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TITLE: Phase 2 Study of Nivolumab in Solid tumors induced by Prior Radiation Exposure

Center: Johns Hopkins University School of Medicine, Sidney Kimmel Comprehensive Cancer Center

Principal Investigator: Name Patrick Forde, MD

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Nivolumab (BMS-936558) (IND#118,458) BMS Inc.

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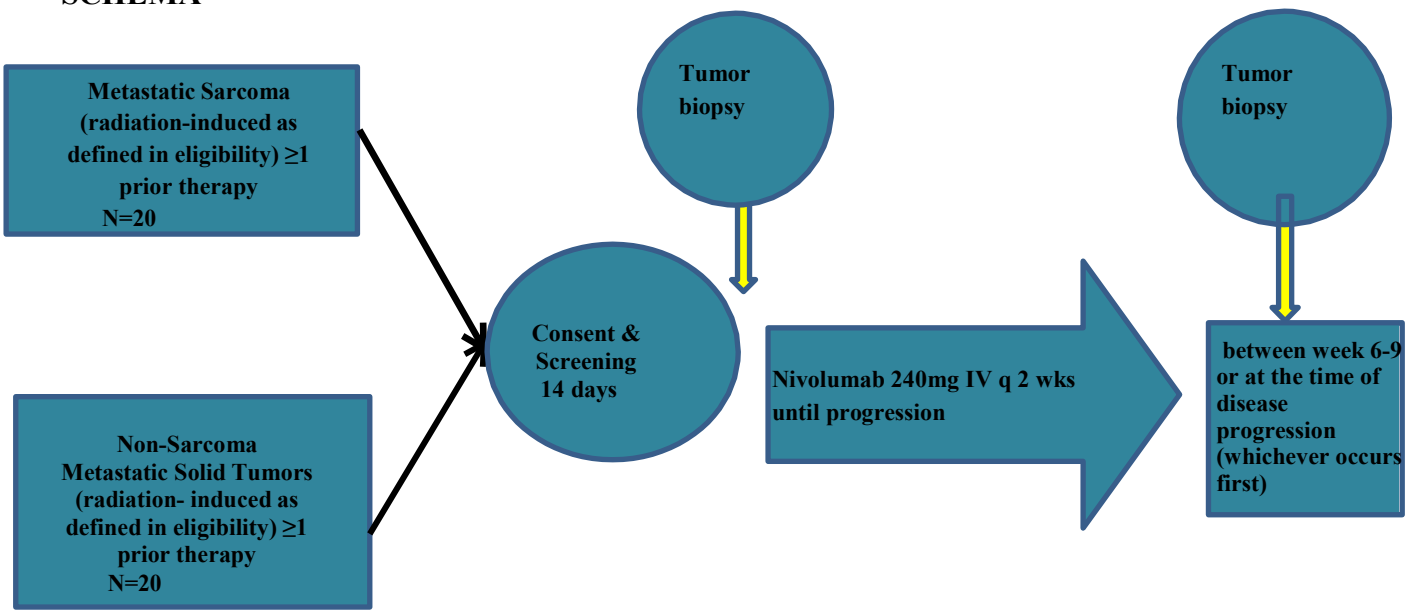
**Sponsor: Patrick Forde, MD.**

**SCHEMA**




This is a pilot phase 2 study of nivolumab in patients with advanced or metastatic solid tumors where the primary tumor has arisen with an area that has been exposed to previous external beam radiation i.e. radiation-induced solid tumors. This study has two arms, allowing for up to 10% non-evaluable patients, one of which will enroll up to 22 patients with sarcomas (these are the most frequent solid tumors induced by previous radiation exposure) while the second arm will enroll up to 22 patients with other radiation-induced solid tumors (it is expected that this arm will enroll predominantly lung cancers, pancreatic cancers and thyroid cancers among other solid tumors).

The primary endpoint for this study will be best overall response rate (BORR) as determined by RECIST 1.1 (evaluation for BORR will continue up to 24 weeks on nivolumab therapy to allow for the unique patterns of response to immune checkpoint therapy). If the BORR in either arm of this study exceeds 50% then we will conclude that the treatment in this patient population is promising enough to lead us to undertake a larger confirmatory study.

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# 1. OBJECTIVES

### 1.1 Primary Endpoint

Best overall response rate (BORR) – evaluation for BORR will continue up to 24 weeks on nivolumab therapy and response will be coded based on RECIST 1.1 criteria

### 1.2 Secondary Endpoints

1. Percentage of patients progression-free at 24 weeks from the time of enrollment: Disease status at 24 weeks will be compared to disease status at the time of enrollment, and response coded based on RECIST 1.1 criteria.
2. Progression-free survival: Progression-free survival will be measured from the time of study enrollment until radiologic or death.
3. Duration of response - The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.
4. Safety and Tolerability – Toxicities observed will be assessed by CTCAE 4.0 criteria
5. Overall survival Overall survival will be measured from the time of enrollment until death.

### 1.3 Exploratory Endpoints

Laboratory correlates of response:
Whole-exome sequencing and assessment of immunologic parameters in mandatory pre-and post-treatment biopsies will be performed. Assessment of tumor baseline PD-L1 expression will be performed. Serial assessment of target gene methylation status in circulating DNA, and of gene expression in peripheral blood mononuclear cells, will be performed.

# 2. BACKGROUND

### 2.1 Role of ionizing radiation in generating genomic instability and driving second cancer tumorigenesis.

Improvements in therapy for a variety of malignancies have led to increasing numbers of people who are long-term cancer survivors. Survivors of both adult and childhood cancers are at risk for developing therapy-related complications, including second cancers[1] and approximately one in every six cancers diagnosed in the United States occurs among the nearly 14 million cancer survivors nationwide[2]. Therapeutic radiation is recognized as an inducing agent in the development of several different malignant neoplasms including second cancers of the breast, thyroid, central nervous system, gastrointestinal track, lung and sarcomas[1,3-10]. Patients undergoing radiotherapy are exposed to a wide range of radiation doses to tissues outside the treatment area because of scatter. A study on adult cancer survivors attributed about 10% of second cancers to radiotherapy alone[11]. Radiotherapy can result in different types of cancers, and the risk is directly proportional to the dose and duration of treatment[12]. In most cases, tumors resulting from radiation occur 10-20 years post-exposure, reflecting the time required for the accumulation of deleterious mutations[1].

Genomic instability is a hallmark of cancer. DNA damage and the associated repair mechanisms play a crucial role in carcinogenesis, as most oncogenic alterations in humans are caused by the inefficient repair of damaged DNA[13]. Radiation induces multiple types of DNA damage, including double-strand breaks[14] which differ from the effects of known mutagen such as UV radiation and tobacco[15]. DNA injury activates the DNA damage response (DDR) pathway ultimately leading to cell death. If the damaged DNA is misrepaired, cells escape DDR-mediated senescence and death, transforming to a cell population that carries deleterious mutations with oncogenic potential[16,17]. Radiation has also been shown to facilitate the formation of gene fusions in prostate cancer cells[18].

The genomic background of radiation-induced second cancers has not been thoroughly investigated. A genome-wide association study of radiation-induced second cancers revealed that a locus on chromosome 6q21 is significantly associated with second cancer risk and subsequent functional studies identified PRDM1, a transcriptional repressor as a radiation- responsive tumor suppressor[19]. Whole-exome sequencing of ionizing radiation-induced malignancies in mouse models has been associated with distinct mutational signatures and a high number of genomic alterations in a majority of tumors[20]. Furthermore, studies on comparing mutational landscapes of carcinogen-induced lung cancer mouse models have shown that oncogene driven lung cancers harbor significantly fewer single nucleotide variants compared to carcinogen-associated tumors[21]. Mutagen-associated malignancies have relatively high mutation rates and foci of localized substitution hypermutation are often found close to genomic rearrangements in human cancers[22]. These foci include up to several thousand mutations and introduction of a DNA double-strand break greatly increases the likelihood of such foci in its vicinity, indicating a role for DNA double-strand breaks in initiating the process[23]. Genomic injury resulting from initial cancer therapy might thus shape cancer evolution and subsequent resistance to cancer therapies.

