Phase 2 Study of Ipilimumab Plus Nivolumab in Combination with Cryotherapy in Metastatic or Locally Advanced Soft Tissue Sarcoma

Coordinating Center
Stanford Cancer Institute
875 Blake Wilbur Drive
Stanford, CA 94305

Protocol Director
Kristen Ganjoo, MD
875 Blake Wilbur Dr
Stanford, CA 94305
650-924-0900
Email: Kganjoo@stanford.edu

Co-Investigators		
Nam Bui, MD 780 Welch Rd Palo Alto, CA 94304 Phone: 650-498-3483; Fax: 650-725-7760 nbui@stanford.edu	David Wang, MD Stanford Interventional Radiology 300 Pasteur Drive, H-3630 Stanford, CA 94305 Phone: 650-723-0728; Fax: 650-725-0533 davidwang@stanford.edu	Gloria Hwang, MD Stanford Interventional Radiology 300 Pasteur Drive, H-3630 Stanford, CA 94305 Phone: 650-723-0728; Fax: 650-725-0533 glhwang@stanford.edu
	Biostatistician Manisha Desai 1070 Arastradero Road, Rm 305 Palo Alto, CA 94304 Phone: 650-725-1946 Fax: 650-725-6906 manisha.desai@stanford.edu	**Study Coordinator** Maria Ahern 800 Welch Rd Palo Alto, CA 94304 Phone: 650-725-6413; Fax: 650-725-7760 mahern@stanford.edu

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PROTOCOL SYNOPSIS

TITLE	Phase 2 Study of Ipilimumab Plus Nivolumab in Combination with Cryotherapy in Metastatic or Locally Advanced Soft Tissue Sarcoma
STUDY PHASE	Phase 2
INDICATION	Soft tissue sarcoma
INVESTIGATIONAL PRODUCT OR PROCEDURE	Ipilimumab plus nivolumab in combination with cryotherapy
PRIMARY OBJECTIVE(S)	• Assess whether the rate of clinical benefit is sufficiently high to merit promise for further study
SECONDARY OBJECTIVE(S)	• Characterize the 6-month progression-free survival rate • Assess whether the treatment yields a reasonably safe and tolerable profile
EXPLORATORY OBJECTIVE(S)	• Correlate the mutation burden by NGS with response • Correlate PDL1 expression with response • Correlate tumor infiltrating lymphocytes/macrophages with response
TREATMENT SUMMARY	• Ipilimumab 1 mg/kg and nivolumab 3 mg/kg every 3 weeks for 4 doses followed by nivolumab 480 mg every 4 weeks until progression or intolerance (maximum 24 months). • Cryoablation of at least one tumor mass between Cycle 1 and 2 of above immunotherapy
SAMPLE SIZE	30
STATISTICAL CONSIDERATIONS	It is anticipated that 3 to 4 subjects will be enrolled per month. It is expected that 12 months will be required to accrue the number of subjects necessary to complete the trial. Treatment response will be assessed for each subject at 16 weeks. The null hypothesis is that the response rate (ie, sum of CR and PR) is ≤ 30%, & alternative hypothesis is that the response rate (RR) is ≥ 60%. The selection of 60% as a threshold for determining the treatment is worthy of further study is due to recent trials of sarcoma FDA approved drugs showing ~60% PFS rate at 3 months (ie, pazopanib, trabectedin, and eribulin).

STATISTICAL CONSIDERATIONS (continued)	A Simon-2-stage design will be used to address primary objective. The null hypothesis that the true response rate is 30% will be tested against a one-sided alternative. Our decision rule works as follows. In the first stage, we will accrue and assess 10 subjects. If 3 or fewer subjects (< 30% of 10 subjects) achieve a clinical response, the trial will be stopped for futility. If more than 3 subjects achieve a clinical response, we will enroll and treat an additional 18 subjects. If 12 or fewer of the 28 treated subjects (< 42.8%) demonstrate a clinical response (either CR or PR), we will conclude the treatment is not worthy of further study. If, however, more than 12 of the 28 treated subjects (≥ 42.8%) responds, we will claim the treatment promising. Assuming a true response rate of 60%, we have 90% power to correctly conclude the treatment promising. In contrast, if the true response rate is only 30%, we have a low probability (0.04) of incorrectly continuing to study the treatment. Rates of toxicity will be characterized by level of toxicity as well as by other subject-level characteristics. To address secondary objectives, we will use Kaplan-Meier methods to graphically depict PFS. This tool will also be used to demonstrate survival across various sub-groups of interest. Generalized linear regression methods (with a logit link) will be utilized to characterize relationships between features such as mutation burden, PDL1 expression, and tumor infiltrating lymphocytes and response.

SCHEMA

Baseline:
(Collect archival tumor tissue and blood)
(Routine labs and Scans)

Cycle 1: Ipilimumab plus nivolumab
(Routine labs and Blood for research)

(CRYOABLATION WITH BIOPSY IMMEDIATELY BEFORE ABLATION:
Procedure may be repeated)

Cycle 2: Ipilimumab plus nivolumab
(Routine labs and blood for research)

Cycle 3: Ipilimumab plus nivolumab
(Routine labs and blood for research)

Cycle 4: Ipilimumab plus nivolumab
(Routine labs and blood for research)

Response Scans

(Continue to maintenance Nivolumab if stable disease or response)
(Routine labs and blood for research)

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ADL	Activities of daily living
AE	Adverse event
BID	Twice daily
BSA	Body surface area
CBC	Complete blood count
CI	Confidence interval
CMAX	Maximum concentration of drug
CNS	Central nervous system
CRF	Case report form
CR	Complete response
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Toxicity
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
GI	Gastrointestinal
Hgb	Hemoglobin
HIV	Human Immunodeficiency Virus
HPF	High-power field
HTN	Hypertension
IRB	Institutional Review Board
irRECIST	Immune–related response evaluation criteria in solid tumors (RECIST)
IV	Intravenous
LLN	Lower limit of normal
MIBI	Multiplexed ion beam imaging
NGS	Next generation sequencing
OS	Overall survival
PLT	Platelets
PD	Progressive disease
PFS	Progression free survival
PR	Partial response
QD	Once daily
RECIST	Response evaluation criteria in solid tumors
RR	Response rate
SAE	Serious adverse event
SD	Stable disease
TTP	Time to progression
ULN	Upper limit of normal
UNK	Unknown
WBC	White blood cell
WHO	World Health Organization

1. OBJECTIVES

1.1. Primary Objectives

a. Assess whether the rate of clinical benefit is sufficiently high to merit promise for further study

1.2. Secondary Objectives

a. Characterize the 6-month progression-free survival (PFS) rate

b. Assess whether the treatment yields a reasonably safe and tolerable profile

1.3. Exploratory Objectives

a. Correlate the mutation burden by NGS with response

b. Correlate PDL1 expression with response

c. Correlate tumor infiltrating lymphocytes/macrophages with response

2. BACKGROUND

Immunotherapy with PD1 antibody, nivolumab, and CTLA-4 antibody, ipilimumab, has been administered safely in subjects with metastatic sarcoma. The combination of these two antibodies improved response rate and overall survival when compared to nivolumab alone (1). Radiation therapy of metastatic melanoma lesions while on immunotherapy has improved response rates both in the irradiated and non-radiated fields (2-5).

We have treated about 40 subjects with metastatic sarcoma with combination ipilimumab and nivolumab in the past 24 months. Many of these subjects had external beam radiation therapy while receiving immunotherapy. However, a minority of subjects were not able to receive radiation due to prior exposure or location of the metastatic lesions. These subjects were treated with cryoablation. We observed tumor responses in both non-ablated and ablated tumors. Theoretically, tumor antigens are released from cryotherapy which can augment the response to immunotherapy. Abdo *et al* proposed that by priming the immune system with CTLA-4 and PD1 inhibitors, cytotoxic CD8+ cell develop after tumor antigens are released by cryotherapy (6), thus facilitating tumor kill by the host's immune system.

In this study, we will prospectively study the response rate, toxicity and outcome in subjects with metastatic sarcoma when treated with cryotherapy while receiving immunotherapy with ipilimumab and nivolumab.

2.1 Study Disease

a. Metastatic or locally advanced soft tissue sarcomas with at least one tumor area amendable to ablation.

b. Subjects able to receive immunotherapy (No autoimmune disorders and not on chronic immunosuppression).

c. Subjects must have archival tumor samples available for Next Generation Sequencing.

2.2 Study Agents and Procedures

a. Ipilimumab

b. Nivolumab

c. Cryoablation

For clinicaltrials.gov compliance

The US Food and Drug Administration (FDA) has not approved ipilimumab nor nivolumab for the treatment of soft tissue sarcoma. An IND will be submitted by the principal investigator of this study; however, it is possible that the FDA may deem this study to be IND-exempt.

2.3 Rationale

Subjects with relapsed or refractory metastatic sarcoma have a dismal prognosis. Response rates to conventional therapy are generally very low, in the range of 10-20%, and new therapeutic approaches are needed for this disease.7-11

Most subjects with soft tissue sarcomas (STS) are treated with gemcitabine and docetaxel, and some with pazopanib if they tolerate the drug. However, subjects do not have many options following these treatments. Pazopanib will give less than a 10% response rate and has many side effects, and it only improves PFS by 3 months.6 Gemcitabine and docetaxel give less than a 20% response rate, and subjects are unable to take these two drugs long-term due to side effects.1 These are not optimal therapies for STS. This is why we are bringing in immunotherapy and cryoablation therapy, as we hope that they will improve outcome. Based on the encouraging results from the early clinical experience in various types of solid tumors, a clinical study which combines anti-PD-1 and anti-CTLA4 IT with ablation to treat subjects with relapsed or refractory metastatic sarcoma shows great promise to enhance antitumor immune response and improve clinical outcomes.

2.4 Study Design

- The primary purpose for the protocol is treatment
- The interventional model is non-randomized single group
- One intervention arm
    - Ipilimumab 1 mg/kg with nivolumab 3 mg/kg every 3 weeks x 4 doses. (One cycle of treatment is 3 weeks)

- Cryoablation of at least one tumor mass between Cycle 1 to 2 of above immunotherapy
- Maintenance therapy with nivolumab 480 mg every 4 weeks (up to 24 months). (Each cycle of maintenance therapy is 4 weeks).
- Additional ablations are allowed per investigator's discretion

- Subject number: 30
- Primary endpoints
  - Clinical benefit (CR, PR, SD) based on the response evaluation criteria in solid tumors (RECIST) criteria v1.1 and Immune-related RECIST (irRECIST), assessed at 16 weeks.
- Secondary endpoints
  - 6-month progression-free survival
  - Safety and tolerability
  - Correlative measures (See below)

2.5 Correlative Studies (Dr van de Rijn and Dr Katherine Ferrera) [12-14]

2.5.1. Archival tumor samples:

Histology, immunohistochemistry for lymphocyte subsets (include multi-marker studies such as MIBI or other) and for Gene Expression profiling with additional analysis of immune subsets by Cibersort and for Gene Expression profiling within the immune cells by CibersortX. The latter dataset can be validated by performing LCM-SMART-3SEQ.

2.5.2 Tumor samples collected throughout study:

Histology, immunohistochemistry for lymphocyte subsets (include multi-marker studies such as MIBI or other) and for Gene Expression profiling (GEP) with additional analysis of immune subsets by Cibersort and for GEP within the immune cells by CibersortX. The latter dataset can be validated by performing LCM-SMART-3SEQ.

2.5.3 Blood collection:

- Determine ctDNA levels
- Gene expression profiling on blood lymphocytes, followed by CibersortX to assess subsets.
  - Gene expression profiling on blood lymphocytes: It is estimated that there will be approximately 20 million white blood cells in 2.5 mL of blood drawn into a PAXgene tube. Two 2.5-mL tubes will be collected at each time point, so approximately 40 million white blood cells will be collected in that volume of blood. Gene expression profiles in blood and tumor may be compared over time.
- Perform flow with purification of T-cells, followed by gene expression profiling

- Perform proteomic analysis for cytokines

3. PARTICIPANT SELECTION AND ENROLLMENT PROCEDURES

3.1 Inclusion Criteria

3.1.1 Unresectable or metastatic soft tissue sarcoma

3.1.2 ≥ 1 prior systemic therapy for sarcoma, including adjuvant systemic therapy

3.1.3 Age ≥ 18 years

3.1.4 Life expectancy > 3 months

3.1.5 Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1

3.1.6 Lab values as below:

- Absolute neutrophil count (ANC) ≥ 1,000/mm^3
- Platelet count ≥ 75,000/mm^3
- Creatinine ≤ 1.5 x upper limit of normal (ULN) OR calculated (calc.) creatinine clearance > 45 mL/min using the lean body mass formula only
- Total bilirubin ≤ 1.5 x ULN in absence of Gilbert disease (total bilirubin ≤ 3 x ULN with Gilbert); also, if hyperbilirubinemia is clearly attributed to liver metastases total bilirubin ≤ 3 x ULN is permitted
- AST/ALT ≤ 3 x ULN
- Thyroid stimulating hormone (TSH) within normal limits (WNL); supplementation is acceptable to achieve a TSH WNL; in subjects with abnormal TSH if free T4 is normal and subject is clinically euthyroid, subject is eligible

3.1.7 Any toxic effects of prior therapy (except alopecia) must be resolved to NCI CTCAE, version 5.0, Grade 1 or less

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

3.1.9 Women of childbearing potential (WOCBP) receiving nivolumab must be willing to adhere to contraception for a period of 5 months after the last dose of nivolumab. Men receiving nivolumab and who are sexually active with WOCBP will be instructed and must be willing to adhere to contraception for a period of 7 months after the last dose of nivolumab.

