Nivolumab as a Non-Castrating Therapy for MMR-deficient and CDK12-Altered Prostate Cancer with PSA Recurrence After Local Therapy

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INVESTIGATOR'S APPROVAL OF PROTOCOL

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SYNOPSIS

Title	Nivolumab as a Non-Castrating Therapy for MMR-deficient and CDK12-altered Prostate Cancer with PSA Recurrence After Local Therapy
Lead site/Sponsor	Johns Hopkins University
IND holder	Mark C. Markowski, MD, Ph.D
Investigational agent	Nivolumab 480mg IV every 4 weeks
Phase	2

Target population	• Adult male ≥18 years of age
	• Histologic or cytologic diagnosis of prostate cancer with an adenocarcinoma component.
	• Prior local therapy with prostatectomy or radiation therapy required.
	• Post-prostatectomy salvage or adjuvant radiation therapy is allowed but not mandated. Radiation therapy must have been completed for at least 3 months.
	• No radiographic evidence of metastatic disease by CT scan and bone scan, performed within the prior 4 weeks.
	• Must have at least one of the following genetic alterations identified using archival tissue (i.e. prostate needle biopsy prior to radiation therapy or prostatectomy specimen):
	  ○ Microsatellite instability (MSI-high) status by clinical grade testing.
	  ○ MMR protein loss (MSH2, MSH6, MLH1, PMS2) by immunohistochemistry.
	  ○ Inactivating mutation of *MSH2, MSH6, MLH1 or PSM2* by clinical grade genomic testing.
	  ○ Tumor mutational burden ≥20 mutations/ megabase (TMB ≥20 muts/Mb) by clinical grade testing.
	  ○ Inactivating mutation (at least monoallelic) of *CDK12* by clinical grade testing
	• Serum PSA at screening ≥ 1.0 ng/mL
	• Serum testosterone level: ≥ 150 ng/dL at time of screening
	• ECOG performance status ≤ 2
	• Adequate bone marrow, renal and liver function (ANC > 1.0K, Plt > 100K, Hgb > 9 g/dL; Cr < 1.5 mg/dL; AST/ALT WNL; Total Bilirubin WNL).

Study centers	2 sites in the United States, within the the PCCTC network (pcctc.org)

Start date/Duration	First patients are expected to be enrolled in Q3 2019. Accrual is estimated to last approximately 12 months with up to 6 months of follow-up after the last patient has been entered.
Expected enrollment	15 patients
Rationale	Disease Background: MMR-deficient cancers of any histologic type appear to be very sensitive to PD-1 blockade with pembrolizumab, and similar data are also beginning to emerge for nivolumab and other immune checkpoint inhibitors. Among the MMR-deficient cancers, the best antitumor responses are often associated with high microsatellite instability (MSI-H status), higher tumor mutational burden (TMB), and higher predicted neoantigen load. Prevalence estimates of MMR deficiency across solid tumor types range from 1% to 20% depending on the type of malignancy. In prostate cancer, 1-3% of unselected cases harbor MMR deficiency and/or microsatellite instability. For men who previously received definitive treatment for prostate cancer and subsequently develop detectable prostate specific antigen (PSA) levels, the clinical state is known as biochemically recurrent prostate cancer. The current standard of care treatment for patients with biochemically recurrent prostate cancer is either surveillance or androgen deprivation therapy (ADT). ADT has not been shown to provide a survival benefit in this setting, and the decision to initiate ADT will depend on patient preference and perceived risks of the disease. A non-hormonal therapy such as nivolumab would provide an alternative to ADT in patients with biomarker selected (i.e. dMMR, MSI-H, high TMB, or CDK12-altered) biochemically recurrent prostate cancer.
Objectives	Primary: To estimate the PSA_{50} response rate, defined as a $\geq$ 50% decline in PSA from baseline, confirmed with a second measurement at least 4 weeks apart (PCWG3). Secondary: • To estimate the median PSA progression-free survival (PSA-PFS)(PCWG3). • To estimate the the proportion of patients that achieve an undetectable PSA (i.e. PSA <0.1) lasting $\geq$ 12 weeks • To estimate median metastasis-free survival (PCWG3) • To estimate median time to initiation of next systemic therapy • To determine the safety and tolerability of nivolumab in the biochemical recurrent prostate cancer population. Correlatives/Tertiary:

Criteria for evaluation

Primary Endpoint

- PSA_{50} response rate, defined as a ≥ 50% decline in PSA from baseline, confirmed with a second measurement at least 4 weeks later (PCWG3).

Secondary Endpoints

- Safety/Tolerability, defined as incidence of CTCAE v.5.0 Grade ≥ 3 toxicities experienced by patients on the trial.
- PSA progression-free survival (PSA-PFS), defined as a time from initiation on nivolumab therapy until PSA increase of 25%, confirmed with another measurement at least 4 weeks later (PCWG3). Patients will be allowed to stay on study until PSA increases 100% (i.e. doubles) from baseline value.
- Metastasis-free survival (PCWG3)
- Undetectable PSA: Defined as a PSA level <0.1 lasting ≥ 12 weeks
- Time to initiation of next systemic therapy

Exploratory Endpoints

- To associate PSA_{50} response to nivolumab with CD4, CD8, FOXP3, PD-1, PD-L1 levels, MMR and CDK12 mutational status by IHC in archival tumor tissue.
- To associate clinical responses to nivolumab with the expansion of anti-tumor peripheral T cell clones.
- To associate clinical responses to nivolumab with generation of tumor associated antigens as determined by PhIP-seq testing.
- To investigate primary and acquired resistance to PD-1 inhibitor therapy in these biomarker-selected patients (JAK1/2 mutations, B2M expression)

Exploratory Analyses

PD-1, PD-L1, FOXP3, CD4, CD8, MSH2, MSH6, MLH1, PSM2, CDK12 protein levels:
Archival tumor specimens including prostatectomy or needle biopsy of primary tumor, will be analyzed. IHC staining for PD-1 and PD-L1 will be performed and scored as <1%, 1-5%, 5-10%, >10% and the results will be associated with responses for both proteins, separately, using descriptive statistics and Fisher's Exact Tests. IHC will be used to quantify the CD4, CD8, and FOXP3 levels. Using analytical microscopy, Dr. Lotan can quantify the number of T cells per surface area of tumor analyzed. The median number of CD4+ and CD8+ intratumor lymphocytes will be compared in PSA_{50} responders versus non-responders using a T-test. We will also correlate CD4+ and CD8+ lymphocyte tumor infiltration in patients that obtain an undetectable PSA versus those with progressive disease as best response. Lastly, we will correlate response to nivolumab with MMR and CDK12 mutational status.

Interrogate for tumor-associated neoantigens (TAAs) and mutation-associated neoantigens (MANAs):
Whole-exome and RNA sequencing will be performed on archival tissue. Exome data will be applied in a neoantigen prediction pipeline that evaluates antigen processing, MHC binding and gene expression to generate neoantigens specific to the patient's HLA haplotype. TCR sequencing will also be performed on intratumor T cells as well as T cells in the peripheral blood. T cells will be collected prior to therapy, after 1 and 3 cycles of nivolumab and at progression. Truncal neoantigens will be identified by

correcting for tumor purity and ploidy. Putative neoantigens will then be used to generate peptides and stimulate autologous T cells. All specimens will be collected and stored. These studies will be conducted at the completion of this study.

Interrogate for auto antigens mimicking TAAs or MANAs:
Peripheral blood samples will be processed using Phase ImmunoPrecipitation Sequencing (PhIP-seq). A human 90-mer peptidome library which encodes 259,346 peptides that tile the complete human proteome has been developed. The oligonucleotide library was printed on a releasable DNA microarray, and cloned into a T7 phage display system. The phage library is mixed with samples containing antibodies, and antibody-bound phage are captured on protein A/G coated magnetic beads. DNA from the enriched phage population is recovered and inserts are PCR amplified with sequencing adapters and sample-specific barcodes for deep DNA sequencing analysis.

Statistical method

Primary Analysis

The primary endpoint of this study is PSA_{50} response, defined as a decrease in the PSA to ≥ 50% less than the baseline PSA upon enrollment in the trial. The decrease must be confirmed by a second measurement at least 4 weeks apart. For purposes of meeting the primary endpoint, patients will be considered to have done so if they have a PSA_{50} response while on nivolumab. PSA values will be measured monthly during the trial. All patients who take at least one dose of nivolumab will be considered evaluable for the primary endpoint. If patients do not have at least one follow-up PSA after initiation of nivolumab due to stopping therapy for toxicity or withdrawing consent, then they will be replaced. PSA_{50} response rate will be estimated along with 95% confidence interval.

Secondary Analysis

Safety:
Patients will be assessed for toxicities at each clinical evaluation. Toxicities will be graded according to CTCAE v.5.0 standardized grading scales. The incidence of grade 3-5 toxicities will be reported. Patients will be assessed for toxicity as long as they are taking nivolumab, and patients will continue to be followed for at least 100 days if treatment is discontinued for toxicity until the toxicities improve to grade 1 or resolve. Toxicities will be reported as a tabulated table by type and grade.

PSA progression-free survival (PSA-PFS):
A standard definition of PSA progression per PCWG3 will be used. PSA-PFS will be defined as an increase in 25% over a nadir value, confirmed by a follow-up PSA at least 4 weeks later. If patients are removed from study prior to PSA progression, then they will be censored at that time. We will use the Kaplan-Meier method to summarize the PSA-PFS.

Undetectable PSA:
A durable undetectable PSA endpoint will be defined as a patient on study with nivolumab who achieves a PSA < 0.1, lasting at least 12 weeks. This endpoint will represent a durable complete biochemical response to nivolumab. This endpoint can only be achieved while on therapy, although the confirmatory values after initially achieving undetectable PSA may be measured while off therapy with nivolumab.

Metastasis-free survival
Metastasis-free survival will be defined at the time from the first dose of nivolumab until the development of radiographic metastatic disease, as determined using CT imaging and/or bone scan.

Time to initiation of next systemic therapy
The time from the first dose of nivolumab to next systemic therapy (i.e. ADT) will be measured.

Sample size/Power calculation:
The sample size of 15 patients will provide a prelimimary evaluation of clinical activity based on PSA response. Two-sided 95% Wilson confidence interval for various scenrios of PSA_{50} response rate is presented in the table below:

Number of PSA_{50} response	Observed PSA_{50} response rate	95% confidence interval
3	20%	7.0 – 45.2%
4	26.7%	10.9 – 52.0%
5	33.3%	15.2 – 58.3%
6	40%	19.8 – 64.3%
7	46.7%	24.8 – 69.9%
8	53.3%	30.1 – 75.2%
9	60%	35.7 – 80.2%
10	66.7%	41.7 – 84.8%
11	73.3%	48.0 – 89.1%
12	80%	54.8 – 93.0%

Safety analysis — Standard safety summaries will be provided for treatment exposure, patient disposition, adverse events leading to discontinuation, serious adverse events, and all events resulting in death, including those up to 100 days after treatment discontinuation. The incidence of adverse events will be tabulated and reviewed for potential significance and clinical importance.

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1. INTRODUCTION

1.1 Disease Background

Prostate cancer is the most commonly diagnosed non-cutaneous malignancy in men, with an estimated 180,000 cases annually in the United States (1). It is the second most common cause of cancer mortality in the United States as well, with over 26,000 deaths in 2016 (1). The discrepancy between the incidence and mortality numbers demonstrate its potential curability if treated while disease is local, as well as the non-lethal nature of some cancers, even if not treated definitively. While many men are cured of their disease, many others will unfortunately progress to incurable and lethal metastatic disease.

1.1.1 Clinical States of Prostate Cancer

The course of prostate cancer from diagnosis to death is best categorized as a series of clinical states (Fig. 1). These states are defined by the extent of disease and status of responsiveness to hormonal therapy. Therapies have been developed for specific states, as each state presents unique risks to the patient and different responsiveness of the disease to therapy.

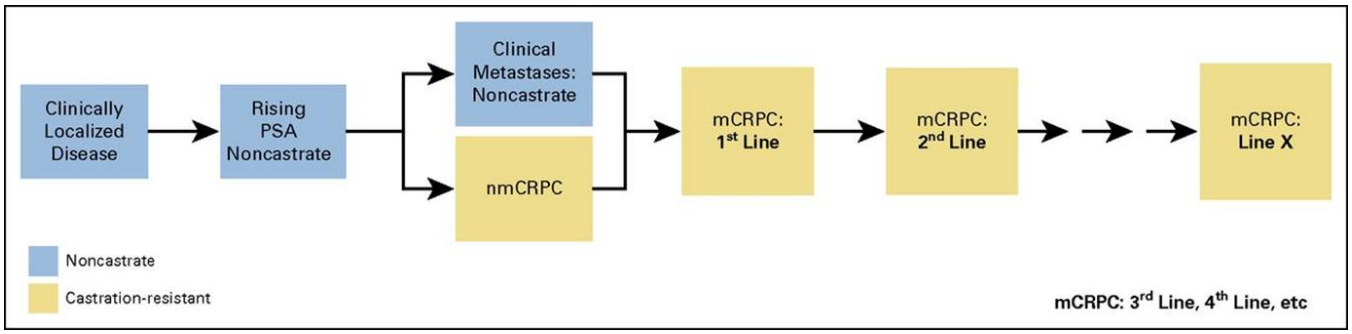


Figure 1 - Clinical states of prostate cancer (2)

1.1.2 The Biochemically Recurrent Disease State

For patients who have undergone definitive local therapy for prostate cancer, for example with a radical prostatectomy, the first signs of recurrent disease are biochemical in nature. For such a patient who develops detectable prostate specific antigen (PSA), defined as confirmed values ≥0.2 for patients post prostatectomy, the clinical state is known as biochemically recurrent prostate cancer (3). This state is further characterized by the lack of evidence of metastatic prostate cancer on traditional imaging, including CT scans of the abdomen and pelvis and NM bone scans of the skeleton. Salvage radiation therapy is potentially curative in some patients with biochemical recurrence, but others will go on to have progressive disease (4).

For patients who were not candidates for salvage radiation therapy or who experience a rising PSA despite that therapy, their natural history will vary significantly depending on

the clinical characteristics of the cancer. The risk of development of metastasis (and thus potentially lethal prostate cancer) has been shown to be dependent on multiple longitudinal clinical characteristics (5). Two of these clinical criteria are Gleason score sum and rate of rise of the PSA—the PSA Doubling time (PSADT). Based upon registry data for hundreds of patients who were treated with radical prostatectomies for localized prostate cancer, and subsequently experienced biochemical recurrence, nomograms have been constructed regarding the risk of developing metastatic disease based upon the Gleason score and/or PSADT. One of the largest cohorts observed was the Johns Hopkins cohort, wherein patients with biochemical recurrence were observed (without hormonal therapy) until the development of radiographic or clinical metastasis, thus demonstrating the natural history of the disease (6).

Post salvage radiotherapy, patients with biochemically recurrent prostate cancer are not cured of their disease. Mismatch repair deficiency and CDK12 inactivation are known predictors of response to immune checkpoint blockade (described below). In this study, we propose take advantage of these biomarkers to identify a patient population that may be predisposed to respond to nivolumab, potentially resulting in long-term remissions of their prostate cancer.

1.2 Treatment Background

1.2.1 Description and mechanism of action

Nivolumab (also referred to as BMS-936558, MDX1106, or ONO-4538) is a human monoclonal antibody (HuMAb; immunoglobulin G4 [IgG4]-S228P) that targets the programmed death-1 (PD-1) cluster of differentiation 279 (CD279) cell surface membrane receptor. PD-1 is a negative regulatory molecule expressed by activated T and B lymphocytes. 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.

OPDIVO (nivolumab) is approved for the treatment of several types of cancer in multiple regions including the United States (US, Dec-2014), the European Union (EU, Jun-2015), and Japan (Jul-2014). Nivolumab is also being investigated in various other types of cancer as monotherapy or in combination with other therapies, and as single-dose monotherapy for the treatment of sepsis.

1.2.3 Preclinical studies

Tumors can express tumor-specific antigens as a result of mutational burden, and ongoing immune surveillance is believed to control the development of many tumors. Tumor progression may depend on the acquisition of mechanisms that permit them to evade an effective immune response. One such mechanism of evasion may be the expression of ligands, which engage inhibitory receptor(s) on anti-tumor T-cells of many tumors. PD-L1

expression has been found on a number of tumors and may be a mechanism by which tumors can directly engage PD-1 to evade an effective anti-tumor immune response. (7-9) Expression of IFN-γ by activated T cells is known to induce PD-L1 expression in tumors.(10) PD-L1 expression has been associated with poor prognoses in renal,(11-13) esophageal,(14) gastric,(15) ovarian,(9) pancreatic,(16) and lung cancers.(17) PD-1 engagement on T-cells by PD-L1-positive APC or PD-L1-positive tumor cells in the tumor microenvironment may limit effective immune responses. Conversely, PD-L1 expression may be a positive prognostic factor as it may indicate infiltration of tumor-specific T cells that secrete IFN-γ, which upregulates PD-L1 expression. Consistent with this hypothesis is the co-localization of lymphoid cell infiltrates and PD-L1 staining observed in human melanoma lesions.(18)

Studies in multiple tumor models using a chimeric murine anti-mouse PD-1 antibody showed that PD-1 blockade has anti-tumor activity.(19) Blocking PD-1 in PD-L1-positive tumors may reverse the inactivation of tumor-specific effector T-cells at the tumor site, as well as activate anti-tumor responses that are limited by PD-L1 expression on "host" DC or APC. The anti-tumor effects of anti-PD-1 observed in several murine models suggest that both PD-L1-positive and PD- L1-negative tumors may be targeted using this approach. In addition, in several tumor models in which anti-PD-1 has proved ineffective, PD-1 blockade can be combined with vaccines or other immunomodulatory antibodies for improved therapeutic efficacy.(20-22) PD-1 blockade by nivolumab is a promising avenue to pursue as an anti-tumor therapy for recurrent or treatment- refractory malignancies.

