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TITLE: A Phase 2 Study of Nivolumab in Patients with High-Risk Biochemically Recurrent Prostate Cancer

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PROTOCOL SIGNATURE PAGE

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A Phase 2 Study of Nivolumab in Patients with High-Risk Biochemically Recurrent Prostate Cancer

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I confirm I have read this protocol, I understand it, and I will work according to this protocol and to the ethical principles stated in the latest version of the Declaration of Helsinki, the applicable guidelines for good clinical practices, whichever provides the greater protection of the individual. I will accept the monitor's overseeing of the study. I will promptly submit the protocol to applicable institutional review board(s).

_____ _____

Signature of Site Investigator Date

_____

Site Investigator Name (printed)

_____

Site Investigator Title

_____

Name of Facility

_____

Location of Facility (City and State)

SYNOPSIS

OBJECTIVES	Primary Objective: Determine the proportion of patients with high-risk biochemically-recurrent (BCR) prostate cancer (PCa) that experiences decline or stabilization in PSA (without symptomatic/radiographic progression) after 12 weeks of nivolumab treatment Secondary Objectives: • Determine the maximal change (proportional) and best decline (absolute) in prostate specific antigen (PSA) during nivolumab treatment • Determine the change in PSA doubling time (PSADT) during nivolumab therapy relative to baseline • Determine the time to development of overt metastatic disease after treatment with nivolumab • Determine the time to initiation of androgen deprivation therapy (ADT) after treatment with nivolumab • Assess the toxicity of nivolumab monotherapy in patients with BCR PCa Exploratory Objectives: • Evaluate whether response to nivolumab treatment is enriched among patients with tumor programmed death-ligand 1 (PD-L1) expression on tumor cells and on tumor-infiltrating lymphocytes and antigen-presenting cells (APCs) • Identify genomic biomarkers associated with response to nivolumab • Evaluate correlations between alterations in DNA-damage-repair genes and PD-L1 expression • Evaluate correlations between tumor-infiltrating lymphocytes and APCs with response to nivolumab • Evaluate subtypes of "exhausted" tumor-infiltrating lymphocytes • Explore whether peripheral T-cells are present that might recognize tumor neoantigens and whether these correlate with responses to nivolumab • Assess for genomic alterations in circulating tumor DNA and correlate with mutations identified in primary tumor specimens • Assess levels of plasma cytokines prior to initiation and during therapy and determine whether levels or changes in specific cytokines may be predictive biomarkers for response • Assess PBMC subsets prior to initiation and during therapy and determine basal features or changes during therapy may be predictive biomarkers for response
STUDY DESIGN	This is a single-arm exploratory phase 2 trial wherein eligible patients high-risk biochemically recurrent prostate cancer will receive

	nivolumab. PD-L1 tumor-cell expression on primary tissue samples (prostatectomy or diagnostic core biopsies) will be prospectively assessed and used to assign subjects to one of two groups (positive vs. negative). Next-generation sequencing will also be performed upon enrollment but will be used only for exploratory studies. If subjects experience PSA stabilization (<10% increase from baseline) or responses, they will continue nivolumab for up to 2 years in the absence of progression to metastatic disease or unacceptable toxicity. Patients who have stabilization of PSA in the absence of radiographic or clinical progression may also continue treatment. Patients who have isolated PSA progression at 12 weeks can be continued on treatment by investigator's discretion if they are believed to be clinically benefitting (for example, if they experience decreased PSA doubling time) and have not demonstrated symptomatic or radiographic evidence of metastatic disease.
ELIGIBILITY CRITERIA	Eligible subjects must meet all of the following inclusion criteria: 1. Patients must have signed an informed-consent form indicating that the patient understands the purpose of and procedures required for the study and is willing to participate in the study. 2. Patients must have a history of prostate adenocarcinoma (adenocarcinoma must be the primary histology; secondary components of variant histologies are acceptable) confirmed on biopsy and treated with primary radical prostatectomy (RP) or definitive radiation (RT). Prior salvage RT is acceptable. 3. Patients must have experienced BCR with the most recent PSA ≥0.5 ng/mL. For patients who underwent primary RT, PSA must have risen to at least 2 ng/mL above nadir value (per RTOG-ASTRO consensus criteria). • No evidence of metastases on conventional imaging. (See Section 9 for definitions of radiographic metastases. As per PCWG3, lymph nodes ≥1.5 cm are considered pathologic and nodes 1.0-<1.5 cm may be considered pathologic but with clinical discretion. Metastases seen on fluciclovine PET, NaFl, or PSMA scans or other advanced imaging but not on conventional imaging are acceptable. Advanced imaging such as fluciclovine PET or PSMA PET with no evidence of metastases may replace conventional imaging for eligibility. NaFl scan would only replace bone scan.) 4. PSADT <10 months • PSA doubling time (PSADT): calculated as per the PCWG3 and Memorial Sloan Kettering Cancer Center calculator (https://www.mskcc.org/nomograms/prostate/psa_doubling_time) with linear regression model of natural logarithm of PSA and time, based on: o At least 3 consecutive PSA values with each value ≥0.2 ng/mL

- Interval between first and last PSA values is ≥8 weeks but ≤12 months

5. Archival tissue is mandatory, either prostatectomy specimen (in patients who underwent RP) or diagnostic core biopsies (in patients who underwent primary RT, in which case at least 3 cores must be involved by tumor). Patients must consent to next-generation sequencing performed on this tissue.
6. ECOG performance status 0-1
7. Age ≥ 18 years
8. Patients must have adequate organ and marrow function:

System	Laboratory Value	
Hematological		
White blood cell (WBC)	≥ 2000/μL	
Absolute Neutrophil Count (ANC)	≥ 1500/μL	
Platelets (Plt)	≥ 100 x10^3/μL	
Hemoglobin (Hgb)	> 9.0 g/dL (with or without transfusion)	
Renal		
Serum Creatinine	≤ 2 x ULN	
Hepatic		
Bilirubin[1]	≤ 1.5× upper limit of normal (ULN)	
Aspartate aminotransferase (AST)	≤ 3 × ULN	
Alanine aminotransferase (ALT)	≤ 3 × ULN	

[1]Except subjects with Gilbert Syndrome, who can have total bilirubin < 3.0 mg/dL

9. Baseline testosterone ≥ 100 ng/dL
10. Recovery from acute toxicity related to prior therapy, including surgery and radiation, or no treatment-related toxicity ≥ grade 2.
11. History of prior malignancy or concurrent separate malignancy is not an exclusion criterion so long as the non-prostate malignancy is stable and does not require any treatment.
12. Able to understand and sign informed consent and adhere to study procedures.
13. Male patients whose female partners are of reproductive potential must agree to use a contraception during the trial period.

Eligible patients must not have any of the following exclusion criteria:

1. Current use of ADT or plan to initiate ADT during trial period
2. Major surgery or radiation therapy within 14 days of starting study treatment
3. Subjects with active autoimmune disease. Patients with a history of autoimmune disease that has not required systemic immunosuppressive therapy or does not threaten vital organ

	function including CNS, heart, lungs, kidneys, skin, and GI tract will be allowed. 4. Known history of immune deficiencies or chronic viral infections including HIV, HBV, and HCV (prior therapy for HBV or HCV is permitted if viral clearance was documented). 5. Concurrent medical condition requiring use of systemic corticosteroids with prednisone >10 mg per day or equivalent. Use of inhaled, nasal, and topical steroids (applied to small body areas) is allowed. 6. Current use (within past 4 weeks) of other prohibited medications including anti-cancer therapies, hormonal therapies, 5-alpha reductase inhibitors, and alternative medications known to alter PSA (e.g. phytoestrogens and saw palmetto). 7. Prior treatment with immune checkpoint inhibitors. Prior cancer vaccines are allowed. 8. Serious intercurrent medical or psychiatric illness that, in the judgment of the investigator, would interfere with patient's ability to carry out the treatment program.
STATISTICAL CONSIDERATIONS	We plan to enroll 34 patients into the study (with 20 in the PD-L1 positive cohort and 14 in the PD-L1 negative cohort). We hypothesize that patients with positive PD-L1 status will have a higher frequency of disease control after 12 weeks of nivolumab; however we do not have sufficient power to compare patients with and without positive PD-L1 status. In the 20 patients with PD-L1-positive status, if 2 or more patients achieved disease control we would consider the study a success. With 20 patients, there is 97% power to detect a 25% disease control rate (compares to a null hypothesis of 1% disease control rate) with one-sided alpha of 0.02 using a one-sample binomial test. With 14 patients in the PD-L1 negative group, the probably of seeing 0 responses is 4.4% if the true disease control rate is 20%. There is 96% power to detect a 20% disease control rate (vs. null of 1% DCR) at one-sided alpha=0.13. We would like to be able to see at least 1 patient with stable disease at 12 weeks in order to consider the regimen promising for future testing in the PD-L1-negative population. The 96% power(a small false negative rate) while allowing larger alpha is to ensure we do not miss any potential drug activity in patients with PD-L1 negative status.
ENDPOINTS	All subjects will be assessed for response to treatment using the recommendations of PCWG3. <u>Primary Endpoint:</u> • Disease control, defined as PSA at 12 weeks less than 10% above baseline, or below baseline, and no symptomatic/radiographic progression, after 12 weeks of nivolumab treatment <u>Secondary Endpoints:</u>

	• Maximal change in prostate specific antigen (PSA) during nivolumab treatment, as a percentage of baseline • Best PSA response during nivolumab treatment, as an absolute change relative to baseline • Change in PSA doubling time (PSADT) at end-of-study relative to baseline • Time from enrollment to PSA progression (10% or greater increase in PSA relative to baseline) • Time from enrollment to development of radiographic metastatic disease • Time from enrollment to initiation of ADT • Safety of nivolumab <u>Exploratory Endpoints:</u> • Correlation of tumor PD-L1 expression with response to nivolumab • Correlation of tumor MSI-high status with response to nivolumab • Correlation of tumor PD-L1 expression with tumor-infiltrating immune cells • Exploration of exhausted tumor-infiltrating T-cell subsets • Correlation of tumor genomic alterations with PD-L1 expression and nivolumab response, especially homologous recombination deficiencies (HRD; e.g. BRCA1, BRCA2, ATM, CHEK2), mismatch repair deficiencies (MMRd; e.g. MSH2 loss or alteration), status of tumor suppressors (e.g. PTEN and RB1), and ETS fusion status • Detection of peripheral T-cells that recognize identified tumor neoantigens • Detection of circulating tumor DNA and correlation of genomic alterations with those found in primary tumor • Changes in soluble biomarkers during treatment
SAFETY EVALUATION	Toxicity will be graded using the NCI-CTCAE version 5.0. Safety analyses will be conducted on all subjects who have received at least one dose of study drug, and will include the frequency of all AEs and laboratory abnormalities as well as the frequency of dose interruptions, dose reductions, and treatment discontinuations. All adverse events (AEs) must be attributed to study drugs unless there is a reasonably acceptable alternate cause for the AEs. Additional safety assessments will include physical examination; medical history; ECOG performance status; weight; vital signs measurements; and clinical laboratory testing.

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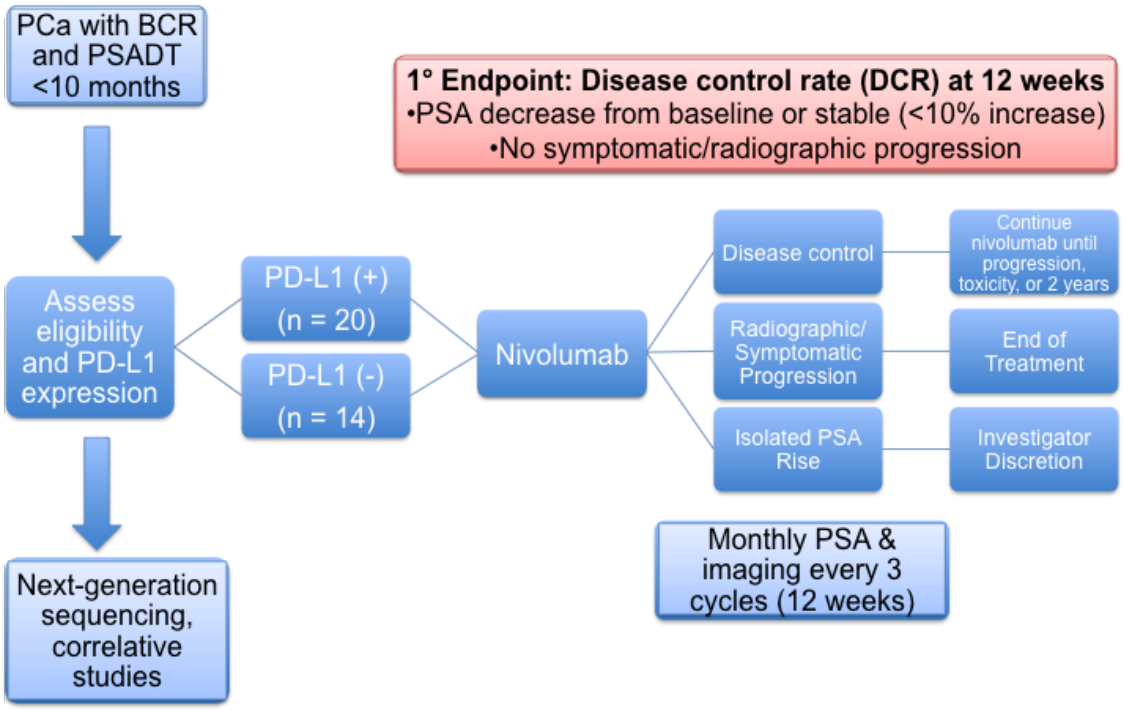
SCHEMA


PCa with BCR and PSADT <10 months
1° Endpoint: Disease control rate (DCR) at 12 weeks
•PSA decrease from baseline or stable (<10% increase)
•No symptomatic/radiographic progression
Assess eligibility and PD-L1 expression
PD-L1 (+) (n = 20)
PD-L1 (-) (n = 14)
Nivolumab
Disease control
Continue nivolumab until progression, toxicity, or 2 years
Radiographic/ Symptomatic Progression
End of Treatment
Isolated PSA Rise
Investigator Discretion
Monthly PSA & imaging every 3 cycles (12 weeks)
Next-generation sequencing, correlative studies