3.2 Exclusion Criteria

3.2.1 Prior therapy with ipilimumab or nivolumab, or any agent targeting programmed cell death 1 (PD-1), PD-L1 or cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)

3.2.2 History of the following:

3.2.2.1 Active known or suspected autoimmune disease

3.2.2.2 Known human immunodeficiency virus (HIV) (Subjects with lymphocytes > 350 cluster of differentiation (CD)4+ cells and no detectable viral load are eligible)

3.2.2.3 Active known Hepatitis B. Testing is unrequired in the absence of history.

Hepatitis B can be defined as:

- Hepatitis B surface antigen (HBsAg) > 6 months
- Serum hepatitis B virus (HBV) deoxyribonucleic acid (DNA) 20,000 IU/mL (10^5 copies/mL), lower values 2,000 to 20,000 IU/mL (10^4 to 10^5 copies/mL) are often seen in hepatitis B-e antigen (HbeAg)-negative chronic hepatitis B
- Persistent or intermittent elevation in alanine aminotransferase (ALT)/alanine aminotransferase (AST) levels.
- Liver biopsy showing chronic hepatitis with moderate or severe necroinflammation

3.2.2.4 Active known Hepatitis C. Testing is unrequired in the absence of history.

- Hepatitis C antibody (Ab) positive
- Presence of hepatitis C virus (HCV) ribonucleic acid (RNA)

3.2.2.5 Known active pulmonary disease with hypoxia defined as:

- Oxygen saturation < 85% on room air or
- Oxygen saturation < 88% despite supplemental oxygen

3.2.3 Systemic treatment with either corticosteroids (> 10 mg daily prednisone equivalents) or other immunosuppressive medications within 7 days of registration

3.2.4 Received any live/attenuated vaccine (eg, varicella, zoster, yellow fever, rotavirus, oral polio and measles, mumps, rubella (MMRI) within 30 days before initiation of treatment on this protocol.

3.2.5 If female, pregnant or lactating. (Women of childbearing potential are required to have a negative pregnancy test within 24 hours prior to the initial administration of study drug)

3.3 Informed Consent Process

All participants must be provided a consent form describing the study with sufficient information for participants to make an informed decision regarding their participation. Participants must sign the IRB approved informed consent prior to participation in any

study specific procedure. The participant must receive a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

3.4 Registration Process

No subject may begin study treatment prior to registration and assignment of a subject identification number.

At registration, Stanford will sequentially assign eligible subjects an identification number. The maximum allowable time between registration and the first administration of study treatment is 14 days. The subject's identification number will be used on all subject-specific Case Report Forms (CRFs) and serious adverse event (SAE) forms. Case report forms will be electronic. Participant information should be entered into OnCore within 5 business days.

3.5 Study Timeline

Primary Completion: Primary completion is the date that the final datum is obtained / expected to be obtained for the primary outcome. The timeframe for primary completion for an individual subject is 16 weeks after initiation of treatment. This datum is expected to be obtained approximately 40 months from the time the study opens to accrual.

Study Completion: The study is expected to reach overall completion (last datum from last subject) approximately 60 months (5 years) from the time the study opens to accrual.

4. TREATMENT PLAN

This is an open-label phase 2 study evaluating the safety and efficacy of ipilimumab/nivolumab in combination with cryotherapy.

The main primary objective of this study is to determine if immunotherapy in combination with cryoablation adds a clinically-significant benefit as measured by the objective response rate (ORR). The response rate will be evaluated by RECIST v1.1 as well as by irRECIST. Imaging by PET-CT or CT scan will be performed at screening, then every 12 weeks ± 7 days until disease progression. Earlier scans will be performed if clinically indicated. Ablated tumors will not be included as part of the treatment response assessment, ie, disease response; measurable disease; or tumor progression, by either RECIST or irRECIST.

Subjects will receive ipilimumab 1 mg/kg IV over 30 minutes $\pm$ 10 minutes and nivolumab 3 mg/kg IV over 30 minutes $\pm$ 10 minutes every 3 weeks for 4 doses followed by nivolumab 480 mg IV over 30 minutes $\pm$ 10 minutes every 4 weeks until progression or intolerance (maximum 24 months). The standard cycle of investigational agent treatment is considered to be 3 weeks. See Study Calendar at Section 9.

Per protocol cryotherapy (cryoablation) will be performed between investigational agent treatment Cycles 1 and 2 (see Section 5.3). Additional cryoablation procedures may be conducted as regular medical care per investigator discretion.

Blood samples will be drawn prior to each immunotherapy administration for routine tests as well as correlative studies. Blood and tissue samples will be retained and used for future research.

After completion of study treatment, a post-treatment biopsy will be obtained if feasible.

4.1 General Concomitant Medication and Supportive Care Guidelines

Subjects will not receive any live/attenuated vaccine (eg, varicella, zoster, yellow fever, rotavirus, oral polio and measles, mumps, rubella (MMRI) during treatment and until 100 days post-last dose.

Otherwise, there are no restrictions on concomitant medications or supportive care medications.

If clinically indicated, palliative radiation therapy to non-target lesions is permitted.

4.2 Criteria for Removal from Study

Subjects will be removed from study if they have disease progression; unacceptable adverse events in the opinion of the subject or the investigator; withdrawal of consent; non-compliance; or if subject is female and has become pregnant.

4.3 Alternatives

Alternatives include other systemic therapies, other clinical trials, or supportive care (NOTE: corticosteroid restriction is only an eligibility criteria and not an on-study concurrent medication restriction).

5. INVESTIGATIONAL AGENT

5.1 Ipilimumab (Yervoy)

Ipilimumab (BMS-734016, MDX010, MDX-CTLA4) is a fully-human monoclonal immunoglobulin (Ig) G1κG1κ specific for human cytotoxic T-lymphocyte antigen 4 (CTLA-4, CD-152), which is expressed on a subset of activated T cells. CTLA-4 is a negative regulator of T-cell activity. Ipilimumab is a monoclonal antibody (mAb) that binds to CTLA-4 and blocks the interaction of CTLA-4 with its ligands, CD80/CD86. Blockade of CTLA-4 has been shown to augment T-cell activation and proliferation, including the activation and proliferation of tumor-infiltrating T-effector cells. Inhibition of CTLA-4 signaling can also reduce T-regulatory cell (Treg) function, which may contribute to a general increase in T-cell responsiveness, including the anti-tumor response.

5.1.1 Effects in Humans

Bristol-Myers Squibb (BMS) and Medarex, Inc (MDX, acquired by BMS in Sep 2009) have co-sponsored an extensive clinical development program for ipilimumab, encompassing more than 22,571 subjects (total number of subjects enrolled in ipilimumab studies) in several cancer types in completed and ongoing studies, including a compassionate use program.

Ipilimumab 3 mg/kg has been approved for use in advanced melanoma in over 47 countries, including the United States (US, 25 Mar 2011), the European Union

(EU, 13 Jul 2011), and Australia (Jul 2011). Ipilimumab 10 mg/kg is approved as adjuvant treatment of unresectable or metastatic melanoma in the US. Ipilimumab is approved in the EU for the treatment of adolescents and in the US for the treatment of melanoma in pediatric subjects.

Additionally, ipilimumab has been approved for the following indications:

- Treatment of subjects with intermediate or poor risk, previously untreated advanced renal cell carcinoma, in combination with nivolumab.
- Treatment of adult and pediatric subjects 12 years of age and older with microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan, in combination with nivolumab. This indication is approved under accelerated approval based on overall response rate and duration of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in confirmatory trials.

5.1.2 Availability

Bristol-Myer Squibb will be providing this agent.

5.1.3 Agent Ordering

This agent will be requested from Bristol-Myers Squibb in advance. A supply of the drug will be available at Stanford investigational pharmacy at all times.

5.1.4 Agent Storage and Accountability

Per the Investigator Brochure, vials of ipilimumab injection must be stored at 2°C to 8°C (36°F to 46°F) and protected from light and freezing. Ipilimumab injection may be stored undiluted (5 mg/mL) or following dilution in 0.9% sodium chloride injection or 5% dextrose injection in PVC, non-PVC/non-DEHP, or glass containers for up to 24 hours at 2°C to 8°C or room temperature/room light.

This agent will be kept secure per Stanford investigational pharmacy SOP.

5.2 Nivolumab (Opdivo)

Nivolumab (also referred to as BMS-936558, MDX1106, or ONO-4538) is a human monoclonal antibody (HuMAb; immunoglobulin G4 [IgG4]-S228P) that targets the programmed death-1 (PD-1) cluster of differentiation 279 (CD279) cell surface membrane receptor. PD-1 is a negative regulatory molecule expressed by activated T and B lymphocytes.1 Binding of PD-1 to its ligands, programmed death–ligands 1 (PD-L1) and 2 (PD-L2), results in the down-regulation of lymphocyte activation. Inhibition of the interaction between PD-1 and its ligands promotes immune responses and antigen-specific T-cell responses to both foreign antigens as well as self-antigens. Nivolumab is expressed in Chinese hamster ovary (CHO) cells and is produced using standard mammalian cell cultivation and chromatographic purification technologies. The clinical study product is a sterile solution for parenteral administration.

5.2.1 Effects in Humans

The pharmacokinetics (PK), clinical activity, and safety of nivolumab have been assessed in subjects with non-small cell lung cancer (NSCLC), melanoma, clear-cell renal cell carcinoma (RCC), classical Hodgkin Lymphoma (cHL), urothelial carcinoma (UC), squamous cell carcinoma of the head and neck (SCCHN), in addition to other tumor types.

Nivolumab monotherapy is approved in multiple regions, including the US and EU, for unresectable or metastatic melanoma, previously treated metastatic NSCLC, previously treated advanced RCC, previously treated relapsed or refractory cHL, previously treated advanced or metastatic UC, and for the treatment of previously treated recurrent or metastatic SCCHN; it is also approved for previously treated CRC, previously treated HCC, and the adjuvant treatment of melanoma in the US.

In addition, nivolumab has been approved for use in combination with ipilimumab for RCC in the US and unresectable melanoma in multiple countries, including the US and EU.

5.2.2 Availability

Bristol-Myers Squibb will be providing this agent.

5.2.3 Agent Ordering

This agent will be requested from Bristol-Myers Squibb in advance. A supply of the drug will be available at Stanford investigational pharmacy at all times.

5.2.4 Agent Storage and Accountability

Per the Investigator Brochure, vials of nivolumab injection must be stored at 2°C to 8°C (36°F to 46°F) and protected from light and freezing. The unopened vials can be stored at room temperature (up to 25°C, 77°F) and room light for up to 48 hours.

This agent will be kept secure per Stanford investigational pharmacy SOP.

5.3 Cryoablation

Cryoablation of the tumors occur between investigational agent treatment Cycles 1 and 2, and will be performed according to standard procedures. The cryotherapy devices will be FDA-approved cryoablation systems that in current use within the Stanford University Medical Center.

Prior to the cryoablation procedure, the interventional radiologist will review cross-sectional imaging studies to formulate a treatment plan, including identifying the target lesions appropriate for treatment; the number and type of cryoablation probes needed; and optimal percutaneous access approach. As the goal of the cryotherapy is to enhance systemic immunotherapy, the cryoablation will most commonly be subtotal. For lesions that are in close proximity to freeze-sensitive tissues, such as bowel or skin, the cryoablation treatment will be performed with a safe distance (≥ 1 cm) between the edge of the tissue to be frozen (the “iceball”) and the freeze-sensitive tissue.

The cryoablation procedure will be performed with moderate sedation or general anesthesia in a CT procedure room. A limited CT will be obtained and the desired

access sites will be marked on the subject's skin. After sterile preparation of the treatment field and application of local anesthesia, cryoablation probes will be sequentially placed percutaneously into the target lesion(s) using CT and/or ultrasound guidance. After all cryoablation probes are positioned appropriately, cryoablation will be performed with 1 or more freeze-thaw cycles. Iceball growth will be monitored by CT. The probes will then be removed and sterile dressings applied.

In brief, a total of 20 minutes of freezing time will be administered to the tumor in 2 or 3 freeze-thaw cycles. Care will be taken to avoid off-target ablation of sensitive structures such as bowel and skin. The procedures will be performed under either moderate sedation, monitored anesthesia care (conscious sedation), or general anesthesia, depending on the subject's ability to tolerate positioning for the anticipated duration of the procedure. The risks of ablation include hemorrhage, infection, pneumothorax, and injury to off-target structures. With any procedure there is a small risk of death. After ablation, the subject can expect discharge to home within 23 hours in most circumstances.

6. DOSE MODIFICATIONS

There will be no dose modifications for the investigational agents.

Per-subject stopping rule

Subjects will be monitored for toxicity at every study visit during the treatment phase (through 10 weeks). As defined by the current US package inserts (USPIs) for nivolumab and ipilimumab, treatment will be withheld for the related adverse events (toxicities) specified therein, and additionally by the discretion of the investigator. Treatment may be resumed in accordance with the USPIs. In the event that toxicity is judged to be excessive, treatment will be terminated with one or both agents. Particular attention will be given to events that might be an overlapping toxicity.

7. ADVERSE EVENTS AND REPORTING PROCEDURES

7.1 Potential Adverse Events

7.1.1 Ipilimumab (see appendix D for treatment algorithm)

Inflammatory Events (Immune-related Adverse Events or Immune-mediated Adverse Reactions)

Blockade of CTLA-4 by ipilimumab leads to T-cell activation, with the potential for clinical inflammatory AEs primarily involving the skin (dermatitis/pruritus), GI tract (diarrhea/colitis), liver (hepatitis), endocrine glands (eg, hypophysitis and adrenal or thyroid abnormalities), and other less frequent organs (eg, uveitis/episcleritis). The majority of these inflammatory AEs initially manifest during treatment; however, a minority occur weeks to months after discontinuation of ipilimumab. The majority of the inflammatory AEs are reversible with the guidance issued below.

- Gastrointestinal Toxicities

The most common site for ipilimumab-induced GI toxicity was the lower GI tract, and the most common presentation was mild to severe diarrhea or colitis with occasional bloody stools. In some cases, diarrhea began as mild and then worsened. Constipation was

rarely associated with ipilimumab administration. Delay in corticosteroid treatment may be associated with a poor outcome for subjects with high-grade diarrhea.

- Liver Toxicities

Subjects receiving ipilimumab may develop elevations in LFTs in the absence of clinical symptoms. Occasionally, subjects may present with symptoms, including right upper quadrant abdominal pain or unexplained vomiting. Most cases of inflammatory hepatitis responded to high-dose corticosteroids (IV route recommended).

- Endocrine Toxicities

The most common inflammatory endocrine toxicities are hypophysitis and hypopituitarism, secondary cortisol deficiency (hypoadrenalism), hypothyroidism or thyroiditis. Symptoms of hypopituitarism and other endocrine toxicities were generally controlled with appropriate hormone replacement.

- Skin Toxicities

The most common inflammatory skin toxicities are rash and pruritus, mostly mild to moderate in severity. Two cases of fatal treatment-related toxic epidermal necrolysis have been reported in clinical trials.

- Neurological Toxicities

Neurological manifestations may include motor and/or sensory neuropathy. Fatal Guillain-Barre syndrome and cases of myasthenia gravis have been reported in clinical trials. Unexplained motor neuropathy, muscle weakness, or sensory neuropathy should be evaluated, and non-inflammatory causes such as infections, metabolic disorders, and medications should be excluded.