1.2.4 Clinical studies

This updated IB references the most recent USPI and EU SmPC as the basis for the current state of knowledge on nivolumab for use in humans with cancer. The approved USPI and SmPC are provided in Appendix 1 and Appendix 2 of the IB, respectively. The USPI and SmPC summarize nivolumab monotherapy clinical data for melanoma (based on CA209037 and CA209066), SQ NSCLC (based on CA209063 and CA209017), NSQ NSCLC (based on CA209057), RCC (based on CA209025), Classical Hodgkin Lymphoma (cHL; based on CA209205 and CA209039), and urothelial carcinoma (UC; based on CA209275 and CA209032), as well as clinical data for nivolumab in combination with ipilimumab for melanoma (based on CA209004, CA209069, and CA209067). In addition, the USPI describes nivolumab monotherapy clinical data for SCCHN (based on CA209141). Data from clinical studies that are relevant to ongoing clinical investigations in oncology that are not in the approved USPI and SmPC or to subjects with sepsis are included in this updated IB.
The PK, clinical activity, and safety of nivolumab have been assessed in approximately 75 clinical studies sponsored by BMS or ONO. The description and status of studies with reference safety information are provided in Appendix 4 of the IB. Across those studies, approximately 16,900 subjects have received nivolumab monotherapy in single- or multiple-dose Phase 1/2/3 studies or studies with nivolumab in combination with other therapeutics (ipilimumab, cytotoxic chemotherapy, anti-angiogenics, and targeted therapies). Results from the ongoing studies are preliminary and are subject to change.

Nivolumab has demonstrated clinical activity in NSCLC, melanoma, RCC, cHL, SCCHN, UC (approved indications) and other tumor types (Section 5.4 of the IB) as monotherapy or in combination with ipilimumab or other therapeutics. The majority of responses were durable and exceeded 6 months. In randomized, controlled studies, nivolumab

monotherapy demonstrated statistically significant improvement in OS over standard of care in subjects with advanced or metastatic melanoma, subjects with advanced or metastatic NSCLC, subjects with advanced RCC, and subjects with recurrent or metastatic SCCHN. In randomized, controlled studies, nivolumab in combination with ipilimumab demonstrated statistically significant improvement in PFS and ORR over ipilimumab monotherapy in subjects with advanced or metastatic melanoma.

All available data suggest that nivolumab monotherapy has a consistent AE profile across tumor types. The safety profile is generally consistent across completed and ongoing clinical trials, with no maximum tolerated dose (MTD) reached at any monotherapy dose tested up to 10 mg/kg. There was no pattern in the incidence, severity, or causality of AEs to nivolumab dose level. The safety profile of nivolumab in combination with ipilimumab was consistent with the mechanisms of action of nivolumab and ipilimumab. The nature of the AEs was similar to that observed with either agent used as monotherapy; however, both frequency and severity of most AEs were increased with the combination. A dose of 3 mg/kg nivolumab/3 mg/kg ipilimumab exceeded the MTD, and both 1 mg/kg nivolumab/3-mg/kg ipilimumab and 3 mg/kg nivolumab/1 mg/kg ipilimumab were identified as the MTD.70 Across all studies conducted to date, drug-related AEs have included pulmonary toxicity, renal toxicity (including acute renal failure), endocrine abnormalities, GI toxicity, dermatologic toxicity (including rash), and hepatotoxicity. For nivolumab monotherapy and combination therapy, the majority of these AEs have been managed successfully with supportive care and, in more severe cases, a combination of dose delay, permanent discontinuation, and/or use of corticosteroids or hormone replacement therapy (endocrinopathies) as instructed in the management guidelines provided in Appendix 3 of the IB.

In addition to BMS-sponsored ongoing studies, 22 studies sponsored by ONO Pharmaceuticals, Ltd. and conducted in Japan, Korea, and/or Taiwan are included in the reference safety information in Section 5.6 of the IB. Brief descriptions of these studies are provided in Appendix 4 of the IB. The studies are not under any US investigational new drug application. Efficacy and safety information from ONO studies (ONO-4538-01, ONO-4538-04, ONO-4538-07, ONO-4538-12, ONO-4538-13, and ONO-4538-14) are provided in Section 5.4 and Section 5.5 of the IB.

All studies were conducted in accordance with Good Clinical Practice, as defined by the International Conference on Harmonisation and in accordance with the ethical principles underlying European Union Directive 2001/20/EC and the US Food and Drug Administration Code of Federal Regulations (CFR), Title 21, Part 50 (21CFR50).

1.2.5 Clinical safety summary

The overall safety experience with nivolumab, as a monotherapy, is based on experience in approximately 11,000 subjects treated to date. For monotherapy, the safety profile is similar across tumor types. The only exception is pulmonary inflammation adverse events (AEs), which may be numerically greater in subjects with NSCLC, because in some cases, it can be difficult to distinguish between nivolumab-related and unrelated causes of pulmonary symptoms and radiographic changes. There is no pattern in the incidence, severity, or causality of AEs to nivolumab dose level. A detailed list of AE for patients treated with nivolumab as monotherapy on clinical trials can be found in Table 5.6.1-1 in the IB.

In several ongoing clinical trials, the safety of nivolumab in combination with other therapeutics such as ipilimumab, cytotoxic chemotherapy, anti-angiogenics, and targeted therapies is being explored. Most studies are ongoing and, as such, the safety profile of nivolumab combinations continues to evolve.

1.3 Rationale

1.3.1 Rationale for conducting the study

Two randomized phase III trials involving single-agent checkpoint (CTLA-4) inhibitors in mCRPC patients did not meet their primary endpoints.(23,24) However, a small subset (~20%) of mCRPC patients did derive clinical benefit, although the molecular characteristics of these patients are unknown. Subsequent studies using immune checkpoint blockage have shown limited efficiacy when used in combination with the anti-androgen, enzalutamide(25). Many studies involving immune checkpoint blockade and metastatic prostate cancer are ongoing.

Mismatch repair (MMR)-deficient cancers of any histologic type appear to be very sensitive to PD-1/PD-L1 blockade with pembrolizumab (26), and similar data are also beginning to emerge for nivolumab and other immune checkpoint inhibitors. Among the MMR-deficient (dMMR) cancers, the best antitumor responses are often associated with high microsatellite instability (MSI-H status), higher tumor, mutational burden (TMB), and higher predicted neoantigen load. Prevalence estimates of MMR deficiency across solid tumor types range from 1% to 20% depending on the type of malignancy.(27)

In prostate cancer, 1-3% of unselected cases harbor MMR deficiency and/or microsatellite instability.(28) Additionally, cyclin dependent kinase 12 (CDK12) has been shown to be mutated in a subgroup of prostate cancers leading to the formation of neoantigens and response to immune checkpoint blockade.(29,30) Preliminary evidence to date suggests that even in dMMR/MSI-H advanced prostate cancers (*i.e.* metastatic castrate-resistant prostate cancers [mCRPC]), response rates to PD-1/PD-L1 inhibitors are only about 40% and durations of response typically range from 6 to 9 months, arguably much shorter than seen in other cancer types.(31) One hypothesis that may explain this suboptimal response of dMMR mCRPC to PD-1/PD-L1 blockade is the potential immunosuppressive role of ADT (*i.e.* castration) on the tumor microenvironment. A second factor might be due to the larger extent of disease, the greater intratumoral heterogeneity, and the emergence of immune tolerance.

Biochemically-recurrent prostate cancer (*i.e.* manifesting by a rising PSA level after local therapy), represents an ideal space for deploying immunotherapy in this disease. These patients have not yet received androgen deprivation therapies (*i.e.* are non-castrate), and do not have radiographic evidence of metastases, yet are incurable and will eventually develop both metastatic and castrate-resistant prostate cancer. This population also represents an ideal setting to study a non-castrating systemic therapy, which will not be influenced by androgen levels or their suppression. To maximize the chance for success, we propose to focus on the dMMR/MSI-H patient population for treatment with nivolumab (without ADT).

While the overall prevalence of dMMR/MSI-H prostate cancer is 1-3%, our group has shown that there may be enrichment in certain histologic subtypes. For example, the prevalence of dMMR/MSI-H prostate cancer increases to 8-15% among patients with primary Gleason pattern 5 (*i.e.* Gleason scores 5+5 or 5+4), those with intraductal or ductal histology (as opposed to typical acinar histology), and those with neuroendocrine small cell prostate cancer (unpublished data). By targeting our prescreening efforts on these histologic subtypes, it becomes much more feasible to contemplate a study specifically in dMMR prostate cancer.

We hypothesize that nivolumab monotherapy (without ADT) will have clinical activity in a significant proportion of dMMR/MSI-H prostate cancers with a PSA recurrence following local therapy. Further, we hypothesize that the most favorable responses to nivolumab will be seen in patients with high tumoral lymphocytic infiltrate, positive PD-1 and PD-L1 expression, and high tumor mutational burden (TMB).

1.3.2 Rationale for dosage selection

Nivolumab will be administered at a flat dose of 480mg IV given every 4 weeks until progression. Nivolumab has been extensively studied in humans at doses ranging from 1-10mg/kg q2 weeks to 0.3-10mg/kg q3 weeks. Using these doses, the clinical pharmacology of nivolumab is well-established. Based on the exposure-response relationships for efficacy and safety, the benefit-risk profile of nivolumab 480mg given every 4 weeks is predicted to be similar to the 3mg/kg dosing across multiple tumor types.(32)

2. OBJECTIVES

2.1 Primary Objective

The primary objective is to estimate the PSA response rate (PSA_{50}) in patients with BCR prostate cancer with underlying mismatch repair deficiency. This will serve as an initial exploration of this drug's activity in this disease state.

2.2 Secondary Objectives

- Safety/Tolerability, defined as incidence of CTCAE v.5.0 grade ≥ 3 toxicities experienced by patients on the trial.
- PSA progression-free survival, defined as a time from initiation on nivolumab until PSA increase of 25%, confirmed with another measurement at least 4 weeks later (PCWG3).
- Undetectable PSA, defined as a PSA $\leq$ 0.1 lasting $\geq$ 12 weeks

2.3 Correlative/Exploratory/Tertiary Objectives

- To measure IHC-based immune cell infiltrates (CD4, CD8, FOXP3) and markers (PD-1, PD-L1), MMR and CDK12 mutational status on archived tissue (prostatectomy or needle biopsy of primary tumor.
- To investigate any association between PD-1/PD-L1 expression, CD4/CD8 T cell infiltration in tumor specimens and PSA_{50} response rates.
- To perform deep sequencing of the T cell Receptor CDR3 region (TCRseq) from peripheral blood and arrived tissues.

- To explore the relationship between clonal T cell expansion in peripheral blood and clinical outcomes.
- To perform whole exome sequencing (WES) and RNAseq on archived tissue.
- To utilize predictive algorithms based on WES/RNAseq/TCRseq to identify tumor associated neoatigens
- To utilize Phage ImmunoPrecipitation sequencing (PhIP-Seq) to identify underlying T cells responses against tumor associated antigens .

3. PATIENT SELECTION

3.1 Target Population

The target population is men with biochemically recurrent prostate cancer (i.e. rising PSA after local therapy with either prostatectomy or EBRT/brachytherapy). This population cannot have evidence of metastatic disease on CT and NM bone scan. An absolute PSA value of ≥ 1.0 ng/ml is required.

3.2 Expected Enrollment

A total of 15 patients will be included in this study. The first patients are expected to be enrolled in Q3 2019. Accrual is expected to be completed in 12 months once the protocol has been approved by the IRB at each participating institution. After the last patient is enrolled, we expect approximately 6 months additional followup.

3.3 Inclusion Criteria

To be included in this study, patients should meet all of the following criteria:

- Willing and able to provide signed informed consent and HIPAA authorization for the release of personal health information.

  NOTE: HIPAA authorization may be either included in informed consent or obtained separately.

- Males aged 18 years of age and above
- Prior local therapy with prostatectomy or EBRT/Brachytherapy is required.
- Prior salvage or adjuvant radiation therapy is allowed but not mandated. Radiation therapy must have been completed for at least 6 months.
- Absolute PSA ≥ 1.0 ng/ml at screening.
- Must have at least one of the following geneic alternations identified using archial tissue (i.e. prostate needle biopsy prior to radiation therapy or prostatectomy specimen):
  - Microsatellite instability (MSI-high) status by clinical grade testing
  - MMR protein loss (MSH2, MSH6, MLH1, PMS2) by immunohistochemistry
  - Inactivating mutation of *MSH2, MSH6, MLH1 or PSM2* by clinical grade genomic testing.
  - Tumor mutational burden ≥20 mutations/megabase (TMB ≥20 muts/Mb) by clinical grade testing.
  - Inactivating mutation (at least monoallelic) of *CDK12* by clinical grade testing

- Serum testosterone ≥ 150 ng/dl

- No radiographic evidence of metastatic disease by CT scan and bone scan, performed within the prior 8 weeks
- Karnofsky Performance Status (KPS): ≥ 70% within 14 days before start of study treatment (ECOG ≤ 1) (See Appendix B).
- Participants must have normal organ and bone marrow function measured within 28 days prior to administration of study treatment as defined below:
  - Hemoglobin ≥ 9.0 g/dL with no blood transfusion in the past 28 days
  - Absolute neutrophil count (ANC) ≥ $1.0x10^9$/L
  - Total bilirubin within institutional upper limit of normal (ULN) (In patients with Gilbert's syndrome, total bilirubin < 1.5x institutional ULN will be acceptable)
  - Aspartate aminotransferase (AST)(Serum Glutamic Oxaloacetic Transaminase (SGOT)) / Alanine aminotransferast (ALT) (Serum Glutamic Pyruvate Transaminase (SGPT)) within institutional upper limit of normal
- Participants must have creatinine clearance estimated using the Cockcroft-Gault equation of ≥40 mL/min:

  $$\text{Estimated creatinine clearance} = \frac{(140\text{-age [years]}) \times \text{weight (kg)}}{\text{serum creatinine (mg/dL)} \times 72}$$

- Participants must have a life expectancy ≥ 6 months
- Male participants and their partners, who are sexually active and of childbearing potential, must agree to the use of two highly effective forms of contraception in combination (see Appendix D for acceptable methods), throughout the period of taking study treatment and for 7 months after the last dose of nivolumab to prevent pregnancy in a partner.
- No evidence (within 5 years) of prior malignancies (except successfully treated basal cell or squamous cell carcinoma of the skin).

3.4 Exclusion Criteria

Patients must meet any of the criteria listed below will not be eligible for study entry:

- Metastatic disease or currently active second malignancy
- Prior ADT in the past 6 months. Prior ADT in context of neoadjuvant/adjuvant primary; prior AdT for biochemical recurrence is allowed, as long as no ADT has been administered in past 6 months and testosterone has recovered (>150 ng/dl).
- Prior oral anti-androgen (e.g. bicalutamide, nilutamide, enzalutamide, apalutamide), or androgen syntehesis inhibitor (e.g. abiraterone, orteronel) within the past 2 weeks is not permitted. 5-alpha reductase inhibitor therapy (e.g. finasteride, dutasteride) is allowed, as long as subject has been stable on medication for past 6 months.

- Involvement in the planning and/or conduct of the study (applies to both BMS staff and/or staff at the study site).
- Participation in another clinical study with an investigational product during the last 4 weeks/28 days.
- Patients should be excluded if they have had prior systemic treatment with an anti-PD-1 , anti-PD-L1, anti-PD-L2, anti-CTLA-4 antibody, or any other antibody or drug specifically targeting T-cell costimulation or immune checkpoint pathways.
- Concurrent use of other anticancer agents or treatments, including but not limited to androgen deprivation therapy (ADT).
- Any treatment modalities involving major surgery within 4 weeks prior to the start of study treatment.
- Patients should be excluded if they have an active, known or suspected autoimmune disease (e.g. inflammatory bowel disease, rheumatoid arthritis, autoimmune hepatitis, lupus, celiac disease). Subjects are permitted to enroll if they have vitiligo, type I diabetes mellitaus, residual hypothyroidism due to autoimmune condition only requiring hormone replacement, psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger.
- Patients should be excluded if they have a condition requiring systemic treatment with either corticosteroids (>10 mg daily prednisone equivalents) or other immunosuppressive medications within 14 days of study drug administration. Inhaled or topical steroids and adrenal replacement doses >10mg daily prednisone equivalents are permitted in the absence of active autoimmune disease.
- Permitted therapies include topical, ocular, intra-articular, intranasal, and inhalational corticosteroids (with minimal systemic absorption). Physiologic replacement doses of systemic corticosteroids are permitted, even if >10mg/day prednisone equivalents. A brief course of corticosteroids for prophylaxis (e.g. contrast dye allergy) or for treatment of non-autoimmune conditions (e.g. delayed-type hypersensitivity reaction caused by contact allergen) is permitted.
- As there is potential for hepatic toxicity with nivoluman, drugs with a predisposition to hepatotoxicity should be used with caution in patients treated with nivolumab-containing regimen.
- Patients should be excluded if they have known history of testing positive for human immunodeficiency vierus (HIV) or known acquired immunodeficiency syndrome (AIDS).
- History of allergy to study drug components.
- History of severe hypersensitivity reaction to any monoclonal antibody
- Any other serious illness or medical condition that would, in the opinion of the investigator, make this protocol unreasonably hazardous, including but not limited to:
    - Any uncontrolled major infection
    - Cardiac failure NYHA (New York Heart Association) III or IV.
    - Crohn's disease or ulcerative colitis

  - Bone marrow dysplasia
  - Known allergy to any of the compounds under investigation
  - Unmanageable fecal incontinence.
- Poor medical risk due to a serious, uncontrolled medical disorder, non-malignant systemic disease or active, uncontrolled infection. Examples include, but are not limited to, uncontrolled ventricular arrhythmia, recent (within 6 months) myocardial infarction, uncontrolled major seizure disorder, extensive interstitial bilateral lung disease, or any psychiatric disorder that prohibits obtaining informed consent.