1. BACKGROUND AND RATIONALE

1.1. Biochemically Recurrent Prostate Cancer

Prostate cancer (PCa) is the second most frequently diagnosed cancer and fifth most common cause of cancer death among men, causing an estimated 300,000 deaths worldwide in 2012.[1] Although localized PCa is highly treatable with radical prostatectomy (RP) or radiation therapy (RT), approximately 20-40% of patients undergoing RP and 30-50% of patients undergoing RT will develop a biochemical recurrence (BCR) manifested as rising prostate-specific antigen (PSA) within 10 years.[2-4] Despite salvage RT, many patients will continue to experience BCR. For BCR, no clear standard of care exists. The role and timing of androgen deprivation therapy (ADT) is controversial but is often considered for patients with high risk of developing metastatic disease (mPCa), especially those with PSA doubling times (PSADT) of less than 3-9 months, despite absence of strong data to support improved outcomes.[5] In two observational studies, there was no statistically significant survival benefit conferred by use of ADT for BCR compared with delaying ADT until metastatic disease.[6,7] The TOAD trial attempted to randomize patients between early ADT versus ADT at progression and did demonstrate some improvement in overall survival with early ADT, but it was significantly underenrolled and too underpowered given the small number of events to make any definitive conclusions.[8] NCT00439751 is an ongoing trial also investigating this question.There are clear downsides to early ADT.[9] Use of ADT in this setting may merely prolong ADT-related toxicities, including fatigue, hot flashes, weight gain, muscle loss, bone thinning, and loss of libido, thereby worsening quality of life without any downstream improvement in survival or quality of life, and therefore many patients are understandably reluctant to begin ADT in this setting. Treatment intensification with early use of docetaxel[10] or abiraterone acetate[11] (prior to castration resistance) is under investigation in this population in the STAMPEDE trial, but as of 2018, too few events have occurred in patients with high-risk BCR to change current practice. Alternatively, patients may be monitored until development of symptomatic or radiographic mPCa. Importantly for the design of this study, patients with clearly rising PSA and especially those with high-risk PSADTs are not expected to have spontaneous stabilization of PSA values. In addition, given the PSADT required for entry in this trial, variation in the PSA assay itself would not be expected to result in a stable PSA at the 12-week endpoint used in this trial (see Primary Endpoint, Section 2.2.1).

1.2. Nivolumab Summary

Nivolumab (also referred to as BMS-936558 or MDX1106) is a human monoclonal antibody (HuMAb; immunoglobulin G4 [IgG4]-S228P) that targets the programmed death-1 (PD-1) cluster of differentiation 279 (CD279) cell surface membrane receptor. PD-1 is a negative regulatory molecule expressed by activated T and B lymphocytes.[12] Binding of PD-1 to its ligands, programmed death–ligands 1 (PD-L1) and 2 (PD-L2), results in the down-regulation of lymphocyte activation. Inhibition of the interaction between PD-1 and its ligands promotes immune responses and antigen-specific T-cell responses to both foreign antigens as well as self-antigens. Nivolumab is expressed in

Chinese hamster ovary (CHO) cells and is produced using standard mammalian cell cultivation and chromatographic purification technologies. The clinical study product is a sterile solution for parenteral administration. Nivolumab is approved for use in multiple countries including the United States (US, Dec-2014), the European Union (EU, Jun-2015), and Japan (Jul-2014).

1.3. Non-Clinical Studies

Nivolumab has been shown to bind specifically to the human PD-1 receptor and not to related members of the CD28 family.[13,14] Nivolumab inhibits the interaction of PD-1 with its ligands, PD-L1 and PD-L2, resulting in enhanced T-cell proliferation and interferon-gamma (IFN-γ) release in vitro.[14-16] Nivolumab binds with high affinity to activated human T-cells expressing cell surface PD-1 and to cynomolgus monkey PD-1. In a mixed lymphocyte reaction (MLR), nivolumab promoted a reproducible concentration-dependent enhancement of IFN- γ release.[17] In intravenous (IV) repeat-dose toxicology studies in cynomolgus monkeys, nivolumab was well tolerated at doses up to 50 mg/kg, administered weekly for 5 weeks, and at doses up to 50 mg/kg, administered twice weekly for 27 doses. While nivolumab alone was well tolerated in cynomolgus monkeys, combination studies have highlighted the potential for enhanced toxicity when combined with other immunostimulatory agents.[18] In addition, an enhanced pre- and postnatal development (ePPND) study in pregnant cynomolgus monkeys with nivolumab was conducted.[19] Administration of nivolumab at up to 50 mg/kg 2QW was well tolerated by pregnant monkeys; however, nivolumab was determined to be a selective developmental toxicant when administered from the period of organogenesis to parturition at > 10 mg/kg). Specifically, increased developmental mortality (including late gestational fetal losses and extreme prematurity with associated neonatal mortality) was noted in the absence of overt maternal toxicity. There were no nivolumab-related changes in surviving infants tested throughout the 6-month postnatal period. Although the cause of these pregnancy failures was undetermined, nivolumab-related effects on pregnancy maintenance are consistent with the established role of PD-L1 in maintaining fetomaternal tolerance in mice.[20]

1.4. Effects in Humans

The PK, clinical activity, and safety of nivolumab have been assessed in subjects with non-small cell lung cancer (NSCLC), melanoma, renal cell carcinoma (RCC), and PCa in addition to other tumor types. Nivolumab is being investigated both as monotherapy and in combination with chemotherapy, targeted therapies, and other immunotherapies. Nivolumab is approved in multiple countries including the US for treatment of previously treated, unresectable or metastatic melanoma and previously treated, metastatic NSCLC, the EU for treatment of previously treated, unresectable or metastatic melanoma, and Japan for treatment of unresectable melanoma.

1.5. Clinical Pharmacokinetics

The pharmacokinetics (PK) of nivolumab was studied in subjects over a dose range of 0.1 to 10 mg/kg administered as a single dose or as multiple doses of nivolumab every 2 or 3 weeks. The geometric mean (% CV%) clearance (CL) was 9.5 mL/h (49.7%), geometric mean volume of distribution at steady state (Vss) was 8.0 L (30.4%), and geometric mean elimination half-life (t1/2) was 26.7 days (101%). Steady-state concentrations of nivolumab were reached by 12 weeks when administered at 3 mg/kg Q2W, and systemic accumulation was approximately 3-fold. The exposure to nivolumab increased dose proportionally over the dose range of 0.1 to 10 mg/kg administered every 2 weeks. The clearance of nivolumab increased with increasing body weight. A PK analysis suggested that the following factors had no clinically important effect on the CL of nivolumab: age (29 to 87 years), gender, race, baseline LDH, PD-L1. A PK analysis suggested no difference in CL of nivolumab based on age, gender, race, tumor type, baseline tumor size, and hepatic impairment. Although ECOG status, baseline glomerular filtration rate (GFR), albumin, body weight, and mild hepatic impairment had an effect on nivolumab CL, the effect was not clinically meaningful. When nivolumab is administered in combination with ipilimumab, the CL of nivolumab was increased by 24%, whereas there was no effect on the clearance of ipilimumab. Additionally, PK and exposure response analyses have been performed to support use of 240 mg Q2W dosing as a substitute for the previously studied 3 mg/kg Q2W regimen. Using a PK model, exposure of nivolumab at 240 mg flat dose was identical to a dose of 3 mg/kg for subjects weighing 80 kg, which was the approximate median body weight in nivolumab clinical trials. The 240 mg q 2 week and 480 mg q 4 week flat dose of nivolumab monotherapy have received FDA approval for various indications.. The less frequent dosing regimen results in similar drug exposure to the more frequent regimen and is designed to afford more convenience to the target patient population.

1.6. Clinical Efficacy

Nivolumab has demonstrated durable responses exceeding 6 months as monotherapy in several tumor types, including NSCLC, melanoma, RCC, and some lymphomas. In confirmatory trials, nivolumab as monotherapy demonstrated a statistically significant improvement in OS as compared with the current standard of care in subjects with advanced or metastatic NSCLC and in subjects with unresectable or metastatic melanoma. Despite the successes of nivolumab in other solid tumors, no objective responses were observed in phase I trials in advanced metastatic castration-resistant and treatment-refractory PCa.[21,22]

1.7. Clinical Safety

The overall safety experience with nivolumab, as a monotherapy or in combination with other therapeutics, is based on experience in approximately 16,900 subjects treated to date. For monotherapy, the safety profile is similar across tumor types. There is no pattern in the incidence, severity, or causality of AEs to nivolumab dose level. In Phase 3 controlled studies, the safety profile of nivolumab monotherapy is acceptable in the context of the observed clinical efficacy, and manageable using established safety guidelines. Clinically relevant AEs typical of stimulation of the immune system were

infrequent and manageable by delaying or stopping nivolumab treatment and timely immunosuppressive therapy or other supportive care.

1.8. Biomarkers for Response and Resistance

Several prognostic clinical factors and models (e.g. MSKCC, Heng) have been developed to risk stratify survival outcomes for patients with other tumor types (e.g. RCC). However, predictive biomarker development is critical to identify patient subgroups who are likely to experience frequent and/or durable responses to particular therapies. The importance of this unmet need is heightened by the expense, prolonged duration of exposure to, or toxicities of current therapeutic agents. Tumor associated PD-L1 expression has been proposed as a potential biomarker for sensitivity to PD-1 pathway inhibitors in other tumors.

In the phase I trial of nivolumab in patients with advanced solid tumors, 9 of 25 patients with PD-L1 positive tumors responded compared with 0 of 17 patients with PD-L1 negative tumors (p=0.006).[21] In the Checkmate 025 registration Phase III trial of nivolumab versus everolimus in RCC, tumor PD-L1 membrane expression (≥1% vs. <1% and ≥5% vs. <5%) was evaluated centrally using the Dako PD-L1 immunohistochemical stain.[23] Among 756 patients with quantifiable PD-L1 expression, 181 (24%) had > 1% PD-L1 expression in at least 100 tumor cells. However, the benefit of nivolumab relative to everolimus on overall survival did not differ in these two PDL1 defined subgroups at either this cutoff or when a 5% cut off was used. The lack of predictive value for PD-L1 expression in RCC diverges from data for nivolumab in lung cancer and melanoma.

1.9. Rationale for the Current Trial

Despite the successes of programmed death-1 (PD-1)/programmed-death ligand-1 (PD-L1) inhibitors in other solid tumors, no objective responses were observed in phase I trials in prostate cancers.[21,22] Treatments were well tolerated, with grade 3-4 drug-related adverse events occurring in 18% of patients at most (many of these being laboratory, not clinical events). One concern was that PD-L1 might be insufficiently expressed, as suggested in early reports in primary[24] and in metastatic castration-resistant prostate cancer (mCRPC).[21,25] Prostate cancer also has been suggested to have a relatively low overall mutation rate compared to solid tumors that demonstrate response, such as bladder and lung cancer. However, subsequent studies—in primary PCa rather than mCRPC, with much larger sample sizes, and using newer antibodies –suggested PD-L1 expression rates of 20-60%[26,27] or as high as 92%.[28] Additionally, tumor PD-L1 expression significantly correlated with risk of BCR,[29] suggesting the BCR population might be especially enriched for PD-L1 expression and that inhibition of innate immune function could be associated with more aggressive disease. Similarly, PD-L1 expression may be affected by hormonal manipulation[30]; in particular, it may decrease after ADT with abiraterone,[31] suggesting that hormone-naïve PCa could be enriched for PD-L1 expression. Finally, immune-suppressive immune cells such as myeloid-derived suppressor cells may be increased in the setting of castration resistance,[32] suggesting that

the tumor microenvironment may be quite different in earlier stages of disease and therefore justifying a new trial in this space. The presence of immune-suppressive immune cells could also represent a potential mechanism of resistance to this strategy, as well as a target for future interventions.

Deficiencies in DNA repair leading to microsatellite instability (MSI-high)/mismatch-repair (MMR)-deficiency are associated with particular responsiveness to PD-1 inhibition. A phase II trial of pembrolizumab in MSI-high tumors of several histologies demonstrated objective response rates up to 70%,[33] and in combination with data from 5 trials total, led to an FDA approval for pembrolizumab for MSI-high tumors regardless of histology. The prevalence of MSI-high PCa may be as high as 11% in mPCa.[34] Alterations in mismatch-repair genes, which can lead to an MSI-high phenotype, have been found in approximately 2% of samples depending on series and disease status.[35-38] Importantly for the current project (since it is based on historical tissue samples), hypermutation status between primary and metastatic sites was entirely concordant in two patients with matched samples available.[34] In our own experience with one patient with highly treatment-refractory but MSI-high mCRPC, treatment with pembrolizumab resulted in rapid clinical, PSA, and radiographic response.

In this exploratory trial, we aim to collect data to ultimately support a larger trial aimed at addressing several critical unanswered questions including: (1) Does PD-1 inhibition in patients with BCR PCa result in better disease-control rates than seen in more advanced and heavily pre-treated patients? Can this strategy prevent or delay progression of disease and/or the need to start ADT? (2) Is tumor PD-L1 expression (in primary tumors) associated with increased disease-control rates with nivolumab? And is PD-L1 expression associated with alterations in DNA-damage-repair genes? (3) Is infiltration of CD8+ T-cells in primary tumors and expression of PD-L1 by tumor-infiltrating immune cells associated with response to nivolumab? Do specific subtypes of tumor-infiltrating T-cells, e.g. “exhausted” subtype, correlate with disease control with nivolumab? (4) Is MSI status and/or tumor mutation burden in primary tumors associated with disease-control with nivolumab? (5) Are there other genomic alterations—in particular, deficiencies in homologous recombination repair, loss of gene copies of phosphatase and tensin homolog (PTEN) or retinoblastoma (RB1), or E26 transformation-specific (ETS) fusions—that are associated with disease control with nivolumab?

2. STUDY OBJECTIVES AND ENDPOINTS

2.1. Objectives

2.1.1. Primary Objective

The primary objective is to determine the proportion of patients with high-risk biochemically-recurrent (BCR) prostate cancer (PCa) that experiences decline or stabilization in PSA (without symptomatic/radiographic progression) after 12 weeks of nivolumab.