- Other Toxicities

Ocular inflammation can be manifested as episcleritis or uveitis. Other presumed inflammatory events reported include, arthritis/arthralgia, pneumonitis, pancreatitis, autoimmune (aseptic) meningitis, autoimmune nephritis, pure red cell aplasia, noninfective myocarditis, polymyositis, eosinophilia, pericarditis, urticaria (2%), large intestinal ulcer, esophagitis, acute respiratory distress syndrome, renal failure, infusion reactions, and myasthenia gravis.

7.1.2 Nivolumab (see appendix D for treatment algorithm)

- Pulmonary toxicities

Subjects can present with either asymptomatic radiographic changes (eg, focal ground glass opacities and patchy infiltrates) or with symptoms of dyspnea, cough, or fever. All subjects with Grade 3 to 4 pneumonitis should discontinue nivolumab and initiate treatment with high doses of corticosteroids.

- Gastrointestinal toxicities

Most cases present with diarrhea. However diverticular perforation should also be considered when subjects present with abdominal pain and fevers.

- Hepatic toxicities

Early recognition and treatment of elevated LFTs and drug-induces liver injury are critical to their management. The principal treatment for high-grade hepatic AEs is corticosteroids.

- Endocrinopathies

The events are typically identified through either routine periodic monitoring of specific laboratories (eg, TSH) or as part of a work-up for associated symptoms (eg, fatigue). Moderate- to high-grade cases were managed with hormone replacement therapy and, in some cases, with the addition of corticosteroids.

- Skin toxicities

The most common skin toxicities include rash and pruritus. Rashes are typically focal with a maculopapular appearance occurring on the trunk, back, or extremities. Topical corticosteroids can be used for some cases of rash. Anti-histamines can be used for some cases of pruritus.

- Renal toxicities

Elevated creatinine and biopsy-confirmed tubulointerstitial nephritis and allergic nephritis are seen infrequently.

- Neurologic toxicities

Neurologic AEs can manifest as central abnormalities (eg, aseptic meningitis, encephalopathy, or encephalitis) or peripheral sensory/motor neuropathies (eg, Guillain-Barre Syndrome, myasthenia gravis complicated with sepsis and fatality). The onset has been observed as early as after a single treatment with the nivolumab + ipilimumab combination.

- Infusion Reactions

Infusion reactions, including high-grade hypersensitivity reactions, following administration of nivolumab are uncommon. Investigators are advised to monitor for fever, chills, shakes, itching, rash, hypertension or hypotension, or difficulty in breathing during and immediately after administration of nivolumab. Study protocols provide explicit guidance on the management of infusion-related reactions.

- Lipase/Amylase Elevations

In studies evaluating the safety of the nivolumab + ipilimumab combination in multiple tumor types, lipase and amylase levels were systematically monitored, and elevations in any grade of lipase/amylase were consistently noted in approximately 10% to 30% of subjects. Very few subjects reported associated symptoms.

- Uveitis and Visual Complaints

Uveitis may occur more frequently with nivolumab + ipilimumab combination therapy than with nivolumab monotherapy or nivolumab in combination with other therapies. An ophthalmologist should evaluate visual complaints with examination of the conjunctiva, anterior and posterior chambers, and retina. Topical corticosteroids may be used to

manage low-grade events. Low-grade events that do not resolve and high-grade events should be managed with systemic corticosteroids. Rare cases of granulomatous panuveitis with exudative retinal detachment have been observed and are often associated with neurologic and cutaneous manifestations.

- Other Immune-mediated Adverse Events

Rare cases of myotoxicity (myositis, myocarditis, and rhabdomyolysis), some with fatal outcome, have been reported with nivolumab or nivolumab in combination with ipilimumab.

For Grade 3 myocarditis, nivolumab or nivolumab in combination with ipilimumab should be permanently discontinued.

7.2 Adverse Event Reporting

Adverse events will be graded according to CTCAE v5. Adverse events will be recorded from the time the subject signs informed consent and for 30 days after the last dose of study drug. Adverse Events will be clearly noted in source documentation and listed on study specific Case Report Forms (CRFs). The Protocol Director (PD) or designee will promptly assess each Adverse Event (AE) to determine whether it is unexpected according to the Investigator's Brochure; this protocol, and/or the informed consent document, and related to the both the study drug and the investigation itself. All Serious Adverse Events (SAEs) will be tracked until resolution, or until 100 days after the last dose of the study drug.

Reporting Serious Adverse Events (SAEs)

Any adverse event meeting the definition of a 21CFR§312.32 serious adverse event (SAE) that occurs during the clinical study and for 100 days after the last dose of study drug must be recorded as an on-study SAE. Attribution of the SAE to study drug should follow the standard attribution and grading system as specified by CTEP (http://ctep.cancer.gov/reporting/adeers.html, see table below).

Attribution of Adverse Events

Code	Descriptor	Definition
1	Unrelated	The adverse event is clearly not related to the investigational agent(s)
2	Unlikely	The adverse event is doubtfully related to the investigational agent(s)
3	Possible	The adverse event may be related to the investigational agent(s)
4	Probable	The adverse event is likely related to the investigational agent(s)
5	Definite	The adverse event is clearly related to the investigational agent(s)

FDA

Events that meet the 21CFR§312.32 definition of a serious, unexpected, suspected adverse reaction (SUSAR, ie, serious, unexpected, and related SAE) will be reported to the investigator's IND as a serialized submission on the Form FDA 3500A (MedWatch

form for Mandatory Reporting), in accordance with the timelines defined at 21CFR§312.32.

Drug Manufacturer (Bristol Myers Squib)

SAEs, whether related or not related to study drug, and any on-study pregnancies, will be reported within 24 hours to Bristol-Myers Squibb (**SAE Email Address: Worldwide.Safety@BMS.com; SAE Facsimile Number:** +1-609-818-3804). The SAE will be reported on the Form FDA 3500A (MedWatch form for Mandatory Reporting) if used per the above; or the Form FDA 3500 (MedWatch form for Voluntary Reporting) or the Council for International Organizations of Medical Sciences (CIOMS) form.

Stanford Cancer Institute (SCI) Data Safety Monitoring Committee (DSMC)

SAEs, CTCAE Grade 3 and above, whether related or not related to study drug; all on-study pregnancies; and all subsequent follow-ups must be reported to the Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) via **CCTO-Safety@Stanford.edu** within 24 hours (1 business day) of becoming aware of the event. The SAE will be reported using the CCTO SAE CRF plus the Form FDA 3500A (MedWatch form) if prepared per above.

Institutional Review Board (IRB)

Events that meet the 21CFR§312.32 definition of a serious, unexpected, suspected adverse reaction (SUSAR) which occurred as a result of the study intervention(s) or protocol-specific procedures, including diagnostic procedures, will be reported to the local IRB of record. If a Form FDA 3500A (MedWatch form) was prepared per the above, this should be part of the report.

Following review by the SCI DSMC, the committee may issue an assessment that an event met the criteria of an IRB definition of “Unanticipated Problem (UP).” Upon notification of such determination, the investigator will, if not previously reported to the IRB as a UP, submit a UP report of the event within 10 working days of DSMC notification, or within 5 working days for deaths or life-threatening events.

8. CORRELATIVE/SPECIAL STUDIES

8.1 Archival tumor samples:

Histology, immunohistochemistry for lymphocyte subsets (include multi-marker studies such as MIBI or other) and for Gene Expression profiling with additional analysis of immune subsets by Cibersort and for Gene Expression profiling within the immune cells by CibersortX. The latter dataset can be validated by performing LCM-SMART-3SEQ.

8.2 Tumor samples collected throughout study:

Histology, immunohistochemistry for lymphocyte subsets (include multi-marker studies such as MIBI or other) and for gene expression profiling (GEP) with additional analysis of immune subsets by Cibersort and for GEP within the immune cells by CibersortX. The latter dataset can be validated by performing LCM-SMART-3SEQ.

8.3 Blood collection:

a) Determine ctDNA levels

- Draw blood into two Streck Cell-free DNA BCT tubes (10 mL each)
- Cell-free DNA will be extracted from plasma using QIAamp Circulating Nucleic Acid kit (Qiagen), according to the manufacturer's protocol. Germline DNA will be extracted from peripheral blood cells using DNeasy Blood & Tissue kit (Qiagen), following the manufacturer's protocol. Depending on the histological subtype of sarcoma, cell-free DNA will be analyzed for the presence of tumor-type specific point mutations and/or DNA copy number alterations. For the point mutation analysis, we will apply deep targeted sequencing by Cancer Personalized Profiling by deep Sequencing (CAPP-Seq). For detection of DNA copy number alterations, we will perform shallow whole genome sequencing. We have previously successfully applied both methods to profile tumor-derived genomic aberrations in cell-free DNA extracted from plasma of subjects diagnosed with leiomyosarcoma (Przybyl J, Chabon JJ, *et al*. Combination Approach for Detecting Different Types of Alterations in Circulating Tumor DNA in Leiomyosarcoma. *Clin Cancer Res*. 2018 Jun 1;24(11):2688-2699.)

b) Gene expression profiling on blood lymphocytes, followed by CibersortX to assess subsets. This data can then be compared to c)

- For gene expression profiling of blood cells, collect blood into two PAXgene Blood RNA tubes (2.5 mL each) manufactured by BD biosciences that are specifically optimized for RNA extraction from the blood cells.

c) Perform flow with purification of T-cells, followed by Gene Expression profiling

d) Perform proteomic analysis for cytokines

8.3.1 Blood Specimen Handling Instructions:

a) Prior to Collection:

- Using a permanent marker pen, record the required information (subject number) on the blood collection tube label.

b) Sample Collection and Handling:

- Draw blood into two Streck BCT tubes (10 mL each), and draw blood into two PAXgene Blood RNA tubes (2.5 mL each). Invert gently 10 times to mix.
- Blood drawn into PAXgene tubes should be frozen at -80C and sent directly to van de Rijn lab on dry ice in batches of 40 tubes (so not each one separately).
- Blood drawn in the Streck BCT tubes should be sent to van de Rijn laboratory of the Stanford University Medical Center for processing.

9. STUDY CALENDAR

	Pre-Study/ Baseline	Wk 1	Wk 4 (± 3 d)	Wk 7 (± 3 d)	Wk 10 (± 3 d)	Maintenance Every 4W± 1W	Off-study (post-30 dy ± 1 wk)	Follow-up [i]
Investigational Agents		X	X	X	X	X		
Cryoablation			X					
Informed Consent	X							
Demographics	X							
Medical History	X							
Concomitant medications	X	X	X	X	X	X	X	
Physical exam	X	X	X	X	X	X	X	
Vital signs	X	X	X	X	X	X	X	
Height	X							
Weight	X	X	X	X	X	X	X	
Performance status	X	X	X	X	X	X	X	
PRO-CTCAE Questionnaire [a]		X	X	X	X	X	X	
CBC with differential and platelets [b]	X	X	X	X	X	X	X	
Serum chemistry [b]	X	X	X	X	X	X	X	
EKG	X	(as clinically indicated)						
Adverse event evaluation [c]	X	X	X	X	X	X	X	
Tumor measurements [d]	X				X	X		
Radiologic evaluation (CT, MRI, X-ray) [d]	X				X	X [d]		
β-HCG [e]	X	X	X [e]	X	X	X [e]		
Other tests, as appropriate [f]	X		X					
Other correlative studies [g]		X	X	X	X		X	

a. PRO-CTCAE Questionnaire will be completed by patients at each study visit before any assessments are performed. Questionnaires in English, Spanish, and traditional Chinese are attached in Appendix F.

b. Metabolic comprehensive includes: albumin, alkaline phosphatase, ALT (SGPT), AST (SGOT), urea nitrogen, calcium, chloride, CO_2, creatinine, glucose, globulin, potassium, sodium, total bilirubin, total protein. These labs should be done at baseline within 14 days of first treatment, and within 72 hours of day 1 of each subsequent treatment.

c. Adverse events are based on Common Terminology Criteria for Adverse Events (CTCAE) v5.0 Publish Date: 27 November 2017. Adverse events will be recorded from the time the subject signs informed consent through and including 30 days after the last dose of study drug. All serious adverse events will be tracked until resolution, or until 100 days after the last dose of study drug.

d. Tumor measurements are based on RECIST v1.1 and irRECIST criteria (Appendices C & D). Scans will be done at baseline within 28 days of 1st treatment and then after dose 4 of immunotherapy (W 10 dose). This scan should be done at week 14 ± 1 week and every 12 weeks ± 1 week, once subjects start maintenance nivolumab. Ablated tumors will not be included as part of the treatment response assessment, ie, disease response; measurable disease; or tumor progression, by either RECIST or irRECIST. The baseline measurements will be done before treatment, and follow-up measurements will be done after 4 doses of ipilimumab and nivolumab (12 weeks), then every 12 weeks thereafter. At Investigator's discretion, imaging of known sites of disease is recommended.

e. Serum β-HCG: Women of child-bearing potential are required to have a negative pregnancy test within 24 hours (eligibility criteria) prior to the initial administration of study drug, then every 3 to 4 weeks (± 1 week) during the study (this test can be omitted if subject is post-menopausal by either surgery or elevated FSH).

f. ACTH, serum cortisol, TSH, free T4, CRP, ESR.

g. Archival tumor sample must be available at baseline. All subsequent biopsies will be collected in paraffin for research purposes and standard diagnostics. Blood will be collected at baseline, and prior to treatment dose on weeks 1, 4, 7, 10 and during the off-study visit. Off study visit with a ± 1 week.

h. Per protocol cryoablation is required to be performed before Week 4 administration of ipilimumab and nivolumab. (between Cycle 1 and Cycle 2), at the discretion of the of the investigator.

i. Safety follow-up: After the off-study visit, there will be two additional follow-up phone call/or retrieval of medical records that occur every 35 days ± 1 week to collect information on participants' health status.

10. MEASUREMENTS

10.1 Primary and Secondary Outcome measures

Primary Outcome

- Outcome 1 (Primary) Title: Clinical Response
- Outcome Description: Clinical response was assessed per the Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 criteria.
    - Complete response (CR) = Disappearance of all target lesions; all lymph nodes < 10 mm on the short axis; no new lesions.
    - Partial response (PR) = ≥ 30% decrease in the sum of the longest diameter of target lesions; no new lesions.
    - Stable disease (SD) = Small changes that do not meet any of the above criteria; no new lesions.
    - Progressive disease (PD) = 20% increase in the sum of the longest diameter of target lesions, and/or the appearance of one or more new lesion(s).