4. PATIENT REGISTRATION AND ENROLLMENT PLAN

4.1 Registration Procedure

After eligibility screening and confirmation that a patient is eligible, patients who are selected to participate will be registered with the Lead Center Johns Hopkins University, with their local study site/institution, and if applicable, in the online centralized PCCTC database. A record of patients who fail to meet entry criteria (i.e. screen failures) will be maintained. Patient registration must be complete before beginning any treatment or study activities. A complete, signed study consent is required for registration.

4.1.1 Registration at Johns Hopkins University

Confirm eligibility as defined in ***Section 3. Patient Selection***.

Obtain informed consent, by following procedures in ***Section 12.3 Written Informed Consent***.

Patient will be entered into CRMS system and enrolled in trial.

4.1.2 Multicenter/Participating site registration

Central registration for this study will take place at Johns Hopkins University.

Patient registration at each study site/institution will be conducted according to the institution's established policies. Before registration, patients will be asked to sign and date an Institutional Review Board (IRB)-approved consent form. Patients must be registered with their local site/institution and also with the Lead Site before beginning any treatment or study activities.

5. TREATMENT/INTERVENTION PLAN

The following assessments and procedures will occur during the study. A schedule of assessments is provided in Table 1.

Table 1 Study Calendar

	Pre-study	Study Period					
		On Treatment Assessments (Every 28 days)				End of Treatment Visit	Off study follow-up
	Day -28 to -1[a]	C_1 D_1 (±5 d)	C_2 D_1 (±5 d)	C_3 D_1 (±5 d)	C_N D_1 (±5 d)	28 days after last dose (±5 d)	Every 28 days[d] & 100 days after last dose (±5 d)
Informed consent	X						
Demographics	X						
Complete Medical history	X						
EKG	X						
Height	X[g]						
Weight	X[g]	X	X	X	X	X	X
Nivolumab		X	X	X	X		
Focused Medical history	X	X	X	X	X	X	X
Concomitant meds	X	X	X	X	X	X	
ECOG Performance status	X	X	X	X	X	X	X
Physical exam	X[g]	X[g]	X[g]	X[g]	X[g]	X[g]	X[g]
Vital signs (P, BP, RR, T)	X[g]	X[g]	X[g]	X[g]	X[g]	X[g]	X[g]
Toxicity assessment		X	X	X	X	X	X
CBC w/ diff[e]	X	X[f]	X	X	X	X	
CMP[e]	X	X[f]	X	X	X	X	
PSA[e]	X	X[f]	X	X	X	X	
Serum Testosterone[e]	X	X[f]	X	X	X	X	
Serum Amylase/Lipase/ CK/TSH with Reflex T3/T4[e]	X	X[f]	X	X	X	X	
Hepatitis B/C; PTT, PT/INR, Urinalysis	X						
Radiologic tests[b]	X				X[b]	X[b]	
Archival Tissue/DNA/RNA studies	X						
Plasma for PBLs/Phipseq		X[c]	X[c]		X[c]	X[c]	
Adverse events			X	X	X	X	X[d]

Abbreviations: CBC, complete blood count; CT, computerized tomography; MRI, magnetic resonance imaging; PSA, prostate-specific antigen; PBLs, peripheral blood leukocytes; CK, creatine kinase; TSH, thyroid stimulating hormone

[a] Informed consent must be obtained within 4 weeks and radiologic assessments should be obtained within 8 weeks of study start date.

[b] Radiographic evaluations (CT C/A/P and NM Bone Scan every 6 months while enrolled in the study). They are needed within 8 weeks of screening; if previously performed then they are to be performed at screening. CT C/A/P and NM Bone Scan are to be performed at the end of treatment visit if they have not been performed within the prior month. If PSA becomes undetectable, CT C/A/P can be obtained in a timeframe at PI discretion

[c] Plasma for PBLs/PHipSeq will be drawn before treatment administration at C1D1, C2D1, C4D1, and at progression (EOT).

[d] Subjects will be followed after completion of nivolumab therapy monthly until toxicities resolve to grade 0-1 if they withdrew because of AEs. Otherwise, subjects will be followed at 100 days after last dose for AE reassessment. This 100 day visit may take place via telephone.

[e] All labs should be drawn before treatment administration.

[f] Labs do not need to be repeated if done within 7 days of screening.

[g] Physical exam (including vital signs/height/weight) may be missed if not clinically-indicated, or in the event of a telemedicine visit. In order to minimize the need for research-only in-person visits, telemedicine visits may be substituted for in-person clinical trial visits or portions of clinical trial visits where determined to be appropriate and where determined by the investigator not to increase the participants risks. Prior to initiating telemedicine for study visits the study team will explain to the participant, what a telemedicine visit entails and confirm that the study participant is in agreement and able to proceed with this method. Telemedicine acknowledgement will be obtained in accordance with the Guidance for Use of Telemedicine in Research. In the event telemedicine is not deemed feasible, the study visit will proceed as an in-person visit. Telemedicine visits will be conducted using HIPAA compliant method approved by the Health System and within licensing restrictions.

5.1 Screening/Pretreatment Assessment (Day -28 to Day -1)

Before initiating any screening activities, the scope of the study should be explained to each patient. Patients should be advised of any known risks inherent in the planned procedures, any alternative treatment options, their right to withdraw from the study at any time for any reason, and their right to privacy. After this explanation, patients should be asked to sign and date an IRB-approved statement of informed consent that meets the requirements of the Code of Federal Regulations (Federal Register Vol. 46, No. 17, January 27, 1981, part 50).

The screening visit will determine patient eligibility according to the inclusion and exclusion criteria (Sections 3.3 Inclusion Criteria & 3.4 Exclusion Criteria). The following assessments will be performed at this visit:

- Obtain informed consent and research authorization.
- Record demographics (including age) and medical history (including prior treatment for prostate carcinoma).
- Conduct physical exam (including vital signs, height/weight). *This may be missed if visit is taking place over telemedicine.*
- Obtain histologic confirmation of disease. If radiographic studies have not been performed in prior 8 weeks, they must be obtained as part of screening.
- Obtain history regarding prior treatment history for prostate cancer (including history of ADT, history of radiation therapy or other local therapy).
- Must have at least one of the following genetic alternations identified using archival tissue (i.e. prostate needle biopsy prior to radiation therapy or prostatectomy specimen):
    - Microsatellite instability (MSI-high) status by clinical grade testing.
    - MMR protein loss (MSH2, MSH6, MLH1, PMS2) by immunohistochemistry.
    - Inactivating mutation of *MSH2, MSH6, MLH1 or PSM2* by clinical grade genomic testing.
    - Tumor mutational burden ≥20 mutations/ megabase (TMB ≥20 muts/Mb) by clinical grade testing.
    - Inactivating mutation (at least monoallelic) of *CDK12* by clinical grade testing
- Perform laboratory tests (Complete blood count w/ Diff, PSA, Comprehensive metabolic panel, urinalysis, PT/INR, PTT, testosterone, TSH with reflexive T3/T4 testing, serum CK, amylase/lipase, and Hepatitis B/C panel).
- Assess performance status (ECOG). (Appendix B).
- Perform 12-lead EKG. ECGs are required within 14 days prior to starting study treatment and when clinically indicated.
    - Twelve-lead ECGs will be obtained after the patient has been rested in a supine position for at least 5 minutes in each case. The Investigator or designated physician will review the paper copies of each of the timed 12-lead ECGs on each of the study days when they are collected.

- ECGs will be recorded at 25 mm/sec. All ECGs should be assessed by the investigator as to whether they are clinically significantly abnormal or not clinically significantly abnormal. If there is a clinically significant abnormal finding, the Investigator will record it as an AE on the eCRF. The original ECG traces must be stored in the patient medical record as source data.

- Determine suitability for nivolumab.
- Discuss concurrent medications.

Relevant information should be documented. The institutional registration should be finalized, and appropriate documents (i.e. signed informed consent and supporting source documentation for eligibility questions) emailed to the Lead site.

Information for patients who do not meet the eligibility criteria to participate in this study (i.e. screening failures) should be captured in the CRMS database at the pretreatment assessment.

5.2 Treatment/Intervention Period (Day 1 of each 28 day cycle +/- 3 days, (C1D1, C2D1, C3D1, CnD1))

Patients will be seen on Day 1 of each cycle of nivolumab (consisting of 28 days, +/- 3 days).

The following assessments will be performed at each visit:

- Conduct physical exam (including vital signs, weight). *This may be missed if visit is taking place over telemedicine.*
- Obtain any medical history changes from prior assessment.
- Assess performance status (ECOG). (Appendix B).
- Review concurrent medications.

5.2.1 Clinical and laboratory assessments

On Day 1 of each cycle, before treatment administration, patients will have a non-fasting blood drawn for the following values: Repeat labs are not needed on C1D1 as long as visit is within 7 days of screening values.

- CBC w/ Diff
- Comprehensive chemistry panel
- PSA
- Testosterone
- TSH w/ reflexive T3/T4
- Amylase/lipase
- Serum CK
- PBML sample blood draw (120mL; C1D1, C2D1, C4D1, End of Treatment)

Laboratory Safety Assessments:

Full hematology assessments for safety (hemoglobin, red blood cells [RBC], platelets, mean cell volume [MCV], mean cell hemoglobin concentration [MCHC], mean cell hemoglobin [MCH], white blood cells [WBC], absolute differential white cell count (neutrophils, lymphocytes, monocytes, eosinophils and basophils) and absolute neutrophil count or segmented neutrophil count and Band forms should be performed at each visit and when clinically indicated. If absolute differentials not available, will check % differentials. Coagulation [activated partial thromboplastin

time (APTT) and international normalized ratio (INR)] will be performed at baseline and if clinically indicated.

Biochemistry assessments for safety (sodium, potassium, calcium, fasting glucose, creatinine, total bilirubin, alkaline phosphatase [ALP], aspartate transaminase [AST], alanine transaminase [ALT], urea or blood urea nitrogen [BUN], total protein, albumin.

Urinalysis by dipstick should be performed at baseline and then only if clinically indicated. Microscopic analysis should be performed by the hospital's local laboratory if required.

These tests will be performed by the hospital's local laboratory. Additional analyses may be performed if clinically indicated.

Any clinically significant abnormal laboratory values should be repeated as clinically indicated and recorded on the eCRF.

In case a subject shows an AST **or** ALT ≥ 3xULN **or** total bilirubin ≥ 2xULN please refer to Appendix E *"Actions required in cases of combined increase of Aminotransferase and Total Bilirubin – Hy's Law"*, for further instructions.

5.2.2 Radiographic assessments:
Every 6 cycles, patients will have radiographic studies beginning C7D1:

- CT Chest, Abdomen and Pelvis with contrast.
- NM Bone scan.

5.2.3 Safety assessments
Adverse events (AEs) will be monitored at each scheduled visit and throughout the study. Toxicity will be assessed using the most recent National Cancer Institute (NCI) guidance: the most recent version of Common Terminology Criteria for Adverse Events (CTCAE).

5.3 End of Treatment/Treatment Discontinuation Visit (28 days after last dose +/- 3 days)

- Conduct physical exam (including vital signs, weight). *This may be missed if visit is taking place over telemedicine.*
- Perform laboratory tests: Complete blood count w/Diff, PSA, Comprehensive metabolic panel.
- Assess performance status (ECOG). (Appendix B).
- Review concurrent medications.
- Assess AEs.
- If patient is discontinuing participation in study, perform radiographic tests: CT C/A/P and NM Bone Scan (if these have not been performed in the prior month).
- The following non-fasting blood draw will include:
    - CBC w/ Diff
    - Comprehensive chemistry panel
    - PSA
    - Testosterone
    - TSH w/ reflexive T3/T4

- Amylase/lipase
- Serum CK
- PBML blood collections (120ml)

5.4 Follow-up (Every 28 days while active AEs, 100 days after last dose & Survival status +/- 3 days)

Patients will be followed every 28 days beyond the end of treatment visit if they have withdrawn from study because of AEs. They will be followed until the adverse event has either resolved or stabilized (Grade ≤ 1). Reasons for premature withdrawal should be determined and noted. All patients who received nivolumab will be contacted via telephone at 100 days after last dose +/- 3 days. Reassessment for AE will be conducted at each visit or via telephone.

5.5 Correlative/Special Studies

5.5.1 Immunohistory chemotherapy analysis

FFPE biopsy or prostatectomy specimens will be sent to the Lotan Laboratory for analysis

5.5.2. Whole exome sequencing/ RNA seq

FFPE biopsy or prostatectomy specimens will be sent to the Lotan Laboratory for whole exome sequencing/RNAseq in collaboration with the Next Generation Sequencing Core Lab.

5.5.3. PhIP seq analysis

Plasma will be obtained at C1D1, C2D1, C4D1, and at progression (EOT) and sent to the Immune Processing Core for analysis.

5.5.4. TCR seq

PBMLs will be obtained at C1D1, C2D1, C4D1, and at progression (EOT). These PBMLs will be processed and stored in the Immune Processing Core at JHU.

6 STUDY DRUGS

6.1 Description of Treatments

The drug to be tested in this clinical protocol is nivolumab. Nivolumab will be supplied by Bristol-Myers Squibb, Co.

6.2 Administration, Supply, and Storage

Nivolumab will be dosed at 480mg IV every 4 weeks (+/- 3 days) beginning on C1D1. Treatment will be administered on an outpatient basis.

PRODUCT INFORMATION TABLE: Please also see Nivolumab Investigator Brochure.

Product Description and Dosage Form	Potency	Primary Packaging (Volume)	Appearance	Storage Conditions (per label)
Nivolumab BMS-936558-01 Solution for Injection	100 mg (10 mg/mL)	10 mL vial	Clear to opalescent colorless to pale yellow liquid. May contain particles.	2 to 8°C. Protect form light and freezing.

*Nivolumab may be labeled as BMS-936558-01 Solution for Injection

If stored in a glass front refrigerator, vials should be stored in the carton. Recommended safety measures for preparation and handling of nivolumab include laboratory coats and gloves.

For additional details on prepared drug storage and use time of nivolumab under room temperature/light and refrigeration, please refer to the BMS-936558 (nivolumab) Investigator Brochure section for "Recommended Storage and Use Conditions".

7 DOSE ADJUSTMENT AND DELAY, TREATMENT DISCONTINUATION, WITHDRAWAL, AND TERMINATION CRITERIA

7.1 Dosing and Dose Modifications

7.1.1 Dosing
Nivolumab 480mg will administered intravenously every 4 weeks approximately (+/-3 days) over an approximate 30 minute infusion time beginning on C1D1 until progression or end of study.

7.1.2 Dose Modifications
There will be no dose modifications permitted.

7.2 Dose delay and treatment discontinuation

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

7.3 Dose Delay Criteria

Nivolumab administration should be delayed for the following:

- Any Grade ≥ 2 non-skin, drug-related adverse event, with the following exceptions:
    - Grade 2 drug-related fatigue or laboratory abnormalities do not require a treatment delay.
- Any Grade 3 skin, drug-related adverse event.
- Any Grade 3 drug-related laboratory abnormality, with the following exceptions for AST, ALT, or total bilirubin:
    - Delay dosing for drug-related AST, ALT, or total bilirubin grade ≥ 2 toxicity.
- Grade 3 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis do not require a dose delay.
- Grade 3 lymphopenia does not require a dose delay.
- Any adverse event, laboratory abnormality, or intercurrent illness, which, in the judgment of the investigator, warrants delaying the dose of study medication.

Because of the potential for clinically meaningful nivolumab-related AEs requiring early recognition and prompt intervention, management algorithms have been developed for suspected pulmonary toxicity, GI, hepatotoxicity, endocrinopathy, skin toxicity, neurological toxicity and renal toxicity. The recommendations are to follow the nivolumab IB adverse event algorithm (Appendix A).

7.4 Criteria to Resume Treatment

Missed doses of nivolumab should be administered when subject meets criteria to resume treatment. If a dose has been missed, the subject should wait until the next scheduled dosing date.