### 2.2 Nivolumab

Nivolumab (BMS-936558 (MDX-1106)) is a fully human IgG4:Ƙ monoclonal antibody that blocks PD-1, an inhibitory receptor expressed on activated T and B cells. PD-L1 or PD-L2 expressed on tumors or non-transformed cells in the tumor microenvironment can bind to PD-1 on the surface of CD4 helper and CD8 cytotoxic T cells, suppressing anti-tumor cytolytic responses[24]. Nivolumab has demonstrated durable responses exceeding 12 months as monotherapy in several tumor types, including NSCLC, melanoma and renal cell carcinoma, and has demonstrated improved overall survival for pretreated patients with these tumor types compared with standard cytotoxic or targeted therapies[25-27]. Nivolumab has been generally well tolerated in studies to date with grade 3-4 toxicities occurring in 10-19% of patients treated[25-27].

### 2.3 Rationale for nivolumab treatment for radiation-induced solid tumors

There is a recognized association between high tumor somatic mutational burden and neoantigen load with improved objective response and clinical benefit from immune checkpoint blockade[28-30]. Non-synonymous mutations are foreign to the immune system and therefore could represent tumor-specific antigens and immunogenicity resulting from missense mutations has been demonstrated in preclinical models[31]. Any genetic alteration affecting a protein-coding region has the potential to generate mutated peptides that are presented by surface MHC class I proteins to cytotoxic T cells and several whole

exome sequencing studies have used computational algorithms to predict candidate tumor neoantigens generated by missense somatic mutations in a variety of solid tumors[29,32-34]. The importance of tumor genetics in delineating therapeutic benefit from immune checkpoint inhibitors has been shown in melanoma, where mutational load and distinct somatic neoepitope signatures were found to be highly predictive of long- term clinical benefit from ipilimumab[29]. The role of neoantigen signatures in predicting response to CTLA-4 blockade remains controversial[30] however high somatic mutational density has been consistently correlated with response to immunotherapies[28-30]. High nonsynonymous mutational load was recently shown to correlate with durable benefit from treatment with the anti-PD-1 antibody pembrolizumab in NSCLC however a predictive neoantigen signature has not been identified yet[35].

At our institution we have treated two never smoker patients with radiation induced NSCLC who have had dramatic clinical and radiological responses to anti-PD-1 treatment with clinical improvement after one dose and radiological response at first restaging (the patients both had prior chest radiation exposure during treatment for Hodgkin Lymphoma and seminoma, 20 and 30 years ago respectively and developed lung cancers within the previous radiation field). A third never smoker patient with radiation-induced NSCLC is currently on therapy and awaiting first restaging. In general the expected objective response rate to anti-PD1 therapy in NSCLC patients who are never smokers is low, ranging from 0-10% in several retrospective analyses[36-38].

Our hypothesis is that these patients with radiation-induced tumors have a high mutational burden related to previous radiation exposure with consequent propensity to neo-antigen expression. We further hypothesize that high somatic mutational density and related neoantigen signatures are driving response to immune checkpoint blockade and that other patients with diverse radiation- induced malignancies may be likely to respond to nivolumab.

Radiation-induced malignancies are recognized as a significant unmet medical need and may follow an aggressive disease course with rapid progression on standard chemotherapy. This pilot study will evaluate nivolumab as a therapy for diverse solid tumors which have been induced by prior radiation exposure. In addition we will perform detailed genomic and immunologic analyses to identify predictors of response with the goal of performing larger confirmatory studies should we note a significant treatment effect in this initial study.

# 3. PATIENT SELECTION

## 3.1 Eligibility Criteria

3.1.1 Patients must have a histologically confirmed solid tumor that is metastatic or unresectable and for which standard curative or palliative measures do not exist or are no longer effective. Additionally, in the opinion of the investigator the primary site of the metastatic or unresectable tumor must have arisen within a previously irradiated site and be considered a radiation-induced tumor.

3.1.2 Patients must have a pre-treatment tumor specimen available for correlative studies, either core needle biopsy or equivalent amount or via excisional specimen (cytology specimen not acceptable for this purpose). If an archival specimen is to be used then no interceding anticancer therapy (systemic therapy or radiation to the biopsied lesion) should have been

administered since that specimen was obtained. Patients with no available archived specimen must be willing to undergo a pre-treatment tumor biopsy.

3.1.3 Patients must have measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded for non-nodal lesions and short axis for nodal lesions) as ≥20 mm with conventional techniques or as ≥10 mm with spiral CT scan, MRI, or calipers by clinical exam for non-nodal lesions, or as ≥15mm short axis for nodal lesions. The site of measurable disease should not have been irradiated for the treatment of the current cancer. See Section 11 for the evaluation of measurable disease.

3.1.4 If an approved first-line standard therapy for the patient's tumor is available, subjects must have failed, be intolerant to, be ineligible for, or have refused that treatment. Enrollment of patients for whom no standard therapy exists or who decline standard therapy should be discussed with the Principal Investigator prior to enrollment. Patients must have progressive disease on study entry.

3.1.4.1 Patients who received adjuvant or neoadjuvant chemotherapy (after surgery and/or radiation therapy) and developed recurrent or metastatic disease within 6 months of completing therapy are eligible and the adjuvant or neoadjuvant chemotherapy will count as a line of therapy as above.

3.1.5 Age ≥18 years.

3.1.6 ECOG performance status ≤2

3.1.7 Life expectancy of greater than 3 months

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

- WBC ≥2,000/mcL
- Absolute neutrophil count ≥1,500/mcL
- Platelets ≥100,000/mcL
- Hemoglobin ≥9.0 g/dL
- Total bilirubin ≤1.5 times the institutional upper limit of normal (ULN) (except subjects with Gilbert Syndrome, who can have total bilirubin < 3.0 mg/dL)
- AST(SGOT)/ALT(SGPT) ≤3 X institutional ULN
- Creatinine ≤1.5 X institutional ULN OR
- Creatinine clearance ≥40 mL/min for patients with creatinine

Levels above institutional normal (calculated using the Cockcroft-Gault formula below).

- *Female* CrCl = $\frac{(140 - \text{age in years}) \times \text{weight in kg} \times 0.85}{72 \times \text{serum creatinine in mg/dL}}$

- *Male* CrCl = $\frac{(140 - \text{age in years}) \times \text{weight in kg} \times 1.00}{72 \times \text{serum creatinine in mg/dL}}$

3.1.9 Women of childbearing potential (WOCBP) must use highly effective method(s) of contraception. WOCBP should use a highly effective method to avoid pregnancy for the duration of this study and for at least 5 months after the last dose of nivoluab. . The effects of

nivolumab, on the developing human fetus are unknown. For this reason WOCBP and men must agree to use a highly effective contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation and for at least 5 months from the last dose of nivolumab. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men who are sexually active with WOCBP must also use a highly effective contraceptive method for at least 7 months after the last dose of nivolumab.

3.1.10 Women of childbearing potential must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 24 hours prior to the start of nivolumab. For WOCBP, follow up pregnancy tests will be performed every 4 weeks (+/-1 week) during the study regardless of dosing schedules.