    The outcome is expressed as the total number of participants who achieve a RECIST clinical response, defined as CR + PR, by 16 weeks, a number without dispersion. The outcome is expressed as the total number of participants who achieve a clinical response by 16 weeks, a number without dispersion.
- Outcome Timeframe: 16 weeks

Note: Per guidance from ClinicalTrials.gov, a single Primary Outcome is declared. Other primary and secondary objectives are reflected as secondary outcomes.

Secondary Outcomes

- Outcome 2 (Secondary) Title: Partial Response (PR) Rate
  - Outcome Description: Clinical response was assessed per the Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 criteria.
    - Complete response (CR) = Disappearance of all target lesions; no new lesions.
    - Partial response (PR) = ≥ 30% decrease in the sum of the longest diameter of target lesions; no new lesions.
    - Stable disease (SD) = Small changes that do not meet any of the above criteria; no new lesions.
    - Progressive disease (PD) = 20% increase in the sum of the longest diameter of target lesions, and/or the appearance of one or more new lesion(s)

    The outcome is expressed as the total number of participants who achieve PR by 16 weeks, a number without dispersion.
  - Outcome Timeframe: 16 weeks
- Outcome 3 (Secondary) Title: Stable Disease (SD) Rate
  - Outcome Description: Clinical response was assessed per the Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 criteria.
    - Complete response (CR) = Disappearance of all target lesions; no new lesions.
    - Partial response (PR) = ≥ 30% decrease in the sum of the longest diameter of target lesions; no new lesions.
    - Stable disease (SD) = Small changes that do not meet any of the above criteria; no new lesions.
    - Progressive disease (PD) = 20% increase in the sum of the longest diameter of target lesions, and/or the appearance of one or more new lesion(s)

    The outcome is expressed as the total number of participants with SD at 16 weeks, a number without dispersion.
  - Outcome Timeframe: 16 weeks
- Outcome 4 (Secondary) Title: Related Adverse Events (Toxicity)
  - Outcome Description: Adverse events were assessed per CTCAE version 5. The outcome is expressed as the total number of possibly, probably, or definitely-related adverse events experienced by participants, a number without dispersion.
  - Outcome Timeframe: 24 months

- Outcome 5 (Secondary) Title: Immune-related Clinical Response (irRECIST)
  - Outcome Description: The Immune-related Response Evaluation Criteria in Solid Tumors (irRECIST) vary from the RECIST criteria in order to distinguish cellular anti-tumor responses from tumor progression. This is primarily accomplished by specifying the minimum size to be considered a new tumor lesion. Clinical response was assessed per the irRECIST criteria.
    - Complete response (CR) = Disappearance of all target lesions; no new lesions > 5 × 5 mm in size.
    - Partial response (PR) = ≥ 30% decrease in the sum of the longest diameter of target lesions; no new lesions > 5 × 5 mm in size.
    - Stable disease (SD) = Small changes that do not meet any of the above criteria; no new lesions > 5 × 5 mm in size.
    - Progressive disease (PD) = 20% increase in the sum of the longest diameter of target lesions, and/or the appearance of one or more new lesion(s).

    The outcome is expressed as the total number of participants who achieve an irRECIST clinical response, defined as CR + PR, by 16 weeks, a\ number without dispersion.
  - Outcome Timeframe: 16 weeks
- Outcome 6 (Secondary) Title: Progression-free survival (PFS)
  - Outcome Description: 6-month Progression-free survival (PFS) as measured from the time of consent until death or disease progression. The outcome is expressed as the total number of participants remaining alive without disease progression at 6 months after consent, a number without dispersion.
  - Outcome Timeframe: 6 months

11. REGULATORY CONSIDERATIONS

11.1 Regulatory Oversight

The US Food and Drug Administration (FDA) has not approved ipilimumab nor nivolumab for the treatment of soft tissue sarcoma, however ipilimumab and nivolumab are approved for use in combination by the FDA for several tumor settings. Although the investigator will submit an IND for this study, the FDA may deem the study to be IND-exempt.

This study will only be initiated and conducted after review and approval by the local IRB of record.

11.2 Monitoring plan

The Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) will be the monitoring entity for this study.

The DSMC will audit study-related activities approximately once per year to determine whether the study has been conducted in accordance with the protocol, local standard operating procedures, FDA regulations, and Good Clinical Practice (GCP). This may include review of the following types of documents participating in the study: regulatory binders, case report forms, eligibility checklists, and source documents. In addition, the DSMC will regularly review serious adverse events and protocol deviations associated with the research to ensure the protection of human subjects. Results of the DSMC audit will be communicated to the IRB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as needed.

11.3 Protocol Review and Amendments

The protocol, the proposed informed consent and all forms of participant information related to the study (eg, advertisements used to recruit participants) will be reviewed and approved by the Stanford IRB and Stanford Cancer Institute Scientific Review Committee (SRC). Any changes made to the protocol will be submitted as a modification and will be approved by the IRB prior to implementation.

11.4 Data management

The research coordinator will be responsible for database records of subject data. The data will be kept in an OnCore online database, under password protection with access limited to specific areas of the database. A chart with all of the relevant research subject information will be maintained for each subject by the research coordinator. Subject charts will be reviewed by Stanford PI and Study Coordinator for yearly audits.

11.5 Study Documentation

The Protocol Director must maintain adequate and accurate participant case histories with observations and other data pertinent to the study. Original source documents should be transcribed to Case Report Forms (CRFs). Source documents include hospital records, clinical charts, laboratory and pharmacy records, and recorded electronic data.

The Principal Investigator will be responsible for maintaining the clinical protocol and subjects' study charts, reporting adverse events, assuring that consent is obtained and documented, and reporting the status of the trial in continuing renewals submitted to their IRB and trial monitoring group(s).

12. STATISTICAL CONSIDERATIONS

We anticipate enrolling no more than 30 subjects to achieve our aims and this is feasible. Specifically, it is anticipated that 3 to 4 subjects per month may be enrolled onto this trial. Thus, it is expected that 12 months will be required to accrue the number of subjects necessary to complete the trial.

More specifically, clinical benefit (CR/PR/SD) will be assessed for each subject at 16 weeks. Our null hypothesis is that the response rate is ≤ 30%, and our alternative

hypothesis is that the response rate is ≥ 60%. The selection of 60% as a threshold for determining the treatment is worthy of further study is due to recent trials of sarcoma FDA-approved drugs showing ~60% PFS rate at 3 months (ie, pazopanib, trabectedin, eribulin).

We will employ a Simon 2-stage design to address our primary objective. The type 1 error rate assumed for the Simon 2-stage design is 0.04. The null hypothesis that the true response rate (ie, sum of CR and PR) is 30% or less will be tested against a one-sided alternative. Our decision rule works as follows. In the first stage, we will accrue and assess 10 subjects. If 3 or fewer subjects (< 30% of 10 subjects) achieve a clinical response, the trial will be stopped for futility. If more than 3 subjects achieve a clinical response, we will enroll and treat an additional 18 subjects. If 12 or fewer of the 28 treated subjects (< 42.8%) demonstrate a clinical response (either CR or PR), we will conclude the treatment is not worthy of further study. If, however, more than 12 of the 28 treated subjects (≥ 42.8%) responds, we will claim the treatment promising. Assuming a true response rate of 60%, we have 90% power to correctly conclude the treatment promising. In contrast, if the true response rate is only 30%, we have a low probability (0.04) of incorrectly continuing to study the treatment.

Additionally, we will characterize rates of toxicity by level of toxicity as well as by other subject-level characteristics.

To address secondary objectives, we will use Kaplan-Meier methods to graphically depict progression-free survival. This tool will also be used to demonstrate survival across various sub-groups of interest. Generalized linear regression methods (with a logit link) will be utilized to characterize relationships between features such as mutation burden, PDL1 expression, and tumor infiltrating lymphocytes and response.

13. REFERENCES

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APPENDICES

APPENDIX A: Participant Eligibility Checklist

A Participant Eligibility Checklist must be completed in its entirety for each subject prior to registration. The completed, signed, and dated checklist must be retained in the subject's study file and the study's Regulatory Binder.

Protocol Title:	Phase II Study of Ipilimumab Plus Nivolumab in Combination with Cryotherapy in Metastatic or Locally Advanced Soft Tissue Sarcoma
Protocol Number:	IRB-50853
Principal Investigator:	Kristen Ganjoo, MD

II. Subject Information:

Subject Name/ID:
Gender: ☐ Male ☐ Female

III. Study Information:

SRC Approved ☐ IRB Approved ☐

III. Inclusion/Exclusion Criteria

Inclusion Criteria (From IRB approved protocol)	**Yes**	**No**	**Supporting Documentation***
1. Unresectable or metastatic soft tissue sarcoma	☐	☐	
2. ≥ 1 prior systemic therapy for sarcoma, including adjuvant systemic therapy	☐	☐	
3. Age ≥ 18 years	☐	☐	
4. Life expectancy > 3 months	☐	☐	
5. Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1	☐	☐	
6. Absolute neutrophil count (ANC) ≥ 1,000/mm^3	☐	☐	
7. Platelet count ≥ 75,000/mm^3	☐	☐	
8. Creatinine ≤ 1.5 x upper limit of normal (ULN) OR calculated (calc.) creatinine clearance > 45 mL/min using the lean body mass formula only	☐	☐	
9. Total bilirubin ≤ 1.5 x ULN in absence of Gilbert disease (total bilirubin ≤ 3 x ULN with Gilbert); also, if hyperbilirubinemia is clearly attributed to liver metastases total bilirubin ≤ 3 x ULN is permitted	☐	☐	
10. AST/ALT ≤ 3 x ULN	☐	☐	

11. Thyroid stimulating hormone (TSH) within normal limits (WNL); supplementation is acceptable to achieve a TSH WNL; in subjects with abnormal TSH if free T4 is normal and subject is clinically euthyroid, subject is eligible	☐	☐	
12. Any toxic effects of prior therapy (except alopecia) must be resolved to NCI CTCAE, version 5.0, Grade 1 or less	☐	☐	
13. Ability to understand and the willingness to sign a written informed consent document	☐	☐	
14. Women of childbearing potential (WOCBP) receiving nivolumab must be willing to adhere to contraception for a period of 5 months after the last dose of nivolumab. Men receiving nivolumab and who are sexually active with WOCBP will be instructed and must be willing to adhere to contraception for a period of 7 months after the last dose of nivolumab	☐	☐	
Exclusion Criteria (From IRB approved protocol)			
1. Prior therapy with ipilimumab or nivolumab, or any agent targeting programmed cell death 1 (PD-1), PD-L1 or cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)	☐	☐	
2. Active known or suspected autoimmune disease	☐	☐	
3. Subjects with known human immunodeficiency virus (HIV) (Subjects with lymphocytes > 350 cluster of differentiation (CD)4+ cells and no detectable viral load are eligible)	☐	☐	

4. Active known Hepatitis B. Testing is unrequired in the absence of history. Hepatitis B can be defined as: • Hepatitis B surface antigen (HBsAg) > 6 months. • Serum hepatitis B virus (HBV) deoxyribonucleic acid (DNA) 20,000 IU/mL (10^5 copies/mL), lower values 2,000 to 20,000 IU/mL (10^4 to 10^5 copies/mL) are often seen in hepatitis B-e antigen (HbeAg)-negative chronic hepatitis B • Persistent or intermittent elevation in alanine aminotransferase (ALT)/alanine aminotransferase (AST) levels. • Liver biopsy showing chronic hepatitis with moderate or severe necroinflammation	☐	☐	
5. Active Known Hepatitis C. Testing is unrequired in the absence of history. • Hepatitis C antibody (Ab) positive • Presence of hepatitis C virus (HCV) ribonucleic acid (RNA)	☐	☐	
6. Known active pulmonary disease with hypoxia defined as: • Oxygen saturation < 85% on room air or • Oxygen saturation < 88% despite supplemental oxygen	☐	☐	
7. Systemic treatment with either corticosteroids (> 10 mg daily prednisone equivalents) or other immunosuppressive medications within 7 days of registration	☐	☐	
8. Received any live/attenuated vaccine (eg, varicella, zoster, yellow fever, rotavirus, oral polio and measles, mumps, rubella (MMRI) within 30 days before initiation of treatment on this protocol	☐	☐	
9. If female, pregnant or lactating. (Women of childbearing potential are required to have a negative pregnancy test within 24 hours prior to the initial administration of study drug)	☐	☐	

*****All subject files must include supporting documentation to confirm subject eligibility. The method of confirmation can include, but is not limited to, laboratory test results, radiology test results, subject self-report, and medical record review.

IV. Statement of Eligibility

By signing this form of this trial, I verify that this subject is [☐ **eligible** / ☐ **ineligible**] for participation in the study. This study is approved by the Stanford Cancer Institute Scientific Review Committee, the Stanford IRB, and has finalized financial and contractual agreements as required by Stanford School of Medicine's Research Management Group.

Treating Physician Signature:	Date:
Printed Name:	

Secondary Reviewer Signature:	Date:
Printed Name:	

Study Coordinator Signature:	Date:
Printed Name:	

APPENDIX B: Revised Response Evaluation Criteria in Solid Tumors (RECIST) 1.1

1. Measurability of tumor at baseline

1.1. Definitions

At baseline, tumor lesions/lymph nodes will be categorized measurable or non-measurable as follows:

1.1.1. Measurable

Tumor lesions: Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of 10 mm by CT scan (CT scan slice thickness no greater than 5 mm). 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable). 20 mm by chest X-ray.

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

1.1.2. Non-measurable

All other lesions, including small lesions (longest diameter < 10 mm or pathological lymph nodes with ≥ 10 to < 15 mm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

1.1.3. Special considerations regarding lesion measurability

Bone lesions, cystic lesions, and lesions previously treated with local therapy require particular comment:

Bone lesions: Bone scan, PET scan or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions. Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be considered as measurable lesions if the soft tissue component meets the definition of measurability described above. Blastic bone lesions are non-measurable.

Cystic lesions: 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 subject, these are preferred for selection as target lesions.

Lesions with prior local treatment: Tumor lesions situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not considered

measurable unless there has been demonstrated progression in the lesion. Study protocols should detail the conditions under which such lesions would be considered measurable.