2.1.2. Secondary Objectives

- Determine the maximal change (proportional) and best decline (absolute) in prostate specific antigen (PSA) during nivolumab treatment
- Describe the change in PSA doubling time (PSADT) during nivolumab therapy relative to baseline
- Determine the time to development of overt metastatic disease after treatment with nivolumab
- Determine the time to initiation of androgen deprivation therapy (ADT) after treatment with nivolumab
- Assess the toxicity of nivolumab monotherapy in patients with BCR PCa

2.1.3. Exploratory Objectives

- Evaluate whether response to nivolumab treatment is enriched among patients with tumor programmed death-ligand 1 (PD-L1) expression on tumor cells and on tumor-infiltrating lymphocytes and antigen-presenting cells (APCs)
- Identify genomic biomarkers associated with response to nivolumab
- Evaluate correlations between alterations in DNA-damage-repair genes and PD-L1 expression
- Evaluate correlations between tumor-infiltrating lymphocytes and APCs with response to nivolumab
- Evaluate subtypes of "exhausted" tumor-infiltrating lymphocytes
- Explore whether peripheral T-cells are present that might recognize tumor neoantigens and whether these correlate with responses to nivolumab
- Assess for genomic alterations in circulating tumor DNA and correlate with mutations identified in primary tumor specimens
- Assess levels of plasma cytokines prior to initiation and during therapy and determine whether levels or changes in specific cytokines may be predictive biomarkers for response
- Assess PBMC subsets prior to initiation and during therapy and determine basal features or changes during therapy may be predictive biomarkers for response

2.2. Endpoints

2.2.1. Primary Endpoint

The primary endpoint is disease control, defined as PSA at 12 weeks less than 10% above baseline, or below baseline, and no symptomatic/radiographic progression, after 12 weeks of nivolumab treatment.

The expected increase in PSA at 12 weeks depends on doubling time. Based on the doubling time formula, doubling time = log(2)/log(1 + growth rate), a patient with a 10-month doubling time (the longest allowed under the exclusion criteria) would be expected to have an approximately 7.18% growth rate per month. PSA increase over 3 months (12 weeks) without intervention would be expected to be 23%. Faster doubling time would result in faster growth rate. Therefore, disease stability will be considered a PSA rise of less than 10% above baseline at 12 weeks. (This is in contrast with PCWG3, which considers PSA progression to be rise of at least 25% of baseline; as noted above, this criterion is too lenient in this setting given the expected PSA rise in the absence of intervention).

2.2.2. Secondary Endpoints

- Maximal change in prostate specific antigen (PSA) during nivolumab treatment, as a percentage of baseline
- Best PSA response during nivolumab treatment, as an absolute change relative to baseline
- Change in PSA doubling time (PSADT) at end-of-study relative to baseline
- Time from enrollment to PSA progression (10% or greater increase in PSA relative to baseline)
- Time from enrollment to development of radiographic metastatic disease
- Time from enrollment to initiation of ADT
- Safety of nivolumab

2.2.3. Exploratory Endpoints

- Correlation of tumor PD-L1 expression with response to nivolumab
- Correlation of tumor MSI-high status with response to nivolumab
- Correlation of tumor PD-L1 expression with tumor-infiltrating immune cells
- Exploration of exhausted tumor-infiltrating T-cell subsets
- Correlation of tumor genomic alterations with PD-L1 expression and nivolumab response, especially homologous recombination deficiencies (HRD; e.g. BRCA1, BRCA2, ATM, CHEK2), mismatch repair deficiencies (MMRd; e.g. MSH2 loss or alteration), status of tumor suppressors (e.g. PTEN and RB1), and ETS fusion status
- Detection of peripheral T-cells that recognize identified tumor neoantigens
- Detection of circulating tumor DNA and correlation of genomic alterations with those found in primary tumor
- Changes in soluble biomarkers during treatment

3. ELIGIBILITY CRITERIA

3.1. Inclusion Criteria

Eligible subjects must meet all of the following inclusion criteria:

1. Patients must have signed an informed-consent form indicating that the patient understands the purpose of and procedures required for the study and is willing to participate in the study.
2. Patients must have a history of prostate adenocarcinoma (adenocarcinoma must be the primary histology; secondary components of variant histologies are acceptable) confirmed on biopsy and treated with primary radical prostatectomy (RP) or definitive radiation (RT). Prior salvage RT is acceptable.
3. Patients must have experienced biochemical recurrence (BCR) with most recent PSA ≥0.5 ng/mL. For patients who received primary RT, PSA must have risen to ≥2 ng/mL above the nadir (per RTOG-ASTRO consensus criteria).
    a. No evidence of metastases on conventional imaging. (See Section 9 for definitions of radiographic metastases. As per PCWG3, lymph nodes ≥1.5 cm are considered pathologic and nodes 1.0-<1.5 cm may be considered pathologic but with clinical discretion. Metastases seen on fluciclovine PET, NaFl, or PSMA scans or other advanced imaging but not on conventional imaging are acceptable. Advanced imaging such as fluciclovine PET or PSMA PET with no evidence of metastases may replace conventional imaging for eligibility. NaFl scan would only replace bone scan.)
4. PSA doubling time (PSADT) <10 months
    a. PSADT: calculated as per PCWG3 and the Memorial Sloan Kettering Cancer Center calculator: (https://www.mskcc.org/nomograms/prostate/psa_doubling_time) With linear regression model of normal logarithm of PSA and time, based on:
        i. At least 3 consecutive PSA values with each value ≥0.2 ng/mL
        ii. Interval between first and last PSA values is ≥8 weeks but ≤12 months
5. Archival tissue is mandatory, either prostatectomy specimen or (in patients who received primary RT) diagnostic core biopsies. Patients must consent to next-generation sequencing performed on this tissue.
    a. If diagnostic core biopsies are only available tissue, at least 3 cores must be involved by tumor
6. ECOG performance status 0-1
7. Age ≥18 years
8. Adequate organ and marrow function:

System	Laboratory Value
Hematological	
White blood cell (WBC)	≥ 2000/μL
Absolute Neutrophil Count (ANC)	≥ 1500/μL
Platelets (Plt)	≥ 100 x10^3/μL
Hemoglobin (Hgb)	> 9.0 g/dL (with or without transfusion)
Renal	
Serum Creatinine	≤ 2 x ULN
Hepatic	

Bilirubin[1]	≤ 1.5× upper limit of normal (ULN)
Aspartate aminotransferase (AST)	≤ 3 × ULN
Alanine aminotransferase (ALT)	≤ 3 × ULN

[1]Except subjects with Gilbert Syndrome, who can have total bilirubin < 3.0 mg/dL

9. Baseline testosterone ≥100 ng/dL
10. Recovery from acute toxicity related to prior therapy, including surgery and radiation, or no treatment-related toxicity ≥ grade 2.
11. History of prior malignancy or concurrent separate malignancy is not an exclusion criterion so long as the non-prostate malignancy is stable and does not require any treatment.
12. Able to understand and sign informed consent and adhere to study procedures.
13. Male patients whose female partners are of reproductive potential must agree to use a contraception during the trial period.

3.2. Exclusion Criteria

Eligible patients must not have any of the following exclusion criteria:

1. Current use of ADT or plan to initiate ADT during trial period
2. Major surgery or radiation therapy within 14 days of starting study treatment
3. Subjects with active autoimmune disease. Patients with a history of autoimmune disease that has not required systemic immunosuppressive therapy or does not threaten vital organ function including central nervous sytem, heart, lungs, kidneys, skin, and gastrointestinal tract will be allowed.
4. Known history of immune deficiencies or chronic viral infections including HIV, HBV, and HCV (patients with prior therapy for HBV or HCV is permitted if viral clearance was documented).
5. Concurrent medical condition requiring use of systemic corticosteroids with prednisone >10 mg per day or equivalent. Use of inhaled, nasal, and topical steroids (applied to small body areas) is allowed.
6. Current use (within past 4 weeks) of other prohibited medications including anti-cancer therapies, hormonal therapies, 5-alpha reductase inhibitors, and alternative medications known to alter PSA (e.g. phytoestrogens and saw palmetto).
7. Prior treatment with immune checkpoint inhibitors. (Prior cancer vaccines are allowed.)
8. Serious intercurrent medical or psychiatric illness that, in the judgment of the investigagor, would interfere with patient's ability to carry out the treatment program.

4. SUBJECT REGISTRATION

4.1. General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of any protocol-specific therapy or intervention. Any participant not registered to the protocol before protocol-

specific therapy or intervention begins will be considered ineligible and registration will be denied.

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

Following registration, participants may begin protocol-specific therapy and/or intervention. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If the subject does not receive protocol therapy following registration, the subject must be taken off-study in the CTMS (OnCore) with an appropriate date and reason entered.

Applicable DF/HCC policy (REGIST-101) must be followed.

5. TREATMENT PLAN

5.1. Screening and Biomarker Cohorts

Once subjects have been registered for the study, primary tissue (from RP or diagnostic core biopsies) will be obtained, including blocks and any slides. For non-BIDMC institutions, a BIDMC clinical research assistant should be contacted by email (**GUOncologyTrials@caregroup.harvard.edu**) with the subject line including protocol number and "Tissue Collection."

Dr. Huihui Ye will perform screening PD-L1 immunohistochemistry at BIDMC. "Positive" PD-L1 expression is defined for this study by ≥1% Combined Positive Score (CPS), which is the number of PD-L1-staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multipled by 100. For any cases deemed positive, tissue will be sent to Acupath Laboratories, Inc., a CLIA-certified laboratory, for confirmation immunohistochemistry. For cases deemed negative, these will also be sent to Acupath for confirmation, but only so long as the PD-L1-negative cohort is open; once the target accrual of 14 patients in this cohort is reached, subsequent cases deemed to be PD-L1 negative by Dr. Ye will not be sent for Acupath confirmation and these subjects would not be eligible for the study.

Specifications for shipment of tissue to Acupath are:

- Slides must be 4-5 microns per section, unbaked, placed in a slide holder box so slide is on its side
- Slides must be accompanied by an information sheet (please see Appendix 2)

Ship via FedEx to:

Steve Kamalic
Acupath Labs
S Terminal Dr
Plainview, NY 11803
Cell: 516-492-4549

Office: 516-775-8103 Ext 5605
Fax: 516-908-6040

As noted in this trial schema, this study involves two cohorts, a PD-L1-positive cohort and a PD-L1-negative cohort. Subjects will be assigned to one of these cohorts once results of immunohistochemistry (with confirmation from Acupath) are available. Until the PD-L1-negative cohort has accrued 13 subjects, study treatment may begin once the lead site has received archival tissue; PD-L1 status may be assigned retroactively. Once the 13th PD-L1-negative subject has been accrued, then all subjects will need to have PD-L1 status assigned before they may start study treatment. Once a cohort reaches maximum enrollment (20 subjects for the PD-L1-positive cohort; 14 subjects for the PD-L1-negative cohort), no further subjects may be enrolled to that cohort. It is likely that the PD-L1-negative cohort will close first. At this point, subjects may still be screened, but any deemed to be PD-L1-negative by Dr.Ye will be excluded from the trial without confirmation from Acupath.

After the PD-L1-negative cohort closes, a tissue pre-screening consent will occur. Potential participants will be consented to have their tissue evaluated for PD-L1 expression. If they are found to be PD-L1-negative, no further study-related activities will occur. If they are found to be PD-L1-positive, then full informed consent will occur and this will begin the study screening period.

After review and selection of tissue for PD-L1 testing, remaining tissue will be used for next-generation sequencing and exploratory studies.

In the event of limited quantity of tissue, priority order for tissue specimens is:

1. PD-L1 expression assessment
2. Next-generation sequencing
3. Exploratory studies

5.2. Nivolumab Administration

Nivolumab will be given on day 1 of a 28-day cycle at a dose of 480 mg intravenously (IV). All visits have a window of +/- 3 days (see Study Calendar, section 7).

The administration of nivolumab infusion must be completed within 24 hours of preparation. If not used immediately, the infusion solution may be stored under refrigeration conditions (2°C to 8°C, 36°F to 46°F) for up to 24 hours, and a maximum of 8 hours of the total 24 hours can be at room temperature (20°C to 25°C, 68°F to 77°F) and room light. The maximum of 8 hours under room temperature and room light conditions includes the product administration period. The unopened vials can be stored at room temperature (up to 25°C, 77°F) and room light for up to 48 hours.

Nivolumab injection is to be administered as an IV infusion over 30 minutes (acceptable window is 25 minutes to 40 minutes) through a 0.2-micron to 1.2-micron pore size, low-protein binding (polyethersulfone membrane) in-line filter at the protocol-specified doses

and infusion times. It is not to be administered as an IV push or bolus injection. Nivolumab can be infused undiluted or diluted so as not to exceed a total infusion volume of 1**6**0 mL. For patients weighing less than 40 kilograms (kg), the total volume of infusion must not exceed 4 mL per kg of patient weight.

5.3. Concomitant Medications

5.3.1. Allowed Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care.

5.3.2. Prohibited Concomitant Medications

Concomitant systemic or local anti-cancer medications or treatments are prohibited in this study while receiving nivolumab treatments.

Patients may not use any of the following therapies during the study or for the 4 weeks preceding the PSA values used to determine subject eligibility:

- Any non-study anti-cancer agent (investigational or non-investigational), including but not limited to bicalutamide or other anti-androgens; GnRH analogs/antagonists; dutasteride, finasteride, or other 5 alpha-reductase inhibitors; or alternative medications known to alter PSA (e.g. pytoestrogens and saw palmetto)
- Any other investigational agents
- Immunosuppressive agents (except as needed to treat toxicities that develop on therapy)
- Physiologic replacement doses of corticosteroids are permitted if required
- Any non-oncology vaccine therapies used for the prevention of infectious diseases (for up to 30 days prior to or after any dose of study drug)
  NOTE: Patients are permitted to receive the seasonal influenza vaccine. If seasonal influenza vaccine is considered, killed vaccines should be recommended. Patients are permitted to receive all COVID-19 vaccines.

6. TOXICITIES AND DOSE DELAYS

Please see Section 10 for expected toxicities related to nivolumab therapy.

6.1. Management of Immune-Related Adverse Events

Please see Appendix 1 for tables summarizing management of immune-related adverse events plus management algorithms for specific organ systems.

6.2. Dose Modifications

There will be no dose modifications permitted. Dose reductions or dose escalations are not permitted.

