping label to accommodate the variable dry ice weight of the shipment package. Use only FedEx Priority Overnight Shipping. NCI-F PD Support will provide a FedEx account number to cover the cost of the shipment. By linking the NCI-F FedEx account number provided to the biopsy shipment, NCI-F PD Support can closely monitor the shipment and cover all shipping costs.

PADIS Contact(s):

Please contact [redacted] (office: 301-846-6747, cell: [redacted]) or [redacted] (office: 301-846-1951, cell: [redacted]) (email: NCI_PD_Support@mail.nih.gov) **to obtain the PADIS FedEx account number** or to ask any questions regarding storage or shipment of the baseline and C3D1 biopsy specimens.

Special Shipping Instructions:

Email the NCI-F PADIS group at NCI_PD_Support@mail.nih.gov prior to shipping with expected arrival date, protocol number, specimen IDs, histologic classification of the primary tumor, tracking information, and site information. PADIS needs to be notified as soon as possible of all protocol deviations or issues, prior to shipment of specimens(s), and these must be noted on the sample shipping manifest and batch record, both of which are included in SOP340507. One batch record is completed per patient's biopsy specimens, with all required information noted. If shipping multiple patient biopsies, one shipping manifest can be completed, including all patient specimens within the shipment.

Specimens should be shipped on dry ice. **Do not ship frozen biopsy specimens on Fridays or one day prior to any Federal holiday** as FNLCR receiving is closed and unable to receive samples on weekends and on all Federal holidays. This prevents the risk of dry ice sublimation and loss of sample integrity.

9.2.4.2 Shipping Baseline and C3D1 Biopsy Specimens from the NIH Clinical Center

For NIH Clinical Center specimens only: Baseline and post-treatment biopsy samples should be held at -80ºC or colder and shipped to PADIS together upon completion of the second biopsy. Shipment should be by CSP Courier and may be arranged by contacting [redacted] FNLCR, Tel.: 301-846-5893. Non-NIH sites should not contact this number; please contact NCI PD Support as described in Section 9.2.4.1.

9.2.5 Optional Restaging Follow-Up or Progression Biopsy Specimens

Biopsies will be collected and flash frozen as above (Section 9.2.3).

The sample shipping manifest and batch record, both of which are included in NCI DCTD SOP340507, should be completed and included with the shipment. NCI DCTD SOP340507 for biopsy collection, processing, and shipment; the SOP can be found by following the link below.
http://dctd.cancer.gov/ResearchResources/biomarkers/docs/par/SOP340507_Biopsy_Frozen.pdf

Biopsy specimens should be labeled with:

- Sample type (e.g., Biopsy)
- Time point (e.g., Restaging)
- Collection date and time
- Sample ID, containing:
  - CTEP protocol number
  - Unique patient ID (Do NOT include patient name, medical record number, or initials)
  - 500-series sample collection number (e.g., 500, 501, 502, etc.)
  - Pass identifier (e.g., A, B, C, etc.)

For example, the sample ID for the first core of a restaging follow-up sample collected for NCI DTC patient #[redacted] should be: 10398-NCIDTC [redacted]-502A (if C1D1 and C3D1 were already collected as 500 and 501, respectively).

9.2.5.1 Shipping Optional Restaging Follow-Up or Progression Biopsy Specimens from Non-NIH Sites

The first 2 cores

Shipping Address:

The **first 2 cores** should be shipped on dry ice via FedEx (using MoCha account number) to:
Attn: [redacted]

MoCha Histology Lab
Frederick National Laboratory for Cancer Research
Leidos Biomedical Research, Inc.
1050 Boyles Street
Building 321 Room [redacted]
Frederick, MD 21702

Shipping Method:

The **first 2 cores** should be shipped on dry ice on the day of biopsy collection to the MoCha Laboratory at FNLCR for genomic analysis using the OCAv3 assay. Participating sites are required to create a FedEx shipping label to accommodate the variable dry ice weight of the shipment package. Use only FedEx Priority Overnight Shipping. The MoCha Lab will provide a FedEx account number to cover the cost of the shipment.

MoCha Contact(s):

Please contact [redacted] at [redacted] (email: [redacted] **to obtain the MoCha FedEx account number** or to ask any questions regarding storage or shipment of the restaging or progression biopsy specimens.

Special Shipping Instructions:

Email the MoCha Histology group at [redacted] **prior to shipping** with expected arrival date, protocol number, specimen IDs, histologic classification of the primary tumor, tracking information, and site information. MoCha should be notified as soon as possible of all protocol deviations or issues, prior to shipment of specimens(s).

Specimens should be shipped on dry ice. **Do not ship frozen biopsy specimens on Fridays or one day prior to any Federal holiday** as FNLCR receiving is closed and unable to receive samples on weekends and on all Federal holidays. This prevents the risk of dry ice sublimation and loss of sample integrity.

Any remaining cores

Any remaining cores (beyond the first 2 cores) should be shipped to PADIS as described in Section 9.2.4.1.

9.2.5.2 Shipping Optional Restaging Follow-Up or Progression Biopsy Specimens from the NIH Clinical Center

For NIH Clinical Center specimens only: Shipment should be by CSP Courier and may be arranged by contacting [redacted] FNLCR, Tel.: 301-846-5893. Non-NIH sites should not contact this number; please contact the MoCha Lab as described in Section 9.2.5.1.

The first 2 cores should be shipped to MoCha. **Any remaining cores** should be

shipped to PADIS.

9.3 Correlative Assays in Blood

9.3.1 Genetic Analysis – All Sites

Blood collection for genetic analysis is mandatory at all sites for patients ≥12 years of age.

9.3.1.1 Blood Collection for Genetic Analysis (Performed at All Sites)

Two whole blood samples (at least 7.5 mL each in 10-mL Streck tubes) will be collected at each of the following time points from patients ≥12 years of age and shipped at ambient temperature to MoCha within 2 days for ctDNA analysis:

- Baseline
- C2D1
- C3D1 (±3 days, or sooner if there is clinical evidence that the patient is responding to drug);
- at the first two restaging visits (end of C3 and end of C5);
- every two restaging visits after that (e.g., C9, C13, etc.); and
- at progression.

Blood specimens should be labeled with:

- Sample type (e.g., Whole blood)
- Time point (e.g., C1D1 pre dose)
- Collection date and time
- Sample ID, containing:
  - CTEP protocol number
  - Unique patient ID (Do NOT include patient name, medical record number, or initials)
  - 800-series sample collection number (e.g., 800, 801, 802, etc.)

For example, the sample ID for the baseline sample collected for NCI DTC patient #[redacted] should be: 10398_NCIDTC_[redacted]_800.

Mononuclear cells will be isolated from whole blood by the MoCha Laboratory for nucleic acid extraction and exploratory WES to allow for germline and somatic variants to be accurately identified, and to enable the accurate assessment of mutational signatures in the tumor specimen and, possibly, cell-free DNA. The results of these studies will not be returned to patients.

Circulating tumor DNA (ctDNA) monitoring will be performed with the TSO500 assay and, potentially, WES. Patient plasma will be analyzed for ctDNA by the TSO500 panel and, if a sufficient proportion of the circulating, cell free DNA (cfDNA) is ctDNA, then WES of cfDNA will also be considered to uncover additional relevant changes in the tumor genome; the results of these studies will not

be returned to patients. Once a patient has come off study, ctDNA from blood samples correlating to clinically informative time points (minimally, before treatment and at restaging scans suggesting tumor growth) will be analyzed.

9.3.1.2 Shipping Blood for Genetic Analysis from Non-NIH Sites

Shipping Address:

Ship blood specimens at ambient temperature via FedEx (using MoCha account number) to:

Attn: [redacted]
MoCha Histology Lab
Frederick National Laboratory for Cancer Research
Leidos Biomedical Research, Inc.
1050 Boyles Street
Building 321 Room [redacted]
Frederick, MD 21702

Shipping Method:

Blood samples should be shipped at ambient temperature to the MoCha Laboratory at FNLCR via FedEx First Overnight or FedEx Priority Overnight. The MoCha Lab will provide a FedEx account number to cover the cost of the shipment. The sample shipping manifest, which can be found in Appendix F, should be completed and included with the shipment.

MoCha Contact(s):

Please contact [redacted] at [redacted] (email: [redacted]) **to obtain the MoCha FedEx account number** or to ask any questions regarding storage or shipment of these specimens.

Special Shipping Instructions:

Email the MoCha Histology group at [redacted] prior to shipping with expected arrival date, protocol number, specimen IDs, histologic classification of the primary tumor, tracking information, and site information. MoCha should be notified as soon as possible of all protocol deviations or issues, prior to shipment of specimens(s).

Samples should **arrive at MoCha within 2 days of collection when possible; arrival within 3 days of collection is permissible when necessary**. Note that FNLCR receiving is closed and unable to receive samples on weekends and on all Federal holidays. **Therefore, these blood samples should not be collected on a Friday, or the day before a Federal holiday.**

9.3.1.3 Shipping Blood for Genetic Analysis from the NIH Clinical Center

For NIH Clinical Center specimens only: Shipment to MoCha should be by CSP Courier and may be arranged by contacting [redacted] FNLCR, Tel.: 301-846-

5893. Non-NIH sites should not contact this number; please contact the MoCha Lab as described in Section 9.3.1.2.

9.3.2 Pharmacodynamic Analysis – NCI Only

At **NCI only**, collection of blood samples for ImmunoPD studies will be mandatory for patients ≥12 years of age.

These samples will be used for the analysis of immune cell subsets and measurement of T-cell activation in the periphery. The activation status of peripheral T cell subsets will be determined based phosphomarker expression (pZAP70, pCD3ζ, pSHP1/SHP2) assessed through intracellular staining and flow cytometry). These studies will allow us to follow changes in the T-cell population longitudinally and potentially correlate increases in T-cell activation with reduction in tumor burden.