1.2. Specifications by methods of measurements

1.2.1. Measurement of lesions

All measurements should be recorded in metric notation, using calipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

1.2.2. Method of assessment

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging based evaluation should always be done rather than 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 and $\geq$ 10 mm diameter as assessed using calipers (eg, skin nodules). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is suggested. As noted above, when lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study.

Chest X-ray: Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.

CT, MRI: CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. When CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (eg, for body scans).

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, they can be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response or surgical resection is an endpoint.

2. Tumor response evaluation

2.1. Assessment of overall tumor burden and measurable disease

To assess objective response or future progression, it is necessary to estimate the overall tumor burden at baseline and use this as a comparator for subsequent measurements. Only subjects with measurable disease at baseline should be included. Measurable disease is defined by the presence of at least one measurable lesion (as detailed above in Section 3).

2.2. Baseline documentation of 'target' and 'non-target' lesions

When more than one measurable lesion is present at baseline all lesions up to a maximum of five lesions total (and a maximum of 2 lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline (this means in instances where subjects have only 1 or 2 organ sites involved a maximum of 2 and 4 lesions respectively will be recorded).

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.

Lymph nodes merit special mention since they are normal anatomical structures which may be visible by imaging even if not involved by tumor. As noted in Section 3, pathological nodes which are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of ≥ 15 mm by CT scan. Only the short axis of these nodes will contribute to the baseline sum. The short axis of the node is the diameter normally used by radiologists to judge if a node is involved by solid tumor. Nodal size is normally reported as two dimensions in the plane in which the image is obtained (for CT scan this is almost always the axial plane; for MRI the plane of acquisition may be axial, sagittal or coronal). The smaller of these measures is the short axis. For example, an abdominal node which is reported as being 20 mm × 30 mm has a short axis of 20 mm and qualifies as a malignant, measurable node. In this example, 20 mm should be recorded as the node measurement. All other pathological nodes (those with short axis ≥ 10 mm but < 15 mm) should be considered non-target lesions. Nodes that have a short axis < 10 mm are considered non-pathological and should not be recorded or followed.

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 as noted above, 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.

All other lesions (or sites of disease) including pathological lymph nodes should be identified as non-target lesions and should also be recorded at baseline.

Measurements are not required, and these lesions should be followed as 'present', 'absent', or in rare cases 'unequivocal progression' (more details to follow). In addition, it is possible to record multiple non-target lesions involving the same organ as a single item on the case record form (eg, 'multiple enlarged pelvic lymph nodes' or 'multiple liver metastases').

2.3. Response criteria

This section provides the definitions of the criteria used to determine objective tumor response for target lesions.

2.3.1. Evaluation of target lesions

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

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

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

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.

2.3.2. Special notes on the assessment of target lesions

<u>Lymph nodes</u>

Lymph nodes identified as target lesions should always have the actual short axis measurement recorded (measured in the same anatomical plane as the baseline examination), even if the nodes regress to below 10 mm on study. This means that when lymph nodes are included as target lesions, the 'sum' of lesions may not be zero even if complete response criteria are met, since a normal lymph node is defined as having a short axis of < 10 mm. Case report forms or other data collection methods may therefore be designed to have target nodal lesions recorded in a separate section where, in order to qualify for CR, each node must achieve a short axis < 10 mm. For PR, SD and PD, the actual short axis measurement of the nodes is to be included in the sum of target lesions.

<u>Target lesions that become 'too small to measure'</u>

While on study, all lesions (nodal and non-nodal) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (eg, 2 mm). However, sometimes lesions or lymph nodes which are recorded as target lesions at baseline become so faint on CT scan that the radiologist may not feel comfortable assigning an exact measure and may report them as being 'too small to measure'. When this occurs, it is important that a value be recorded on the case report form. If it is the opinion of the radiologist that the lesion has likely disappeared, the

measurement should be recorded as 0 mm. If the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned (Note: It is less likely that this rule will be used for lymph nodes since they usually have a definable size when normal and are frequently surrounded by fat such as in the retroperitoneum; however, if a lymph node is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned in this circumstance as well). This default value is derived from the 5 mm CT slice thickness (but should not be changed with varying CT slice thickness). The measurement of these lesions is potentially non-reproducible, therefore providing this default value will prevent false responses or progressions based upon measurement error. To reiterate, however, if the radiologist is able to provide an actual measure, that should be recorded, even if it is below 5 mm.

Lesions that split or coalesce on treatment

When non-nodal lesions 'fragment', the longest diameters of the fragmented portions should be added together to calculate the target lesion sum. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in obtaining maximal diameter measurements of each individual lesion. If the lesions have truly coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximal longest diameter for the 'coalesced lesion'.

2.3.3. Evaluation of non-target lesions

This section provides the definitions of the criteria used to determine the tumor response for the group of non-target lesions. While some non-target lesions may actually be measurable, they need not be measured and instead should be assessed only qualitatively at the time points specified in the protocol.

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

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): Unequivocal progression (see comments below) of existing non-target lesions. (Note: the appearance of one or more new lesions is also considered progression).

2.3.4 New lesions

The appearance of new malignant lesions denotes disease progression; therefore, some comments on detection of new lesions are important. There are no specific criteria for the identification of new radiographic lesions; however, the finding of a new lesion should be unequivocal: i.e. not attributable to differences in scanning technique, change in imaging modality or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). This is particularly important when the subject's baseline lesions show partial or complete response. For example, necrosis of a liver lesion may be reported on a CT scan report as a 'new' cystic lesion, which it is not.

A lesion identified on a follow-up study in an anatomical location that was not scanned at baseline is considered a new lesion and will indicate disease progression. An example of this is the subject who has visceral disease at baseline and while on study has a CT or MRI brain ordered which reveals metastases. The subject's brain metastases are considered to be evidence of PD even if he/she did not have brain imaging at baseline.

If a new lesion is equivocal, for example because of its small size, continued therapy and follow-up evaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan.

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.

2.4. Evaluation of best overall response

The best overall response is the best response recorded from the start of the study treatment until the end of treatment taking into account any requirement for confirmation. The subject's best overall response assignment will depend on the findings of both target and non-target disease and will also take into consideration the appearance of new lesions.

2.4.1. Time point response

Table 1 on provides a summary of the overall response status calculation at each time point for subjects who have measurable disease at baseline.

Table 1. Overall Response Status Calculation by Time point

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, and NE = inevaluable.

2.4.2. Missing assessments and non-evaluable designation

When no imaging/measurement is done at all at a particular time point, the subject is not evaluable (NE) at that time point. If only a subset of lesion measurements are made at an assessment, usually the case is also considered NE at that time point, unless a convincing argument can be made that the contribution of the individual missing lesion(s) would not change the assigned time point response. This would be most likely to happen in the case of PD. For example, if a subject had a baseline sum of 50 mm with three measured lesions and at follow-up only two lesions were assessed, but those gave a sum of 80 mm, the subject will have achieved PD status, regardless of the contribution of the missing lesion.

2.4.3. Best overall response: all time points

The best overall response is determined once all the data for the subject is known.

Best response determination in this trial where confirmation of complete or partial response IS NOT required: Best response in these trials is defined as the best response across all time points (for example, a subject who has SD at first assessment, PR at second assessment, and PD on last assessment has a best overall response of PR). When SD is believed to be best response, it must also meet the protocol specified minimum time from baseline. If the minimum time is not met when SD is otherwise the best time point response, the subject's best response depends on the subsequent assessments. For example, a subject who has SD at first assessment, PD at second and does not meet minimum duration for SD, will have a best response of PD. The same subject lost to follow-up after the first SD assessment would be considered non-evaluable.

2.4.4. Special notes on response assessment

When nodal disease is included in the sum of target lesions and the nodes decrease to 'normal' size (< 10 mm), they may still have a measurement reported on scans. This measurement should be recorded even though the nodes are normal in order not to overstate progression should it be based on increase in size of the nodes. As noted earlier, this means that subjects with CR may not have a total sum of 'zero' on the case report form (CRF).

Subjects 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 objective progression even after discontinuation of treatment. Symptomatic deterioration is not a descriptor of an objective response: it is a reason for stopping study therapy. The objective response status of such subjects is to be determined by evaluation of target and non-target disease as shown in Table 1, Table 2, and Table 3.

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends upon this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before assigning a status of complete response. 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.

For equivocal findings of progression (eg, very small and uncertain new lesions; cystic changes or necrosis in existing lesions), treatment may continue until the next scheduled assessment. If at the next scheduled assessment, progression is confirmed, the date of progression should be the earlier date when progression was suspected.

APPENDIX C: Immune-related Response Evaluation Criteria in Solid Tumors (Immune-related RECIST, irRECIST)

Conventional response criteria may not be adequate to characterize the anti-tumor activity of immunotherapeutic agents, which can produce delayed responses that may be preceded by initial apparent radiographic progression, including the appearance of new lesions. Therefore, immune-related response criteria have been developed to incorporate new lesions into the assessment of total tumor burden and allow radiographic progression to be confirmed at a subsequent assessment. Immune-related Response Evaluation Criteria in Solid Tumors (irRECIST), as described within this appendix, were adapted from RECIST, Version 1.1 (v1.1) (Eisenhauer, *et al*. 2009), in the same manner that immune-related response criteria were adapted from WHO criteria (Wolchok *et al*. 2009) and RECIST v1.0 (Nishino *et al*. 2014). When not otherwise specified, RECIS v1.1 conventions will apply.

TUMOR MEASURABILITY

At baseline, tumor lesions/lymph nodes will be categorized as measurable or non-measurable as described below. All measurable and non-measurable lesions should be assessed at screening and at subsequent protocol-specified tumor assessment time points. Additional assessments may be performed as clinically indicated for suspicion of progression.

DEFINITION OF MEASURABLE LESIONS

Tumor Lesions:

Tumor lesions must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size as follows:

- 10 mm by computed tomography (CT) or magnetic resonance imaging (MRI) scan (CT/MRI scan slice thickness/interval ≤ 5 mm)
- 10-mm caliper measurement by clinical examination (lesions that cannot be accurately measured with calipers should be recorded as non-measurable)
- 20 mm by chest X-ray malignant lymph nodes

To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in the short axis when assessed by CT scan (CT scan slice thickness recommended to be ≤ 5 mm). At baseline and follow-up, only the short axis will be measured and followed. Additional information on lymph node measurement is provided below (see "Identification of Target and Non-Target Lesions," "New Lesions," and "Calculation of Sum of Diameters").

DEFINITION OF NON-MEASURABLE LESIONS

Non-measurable tumor lesions encompass small lesions (longest diameter < 10 mm or pathological lymph nodes with short axis ≥ 10 mm but < 15 mm) as well as truly non-measurable lesions. Lesions considered truly non-measurable include leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, peritoneal spread, and abdominal

mass/abdominal organomegaly identified by physical examination that is not measurable by reproducible imaging techniques.

SPECIAL CONSIDERATIONS REGARDING LESION MEASURABILITY

Bone lesions, cystic lesions, and lesions previously treated with local therapy require particular comment, as outlined below.

Bone Lesions:

- Technetium-99m bone scans, sodium fluoride positron emission tomography scans, and plain films are not considered adequate imaging techniques for measuring bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.
- Lytic bone lesions or mixed lytic-blastic lesions with identifiable soft tissue components that can be evaluated by cross-sectional imaging techniques such as CT or MRI can be considered measurable lesions if the soft tissue component meets the definition of measurability described above.
- Blastic bone lesions are non-measurable.

Cystic Lesions:

- Lesions that meet the criteria for radiographically defined simple cysts should not be considered malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.
- Cystic lesions thought to represent cystic metastases can be considered measurable lesions if they meet the definition of measurability described above.

  However, if non-cystic lesions are present in the same subject, these are preferred for selection as target lesions.

Lesions with Prior Local Treatment:

- Tumor lesions situated in a previously irradiated area or in an area subjected to other loco-regional therapy are usually not considered measurable unless there has been demonstrated progression in the lesion.

METHODS FOR ASSESSING LESIONS

All measurements should be recorded in metric notation, using calipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start 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 the study. Imaging-based evaluation should always be the preferred option.

CLINICAL LESIONS

Clinical lesions will only be considered measurable when they are superficial and ≥ 10 mm in diameter as assessed using calipers (eg, skin nodules). For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is suggested.

CHEST X-RAY

Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions.

However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.

CT AND MRI SCANS

CT is the best currently available and reproducible method to measure lesions selected for response assessment. In this guideline, the definition of measurability of lesions on

CT scan is based on the assumption that CT slice thickness is ≤ 5 mm. When CT scans have slice thickness of > 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable.

If prior to enrollment it is known that a subject is unable to undergo CT scans with intravenous (IV) contrast because of allergy or renal insufficiency, the decision as to whether a non-contrast CT or MRI (without IV contrast) will be used to evaluate the subject at baseline and during the study should be guided by the tumor type under investigation and the anatomic location of the disease. For subjects who develop contraindications to contrast after baseline contrast CT is done, the decision as to whether non-contrast CT or MRI (enhanced or non-enhanced) will be performed should also be based on the tumor type and the anatomic location of the disease, and should be optimized to allow for comparison with the prior studies if possible. Each case should be discussed with the radiologist to determine if substitution of these other approaches is possible and, if not, the subject should be considered not evaluable from that point forward. Care must be taken in measurement of target lesions and interpretation of non-target disease or new lesions on a different modality, since the same lesion may appear to have a different size using a new modality.

ENDOSCOPY, LAPAROSCOPY, ULTRASOUND, TUMOR MARKERS, CYTOLOGY, HISTOLOGY

Endoscopy, laparoscopy, ultrasound, tumor markers, cytology, and histology cannot be utilized for objective tumor evaluation.

ASSESSMENT OF TUMOR BURDEN

To assess objective response or future progression, it is necessary to estimate the overall tumor burden at baseline and use this as a comparator for subsequent measurements.

IDENTIFICATION OF TARGET AND NON-TARGET LESIONS

When more than one measurable lesion is present at baseline, all lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline. This means that, for instances in which subjects have only 1 or 2 organ sites involved, a maximum of 2 lesions (1 site) and 4 lesions (2 sites), respectively, will be recorded. Other lesions (albeit measurable) in those organs will be considered non-target lesions.

Target lesions should be selected on the basis of their size (lesions with the longest diameter) and be representative of all involved organs, but in addition should 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 that can be measured reproducibly should be selected.