3.1.11 Female patients enrolled in the study, who are not free from menses for >2 years, post hysterectomy / oophorectomy, or surgically sterilized, must be willing to use either 2 highly effective barrier methods *or* a barrier method plus a hormonal method of contraception to prevent pregnancy or to abstain from heterosexual activity throughout the study, starting with Visit 1 and at least 5 months from the last dose of nivolumab. Approved contraceptive methods include for example; intra uterine device, diaphragm with spermicide, cervical cap with spermicide, male condoms, female condoms with spermicide, or oral contraceptives. Spermicides alone are not an acceptable method of contraception.

Women who are not of childbearing potential (ie, who are postmenopausal or surgically sterile as well as azoospermic men do not require contraception.

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

3.1.13 Patients must have biopsiable disease at the time of enrollment as biopsies after progression are required for participation.

**3.2 Exclusion Criteria**

3.2.1 Any active, known or suspected autoimmune disease. Subjects are permitted to enroll if they have vitiligo, type I diabetes mellitus, residual hypothyroidism due to autoimmune condition only requiring hormone replacement, psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger

3.2.2 Patients requiring continuous supplemental oxygen are excluded to avoid possible complications from pneumonitis.

3.2.3 Patients who have had chemotherapy or radiotherapy within 2 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study or those who have not recovered from adverse events due to agents administered more than 2 weeks earlier.

3.2.4 Patients with uncontrolled brain metastases. Patients with brain metastases must have stable neurologic status following local therapy (surgery or radiation) for at least 2 weeks without the use of steroids or on stable or decreasing dose of < 10mg daily prednisone (or

equivalent), and must be without neurologic dysfunction that would confound the evaluation of neurologic and other adverse events. Patients with a history of carcinomatous meningitis are not eligible.

3.2.5 History of allergic reactions attributed to compounds of similar chemical or biologic composition to nivolumab including any history of severe allergic reactions to monoclonal antibody therapy.

3.2.7 Pregnant women are excluded from this study because nivolumab is an agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with nivolumab, breastfeeding should be discontinued if the mother is treated on this protocol.

3.2.8 Patients who are receiving any other anticancer therapy.

3.2.9 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.10 Prior therapy with anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, anti-CTLA-4 antibody therapies, any other antibody or drug specifically targeting T-cell costimulation or checkpoint pathways.

3.2.11 Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).

3.2.12 Patients should be excluded if they have a positive test for hepatitis B virus surface antigen (HBV sAg) or hepatitis C virus ribonucleic acid (HCV antibody) indicating ongoing acute or chronic infection

3.2.13 Women who are breast feeding

3.2.14 Subjects with a condition requiring systemic treatment with either corticosteroids (> 10 mg daily prednisone equivalent) or other immunosuppressive medications within 14 days of study drug administration are excluded.

### 3.3 Inclusion of Women and Minorities

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

## 4. TREATMENT PLAN

### 4.1 Agent Administration

This is a phase 2 study with 2 arms, all consented patients who are successfully screened and have available baseline tissue (either archival or from a research biopsy) will receive nivolumab administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or

therapies other than nivolumab described below may be administered with the intent to treat the patient's malignancy (denosumab or bisphosphonates are permitted for patients with bone metastases or hypercalcemia).

### 4.1.1 Nivolumab:

Nivolumab vials are to be stored at a temperature of 2°C to 8°C and should be protected from light. If stored in a glass front refrigerator, vials should be stored in the carton. Recommended safety measures for preparation and handling of BMS- 936558 include laboratory coats and gloves. After Nivolumab has been prepared for administration, the total storage time (combination of refrigeration and room temperature) is not to exceed 24 hours. Stability data for Nivolumab following dilution and transfer to the IV bag supports either: 24 hours at 2°C to 8°C in the refrigerator, or 4 hours at room temperature/under room light and 18 hours at 2°C to
8°C in the refrigerator. Care must be taken to assure sterility of the prepared solution as the product does not contain any anti-microbial preservative or bacteriostatic agent. No incompatibilities between Nivolumab and polyolefin bags have been observed.
Nivolumab will be administered as a 30-minute IV infusion, using a volumetric pump with a 0.2 micron in-line filter, followed by a saline flush. Dosing calculations should be based on the body weight assessed within 2 weeks of starting each cycle. All doses should be rounded to the nearest milligram. There will be no dose escalations or reductions of nivolumab allowed. Subjects may be dosed no less than 12 days from the previous dose. Dosing is on weeks 1, 3, 5, and 7 of each cycle. There are no premedications recommended for Nivolumab on the first cycle. If an allergic reaction is noted, then acetaminophen 650 mg PO and diphenyhydramine 50 mg PO/IV may be administered prior to Nivolumab infusion.

### 4.1.2 Flat (standardized) dosing of nivolumab:

Nivolumab monotherapy has been extensively studied in a number of tumor types including NSCLC, MEL, RCC, and CRC with body weight normalized dosing (mg/kg). Nivolumab pharmacokinetics (PK) and exposures of subjects in these studies have been characterized by population pharmacokinetic (PPK) analysis of data collected these studies, together with PK data from several phase 1, 2, and 3 clinical studies of nivolumab monotherapy in solid tumors. Population PK (PPK) analyses have shown that the PK of nivolumab are linear, with dose proportional exposures over a dose range of 0.1 mg/kg to 10 mg/kg, and are similar across tumor types. Nivolumab clearance and volume of distribution were found to increase with increasing body weight, but the increase was less than proportional, indicating that a mg/kg dose represents an over-adjustment for the effect of body weight on nivolumab PK. Given the relationship between nivolumab PK and body weight, a flat dose is expected to lead to lower exposures in heavier patients, relative to the exposures in lighter patients.

Using the PPK model, nivolumab steady-state trough, peak and time-averaged concentration (Cminss, Cmaxss, and Cavgss, respectively) were predicted for a flat nivolumab dose of 240 mg Q2W and compared to those following administration of 3 mg/kg Q2W in NSCLC subjects. A dose of 240 mg nivolumab is identical to a dose of 3 mg/kg for subjects weighing 80 kg, which is the approximate median body weight of NSCLC subjects in the 3 Phase 2 and 3 BMS clinical studies of nivolumab monotherapy. The geometric mean values of Cminss, Cmaxss, and Cavgss with flat dosing are slightly (< 15%) higher than that produced by a 3 mg/kg dose, and the coefficient of variation (cv%) in these measures of exposure are only slightly (< 10%) greater than that of 3 mg/kg dosing.

Across the various tumor types in the BMS clinical program, nivolumab has been shown to be be safe and well tolerated up to a dose level of 10 mg/kg, and the relationship between nivolumab exposure produced by 3 mg/kg and efficacy has been found to be relatively flat. Taken together, the PK, safety, and efficacy data indicate that the safety and efficacy profile of 240 mg nivolumab will be similar to that of 3 mg/kg nivolumab.

Thus a flat dose of 240 mg every 2 weeks is recommended for investigation in this study.

## 4.2 General concomitant medication and supportive care guidelines

**4.2.1** No investigational or commercial agents or therapies other than those described above may be administered with the intent to treat the malignancy.

**4.2.2** Contraceptive therapy: Sexually active men and women of child-bearing potential must agree to use effective contraception.

**4.2.3** Immune related AEs should be managed according to the algorithms in the current version of the Investigator's brochure for nivolumab.