9.3.2.1 Blood Sample Collection for ImmunoPD (NCI Only)

Blood samples (4 mL) will be obtained from patients ≥12 years of age at the following times:

- at baseline (on Cycle 1 Day 1 prior to drug administration)
- on Cycle 1 Day 15 (optional)
- on the first day of every subsequent cycle prior to drug administration
- at re-staging follow-up or disease progression

Per NIH Clinical Center CC Policy for pediatric patients, no more than 5 mL/kg may be drawn for research purposes in a single day, and no more than 9.5 mL/kg may be drawn over any eight-week period.

Samples will be collected into NaHep tubes (4 mL of blood into one 6 mL tube at each timepoint) and then added to 1x Lyse/Fix buffer per DCTD SOP LHTP003.08.15. Blood must be mixed with Lyse/Fix buffer within 5 minutes of collection. Once samples are processed, they should be stored in 60% methanol at -20˚C for at least one hour (no longer than 6 hours) and then transferred to -80˚C. **Please see DCTD SOP LHTP003.08.15 for detailed guidance.**

Materials for sample collection and processing will be provided to the clinic's sample collection team.

For NIH Clinical Center specimens only: At least 24 hours prior to blood sample collection, the research nurse will contact the NCI Phase I/II PK/PD Support Group in NIH Building 10: E-mail NCIPK-PDsupportgroup@mail.nih.gov, Pager: 102-12798 Phone: 301-451-1169 Fax: 301-480-5871.

9.3.2.2 Blood Sample Shipping for ImmunoPD (NCI Only)

All samples should be sent on dry ice via courier to PADIS at FNLCR:

Attn: [redacted]
Frederick National Laboratory for Cancer Research
Leidos Biomedical Research, Inc.
1050 Boyles Street
Building 425, Room [redacted]
Frederick, MD 21702
Phone: 301-846-1951 or 301-846-6747

For NIH Clinical Center specimens only: Shipment should be by CSP Courier and may be arranged by contacting [redacted] FNLCR, Tel.: 301-846-5893. Non-NIH sites should not contact this number.

9.4 Sample Collection and Processing

Biospecimens will be collected and processed using validated SOPs that will ensure both specimen quality (analyte preservation) and patient confidentiality pursuant to informed consent provisions. Information about each specimen (e.g., blood, tumor biopsy, per specific protocol) will be recorded on a PK/PD collection worksheet included in Appendix E.

Using a computerized inventory system and a backup hardcopy process, all specimen collection and processing steps will be documented and the specific location of each specimen will be tracked. Each new specimen collected will be assigned a unique barcode identifier that can be linked to the original specimen collected and other relevant information within the inventory system. To ensure patient confidentiality, only containers used for the initial specimen collections will be labeled with patient identifiers.

Only the barcode identifier will be applied to all subsequent specimen containers. When specimens are processed and aliquoted, no patient information will be included on the new containers. Original specimen containers will be discarded. Only barcode-labeled specimens without patient identifiers will be shipped for analysis and/or storage. Specimen labels will indicate: CTEP protocol number, unique patient accession number, 3-digit sample number (see list below), collection time, and total volume collected, as appropriate. Samples from sets of at least three patients will be grouped for scientific analysis.

Standardized 3-digit sample collection numbers:
200 series: blood for PK
300 series: blood for PD
500 series: tumor biopsies
800 series: blood for genetic/genomic analyses
900 series: blood for ImmunoPD (NCI only)

The inventory process contains other security provisions sufficient to safeguard patient privacy and confidentiality. Access to the inventory system and associated documents will be restricted to appropriate individuals. Requests to use specimens stored in the repository must be approved. The only patient information available in the inventory system will be the patient sex, diagnosis,

and level of informed consent given. SOPs ensure that any changes in informed consent made by a patient and relayed to the PI will be reflected in the inventory system to ensure that specimens are destroyed as appropriate. All laboratory personnel will be trained to adhere to SOPs and will be monitored for high-quality performance.

Any new use of these samples will require prospective IRB review and approval. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

NCI patients participating in treatment protocols are often also enrolled on the NCI DTC longitudinal protocol, 17-C-0156, which enables transfer of a patient's research data and specimens longitudinally across multiple trials in which the patient has been enrolled. For NCI patients on the atezolizumab in CS and CCS study who are also enrolled on this longitudinal protocol, any frozen biopsy samples collected on this trial that are not needed to be used as the "baseline" tissue sample to determine the eligibility of the patient for a subsequent treatment-intent protocol (i.e., for which there is the possibility of direct benefit), can at the discretion of the PI be used for additional pharmacodynamic analysis to address questions about tumor responses to treatment. This affords us the opportunity to measure dynamic drug-specific target effects (e.g., changes in epithelial-mesenchymal transition, DNA damage response, or immune cell infiltration) in patients on consecutive clinical trials at the Developmental Therapeutics Clinic; there is tremendous value in being able to evaluate molecular changes before and during disease progression to understand why the tumors initially responded but then became resistant to treatment.

Patients on this study will not be consented to new use of their research tissue samples or informed of the results of these studies because (i) broad consent was obtained under the longitudinal protocol and (ii) there will be no direct benefit or added risk to the patient. No new samples will be collected on this study beyond those specified in the current protocol to meet the primary and secondary objectives. No information beyond that associated with the patient's longitudinal protocol registration ID will be shared with investigators performing the pharmacodynamic studies.

If at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested), and reported as such to the IRB. Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e., broken freezer allows for extensive sample thawing, etc.) will be reported as such to the IRB.

9.5 Privacy Considerations

As patient clinical response data (both for this study and, potentially, after a patient is off study) will be required for comparison to sequencing results, delinking the samples from identifying information is not feasible. A Certificate of Confidentiality has been obtained to help protect the privacy of all study participants. The informed consent document for this protocol contains language informing patients about the performance of genetic studies.

9.6 Management of Genomic Results

The results of the OCAv3 targeted sequencing assay performed by MoCha (under CLIA certification #21D2097127) on restaging follow-up or progression tumor biopsy tissue will be returned to patients in the Oncomine report.

Information regarding the Genomic Data Sharing Plan can be found in Section 12.6.

10. STUDY CALENDAR

Eligibility screening evaluations (see Section 3.3) are to be conducted within 8 days prior to patient enrollment, with the exception of informed consent, echocardiogram (ECHO), and imaging, which must be done within 28 days prior to patient enrollment. Baseline history, physical examination, laboratory evaluations, and EKG are to be conducted within 8 days prior to the start of protocol therapy. If protocol therapy is started within 8 days of the eligibility screening evaluations, values from the screening evaluations may be used as baseline measurements; if >8 days have passed since the screening evaluations, the medical history, physical examination, laboratory evaluations, and EKG must be repeated prior to starting protocol therapy. Baseline imaging scans and ECHO must be done within 28 days prior to the start of protocol therapy.

Each cycle is 21 days (± 3 days due to scheduling conflicts). The start of a new cycle may be delayed up to 2 weeks to accommodate scheduling conflicts and other unexpected events. 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.

	Pre-Study Screening	Cycle 1 Week 1	Cycle 1 Week 2	Cycle 1 Week 3	Cycle 2 Week 1	Cycle 3 Week 1	Cycles 4+ Week 1	Off Treatment
Atezolizumab[a]		A			A	A	A	
Informed consent	X							
Demographics	X							
Medical history	X	X[j]						
Concurrent meds	X	X----------						-----X
Physical exam[b]	X	X[j]			X	X	X	X
Vital signs	X	X[j]			X	X	X	X
Height	X							
Weight	X	X[j]			X	X	X	X
Performance status	X	X[j]			X	X	X	X
CBC w/diff, plts[c]	X	X[j]			X	X	X	X
Serum chemistry[c]	X	X[j]			X	X	X	X
TSH[d]	X	X[j]			X	X	X	X
EKG[e]	X	X[j]						
Echocardiogram[e]	X	X[j]						
Adverse event evaluation		X----------						-----X
Tumor measurements[f]	X	Tumor measurements are repeated every 6 weeks. Documentation (radiologic) must be provided for patients removed from study for progressive disease.						X
B-HCG[g]	X	X[j]			X	X	X	
Biopsy[h]		X				X[h]		X[i]
Blood for PD[k]		X		X[k]	X	X	X	X
Blood for genetics[l]		X	Blood for ctDNA analysis at C2D1, at the time of any biopsy, at the first two restagings (end of C3 and end of C5), and every two restaging visits after that (e.g., C9, C13, etc.)					X

a: Atezolizumab dose as assigned; adults ≥18 years at 1200 mg IV and in pediatric/adolescents ≥2 years at 15 mg/kg (max single dose=1200 mg); drug will be administered once on Day 1 only of each cycle. Each cycle is 21 days (± 3 days). Start of new cycle may be delayed up to 2 weeks to accommodate scheduling conflicts.
b: History and physical at baseline and up to 8 days before the start of each subsequent cycle.
c: CBC w/diff, plts, and serum chemistry (ACTH, albumin, alkaline phosphatase, AM cortisol, amylase, bicarbonate, total bilirubin, BUN, calcium, chloride, creatinine, glucose, LDH, lipase, phosphorus, potassium, pregnancy test per institutional standard at each participating sitetotal protein, SGOT [AST], SGPT [ALT], sodium, free T4) at baseline and then up to 3 days before the start of each subsequent cycle. After baseline, amylase, LDH, lipase, protein, and free T4 do not need to be checked.
d: TSH levels at baseline and then up to 3 days before the start of each subsequent cycle.
e: Baseline and as indicated.
f: CT scan or other imaging test at baseline, at the end of Cycle 3, and every two cycles thereafter (every 3 cycles for patients on study for >1 year; every 4 cycles for patients on study for >2 years).
g: Serum or urine pregnancy test (women of childbearing potential) within 8 days prior to enrollment. If

clinically indicated, serum or urine pregnancy test is required for women of childbearing potential ≤8 days before the start of each treatment cycle[per institutional standard at each participating site]; outside lab results are acceptable.

h: Adult patients (≥18 years of age; mandatory at all sites), at baseline and prior to C3D1 (±3 days) or sooner if there is clinical evidence that the patient is responding to drug, as described in Section 9.2.1.

i: One optional tumor biopsy may be collected following progression or a 10-19% increase in tumor volume at restaging, as described in Section 9.2.1.

j: Eligibility screening results may be used for these baseline measurements if conducted within 8 days (for medical history, physical exam, CBC, serum chemistry, TSH, and EKG) or 28 days (for ECHO) prior to the start of protocol therapy. See Sections 3.3 and 5.

k: **At NCI only:** blood for ImmunoPD (mandatory from patients ≥12 years of age) on C1D1, C1D15 (optional), day one of each cycle, and at restaging follow up or progression as described in Section 9.3.

l: At all sites, blood for genetic analysis is mandatory for patients ≥12 years of age. Two tubes at each of the following time points: baseline, C2D1, C3D1 (±3 days, or sooner if there is clinical evidence that the patient is responding to drug); at the first two restagings (end of C3 and end of C5); every two restaging visits after that (e.g., C9, C13, etc.) as described in Section 9.3.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

Patients should be re-evaluated for response at the end of Cycle 3 (before Cycle 4), and at the end of every two cycles thereafter (before every even-numbered cycle). Restaging scans may be performed less frequently for patients on study for more than 1 year (every three cycles for patients on study for more than 1 year; every four cycles for patients on study more than 2 years). Confirmatory scans should also be obtained ≥4 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [59]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

Patients that are clinically well may continue on therapy following RECIST progression with new lesions or increase in target lesions, provided the criteria in Section 11.4 are met.