Lymph nodes merit special mention since they are normal anatomical structures that may be visible by imaging even if not involved by tumor. As noted above, pathological nodes that are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of ≥ 15 mm by CT scan. Only the short axis of these nodes will contribute to the baseline sum. The short axis of the node is the diameter normally used by radiologists to judge if a node is involved by solid tumor. Lymph node size is normally reported as two dimensions in the plane in which the image is obtained (for CT, this is almost always the axial plane; for MRI, the plane of acquisition may be axial, sagittal, or coronal). The smaller of these measures is the short axis. For example, an abdominal node that is reported as being 20 mm × 30 mm has a short axis of 20 mm and qualifies as a malignant, measurable node. In this example, 20 mm should be recorded as the node measurement. All other pathological nodes (those with short axis ≥ 10 mm but < 15 mm) should be considered non-target lesions. Nodes that have a short axis of < 10 mm are considered non-pathological and should not be recorded or followed.

All lesions (or sites of disease) not selected as target lesions (measurable or non-measurable), including pathological lymph nodes, should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required. It is possible to record multiple non-target lesions involving the same organ as a single item on the Case Report Form (CRF) (eg, "multiple enlarged pelvic lymph nodes" or "multiple liver metastases").

NEW LESIONS

New lesions identified after baseline will be evaluated for measurability with use of the same criteria applied to prospective target lesions at baseline per RECIST (eg, non−lymph node lesions must be ≥ 10 mm on the longest diameter; new lymph nodes must be ≥ 15 mm on the short axis [see note below]). All new lesions (measurable or non-measurable) must be assessed and recorded at the time of identification and at all subsequent tumor assessment time points.

Up to a maximum of five measurable new lesions total (and a maximum of two lesions per organ) can be included in the calculation of tumor burden that is performed as part of the tumor response evaluation. New lesion types that would not qualify as target lesions per RECIST cannot be included in the calculation of tumor burden and thus will not affect overall tumor response evaluation. New lesions that are not measurable at first appearance but meet measurability criteria at a subsequent time point can be included in the tumor response evaluation from that point on, if the maximum number of measurable new lesions has not been reached.

Note regarding new lymph node lesions: If at first appearance the short axis of a lymph node lesion is ≥ 15 mm, it will be considered a measurable new lesion. If at first appearance the short axis of a lymph node lesion is ≥ 10 mm and < 15 mm, the lymph node will not be considered measurable but will still be considered a new lesion and should be identified as a non-measurable new lesion. If at first appearance the short axis of a lymph node is < 10 mm, the lymph node should not be considered pathological and should not be considered a new lesion. A lymph node can subsequently become measurable, when the short axis is ≥ 15 mm.

CALCULATION OF SUM OF DIAMETERS

A sum of the diameters (longest diameter for non−lymph node lesions, short axis for lymph node lesions) will be calculated for all target lesions at baseline as a measure of tumor burden. At each subsequent tumor assessment, a sum of the diameters (longest diameter for non-lymph node lesions, short axis for lymph node lesions) will be calculated for all target lesions plus measurable new lesions (up to five new lesions, with a maximum of two new lesions per organ) that have emerged after baseline. Hence, each net percentage change in tumor burden per assessment accounts for the size and growth kinetics of both old lesions and new lesions as they appear.

Measuring Lymph Nodes

If at first appearance the short axis of a new lymph node lesion is ≥ 15 mm, it will be considered a measurable new lesion and may be included in the sum of the diameters.

If the new lymph node lesion is included in the sum of diameters, it will continue to be measured and included in the sum of diameters at subsequent time points, even if the short axis decreases to < 15 mm (or even < 10 mm). However, if it subsequently decreases to < 10 mm and all other lesions are no longer detectable or have also decreased to a short axis of < 10 mm (if lymph nodes), a response assessment of complete response may be assigned.

Lymph nodes should always have the actual short axis measurement recorded (measured in the same anatomical plane as the baseline examination), even if the node regresses to < 10 mm during the study. Thus, when lymph nodes are included in the sum of diameters, the sum may not be zero even if complete response criteria are met, since a normal lymph node is defined as having a short axis of < 10 mm.

Measuring Lesions That Become Too Small to Measure

During the study, all target lesions and up to five measurable new lesions (lymph node and non−lymph node) should have their actual measurements recorded at each subsequent evaluation, even when very small (eg, 2 mm). However, sometimes lesions or lymph nodes become so faint on CT scan that the radiologist may not feel comfortable assigning an exact measurement and may report them as being too small to measure. When this occurs, it is important that a value be recorded on the CRF, as follows:

- If it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0 mm.

- If the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned and "too small to measure" should be ticked. (Note: It is less likely that this rule will be used for lymph nodes since they usually have a definable size when normal and are frequently surrounded by fat such as in the retroperitoneum; however, if a lymph node is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned in this circumstance as well and "too small to measure" should also be ticked).

To reiterate, however, if the radiologist is able to provide an actual measurement, that should be recorded, even if it is < 5 mm, and in that case "too small to measure" should not be ticked.

Measuring Lesions That Split or Coalesce on Treatment

When non−lymph node lesions fragment, the longest diameters of the fragmented portions should be added together to calculate the sum of diameters. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in obtaining maximal diameter measurements of each individual lesion. If the lesions have truly coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximum longest diameter for the coalesced lesion.

EVALUATION OF NON-TARGET LESIONS AND NON-MEASURABLE NEW LESIONS

Measurements are not required for non-target lesions or non-measurable new lesions.

Non-target lesions should be noted at baseline, and non-measurable new lesions should be noted at the time of identification. At subsequent evaluations, non-target lesions and non-measurable new lesions will be categorized as "present" or "absent."

After baseline, changes in non-target lesions or non-measurable new lesions (or measurable new lesions in excess of five total or two per organ) will contribute only in the assessment of complete response (ie, a complete response is attained only with the complete disappearance of all tumor lesions, including non-target lesions and non-measurable new lesions) and will not be used to assess progressive disease.

RESPONSE CRITERIA

Definitions of the criteria used to determine objective tumor response are provided below:

- Complete response (CR): Disappearance of all lesions. Any pathological lymph nodes must have reduction in short axis to < 10 mm.
- Partial response (PR): At least a 30% decrease in the sum of diameters of all target lesions plus measurable new lesions (up to a maximum of five total or two per organ), taking as reference the baseline sum of diameters, in the absence of CR
- Progressive disease (PD): At least a 20% increase in the sum of diameters of all target lesions plus measurable new lesions (up to a maximum of five total or two per organ), taking as reference the smallest sum of diameters on study (including baseline)

- In addition to the relative increase of 20%, the sum of diameters must also demonstrate an absolute increase of ≥ 5 mm. New lesions alone do not qualify as progressive disease. However, their contribution to total tumor burden is factored into the sum of the diameters, which is used to determine the overall irRECIST tumor response.
- Stable disease (SD): Neither sufficient shrinkage to qualify for CR or PR nor sufficient increase to qualify for PD

MISSING ASSESSMENTS AND NOT-EVALUABLE DESIGNATION

When no imaging/measurement is done at all at a particular time point, the subject is not evaluable at that time point. If measurements are made on only a subset of target or measurable new lesions at a time point, usually the case is also considered not evaluable

at that time point, unless a convincing argument can be made that the contribution of the individual missing lesions would not change the assigned timepoint response. This would be most likely to happen in the case of PD. For example, if a subject had a baseline sum of 50 mm with three measured lesions and during the study only two lesions were assessed, but those gave a sum of 80 mm, the subject will have achieved PD status, regardless of the contribution of the missing lesion.

SPECIAL NOTES ON RESPONSE ASSESSMENT

Subjects with a global deterioration in 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 objective progression even after discontinuation of treatment. Symptomatic deterioration is not a descriptor of an objective response; it is a reason for stopping study therapy. The objective response status of such subjects is to be determined by evaluation of target and non-target lesions, as well as new lesions, as shown in Table 2.

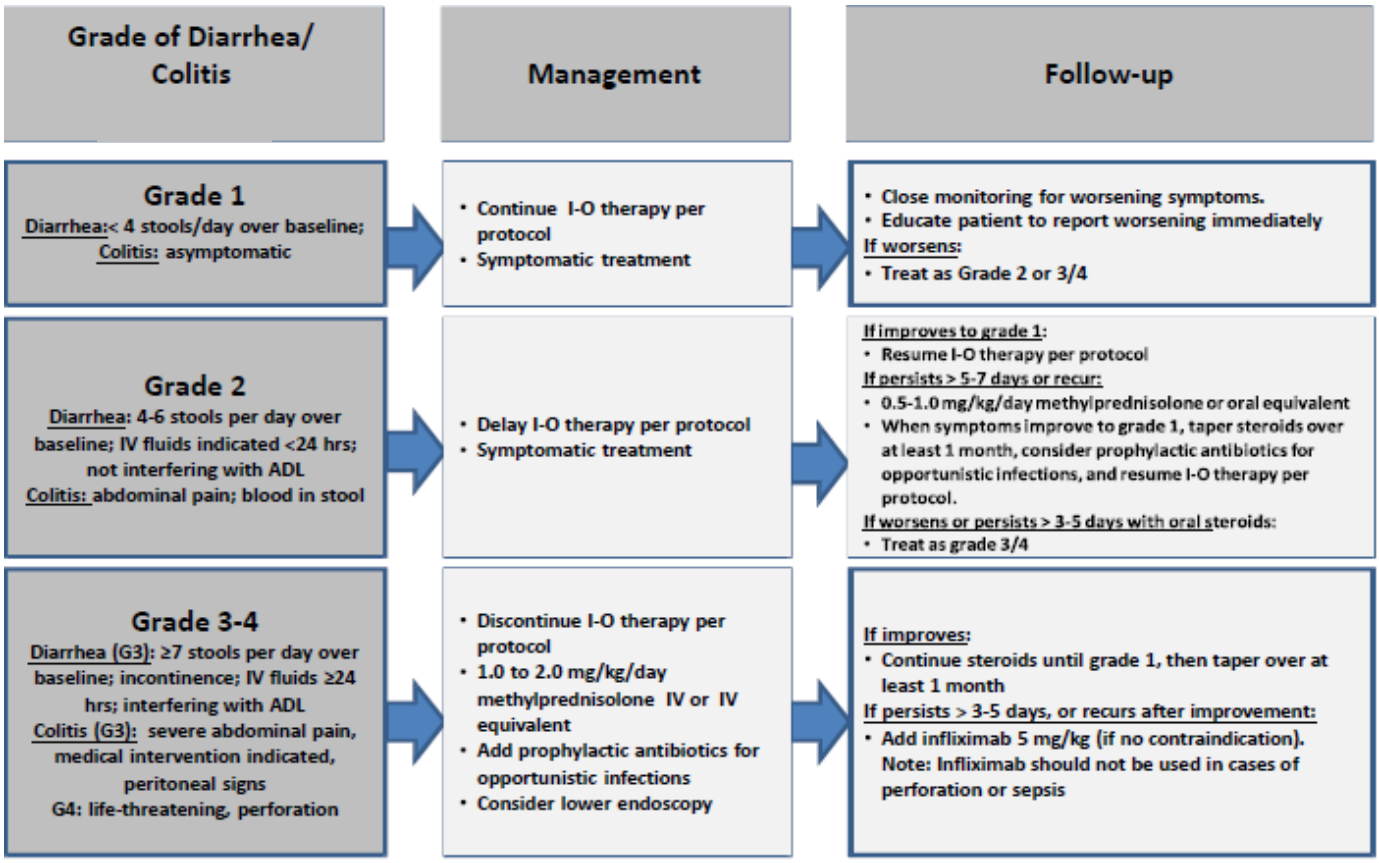
APPENDIX D: Toxicity Management

(From Investigator Brochure nivolumab: BMS-936558/MDX1106/ONO-4538, pg 283-290)

Corticosteroids are a primary therapy for immuno-oncology drug-related adverse events. The oral equivalent of the recommended IV doses may be considered for ambulatory subjects with low-grade toxicity. The lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

GI Adverse Event Management Algorithm

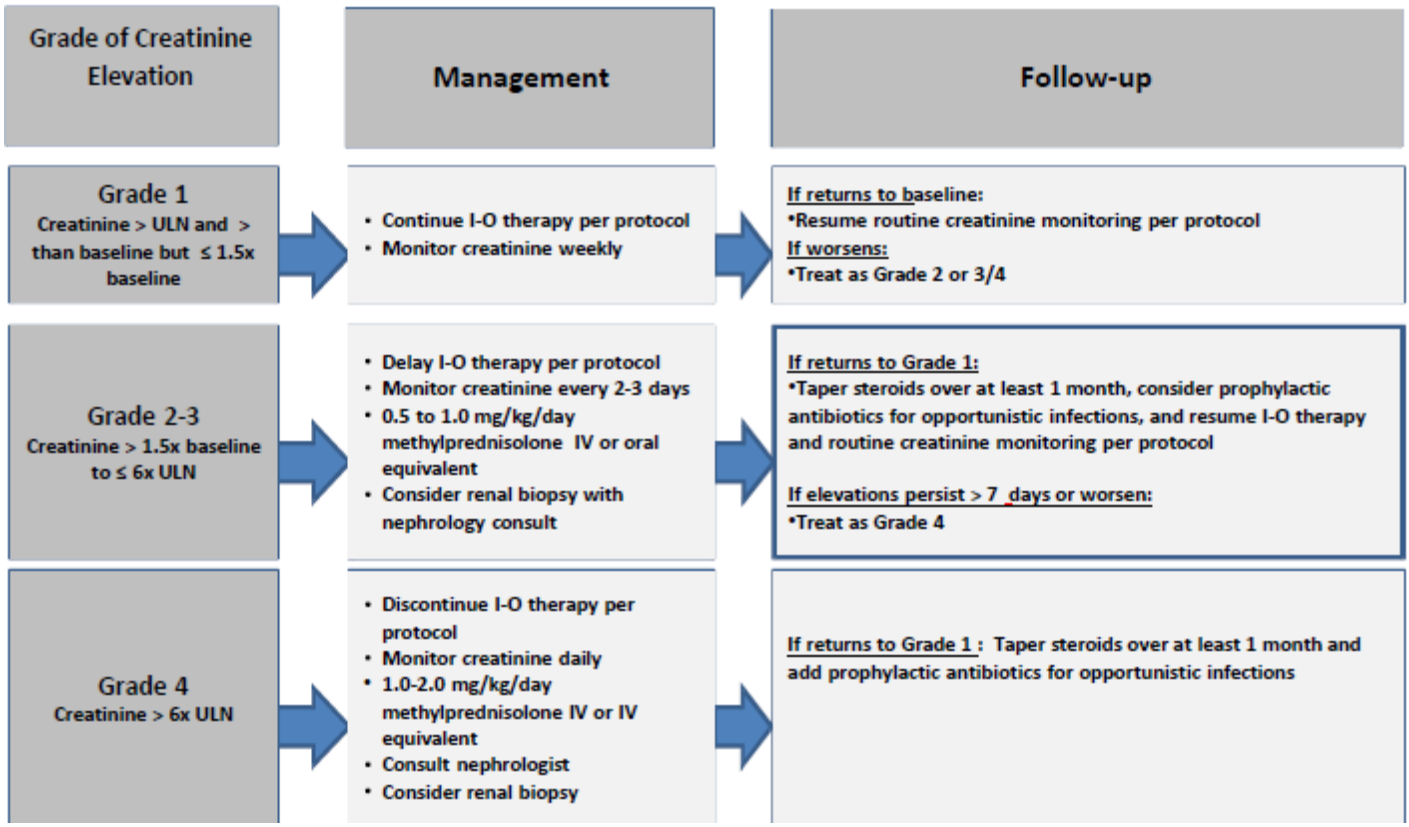
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.