6.3. Dose Delay Criteria

Dose delay criteria apply for all drug-related adverse events.

Nivolumab administration should be delayed for the following:

- Any Grade ≥ 2 non-skin, drug-related adverse event, including grade 2 liver test elevation, with the following exceptions:
    - Grade 2 drug-related fatigue
- Any Grade 3 skin, drug-related adverse event
- Any Grade 3 drug-related laboratory abnormality
- Any new-onset moderate or severe neurologic signs or symptoms. For a diagnosis of immune-mediated encephalitis, permanently discontinue.
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the site investigator, warrants delaying the dose of study medication.

6.4. Criteria to Resume Treatment

Subjects may resume treatment with study drugs when the drug-related AE(s) resolve to Grade ≤ 1 or baseline value, 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
- 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 the criteria to resume treatment are met, the subject should restart treatment at the next scheduled timepoint per protocol (i.e., missed doses are NOT made up). However, if the treatment is delayed past the next scheduled timepoint per protocol, the next scheduled timepoint will be delayed until dosing resumes.

If treatment is delayed > 6 weeks, (from the missed dose) the subject must be permanently discontinued from study therapy, except as specified in discontinuation section below.

6.5. Discontinuation Criteria

Treatment should be permanently discontinued for the following:

- Any Grade 2 drug-related uveitis or 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 drug-related laboratory abnormalities, uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic adverse event, hypersensitivity reactions, and infusion reactions
    - Grade 3 drug-related uveitis, pneumonitis, bronchospasm, neurologic adverse event, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation
    - Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except those noted below
- Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding
- Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:
    - AST or ALT > 5 x ULN (grade 3)
    - Total bilirubin > 3 x ULN (grade 3)
    - AST or ALT >3 x ULN **and** total bilirubin >2 x ULN
- Grade 3 adrenal insufficiency
- Any Grade 4 drug-related adverse event or laboratory abnormality, except for the following events which do not require discontinuation:
    - 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
    - Grade 4 neutropenia lasting 7 days or less
    - Grade 4 lymphopenia or leucopenia
    - Grade 4 drug-related endocrinopathy adverse events, such as ACTH deficiency, hyper- or hypothyroidism, or glucose intolerance, which resolve or are adequately controlled with physiologic hormone replacement (corticosteroids, thyroid hormones) or glucose-controlling agents, respectively, may not require discontinuation after discussion with and approval from the Principal Investigator. However, grade 3 adrenal insufficiency requires nivolumab discontinuation, as above.
- Any dosing interruption lasting > 6 weeks (from scheduled dose) with the following exceptions:
    - Dosing interruptions to allow for prolonged steroid tapers to manage drug-related adverse events are allowed. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the sponsor-investigator must be consulted. 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 if approved by the sponsor-investigator. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the Principal Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted.

- Requirement for 10 mg per day or greater prednisone or equivalent for more than 12 weeks
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the site investigator, presents a substantial clinical risk to the subject with continued nivolumab dosing

6.6. Treatment of Nivolumab-Related Infusion Reactions

Since nivolumab contains only human immunoglobulin protein sequences, it is unlikely to be immunogenic and induce infusion or hypersensitivity reactions. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash, pruritis, arthralgias, hypo- or hypertension, bronchospasm, or other symptoms.

All Grade 3 or 4 infusion reactions should be reported as an SAE if criteria are met. Infusion reactions should be graded according to NCI CTCAE v5.0 guidelines.

Treatment recommendations are provided below and may be modified based on local treatment standards and guidelines as appropriate:

- 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 paracetamol 325 to 1000 mg (acetaminophen) at least 30 minutes before additional nivolumab administrations.
- 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, bronchodilators, 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 paracetamol 325 to 1000 mg (acetaminophen); remain at bedside and monitor subject until resolution of symptoms. Corticosteroid or bronchodilator 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. Administer diphenhydramine 50 mg IV, and remain at bedside and monitor the subject until resolution of symptoms. The amount of study drug infused must be recorded on the electronic case report form (eCRF). The following prophylactic pre-medications are recommended for future infusions:
    - Diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) should be administered at least 30 minutes before additional nivolumab administrations. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.
- Grade 3 or Grade 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 1:1,000 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.*** Site investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery from symptoms. In the case of late-occurring hypersensitivity symptoms (eg, appearance of a localized or generalized pruritis within 1 week after treatment), symptomatic treatment may be given (eg, oral antihistamine, or corticosteroids).

6.7. Protocol Therapy Discontinuation

In addition to discontinuation from therapy related to toxicities as outlined above, a subject will also be discontinued from protocol therapy and followed per protocol under the following circumstances outlined below. The reason for discontinuation of protocol therapy will be documented on the electronic case report form (eCRF).

The Prostate Cancer Working Group 3 (PCWG3)[39] recognizes that favorable effect of experimental therapies on PSA may be delayed for ≥12 weeks and recommends continuing treatment through early rises for a minimum of 12 weeks unless there is other evidence of progression.

PCWG3 recommends continuing experimental therapies for subjects who experience PSA rise after initial decline, in the absence of any radiographic or clinical progression. However, subjects in this trial do have access to a standard-of-care treatment in the form of ADT which can be used to delay development of metastatic disease, although a convincing survival benefit has not been established in this setting. This trial requires treatment discontinuation only for the reasons below, but investigators are advised to use discretion for patients with rapidly increasing PSAs (after 12 weeks of treatment). Patients believed to be clinically benefitting, for example based on decreased PSADT after starting nivolumab, could be continued on treatment at the investigator's discretion.

- Documented metastases on imaging (see Section 9 for definitions).
- Completed 2 years of treatment with nivolumab
- Site investigator determines a change of therapy (i.e. starting ADT) would be in the best interest of the subject
- Subject or legal representative (such as a parent or legal guardian) withdraws consent and requests to discontinue protocol therapy, whether due to unacceptable toxicity or for other reasons
    - In a subject decides to prematurely discontinue protocol therapy ("refuses treatment"), the subject should be asked if he or she may still be contacted for further

scheduled study assessments. The outcome of that discussion should be documented in both the medical records and in the eCRF.

- Unacceptable adverse experiences
- Intercurrent illness that prevents further administration of treatment
- Protocol therapy is interrupted for > 6 weeks (calculated from the date of the first missed dose). Any potential exceptions to this criteria must be discussed with and approved by the PI.
- Noncompliance with protocol treatment or procedure requirements
- The subject is lost to follow-up
- Administrative reasons

6.8. Protocol Discontinuation

If a subject decides to discontinue from the protocol (and not just from protocol therapy) all efforts should be made to complete and report study assessments as thoroughly as possible. A complete final evaluation at the time of the subject's protocol withdrawal should be made with an explanation of why the subject is withdrawing from the protocol. If the reason for removal of a subject from the study is an adverse event, it will be recorded on the eCRF.

7. STUDY CALENDAR & EVALUATIONS

Event	Screening	On Treatment (28-day cycles)	End of Study[i]	Follow-Up
	≤ 28 days prior to enrollment	Day 1[j]	Within 30 days of last dose	Every 3 months until mPCa
Informed consent	X			
Confirmation of all eligibility criteria	X[k]			
Medical history[a]	X		X	X[l]
Prior systemic and radiation therapy[b]	X			
Physical examination[c]	X	X	X	
ECOG performance status	X	X	X	
Concomitant medication review	X	X	X	
Adverse Event review	X	X	X	X[l] (100 days after last nivolumab dose)
Chemistries[d]	X	X		
Hematology[d]	X	X		
TSH (with reflex free T4 testing if abnormal)	X	X		
Prostate-specific antigen[e]	X	X	X	
Archival tissue testing for PD-L1 expression and NGS[f]	X (After tissue pre-screen consent, prior to full informed consent, for patients enrolled under AM2)			
Blood for exploratory studies[g]		Cycles 1 and 2; q4 cycles beyond cycle 4 (C5, C9, etc.). *Optional collection 1 week after cycle 1.*	X	
Radionuclide bone scan and CT of abdomen/pelvis with contrast[h]	X	X (Prior to cycle 4 and every 3rd cycle thereafter)		
Nivolumab administration		X		

Abbreviations: AE=adverse event; CBC=complete blood count; ECOG=Eastern Cooperative Oncology Group (performance score); EOS=end-of-study; mPCa=diagnosis of metastatic prostate cancer; NGS=next-generation sequencing (e.g. Foundation One, OncoPanel).

[a] Medical history: including relevant medical history collection, as well as Gleason score and TNM stage at diagnosis, results of any recent advanced imaging (Axumin, SPECT, choline, sodium fluoride, etc.)

[b] Collect radiation site, administered dose per fraction and treatment duration, as well as any concurrent ADT and duration of ADT.

[c] Physical examination includes height (at screening only), weight, vital signs, and general physical examination

[d] Blood chemistry panel includes sodium, potassium, chloride, bicarbonate, calcium, blood urea nitrogen, creatinine, alkaline phosphatase, total bilirubin, AST, ALT. Testosterone will be drawn during screening to confirm castration but does not need to be repeated after screening. CBC must include white blood cell differential. CBC and chemistry testing may occur up to 48 hours prior to day 1 of each cycle. Labs obtained on cycle 1, day 1 do not need to re-meet eligibility criteria. Treatment may begin at each cycle before TSH result is available.

[e] See requirements for baseline PSA testing in inclusion criteria.

[f] Results of PD-L1 testing (including confirmatory testing if positive) must be obtained so that subject can be assigned to a study cohort BEFORE starting study treatment. Following PD-L1 testing and NGS, remaining tissue will be used for exploratory studies.

[g] Exploratory studies include germline whole-exome sequencing performed at baseline, peripheral immunophenotyping, soluble biomarkers, ctDNA, and T-cell collections at specified intervals. Please see appendix 3 for details of blood collection. Research blood specimens will generally be collected with monitoring labs detailed in footnote d above. If standard labs are collected in advance, then research blood samples may be collected on the treatment day (before or after treatment). If research blood samples are inadvertently missed during collection of standard labs just before treatment, then the research blood samples may also be drawn post-treatment (same day).

[h] Radiographic imaging will be obtained after every 3rd cycle until EOS. Radiographic imaging includes CT abdominal/pelvis with contrast (or MRI if contraindication to intravenous contrast) and bone scan. Radiographic imaging may occur up to 8 days prior to day 1 of the upcoming cycle, or on day 1 itself. Baseline imaging may include advanced imaging modalities as described in the eligibility criteria, assuming there is no radiographic evidence of disease.

[i] End of study (EOS) assessments should be conducted in-person within 30 days after the last dose of nivolumab. AEs will also be assessed at 100 days after the last dose of nivolumab in the Follow-up Period (see footnote l). Patients must contact the study team regarding any significant toxicities or illnesses that occur within 100 days of last nivolumab dose, as SAEs must be reported during this time. For patients experiencing toxicities, EOS visits should occur every 2 weeks (± 3 days) until toxicities resolve. Depending upon the toxicity, the every 2-week assessment may be accomplished via phone call, email or other avenues as appropriate. Mode of assessment will be at site investigator's discretion.

[j] All visits have a window of +/- 3 days.

[k] Baseline imaging may be performed up to 60 days prior to enrollment.

[l] Follow-up period will continue until documentation of metastatic disease (via radiographic imaging and/or biopsies). Follow-up may consist of medical record review (for patients treated at BIDMC) or contact via phone/email (for patients treated at non-BIDMC institutions). Data to be collected includes: date of initiation of any subsequent hormonal therapy, date of documented metastatic disease, and any other therapies received during follow-up period. If contact is made via phone/email with patient, then written documentation must be provided to confirm these dates and the diagnosis of metastatic disease. In addition, the research nurse must attempt to contact the patient (via phone or email depending on patient preference) approximately 100 days after the

last dose of nivolumab, +/- 1 week, to assess AEs. If the patient cannot be reached, attempts should be documented in the medical record and this will not be considered a deviation from protocol.

8. BIOSPECIMEN STUDIES AND PROCEDURES

As discussed above, early trials of PD-1/PD-L1 blockade showed a disappointing efficacy in castration-resistant prostate cancer (CRPC). The failure of PD-1/PD-L1 checkpoint blockade in CRPC has been hypothesized to be due to poor immunogenicity of PCa and subsequently lack of immune resistance in PCa, supported by absence or little PD-L1 expression on prostate cancer cells. [21,40] However, recently there have been a number of reports describing significant PD-L1 positivity in both primary PCa (up to 61.7%) and CRPC (up to 19%), [27,41] presumably due to improvement of antibody and immunostaining sensitivity. In clinical practice, it is not uncommon to encounter primary PCas that are enriched in TILs. Therefore, we have hypothesized that a subset of PCas are immunogenic and upregulate immune checkpoint molecules such as PD-L1. Identification of this fraction of "hot" PCas may correlate with response to immune checkpoint inhibition, a hypothesis we will test in this trial.

8.1. Exploratory Studies

All planned studies are exploratory and intended as hypothesis-generating. These will be performed if responses to nivolumab are observed. Statistical plan is broadly outlined in Section 12.3.

We will first evaluate whether that disease-control rate with nivolumab is enriched among patients with PD-L1 expression and/or CD8+ tumor-infiltrating lymphocytes (TILs). For this analysis, serial sections will be immunostained for PD-L1, CD3 (total T cells), CD4, and CD8. For the assessment of PD-L1 expression, screening immunostains will be carried out in the Ye laboratory at BIDMC, and confirmatory immunostains of qualified cases will be performed by a CLIA-certified commercial laboratory. We will in parallel investigate functional status of these CD8+ T cells in trial patient specimens, including subtypes of exhausted tumor-infiltrating T-cells (T_{EX} cells). We hypothesize that responders retain a higher fraction of "progenitor" subtype of T_{EX} cells that can be reinvigorated by nivolumab; in contrast, non-responders are associated with a predominance of "terminal" subtype of T_{EX} cells. For this analysis we will perform additional immunohistochemistry on serial sections for PD-1, T-bet, Eomes, TIM-3, LAG-3, CTLA-4, granzyme B, and FoxP3. We may also consider use of Cytometry by Time Of Flight (CyTOF) technology for assessment of peripheral T-cell subsets in blood. Finally, we will use immunohistochemistry to assess myeloid cells (macrophage, dendritic cells, myeloid suppressor cells) in the tumor microenvironment, and correlations with T cells, PD-L1 expression, and responses.