Subjects may resume treatment with study drug 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.
- For subjects with Grade 2 AST, ALT, or total bilirubin elevations, dosing may resume when laboratory values return to baseline and management with corticosteroids, if needed, is complete.
- Subjects with combined Grade 2 AST/ALT **AND** total bilirubin values meeting discontinuation parameters should have treatment permanently discontinued.
- Drug-related diarrhea, or colitis, must have resolved to baseline before treatment is resumed.
- Subjects with persistent Grade 1 pneumonitis after completion of a steroid taper over at least 1 month may be eligible for retreatment if discussed with and approved by the investigator.
- Drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment.

If treatment is delayed >10 weeks, the subject must be permanently discontinued from study therapy.

7.5 Discontinuation Criteria

Treatment with nivolumab should be permanently discontinued for any of 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 toxicity, hypersensitivity reactions, and infusion reactions:
    - Grade 3 drug-related uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic toxicity, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation.
    - Grade 3 drug-related endocrinopathies adequately controlled with only physiologic hormone replacement do not require discontinuation, except for Grade 3 adrenal insufficiency which requires permanent discontinuation.
    - Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except:
        - Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding requires discontinuation.
        - Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:

- Grade ≥ 3 drug-related AST, ALT, or total bilirubin requires discontinuation.
- Concurrent AST or ALT > 3xULN and total bilirubin > 2xULN.

- Any Grade 4 drug-related adverse event or laboratory abnormality, except for the following events which do not require discontinuation:
  - Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis.
  - Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset.
  - Grade 4 neutropenia ≤ 7 days.
  - Grade 4 lymphopenia or leukopenia.
  - Grade 4 drug-related endocrinopathy AEs, such as, hyper- or hypothyroidism, or glucose intolerance, that 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 investigator. Grade 4 drug-related adrenal insufficiency or hypophysitis requires discontinuation regardless of control with hormone replacement.
- Any dosing interruption lasting > 10 weeks unless the investigator is consulted and agrees with the rationale for resuming therapy after a delay > 10 weeks. Note that tumor assessments should continue as per protocol even if dosing is interrupted.
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the Investigator, presents a substantial clinical risk to the subject with continued nivolumab dosing.

7.6 Treatment of Nivolumab-Related Infusion Reactions

Since nivolumab contain only human immunoglobulin protein sequences, each 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. Infusion reactions should be graded according to NCI CTCAE v.5.0 guidelines.

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

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

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

For Grade 2 symptoms: (Moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [e.g. antihistamines, non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; prophylactic medications indicated for up to 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 premedications 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 or administrations. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.

For Grade 3 or Grade 4 symptoms: (Severe reaction, Grade 3: prolonged [i.e. not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae [e.g. 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. 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 (e.g. appearance of a localized or generalized pruritis within 1 week after treatment), symptomatic treatment may be given (e.g. oral antihistamine, or corticosteroids).

7.7 Immunotherapy Adverse Event Management

Because of the potential for clinically meaningful nivolumab-related AEs requiring early recognition and prompt intervention, management algorithms have been developed for suspected pulmonary toxicity, GI toxicity, hepatotoxicity, endocrinopathy, skin toxicity, neurological toxicity, and renal toxicity (Nivolumab IB).

These adverse event management algorithms are included in Appendix A.

7.8 Removal of Subjects from the Study, Therapy Assessment

7.8.1 Subject withdrawal

Single subject termination is by definition when the patient is withdrawn or when the patient has died. The study termination page in the eCRF must be completed.

The Investigator also has the right to withdraw subjects from the study in the event of:

- Occurrence of an exclusion criterion, which is clinically relevant and affects the subject's safety, and discontinuation is considered necessary by the Investigator and/or the Sponsor.
- Therapeutic failure requiring urgent additional medication (if applicable)
- Occurrence of AEs, if discontinuation of study medication is considered necessary by the Investigator and/or subject (if applicable).
- Intake of non-permitted concomitant medication as defined in Appendix A where the predefined consequence is withdrawal from the study.
- Progression of disease (subjects will only come off study after meeting PCWG3 criteria for radiographic progression or clinical progression as determined by the treating physician).
- Lack of subject compliance.
- Protocol violation.

7.9 Treatment Compliance

Trained medical personnel will administer nivolumab and dispense other study medications. Treatment compliance will be monitored by drug accountability, as well as by recording administration of all medications in the CRF. The date and time of start and end of infusion and the exact amount given at each infusion will be recorded. Any missed doses will be recorded. In case the treatment has to be interrupted during an infusion and the dosing is not resumed, the medical personnel should evaluate the percentage of dose received by the patient and document it in the patient record. Any reason for non-compliance should also be documented.

7.10 Destruction of Study Drug

Investigator drug destruction is allowed provided the following minimal standards are met:

- On-site disposal practices must not expose humans to risks from the drug.
- On-site disposal practices and procedures are in agreement with applicable laws and regulations, including any special requirements for controlled or hazardous substances.
- Written procedures for on-site disposal are available and followed. The procedures must be filed with the Lead Site/sponsor SOPs and a copy provided to BMS upon request.
- Records are maintained that allow for traceability of each container, including the date disposed of, quantity disposed, and identification of the person disposing the containers. The method of disposal, ie, incinerator, licensed sanitary landfill, or licensed waste disposal vendor must be documented.
- Accountability and disposal records are complete, up-to-date, and available for BMS to review throughout the clinical trial period as per the study agreement. A copy of the drug destruction certificate must be made accessible to BMS at the end of study.

If conditions for destruction cannot be met, please contact BMS.

It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local, and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

7.11 Return of Study Drug

It is the investigator's responsibility to arrange for destruction of drug upon completion or terminal of the study and disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local, and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

8. ADVERSE EVENTS

8.1 Definitions

8.1.1 Adverse Event (AE)

An AE is defined as any untoward medical occurrence in a patient administered a medicinal product that does not necessarily have a causal relationship with this treatment. An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not related to the investigational medicinal product. This includes an exacerbation of pre-existing conditions or events, intercurrent illnesses, drug interaction, or the significant worsening of the indication under investigation that is not recorded elsewhere on the eCRF under specific efficacy assessments. Anticipated fluctuations of pre-existing conditions, including the disease under study, that do not represent a clinically significant exacerbation or worsening are not considered AEs.

It is the responsibility of the investigator to document all AEs that occur during the study. AEs should be elicited by asking the patient a non-leading question (e.g. "Have you experienced any new or changed symptoms since we last asked/since your last visit?"). The existence of an AE may be concluded from a spontaneous report of the patient; from the physical examination; or from special tests such as the ECG, laboratory assessments, or other study-specified procedure (source of AE). Symptoms reported spontaneously by the patient during the physical examination would also qualify as an AE (and hence documented on the AE eCRF, not on the physical examination eCRF, which is reserved for physical signs or findings).

8.1.2 Serious Adverse Event (SAE)

An SAE is any untoward medical occurrence that occurs at any dose (or, occurs after informed consent is given and prior to dosing if the SAE is related to a study procedure) that:

- Results in death. Any event resulting in death during the reporting period (from date of first dose of study drug through 100 days after last dose) must be treated as an SAE and reported as such. An event related to a study procedure that occurs after informed consent, but prior to dosing that results in death must also be reported as an SAE.
- Is life-threatening (patient is at immediate risk of death from the event as it occurred).
- Requires in-patient hospitalization (formal admission to a hospital for medical reasons) or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Results in a congenital anomaly or birth defect.

Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may be considered SAEs when, based on appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home or the development of drug dependency or drug abuse.

8.1.2.1 Events or Outcomes Not Qualifying as Serious Adverse Events

The following are not considered SAEs and therefore do not need to be reported as such:

- Pre-planned or elective hospitalization including social and/or convenience situations (e.g. respite care).
- Hospital visits of less than 24 hours duration (e.g. patient presents to the emergency room, but is not admitted to a ward).
- Overdose of either BMS study drug or concomitant medication unless the event meets SAE criteria (e.g. hospitalization). However, the event should still be captured as a nonserious AE on the appropriate eCRF page.
- Events of progression of the patient's underlying cancer as well as events clearly related to progression of the patient's cancer (signs and symptoms of progression) should not be reported as a serious adverse event unless the outcome is fatal within the safety reporting period. If the event has a fatal outcome within the safety reporting period, then the event of Progression of Disease must be recorded as an AE and as a SAE with CTCAE Grade 5 (fatal outcome) indicated.

8.1.3 Progression of malignancy

Progression of a patient's malignancy should not be considered an AE or SAE, unless in the investigator's opinion, study treatment resulted in an exacerbation of the patient's condition. If disease progression results in death or hospitalization while on study or within 100 days of the last dose, progressive disease will be considered an SAE.

8.1.4 Life-threatening events

A life-threatening event is any AE that places the patient at immediate risk of death from the reaction as it occurs. It is not a reaction that had it occurred in a more severe form, might have caused death.

8.1.5 Hospitalization or prolongation of hospitalization

Hospitalization encompasses any inpatient admission (even for less than 24 hours) resulting from a precipitating, treatment-emergent adverse event. For chronic or long-term patients, inpatient admission also includes transfer within the hospital to an acute or intensive care inpatient unit. Hospitalizations for administrative reasons or a non-worsening preexisting condition should not be considered AEs (e.g. admission for workup of a persistent pretreatment laboratory abnormality, yearly physical exam, protocol-specified admission, elective surgery). Preplanned

treatments or surgical procedures should be noted in the baseline documentation. Hospitalization because of an unplanned event will be deemed an SAE.

Prolongation of hospitalization is any extension of an inpatient hospitalization beyond the stay anticipated or required for the original reason for admission.

8.1.6 Significant disability

Disability is a substantial disruption of the patient's ability to conduct normal life functions.

8.1.7 Pregnancy

Male participants should refrain from fathering a child or donating sperm during the study and for 7 months following the last dose of nivolumab.

Pregnancy of the patient's partners is not considered to be an adverse event. However, all pregnancies must be reported and submitted to BMS within 24 hours/ 1 business day of becoming aware of the event. BMS will perform due diligence follow-up using the BMS Pregnancy Form which the investigator must complete.

8.1.8 Medical significance

An event that is not fatal or life-threatening and that does not necessitate hospitalization may be considered serious if, in the opinion of the investigator, it jeopardizes the patient's status and might lead to medical or surgical intervention to prevent any of the outcomes described in section 7.1.2. Such medically significant events could include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias that do not result in inpatient hospitalization, or the development of drug dependency or abuse.

8.1.9 Deaths

All deaths that occur during the study, or within the protocol-defined 100-day post-study follow-up period after the administration of the last dose of study treatment, must be reported as follows:

- Death that is clearly the result of disease progression should be reported to the study monitor at the next monitoring visit, be documented, and reported as an SAE.
- Where death is not due (or not clearly due) to progression of the disease under study, the AE causing the death must be reported to the study monitor as a SAE within **24 hours**. The report should contain a comment regarding the co-involvement of progression of disease, if appropriate, and should assign main and contributory causes of death.
- Deaths with an unknown cause should be reported as a SAE., but followup should be done to determine the cause of death. If a cause of death is determined, the event term of "death" must be updated at that time. A post mortem maybe helpful in the assessment of the cause of death, and if performed a copy of the post-mortem results should be forwarded to BMS within the usual timeframes.

The most recent version of the NCI CTCAE v.5.0 handbook will be used for adverse event descriptions and grading.

All AEs (including SAEs and AESIs) occurring during the study are to be followed up in accordance with good medical practice until resolved; judged no longer clinically significant; or, if a chronic condition, until fully characterized through 100 days after the last dose of study drug. Any SAEs, AESIs, and treatment-related Grade 3/4 AEs must be followed until resolution or stabilization, or until lost to follow-up. After the 100-day window, treatment-related SAEs and all AESIs, which are believed to be related to the study drug or protocol-specific procedure, should be reported..

8.2 Expectedness

Adverse events can be considered, "expected," or, "unexpected."

8.2.1 Expected Adverse Events

Expected adverse events are those that have been previously identified as resulting from administration of the agent. An adverse event can be considered expected when it appears in the same nature severity and specificity as what is in the current adverse event list of the Investigator's Brochure.

8.2.2 Unexpected Adverse Events

An adverse event can be considered unexpected when the nature, intensity or frequency of which is not consistent with the current adverse event list of the Investigator's Brochure, contact the lead site, principal investigator or sponsor to confirm unexpected adverse events when necessary.

8.3 Recording and Grading

8.3.1 Recording

All observed or volunteered adverse events, regardless of treatment group, severity, suspected causal relationship, expectedness, or seriousness will be documented.

A clinically significant change in a physical examination finding or an abnormal test result (i.e., laboratory, X-ray, EKG) should be recorded as an AE, if it:

- Is associated with accompanying symptoms.
- Is suggestive of organ toxicity.
- Requires additional diagnostic testing or medical or surgical intervention.
- Leads to a change in study dosing or discontinuation from the study.
- Is considered clinically significant by the investigator.

An abnormal test result that is subsequently determined to be in error does not require recording as an adverse event, even if it originally met one or more of the above criteria.

8.3.2 Grading severity

All adverse events will be graded for intensity on a scale of 0 to 5. Severity grades will be recorded and based on the most recent version of the NCI CTCAE v.5.0 handbook.

8.3.3 Attributing causality

Medical judgment should be used to determine the cause of the AE considering all relevant factors such as, but not limited to, the underlying study indication, coexisting disease, concomitant medication, relevant history, pattern of the AE, temporal relationship to the study medication, dechallenge or rechallenge with the study drug (Table 2).

Not Related To Study Drug	• An AE that is clearly due to extraneous causes (e.g. concurrent disease, concomitant medications, disease under study, etc.) • It does not follow a reasonable temporal sequence from administration of the study drug. • It does not follow a known pattern of response to study drug • It does not reappear or worsen when study drug is restarted. • An alternative explanation is likely, but not clearly identifiable.
Related to Study Drug	• An AE that is difficult to assign to alternative causes. • It follows a strong or reasonable temporal sequence from administration of study drug. • It could not be reasonably explained by the patient's clinical state, concurrent disease, or other concomitant therapy administered to the patient. • It follows a known response pattern to study drug. • It is confirmed with a positive rechallenge or supporting laboratory data.

Table 2. *Relationship of Adverse Event to Study Drug*

8.4 Reporting Adverse Events

8.4.1 Reporting serious adverse events

Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, must be collected, including those thought to be associated with protocol-specified procedures. All SAEs must be collected that occur within 100 days of discontinuation of dosing.

All SAEs must be collected that occur during the screening period. If applicable, SAEs must be collected that relate to any protocol-specified procedure (e.g. a follow-up skin biopsy). The investigator should report any SAE that occurs after these time periods that is believed to be related to study drug or protocol-specified procedure.

All SAEs that occur following the subject's written consent to participate in the study through 100 days of discontinuation of dosing must be reported to BMS Worldwide Safety, (Email: *worldwide.safety@BMS.com*; Fax: 1(609) 818-3804) whether related or not related to study drug. If applicable, SAEs must be collected that relate to any later protocol-specified procedure (e.g. a follow-up skin biopsy).

An appropriate SAE form (e.g. ex-US = CIOMS form or USA = Medwatch form) should be used to report SAEs to BMS. The BMS protocol ID number must be included on whatever form is submitted by the Sponsor/Investigator.

- The CIOMS form is available at: http://www.cioms.ch/index.php/cioms-form-i
- The MedWatch form is available at: https://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM048334.pdf

SAEs, whether related or not related to study drug, and pregnancies must be reported to BMS within 24 hours. SAEs must be recorded on either CIOMS or MedWatch form & pregnancies must be reported on a Pregnancy Surveillance Form or can be submitted on the aforementioned SAE form to BMS.

IND application sponsors are required to notify FDA in a written safety report of:

- Any adverse experience associated with the use of the drug that is both serious and unexpected or,
- Any findings from tests in laboratory animals that suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, and carcinogenicity.

Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

Adverse reaction means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions where there is reason to conclude that the drug caused the event.

Unexpected adverse event or suspected adverse reaction refers to an event or reaction that is not listed in the investigator's brochure or is not listed at the specificity or severity that has been observed; or, if an investigator's brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current IND application.

Serious adverse event or suspected adverse reaction refers to an event or reaction that, in the view of either the investigator or sponsor, results in any of the following outcomes:

- Death.
- A life-threatening adverse event.
- In-patient hospitalization or prolongation of existing hospitalization.

- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly or birth defect.

A life-threatening adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or research subject and may require medical or surgical intervention to prevent one of the outcomes listed as serious.

Mandatory Safety Reporting

- Initial reporting: IND application sponsor must report any suspected adverse reaction or adverse reaction to study treatment that is both serious and unexpected.

  Unexpected serious suspected adverse reactions suggesting significant risk to human subjects must be reported to FDA as soon as possible but no later than within 15 calendar days following the sponsor's initial receipt of the information.

  Unexpected fatal or life-threatening suspected adverse reactions represent especially important safety information and must be reported to FDA as soon as possible but no later than 7 calendar days following the sponsor's initial receipt of the information.

- Follow-up reporting: Any relevant additional information obtained by the sponsor that pertains to a previously submitted IND safety report must be submitted as a Follow-up IND Safety Report. Such report should be submitted without delay, as soon as the information is available but no later than 15 calendar days after the sponsor receives the information.

  All IND safety reports must be submitted on Form 3500A and be accompanied by Form 1571. The type of report (initial or follow-up) should be checked in the respective boxes on Forms 3500A and 1571.