**4.2.4** The following medications are prohibited during the study (unless utilized to treat a drug-related adverse event): immunosuppressive agents, immunosuppressive doses of systemic corticosteroids (except as noted in inclusion/exclusion section), Subjects are permitted to use topical, ocular, intra-articular, intranasal, and inhaled corticosteroids (with minimal systemic absorption). Physiologic replacement doses of systemic corticosteroid (eg prednisone < 10 mg/day) are permitted. A brief (less than 3 weeks) course of corticosteroids for prophylaxis (eg, contrast dye allergy) or for treatment of non-autoimmune conditions (eg, delayed-type hypersensitivity reaction caused by a contact allergen) is permitted.

**4.2.5** Non-palliative radiation therapy is not allowed. Palliative radiation therapy is only allowed during Nivolumab therapy if deemed necessary for the patient by the investigator. If palliative radiotherapy is required, then Nivolumab should be withheld for at least 1 week before, during and 1 week after radiation. Patients should be closely monitored for any potential toxicity during and after receiving radiotherapy, and AEs should resolve to grade < 1 prior to resuming Nivolumab.

## 4.3 Management of Nivolumab-related infusion reactions

Since Nivolumab contains only human immunoglobulin protein sequences, it has a low incidence of infusion or hypersensitivity reactions. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash, pruritus, arthalgias, hypotension, hypertension, bronchospasm, or other allergic-like reactions. All Grade 3 or 4 infusion reactions should be reported as an SAE if it meets the criteria. Infusion reactions should be graded according to NCI CTCAE (Version 4.0) guidelines.

Treatment recommendations are provided below:

For Grade 1 symptoms: (Mild reaction; infusion interruption not indicated; intervention not indicated).

• Remain at bedside and monitor subject until recovery from symptoms. The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or acetaminophen/paracetamol 325 to 1000 mg at least 30 minutes before additional Nivolumab administrations.

For Grade 2 symptoms: (Moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [eg, antihistamines, non-steroidal anti- inflammatory drugs, narcotics, corticosteroids, IV fluids]; prophylactic medications indicated for ≤ 24 hours).

• Stop the Nivolumab infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent) and/or acetaminophen/paracetamol 325 to 1000 mg; remain at bedside and monitor subject until resolution of symptoms. Corticosteroid therapy may also be administered as appropriate. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor subject closely. If symptoms recur, then no further Nivolumab will be administered at that visit.
• For future infusions, the following prophylactic premedications are recommended: diphenhydramine 50 mg (or equivalent) and/or acetaminophen 325 to 1000 mg should be administered at least 30 minutes before Nivolumab infusions. If necessary, corticosteroids (up to 25 mg of SoluCortef or equivalent) may be used.

For Grade 3 or 4 symptoms: (Severe reaction, Grade 3: prolonged [ie, not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae [eg, renal impairment, pulmonary infiltrates]. Grade 4: Life-threatening; pressor or ventilatory support indicated).

• Immediately discontinue infusion of Nivolumab. Begin an IV infusion of normal saline and treat the subject as follows: Recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV or equivalent), as needed. Subject should be monitored until the Investigator is comfortable that the symptoms will not recur. Nivolumab will be permanently discontinued. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery of the symptoms.

In case of late-occurring hypersensitivity symptoms (eg, appearance of a localized or generalized pruritus within 1 week after treatment), symptomatic treatment may be given (eg, oral antihistamine or corticosteroids).

### 4.4 Duration of Therapy

Nivolumab treatment may continue up to 24 months (2 years) or until one of the following criteria applies and at that time, nivolumab treatment should be permanently discontinued:

- Any Grade > 2 drug-related uveitis, eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR

requires systemic treatment

- Any Grade 3 non-skin, drug-related adverse event lasting > 7 days, with the following exceptions for laboratory abnormalities, drug-related bronchospasm, hypersensitivity reactions, and infusion reaction:
- Grade 3 drug-related bronchospasm, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation
- Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except:
  - Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding requires discontinuation
  - Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:
    - AST or ALT > 5-10x ULN for > 2 weeks
    - AST or ALT > 10x ULN
    - Total bilirubin > 5x ULN
    - Concurrent AST or ALT > 3x ULN and total bilirubin > 2x ULN
- Any Grade 4 drug-related adverse event or laboratory abnormality, except for the following events which do not require discontinuation:
  - Grade 4 neutropenia < 7 days
  - Grade 4 lymphopenia or leucopenia
  - Isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms \or clinical manifestations of pancreatitis.
  - Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset
- Any dosing interruption lasting > 6 weeks with the following exceptions:
- Dosing interruptions to allow for prolonged steroid tapers to manage drug- related adverse events are allowed after discussion with study PI. Tumor assessments should continue as per protocol even if dosing is interrupted.
- Dosing interruptions > 6 weeks that occur for non-drug-related reasons may be allowed. Tumor assessments should continue as per protocol even if dosing is interrupted.
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, presents a substantial clinical risk to the subject with continued nivolumab dosing
- Confirmed disease progression at time points after week 8 (cycle 1) of nivolumab therapy. This allows for patients to remain on treatment with nivolumab after disease progression if clinically stable. However, once the next disease assessment confirms progression or if clinically unstable, patients must stop treatment. (See section 4.4.1)
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event (s), not able to be managed by steroid/infliximab administration.
- Patient decides to withdraw from the study, or General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

## 4.5 Treatment Beyond Disease Progression

Subjects treatment with nivolumab will be permitted beyond initial RECIST 1.1 defined PD as long as they meet the following criteria:

1. Investigator-assessed clinical benefit, and do not have rapid disease progression
2. Continue to meet all other study protocol eligibility criteria
3. Tolerance of the study drug
4. Stable performance status
5. Treatment beyond progression will not delay an imminent intervention to prevent serious complications of disease progression (e.g. CNS metastasis)

The decision to continue treatment beyond initial progression should be documented in the study records.

A radiographic assessment/scan should be performed at the end of the following cycle of the original PD to determine whether there has been a decrease in the tumor size or continued PD. The assessment of clinical benefit should be balanced by clinical judgment as to whether the subject is clinically deteriorating and unlikely to receive any benefit from continued treatment with nivolumab.

If the investigator feels that the nivolumab subject continues to achieve clinical benefit by continuing treatment, the subject should remain on the trial and continue to receive monitoring according to the Study calendar.

New lesions are considered measurable at the time of initial progression if the longest diameter is at least 10mm (except for pathological lymph nodes which must have a short axis of at least 15 mm). Any new lesion considered non-measurable at the time of initial progression may become measurable and therefore included in the tumor burden volume if the longest diameter increases to at least 10 mm (except for pathological lymph nodes which must have a short axis of at least 15 mm).

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 (i.e. radiographic confirmation) even after discontinuation of treatment.

### 4.6 Duration of Follow Up

Once treatment has been discontinued, patients will be followed with phone calls or in person every 3 months until death or 5 years, whichever occurs first. We will collect data on development of additional cancers, subsequent therapy (chemotherapy, radiation or surgery) for their cancer, and survival. Medical records including laboratory, pathology, operative, and radiology reports will be obtained at the discretion of the principal investigator with permission from the patient. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

### 4.7 Criteria for Removal from Study

A patient will be withdrawn from the study if any of the following events occur while on therapy:

- Interruption of scheduled therapy for greater than 6 weeks except as noted above incsection 4.4.
- Intolerable adverse effects, laboratory abnormality or intercurrent illness that is judged by the

investigator to be either physically or psychologically detrimental to the patient.

- Withdrawal of informed consent (subject's decision to withdraw for any reason).
- Pregnancy.
- Patient non-compliance.
- Unresolved or recurrent Grade 3 or 4 toxicities except as noted above in section 4.4.
- Treatment with other anti-cancer drugs.
- Confirmed disease progression on Nivolumab at time points after week 8.
- Loss of ability to freely provide consent through imprisonment or involuntary incarceration for treatment of either a psychiatric or physical (e.g., infectious disease) illness.
- All subjects who discontinue should comply with protocol specified follow-up and survival procedures as outlined in Section 4.5. The ONLY exception to this requirement is when a subject withdraws consent for all study procedures or loses the ability to consent freely (i.e., is imprisoned or involuntarily incarcerated for the treatment of either a psychiatric or physical illness).