Response will also be explored using iRECIST (see Section 11.3). Increasing clinical experience indicates that traditional response criteria such as RECIST may not be sufficient to fully characterize activity in the new era of target therapies and/or biologics. In studies with therapeutic antibodies, complete response, partial response, or stable disease has been shown to occur after an increase in tumor burden, characterized by progressive disease by traditional response criteria. Therefore, conventional response criteria may not adequately assess the activity of immunotherapeutic agents because progressive disease (by initial radiographic evaluation) does not necessarily reflect therapeutic failure. Long-term effect on the target disease must also be captured. The iRECIST criteria, by incorporating specific response patterns

that have been observed with immunotherapeutic agents, have been developed to address these issues and provide standardized response criteria for use with immmunotherapy [60].

11.2 RECIST Response Assessment

11.2.1 Definitions

Evaluable for Toxicity. All patients will be evaluable for toxicity from the time of their first treatment with atezolizumab.

Evaluable for Objective Response Rate. Only those patients who have measurable disease present at baseline and have received at least one dose of therapy will be considered evaluable for the primary endpoint of objective response rate. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of Cycle 1 will also be considered evaluable.)

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

11.2.2 Disease Parameters

Measurable Disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm (≥2 cm) by chest x-ray or as ≥10 mm (≥1 cm) with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Malignant Lymph Nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm (≥1.5 cm) in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm [0.5 cm]). At baseline and in follow-up, only the short axis will be measured and followed.

Non-Measurable Disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm [<1 cm] or pathological lymph nodes with ≥10 to <15 mm [≥1 to <1.5 cm] short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

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

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are

present in the same patient, these are preferred for selection as target lesions.

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

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

11.2.3 Methods for Evaluation of Measurable Disease

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

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

Clinical Lesions. Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm (≥1 cm) diameter as assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

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

Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm (0.5 cm) or less. If CT scans have slice thickness greater than 5 mm (0.5 cm), the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly

impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

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

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

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

Tumor Markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [61-63]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [64].

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

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is

mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

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

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

11.2.4 Response Criteria

11.2.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm (<1 cm).

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

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm (0.5 cm). (Note: the appearance of one or more new lesions is also considered progressions).

Patients that are clinically well may continue on therapy following RECIST progression with new lesions or increase in target lesions provided the criteria in Section 11.4 are met.

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to

qualify for PD, taking as reference the smallest sum diameters while on study.

11.2.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm [<1 cm] short axis).

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

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

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

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

11.2.4.3 Evaluation of Best Overall Response

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

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

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

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

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

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

11.2.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

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

11.2.6 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

11.2.7 Response Review

For trials where the response rate is the primary endpoint, it is strongly recommended that all responses be reviewed by an expert(s) independent of the study at the study's completion. Simultaneous review of the patients' files and radiological images is the best approach.

11.3 iRECIST Response Assessment

Overall response will also be assessed using iRECIST as an exploratory endpoint, for comparison to RECIST v1.1.

Immunotherapeutics may result in infiltration of immune cells leading to transient increase in the size in malignant lesions, or undetectable lesions becoming detectable. Investigators should continue treatment, as appropriate, in the absence of unacceptable toxicity, until unequivocal disease progression. This is particularly important for patients in whom pseudoprogression may have occurred. Follow up response assessments must be continued until unequivocal disease progression has occurred.

The iRECIST criteria are identical to those of RECIST 1.1 in many respects but have been adapted to account for instances where an increase in tumor burden, or the appearance of new lesions, does not reflect true tumor progression.

Key differences are described below. All responses defined using iRECIST criteria are designated with a prefix. iRECIST time-point and best overall responses will be recorded separately. Unlike RECIST 1.1, iRECIST requires the confirmation of progression and uses the terms iUPD (unconfirmed progression) and iCPD (confirmed progression). Confirmatory scans should be performed at least 4 weeks, but no longer than 8 weeks after iUPD.

Confirming progression: Unlike RECIST 1.1, iRECIST requires the confirmation of progression and uses the terms iUPD (unconfirmed progression) and iCPD (confirmed progression). Confirmatory scans should be performed at least 4 weeks, but no longer than 8 weeks, after iUPD.

iCPD is confirmed if further increase in tumor burden, compared to the last assessment, is seen as evidenced by one or more of the following:

- Continued increase in tumor burden (from iUPD) where RECIST 1.1 definitions of progression had been met (from nadir) in target, non-target disease, or new lesions.
    - Progression in target disease worsens with an increase of at least 5 mm in the absolute value of the sum.
    - Continued unequivocal progression in non-target disease with an increase in tumor burden.
    - Increase in size of previously identified new lesion(s) (an increase of at least 5 mm in the absolute value of the sum of those considered to be target new lesions) or additional new lesions.
- RECIST 1.1 criteria are met in lesions types (target or non-target or new lesions) where progression was not previously identified, including the appearance of additional new lesions.

If iUPD is not confirmed at the next assessment, then the appropriate response will be assigned (iUPD if the criteria are still met, but no worsening, or iSD, iPR, or iCR if those criteria are met compared to baseline). The prior documentation of iUPD does not preclude assigning iCR, iPR,

or iSD in subsequent time-point assessments or as best overall response (BOR) providing that iCPD is not documented at the next assessment after iUPD (*Lancet Oncol* 18:e143-e152, 2017 - Table 2).

New lesions:

New lesions should be assessed and measured as they appear using RECIST 1.1 criteria (maximum of 5 lesions, no more than 2 per site, at least 10 mm in long axis [or 15 mm in short axis for nodal lesions]), and recorded as New Lesions - Target (NLT) and New Lesion - Non-Target (NLNT) to allow clear differentiation from baseline target and non-target lesions.

New lesions may either meet the criteria of NLT or NLNT to drive iUPD (or iCPD). However, the measurements of target lesions should NOT be included in the sum of measures of original target lesions identified at baseline. Rather, these measurements will be collected on a separate table in the case record form.

PD is confirmed in the New Lesion category if the next imaging assessment, conducted at least 4 weeks (but not more than 8 weeks) after iUPD confirms further progression from iUPD with either an increase of at least 5 mm in the absolute value of the sum of NLT OR an increase (but not necessarily unequivocal increase) in the size of NLNT lesions OR the appearance of additional new lesions.

Time-point (TP) iResponse

Target Lesions*	**Non-Target Lesions***	**New Lesions***	**Time Point Response**	
			No prior iUPD**	**Prior iUPD**, *****
iCR	iCR	No	iCR	iCR
iCR	Non-iCR/Non-iUPD	No	iPR	iPR
iPR	Non-iCR/Non-iUPD	No	iPR	iPR
iSD	Non-iCR/Non-iUPD	No	iSD	iSD
iUPD with no change OR decrease from last TP	iUPD with no change OR decrease from last TP	Yes	NA	NLs confirms iCPD if NLs were previously identified and increase in size (≥5 mm in SOM for NLT or any increase for NLNT) or number. If no change in NLs (size or number) from last TP, remains iUPD.

Time-point (TP) iResponse

Target Lesions*	**Non-Target Lesions***	**New Lesions***	**Time Point Response**	
			No prior iUPD**	**Prior iUPD**, *****
iSD	iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based in further increase in size of NT disease (need not meet RECIST 1.1 criteria for unequivocal PD).
iUPD	Non-iCR/Non-iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based on further increase in SOM of at least 5 mm, otherwise remains iUPD.
iUPD	iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based on further increase in: • previously identified T lesion iUPD SOM ≥5 mm and/or • NT lesion iUPD (prior assessment - need not be unequivocal PD)
iUPD	iUPD	Yes	iUPD	Remains iUPD unless iCPD confirmed based on further increase in: • previously identified T lesion iUPD ≥5 mm and/or • previously identified NT lesion iUPD (need not be unequivocal) and/or • size or number of new lesions previously identified
Non-iUPD/PD	Non-iUPD/PD	Yes	iUPD	Remains iUPD unless iCPD confirmed based on increase in size or number of new lesions previously identified.
* Using RECIST 1.1 principles. If no PSPD occurs, RECIST 1.1 and iRECIST categories for CR, PR, and SD would be the same. ** in any lesion category. *** previously identified in assessment immediately prior to this TP.				