Second, we will explore the hypothesis that PD-L1-positive PCas (defined by PD-L1 expression in ≥5% of tumor cells) are associated with genomic instability. Pending funding, we will plan to perform next-generation sequencing on primary tumors from many if not all enrolled patients. We will investigate correlations between PD-L1 positivity (as well as response to nivolumab therapy) with results of next-generation sequencing, including

alterations in mismatch-repair genes, microsatellite instability, deficiencies in homologous recombination genes, and overall mutational burden. Any observed correlations will be hypothesis-generating and not definitive.

Third, we will consider exploratory analyses of tumor neoantigens and peripheral T-cell reactivity. Whole-exome sequencing (WES) of primary tissue may be used to identify potential tumor neoantigens that could be the target of effector T-cell responses. Sequential peripheral blood collections will allow banking of antigen-presenting cells and T-cells. If there are patients with strong responses to nivolumab treatment, we will consider evaluating their banked samples for evidence of T-cell responsiveness to the potential neoantigens identified in the patients' primary tumor WES data.

Fourth, banked blood would allow future analysis of circulating tumor DNA to investigate whether mutations in the primary tissue correlated with mutations at the time of biochemical recurrence.

Finally, we will assess changes in soluble biomarkers (cytokines, chemokines) during treatment, which may correlate with treatment response.

8.2. Tissue Collections

8.2.1. Archival Tumor Tissue

Archival tumor tissue will be requested prior to registration. For patients who underwent primary prostatectomy, prostatectomy slides and blocks will be obtained. For patients who underwent primary radiation therapy, diagnostic core-needle biopsy slides and tissue blocks will be obtained. Slides will be reviewed at BIDMC. For each patient, one to two tissue blocks that contain the greatest dimension of tumor will be used for screening and exploratory studies (please see Section 5.1); the remainder may be used for exploratory studies.

8.2.2. Peripheral Blood

Blood will be collected as outlined in the study calendar (section 7). Please see appendix 3 for details of tubes, volume, and delivery.

8.3. Storage of Biospecimens

All specimens not exhausted in planned exploratory studies for this trial will be stored for future research.

8.4. Confidentiality of Biospecimens

All samples will be labeled with the following identifier system:

- Patient's enrollment number
- Patient's initials

- Protocol number
- Collection timepoint (cycle number)
  Example: 01-AB-18XXX-1

Any material issued to collaborating researchers will be anonymized and only identified by this identifier.

8.5. Collection and Shipping of Research Blood Samples

For patients enrolled at non-BIDMC sites, research tubes as described above will be drawn as per the study calendar. To arrange pick-up, a BIDMC clinical research assistant should be contacted by email (GUOncologyTrials@caregroup.harvard.edu) with the subject line including protocol number and "Blood Collection." Blood samples for germline WES will be brought to the Balk lab at Center for Life Sciences, 4th Floor, 3 Blackfan Circle, Boston, MA. Blood samples for T-cell isolation and banking will be brought to the Avigan/Rosenblatt lab, also at Center for Life Sciences, on the 6th floor and under the care of Dr. Dina Stroopinsky (contact dstroopi@bidmc.harvard.edu in advance to arrange delivery).

9. CRITERIA FOR DISEASE EVALUATION

As per the Prostate Cancer Working Group 3 (PCWG3),[39] a rising PSA is typically the first sign of tumor regrowth, followed later by worsening of disease by imaging and the development of clinical symptoms. Since there may be some interassay variability, subjects should have PSAs measured while on study at a single institution. PSAs from other institutions prior to enrollment are acceptable with the caveat that clear rise must be demonstrated and that interassay variability should be factored into this assessment. For this study, we are using the PCWG3-designated "control/relieve/eliminate" endpoints for therapies expected to be cytotoxic. In limited experience with MSI-high PCa that responds to PD-1 blockade, PSAs have fallen dramatically. It is also possible that stabilization of PSA will be a clinically meaningful endpoint if it delays or prevents development of metastatic disease and/or need for ADT; therefore, PSA stabilization is included in the primary endpoint.

9.1. Definitions

- Disease control rate: please see Study Endpoints (section 2) above
- Maximal PSA change: Best PSA response divided by baseline PSA x 100%]
- Best PSA response: Baseline PSA minus lowest on-study PSA at any time
- PSA doubling time (PSADT): calculated as per the PCWG3 and Memorial Sloan Kettering Cancer Center calculator (https://www.mskcc.org/nomograms/prostate/psa_doubling_time) with linear regression model of natural logarithm of PSA and time, based on:
  - At least 3 consecutive PSA values with each value ≥0.2 ng/mL
  - Interval between first and last PSA values is ≥8 weeks but ≤12 months

PSADT should be calculated based on pre-study PSAs and based on the last 3 PSAs prior to EOT. If subject comes off trial prior to cycle 4, then EOT PSADT cannot be calculated since the first of three values would coincide with baseline value at cycle 1 day 1.

- Radiographic progression to metastatic disease:
    - Lymph nodes must have grown by at least 5 mm in the short axis from baseline or nadir and be ≥1.0 cm in short axis to be considered to have progressed. If the node progresses to ≥1.5 cm in the short axis, it is considered pathologic (metastatic) in the absence of alternative explanation. Nodes that have progressed to between 1.0 and less than 1.5 cm are subject to clinical discretion.
    - New unequivocal visceral lesions are determined by RECIST 1.1 criteria[42]
    - Bone metastases are difficult to assess. PCWG3 recommends recording the date of unequivocal development of new sites on bone scintigraphy, then continuing therapy (assuming it is well tolerated and there is no clinical reason to discontinue it) until two additional new lesions are detected. If two new lesions are subsequently detected, these must be confirmed at the time of next bone scan; if confirmed, date of progression is the date these two new lesions were initially detected. **All cases of progression called on bone scan must be reviewed by the overall PI to confirm adherence to PCWG3 guidelines.** Sites should ask interpreting radiologists to enumerate bone lesions for all such cases.

10. DRUG INFORMATION

10.1. Nivolumab

Nivolumab was selected for dosage form development and is also referred to as BMS-936558-01 or BMS-936558. Nivolumab is a soluble protein consisting of 4 polypeptide chains, which include 2 identical heavy chains and 2 identical light chains.

Other Names Nivolumab, BMS-936558, MDX1106, anti-PD-1
Molecular Wt 146,221 daltons (143,619.17 daltons, protein portion)
Appearance Clear to opalescent, colorless to pale yellow liquid, few particulates may be present

Solution pH 5.5 to 6.5

10.1.1. Supplier/How Supplied

Nivolumab Injection, 100 mg/10 mL (10 mg/mL) vials. There are five nivolumab 100-mg vials per carton.

BMS will supply nivolumab as investigational supply at no charge to subjects participating in this clinical trial.

The site investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution, and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

10.1.2. Preparation

Visually inspect drug product solution for particulate matter and discoloration prior to administration. Nivolumab is a clear to opalescent, colorless to pale-yellow solution. Discard the vial if the solution is cloudy, discolored, or contains extraneous particulate matter other than a few translucent-to-white, proteinaceous particles. Do not shake the vial.

- Withdraw the required volume of nivolumab and transfer into an intravenous container.
- Dilute nivolumab with either 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to prepare an infusion with a final concentration ranging from 1 mg/mL to 10 mg/mL.
- Mix diluted solution by gentle inversion. Do not shake.
- Discard partially used vials or empty vials of nivolumab.
- The maximum allowable volume of final infusion is 120 mL.

10.1.3. Storage and Stability

The product does not contain a preservative.

After preparation, store the nivolumab infusion either:

- At room temperature for no more than 8 hours from the time of preparation. This includes room temperature storage of the infusion in the IV container and time for administration of the infusion or
- Under refrigeration at 2°C to 8°C (36°F to 46°F) for no more than 24 hours from the time of infusion preparation.

Do not freeze.

10.1.4. Administration

Administer the infusion over 30 minutes (acceptable window is 25 minutes to 40 minutes) through an intravenous line containing a sterile, non-pyrogenic, low protein binding in-line filter (pore size of 0.2 micrometer to 1.2 micrometer).

Do not coadminister other drugs through the same intravenous line. Flush the intravenous line at end of infusion.

10.1.5. Handling and Disposal

Preparation should be performed by trained personnel in accordance with good practices rules, especially with respect to asepsis.

10.1.6. Accountability

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

10.1.7. Dispensing

Nivolumab must be dispensed only from official study sites and to eligible subjects under the supervision of the site investigator. Nivolumab should be stored in a secure area according to local regulations. It is the responsibility of the site investigator to ensure that study drug is only dispensed to subjects.

10.1.8. Adverse Events

The most common side effects of nivolumab are:

- Fatigue
- Skin reactions: including rash, itching, hives, redness, and dry skin.
- Diarrhea
- Nausea
- Abdominal pain
- Decreased appetite
- Low red blood cells
- Fever
- Joint pain or stiffness

Less common side effects of nivolumab include:

- Bowel inflammation
- Liver function blood test abnormalities
- Loss of color (pigment) from areas of skin
- Dry mouth
- Vomiting
- Weight loss

- Thyroid gland abnormalities
- Blood chemistry abnormalities, including low blood phosphate, magnesium, and potassium levels.
- High blood uric acid level
- Lung inflammation (pneumonitis - see details below)
- Cough
- Dizziness
- Headache
- Low white blood cells
- Chills
- Muscle soreness, weakness, stiffness spasms or paralysis
- Pain in arms or legs
- Tingling, burning, or numbness in hands and feet
- Shortness of breath
- Abnormal taste
- Flushing
- High or low blood pressure
- Allergic reaction during or between study drug infusions
- Increased sensitivity of skin to sunlight
- Constipation
- Difficulty swallowing
- Heartburn
- Low blood platelets (may increase risk of bleeding)

Rare but potentially serious side effects of nivolumab include:

- Low blood oxygen level
- Acute lung injury or failure
- Collection of fluid around the lungs
- Inflammation of the appendix
- Increase in inflammatory blood proteins (e.g., lipase)
- Adrenal gland abnormalities
- Pituitary gland inflammation
- Changes in vision (including decreased or blurry vision), inflammation of the eye, or bleeding into the eye
- Liver inflammation
- Acute kidney injury or failure
- Abnormal blood cell production
- Inflammation of the mouth and lining of the digestive tract
- Swelling of the face, arms, or legs
- Inflammation of the pancreas
- Back pain
- Autoimmune disorders, including Guillain-Barre syndrome (associated with progressive muscle weakness or paralysis)
- Chest discomfort

- Heart palpitations
- Inflammation of the heart or its lining
- Collection of fluid around the heart
- Increased blood sugar
- Dehydration
- Infections: including sepsis, lung infections, and skin infections.
- Decreased movement of the intestines
- Disorientation
- Swelling of the optic disc
- Inflammation of the optic nerve
- Inflammation or loss of the lining of the brain and spinal cord
- Drug reaction with rash, blood cell abnormalities, enlarged lymph nodes, and internal organ involvement (including liver, kidney, and lung); known as Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS)
- Myasthenia gravis, a nerve disease that may cause weakness of eye, face, breathing, and swallowing muscles.
- Abnormal brain function due to brain inflammation.
- Rhabdomyolysis (muscle fiber released into the blood stream which could damage your kidney) and polymyositis (chronic muscle inflammation with muscle weakness) has been reported in one subject.
- Lung inflammation or pneumonitis

11. ADVERSE EVENTS

The descriptions and grading scales found in the NCI CTCAE version 5.0 will be utilized for AE assessment. A copy of the CTCAE v5 can be downloaded from the CTEP website at http://ctep.cancer.gov. All forms for AE/SAE recording and reporting can be found in OnCore (Documents and Information Tab).

11.1. Definitions

11.1.1. Adverse Event (AE)

An AE is any untoward medical occurrence whether or not considered related to the study drug that appears to change in intensity during the course of the study. The following are examples of AEs:

- Unintended or unfavorable sign or symptom
- A disease temporally associated with participation in the protocol
- An intercurrent illness or injury that impairs the well-being of the subject

Abnormal laboratory values or diagnostic test results constitute AEs only if they induce clinical signs or symptoms or require treatment or further diagnostic tests

Hospitalization for elective surgery or routine clinical procedures that are not the result of an AE (e.g., surgical insertion of central line) should not be recorded as an AE.
Disease progression should not be recorded as an AE, unless it is attributable to the study regimen by the site investigator.

11.1.2. Serious Adverse Event (SAE)

A SAE is an adverse event that:

- Results in death. NOTE: Death due to disease progression should not be reported as a SAE, unless it is attributable by the site investigator to the study drug(s)
- Is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization for >24 hours or prolongation of existing hospitalization. **NOTE:** Hospitalization for anticipated or protocol specified procedures such as administration of chemotherapy, central line insertion, metastasis interventional therapy, resection of primary tumor, or elective surgery, will not be considered serious adverse events.
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above). Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions not resulting in hospitalization; or the development of drug dependency or drug abuse.

11.1.3. Unexpected Adverse Event

For this study, an AE is considered unexpected when it varies in nature, intensity or frequency from information provided in the current IB, prescribing information or when it is not included in the informed consent document as a potential risk. Unexpected also refers to AEs that are mentioned in the IB as occurring with a class of drugs or are anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

11.1.4. Relatedness

AEs will be categorized according to the likelihood that they are related to the study drug(s). Specifically, they will be categorized using the following terms:

Unrelated	Adverse Event is ***not related*** to the study drug(s)
Unlikely	Adverse Event is ***doubtfully related*** to the study drug(s)

Possible	Adverse Event ***may be related*** to the study drug(s)
Probable	Adverse Event is ***likely related*** to the study drug(s)
Definite	Adverse Event is ***clearly related*** to the study drug(s)

11.1.5. Pregnancy

Any pregnancy that occurs in a female partner of a male study participant should be reported within 24 hours of knowledge of the event to BMS WorldWide Safety using either the MedWatch form. Information on this pregnancy will be collected on the Pregnancy Surveillance Form. In order for Sponsor or designee to collect any pregnancy surveillance information from the female partner, the female partner must sign an informed consent form for disclosure of this information. Details on the pregnancy will be collected for safety analysis throughout the pregnancy and for one year following the birth of the infant. After obtaining the subject's consent (or subject and pregnant partner's consent in the case of a male participant), monitoring of the pregnancy and infant should comply the following procedures:

- If the outcome is an abnormal neonate (infant), as much follow up information as possible to permit evaluation of the case will be collected, including where possible, medical confirmation, medical investigations and medical record summary details. Follow up should continue for one year post birth.
- If the outcome is a normal neonate, follow up data should continue for one month

11.1.6. Overdose

An overdose is defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. All occurrences of overdose must be reported as a SAE.