# 5. DOSING DELAYS/DOSE MODIFICATIONS

Dosing delays on this study will be made at the discretion of the treating physician. No dose modifications will be allowed for nivolumab. Adverse symptoms prompting dose delay of greater than 6 weeks will result in study discontinuation except as noted in section 4.4.

## 5.1 Nivolumab dose delay criteria

- Nivolumab administration should be delayed for the following:
- Any Grade > 2 non-skin, drug-related adverse event, with the following exceptions:
- Grade 2 drug-related fatigue or laboratory abnormalities do not require a treatment delay.
- Any Grade 3 skin, drug-related adverse event
- Any Grade 3 drug-related laboratory abnormality, with the following exceptions for lymphopenia, leucopenia, AST, ALT, or total bilirubin:
- Grade 3 lymphopenia or leucopenia does not require dose delay.
- If a subject has baseline AST, ALT, or total bilirubin within the Grade 1 toxicity range,delay dosing for drug-related Grade > 3 toxicity.
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, warrants delaying the dose of study medication.

## 5.2 Criteria to Resume Treatment with Nivolumab

Subjects may resume treatment with nivolumab when the drug-related AE(s) resolve(s) to Grade < or baseline, with the following exceptions:

- Subjects may resume treatment in the presence of Grade 2 fatigue
- Subjects who have not experienced a Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin toxicity.
- Subjects with baseline AST/ALT or total bilirubin in the Grade 1 toxicity range who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of Grade 2 AST/ALT OR total bilirubin.
- Subjects with combined Grade 2 AST/ALT AND total bilirubin values meeting discontinuation parameters (section 4.4) should have treatment permanently discontinued.

- Drug-related pulmonary toxicity, diarrhea, or colitis must have resolved to baseline before treatment is resumed. Drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment
- If treatment is delayed > 6 weeks, the subject must be permanently discontinued from study therapy, except as specified in section 4.4.

# 6. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 6.1) and the characteristics of an observed AE (Section 6.2) will determine whether the event requires expedited reporting in addition to routine reporting.

## 6.1 Adverse effects of Nivolumab

Common adverse effects of Nivolumab, occurring in >10% of patients, include fatigue, rash, diarrhea, nausea, abdominal pain, fever, decreased appetite and joint pain or stiffness.

Less common adverse effects (>1-9%) Include liver enzyme elevation, pneumonitis, hypothyroidism, hyperthyroidism, adrenal insufficiency, decreased appetite, nausea, vomiting, cough, dyspnea, constipation, headache, arthralgia, dizziness, decreased weight, anemia, hypotension, and colitis.

Rare, but serious adverse effects (<1%) have included pneumonitis resulting in hypoxia and death, toxic epidermal necrolysis, respiratory failure, dehydration, malignant neoplasm, peripheral edema, back pain, allergic reactions, pancreatitis, cytopenias, and nephritis.

## 6.2 Adverse Event Characteristics

**CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting.

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

## 6.3 Adverse Event Reporting

The principal investigator will notify the appropriate regulatory agencies of any serious adverse event due to any cause during the course of this investigation. These include the Johns Hopkins Cancer Center Data and Safety Monitoring Committee, and the Johns Hopkins Medical Institutional Review Board (JHM-IRB) of The Johns Hopkins Medical Institutions. The required reporting time period is 3 days for fatal events, and 10 days for all other events.

Adverse event information will be collected for the duration of the study. Patients will be instructed to notify investigators of any symptoms, and investigators will assess patients for adverse events at each visit. All toxicity and adverse events will be recorded on Case Report Forms, graded as to the severity and relationship to the study drug, and reported within the required time frame.

### 6.3.1 Adverse event reporting period

All SAEs, whether related or unrelated to nivolumab and all pregnancies must be reported to BMS within 24 hours of knowledge of the event.

For studies conducted under an **Investigator IND**, any event that is serious, unexpected, and at least possibly related must be reported to the Food and Drug Administration (FDA) as soon as possible and no later than 7 days (for a death or life-threatening event) or 15 days (for all other SAEs) after the investigator's or institution's initial receipt of the information. BMS will be provided with a simultaneous copy of all adverse events filed with the FDA. SAEs should be reported on MedWatch Form 3500A or similar form and be accompanied by Form 1571. It MUST include the institutional AND BMS study ID [per study Agreement]. The type of report (initial or follow-up) should be checked in the respective boxes on Forms 3500A and 1571. The submission must be identified as:

- "IND safety report" for 15-day reports, or
- "7-day IND safety report" for unexpected fatal or life-threatening suspected adverse reaction reports, or
- "Follow-up IND safety report" for follow-up information.

The report must be submitted to an appropriate Review division that has the responsibility to review the IND application under which the safety report is submitted. Each submission to this IND must be provided in triplicate (original plus two copies). Send all submissions to the FDA at the following address:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Oncology Products 1
5901-B Ammendale Road
Beltsville, MD 20705-1266

http://www.fda.gov/Safety/MedWatch/HowToReport/ucm388987.htm

http://www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/InvestigationalNewDrugINDApplication/ucm362555.htm

All SAEs should simultaneously be faxed or e-mailed to BMS at:

Global Pharmacovigilance & Epidemiology
Bristol-Myers Squibb Company
Fax Number: 609-818-3804
**SAE Email Address**: Worldwide.Safety@BMS.com

The study period during which adverse events will be reported is from the initiation of study procedures to the end of the study treatment follow-up, defined as 100 days following the last administration of nivolumab treatment.

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 to BMS, using the same procedure used for transmitting the initial SAE report. In accordance with local regulations, BMS will notify investigators of all SAEs that are suspected (related to their respective investigational product) and unexpected (ie, not previously described in the Investigator Brochure). In the European Union (EU), an event meeting these criteria is termed a Suspected, Unexpected Serious Adverse Reaction (SUSAR). Investigator notification of these events will be in the form of an expedited safety report (ESR).

### 6.3.2 Adverse event definition

An **adverse event** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries are regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- Results in study withdrawal
- Is associated with a serious adverse event
- Is associated with clinical signs or symptoms
- Leads to additional treatment or to further diagnostic tests
- Is considered by the investigator to be of clinical significance

### 6.3.3 Serious adverse event definition

A serious adverse event is any AE that is:

- Fatal
- Life-threatening
- Requires or prolongs hospital stay
- Results in persistent or significant disability or incapacity
- A congenital anomaly or birth defect
- an important medical event

### 6.3.4 Important medical event definition

Important medical events are those that may not be immediately life threatening, but are judged by the study investigator to be of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above.

## 6.4 Data Handling and Record Keeping

### 6.4.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA).

### 6.4.2 Source Documents

Source data include all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial.

### 6.4.3 Case Report Forms

The study case report forms (CRFs) are the primary data collection instrument for the study. All data requested on the CRF will be recorded for each subject. If a procedure was not done or a question was not asked, this will be recorded as "N/D". If the item is not applicable to the individual case, this will be recorded as "N/A". CRFs will be built electronically in CRMS. All data will be entered electronically onto the electronic CRF through CRMS by the Study Coordinator and/ or Data Manager.