The submission must be identified as:

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

The report must be submitted to an appropriate Review division that has the responsibility to review the IND application under which the safety report is

submitted. Each submission to this IND must be provided in triplicate (original plus two copies). Send all submissions to the following address:

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

8.4.2 Reporting SAEs at multi-site/participating institutions
SAEs should be reported to the lead site and BMS.

9. CRITERIA FOR OUTCOME ASSESSMENT/THERAPEUTIC RESPONSE

9.1 Outcome Assessment

All baseline evaluations will be performed as closely as possible to the beginning of treatment (within 7 days). For subsequent evaluations, the method of assessment and techniques will be the same as those used at baseline.

- Tumor markers.
  PSA measurements will be used to assess the primary endpoint.

9.1.1 Primary endpoint
The primary endpoint is defined as a PSA_{50} response, defined as a ≥ 50% decline in PSA from baseline, confirmed with a second measurement at least 4 weeks later.

9.1.2 Secondary endpoints

9.1.2.1. Safety
This endpoint is defined as incidence of grade 3-5 toxicities based upon CTCAE v.5.0 standard grading scales.

9.1.2.2. PSA Progression-Free Survival (PFS)
PSA progression (PSA progression-free survival; PSA–PFS) will be defined per PCWG3 guidelines.

For those subjects showing an initial decline in PSA from baseline, this is defined as an increase in PSA that is ≥ 25% above the nadir, an absolute increase of ≥ 2ng/ml from nadir,which is confirmed by a second value ≥ 3 weeks later (i.e. a confirmed rising trend).

For those subjects with no decline in PSA from baseline, this is defined as an increase in PSA that is ≥ 25% and an absolute increase of ≥ 2ng/ml after 12 weeks.

Patients will be allowed to stay on study beyond PSA progression, as long as they do not have evidence of radiographic or clinical progression as determined by their treating physician.

9.1.2.3. Undectable PSA

This endpoint represents a durable complete response to therapy. It is defined as a PSA of <0.1 confirmed with a repeat measurement at least 12 weeks later.

9.1.2.4 *Metastasis-free survival*

Metastasis-free survival will be defined at the time from the first dose of nivolumab until the development of radiographic metastatic disease as determined by CT imaging and/or bone scan.

9.1.2.5 *Time to initiation of next systemic therapy*

Time to initiation of next systemic therapy will be defined as the time from the first dose of nivolumab until the next systemic therapy is initiated.

9.2 Therapeutic Response

Response and progression will be evaluated in this study using a combination of the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST1.1) Committee(33) and the guidelines for prostate cancer endpoints developed by the Prostate Cancer Clinical Trials Working Group (PCWG3).(2)

Patients will need to be reevaluated for response every cycle according to the guidelines below.

9.2.1 PSA

Perform PSA testing at a minimum of 1-week intervals with the threshold PSA level at 1.0 ng/mL. To report PSA-based outcomes, PCWG3 recommends that the percent of change in PSA from baseline to 12 weeks (or earlier for those who discontinue therapy) and the maximum decline in PSA that occurs at any point after treatment be reported for each patient using a waterfall plot. PSA measurements obtained during the first 12 weeks should not be used as the sole criterion for clinical decision making.

9.3 Response Criteria for Primary and Secondary Endpoints

9.3.1 PSA

For each patient, use a waterfall plot to report the percent change in PSA from baseline to 12 weeks (or earlier for those who discontinue therapy) and the maximum decline in PSA that occurs at any point after treatment. We will also report the proportion of patients to achieve a 50% or greater decrease in PSA from baseline (i.e. PSA_{50} response rate).

9.4 Criteria for Progressive Disease.

9.4.1 Radiographic Progression

Radiographic progression is defined at the development of metastatic disease observed on CT and/or Bone Scan.

10. DATA REPORTING AND REGULATORY REQUIREMENTS

Multicenter Guidelines
The Protocol Chair

The Protocol Chair, Dr. Mark Markowski is responsible for performing the following tasks:

- Coordinating, developing, submitting, and obtaining approval for the protocol as well as its subsequent amendments.
- Assuring that all participating institutions are using the correct version of the protocol.
- Taking responsibility for the overall conduct of the study at all participating institutions and for monitoring the progress of the study.
- Reviewing and ensuring reporting of Serious Adverse Events (SAEs).
- Reviewing data from all sites.

Lead Center

The Lead Center (Johns Hopkins University) is responsible for performing the following tasks:

- Ensuring that IRB approval has been obtained at each participating site prior to the first patient registration at that site, and maintaining copies of IRB approvals from each site.
- Managing central patient registration.
- Collecting and compiling data from each site.
- Establishing procedures for documentation, reporting and submitting of AE's and SAE's to the Protocol Chair and all other applicable parties.
- Facilitating audits by securing selected source documents and research records from participating sites for audit, or by auditing at participating sites.

Participating PCCTC Sites

Participating sites are responsible for performing the following tasks:

- Following the protocol as written, and the guidelines of Good Clinical Practice (GCP).
- Submitting data to the Lead Center.
- Registering all patients with the Lead Center by submitting patient registration form, and signed informed consent promptly.
- Providing sufficient experienced clinical and administrative staff and adequate facilities and equipment to conduct a collaborative trial according to the protocol.
- Maintaining regulatory binders on site and providing copies of all required documents to the Lead Center.
- Collecting and submitting data according to the schedule specified by the protocol.

10.1 Data Entry

Data collected during this study will be entered into a secure database. Staff at Johns Hopkins University will be responsible for the initial study configuration and setup in the CRMS database and for any future changes.

10.1.1 Case report forms completion

Electronic Case report forms will be generated by the coordinating center for the collection of all study data. Investigators will be responsible for ensuring that the CRFs are kept up-to-date.

The paper Eligibility Checklist CRF must be completed using black ink. Any errors must be crossed out so that the original entry is still visible, the correction clearly indicated and then initialed and dated by the individual making the correction.

eCRFs will be completed within 2 weeks of the patient coming to the clinic and all relevant supporting documentation such as scans, progress notes, nursing notes, blood work, pathology reports, etc., will be submitted via email to the SKCCC Coordinating Center Study Manager. All patient names or other identifying information will be removed prior to being sent to the Coordinating Center (SKCCC) or non-redacted source documents can be sent via a password-protected/secured document transfer based on each institution's guidelines.

Authorized representatives of the Coordinating Center (SKCCC) may visit the satellite sites to perform audits or inspections, including source data verification. The purpose of these audits or inspections is to systematically and independently examine all trial-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice (GCP), and any applicable regulatory requirements.

10.1.2 Source documents

Study personnel will record clinical data in each patient's source documents (i.e. the patient's medical record). Source documentation will be made available to support the patient research record. Study monitors will review entries on the CRFs at regular intervals, comparing the content with source documents.

10.1.3 Record retention

The investigator will maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. After study closure, the investigator will maintain all source documents, study-related documents, and the CRFs. Because the length of time required for retaining records depends upon a number of regulatory and legal factors, documents should be stored until the investigator is notified that the documents may be destroyed. In this study, records are to be retained and securely stored for a minimum of 5 years after the completion of all study activities.

10.2 Data Management

10.2.1 Lead research program coordinators

A Lead research program coordinator at the coordinating center will be assigned to the study. A Lead Research Program Coordinator will manage the study activities at each of the participating sites. The responsibilities of the Lead Research Program Coordinator include project compliance, data collection, data entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordination of the activities of the protocol team.

10.3 Study Monitoring and Quality Assurance

Regularly scheduled registration reports will be generated to monitor patient accruals and the completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and the extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period, and potential problems will be brought to the attention of the principal investigator for discussion and action.

Random-sample data quality and protocol compliance audits will be conducted by the study team at least once a year, more frequently if indicated. Audits by the coordinating center may entail (1) shipping source documents and research records for selected patients from participating sites to the coordinating center for audit, or (2) on-site auditing of selected patient records at participating sites.

All clinical work conducted under this protocol is subject to Good Clinical Practice (GCP) guidelines. This includes inspection of study-related records by the lead site, sponsor, its designee, or health authority representatives at any time.

Data and Safety Monitoring:

The SKCCC Compliance Monitoring Program will provide external monitoring for JHU affiliated sites in accordance with SKCCC DSMP (Version 6.0, 02/21/2019). The SMC Subcommittee will determine the level of patient safety risk and level/frequency of monitoring. Additionally, scheduled meetings will take place monthly and will include the protocol principal investigator, research nurse, data manager, and, when appropriate, the collaborators, subinvestigators, and biostatistician involved with the conduct of the protocol.

Lead site/sponsor will conduct a quarterly reconciliation of SAEs with the BMS safety database by submitting an email request to aepbusinessprocess@bms.com and requesting the SAE reconciliation report followed by communication of any findings.

During these meetings the investigators will discuss matters related to: safety of protocol participants, validity and integrity of the data, enrollment rate relative to expectation, characteristics of participants, retention of participants, adherence to protocol (potential or real protocol violations), data completeness, and progress of data for secondary objectives.

10.4 Clinical Trial Agreement

This trial is being conducted under one or more clinical trial agreements that contain, among other terms, the publication policy, indemnity agreements, and financial arrangements for the study.

11. STATISTICAL CONSIDERATIONS

The sample size of 15 patients will provide a prelimimary evaluation of clinical activity based on PSA response. Two-sided 95% Wilson confidence interval for various scenrios of PSA_{50} response rate is presented in the table below:

Number of PSA_{50} response	Observed PSA_{50} response rate	95% confidence interval
3	20%	7.0 – 45.2%
4	26.7%	10.9 – 52.0%
5	33.3%	15.2 – 58.3%
6	40%	19.8 – 64.3%
7	46.7%	24.8 – 69.9%
8	53.3%	30.1 – 75.2%
9	60%	35.7 – 80.2%
10	66.7%	41.7 – 84.8%
11	73.3%	48.0 – 89.1%
12	80%	54.8 – 93.0%

11.1 Study Endpoints

11.1.1 Analysis of the primary endpoint

The primary endpoint of this study is PSA_{50} response, defined as a decrease in the PSA to ≥ 50% less than the baseline PSA upon enrollment in the trial. The decrease must be confirmed by a second measurement at least 4 weeks apart. For purposes of meeting the primary endpoint, patients will be considered to have done so if they have a PSA_{50} response while on nivolumab. PSA values will be measured monthly during the trial. All patients who take at least one dose of nivolumab will be considered evaluable for the primary endpoint. If patients do not have at least one follow-up PSA after initiation of nivolumab due to stopping therapy for toxicity or withdrawing consent, then they will be replaced. PSA_{50} response rate will be estimated along with 95% confidence interval.

We will estimate the PSA_{50} response rate, along with the exact 95% confidence interval, for the population of patients.

11.1.2 Analysis of secondary endpoints

11.1.2.1. Safety

Patients will be assessed for toxicities at each clinical evaluation. Toxicities will be graded according to CTCAE v.5.0 standardized grading scales. The incidence of

grade 3-5 toxicities will be reported. Patients will be assessed for toxicity as long as they are taking nivolumab, and patients will continue to be followed if treatment is discontinued for toxicity until the toxicities improve to grade 1 or resolve.

Toxicities will be reported as a tabulated table by type and grade.

11.1.2.2. PSA progression-free survival (PSA-PFS)
A standard definition of PSA progression per PCWG3 will be used. PSA PFS will be defined as an increase in 25% over a nadir value, confirmed by a follow-up PSA at least 4 weeks apart. If patients are removed from study prior to PSA progression, then they will be censored at that time.

We will use the Kaplan-Meier method to summarize the PSA PFS.

11.1.2.3. Undetectable PSA
Undetectable PSA will be defined as the proportion of patients that achieve a PSA <0.1 ng/ml last $\geq$ 12 weeks.

11.1.2.4 Metastasis-free survival
Metastasis-free survival will be defined at the time from the first dose of nivolumab until the development of radiographic metastatic disease as determined by CT imaging and/or bone scan. If a patient is removed from the study prior to the development of metastatic disease, they will be censored at that time.

We will use the Kaplan-Meier method to summarize the metastasis-free survival.

11.1.2.5 Time to initiation of next systemic therapy
Time to initiation of next systemic therapy will be defined as the time from the first dose of nivolumab until the next systemic therapy is initiated. For patients that withdraw consent or are lost to follow-up, they will be censored at the time of last follow-up.

Will use the Kaplan-Meier method to summarize the time to initiation of next systemic therapy.

11.1.3 Analysis of exploratory endpoints

11.1.3.1 PD-1, PD-L1, CD4, CD8, FOXP3, MSH2, MSH6, MLH1, PSM2, CDK12 protein levels:
Archival tumor specimens including prostatectomy or needle biopsy of primary tumor, will be analyzed. IHC staining for PD-1 and PD-L1 will be performed and scored as <1%, 1-5%, 5-10%, >10% and the results will be associated with responses for both proteins, separately, using descriptive statistics and Fisher's Exact Tests. IHC will be used to quantify the CD4, CD8, and FOXP3 levels. Using analytical microscopy, the lead pathologist, Dr. Tomara Lotan can quantify the number of T cells per surface area of tumor analyzed. The median number of CD4+ and CD8+ intratumor lymphocytes will be compared in PSA_{50} responders versus non-responders using a T-test. We will also correlate CD4+ and CD8+ lymphocyte tumor infiltration in patients that obtain an undetectable PSA versus those with

progressive disease as best response. Lastly, we will correlate response to nivolumab with MMR and CDK12 mutational status.

11.1.3.2 Interrogate for tumor-associated neoantigens (TAAs):

Whole-exome and RNA sequencing will be performed on archival tissue. Exome data will be applied in a neoantigen prediction pipeline that evaluates antigen processing, MHC binding and gene expression to generate neoantigens specific to the patient's HLA haplotype. TCR seuqnecing will also be performed on intratumor T cells as well as T cells in the peripheral blood. T cells will be collected prior to therapy, after 1 and 3 cycles of nivolumab and progression. Truncal neoantigens will be identified by correcting for tumor purity and ploidy. Putative neoantigens will then be used to generate peptides and stimulate autologous T cells.

11.1.3.3 Interrogate for auto antigens mimicking TAAs:

Peripheral blood samples will be processed using Phase ImmunoPrecipitation Sequencing (PhIP-seq). A human 90-mer peptidome library which encodes 259,346 peptides that tile the complete human proteome has been developed. The oligonucleotide library was printed on a releasable DNA microarray, and cloned into a T7 phage display system. The phage library is mixed with samples containing antibodies, and antibody-bound phage are captured on protein A/G coated magnetic beads. DNA from the enriched phage population is recovered and inserts are PCR amplified with sequencing adapters and sample-specific barcodes for deep DNA sequencing analysis.

11.2 Analysis Populations

11.2.1 Intent-to-treat/Response-to-treatment/Evaluable population

All patients who meet eligibility criteria and receive at least 1 dose of nivolumab will be included in the main analysis of the response rate, even if there are major protocol deviations (e.g. incorrect treatment schedule or drug administration).

Conclusions are to be based on the population of all eligible patients. Subanalyses may be performed on various subsets of patients, such as those with no major protocol deviations or those who continued in the study for the entire treatment period (i.e. did not withdraw prematurely). Subanalysis will not serve as the basis for drawing conclusions concerning treatment efficacy.

11.2.2 Safety population

All patients enrolled in the study will be included in the safety analysis population and considered evaluable for toxicity and safety from the time of their first dose. Demographic and baseline characteristics for the safety population will be summarized by number and percent for categorical data (e.g. sex, race/ethnicity) and by descriptive statistics for continuous data (e.g. weight, vital signs, EKG readings, disease status).

11.3 Safety Analysis

11.3.1 Evaluation of adverse events

Treatment-emergent adverse events will be translated from investigator terms to MedDRA v.20.1 terminology and summarized (number and percentage of patients) for all patients who receive at least 1 dose. Adverse event summaries will be organized by body system, frequency of occurrence, intensity (i.e. severity grade), and causality or attribution. Patients who experience an adverse event more than once will be counted only once. The occurrence with the maximum severity will be used to calculate intensity.

11.3.2 Evaluation of serious adverse events and premature withdrawals

Adverse events deemed serious and those resulting in treatment withdrawal or death will be summarized separately. Narrative paragraphs will be generated to describe the circumstances surrounding each SAE and death.

11.3.3 Evaluation of laboratory parameters and assays

Selected clinical laboratory parameters will be summarized and clinically significant changes from baseline will be discussed.

11.3.4 Extent of exposure

Treatment exposure will be summarized for all patients, including dose administration, number of cycles, dose modifications or delays, and duration of therapy.

12. PROTECTION OF HUMAN SUBJECTS

12.1 Ethical Considerations

This study will be conducted in compliance with the protocol, GCP guidelines established by the International Conference on Harmonization, and the ethical standards set forth in the Declaration of Helsinki 2004 (available at: www.laakariliitto.fi/e/ethics/helsinki.html).

12.2 Protocol Amendments

Before starting the study, the protocol must be approved by each institution's IRB or Independent Ethics Committee (IEC). Amendments to the protocol may be made only with consent of the lead site/sponsor and principal investigator and are subject to IRB approval before instituting.

12.3 Written Informed Consent

Before obtaining consent, members of the study team will review the rationale for the treatment program with the patient. The discussion will review the alternatives available (including hormonal therapy, chemotherapy, or supportive care as appropriate), the potential benefits of this program, the risks and the probability of their occurrence, and the procedures to minimize these risks. Should an adverse event occur, the provisions available to ensure medical intervention will also be reviewed. Why the risks are reasonable in relation to the anticipated benefits, incentives, or costs that will or may be incurred as a result of participating in the study, as well as the efforts to maintain confidentiality, will also be discussed with the patient.