11.2. Reporting

Each participating investigator is required to abide by the reporting requirements set by the DF/HCC. The study must be conducted in compliance with FDA regulations, local safety reporting requirements, and reporting requirements of the principal investigator.

Each investigative site will be responsible to report SAEs that occur at that institution to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

11.2.1. Adverse Events

- AEs will be recorded from time of signed informed consent until 100 days after discontinuation of study drug(s).
- AEs will be recorded regardless of whether or not they are considered related to the study drug(s).
- All AEs will be recorded in the subject's medical record and on the appropriate study specific eCRF form within OnCore.
- AEs considered related to study drug(s) will be followed until resolution to Grade ≤ 1 or baseline, deemed clinically insignificant, and/or until a new anti-cancer treatment starts, whichever occurs first.

11.2.2. Serious Adverse Events (SAEs)

11.2.2.1. Site Requirements for Reporting SAEs

All serious adverse events that occur after the subject's written consent to participate in the study, during treatment, or within 100 days of the last dose of treatment must be reported to the DF/HCC Overall Principal Investigator and BMS WorldWide Safety on the local institutional SAE form, whether or not related to nivolumab treatment and including those thought to be associated with protocol-specified procedures.

Note: If the participant is in long term follow up, report the death at the time of continuing review.

Participating investigators must report each serious adverse event to the DF/HCC Overall Principal Investigator as well as the Bristol-Myers Squibb Safety Department within 24 hours of learning of the occurrence. In the event that the participating investigator does not become aware of the serious adverse event immediately (e.g., participant sought treatment elsewhere), the participating investigator is to report the event within 24 hours after learning of it and document the time of his or her first awareness of the adverse event. The local institutional SAE form or Medwatch SAE form (https://www.accessdata.fda.gov/scripts/medwatch/index.cfm?action=reporting.home) should be completed for any event where doubt exists regarding its status of seriousness. Note: Please include the BMS Protocol number on the SAE form or on the cover sheet with the SAE form transmission

Report serious adverse events by email or facsimile to both:

DF/HCC Overall Principal Investigator and Medical Monitor:
David J. Einstein, MD
Phone 617-667-2100
deinstei@bidmc.harvard.edu
Fax 617-735-2060

AND

Bristol-Myers Squibb:
SAE Email Address: Worldwide.Safety@BMS.com
SAE Facsimile Number: 609-818-3804

Within the following 24-48 hours, the participating investigator must provide follow-up information on the serious adverse event. Follow-up information should describe whether the event has resolved or continues, if and how the event was treated, and whether the participant will continue or discontinue study participation.

Participating investigators will report to their local Risk Management office any participant safety reports, sentinel events or unanticipated problems that require reporting per institutional policy.

11.2.3. Monitoring of Adverse Events and Period of Observation

All adverse events, both serious and non-serious, and deaths that are encountered from initiation of study intervention, throughout the study, and within 100 days of the last study intervention should be followed to their resolution, or until the participating investigator assesses them as stable, or the participating investigator determines the event to be irreversible, or the participant is lost to follow-up. The presence and resolution of AEs and SAEs (with dates) should be documented on the appropriate case report form and recorded in the participant's medical record to facilitate source data verification.

For some SAEs, the Principal Investigator or designee may follow-up by telephone, fax, and/or monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. Participating investigators should notify the DF/HCC Overall Principal Investigator of greater or equal to grade 3 events and the IRB of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

At the end of the study period, Bristol-Myers Squibb Company will not continue to supply study drug to subjects/investigators. The investigator is responsible to ensure that the subject receives appropriate standard of care or other appropriate treatment in the independent medical judgement of the Investigator to treat the condition under study.

11.2.4. Reporting to the Food and Drug Administration (FDA)

The Overall PI, as study sponsor, will be responsible for all communications with the FDA. The Overall PI will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

12. STATISTICAL METHODS

12.1. Power Calculation and Sample Size Justification

We plan to enroll 34 patients into the study (with 20 in the PD-L1-positive cohort and 14 in the PD-L1-negative cohort). We hypothesize that patients with positive PD-L1 status will respond better to therapy, but we do not have enough power for formal comparisons between patients with and without positive PD-L1 status.

In 20 patients with PD-L1-positive status, if 2 or more patients achieved disease control at 12 weeks, we would consider the study a success. With 20 patients, there is 97% power to detect a 25% disease control rate (from null of 1%) with one-sided alpha of 0.02 using a one-sample binomial test. A null hypothesis of 1% was chosen since we do not expect any patients to achieve disease control without receiving any intervention.

With 14 patients having PD-L1-negative status, the probably of seeing 0 response is 4.4% if the true rate of disease control is 0.2. There is 96% power to detect a 20% disease control rate vs. null of 1% DCR at one-sided alpha=0.13 (target alpha 0.15, target power 0.95). We would like to be able to see at least 1 patient achieving disease control at 12 weeks in order to consider the regimen promising for future testing in the PD-L1-negative population. The 96% power (a small false negative rate) while allowing larger alpha is to ensure we do not miss any potential drug activity in patients with PD-L1-negative status.

12.2. Assessment of Efficacy

12.2.1. Primary analysis

Patients with disease control at 12 weeks will be summarized as number and proportion with 90% CI for the 20 patients with PD-L1 positive status and 14 patients with PD-L1 negative status separately.

Patients that are considered un-evaluable for the primary endpoint by never starting treatment will be replaced.

12.2.2. Secondary analysis and analysis of safety

Maximum PSA change and best PSA response will be summarized descriptively and graphically in waterfall plots. PSA doubling time will be calculated with linear regression model of natural logarithm of PSA and time. PSADT at baseline and

EOS will be summarized, and change in PSADT, as captured by the change in PSA slope, will be summarized descriptively as well. Time to event endpoints will be summarized using Kaplan Meier estimate.

All reported toxicities, regardless of attribution, will be summarized by toxicity type and maximum grade. The maximum grade consolidates the reports of a given toxicity for a patient over time by taking the maximum across time. Immune modulatory medication used for toxicities will also be summarized descriptively.

12.3. Exploratory Analyses

The exploratory analyses will proceed with all 34 enrolled patients combined if responses are observed. The markers of interest are described in Section 8.1. Some objectives will explore the relation among markers, and some will explore markers (e.g., PD-L1 expression, CD8+ TILs, change in plasma cytokines or PBMC subsets from pre-treatment to post-1 cycle) in relation to with disease control on nivolumab. With the given sample size and low expected disease control rate, all results will be considered as preliminary. Analyses will include descriptive and graphical summaries, using scatterplots and estimating correlation coefficients among continuous markers (including the % change in PSA at 12 weeks as continuous value), and using boxplots of marker levels according to the disease control endpoint. Hypothesis testing may be undertaken if there are several patients who experience disease control (more than say 4 or 5) of the hypothesis that greater expression of PD-L1 and/or CD8+ TILs is associated with higher probability of disease control, using Wilcoxon rank sum tests if the marker has a continuous distribution, or Fisher's exact test if an established cutpoint of the distribution exists.

13. TRIAL MANAGEMENT

13.1. Data Reporting

13.1.1. Method

The Office of Data Quality (ODQ) will collect, manage, and monitor data for this study.

13.1.2. Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the Office of Data Quality (ODQ) in accordance with DF/HCC policies.

The schedule for completion and submission of case report forms (paper or electronic) is as follows:

Form	Submission Timeline
Eligibility Checklist	Complete prior to registration in OnCore
On Study Form	Within 14 days of registration
Baseline Assessment Form	Within 14 days of registration
Treatment Form	Within 10 days of the last day of the cycle
Adverse Event Report Form	Within 10 days of the last day of the cycle
Response Assessment Form	Within 10 days of the completion of the cycle required for response evaluation
Follow up/Survival Form	Within 14 days of the protocol defined follow up visit date or call

When a participant is removed from protocol therapy and/or is off of the study, the relevant Off-Treatment/Off-Study information will be updated in OnCore.

13.2. Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of medical oncologists, research nurses, pharmacists and biostatisticians with direct experience in cancer clinical research. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

The DSMC will review each protocol up to four times a year with the frequency determined by the outcome of previous reviews.. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 100 days of intervention; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

13.3. Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the sponsor-investigator of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. All results of primary and secondary objectives must be posted to CT.gov within a year of completion. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and study site contact information.

14. ETHICS

14.1. Ethical Conduct of the Study

The study will be performed in accordance with ethical principles originating from the Declaration of Helsinki. Conduct of the study will be in compliance with ICH Good Clinical Practice, and with all applicable federal (including 21 CFR parts 56 & 50), state, or local laws, as well as DF/HCC research policies and procedures (http://www.dfhcc.harvard.edu/clinical-research-support/clinical-research-unit-cru/policies-and-procedures/).

14.2. Informed Consent Process

The site investigator will ensure the subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study. Subjects must also be notified they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.

The subject's signed and dated informed consent must be obtained before conducting any procedure specifically for the study. The site investigator must store the original, signed informed consent form. A copy of the signed informed consent form must be given to the subject.

14.3. Human Subject Protections

14.3.1. Rationale for Subject Selection

14.3.1.1. Selection Based on Gender, Ethnicity, and Race

Subjects from all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one group compared with another. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on one hand and the need to explore ethnic aspects of clinical research on the other hand. If differences in outcome that correlate with ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully. Women are not eligible for this study as this diease occurs only in men.

14.3.1.2. Strategies/Procedures for Recruitment

Patient accrual for this protocol will consist of outreach to medical oncologists caring for patients at DFHCC. We will also reach out to the Boston Prostate Cancer Support Group with information for interested patients. This protocol will be listed on www.clinicaltrials.gov.

14.3.1.3. Justification for Exclusions

Due to impaired cellular immunity, this protocol excludes patients with chronic viral infections including HIV, HBV, and HCV.

14.3.1.4. Participation of Children

Men under the age of 18 will not be eligible for participation in this study based on the fact that patients under 18 are unlikely to have PCa and the toxicities of nivolumab in pediatric patients is unknown.

14.3.2. Evaluation of Benefits/Risks/Discomforts

There is no firmly established standard-of-care therapy for patients with PCa and rising PSA following local definitive therapy. Potential risks of nivolumab and in this patient population include the range of side effects outlined in section 10.1.8.

14.3.2.1. Procedures to Eliminate or Minimize Potential Risks

All patients will be given blood tests, physical examinations, and scans, as described in the study calendar (section 7), and must have a local physician to provide long-term care and monitoring for complications. No compensation is available, but any injury will be evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations.

14.3.2.2. Provisions for Monitoring Data Collection to Ensure Subject Safety

As information is gathered from this trial, clinical results will be shared with patients. Laboratory and clinical data will be frequently gathered and any new significant finding(s) found during the course of the research, which may affect a patient's willingness to participate further, will be explained.

Confidentiality of information concerning participants will be maintained, including in all publications and presentations resulting from this study. Names of participants or material identifying participants will not be released without permission, except as such release is required by law. Records at the National Cancer Institute are maintained according to current legal requirements, and are made available for review, as required by the Food and Drug Administration or other authorized users, only under the guidelines established by the Federal Privacy Act.

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16. **APPENDICES**

Appendix 1: Management of immune-related Adverse Events

Appendix 2: Information sheet for shipment of preliminary PD-L1-positive cases

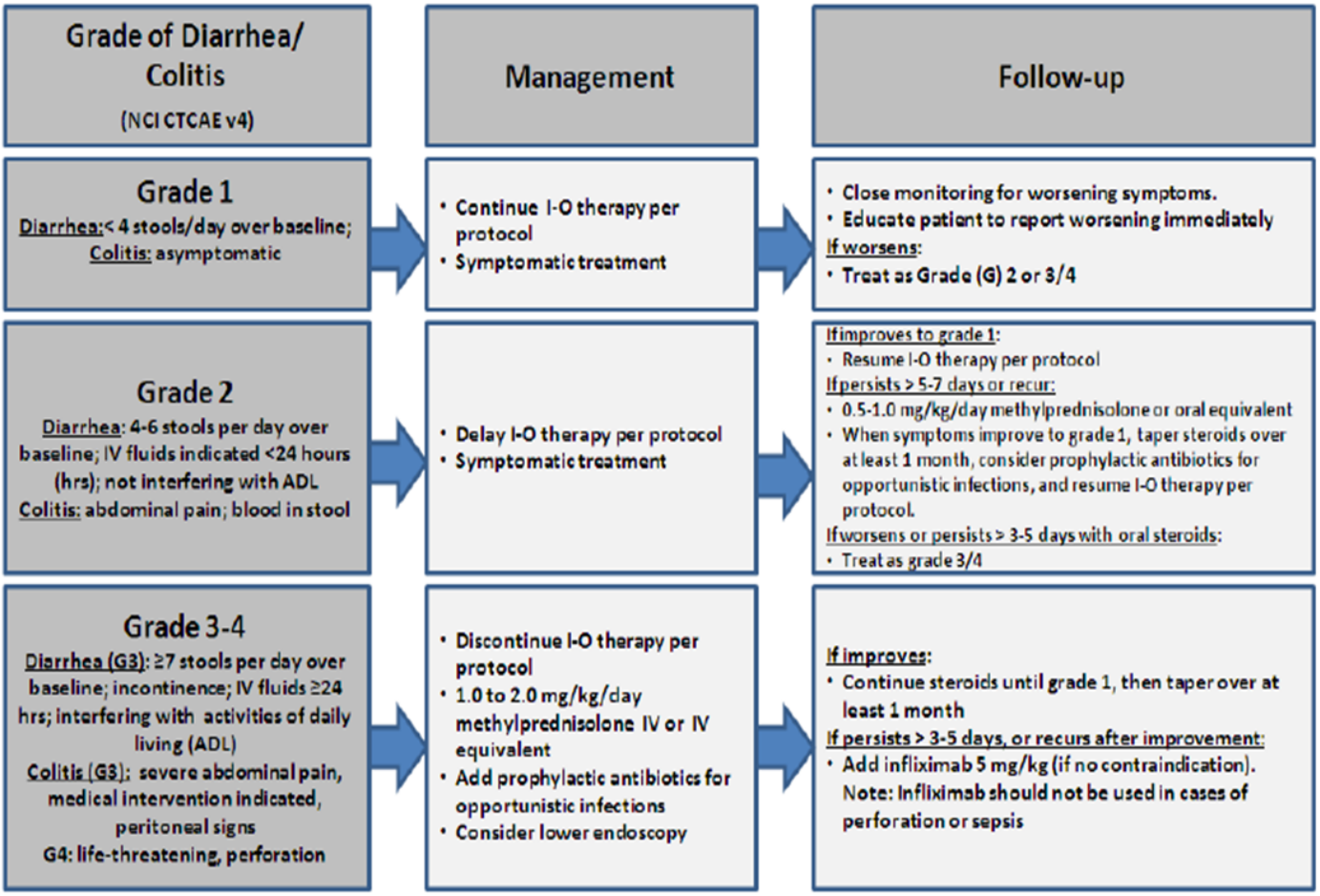
Appendix 3: Instructions for peripheral blood collection for exploratory studies