### 6.4.4 Auditing and Monitoring

The investigator will permit study-related monitoring, audits, and inspections by the IRB, government regulatory bodies, University compliance and quality assurance groups of all study related documents. The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

This is a DSMP Level II study under the SKCCC Data Safety Monitoring Plan (12/06/2012). Data monitoring of this protocol will occur on a regular basis with the Frequency dependent on the rate of subject accrual and the progress of the study. The protocol will be monitored internally at SKCCC by the Principal Investigator and externally by the SKCCC CRO in accordance with SKCCC guidelines. Trial monitoring and reporting will be done through the Safety Monitoring Committee (SMC) at SKCCC.

### 6.5 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct.

The decision of the EC/IRB concerning the conduct of the study will be made to the investigator before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. The consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent. Patients will also be required to reconsent for treatment beyond disease progression while on nivolumab.

## 7. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with nivolumab can be found in Section 6.1.

## 7.1 Nivolumab

### 7.1.1 Fully human anti-PD-1 IgG4: monoclonal antibody

Other names: BMS-936558, MDX-1106
Classification: Monoclonal antibody; impedes PD-1/PD-L1 interaction

**7.1.2 How Supplied:** Nivolumab is provided as a sterile liquid in vials each containing 100mg/10ml of the monoclonal antibody.

**7.1.3 Mechanism of Action:** Nivolumab is a monoclonal antibody that binds to the cell surface protein PD-1, a key regulator of cytotoxic T lymphocyte activation. Nivolumab inhibits PD-1/PD-L1 and PD-1/PD-L2 interaction, facilitating CTL reactivity and promoting an anticancer cytolytic response.

**7.1.4 Route of Administration:** Nivolumab is to be administered as an intravenous infusion, using a volumetric pump with a 0.2 micron in-line filter at the protocol-specified doses. It is not to be administered as an intravenous push or bolus injection. At the end of the infusion, the line should be flushed with a sufficient quantity of normal saline. A 60 minute infusion, can be diluted with 0.9% NS for delivery but the total drug concentration of the solution cannot be below 0.35mg/ml.

**7.1.5 Storage and Stability:** Nivolumab vials must be stored at a temperature of 2°C to 8°C and should be protected from light and freezing. If stored in a glass front refrigerator, vials should be stored in the carton. Recommended safety measures for preparation and handling of Nivolumab include laboratory coats and gloves. After Nivolumab has been prepared for administration, the total storage time (combination of refrigeration and room temperature) is not to exceed 24 hours. Stability data for Nivolumab following dilution and transfer to the IV bag supports either: 24 hours at 2°C to 8°C in the refrigerator, or 4 hours at room temperature/under room light and 18 hours at 2°C to 8°C in the refrigerator. Care must be taken to assure sterility of the prepared solution as the product does not contain any anti-microbial preservative or bacteriostatic agent. No incompatibilities between Nivolumab and polyolefin bags have been observed.

**7.1.6 Potential Drug Interactions**: Studies examining interactions between Nivolumab and other agents are ongoing.

**7.1.7 Nivolumab will be supplied directly from BMS Inc..** The IDS Pharmacy will be responsible for ordering the study drug directly from BMS Inc.

**7.1.8 Pharmacy:** Prepare in Non-PVC bag. Final concentration will be > 0.35mg/ml. Mix to total volume of 60ml NSS. If dose volume > 60ml, use straight drug. Prime tubing with NSS and attach to bag. Infuse via 0.2 micron filter. Send inline filter to clinic.
Nurse: Attach inline filter to patient side of pump.

## 8. STUDY CALENDAR

All assessments for screening should be completed within 28 days prior to first dose. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy. ***N.b.: Besides the window for labs is 5 days, all other time points on study have leeway of plus or minus 3 days.***

| | 8 week cycles (repeat) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-Study | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 | Week 7 | Week 8 | End of Treatment |
| Nivolumab[1] | | **X** | | **X** | | **X** | | **X** | | |
| Informed Consent | **X** | | | | | | | | | |
| History & Physical[2] | **X** | **X** | | **X** | | **X** | | **X** | | X |
| Concurrent Meds | **X** | **X** | | **X** | | **X** | | **X** | | X |
| CBC and Chemistry[3] | **X** | **X** | | **X** | | **X** | | **X** | | |
| TSH/T4[4] | **X** | **X[4]** | | | | **X** | | | | |
| Hepatitis B & C | **X** | | | | | | | | | |
| Research Bloods[5] | | X[5] | | | | **X** | | | | X |
| B-HCG[6] | **X** | **X[6]** | | | | **X[6]** | | | | |
| AE assessment | **X** | **X** | | **X** | | **X** | | **X** | | X |
| MRI or CT (with contrast) of brain* | **X** | | | | | | | | | |
| CT or MRI for Tumor Assessment[7] | **X** | **X[7]** | | | | | | | | |
| Tumor Biopsy[8] | **X** | | | | | | **X[8]** | | | |
| Follow-up/ Survival[9] | | | | | | | | | | **X[9]** |

1. 240mg IV
2. History and physical, includes interval history, demographics, vital signs, oxygenation at rest and walking, weight, height (at screening only), and ECOG performance status
3. Complete blood count and differential, and comprehensive metabolic panel
4. TSH, Free T4 once every 4 weeks. Note: labs performed during screening (other than CBC and Chemistry) do not need to be repeated on C1D1.
5. Plasma for assessment of cell-free DNA will be collected pre-study and every 4 weeks on-study (i.e.,

Cycle 1 Week 5, and then Weeks 1 and 5 in subsequent cycles). Peripheral blood lymphocytes for assessment of gene expression will be collected pre-study, at Week 17 (i.e., Cycle 3 Week 1), and at the time of progression or study discontinuation whichever occurs first. Pre-study research bloods may be taken at screening or prior to treatment on C1D1.

6. Women of childbearing potential must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 24 hours prior to the start of nivolumab. For WOCBP, follow up pregnancy tests will be performed every 4 weeks (+/-1 week) during the study regardless of dosing schedules.

7. Tumor assessment includes radiologic assessment for RECIST evaluation. Repeat radiologic assessment pre-study, week 1 of cycle 2 and all subsequent cycles.

8. Tumor biopsy is required prior to starting nivolumab (if there is no adequate recent archival tumor available) and between week 6-9. If a patient has early disease progression and has to come off study prior to week 6, a biopsy should be performed at the time of confirmed disease progression.

9. Once treatment has been discontinued, patients will be followed with phone calls or in person every 3 months until death or 5 years, whichever occurs first. We will collect data on development of additional cancers, subsequent therapy (chemotherapy, radiation or surgery) for their cancer, and survival. Medical records including laboratory, pathology, operative, and radiology reports will be obtained at the discretion of the principal investigator with permission from the patient. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

*only if symptomatic

# 9. MEASUREMENT OF EFFECT

## 9.1 Antitumor Effect – Solid Tumors

On this study, planned radiologic evaluations will be at baseline (within 4 weeks prior to starting nivolumab), and then every 8 weeks while on Nivolumab. Confirmatory scans should be obtained not less than 4 weeks following initial documentation of objective response, and will typically be obtained at the time of the next planned evaluation (i.e. 8 weeks following).

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009], Changes in the longest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

### 9.1.1 Treatment beyond progression

Accumulating evidence indicates a minority of subjects treated with immunotherapy may derive clinical benefit despite initial evidence of PD.

Subjects will be permitted to continue treatment beyond initial RECIST 1.1 defined PD as long as they meet the following criteria determined by the investigator:

- Investigator-assessed clinical benefit and subject is tolerating study drug.
- Tolerance of study drug
- Stable performance status

The assessment of clinical benefit should take into account whether the subject is clinically deteriorating and unlikely to receive further benefit from continued treatment.