All patients will have their iBOR from the start of study treatment until the end of treatment

classified as outlined below.

iRECIST best overall response (iBOR)

TPR 1	TPR 2	TPR 3	TPR 4	TPR 5	iBOR
iCR	iCR, iPR, iUPD, NE	iCR, iPR, iUPD, NE	iUPD	iCPD	iCR
iUPD	iPR, iSD, NE	iCR	iCR, iPR, iSD, iUPD, NE	iCR, iPR, iSD, iUPD, iCPD, NE	iCR
iUPD	iPR	iPR, iSD, iUPD, NE	iPR, iSD, iUPD, NE, iCPD	iPR, iSD, iUPD, NE, iCPD	iPR
iUPD	iSD, NE	PR	iPR, iSD, iUPD, NE	iPR, iSD, iUPD, iCPD, NE	iPR
iUPD	iSD	iSD, iUPD, NE	iSD, iUPD, iCPD, NE	iSD, iUPD, ICPD, NE	iSD
iUPD	iCPD	Anything	Anything	Anything	iCPD
iUPD	iUPD	iCPD	Anything	Anything	iCPD
iUPD	NE	NE	NE	NE	iUPD

Table assumes a randomized study where confirmation of CR or PR is not required.
- NE = not evaluable that cycle.
- Designation "I" for BOR can be used to indicate prior iUPD to aid in data interpretation.
- For patients with non-target disease only at baseline, only CR or non-CR/non-PD can be assigned at each TPR but is not shown in the table for ease of presentation.

11.4 Treatment Beyond RECIST Progression

During treatment, patients who show evidence of clinical benefit will be permitted to continue atezolizumab treatment following progression (as defined by RECIST v1.1), as long as the increase in disease burden does not meet the definition of confirmed PD by immune response criteria and if they meet all of the following additional criteria:

- Evidence of clinical benefit as assessed by the investigator and agreed upon by the Medical Monitor
- Absence of symptoms or signs (including worsening laboratory values) indicating unequivocal progression of disease
- No significant decline in performance status that can be attributed to disease progression
- Absence of tumor progression at critical anatomical sites (e.g., leptomeningeal disease) that cannot be managed by protocol-allowed medical interventions

The investigator must discuss with the patient the risks and benefits of treatment continuation after possible worsening disease and must document the patient's agreement to continue

receiving atezolizumab in the medical record.

If the investigator and patient decide to continue treatment, confirmatory scans should be performed at least 4 weeks, but no longer than 8 weeks after the assessment in which the RECIST 1.1 definition of PD was met. If this consecutive scan confirms PD, the patient should come off treatment.

12. STUDY OVERSIGHT AND DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.

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.

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

Medidata Rave is a clinical data management system being used for data collection for this trial/study. Access to the trial in Rave is controlled through the CTEP-IAM system and role assignments. To access Rave via iMedidata:

- Site staff will need to be registered with CTEP and have a valid and active CTEP-IAM account, and
- Assigned one of the following Rave roles on the relevant Lead Protocol Organization (LPO) or Participating Organization roster at the enrolling site: Rave CRA, Rave Read Only, Rave CRA (LabAdmin), Rave SLA, or Rave Investigator. Refer to https://ctep.cancer.gov/investigatorResources/default.htm for registration types and documentation required.
    - To hold Rave CRA or Rave CRA (Lab Admin) role, site staff must hold a minimum of an AP registration type,
    - To hold Rave Investigator role, the individual must be registered as an NPIVR or IVR, and

- To hold Rave Read Only role, site staff must hold an Associates (A) registration type.

If the study has a DTL, individuals requiring write access to Rave must also be assigned the appropriate Rave tasks on the DTL.

Upon initial site registration approval for the study in Regulatory Support System (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 staff must log in to the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM username and password, and click on the *accept* link in the upper right-corner of the iMedidata page. Site staff 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. If an eLearning is required and has not yet been taken, the link to the eLearning will appear under the study name in iMedidata instead of the *Rave EDC* link; once the successful completion of the eLearning has been recorded, access to the study in Rave will be granted, and a *Rave EDC* link will display under the study name.

Site staff 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 in the Rave section under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website in the Data Management > Rave section at www.ctsu.org/RAVE/ 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 three times annually (one annual site visit and two data audits). For CTMS monitored studies, after users have activated their accounts, please contact the Theradex Help Desk at (609) 619-7862 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.

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).

Study participants are responsible for submitting CDUS data and/or data forms to either the Coordinating Center or to the Lead Organization on the study quarterly. The date for submission to the Coordinating Center or to the Lead Organization will be set by them. CDUS does not accept data submissions from the participants on the study. When setting the dates, allow time for Coordinating Center compilation, Principal Investigator review, and timely submission to CTEP by the quarterly deadlines (see Section 12.2.1). For trials monitored by CTMS, a quarterly report of data will be provided by Theradex to the Coordinating Center.

Either the Coordinating Center or the Lead Organization is responsible for compiling and submitting CDUS data to CTEP for all participants and for providing the data to the Principal Investigator for review.

12.3 Data Quality Portal

The Data Quality Portal (DQP) provides a central location for site staff to manage unanswered queries and form delinquencies, monitor data quality and timeliness, generate reports, and review metrics.

The DQP is located on the CTSU members' website under Data Management. The Rave Home section displays a table providing summary counts of Total Delinquencies and Total Queries. DQP Queries, DQP Delinquent Forms, and the DQP Reports modules are available to access details and reports of unanswered queries, delinquent forms, and timeliness reports. Review the DQP modules on a regular basis to manage specified queries and delinquent forms.

The DQP is accessible by site staff that are rostered to a site and have access to the CTSU website. Staff that have Rave study access can access the Rave study data using a direct link on the DQP.

To learn more about DQP use and access, click on the Help icon displayed on the Rave Home, DQP Queries, and DQP Delinquent Forms modules.

Note: Some Rave protocols may not have delinquent form details or reports specified on the DQP. A protocol must have the Calendar functionality implemented in Rave by the Lead Protocol Organization (LPO) for delinquent form details and reports to be available on the DQP. Site staff should contact the LPO Data Manager for their protocol regarding questions about Rave Calendaring functionality.

12.4 Human Data Sharing Plan

What data will be shared?
We will share human data generated in this research for future research as follows:
- x De-identified data in an NIH-funded or approved public repository
- x Identified data in BTRIS (automatic for activities in the NIH Clinical Center)
- x De-identified or identified data with approved outside collaborators under appropriate agreements

How and where will the data be shared?
Data will be shared through:
- x An NIH-funded or approved public repository: clinicaltrials.gov
- x BTRIS (automatic for activities in the NIH Clinical Center)
- x Approved outside collaborators under appropriate individual agreements
- x Publication and/or public presentations

When will the data be shared?
- x At the time of publication or shortly thereafter

12.5 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.

12.6 Genomic Data Sharing Plan

The NIH Genomic Data Sharing (GDS) Policy ensures broad and responsible sharing of genomic research data from all NIH-funded research (https://gds.nih.gov/index.html). Providing that patient consent has been obtained, targeted sequencing, whole-exome sequencing, and RNA-seq data generated for research purposes will be submitted to the Database of Genotypes and Phenotypes (dbGaP) for controlled-access use within a time frame consistent with data publication. The identities of research participants will not be disclosed to dbGaP or to secondary users of the data.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

This study will enroll adult and pediatric patients, but only adults will be included in the primary and interim analyses. For adult patients, a Simon two stage design will be employed within each of the three study cohorts, with a maximum of 17 evaluable patients per cohort. For each cohort, accrual will be halted at 9 patients to evaluate for response before further enrollment. If within 9

months of the ninth patient being enrolled to a cohort no responses are observed among the initial 9 patients in that cohort, the cohort will be terminated early and declared negative; if at least one response is observed, accrual to that cohort will continue. If at least 3 responses (at least 17.6%) are observed among the initial 17 evaluable patients in a disease cohort, this regimen will be considered worthy of further testing in that disease. This design yields at least 81% power to detect a true response rate of at least 25% in each cohort. It yields at least .95 probability of a negative result if the true response rate in each cohort is no more than 5%, with at least .63 probability of early negative stopping for each cohort. Per the RECIST v 1.1 guidelines (Section 13.4.2), all eligible patients who are treated on study will be included in the primary and interim response analyses.

The trial will remain open to pediatric patients with CCS and CS for as long the study is accruing, up to a maximum of 8 pediatric patients across the three study histologies. These patients will not provide research biopsies, but will have the opportunity to be treated due to the possibility of therapeutic benefit. Within each cohort, a maximum of 2 pediatric patients will be enrolled prior to the completion of the first-stage futility analysis. If none of the initial 9 adult patients enrolled to a cohort respond within 9 months of the ninth adult patient being enrolled, no additional adult or pediatric patients with that histology will be enrolled. If accrual of adult patients proceeds to the second stage within a cohort, additional pediatric patients with that histology may be enrolled until the 17th adult patient is enrolled (as long as the limit of 8 total pediatric patients across all cohorts has not been reached), at which point all accrual of patients with this histology will be halted.

If the study is found to be positive (i.e. at least 3 objective responses are observed) within a cohort and the accrual limit for that cohort has been reached prior to obtaining 10 evaluable biopsy pairs, an amendment will be submitted to CTEP to increase the accrual limit for this cohort so that ten evaluable biopsy pairs can be obtained (if feasible).

13.2 Sample Size/Accrual Rate

Each of the three cohorts, made up of adult and pediatric patients, will have up to 17 evaluable patients. To allow for a small number of patients who may not be evaluable, the accrual ceiling will be set at 68. We anticipate that 1-2 patients per month will be enrolled to study. The table below includes accrual estimates for the duration of the study.

PLANNED ENROLLMENT REPORT

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native					
Asian	3	4			7

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
Native Hawaiian or Other Pacific Islander	1	1			**2**
Black or African American	8	8		1	**17**
White	14	17	4	5	**40**
More Than One Race	1	1			**2**
Total	**27**	**317**	**4**	**6**	**68**

13.3 Analysis of Secondary Endpoints

Mandatory biopsies will be collected for pharmacodynamic analysis from adult patients only. As there are differences in biological characteristics among sarcoma subtypes, pharmacodynamic comparisons will only be made within the CS and CCS sarcoma cohorts, rather than CCS and CS samples being pooled for analysis. Biopsy pairs will be collected from 15 adult patients from each disease cohort. With 15 biopsy pairs collected from each cohort, there will be 95% likelihood of getting at least 10 evaluable biopsy pairs from each cohort. For a given PD endpoint within each cohort, 10 evaluable biopsy pairs will yield 86% power to detect a difference in change from baseline to post-treatment, between the clinical responders and the non-responders, corresponding to at least 2.5 SD's (with respect to the changes from baseline within each subgroup), at the 1-sided .01 significance level (to accommodate multiple comparisons).