Appendix 1: Management of Immune-Related Adverse Events

Immune-related adverse reaction	Severity	Treatment modification
Immune-related pneumonitis	Grade 2 pneumonitis	Withhold dose(s) until symptoms resolve, radiographic abnormalities improve, and management with corticosteroids is complete
	Grade 3 or 4 pneumonitis	Permanently discontinue treatment
Immune-related colitis	Grade 2 diarrhoea or colitis	Withhold dose(s) until symptoms resolve and management with corticosteroids, if needed, is complete
	Grade 3 diarrhoea or colitis	Withhold dose(s) until symptoms resolve and management with corticosteroids is complete
	Grade 4 diarrhoea or colitis	Permanently discontinue treatment
Immune-related hepatitis	Grade 2 elevation in aspartate aminotransferase (AST), alanine aminotransferase (ALT), or total bilirubin	Withhold dose(s) until laboratory values return to baseline and management with corticosteroids, if needed, is complete
	Grade 3 or 4 elevation in AST, ALT, or total bilirubin	Permanently discontinue treatment
Immune-related nephritis and renal dysfunction	Grade 2 or 3 creatinine elevation	Withhold dose(s) until creatinine returns to baseline and management with corticosteroids is complete
	Grade 4 creatinine elevation	Permanently discontinue treatment
Immune-related endocrinopathies	Symptomatic Grade 2 or 3 hypothyroidism, hyperthyroidism, hypophysitis, Grade 2 adrenal insufficiency Grade 3 diabetes	Withhold dose(s) until symptoms resolve and management with corticosteroids (if needed for symptoms of acute inflammation) is complete. Treatment should be continued in the presence of hormone replacement therapy[a] as long as no symptoms are present
	Grade 4 hypothyroidism Grade 4 hyperthyroidism Grade 4 hypophysitis Grade 3 or 4 adrenal insufficiency Grade 4 diabetes	Permanently discontinue treatment
Immune-related skin adverse reactions	Grade 3 rash	Withhold dose(s) until symptoms resolve and management with corticosteroids is complete
	Grade 4 rash	Permanently discontinue treatment
	Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN)	Permanently discontinue treatment
Other immune-related adverse reactions	Grade 3 (first occurrence)	Withhold dose(s)
	Grade 3 myocarditis	Permanently discontinue treatment
	Grade 4 or recurrent Grade 3 ; persistent Grade 2 or 3 despite treatment modification ; inability to reduce	Permanently discontinue treatment

GI Adverse Event Management Algorithm

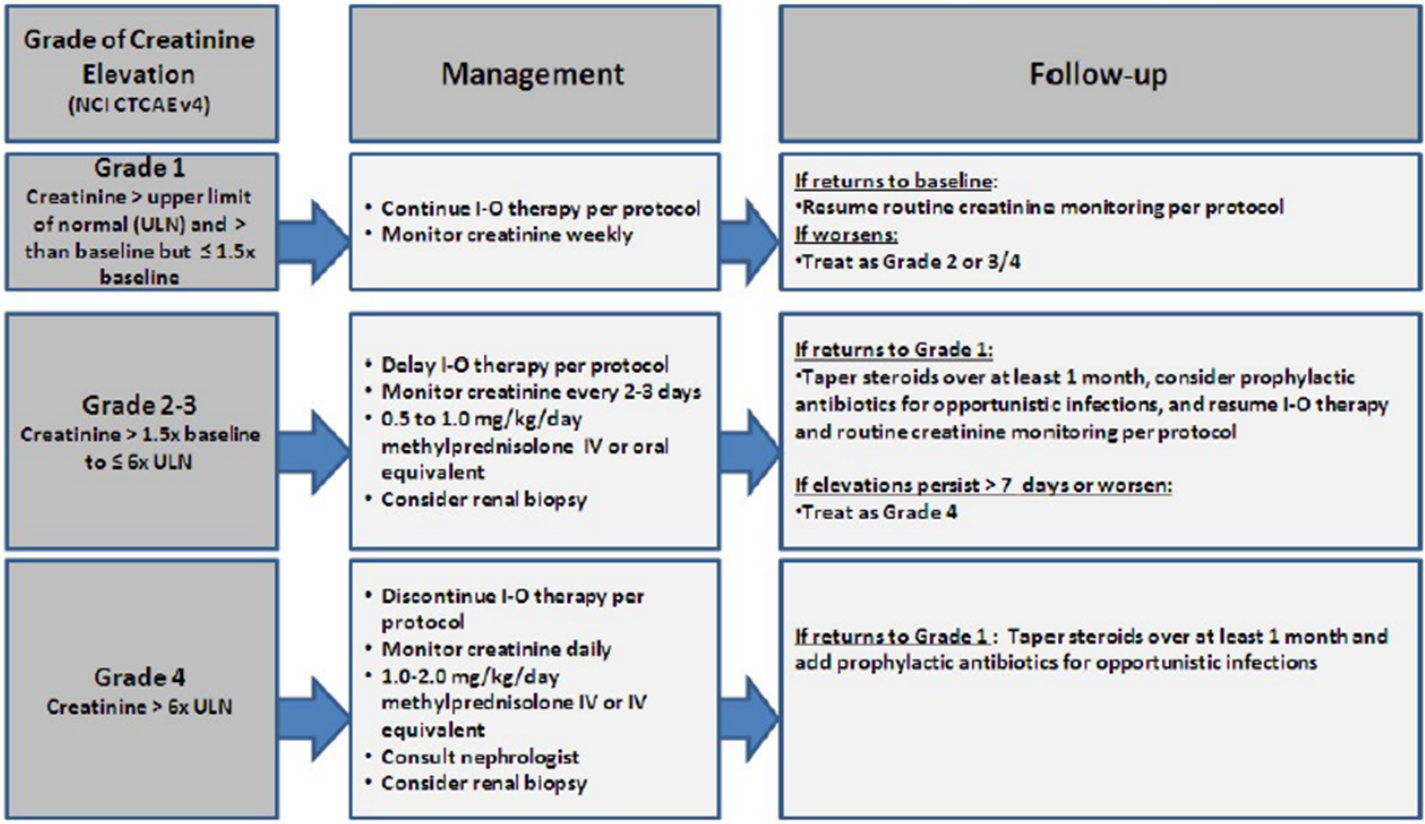
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.



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

Renal Adverse Event Management Algorithm

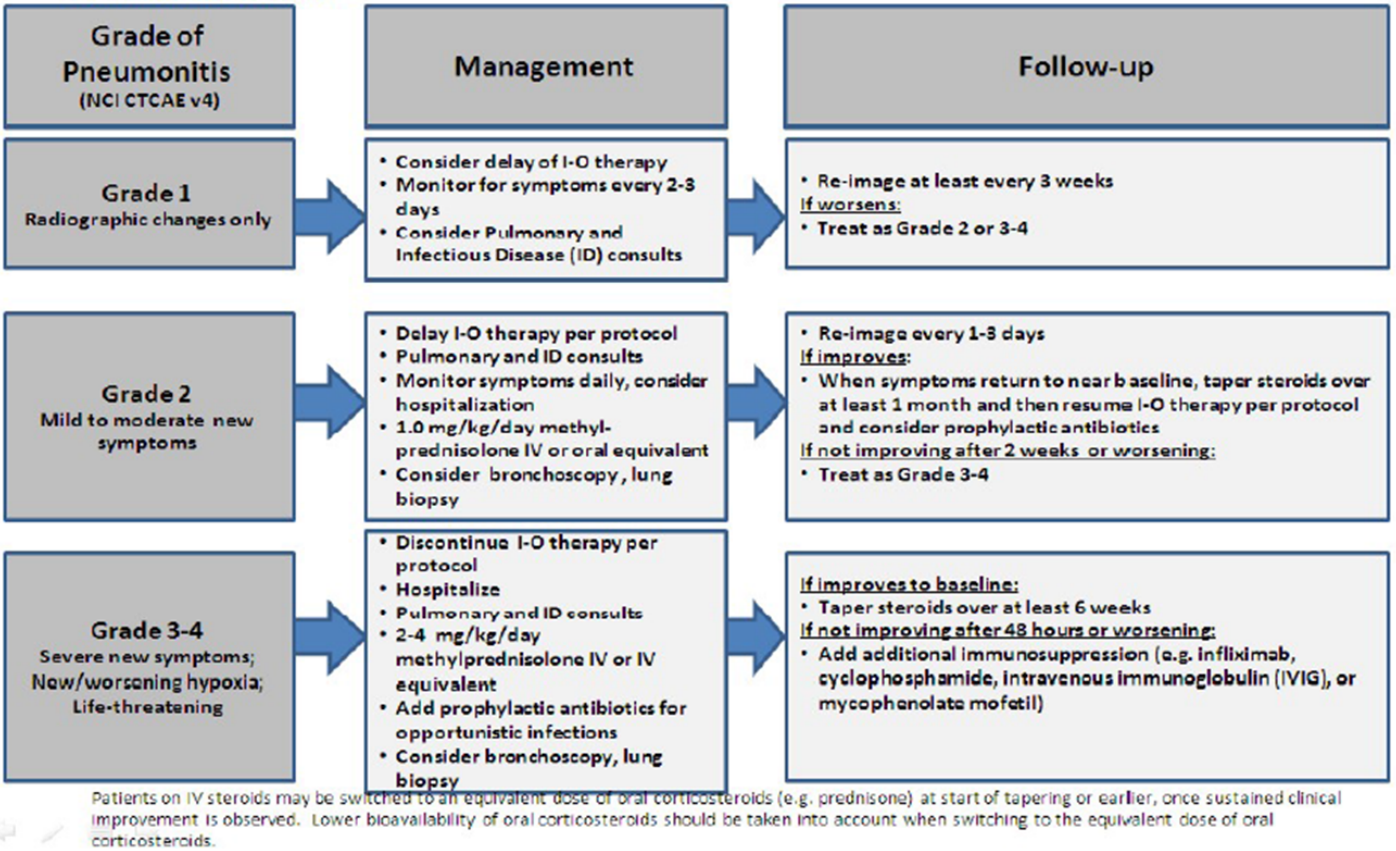
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy



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

Pulmonary Adverse Event Management Algorithm

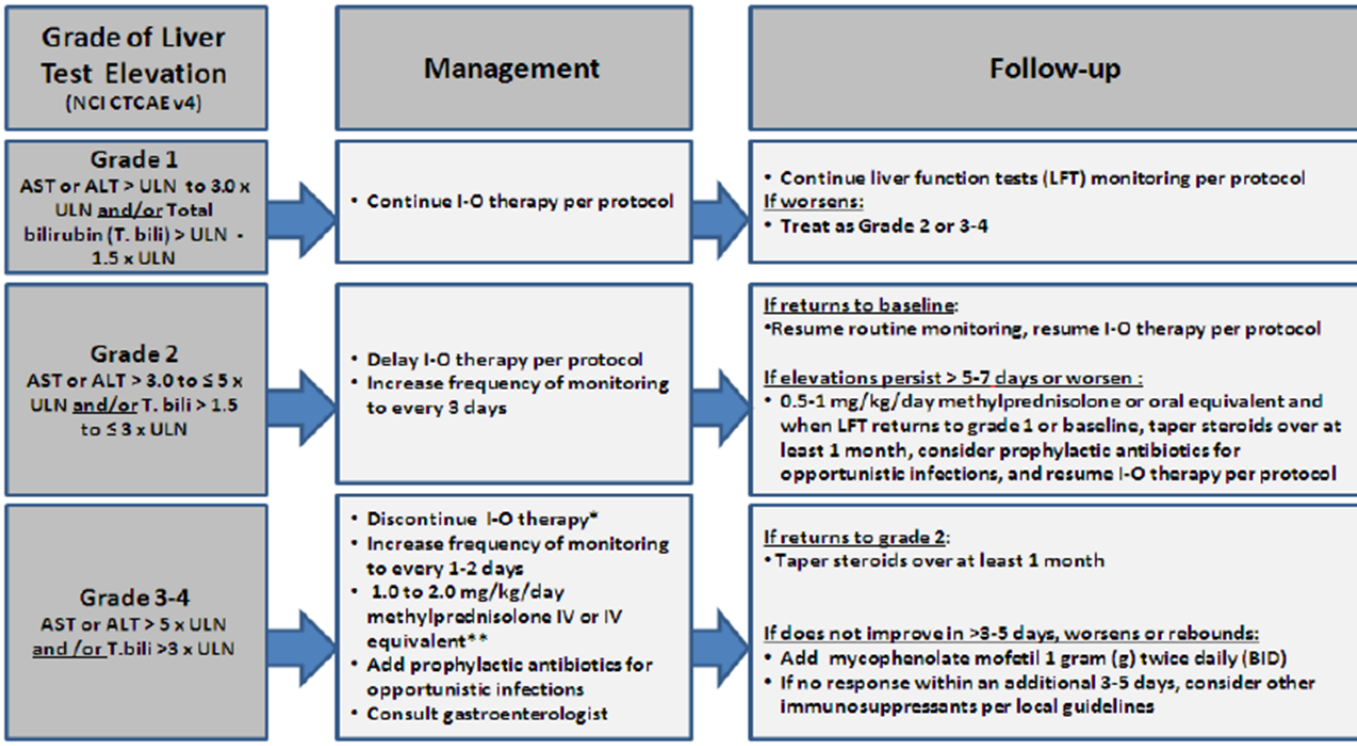
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.