Subjects on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Renal Adverse Event Management Algorithm

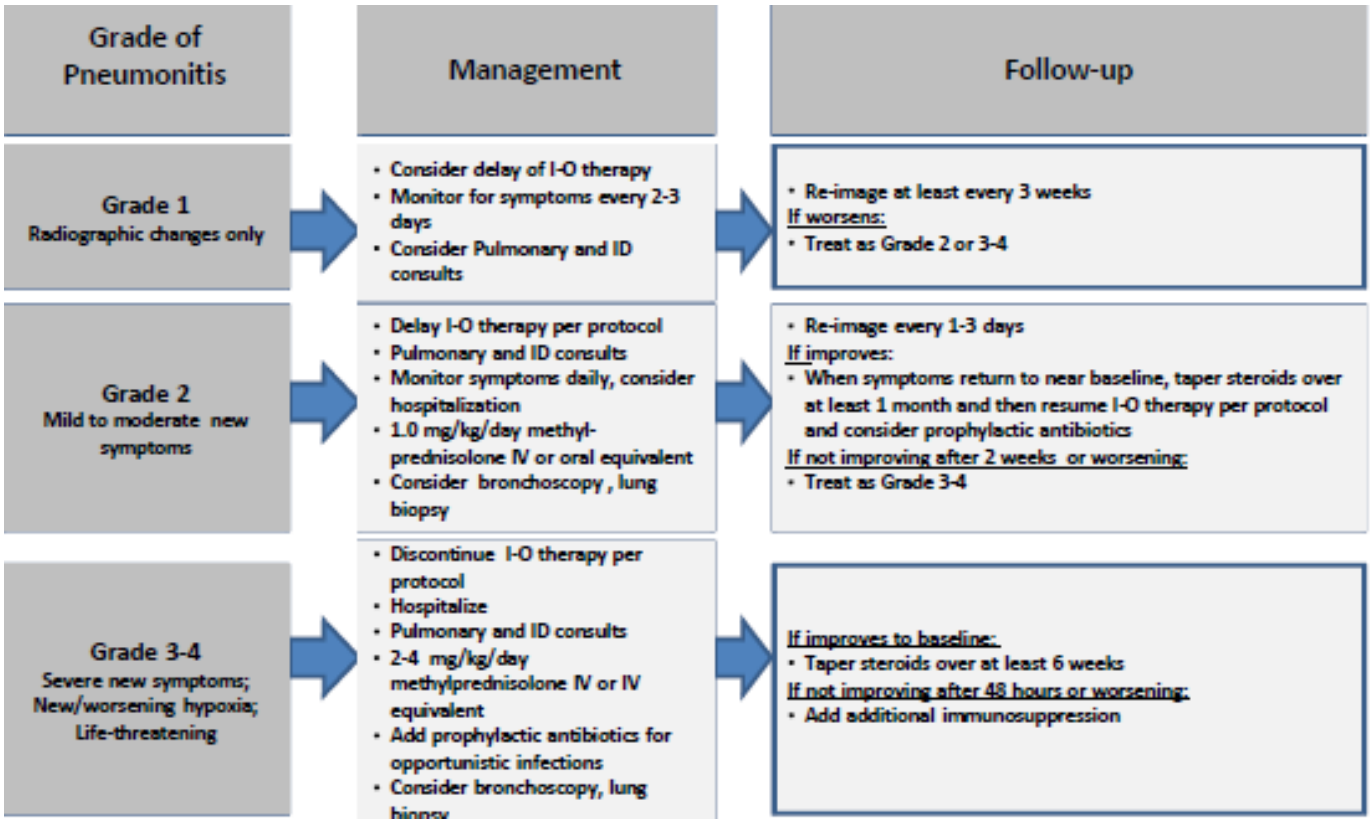
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



Subjects on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Pulmonary Adverse Event Management Algorithm

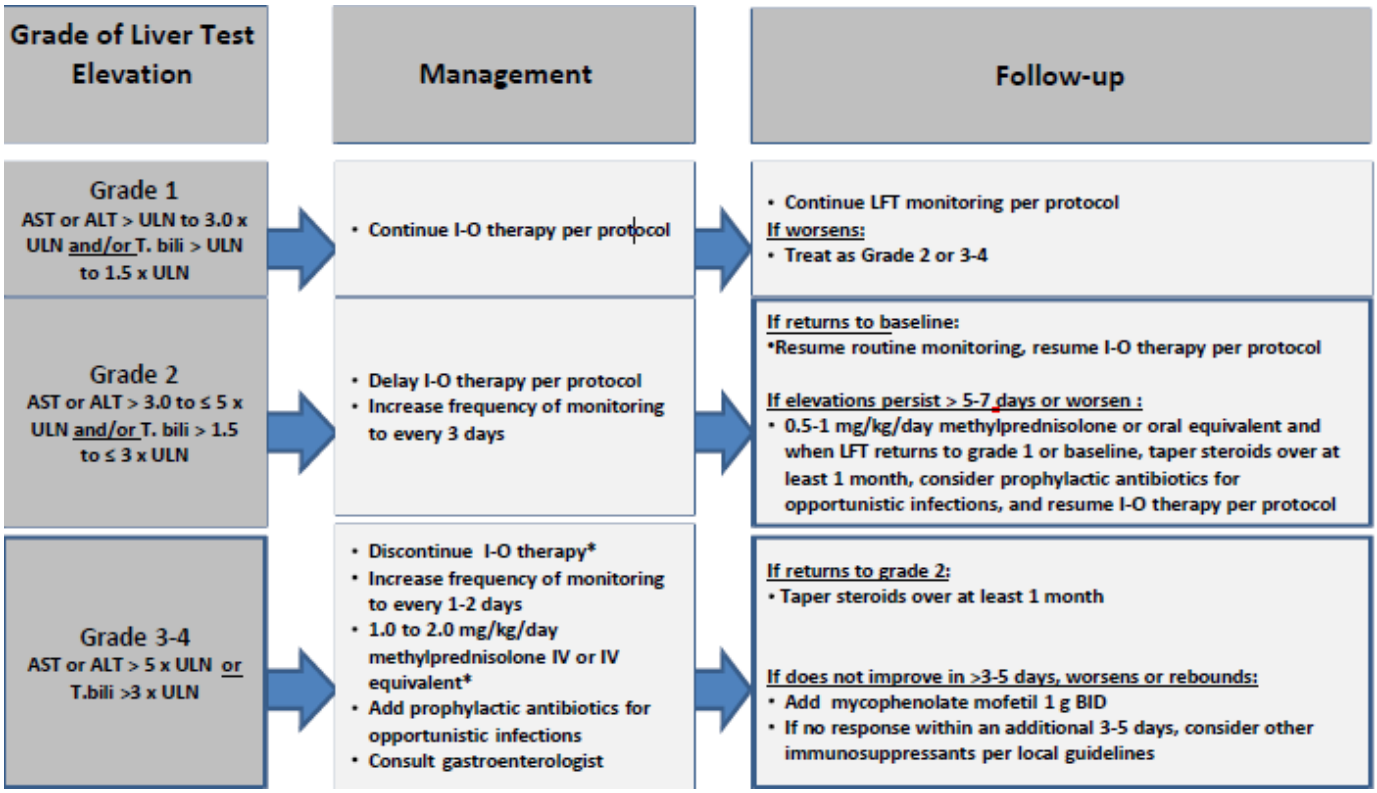
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.



Subjects on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids

Hepatic Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider imaging for obstruction.

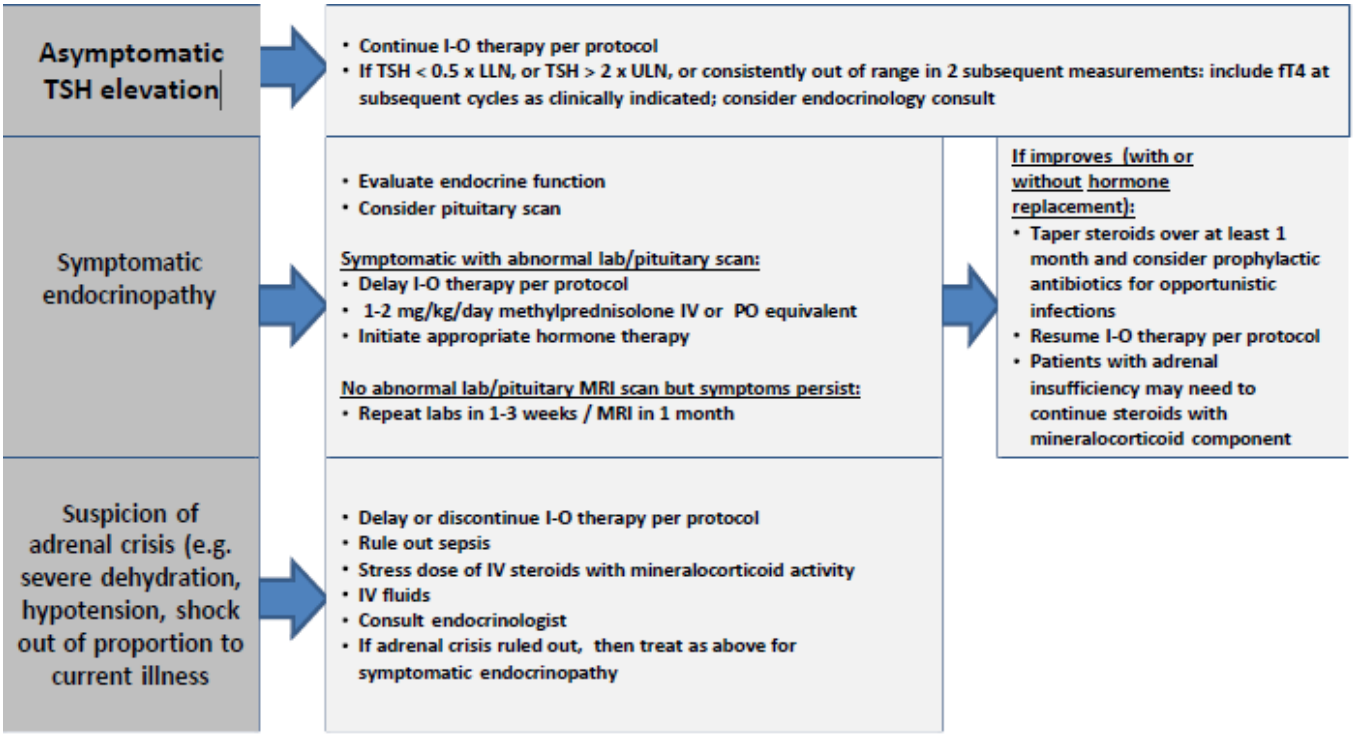


Subjects on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

*The recommended starting dose for Grade 4 hepatitis is 2 mg/kg/day methylprednisolone IV.

Endocrinopathy Adverse Event Management Algorithm

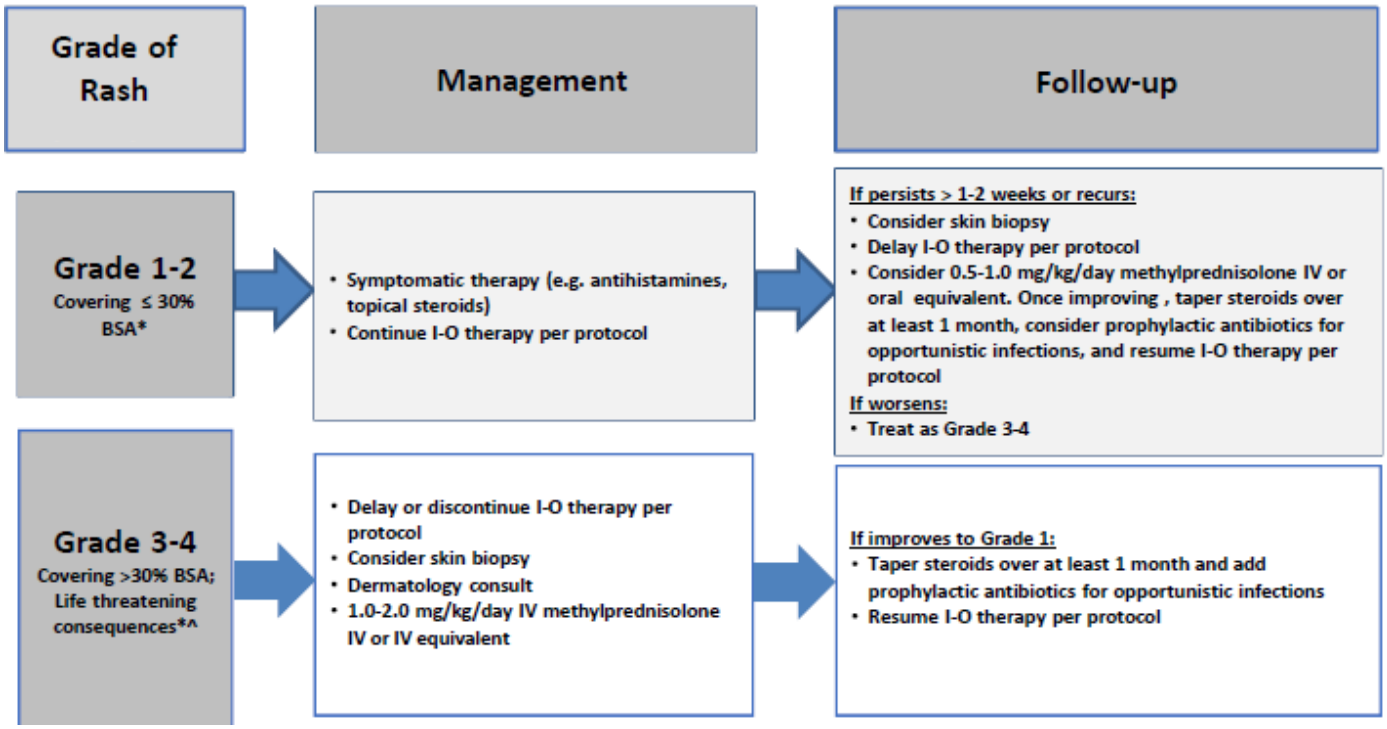
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider visual field testing, endocrinology consultation, and imaging.