Patients will be required to sign and date (in duplicate) a statement of informed consent that meets the requirements of the Code of Federal Regulations (Federal Register Vol. 46, No. 17, January 27, 1981, part 50) and the IRB. The medical record will include a statement that written informed consent was obtained (and document the date that it was obtained) before the patient is enrolled in the study. The original signed document will become part of the patient's medical record, a copy will be forwarded to the lead site/sponsor pursuant to sponsor registration, and a copy will be sent home with each patient.

The consent form will include the following:

- The nature and objectives, potential toxicities, and benefits of the intended study.
- The length of therapy and likely follow-up required.
- Alternatives to the proposed therapy (including available standard and investigational therapies).
- The name of the investigator(s) responsible for the protocol.
- The right of the patient to accept or refuse treatment and to withdraw from participation in this study.
- Text regarding the coordinating center should be added to all institutional informed consent documents.

12.4 Protection of Privacy

Patients will be informed of the extent to which their confidential health information generated from this study may be used for research purposes. After this discussion, they will be asked to sign a Notice of Privacy Practice research authorization/HIPAA form. The original signed documents will become part of the patient's medical records, and each patient will receive a copy of the signed documents. The use and disclosure of protected health information will be limited to the individuals described in the research authorization form. The research authorization form must be completed by the principal investigator and approved by the IRB.

12.5 Terminating or Modifying the Study

Adverse event and laboratory data from this trial will be assessed by the medical monitor (Dr. Mark Markowski) on an ongoing basis. At least quarterly, data from the clinical database will be reviewed. The results of this review will be shared with all investigators either in writing or as part of a teleconference. SAEs will be reviewed as they are reported to the lead site/sponsor, and the medical monitor will make an assessment regarding the safety of continuing or modifying the study. This assessment will be shared with the investigators either in writing or as part of a teleconference as well as with BMS. Should the assessment of either the lead site/sponsor or the principal investigator be that the study should be terminated, the study will be closed to further accrual. Patients who are receiving treatment will be assessed individually by the investigator to see if it is in the patients' best interest to continue, which might be the case for a patient that is responding to the intervention. Follow-up safety assessments will be performed for all patients who are terminated from the study prematurely. Any planned data disclosures will also be discussed with BMS.

13. REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA: a cancer journal for clinicians 2016;66(1):7-30.
2. Scher HI, Morris MJ, Stadler WM, Higano C, Basch E, Fizazi K, Antonarakis ES, Beer TM, Carducci MA, Chi KN, Corn PG, de Bono JS, Dreicer R, George DJ, Heath EI, Hussain M, Kelly WK, Liu G, Logothetis C, Nanus D, Stein MN, Rathkopf DE, Slovin SF, Ryan CJ, Sartor O, Small EJ, Smith MR, Sternberg CN, Taplin ME, Wilding G, Nelson PS, Schwartz LH, Halabi S, Kantoff PW, Armstrong AJ, Prostate Cancer Clinical Trials Working G. Trial Design and Objectives for Castration-Resistant Prostate Cancer: Updated Recommendations From the Prostate Cancer Clinical Trials Working Group 3. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2016;34(12):1402-1418.
3. Bubley GJ, Carducci M, Dahut W, Dawson N, Daliani D, Eisenberger M, Figg WD, Freidlin B, Halabi S, Hudes G, Hussain M, Kaplan R, Myers C, Oh W, Petrylak DP, Reed E, Roth B, Sartor O, Scher H, Simons J, Sinibaldi V, Small EJ, Smith MR, Trump DL, Vollmer R, Wilding G. Eligibility and response guidelines for phase II clinical trials in androgen-independent prostate cancer: Recommendations from the prostate-specific antigen working group. J Clin Oncol 1999;17(11):3461-3467.
4. Stephenson AJ, Scardino PT, Kattan MW, Pisansky TM, Slawin KM, Klein EA, Anscher MS, Michalski JM, Sandler HM, Lin DW, Forman JD, Zelefsky MJ, Kestin LL, Roehrborn CG, Catton CN, DeWeese TL, Liauw SL, Valicenti RK, Kuban DA, Pollack A. Predicting the outcome of salvage radiation therapy for recurrent prostate cancer after radical prostatectomy. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2007;25(15):2035-2041.
5. Nguyen T, Boldt RG, Rodrigues G. Prognostic Factors for Prostate Cancer Endpoints Following Biochemical Failure: A Review of the Literature. Cureus 2015;7(1):e238.
6. Antonarakis ES, Feng ZY, Trock BJ, Humphreys EB, Carducci MA, Partin AW, Walsh PC, Eisenberger MA. The natural history of metastatic progression in men with prostate-specific antigen recurrence after radical prostatectomy: long-term follow-up. Bju Int 2012;109(1):32-39.
7. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, Roche PC, Lu J, Zhu G, Tamada K, Lennon VA, Celis E, Chen L. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nature medicine 2002;8(8):793-800.
8. Wintterle S, Schreiner B, Mitsdoerffer M, Schneider D, Chen L, Meyermann R, Weller M, Wiendl H. Expression of the B7-related molecule B7-H1 by glioma cells: a potential mechanism of immune paralysis. Cancer research 2003;63(21):7462-7467.
9. Dong H, Chen L. B7-H1 pathway and its role in the evasion of tumor immunity. Journal of molecular medicine 2003;81(5):281-287.
10. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nature reviews Cancer 2012;12(4):252-264.
11. Thompson RH, Gillett MD, Cheville JC, Lohse CM, Dong H, Webster WS, Krejci KG, Lobo JR, Sengupta S, Chen L, Zincke H, Blute ML, Strome SE, Leibovich BC, Kwon ED. Costimulatory B7-H1 in renal cell carcinoma patients: Indicator of tumor aggressiveness and potential therapeutic target. Proceedings of the National Academy of Sciences of the United States of America 2004;101(49):17174-17179.
12. Thompson RH, Gillett MD, Cheville JC, Lohse CM, Dong H, Webster WS, Chen L, Zincke H, Blute ML, Leibovich BC, Kwon ED. Costimulatory molecule B7-H1 in primary and metastatic clear cell renal cell carcinoma. Cancer 2005;104(10):2084-2091.

13. Thompson RH, Kuntz SM, Leibovich BC, Dong H, Lohse CM, Webster WS, Sengupta S, Frank I, Parker AS, Zincke H, Blute ML, Sebo TJ, Cheville JC, Kwon ED. Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up. Cancer research 2006;66(7):3381-3385.
14. Ohigashi Y, Sho M, Yamada Y, Tsurui Y, Hamada K, Ikeda N, Mizuno T, Yoriki R, Kashizuka H, Yane K, Tsushima F, Otsuki N, Yagita H, Azuma M, Nakajima Y. Clinical significance of programmed death-1 ligand-1 and programmed death-1 ligand-2 expression in human esophageal cancer. Clinical cancer research : an official journal of the American Association for Cancer Research 2005;11(8):2947-2953.
15. Wu C, Zhu Y, Jiang J, Zhao J, Zhang XG, Xu N. Immunohistochemical localization of programmed death-1 ligand-1 (PD-L1) in gastric carcinoma and its clinical significance. Acta histochemica 2006;108(1):19-24.
16. Nomi T, Sho M, Akahori T, Hamada K, Kubo A, Kanehiro H, Nakamura S, Enomoto K, Yagita H, Azuma M, Nakajima Y. Clinical significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer. Clinical cancer research : an official journal of the American Association for Cancer Research 2007;13(7):2151-2157.
17. Zitvogel L, Tesniere A, Kroemer G. Cancer despite immunosurveillance: immunoselection and immunosubversion. Nature reviews Immunology 2006;6(10):715-727.
18. Taube JM, Anders RA, Young GD, Xu H, Sharma R, McMiller TL, Chen S, Klein AP, Pardoll DM, Topalian SL, Chen L. Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Science translational medicine 2012;4(127):127ra137.
19. Iwai Y, Terawaki S, Honjo T. PD-1 blockade inhibits hematogenous spread of poorly immunogenic tumor cells by enhanced recruitment of effector T cells. International immunology 2005;17(2):133-144.
20. Hirano F, Kaneko K, Tamura H, Dong H, Wang S, Ichikawa M, Rietz C, Flies DB, Lau JS, Zhu G, Tamada K, Chen L. Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. Cancer research 2005;65(3):1089-1096.
21. Li B, VanRoey M, Wang C, Chen TH, Korman A, Jooss K. Anti-programmed death-1 synergizes with granulocyte macrophage colony-stimulating factor--secreting tumor cell immunotherapy providing therapeutic benefit to mice with established tumors. Clinical cancer research : an official journal of the American Association for Cancer Research 2009;15(5):1623-1634.
22. Curran MA, Montalvo W, Yagita H, Allison JP. PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. Proceedings of the National Academy of Sciences of the United States of America 2010;107(9):4275-4280.
23. Beer TM, Kwon ED, Drake CG, Fizazi K, Logothetis C, Gravis G, Ganju V, Polikoff J, Saad F, Humanski P, Piulats JM, Gonzalez Mella P, Ng SS, Jaeger D, Parnis FX, Franke FA, Puente J, Carvajal R, Sengelov L, McHenry MB, Varma A, van den Eertwegh AJ, Gerritsen W. Randomized, Double-Blind, Phase III Trial of Ipilimumab Versus Placebo in Asymptomatic or Minimally Symptomatic Patients With Metastatic Chemotherapy-Naive Castration-Resistant Prostate Cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2017;35(1):40-47.
24. Kwon ED, Drake CG, Scher HI, Fizazi K, Bossi A, van den Eertwegh AJ, Krainer M, Houede N, Santos R, Mahammedi H, Ng S, Maio M, Franke FA, Sundar S, Agarwal N, Bergman AM, Ciuleanu TE, Korbenfeld E, Sengelov L, Hansen S, Logothetis C, Beer TM, McHenry MB,

Gagnier P, Liu D, Gerritsen WR, Investigators CA. Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): a multicentre, randomised, double-blind, phase 3 trial. The lancet oncology 2014;15(7):700-712.

25. Graff JN, Alumkal JJ, Drake CG, Thomas GV, Redmond WL, Farhad M, Cetnar JP, Ey FS, Bergan RC, Slottke R, Beer TM. Early evidence of anti-PD-1 activity in enzalutamide-resistant prostate cancer. Oncotarget 2016;7(33):52810-52817.
26. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Duffy SM, Goldberg RM, de la Chapelle A, Koshiji M, Bhaijee F, Huebner T, Hruban RH, Wood LD, Cuka N, Pardoll DM, Papadopoulos N, Kinzler KW, Zhou S, Cornish TC, Taube JM, Anders RA, Eshleman JR, Vogelstein B, Diaz LA, Jr. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. The New England journal of medicine 2015;372(26):2509-2520.
27. Cortes-Ciriano I, Lee S, Park WY, Kim TM, Park PJ. A molecular portrait of microsatellite instability across multiple cancers. Nature communications 2017;8:15180.
28. Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM, Montgomery B, Taplin ME, Pritchard CC, Attard G, Beltran H, Abida W, Bradley RK, Vinson J, Cao X, Vats P, Kunju LP, Hussain M, Feng FY, Tomlins SA, Cooney KA, Smith DC, Brennan C, Siddiqui J, Mehra R, Chen Y, Rathkopf DE, Morris MJ, Solomon SB, Durack JC, Reuter VE, Gopalan A, Gao J, Loda M, Lis RT, Bowden M, Balk SP, Gaviola G, Sougnez C, Gupta M, Yu EY, Mostaghel EA, Cheng HH, Mulcahy H, True LD, Plymate SR, Dvinge H, Ferraldeschi R, Flohr P, Miranda S, Zafeiriou Z, Tunariu N, Mateo J, Perez-Lopez R, Demichelis F, Robinson BD, Schiffman M, Nanus DM, Tagawa ST, Sigaras A, Eng KW, Elemento O, Sboner A, Heath EI, Scher HI, Pienta KJ, Kantoff P, de Bono JS, Rubin MA, Nelson PS, Garraway LA, Sawyers CL, Chinnaiyan AM. Integrative clinical genomics of advanced prostate cancer. Cell 2015;161(5):1215-1228.
29. Wu YM, Cieslik M, Lonigro RJ, Vats P, Reimers MA, Cao X, Ning Y, Wang L, Kunju LP, de Sarkar N, Heath EI, Chou J, Feng FY, Nelson PS, de Bono JS, Zou W, Montgomery B, Alva A, Team PSCIPCD, Robinson DR, Chinnaiyan AM. Inactivation of CDK12 Delineates a Distinct Immunogenic Class of Advanced Prostate Cancer. Cell 2018;173(7):1770-1782 e1714.
30. Antonarakis ES. Cyclin-Dependent Kinase 12, Immunity, and Prostate Cancer. The New England journal of medicine 2018;379(11):1087-1089.
31. Abida W, Cheng ML, Armenia J, Middha S, Autio KA, Vargas HA, Rathkopf D, Morris MJ, Danila DC, Slovin SF, Carbone E, Barnett ES, Hullings M, Hechtman JF, Zehir A, Shia J, Jonsson P, Stadler ZK, Srinivasan P, Laudone VP, Reuter V, Wolchok JD, Socci ND, Taylor BS, Berger MF, Kantoff PW, Sawyers CL, Schultz N, Solit DB, Gopalan A, Scher HI. Analysis of the Prevalence of Microsatellite Instability in Prostate Cancer and Response to Immune Checkpoint Blockade. JAMA oncology 2018.
32. Long GV, Tykodi SS, Schneider JG, Garbe C, Gravis G, Rashford M, Agrawal S, Grigoryeva E, Bello A, Roy A, Rollin L, Zhao X. Assessment of nivolumab exposure and clinical safety of 480 mg every 4 weeks flat-dosing schedule in patients with cancer. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO 2018;29(11):2208-2213.
33. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. Journal of the National Cancer Institute 2000;92(3):205-216.

APPENDIX A: MANAGEMENT ALGORITHM FOR IMMUNO-ONCOLOGY AGENTS

These general guidelines constitute guidance to the Investigator. The guidance applies to all immuno-oncology agents and regimens.

A general principle is that differential diagnoses should be diligently evaluated according to standard medical practice. Non-inflammatory etiologies should be considered and appropriately treated.

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

For subjects expected who require more than 4 weeks of corticosteroids or other immunosuppressants to manage an adverse event, consider the following recommendations:

- Antimicrobial/antifungal prophylaxis per institutional guidelines to prevent opportunistic infections such as Pneumocystis jiroveci (PJP) and fungal infections.

- Early consultation with an infectious disease specialist should be considered. Depending on the presentation, consultation with a pulmonologist for bronchoscopy or a gastroenterologist for endoscopy may also be appropriate.

- In patients who develop recurrent adverse events in the setting of ongoing or prior immunosuppressant use, an opportunistic infection should be considered in the differential diagnosis.

- Consultation with a medical or surgical specialist, especially prior to an invasive diagnostic or therapeutic procedure, is recommended.

The frequency and severity of the related adverse events covered by these algorithms will depend on the immuno-oncology agent or regimen being used.

GI Adverse Event Management Algorithm

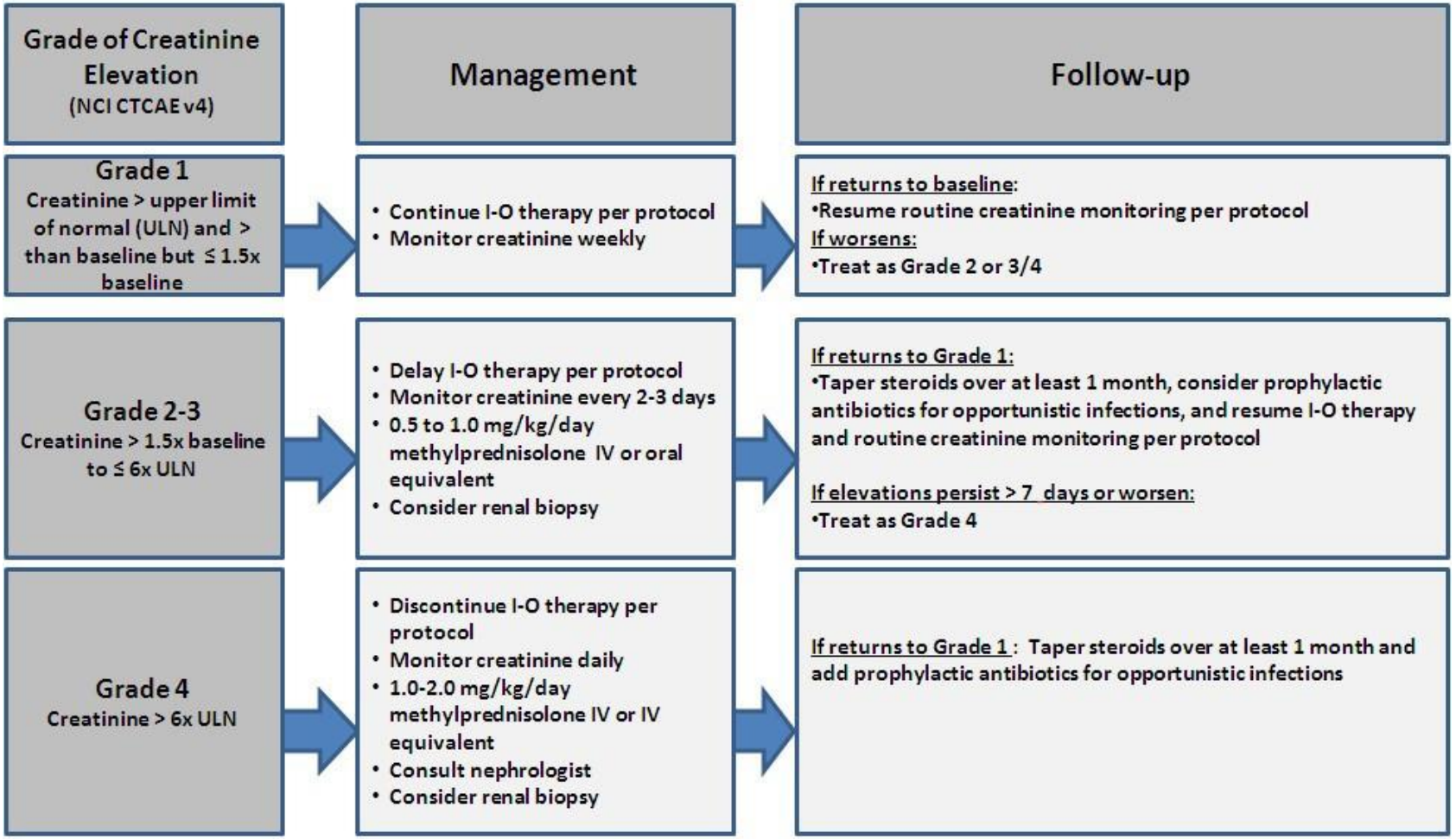
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.