If the study is found to be positive (i.e., at least 3 objective responses are observed) within a cohort and the accrual limit for that cohort has been reached prior to obtaining 15 biopsy pairs (or 10 evaluable biopsy pairs), an amendment will be submitted to CTEP to increase the accrual limit for this cohort so that 10 evaluable biopsy pairs can be obtained (if feasible).

Exploratory evaluations will also be performed, with results reported with appropriate caveats about the exploratory nature of the analysis, and without formal adjustment for multiple comparisons.

13.4 For phase 2 protocols only: Reporting and Exclusions

13.4.1 Evaluation of Toxicity

All patients will be evaluable for toxicity from the time of their first treatment with atezolizumab.

13.4.2 Evaluation of Response

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 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.]

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.

14. HUMAN SUBJECTS PROTECTION

14.1 Rationale for Subject Selection

This study will be open to all individuals regardless of gender, ethnicity, or race provided that the aforementioned inclusion and exclusion criteria are met. For safety reasons, pregnant women are excluded from this study. Patients for this study will be recruited through internal referral, our physician referral base, and through various cancer information hotlines (i.e., Clinical Studies Support Center, 1-800-4Cancer). Participants should realize that there is no guarantee of benefit to them from participation in this trial. The results of this trial may benefit future cancer patients. To date, there is no information that suggests that differences in drug metabolism or effect on tumor would be expected in one ethnic group compared to another. Efforts will be made to extend accrual to each representative population, but a balance must be struck between participant safety considerations and limitations on the number of individuals exposed to potentially ineffective treatments on the one hand and the need to explore racial/ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to ethnic identity are noted, a follow-up study may be written to investigate those differences more fully.

Inclusion of Women and Minorities

This study will be open to all individuals regardless of gender, ethnicity, or race provided that the aforementioned inclusion and exclusion criteria are met. Accrual estimates can be found in the Planned Enrollment Report in Section 13.2.

14.2 Justification for Exclusions

14.2.1 Pregnant Women

Pregnant women are excluded from this study because atezolizumab has known abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with these agents, breastfeeding should be discontinued. Participants with unstable or serious medical conditions or psychiatric illness/social situations that would limit compliance with study requirements are excluded due to the possibility that the underlying condition may obscure the attribution of effect and adverse events and may limit study compliance.

14.2.2 Children <2 years of age

This study includes patients ≥2 years of age at the NCI Clinical Center. Because insufficient dosing or adverse event data are currently available on the use of atezolizumab in younger pediatric patients, children <2 years of age are excluded from this study, but may be eligible for future pediatric trials.

14.3 Evaluation of Benefits and Risks/Discomforts

There may or may not be any clinical benefit to a patient from participation in this trial. Their participation will benefit future cancer patients. Potential risks include the possible occurrence of any of a range of side effects that are listed in the consent document. The procedure for protecting against or minimizing risks will be to medically evaluate patients as described in Section 5 and Section 6. Although no compensation is available, any injury will be fully evaluated and treated in keeping with the benefits or care to which participants are entitled under applicable regulations. A Certificate of Confidentiality has been obtained to help protect the privacy of all study participants.

14.4 Consent and Assent Process and Documentation

14.4.1 Written Consent

An associate or principal investigator on the trial will inform patients or the patient's parents or guardian if he/she is a child, of the purpose, alternatives, drug administration plan, research objectives, and follow-up of this trial. The patient will be provided a CIRB-approved consent for review and signature and his/her questions will be answered. After a decision is made to enroll into the study, a signature will be obtained from the patient. The original signed consent goes to

each participating site's Medical Records; a copy will be placed in the research record.

All patients must have a signed informed consent form and an on-study (confirmation of eligibility) form filled out and signed by a participating investigator before entering on study.

This protocol is open to pediatric patients ≥2 years of age at the NCI clinical center and ≥12 years at other participating sites. The investigators are requesting a waiver from the CIRB to allow only one parent to sign the informed consent to enter a child on the protocol. Because many patients must travel to the NIH from long distances at substantial expense, requiring both parents to be present for the consent process could be a financial hardship for many families. Where deemed appropriate by the clinician and the child's parents or guardian, the child will also be included in all discussions about the trial and verbal or written assent will be obtained depending on the age of the child.

In situations where there is joint custody of a child, both parents must sign consent. If only one parent can be present, the other parent's consent can be obtained by telephone via the procedure described in Section 14.4.3. When guardianship status of the child is uncertain, documentation of custody status must be obtained.

14.4.2 Assent

Where deemed appropriate by the clinician and the child's parents or guardian, the child will also be included in all discussions about the trial and assent will be obtained. Investigators may refer to or distribute the pediatric information sheets provided in Appendix C and Appendix D when explaining the study to patients ages 7-17. As appropriate, verbal assent will be obtained from patients ages 7-13 and written assent will be obtained from patients ages 14-17. Assent of children age 14 and older is a necessary condition for proceeding with the research; these patients (ages 14-17) should be informed about the study using the pediatric informed consent document. Children under the age of 14 will not be required to provide assent because their capacity is so limited. Regardless of the age of the child, the parent or guardian will sign the designated line on the pediatric informed consent granting permission for the child's participation and, for patients 7 years of age and older, attesting to the fact that the child has given assent.

All children will be contacted after they have reached the age of 18 to determine whether they wish to continue on the trial and informed consent will be obtained from them at that time. The signature process for obtaining pediatric consent and assent may be adapted as necessary by participating institutions to adhere to local guidelines.

14.4.3 Telephone Consent

In situations where there is joint custody of a child, and only one parent can be present, the other parent's consent can be obtained by telephone via the remote consent procedure outlined on the NCI CIRB's website: https://ncicirb.org/content/frequently-asked-questions-regarding-remote-consent-procedures.

The parent must receive a copy of the informed consent document (e.g., via mail, fax or email) in advance of discussion regarding the study. The investigator/designee must implement a method to ensure the identity of the parent. The investigator/designee must have the same consent discussion via telephone/video conferencing that they would have during an in-person meeting and there must be a witness who can hear both sides of the conversation. The witness's signature (if required by the site) or name and the date of the original consenting phone call should be recorded in the research records to document the participation of the witness.

Once the research team receives the signed informed consent document from the parent, the investigator/designee who conducted the consent process must sign and date the document using the current date. Under the signature line, the investigator/designee must document whether consent was obtained over the telephone or video conferencing, the date of the telephone/video conference, the date the signed consent was received, and the reason the remote consent procedure was utilized. The date the investigator/designee signs the informed consent document, not the date the consent discussion with the parent took place, is the official date of informed consent for the participant on the trial.

The final informed consent document must be filed in the designated investigator/site regulatory file location. A copy of the final informed consent document, signed by the parent, the investigator, and the witness (if applicable), must be sent back to the participant via email/scan, fax, or postal mail. No research activities related to the study can begin until all steps of the informed consent process are complete.

14.4.4 Participation of Subjects Unable to Give Consent

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary, and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason, and because there is a prospect of direct benefit from research participation, all subjects ≥ age 18 **at the NCI only** will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team for evaluation. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MEC Policy 87-4 for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

14.4.5 Informed Consent of Non-English Speaking Subjects (at the NCI only)

We do not anticipate consistent enrollment of any particular group of non-English speaking research participants into this study. In the event there is consistent enrollment of one non-English speaking group (i.e., more than 5 subjects from one non-English speaking group), the IRB approved full consent document will be translated into that language in accordance with the Clinical MAS Policy M77-2 at that time.

If there is an unexpected enrollment of a research participant for whom there is no translated extant IRB approved consent document, the PI and/or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in NCI Clinical SOP PM-2, MAS Policy M77-2, OSHRP SOP 12, 45 CFR 46.117 (b) (2), 21 CFR 50.27 (b) (2)). The summary that will be used is the English version of the extant IRB approved consent document. Signed copies of both the English version of the consent and the translated short form will be given to the subject or their legally authorized representative and the signed original will be filed in the medical record.

Unless the authorized individual obtaining consent is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation (using either the long translated form or the short form). Preferably someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of the relevant consent documents well before the consent conversation with the subject (24 to 48 hours if possible).

We request prospective IRB approval of the use of the short form process for non-English speaking subjects and will notify the IRB at the time of continuing review of the frequency of use of the Short Form. The Short Form process will be used no more than 5 times per language, after which the full consent document will be translated into that language.

14.5 Patient Advocate

At the NCI only, the patients' rights representative is available to patients receiving treatment on this protocol at the NIH Clinical Center at (301) 496-2626 in Building 10 of the Clinical Research Center, Room [redacted], on the Bethesda NIH campus. Patients will be informed that they can contact the study PI or RN at any time with questions about their medical care, and that the patients' rights representative is also available to answer non-medical questions about the study.