Subjects on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Skin Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



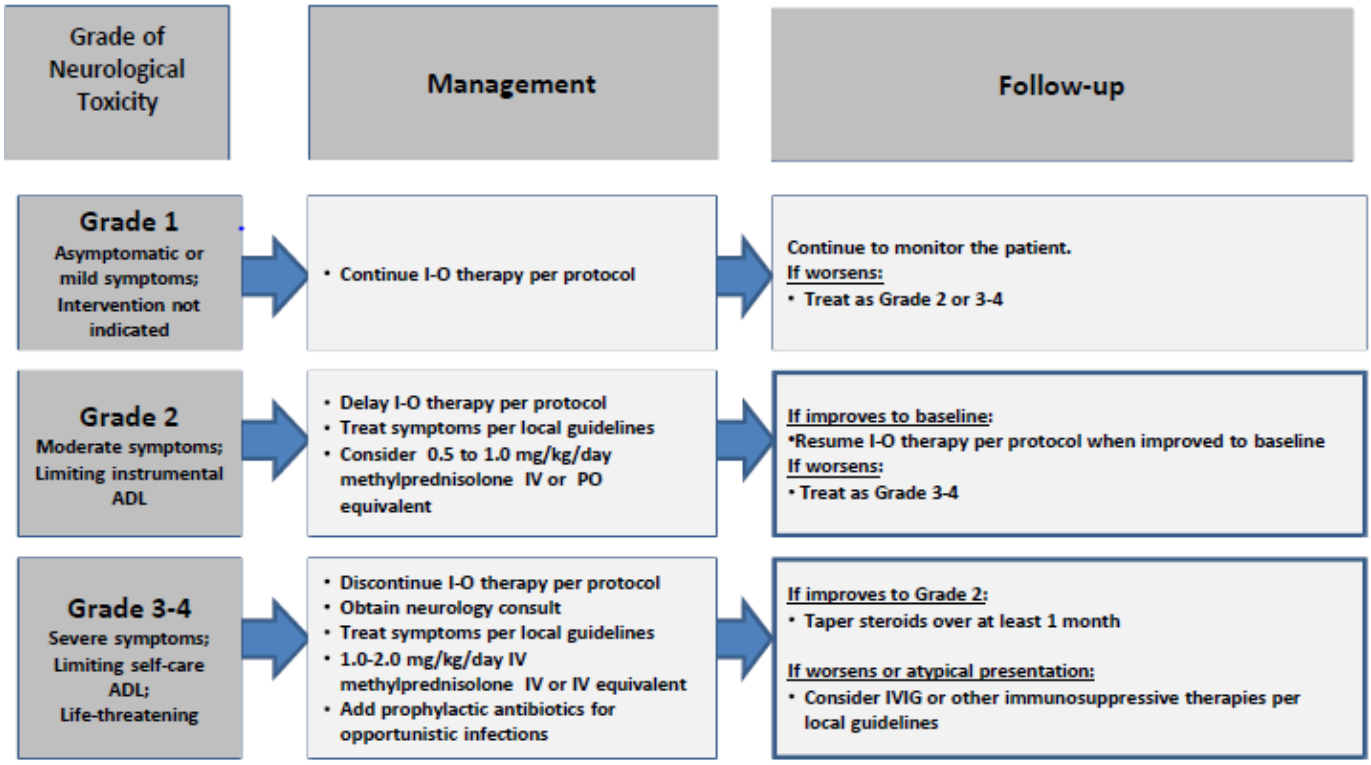
Subjects on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

*Refer to NCI CTCAE v5 for term-specific grading criteria.

^If SJS/TEN is suspected, withhold I-O therapy and refer subject for specialized care for assessment and treatment. If SJS or TEN is diagnosed, permanently discontinue I-O therapy.

Neurological Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



Subjects on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Appendix E: Definitions and Adverse Event Collection and Reporting Information for Interventional Protocols:

DEFINITIONS

ADVERSE EVENTS

An Adverse Event (AE) is defined as any new untoward medical occurrence or worsening of a preexisting medical condition in a clinical investigation participant administered study drug and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (such as an abnormal laboratory finding), symptom, or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product.

A ***non-serious adverse event*** is an AE not classified as serious.

The protocol must include a definition for Serious Adverse Events (SAE).

SERIOUS ADVERSE EVENTS

A ***Serious Adverse Event (SAE)*** is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening (defined as an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- requires in-subject hospitalization or causes prolongation of existing hospitalization (see **NOTE** below)
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [eg, medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)
- Suspected transmission of an infectious agent (eg, pathogenic or nonpathogenic) via the study drug is an SAE.
- Unusual Failure in Efficacy (for Phase IV Canadian studies)

Although pregnancy and potential drug-induced liver injury (DILI), are not always serious by regulatory definition, however, these events must be reported within the SAEs timeline.

Any component of a study endpoint that is considered related to study therapy should be reported as an SAE (eg, death is an endpoint, if death occurred due to anaphylaxis, anaphylaxis must be reported).

The following hospitalizations are not considered SAEs in BMS clinical studies:

- a visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered an important medical or life-threatening event)
- elective surgery, planned prior to signing consent
- admissions as per protocol for a planned medical/surgical procedure
- routine health assessment requiring admission for baseline/trending of health status (eg, routine colonoscopy)
- Medical/surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation is required in these cases.
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (eg, lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason).
- Admission for administration of anticancer therapy in the absence of any other SAEs (applies to oncology protocols)

ADVERSE EVENT Collection and REPORTING INFORMATION:

- All Serious Adverse Events (SAEs) that occur following the subject's written consent to participate in the study through 100 days of discontinuation of dosing must be reported to BMS Worldwide Safety, whether related or not related to study drug. If applicable, SAEs must be collected that relate to any later protocol-specified procedure (eg, a follow-up skin biopsy).
- Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, are collected, including those thought to be associated with protocol-specified procedures. The investigator should report any SAE occurring after these aforementioned time periods, which is believed to be related to study drug or protocol-specified procedure.
- An SAE report should be completed for any event where doubt exists regarding its seriousness;
- If the investigator believes that an SAE is not related to study drug, but is potentially related to the conditions of the study (such as withdrawal of previous therapy or a complication of a study procedure), the relationship should be specified in the narrative section of the SAE Report Form.

An appropriate SAE form (Medwatch form) should be used to report SAEs to BMS. If you prefer to use your own Institutional form, it must be reviewed by the BMS Protocol Manager prior to study initiation to ensure that at a minimum all of the data elements on

the CIOMS form are present. Note: Please include the BMS Protocol number on the SAE form or on the cover sheet with the SAE form transmission.

- ✓ The CIOMS form is available at: http://www.cioms.ch/index.php/cioms-form-i
- ✓ The MedWatch form is available at: MedWatch 3500 Form

- The Sponsor will reconcile the clinical database AE cases (**case level only**) transmitted to BMS Global Pharmacovigilance (Worldwide.Safety@bms.com).
  - ✓ The Investigator will request from BMS GPV&E, aepbusinessprocess@bms.com the SAE reconciliation report and include the BMS protocol number every 3 months and prior to data base lock or final data summary
  - ✓ GPV&E will send the investigator the report to verify and confirm all SAEs have been transmitted to BMS GPV&E.
  - ✓ The data elements listed on the GPV&E reconciliation report will be used for case identification purposes. If the Investigator determines a case was not transmitted to BMS GPV&E, the case should be sent immediately to BMS (Worldwide.Safety@bms.com).
- In addition to the Sponsor Investigator's responsibility to report events to their local HA, suspected serious adverse reactions (whether expected or unexpected) shall be reported by BMS to the relevant competent health authorities in all concerned countries according to local regulations (either as expedited and/or in aggregate reports).
- In accordance with local regulations, BMS will notify sponsor investigators of all reported SAEs that are suspected (related to the investigational product) and unexpected (ie, not previously described in the IB). An event meeting these criteria is termed a Suspected, Unexpected Serious Adverse Reaction (SUSAR). Sponsor investigator notification of these events will be in the form of either a SUSAR Report or a Semi-Annual SUSAR Report.
  - ✓ Other important findings which may be reported by BMS as an Expedited Safety Report (ESR) include: increased frequency of a clinically significant expected SAE, an SAE considered associated with study procedures that could modify the conduct of the study, lack of efficacy that poses significant hazard to study subjects, clinically significant safety finding from a nonclinical (eg, animal) study, important safety recommendations from a study data monitoring committee, or sponsor or BMS decision to end or temporarily halt a clinical study for safety reasons.
  - ✓ Upon receiving an ESR from BMS, the investigator must review and retain the ESR with the IB. Where required by local regulations or when there is a central IRB/IEC for the study, the sponsor will submit the ESR to the appropriate IRB/IEC. The investigator and IRB/IEC will determine if the

informed consent requires revision. The investigator should also comply with the IRB/IEC procedures for reporting any other safety information.

SAEs, whether related or not related to study drug, and pregnancies must be reported to BMS within 24 hours \ 1 Business Day of becoming aware of the event. SAEs must be recorded on either CIOMS, MedWatch, or approved site SAE form.

Pregnancies must be reported and submitted to BMS. BMS will perform due diligence follow-up using the BMS Pregnancy Form which the investigator must complete.

SAE Email Address: Worldwide.Safety@BMS.com

SAE Facsimile Number: +1-609-818-3804

If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported.)

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours \ 1 Business Day to BMS using the same procedure used for transmitting the initial SAE report.

All SAEs should be followed to resolution or stabilization.

The causal relationship to study drug is determined by a physician and should be used to assess all adverse events (AE). The casual relationship can be one of the following:

Related: There is a reasonable causal relationship between study drug administration and the AE.

Not related: There is not a reasonable causal relationship between study drug administration and the AE.

The term "reasonable causal relationship" means there is evidence to suggest a causal relationship.

Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. (In order to prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more AEs.)

NONSERIOUS ADVERSE EVENT

- Non-serious Adverse Events (AE) are to be provided to BMS in aggregate via interim or final study reports as specified in the agreement or, if a regulatory requirement [eg, IND US trial] as part of an annual reporting requirement.
- Non-serious AE information should also be collected from following the subject's written consent to participate in the study.

Non-serious Adverse Event Collection and Reporting

The collection of non-serious AE information should begin following the subject's written consent to participate in the study. All non-serious adverse events (not only those

deemed to be treatment-related) should be collected continuously during the treatment period and for a minimum of 30 days following the last dose of study treatment.

Non-serious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up is also required for non-serious AEs that cause interruption or discontinuation of study drug and for those present at the end of study treatment as appropriate.

Laboratory Test Abnormalities

All laboratory test results captured as part of the study should be recorded following institutional procedures. Test results that constitute SAEs should be documented and reported to BMS as such.

The following laboratory abnormalities should be documented and reported appropriately:

- any laboratory test result that is clinically significant or meets the definition of an SAE
- any laboratory abnormality that required the participant to have study drug discontinued or interrupted
- any laboratory abnormality that required the subject to receive specific corrective therapy.

It is expected that wherever possible, the clinical rather than laboratory term would be used by the reporting investigator (eg, anemia versus low hemoglobin value).

Pregnancy

If, following initiation of the investigational product, it is subsequently discovered that a study participant is pregnant or may have been pregnant at the time of investigational product exposure, including during at least 5 half-lives after product administration, the investigational product will be permanently discontinued in an appropriate manner (eg, dose tapering if necessary for participant).

The investigator must immediately notify Worldwide.Safety@bms.com of this event and complete one of the following forms within 24 hours of awareness of the event via either the CIOMS, MedWatch or appropriate Pregnancy Surveillance Form in accordance with SAE reporting procedures.

Protocol-required procedures for study discontinuation and follow-up must be performed on the participant.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the CIOMS, MedWatch, BMS Pregnancy Surveillance Form, **or** approved site SAE form. A BMS Pregnancy Surveillance Form may be provided upon request.

Any pregnancy that occurs in a female partner of a male study participant should be reported to BMS. Information on this pregnancy will be collected on the Pregnancy Surveillance Form. In order for Sponsor or designee to collect any pregnancy

surveillance information from the female partner, the female partner must sign an informed consent form for disclosure of this information.

Other Safety Considerations

Any significant worsening noted during interim or final physical examinations, electrocardiograms, X-rays, and any other potential safety assessments, whether or not these procedures are required by the protocol, should also be recorded as a non-serious or serious AE, as appropriate, and reported accordingly.

Appendix F: Patient-reported Outcome (PRO) Questionnaires:

Provided on the following pages:

- National Cancer Institute (NCI) patient-reported outcomes (PRO) questionnaire (PRO-CTCAE), English
- National Cancer Institute (NCI) patient-reported outcomes (PRO) questionnaire (PRO-CTCAE), Spanish
- National Cancer Institute (NCI) patient-reported outcomes (PRO) questionnaire (PRO-CTCAE), Chinese (traditional)

APPENDIX REFERENCES

Appendix B: Eisenhauer EA, Therasse P, Bogaerts J, *et al*. New response evaluation criteria in solid tumors: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228−47.

Appendix C: Nishino M, Gargano M, Suda M, *et al*. Optimizing immune-related tumor response assessment: does reducing the number of lesions impact response assessment in melanoma subjects treated with ipilimumab? J Immunother Can 2014;2:17.

Appendix C: Wolchok JD, Hoos A, O'Day S, *et al*. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Can Res 2009;15:7412−20.

Appendix D: Bristol-Myers Squibb Research and Development. Investigator Brochure nivolumab: BMS-936558/MDX1106/ONO-4538, version no: 17, Version date: 27Jun2018; pg 283-290.

Appendix E: Bristol-Myers Squibb. ISR Protocol and AE Reporting Guidance_v8_November2018; pg 9-13.