Grade of Diarrhea/ Colitis (NCI CTCAE v4)	Management	Follow-up
Grade 1 Diarrhea:< 4 stools/day over baseline; Colitis: asymptomatic	• Continue I-O therapy per protocol • Symptomatic treatment	• Close monitoring for worsening symptoms. • Educate patient to report worsening immediately If worsens: • Treat as Grade (G) 2 or 3/4
Grade 2 Diarrhea: 4-6 stools per day over baseline; IV fluids indicated <24 hours (hrs); not interfering with ADL Colitis: abdominal pain; blood in stool	• Delay I-O therapy per protocol • Symptomatic treatment	If improves to grade 1: • Resume I-O therapy per protocol If persists > 5-7 days or recur: • 0.5-1.0 mg/kg/day methylprednisolone or oral equivalent • When symptoms improve to grade 1, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol. If worsens or persists > 3-5 days with oral steroids: • Treat as grade 3/4
Grade 3-4 Diarrhea (G3): ≥7 stools per day over baseline; incontinence; IV fluids ≥24 hrs; interfering with activities of daily living (ADL) Colitis (G3): severe abdominal pain, medical intervention indicated, peritoneal signs G4: life-threatening, perforation	• Discontinue I-O therapy per protocol • 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections • Consider lower endoscopy	If improves: • Continue steroids until grade 1, then taper over at least 1 month If persists > 3-5 days, or recurs after improvement: • Add infliximab 5 mg/kg (if no contraindication). Note: 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

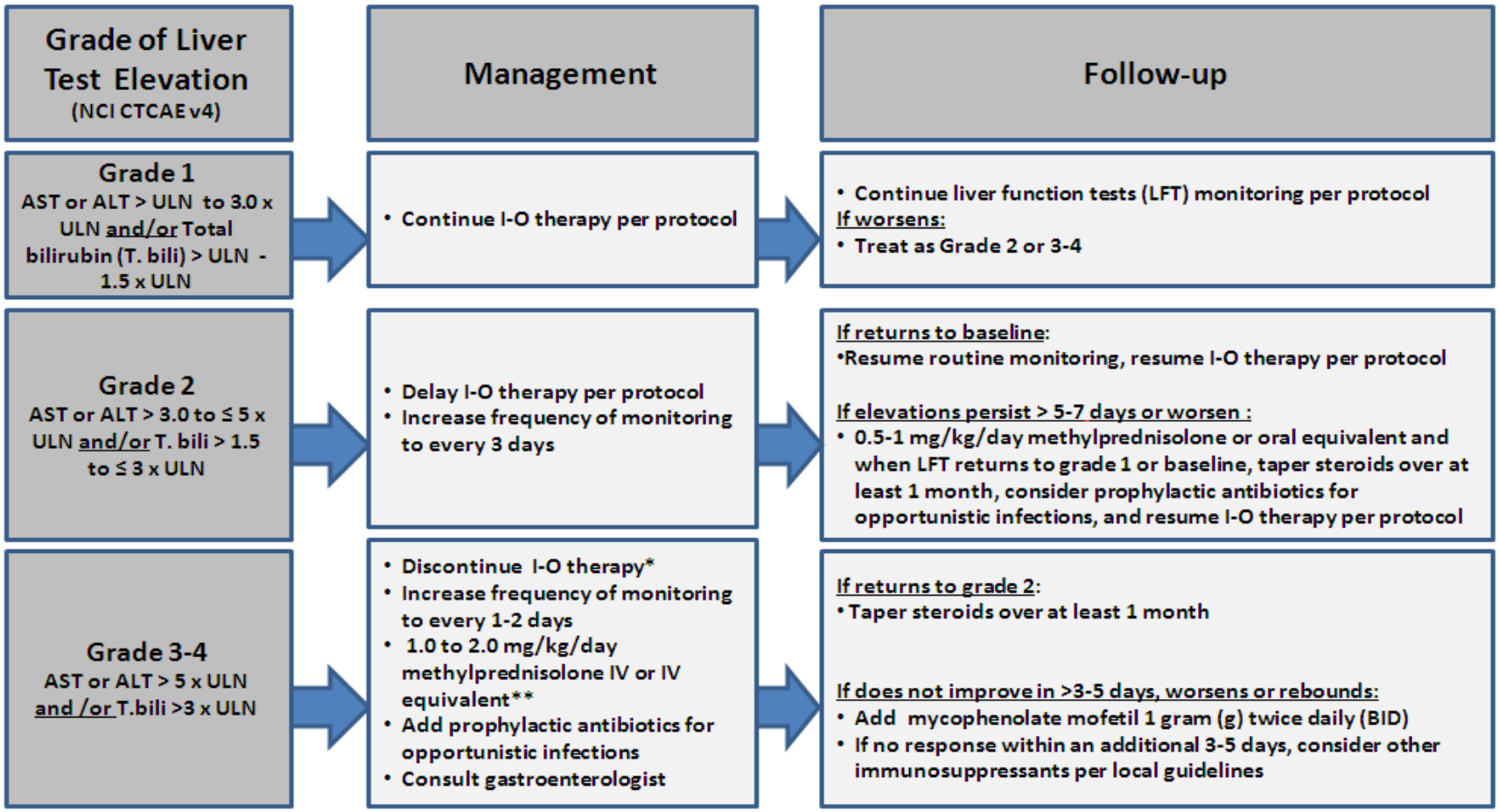
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.

Grade of Pneumonitis (NCI CTCAE v4)	Management	Follow-up
Grade 1 Radiographic changes only	• Consider delay of I-O therapy • Monitor for symptoms every 2-3 days • Consider Pulmonary and Infectious Disease (ID) consults	• Re-image at least every 3 weeks If worsens: • Treat as Grade 2 or 3-4
Grade 2 Mild to moderate new symptoms	• Delay I-O therapy per protocol • Pulmonary and ID consults • Monitor symptoms daily, consider hospitalization • 1.0 mg/kg/day methyl-prednisolone IV or oral equivalent • Consider bronchoscopy , lung biopsy	• Re-image every 1-3 days If improves: • When symptoms return to near baseline, taper steroids over at least 1 month and then resume I-O therapy per protocol and consider prophylactic antibiotics If not improving after 2 weeks or worsening: • Treat as Grade 3-4
Grade 3-4 Severe new symptoms; New/worsening hypoxia; Life-threatening	• Discontinue I-O therapy per protocol • Hospitalize • Pulmonary and ID consults • 2-4 mg/kg/day methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections • Consider bronchoscopy, lung biopsy	If improves to baseline: • Taper steroids over at least 6 weeks If not improving after 48 hours or worsening: • Add additional immunosuppression (e.g. infliximab, cyclophosphamide, intravenous immunoglobulin (IVIG), or mycophenolate mofetil)

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.



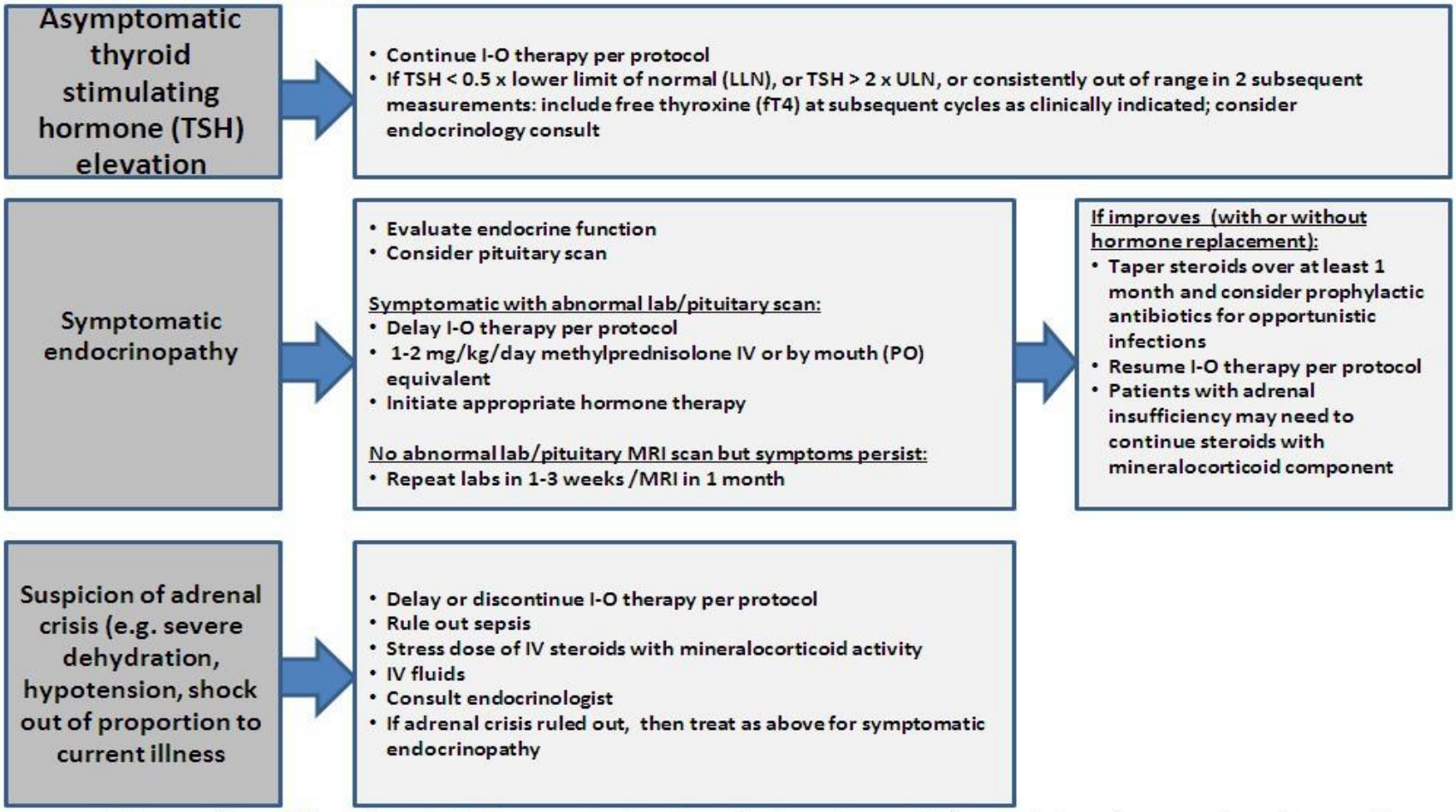
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

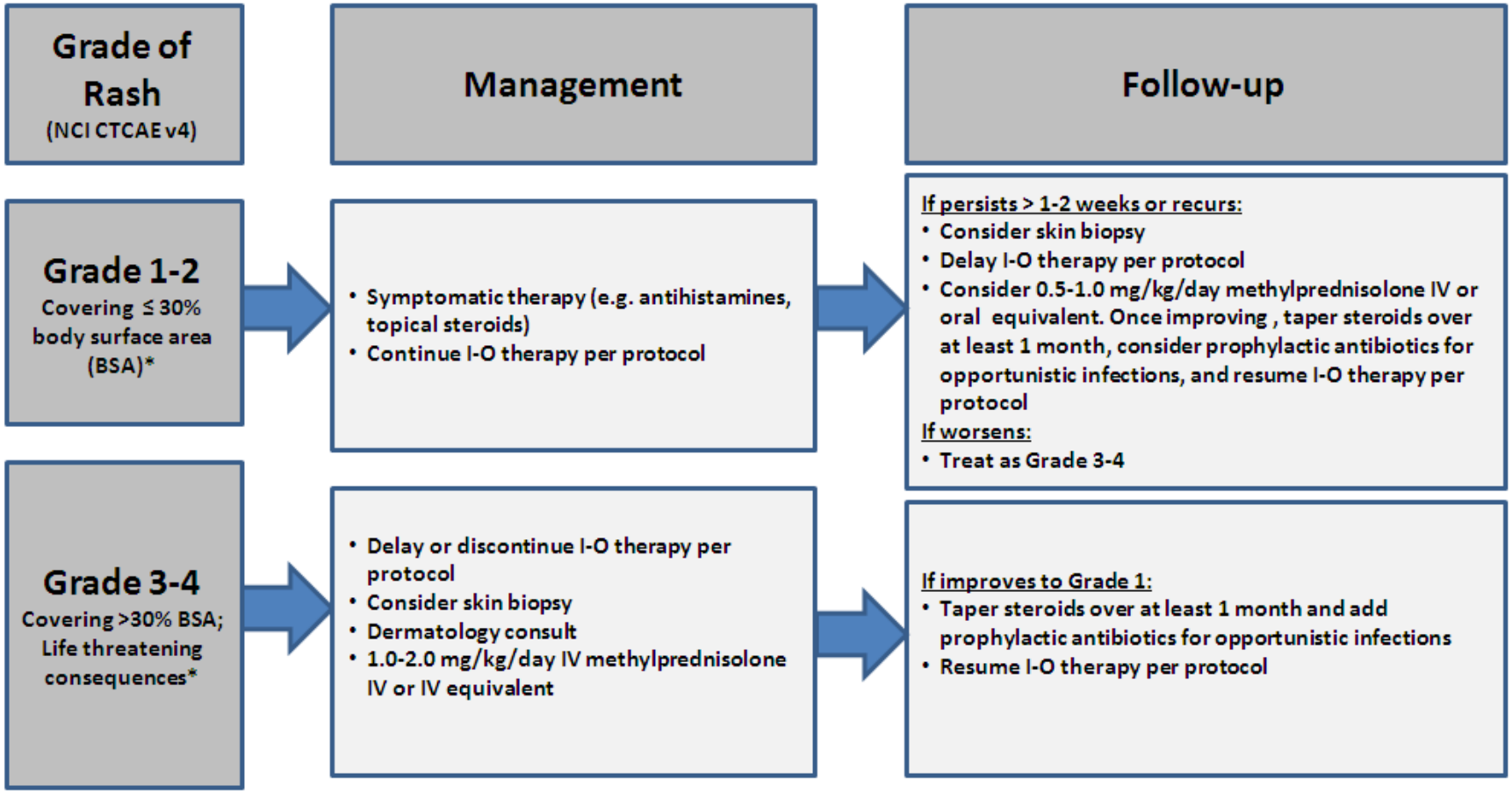
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider visual field testing, endocrinology consultation, and imaging.