Treatment beyond progression will not delay an imminent intervention to prevent serious complications of disease progression (eg, CNS metastases).

A radiographic assessment/ scan should be performed between 4-6 weeks (and no longer than 6 weeks) after initial investigator-assessed progression to determine whether there has been a decrease in the tumor size or continued PD. The assessment of clinical benefit should be balanced by clinical judgment as to whether the subject is clinically deteriorating and unlikely to receive any benefit from continued treatment with nivolumab

For the subjects who continue nivolumab study therapy beyond progression, further progression is defined as an additional 10% increase in tumor burden from time of initial PD. This includes an increase in the sum of diameters of all target lesions and/ or the diameters of new measurable lesions compared to the time of initial PD. Nivolumab treatment should be discontinued permanently upon documentation of further progression.

New lesions are considered measureable at the time of initial progression if the longest diameter is at least 10 mm (except for pathological lymph nodes which must have a short axis of at least 15 mm). Any new lesion considered non-measureable at the time of initial progression may become measureable and therefore included in the tumor burden if the longest diameter increases to at least 10 mm (except for

pathological lymph nodes which must have a short axis of at least 15 mm). In situations where the relative increase in total tumor burden by 10% is solely due to inclusion of new lesions which become measurable, these new lesions must demonstrate an absolute increase of at least 5 mm.

Subjects who continue treatment beyond initial investigator-assessed, RECIST 1.1-defined progression will be considered to have investigator-assessed progressive disease at the time of the initial progression event.

Summary of Treatment Beyond Progression

Subjects with PD should be managed in the study as follows:

− PD while being treated with Nivolumab: In the absence of clinical deterioration, subjects may continue treatment until repeat imaging 4-6 weeks later. If there is a question of clinical deterioration, the decision whether to stop treatment should be discussed with the Principal Investigator.

− If, at each subsequent imaging evaluation, there is no ≥10% increase in the SLD, no unequivocal increase in non-target lesions, and no additional new lesions develop (non- worsening PD), and the subject's clinical status remains stable or has improved, treatment should be continued and further imaging timepoints will follow the study calendar

− If at any subsequent imaging evaluation, there is ≥10% increase in the SLD, unequivocal increase in non-target lesions, or development of additional new lesions (worsening PD) as discussed previously, the subject should stop treatment.

### 9.1.2 Definitions

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment on protocol.

Evaluable for objective response: All patients enrolled in this study should have measurable disease by RECIST 1.1. Patients who have received at least one cycle of therapy and have had their disease re-evaluated will be considered evaluable for response.
These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of the first cycle of therapy will also be considered evaluable.

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

### 9.1.3 Disease Parameters

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. Measurable disease is defined by the presence of at least one measurable lesion.

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

Note: Tumor lesions that are situated in a previously irradiated area are not considered measurable unless there has been demonstrated progression in the lesion after radiation therapy and prior to enrollment on this study.

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

Non-measurable disease: All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitic involvement of the skin or lung, inflammatory breast disease, and abdominal masses/abdominal organomegaly (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor nonmeasurable) since they are, by definition, simple cysts. Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

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

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

### 9.1.4 Methods for Evaluation of Measurable Disease

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

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

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

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

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If 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 (e.g. for body scans). Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement.

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

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

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

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

Tumor markers: Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

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

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

FDG-PET: While FDG-PET response assessments related to solid tumor management and to immunotherapy need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of tumor progression (particularly possible 'new' disease) or regression (evaluation of potential CR). Lesions can be assessed on FDG-PET imaging according to the following algorithm:
Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDGPET 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.
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. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

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

### 9.1.5 Response Criteria

#### 9.1.5.1 Evaluation of Target Lesions

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

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

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

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

Special notes on the assessment of target lesions:

**Lymph nodes**: Lymph nodes identified as target lesions should always have the actual short axis measurement recorded and should be measured in the same anatomical plane as the baseline examination, even if the nodes regress to below 10mm 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.

**Target lesions that become 'too small to measure':** All lesions (nodal and nonnodal) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (eg, 2mm). If the radiologist is able to provide an actual measure, that should be recorded, even if it is below 5mm. When such a lesion becomes difficult to assign an exact measurement, it is recommended to: If it is in 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: in case of a lymph node believed to be present and 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).

**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 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'.

### 9.1.5.2 Evaluation of Non-Target Lesions

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

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

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

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

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator). *When the patient also has measurable disease:* To achieve 'unequivocal progression' on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest 'increase' in size of one or more non-target lesions is usually not sufficient to qualify for unequivocal progression status. *When the patients has only non-measurable disease:* To achieve 'unequivocal progression' on the basis of the non-target disease, there must be an overall level of substantial worsening such that the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest 'increase' in the size of one or more non-target lesions is usually not sufficient to qualify for unequivocal progression status. Because worsening in non-target disease cannot be easily quantified (by definition: if all lesions are non-measurable) a useful test that can be applied when assessing patients for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease: i.e., an increase in tumor burden representing an additional 73% increase in 'volume' (which is equivalent to a 20% increase diameter in a measurable lesion). Examples include an increase in a pleural effusion from 'trace' to 'large', an increase in lymphangitic disease from localized to widespread, or may be described in protocols as 'sufficient to require a change in therapy'. If 'unequivocal progression' is seen, the patient should be considered to have had overall PD at that point.

**9.1.5.3 Evaluation of Best Overall Response**

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

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

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

disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration*."

Every effort should be made to document the objective progression even after discontinuation of treatment.

### 9.1.6 Duration of Response

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

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

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

### 9.1.7 Progression-Free Survival

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

## 10. STATISTICAL CONSIDERATIONS

### 10.1 Study Design/Endpoints

### 10.1.1 Study Design

This is a pilot phase 2 study of nivolumab in patients with advanced or metastatic solid tumors where the primary tumor has arisen within an area that has been exposed to previous external beam radiation i.e. radiation-induced solid tumors. This study has two groups. One group is for metastatic sarcomas (these are the most frequent solid tumors induced by previous radiation exposure) while the second group is for other radiation-induced solid tumors (it is expected that this arm will enroll predominantly lung cancers, pancreatic cancers and thyroid cancers among other solid tumors).

The primary endpoint for this study will be best overall response rate (BORR) as determined by RECIST 1.1 (evaluation for BORR will continue up to 24 weeks on nivolumab therapy to allow for the unique patterns of response to immune checkpoint therapy). The study is aimed to provide preliminary efficacy evaluation based on the BORR with a reasonable precision such that we may determine if the treatment in either or both patient populations is promising enough to lead us to undertake a larger confirmatory study.

### 10.1.2 Endpoints

**Primary Endpoint**

Best overall response rate (BORR) – evaluation for BORR will continue up to 6 months on nivolumab therapy and response will be coded based on RECIST 1.1 criteria. The details of the evaluation of BORR are in Section 9.1.5. Patients who have achieved a best overall response within 6 months of treatment initiation as either complete response (CR) or a partial response (PR) based on RECIST 1.1 criteria will be considered as a "success".

**Secondary Endpoints**

1. Percentage of patients progression-free at 24 weeks from the time of enrollment: Disease status at 24 weeks will be compared to disease status at the time of enrollment, and response coded based on RECIST 1.1 criteria.
2. Progression-free survival: Progression-free survival will be measured from the time of study enrollment until radiologic or death.
3. Duration of response - The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.
4. Safety and Tolerability – Toxicities observed will be assessed by CTCAE 4.0 criteria
5. Overall survival Overall survival will be measured from the time of enrollment until death.