15. REFERENCES

1. Dim, D.C., L.D. Cooley, and R.N. Miranda, *Clear cell sarcoma of tendons and aponeuroses: a review.* Arch Pathol Lab Med, 2007. **131**(1): p. 152-6.
2. Ozuguz, P., et al., *Clear cell sarcoma.* Indian Dermatol Online J, 2014. **5**(4): p. 488-90.
3. Juel, J. and R.M. Ibrahim, *A case of clear cell sarcoma-A rare malignancy.* Int J Surg Case Rep, 2017. **36**: p. 151-154.
4. Hocar, O., et al., *Clear cell sarcoma (malignant melanoma) of soft parts: a clinicopathologic study of 52 cases.* Dermatol Res Pract, 2012. **2012**: p. 984096.
5. Antonescu, C.R., et al., *EWS-CREB1: a recurrent variant fusion in clear cell sarcoma--association with gastrointestinal location and absence of melanocytic differentiation.* Clin Cancer Res, 2006. **12**(18): p. 5356-62.
6. Gonzaga, M.I., et al., *The epidemiology and survivorship of clear cell sarcoma: a National Cancer Database (NCDB) review.* Journal of Cancer Research and Clinical Oncology, 2018. **144**(9): p. 1711-1716.
7. Ferrari, A., et al., *Clear cell sarcoma of tendons and aponeuroses in pediatric patients - A report from the Italian and German Soft Tissue Sarcoma Cooperative Group.* Cancer, 2002. **94**(12): p. 3269-3276.
8. Li, A.B., et al., *Prognostic Factors for Survival in Patients with Clear Cell Sarcoma: An Analysis of the Surveillance, Epidemiology, and End Results (SEER) Database.* Medical Science Monitor, 2019. **25**: p. 6939-6945.
9. Wagner, M.J., et al., *Chemotherapy for Bone Sarcoma in Adults.* J Oncol Pract, 2016. **12**(3): p. 208-16.
10. Giuffrida, A.Y., et al., *Chondrosarcoma in the United States (1973 to 2003): an analysis of 2890 cases from the SEER database.* J Bone Joint Surg Am, 2009. **91**(5): p. 1063-72.
11. Angelini, A., et al., *Clinical outcome of central conventional chondrosarcoma.* J Surg Oncol, 2012. **106**(8): p. 929-37.
12. Mercuri, M., et al., *Dedifferentiated chondrosarcoma.* Skeletal Radiol, 1995. **24**(6): p. 409-16.
13. Amary, M.F., et al., *IDH1 and IDH2 mutations are frequent events in central chondrosarcoma and central and periosteal chondromas but not in other mesenchymal tumours.* J Pathol, 2011. **224**(3): p. 334-43.
14. Lugowska, I., et al., *IDH1/2 Mutations Predict Shorter Survival in Chondrosarcoma.* J Cancer, 2018. **9**(6): p. 998-1005.
15. Nicolle, R., et al., *Integrated molecular characterization of chondrosarcoma reveals critical determinants of disease progression.* Nature Communications, 2019. **10**.
16. Zhu, G.G., et al., *Genomic Profiling Identifies Association of IDH1/IDH2 Mutation with Longer Relapse-Free and Metastasis-Free Survival in High-Grade Chondrosarcoma.* Clinical Cancer Research, 2020. **26**(2): p. 419-427.
17. Behjati, S., et al., *Distinct H3F3A and H3F3B driver mutations define chondroblastoma and giant cell tumor of bone.* Nat Genet, 2013. **45**(12): p. 1479-82.
18. Wu, A.M., et al., *Chondrosarcoma in a paediatric population: a study of 247 cases.* Journal of Childrens Orthopaedics, 2019. **13**(1): p. 89-99.
19. Serlo, J., et al., *Incidence, treatment and survival of paediatric patients with bone sarcomas in Finland from 1991 to 2005.* Acta Paediatrica, 2015. **104**(7): p. 738-745.

20. Thorkildsen, J., et al., *Chondrosarcoma in Norway 1990-2013; an epidemiological and prognostic observational study of a complete national cohort.* Acta Oncologica, 2019. **58**(3): p. 273-282.
21. Butte, M.J., et al., *Programmed Death-1 Ligand 1 Interacts Specifically with the B7-1 Costimulatory Molecule to Inhibit T Cell Responses.* Immunity, 2007. **27**(1): p. 111-122.
22. Herbst, R.S., et al., *Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients.* Nature, 2014. **515**(7528): p. 563-567.
23. Thompson, R.H., et al., *Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up.* Cancer Res, 2006. **66**(7): p. 3381-5.
24. Hamanishi, J., et al., *Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer.* Proc Natl Acad Sci U S A, 2007. **104**(9): p. 3360-5.
25. Okazaki, T. and T. Honjo, *PD-1 and PD-1 ligands: from discovery to clinical application.* Int Immunol, 2007. **19**(7): p. 813-24.
26. Hino, R., et al., *Tumor cell expression of programmed cell death-1 ligand 1 is a prognostic factor for malignant melanoma.* Cancer, 2010. **116**(7): p. 1757-66.
27. Blank, C., T.F. Gajewski, and A. Mackensen, *Interaction of PD-L1 on tumor cells with PD-1 on tumor-specific T cells as a mechanism of immune evasion: implications for tumor immunotherapy.* Cancer Immunol Immunother, 2005. **54**(4): p. 307-14.
28. Keir, M.E., et al., *PD-1 and its ligands in tolerance and immunity.* Annu Rev Immunol, 2008. **26**: p. 677-704.
29. Blank, C. and A. Mackensen, *Contribution of the PD-L1/PD-1 pathway to T-cell exhaustion: an update on implications for chronic infections and tumor evasion.* Cancer Immunology, Immunotherapy, 2007. **56**(5): p. 739-745.
30. Chen, D.S., B.A. Irving, and F.S. Hodi, *Molecular Pathways: Next-Generation Immunotherapy—Inhibiting Programmed Death-Ligand 1 and Programmed Death-1.* Clinical Cancer Research, 2012. **18**(24): p. 6580-6587.
31. Sznol, M. and L. Chen, *Antagonist Antibodies to PD-1 and B7-H1 (PD-L1) in the Treatment of Advanced Human Cancer—Response.* Clinical Cancer Research, 2013. **19**(19): p. 5542.
32. Iwai, Y., et al., *Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade.* Proceedings of the National Academy of Sciences, 2002. **99**(19): p. 12293-12297.
33. Strome, S.E., et al., *B7-H1 Blockade Augments Adoptive T-Cell Immunotherapy for Squamous Cell Carcinoma.* Cancer Research, 2003. **63**(19): p. 6501-6505.
34. Powles, T., et al., *MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer.* Nature, 2014. **515**(7528): p. 558-62.
35. Cornillie, J., et al., *Biology and management of clear cell sarcoma: state of the art and future perspectives.* Expert Review of Anticancer Therapy, 2016. **16**(8): p. 839-845.
36. Scheinberg, T., et al., *PD-1 blockade using pembrolizumab in adolescent and young adult patients with advanced bone and soft tissue sarcoma*. 2017, American Society of Clinical Oncology.
37. Marcrom, S., J.F. De Los Santos, and R.M. Conry, *Complete response of mediastinal clear cell sarcoma to pembrolizumab with radiotherapy.* Clin Sarcoma Res, 2017. **7**: p. 14.
38. Merchant, M.S., et al., *Phase I Clinical Trial of Ipilimumab in Pediatric Patients with*

Advanced Solid Tumors. Clin Cancer Res, 2016. **22**(6): p. 1364-70.
39. MacDonald, I.J., et al., *An update on current and future treatment options for chondrosarcoma.* Expert Review of Anticancer Therapy, 2019. **19**(9): p. 773-786.
40. Thanindratarn, P., et al., *Advances in immune checkpoint inhibitors for bone sarcoma therapy.* Journal of Bone Oncology, 2019. **15**.
41. Kostine, M., et al., *Analysis of PD-L1, T-cell infiltrate and HLA expression in chondrosarcoma indicates potential for response to immunotherapy specifically in the dedifferentiated subtype.* Mod Pathol, 2016. **29**(9): p. 1028-37.
42. Yang, X., et al., *Expression of PD-L1/PD-L2 is associated with high proliferation index of Ki-67 but not with TP53 overexpression in chondrosarcoma.* International Journal of Biological Markers, 2018. **33**(4): p. 507-513.
43. Simard, F.A., et al., *Description of the immune microenvironment of chondrosarcoma and contribution to progression.* Oncoimmunology, 2017. **6**(2).
44. Wagner, M.J., et al., *Response to PD1 inhibition in conventional chondrosarcoma.* J Immunother Cancer, 2018. **6**(1): p. 94.
45. Paoluzzi, L., et al., *Response to anti-PD1 therapy with nivolumab in metastatic sarcomas.* Clin Sarcoma Res, 2016. **6**: p. 24.
46. Tawbi, H.A., et al., *Pembrolizumab in advanced soft-tissue sarcoma and bone sarcoma (SARC028): a multicentre, two-cohort, single-arm, open-label, phase 2 trial.* Lancet Oncol, 2017. **18**(11): p. 1493-1501.
47. Potter, J.W., K.B. Jones, and J.J. Barrott, *Sarcoma-The standard-bearer in cancer discovery.* Crit Rev Oncol Hematol, 2018. **126**: p. 1-5.
48. Przybyl, J., et al., *Metastatic potential is determined early in synovial sarcoma development and reflected by tumor molecular features.* Int J Biochem Cell Biol, 2014. **53**: p. 505-13.
49. Emens, L.A., et al., *Toward integrative cancer immunotherapy: targeting the tumor microenvironment.* J Transl Med, 2012. **10**: p. 70.
50. Srivastava, A.K., et al., *Effect of a Smac Mimetic (TL32711, Birinapant) on the Apoptotic Program and Apoptosis Biomarkers Examined with Validated Multiplex Immunoassays Fit for Clinical Use.* Clinical Cancer Research, 2016. **22**(4): p. 1000-1010.
51. Parchment, R.E., et al., *Immuno-Pharmacodynamics (Immuno-PD) for Evaluating Mechanism of Action and Developing Immunotherapy Combinations.* Seminars Oncology, in press, 2016.
52. Yudushkin, I.A. and R.D. Vale, *Imaging T-cell receptor activation reveals accumulation of tyrosine-phosphorylated CD3ζ in the endosomal compartment.* Proceedings of the National Academy of Sciences, 2010. **107**(51): p. 22128-22133.
53. Amin, S., et al., *Breast Cancer Cells Proliferation Is Regulated by Tyrosine Phosphatase SHP1 through c-jun N-Terminal Kinase and Cooperative Induction of RFX-1 and AP-4 Transcription Factors.* Molecular Cancer Research, 2011. **9**(8): p. 1112-1125.
54. Wang, H., et al., *ZAP-70: An Essential Kinase in T-cell Signaling.* Cold Spring Harbor Perspectives in Biology, 2010. **2**(5): p. a002279.
55. Roszik, J., et al., *Novel algorithmic approach predicts tumor mutation load and correlates with immunotherapy clinical outcomes using a defined gene mutation set.* BMC Medicine, 2016. **14**: p. 168.
56. Johnson, D.B., et al., *Targeted Next Generation Sequencing Identifies Markers of Response to PD-1 Blockade.* Cancer Immunology Research, 2016. **4**(11): p. 959-967.