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

Hepatic Adverse Event Management Algorithm

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



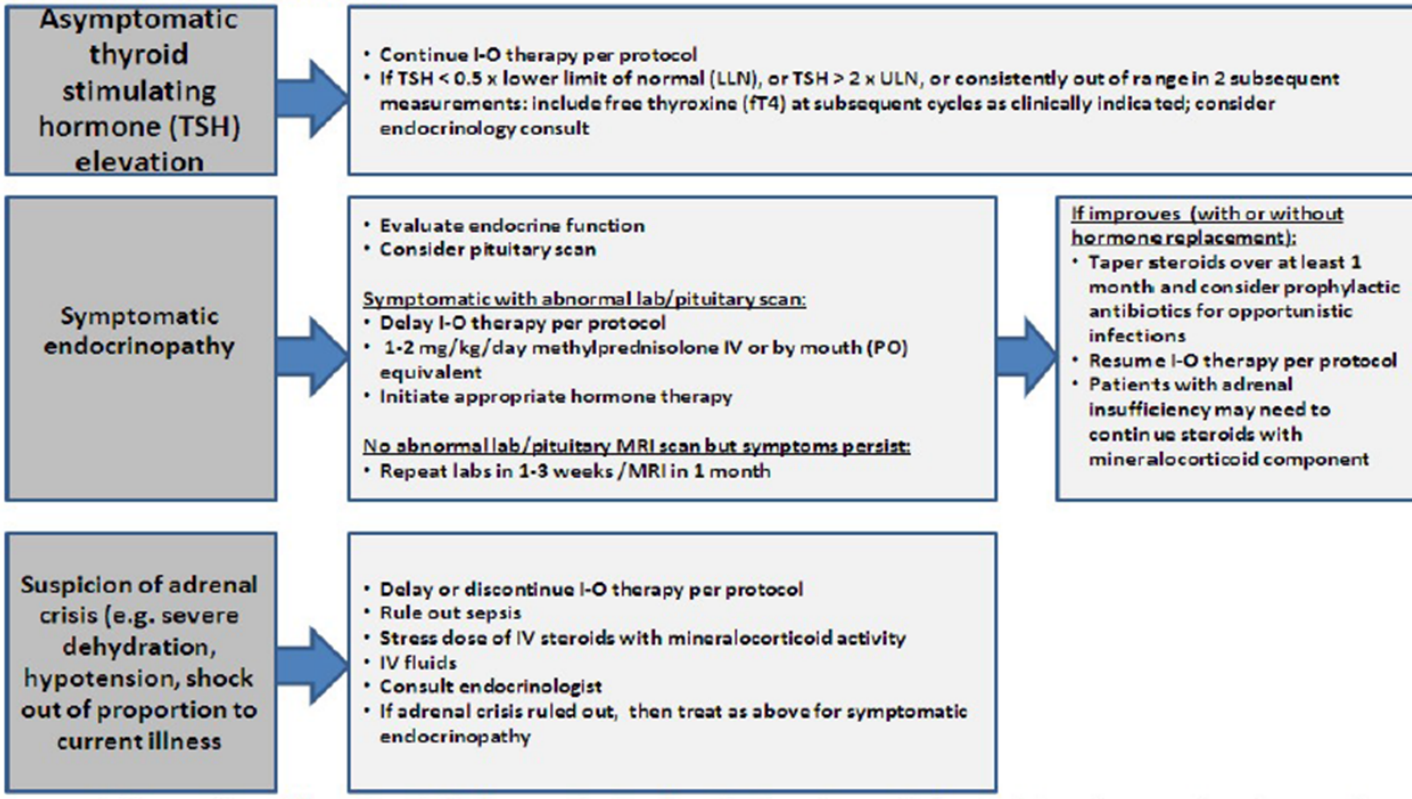
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

*I-O therapy may be delayed rather than discontinued if AST/ALT ≤ 8 x ULN and T.bili ≤ 5 x ULN.

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

Endocrinopathy Management Algorithm

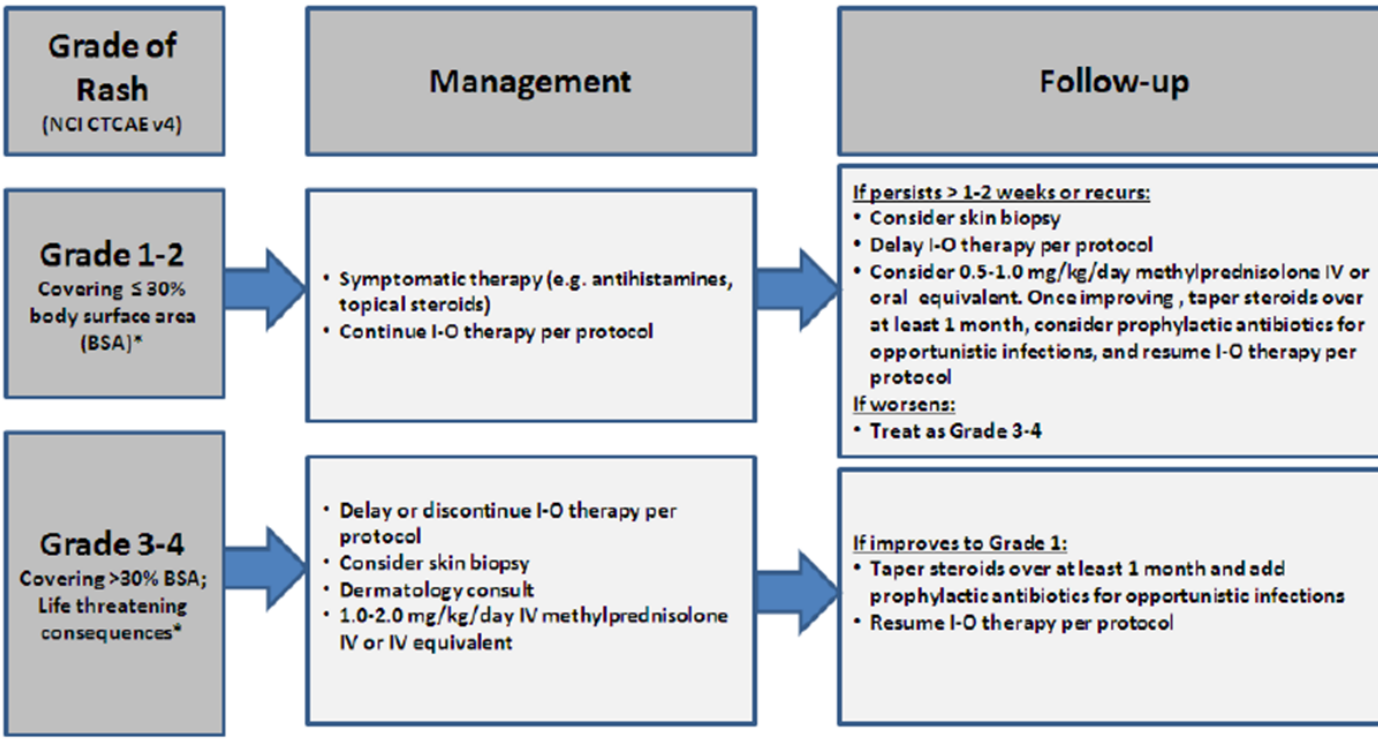
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider visual field testing, endocrinology consultation, and imaging.



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

Skin Adverse Event Management Algorithm

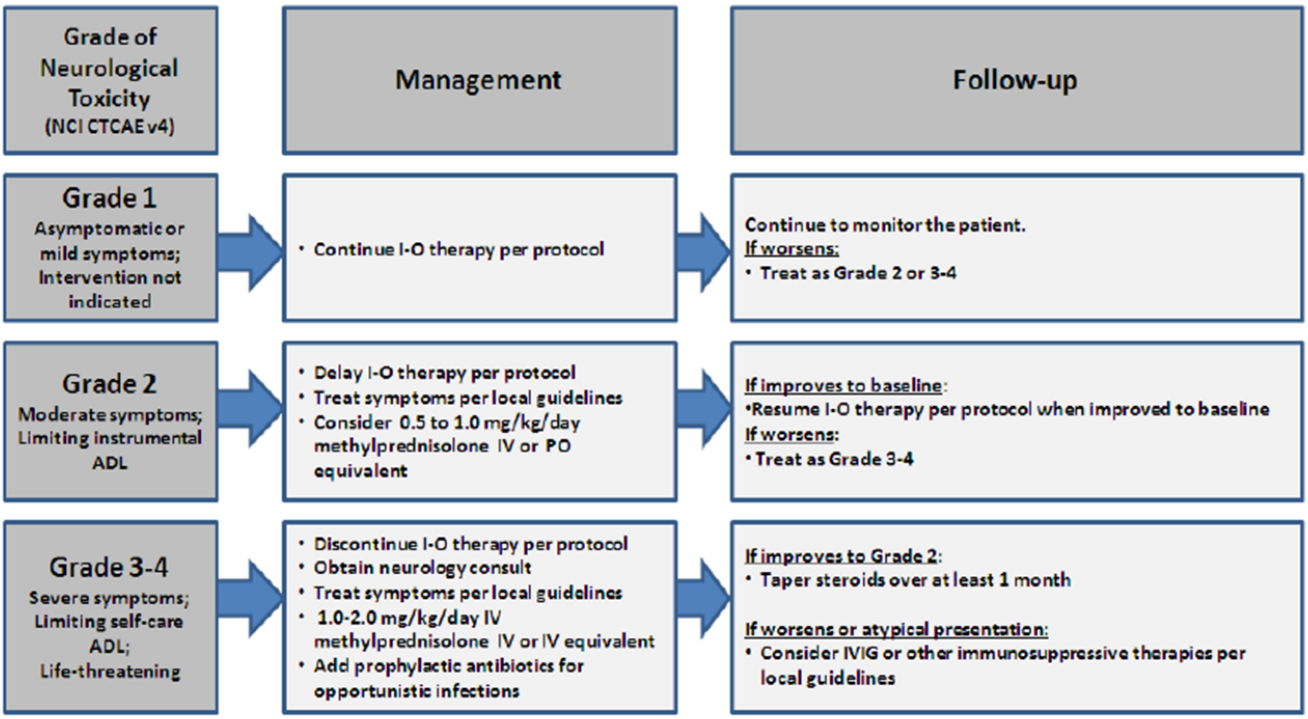
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
*Refer to NCI CTCAE v4 for term-specific grading criteria.

Neurological Adverse Event Management Algorithm

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



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

Appendix 2: Information sheet for shipment of preliminary PD-L1-positive cases

ACUPATH LABORATORIES, INC
RESEARCH PRJOECTS
28 S TERMINAL DR
PLAINVIEW NY 11803

RESEARCH FACILTY CONTACT
Dr. Carla Calagua
Slosberg Building SL142
Department of Pathology,
Beth Israel Deaconess Medical Center
330 Brookline Ave, Boston MA 02215
Phone: 617-667-7834

SPECIMEN TYPE_____ LOCATION_____
DATE OBTAINED_____ UNBAKED___ BAKED_____
STAIN REQUIREMENTS_____
SEND BACK TO RESEARCH FACILTY_____ READ AT ACUPATH_____
ANY OTHER REQUIREMENTS:

RESEARCH ID INFORMATION _____

COMMENTS:

ACUPATH ACCESSION NUMBER_____

Appendix 3: Instructions for peripheral blood collection for exploratory studies

As outlined in the study calendar (section 7), peripheral blood collections will be mandatory for subjects participating in this study.

- Cycle 1 day 1:
    - 5-10 mL in one 10-mL EDTA (purple top) tube at room temperature for germline WES
        - Email Olga Voznesensky (ovoznese@bidmc.harvard.edu) in advance to notify Balk lab of collection
        - For DFCI patients, email BIDMC CRAs (GUoncologytrials@caregroup.harvard.edu) one week in advance to arrange collection
    - 30 mL in three-to-four 10-mL EDTA tubes at room temperature for soluble biomarkers and immunophenotyping
        - Email Mariano Severgnini (Mariano_Severgnini@DFCI.HARVARD.EDU) one week to notify Immune Assessment lab of collection
        - For BIDMC patients, blood will need to be brought to the Immune Assessment Lab by a BI CRA
        - For DFCI patients, blood can be sent via tube system
- Cycle 2 day 1 and End-of-Treatment visit, plus optional collection at 1 week after cycle 1:
    - 30 mL in three-to-four 10-mL EDTA tubes at room temperature for soluble biomarkers and immunophenotyping
        - Email Mariano Severgnini (Mariano_Severgnini@DFCI.HARVARD.EDU) one week to notify Immune Assessment lab of collection
        - For BIDMC patients, blood will need to be brought to the Immune Assessment Lab by a BI CRA
        - For DFCI patients, blood can be sent via tube system
- Cycle 5 day 1 and q4 cycles beyond (cycle 9 day 1, cycle 13 day 1, etc.):
    - 80 mL TOTAL in eight-to-eleven 10-mL EDTA tubes at room temperature, divided as follows:
        - 50 mL in five-to-seven 10-mL EDTA tubes at room temperature for PBMC banking for assessment of T-cell responsiveness to neoantigens
            - Email Dina Stroopinsky (dstroopi@bidmc.harvard.edu) in advance to notify Avigan/Rosenblatt lab of collection
            - For DFCI patients, email BIDMC CRAs (GUoncologytrials@caregroup.harvard.edu) one week in advance to arrange collection
        - 30 mL in three-to-four 10-mL EDTA tubes at room temperature for soluble biomarkers and immunophenotyping PLUS PMBC banking for assessment of T-cell responsiveness to neoantigens
            - Email Mariano Severgnini (Mariano_Severgnini@DFCI.HARVARD.EDU) one week to notify Immune Assessment lab of collection

- For BIDMC patients, blood will need to be brought to the Immune Assessment Lab by a BI CRA
- For DFCI patients, blood can be sent via tube system