**Exploratory Endpoints**

Laboratory correlates of response, See section 11. These analyses are considered exploratory. Whole-exome sequencing and assessment of immunologic parameters in mandatory pre- and post-treatment biopsies will be performed. Assessment of tumor baseline PD-L1 expression will be performed. Serial assessment of circulating plasma cell-free tumor DNA, and of gene expression in peripheral blood mononuclear cells, will be performed.

### 10.2 Sample size, Analysis, and Accrual Rate

This study is intended to be a pilot study that will be hypothesis supporting and meant to determine the preliminary clinical activity of nivolumab in the two patient populations based on best observed response rate. A best overall response rate of 50% or more in each of this cohorts this would spur larger confirmatory studies in defined patient populations, e.g. radiation-induced sarcoma, radiation-related cancers etc..

For each cohort, the sample size is justified by Clopper-Pearson's exact binomial confidence interval method in terms of the respective lower boundaries. We determine a sample size of 20 analyzable patients for each group provides an appropriate level of estimation accuracy for the first study in the respective patient populations. The following table summarizes the exact 90% confidence intervals

when the observed response rates are 40%- 70% with 20 analyzable cases. For example, when the observed response rate is 50%, a sample size of 20 analyzable cases will provide a two-sided 90% confidence interval [30.2%, 69.8%]. Guarding against a 10% of ineligible and drop-out rate, the final sample size is 44 patients, with 22 patients in each group. If a higher rate of ineligible and drop-out rate is observed, the protocol may be amended to ensure at least 20 analyzable cases observed for each group.

| Observed number and proportion of patients with response | 90% exact confidence interval | |
|---|---|---|
| | Lower (%) | Upper (%) |
| 8 (40%) | 21.7 | 60.6 |
| 9 (45%) | 25.9 | 65.3 |
| 10 (50%) | 30.2 | 69.8 |
| 11 (55%) | 34.7 | 74.1 |
| 12 (60%) | 39.4 | 78.3 |
| 13 (65%) | 44.2 | 82.3 |
| 14 (70%) | 49.2 | 86.0 |

The best overall response rate is calculated as the proportion of all analyzable patients, along with a 90% confidence interval using Clopper-Pearson's method. Analyzable patients are defined as eligible patients who receive at least one dose of nivolumab. Formal statistical testing will not be performed.

The anticipated accrual rate for this study is 1-2 patients per month.

**10.3 Analysis of secondary endpoints**

All secondary endpoints will be analyzed based on analyzable cases. The percentage of progression-free at 24 weeks from enrollment is calculated as the proportion of alive patients who are progression-free among all analyzable patients. The associated 95% exact confidence interval will be reported.

Progression-free survival is defined as the time from enrollment to the first occurrence of radiologic progression, clinical progression, or death, whichever occurs first. Overall survival is defined as the time from enrollment to death due to any causes. Kaplan-Meier method will be used to estimate the rates of progression-free survival, duration of response and overall survival at different time points. The associated 95% confidence intervals will be estimated by Greenwood's method.

Toxicities will be tabulated by type, grade and attributions, using CTCAE v4.0. All patients, regardless of eligibility, who receive at least one dose of nivolumab will be evaluable for toxicity from the time of their first treatment with study therapy.

All toxicities, including possibly, probably and definitely treatment-related toxicities, will be closely monitored by the study team and investigators. The adverse event reporting will follow the prospective plans delineated in Section 6.3. No specific interim analysis for toxicities is planned.

### 10.4 Analysis of the exploratory endpoints (correlative studies)

Please see section 11.

## 11. CORRELATIVE/SPECIAL STUDIES – Please see lab manual for full details.

### 11.1 Tumor Tissue Studies

Baseline tumor will be analyzed in all subjects. Archived tumor biopsies (core needle or excision) may be used provided they were obtained after any previous systemic therapy, and/or a new biopsy may be performed. Tumor biopsy at week 6-9 or at the time of progression, whichever occurs first, is required.

The following studies will be performed depending on tissue quality and availability.

-Whole exome sequencing to detect somatic genomic alterations. Using genome-wide methods[39-41] we will perform whole-exome sequencing in pre-treatment tumors for identification of genomic correlates of response to nivolumab. Similar next generation sequencing approaches will be performed on post-progression tumor samples in order to determine genomic mechanisms of acquired resistance to nivolumab. Nonsynonymous missense mutations identified will be used to predict mutant peptides and generate a neoantigen signature for each tumor using a computational pipeline we have developed. A separate targeted capture and sequencing analysis of the T cell receptor will be performed to assess T cell clonality.

- Epigenetic analyses of tumors. Expression and/or methylation analyses of pre- and post- treatment tumors will be performed using RNA-seq and microarray approaches.

- Immunohistochemical (IHC) staining. We will perform IHC of tumor samples to assess PD-L-1 expression in tumor or tumor-infiltrating immune cells using the PD-L1 IHC 28-8 assay. Markers for further characterization of immune cell subsets (CD3, CD4, CD8, CD20, CD68), T regulatory cells-Treg (FOXP3) as well as other immune checkpoints (LAG3, TIM3) will be assessed.

### 11.2 Peripheral Blood Lymphocytes

PBMCs will be obtained at baseline and at time-points indicated in the study calendar and used as matched normal for the tumor exome analyses as well as evaluated for immunologic markers of response.

### 11.3 Plasma Marker Studies

Plasma samples will be obtained at time-points indicated in the study calendar. We will employ non-invasive genomic approaches in order to determine somatic mutations that detect residual disease in the patient's circulation. Using blood samples collected prospectively after initiation of nivolumab, we will determine whether measurements of cancer-specific genomic alterations in circulating cell free DNA (ctDNA) can predict disease progression prior to conventional CT imaging. For each patient, 5-10 ml of plasma will be genomically analyzed for detection of somatic mutations in ctDNA and the sensitivity and specificity of the plasma analysis approach for detection of somatic alterations will be evaluated by comparison of the mutation data from the plasma to the sequence information obtained from analysis of the matching tumor samples. Tumor- specific mutations found in the circulation after disease

progression will be compared to the genomic profile of the recurrent tumors and we will confirm the presence of matching resistance mutations in ctDNA.

### 11.4 Analysis of correlative studies

We will investigate the role of somatic mutations and overall tumor mutational burden in predicting response to nivolumab. Based on previous studies the number of recurrent mutations is expected to be in the range of 5-10, therefore statistical significance will be assessed with a false discovery rate of 5%[42,43]. We will evaluate the impact of genomic alterations in tumor tissue on overall survival. We will use overall survival as the primary endpoint, as it is more suitable to fully capture the clinical benefit and unique response patterns of nivolumab[44]. Survival differences will be evaluated by log rank statistics and graphically represented with Kaplan Meier curves. Differences in the mutational load between responders and non-responders will be assessed by the Wilcoxon rank sum test. The difference between the mutational burden pre- and post-treatment will be compared using paired Student's t-test. Similar analyses will be performed for other biomarker parameters. Relationships between changes in gene re-expression and other correlates with clinical response will be assessed using Fisher's exact test. In addition, estimates of the variance in the biological correlative parameters will be made in this group to be used in planning the integration of these studies in future trials.

For neoantigen analysis we will assess random forests[45] and logic regression for building a neoantigen-based predictive classifier[46,47]. Somatic genomic alterations in ctDNA will be identified and used to stratify patients independently of patient outcomes. Cutoffs used to classify genomic alterations in tumor and ctDNA have been previously described[42]. Any somatic genomic alteration identified in the circulation that matches genomic alterations identified in the primary tumor will be used to classify patients as ctDNA positive. Curves for overall survival will be constructed using the Kaplan-Meier method and compared between groups defined by ctDNA status using the log-rank test. Stratified analyses or multivariate Cox proportional hazards models will be used for overall survival as appropriate.

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