57. Schwartz, G.J. and B. Gauthier, *A simple estimate of glomerular filtration rate in adolescent boys.* J Pediatr, 1985. **106**(3): p. 522-6.
58. Di Giacomo, A.M., M. Biagioli, and M. Maio, *The emerging toxicity profiles of anti-CTLA-4 antibodies across clinical indications.* Semin Oncol, 2010. **37**(5): p. 499-507.
59. Eisenhauer, E.A., et al., *New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1).* Eur J Cancer, 2009. **45**(2): p. 228-47.
60. Seymour, L., et al., *iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics.* Lancet Oncol, 2017. **18**(3): p. e143-e152.
61. Bubley, G.J., et al., *Eligibility and response guidelines for phase II clinical trials in androgen-independent prostate cancer: recommendations from the Prostate-Specific Antigen Working Group.* J Clin Oncol, 1999. **17**(11): p. 3461-7.
62. Rustin, G.J., et al., *Re: New guidelines to evaluate the response to treatment in solid tumors (ovarian cancer).* J Natl Cancer Inst, 2004. **96**(6): p. 487-8.
63. Scher, H.I., et al., *Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group.* J Clin Oncol, 2008. **26**(7): p. 1148-59.
64. Vergote, I., et al., *Re: new guidelines to evaluate the response to treatment in solid tumors [ovarian cancer]. Gynecologic Cancer Intergroup.* J Natl Cancer Inst, 2000. **92**(18): p. 1534-5.

APPENDIX A: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale		Lansky Scale (age <16 years)	
Grade	Description	Percent	Description	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.	100	Fully active.
		90	Able to carry on normal activity; minor signs or symptoms of disease.	90	Minor restriction in physically strenuous play.
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.	80	Restricted in strenuous play, tires more easily, otherwise active.
		70	Cares for self, unable to carry on normal activity or to do active work.	70	Both greater restrictions of, and less time spent, in active play
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.	60	Ambulatory up to 50% of time, limited active play with assistance/ supervision
		50	Requires considerable assistance and frequent medical care.	50	Considerable assistance required for any active play, fully able to engage in active play
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.	40	Able to initiate quiet activities
		30	Severely disabled, hospitalization indicated. Death not imminent.	30	Needs considerable assistance for quiet activity
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.	20	Limited to very passive activity initiated by others (e.g., TV)
		10	Moribund, fatal processes progressing rapidly.	10	Completely disabled, not even passive play
5	Dead.	0	Dead.	0	Dead.

APPENDIX B: PATIENT DRUG INFORATION 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. 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:

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. Immunostimulatory agents, including but not limited to interferon alfa, interferon gamma, tumor necrosis factor-alpha blockers, or interleukin-2 (aldesleukin), 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. Live vaccines and live, attenuated vaccines (prohibited during the study and for 100 days after the last dose of study drug) are prohibited.

Initiation of granulocyte colony-stimulating factors (e.g., filgrastim and biosimilar products, sargramostim and/or pegfilgrastim) should be discussed with the study's Principal Investigator. 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 and they 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:

APPENDIX C: CS PEDIATRIC INFORMATION SHEET (FOR PATIENTS 7-17 YEARS)

INFORMATION SHEET FOR RESEARCH STUDY P10398

A research study testing a new drug, atezolizumab, in your type of cancer called chondrosarcoma

1. We have been talking with you about your illness, a type of cancer called chondrosarcoma. This type of cancer grows in your bones. It can grow in different parts of your body like your legs, arms, toes, fingers, pelvis, and shoulders. It can cause pain and swelling.

2. We want to help you and other young patients with chondrosarcoma by doing a research study. A research study is a way to learn more about a new drug such as atezolizumab and its uses. We are asking you to take part because we want to test the drug in chondrosarcoma. We will test the drug by giving it to people with chondrosarcoma and watching to see if their cancer shrinks. We do not yet know how well the new drug will work in children, teens, or adults.

3. You do not have to be in this research study if you do not want to be. You can say no and no one will be mad at you. If you decide to stop after we begin, that's okay too. If you do not want to be in this research study, we will tell you what other kinds of treatments there are for you (or other research studies).

4. There are some things about this study that you should know. There are things that take a long time, things that might make you uncomfortable, and things that share some of your private information with the researchers. If you agree to take part in this research study, we will give you a newly invented, experimental drug called atezolizumab. A newly invented, experimental drug is one that is not usually given to anyone with your type of cancer yet. You will be given the drug through a vein in your arm (sometimes people call this way of getting a drug "IV"). The drug will be given once every three weeks. Atezolizumab might have some side effects on your body. It is not possible to know now if atezolizumab will shrink your cancer compared to the usual approach.

5. Atezolizumab works by helping your immune system. Your immune system is the part of your body that fights germs and prevents you from getting sick. We hope that giving you atezolizumab will make your immune system fight your chondrosarcoma so that your tumors will stop growing.

6. During the study, you will have imaging tests (like CT scans or X-ray scans) to help the doctors see inside of you. This will help your doctors to figure out if the chondrosarcoma is getting worse, staying the same, or getting better. You will also have your blood drawn every week for three weeks so that we can check if the drug is still safe for you. Later, you will have your blood drawn once every three weeks to check if parts of your body, like your liver and kidneys, are still working right. You have probably had these types of tests already.

7. If you are 12 years of age or older, you will need to have extra blood samples collected for the study. This blood will be used for research. It will help the researchers understand how atezolizumab is working. Your doctor will tell you if your research blood collections will happen approximately every 3 weeks or

every 6 weeks. Each time we collect research blood it will be about 1 or 2 teaspoons of blood. Sometimes we will collect this research blood at the same time that we draw blood for safety tests. There might be other times when we only collect research blood from you. Each time we collect your blood, there is a chance that you will have mild pain, bleeding, bruising, or infection at the place where the needle poked your skin. Fainting or light-headedness can sometimes happen, but they usually last only a few minutes.

8. Not everyone who takes part in this study will benefit. A benefit means that something good happens to you. We hope that a benefit to you for being part of this study will be that your symptoms improve, and the chondrosarcoma tumor shrinks. But we do not know for sure if there is any benefit of being part of this study.

9. All drugs have some bad effects. If you take part in this research study, you may have some of these effects or you may have very few. Some things you might have are fever, throwing up, and feeling tired. Your doctor will talk to you about other things that can happen, and it is okay to ask questions about bad effects. Tell your doctors and nurses about any bad effects you have.

10. If you are 12 years old or older, we will do research tests called genetic sequencing to read the "instruction books" called genes that are in your normal blood cells and cancer cells. We will not tell you or your parents anything about your genes.

11. Please talk this over with your parents before you decide whether or not to take part. We will also ask your parents to give their permission for you to take part in this study. But even if your parents say "yes" you can still decide not to do this. You and your family can choose to be part of this study or not. You and your family can also decide to stop being in this study at any time, even after you start.

12. There may be other treatments or research studies for your chondrosarcoma that your doctors can tell you about. Your doctors will continue to treat you whether or not you participate in this study. Make sure to ask your doctors any questions that you have. <u>It is always okay to ask questions.</u> If you have a question later that you didn't think of now, you can call me at _____ *(insert telephone number)* or ask me next time.

APPENDIX D: CCS PEDIATRIC INFORMATION SHEET (FOR PATIENTS 7-17 YEARS)

INFORMATION SHEET FOR RESEARCH STUDY P10398

A research study testing a new drug, atezolizumab, in your type of cancer called clear cell sarcoma

1. We have been talking with you about your illness, a type of cancer called clear cell sarcoma ("CCS cancer"). This type of cancer can grow in different places in your body. It can grow on your legs, feet, arms, hands, and even around your tummy, head, or neck. It can make bumps under your skin.

2. We want to help you and other young patients with CCS cancer by doing a research study. A research study is a way to learn more about a new drug such as atezolizumab and its uses. We are asking you to take part because we want to test the drug in CCS cancer. We will test the drug by giving it to people with CCS cancer and watching to see if their cancer shrinks. We do not yet know how well the new drug will work in children, teens, or adults.

3. You do not have to be in this research study if you do not want to be. You can say no and no one will be mad at you. If you decide to stop after we begin, that's okay too. If you do not want to be in this research study, we will tell you what other kinds of treatments there are for you (or other research studies).

4. There are some things about this study that you should know. There are things that take a long time, things that might make you uncomfortable, and things that share some of your private information with the researchers. If you agree to take part in this research study, we will give you a newly invented, experimental drug called atezolizumab. A newly invented, experimental drug is one that is not usually given to anyone with your type of cancer yet. You will be given the drug through a vein in your arm (sometimes people call this way of getting a drug "IV"). The drug will be given once every three weeks. Atezolizumab might have some side effects on your body. It is not possible to know now if atezolizumab will shrink your cancer compared to the usual approach.

5. Atezolizumab works by helping your immune system. Your immune system is the part of your body that fights germs and prevents you from getting sick. We hope that giving you atezolizumab will make your immune system fight your CCS cancer so that your tumors will stop growing.

6. During the study, you will have imaging tests (like CT scans or X-ray scans) to help the doctors see inside of you. This will help your doctors to figure out if the CCS cancer is getting worse, staying the same, or getting better. You will also have your blood drawn every week for three weeks so that we can check if the drug is still safe for you. Later, you will have your blood drawn once every three weeks to check if parts of your body, like your liver and kidneys, are still working right. You have probably had these types of tests already.

7. If you are 12 years of age or older, you will need to have extra blood samples collected for the study. This blood will be used for research. It will help the researchers understand how atezolizumab is working. Your doctor will tell you if your research blood collections will happen approximately every 3 weeks or

every 6 weeks. Each time we collect research blood it will be about 1 or 2 teaspoons of blood. Sometimes we will collect this research blood at the same time that we draw blood for safety tests. There might be other times when we only collect research blood from you. Each time we collect your blood, there is a chance that you will have mild pain, bleeding, bruising, or infection at the place where the needle poked your skin. Fainting or light-headedness can sometimes happen, but they usually last only a few minutes.

8. Not everyone who takes part in this study will benefit. A benefit means that something good happens to you. We hope that a benefit to you for being part of this study will be that your symptoms improve, and the CCS tumor shrinks. But we do not know for sure if there is any benefit of being part of this study.

9. All drugs have some bad effects. If you take part in this research study, you may have some of these effects or you may have very few. Some things you might have are fever, throwing up, and feeling tired. Your doctor will talk to you about other things that can happen, and it is okay to ask questions about bad effects. Tell your doctors and nurses about any bad effects you have.

10. If you are 12 years old or older, we will do research tests called genetic sequencing to read the "instruction books" called genes that are in your normal blood cells and cancer cells. We will not tell you or your parents anything about your genes.

11. Please talk this over with your parents before you decide whether or not to take part. We will also ask your parents to give their permission for you to take part in this study. But even if your parents say "yes" you can still decide not to do this. You and your family can choose to be part of this study or not. You and your family can also decide to stop being in this study at any time, even after you start.

12. There may be other treatments or research studies for your CCS cancer that your doctors can tell you about. Your doctors will continue to treat you whether or not you participate in this study. Make sure to ask your doctors any questions that you have. <u>It is always okay to ask questions.</u> If you have a question later that you didn't think of now, you can call me at _____ *(insert telephone number)* or ask me next time.

APPENDIX E: BLOOD AND TISSUE COLLECTION WORKSHEETS/ADULT AND PEDIATRIC OVER 12

Date:	**SAMPLE COLLECTION SHEET: Baseline, up to 8 days prior to starting treatment**		
CTEP Protocol 10398 **Atezolizumab Dose:** **Patient ID:** *****900 series instructions: DCTD SOP LHTP003.08.15*****	**Ht:** **Wt:** **BSA:**	**See Section 9 for shipping details & contact info**	**Research Nurse:** Phone: **PI:** Phone:

PLEASE LABEL EACH TUBE WITH ACTUAL DATE AND TIME OF SAMPLE COLLECTION

Day	Time	Instructions	Ideal Time	Actual Time	Record comments (i.e., if collection missed), and sign each time you collect a sample
	Prior to drug administration; (at time of baseline tumor biopsy if biopsied)	**NCI Only** **PD 900** 6 mL NaHep Label tube: sample number, date and time **Room temperature**			
	Prior to drug administration; (at time of baseline tumor biopsy if biopsied)	**GN 800** Two 10 mL Streck (Germline and ctDNA) Label tube: sample number, date and time			
	Prior to drug administration	**PD 500** tumor biopsy Label tube: sample number, date and time			

Date:	**SAMPLE COLLECTION SHEET: Cycle 1**				
CTEP Protocol 10398 **Atezolizumab Dose:** **Patient ID:** *****900 series instructions: DCTD SOP LHTP003.08.15*****		**Ht:** **Wt:** **BSA:**	**See Section 9 for shipping details & contact info**		**Research Nurse:** Phone: **PI:** Phone:
PLEASE LABEL EACH TUBE WITH ACTUAL DATE AND TIME OF SAMPLE COLLECTION					
Day	**Time**	**Instructions**	**Ideal Time**	**Actual Time**	**Record comments (i.e., if collection missed), and sign each time you collect a sample**
Day 15	**Prior to drug administration**	**NCI Only** **PD 901** 6 mL NaHep Label tube: sample number, date and time **Room temperature**			

Date:	**SAMPLE COLLECTION SHEET: Cycle 2**				
CTEP Protocol 10398 **Atezolizumab Dose:** **Patient ID:** *****900 series instructions: DCTD SOP LHTP003.08.15*****		**Ht:** **Wt:** **BSA:**	**See Section 9 for shipping details & contact info**		**Research Nurse:** Phone: **PI:** Phone:
PLEASE LABEL EACH TUBE WITH ACTUAL DATE AND TIME OF SAMPLE COLLECTION					
Day	**Time**	**Instructions**	**Ideal Time**	**Actual Time**	**Record comments (i.e., if collection missed), and sign each time you collect a sample**
Day 1	**Prior to drug administration**	**NCI Only** **PD 902** 6 mL NaHep Label tube: sample number, date and time **Room temperature**			
Day 1	**Prior to drug administration**	**GN 801** Two 10 mL Streck (ctDNA)			

Date:	**SAMPLE COLLECTION SHEET: Cycle 3**				
CTEP Protocol 10398 **Atezolizumab Dose:** **Patient ID:** *****900 series instructions: DCTD SOP LHTP003.08.15*****		**Ht:** **Wt:** **BSA:**	**See Section 9 for shipping details & contact info**		**Research Nurse:** Phone: **PI:** Phone:

PLEASE LABEL EACH TUBE WITH ACTUAL DATE AND TIME OF SAMPLE COLLECTION

Day	Time	Instructions	Ideal Time	Actual Time	Record comments (i.e., if collection missed), and sign each time you collect a sample
Day 1	**Prior to drug administration**	**NCI Only** **PD 903** 6 mL NaHep Label tube: sample number, date and time **Room temperature**			
Day 1	**Prior to drug administration; at time of on-treatment tumor biopsy (if biopsied)**	**GN 802** Two 10 mL Streck (ctDNA) Label tube: sample number, date and time			
Day 1 (± 3 days)	**Prior to drug administration**	**PD 501** tumor biopsy Label tube: sample number, date and time			
Day 21	**Restaging visit**	**GN 803** Two 10 mL Streck (ctDNA) Label tube: sample number, date and time			

Date:	**SAMPLE COLLECTION SHEET: Cycle 4**				
CTEP Protocol 10398 **Atezolizumab Dose:** **Patient ID:** *****900 series instructions: DCTD SOP LHTP003.08.15*****		**Ht:** **Wt:** **BSA:**			**Research Nurse:** Phone: **PI:** Phone:

PLEASE LABEL EACH TUBE WITH ACTUAL DATE AND TIME OF SAMPLE COLLECTION

Day	Time	Instructions	Ideal Time	Actual Time	Record comments (i.e., if collection missed), and sign each time you collect a sample
Day 1	**Prior to drug administration**	**NCI Only** **PD 904** 6 mL NaHep Label tube: sample number, date and time **Room temperature**			

Date:	**SAMPLE COLLECTION SHEET: Cycle 5**				
CTEP Protocol 10398 **Atezolizumab Dose:** **Patient ID:** *****900 series instructions: DCTD SOP LHTP003.08.15*****		**Ht:** **Wt:** **BSA:**	**See Section 9 for shipping details & contact info**		**Research Nurse:** Phone: **PI:** Phone:
PLEASE LABEL EACH TUBE WITH ACTUAL DATE AND TIME OF SAMPLE COLLECTION					
Day	**Time**	**Instructions**	**Ideal Time**	**Actual Time**	**Record comments (i.e., if collection missed), and sign each time you collect a sample**
Day 1	**Prior to drug administration**	**NCI Only** **PD 905** 6 mL NaHep Label tube: sample number, date and time **Room temperature**			
Day 21	**Restaging visit**	**GN 804** Two 10 mL Streck (ctDNA) Label tube: sample number, date and time			

Date:	**SAMPLE COLLECTION SHEET: Cycle 6 and onwards**				
CTEP Protocol 10398 **Atezolizumab Dose:** **Patient ID:** *****900 series instructions: DCTD SOP LHTP003.08.15*****		**Ht:** **Wt:** **BSA:**	**See Section 9 for shipping details & contact info**		**Research Nurse:** Phone: **PI:** Phone:
PLEASE LABEL EACH TUBE WITH ACTUAL DATE AND TIME OF SAMPLE COLLECTION					
Day	**Time**	**Instructions**	**Ideal Time**	**Actual Time**	**Record comments (i.e., if collection missed), and sign each time you collect a sample**
Day 1	**Prior to drug administration**	**NCI Only** **PD 90X** 6 mL NaHep Label tube: sample number, date and time **Room temperature**			
End of C9, C13, etc.	**Every other restaging visit**	**GN 80X** Two 10 mL Streck (ctDNA) Label tube: sample number, date and time			

Date:	**SAMPLE COLLECTION SHEET: Day of restaging follow-up biopsy or progression biopsy**				
CTEP Protocol 10398 **Atezolizumab Dose:** **Patient ID:** *****900 series instructions: DCTD SOP LHTP003.08.15*****		**Ht:** **Wt:** **BSA:**	**See Section 9 for shipping details & contact info**		**Research Nurse:** Phone: **PI:** Phone:
PLEASE LABEL EACH TUBE WITH ACTUAL DATE AND TIME OF SAMPLE COLLECTION					
Day	**Time**	**Instructions**	**Ideal Time**	**Actual Time**	**Record comments (i.e., if collection missed), and sign each time you collect a sample**
	Prior to drug administration	**NCI Only** **PD 90X** 6 mL NaHep Label tube: sample number, date and time **Room temperature**			
	Prior to drug administration; at time of tumor biopsy (if biopsied)	**GN 80X** Two 10 mL Streck (ctDNA) Label tube: sample number, date and time			
	Biopsy optional. Prior to drug administration	**PD 503** tumor biopsy Label tube: sample number, date and time			

APPENDIX F: SHIPPING MANIFEST FOR BLOOD SAMPLES FOR GENETIC ANALYSIS

Protocol 10398	Shipping date:	**Carrier:** FedEx (MoCha Account number)	
Ship FROM:	Ordering Physician/PI:	**Ship TO:**	MoCha Laboratory
Address:		**Address:**	Attn: [redacted] MoCha Histology Lab Frederick National Laboratory for Cancer Research Leidos Biomedical Research, Inc. 1050 Boyles Street Building 321 Room [redacted] Frederick, MD 21702
Contact Name:		**Contact Name:**	
Telephone:		**Telephone:**	
Email:		**Email:**	[redacted]

Item	Sample ID (Study#_SiteID_Patient#_Specimen Series ID)	Diagnosis	No. Tubes (w/ volumes)	Collection: Date	Collection: Time	Collection: Time Point	Storage Temp
1							
2							
3							
4							
5							
6							
7							
8							
		TOTAL No. ITEMS					

COMMENTS: *DO NOT include patient identifiers