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

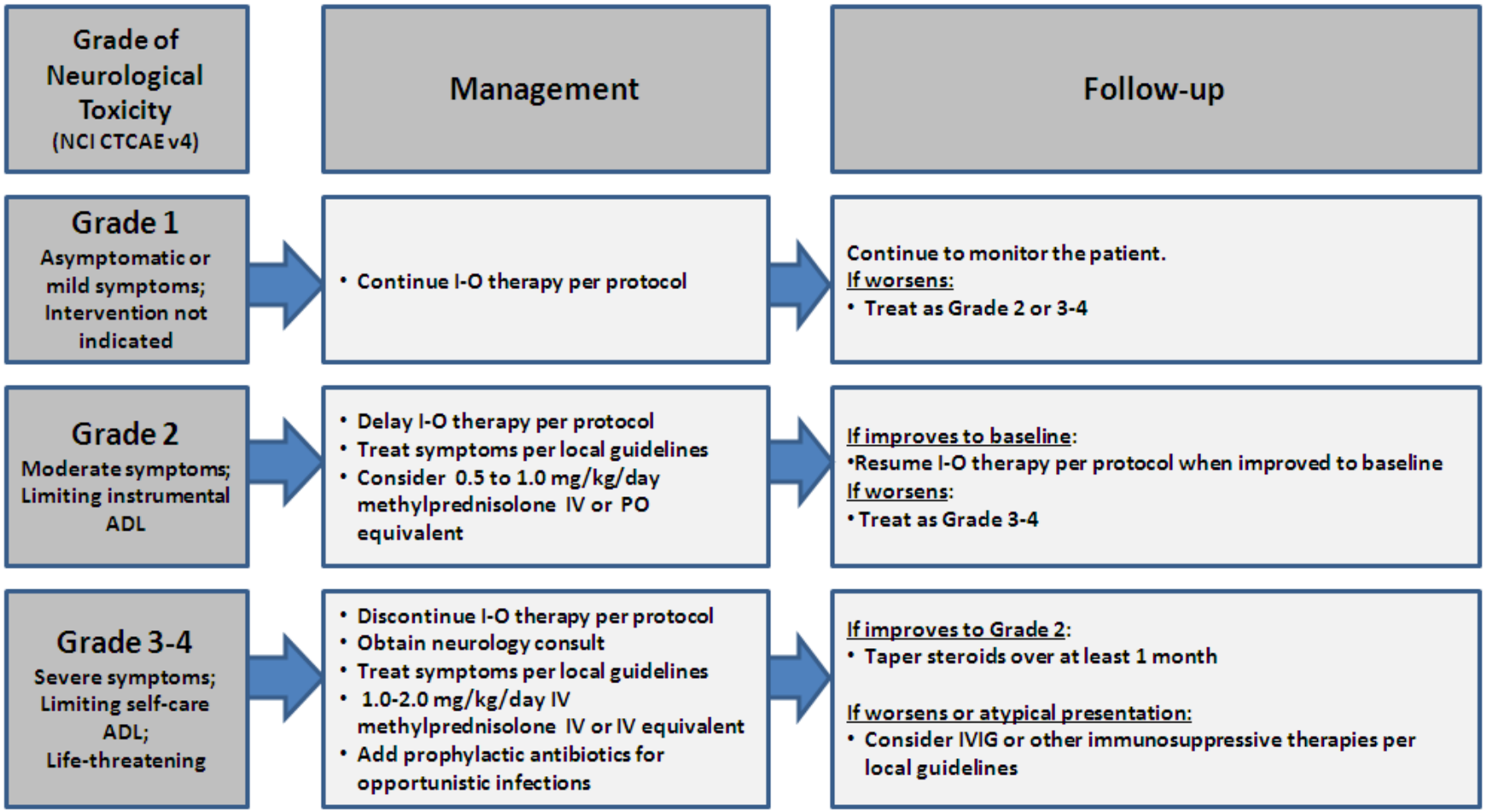
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



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 B: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	**Description**	**%**	**Description**
0	Normal activity. Fully active, able to continue all predisease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity, minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature (e.g. light housework, office work).	80	Normal activity with effort, some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed < 50% of the time. Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance but is able to care for most needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed > 50% of the time. Capable of only limited self-care, confined to bed or chair > 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled, cannot carry on any self-care, totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX C: GLOSSARY OF ABBREVIATIONS AND ACRONYMS

17-AAG	17-allylamino-17-demethoxygeldanamycin
17-DMAG	17-dimethylaminoethylamino-17-demethoxygeldanamycin
2-MPPA	2-(3-mercaptopropyl) Pentanedioic acid
AdEERS	Adverse Event Expedited Reporting System
ADR	Adverse drug reaction
ADT	Androgen-deprivation therapy
AE	Adverse event
AGA	Androgenetic alopecia
AI	Accumulation index
ALT	Alanine aminotransferase
AML	Acute myeloid leukemia
ANC	Absolute neutrophil count
ANOVA	Analysis of variance
APTT	Activated partial thromboplastin time
AR	Androgen receptor
ASAEL	Agent Specific Adverse Event List
AST	Aspartate aminotransferase
AUC	Area under the plasma concentration-time curve
AUC(INF)	Area under the concentration-time curve from time zero extrapolated to infinite time
AUC(0-T)	Area under the concentration-time curve from time zero to the time of the last quantifiable concentration
AUC(TAU)	Area under the concentration-time curve in one dosing interval
AUMC(INF)	Area under the moment concentration time curve extrapolated to infinity
A-V	Atrioventricular
β-HCG	Beta-human chorionic gonadotrophin
%BE	Percent biliary excretion
bid	bis in die (twice a day)
BLQ	Below limit of quantification
BMI	Body mass index
BP	Blood pressure
BSA	Body Surface Area
BUN	Blood urea nitrogen
C	Celsius
Ca++	Calcium
caBIG	Cancer Biomedical Informatics Grid
CAEPR	Comprehensive Adverse Event and Potential Risks
CALGB	Cancer and Leukemia Group B

CBC	Complete blood count
CCC	Clinical Consortium Committee
CCD	Central Consortium Database
CDE	Common data element
CDUS	Clinical Data Update System
CFR	Code of Federal Regulations
CI	Confidence interval
Cl-	Chloride
Clcr	Creatinine clearance
CLNR	Nonrenal clearance
CLR	Renal clearance
CLT	Total body clearance
CLT/F	Apparent total body clearance
Cm	Centimeter
Cmax	Maximum plasma concentration
Cmin	Trough observed concentration
COSTART	Coding Symbols for Thesaurus of Adverse Reaction Terms
CNS	Central nervous system
CR	Complete response
CRC	Clinical Research Center
CRDB	Clinical Research Database
CRF	Case report form
CRMIS	Clinical Research Management Information System
CRPC	Castration resistant prostate cancer
CT	Computerized tomography
CTC	Circulating tumor cell
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
CTMS	Clinical Trials Monitoring Service
CTO	Clinical Trials Office
CV	Coefficient of variation
CYP	Cytochrome p-450
DCTD	Division of Cancer Treatment and Diagnosis
DEV	Deviation from the nominal value
%DEV	Percent deviation
dL	Deciliter
DHEA	Dehydroepiandrosterone
DHEA-S	Dehydroepiandrosterone sulfate
DHT	Dihydrotestosterone
DLT	Dose-limiting toxicity

DSM	Data and safety monitoring
EA	Extent of absorption
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EDC	Electronic data capture
EEG	Electroencephalogram
EKG	Electrocardiogram
EORTC	European Organization for Research and Treatment of Cancer
ESF	Eligibility screening form
ESR	Expedited safety report
F	Bioavailability
FDA	Food and Drug Administration
FDG-PET	2-[18F]fluoro-2-deoxyglucose positron emitting tomography
FDHT	18-fluoro-dehydrotestosterone
%FE	percent fecal excretion
FISH	Fluorescence in situ hybridization
FSH	Follicle stimulating hormone
GAPDH	Glyseraldehayde-3-phophate dehydrogenase
GC	Gas chromatography
GCP	Good clinical practice
GCPII	Glutamate carboxypeptidase II enzyme
GFR	Glomerular filtration rate
GGT	Gamma-glutamyl transferase
GnRH	Gonadotropin-releasing hormone
HAT	Histone acetyltransferases
HCO3-	Bicarbonate
HDAC	Histone deacetylase
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
HL7	American National Standards Institute's Health Level Seven
HPF	High power field
HPLC	High-performance liquid chromatography
HR	Heart rate
HRPC	Hormone-refractory prostate cancer
HRT	Hormone replacement therapy
HSP90	Heat-shock protein 90
ICD	International Classification of Diseases
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IHC	Immunochemical

IM	Intramuscular
IMSL	International Mathematical Statistical Library
IND	Investigational new drug
INR	International normalized ratio
IP	Intraperitoneal
IRB	Institutional Review Board
ITT	Intent-to-treat population
IV	Intravenous
K	Slope of the terminal phase of the log concentration-time curve
K+	Potassium
K3EDTA	Potassium ethylenediaminetetraacetic acid
KLK1	Kallikrein 1
LBD	Ligand-binding domain
LC	Liquid chromatography
LCM	Laser capture microdissection
LC-MS	Liquid chromatography/mass spectrometry
LD	Longest diameter
LDH	Lactate dehydrogenase
LLQ	Lower limit of quantitation
ln	Natural logarithm
LOCF	Last observation carried forward
LOI	Letter of intent
LPF	Low power field
MAD	Maximum administered dose
MDS	Myelodysplasia
MedDRA	Medical Dictionary for Regulatory Activities
MIC	Minimum inhibitory concentration
MMP	Matrix metalloproteinase
MRI	Magnetic resonance imaging
MRT	Mean residence time
MRT(INF)	Mean residence time adjusted for infusion time
MRT(PO)	Mean residence time following oral administration
MRT(SS)	Mean residence time at steady-state
MSKCC	Memorial Sloan-Kettering Cancer Center
MS	Mass spectrometry
MTD	Maximum tolerated dose
N	Number of subjects or observations
NA	Not applicable
N/A	Not available
NBN	National Biospecimen Network

NCI	National Cancer Institute
NIH	National Institutes of Health
NOAEL	No observed adverse effect level
NOS	Not otherwise specified
NSAID	Nonsteroidal anti-inflammatory drug
NTX	N-telopeptide cross-link
NVB	Neurovascular bundle
OCR	Office of Clinical Research at MSKCC
PCCTC	Prostate Cancer Clinical Trials Consortium
PCRP	Department of Defense Prostate Cancer Research Program
PD	Progressive disease
PET	Positron emission tomography
PFS	Progression-free survival
PI	Principal investigator
PIN	Prostatic intraepithelial neoplasia
PK	Pharmacokinetics
PMB	Pharmaceutical Management Branch
PO	per os (by mouth)
PR	Partial response
PSA	Prostate-specific antigen
PSA-DT	Prostate-specific antigen doubling time
PSMA	Prostate specific membrane antigen
PT	Prothrombin time
PTT	Partial thromboplastin time
QC	Quality control
qd	quaque die (every day)
qRT-PCR	Quantitative reverse transcription-polymerase chain reaction
QOL	Quality of life
RBC	Red blood cell
RC	Research Council
RDBMS	Relational Database Management System
RDRC	Radioactive Drug Research Committee
RECIST	Response Evaluation Criteria in Solid Tumors
RP	Radical prostatectomy
RPC	eResearch Program Coordinator
RSA	Research Study Assistant
RSD	Relative standard deviation
%RSD	Percent relative standard deviation
SAE	Serious adverse event
SAHA	Suberoylanilide hydroxamic acid

SC	Subcutaneous
SD	Standard deviation
SD	Stable disease
Seq	Sequence
SHBG	Sex hormone binding globulin
SKI	Sloan-Kettering Institute for Cancer Research
SMD	Stable metabolic disease
SOP	Standard Operating Procedures
SPORE	Specialized Programs of Research Excellence
STAR	Symptom Tracking and Reporting
SUV	Standardized uptake value
t	Temperature
t1/2	Terminal half-life
T	Time
TAUC(TAU)	Trapezoidal area under the concentration-time curve in one dosing interval
TAUC(0-T)	Trapezoidal area under the concentration-time curve from time zero to the time of the last quantifiable concentration
TDP	Time to disease progression
TGP	Prostate-specific transglutaminase
tid	ter in die (3 times a day)
TMA	Tissue microarray
Tmax	Time of maximum observed concentration
TMPRSS2	Transmembrane protease, serine 2
TNM	Tissue, lymph node, metastases
TX	Treatment
ULN	Upper limit of normal
ULQ	Upper limit of quantitation
UR	Urinary recovery
VEGF	Vascular endothelial growth factor
Vss	Volume of distribution at steady-state
WBC	White blood cell
WHO	World Health Organization

APPENDIX D. ACCEPTABLE BIRTH CONTROL METHODS

Subjects with partners of childbearing potential, who are sexually active, must agree to the use of TWO highly effective forms of contraception (defined as a method that can achieve a failure rate of < 1% per year when used consistently and correctly) in combination [as listed below], throughout the period of taking study treatment and for at least 7 months after last dose of study drug(s), or they must totally/truly abstain from any form of sexual intercourse [see below].

Highly Effective Non-hormonal birth control methods include:

- Total sexual abstinence. Abstinence must continue for the total duration of study treatment and for at least 1 month after the last dose. Periodic abstinence (e.g. calendar ovulation, symptothermal post ovulation methods) and withdrawal are not acceptable methods of contraception.
- Vasectomised sexual partner PLUS male condom. With participant assurance that partner received post-vasectomy confirmation of azospermia.
- Tubal occlusion PLUS male condom.
- IUD PLUS male condom. Provided coils are copper-banded.

Highly Effective hormonal methods:

- Normal and low dose combined oral pills PLUS male condom.
- Cerazette (desogestrel) PLUS male condom. Cerazette is currently the only highly efficacious progesterone based pill.
- Hormonal shot or injection (e.g. Depo-Provera) PLUS male condom.
- Etonogestrel implants (e.g. Implanon, Norplant) PLUS male condom.
- Norelgestromin / EE transdermal system PLUS male condom.
- Intrauterine system [IUS] device (e.g. levonorgestrel releasing IUS - Mirena®) PLUS male condom.
- Intravaginal device (e.g. EE and etonogestrel) PLUS male condom.

APPENDIX E. ACTIONS REQUIRED IN CASES OF COMBINED INCREASE OF AMINOTRANSFERASE AND TOTAL BILIRUBIN – HY'S LAW

1. INTRODUCTION

During the course of the study the Investigator will remain vigilant for increases in liver biochemistry. The investigator is responsible for determining whether a patient meets potential Hy's Law (PHL) criteria at any point during the study. The Investigator participates, together with BMS clinical project representatives, in review and assessment of cases meeting PHL criteria to agree whether Hy's Law (HL) criteria are met. HL criteria are met if there is no alternative explanation for the elevations in liver biochemistry other than Drug Induced Liver Injury (DILI) caused by the Investigational Medicinal Product (IMP). The Investigator is responsible for recording data pertaining to PHL/HL cases and for reporting Adverse Events (AE) and Serious Adverse Events (SAE) according to the outcome of the review and assessment in line with standard safety reporting processes.

2. DEFINITIONS

Potential Hy's Law (PHL):

- Aspartate Aminotransferase (AST) or Alanine Aminotransferase (ALT) ≥ 3x Upper Limit of Normal (ULN) and Total Bilirubin (TBL) ≥ 2xULN at any point during the study irrespective of an increase in Alkaline Phosphatase (ALP).

Hy's Law (HL):

- AST or ALT ≥ 3xULN and TBL ≥ 2xULN, where no other reason, other than the IMP, can be found to explain the combination of increases, e.g. elevated ALP indicating cholestasis, viral hepatitis, another drug.

3. IDENTIFICATION OF POTENTIAL HY'S LAW CASES

In order to identify cases of PHL it is important to perform a comprehensive review of laboratory data for any patient who meets any of the following identification criteria in isolation or in combination:

- ALT ≥ 3xULN
- AST ≥ 3xULN
- TBL ≥ 2xULN

The Investigator will without delay review each new laboratory report and if the identification criteria are met will:

- Determine whether the patient meets PHL criteria (see Section 2 of this Appendix for definition) by reviewing laboratory reports from all previous visits.
- Promptly enter the laboratory data into the laboratory CRF.

4. FOLLOW-UP

Potential Hy's Law Criteria not met
If the patient does not meet PHL criteria the Investigator will:

- Perform follow-up on subsequent laboratory results according to the guidance provided in the Clinical Study Protocol.

Potential Hy's Law Criteria met
If the patient does meet PHL criteria the Investigator will:

- Notify the BMS representative who will then inform the central Study Team.
- The Study Physician contacts the Investigator, to provide guidance, discuss and agree an approach for the study patients' follow-up and the continuous review of data. Subsequent to this contact the Investigator will:
    - Monitor the patient until liver biochemistry parameters and appropriate clinical symptoms and signs return to normal or baseline levels, or as long as medically indicated.
    - Investigate the etiology of the event and perform diagnostic investigations as discussed with the Study Physician.
    - Complete the three Liver CRF Modules as information becomes available.
    - If at any time (in consultation with the Study Physician) the PHL case meets serious criteria, report it as an SAE using standard reporting procedures.

5. REVIEW AND ASSESSMENT OF POTENTIAL HY'S LAW CASES

The instructions in this Section should be followed for all cases where PHL criteria are met. No later than 3 weeks after the biochemistry abnormality was initially detected, the Study Physician contacts the Investigator in order to review available data and agree on whether there is an alternative explanation for meeting PHL criteria other than DILI caused by the IMP. BMS and the Principal Investigator will also be involved in this review together with other subject matter experts as appropriate. According to the outcome of the review and assessment, the Investigator will follow the instructions below:

If there is an agreed alternative explanation for the ALT or AST and TBL elevations, a determination of whether the alternative explanation is an AE will be made and subsequently whether the AE meets the criteria for a SAE:

- If the alternative explanation is not an AE, record the alternative explanation on the appropriate CRF.
- If the alternative explanation is an AE/SAE, record the AE/SAE in the CRF accordingly and follow BMS standard processes.

If it is agreed that there is no explanation that would explain the ALT or AST and total bilirubin level elevations other than the IMP:

- Report an SAE (report term 'Hy's Law') according to BMS standard processes.
    - The 'Medically Important' serious criterion should be used if no other serious criteria apply.
    - As there is no alternative explanation for the HL case, a causality assessment of "related" should be assigned.

If, there is an unavoidable delay of over 3 weeks in obtaining the information necessary to assess whether or not the case meets the criteria for HL, then it is assumed that there is no alternative explanation until such time as an informed decision can be made:

- Report an SAE (report term "Potential Hy's Law") applying serious criteria and causality assessment as per above.
- Continue follow-up and review according to agreed plan. Once the necessary supplementary information is obtained, repeat the review and assessment to determine whether HL criteria are met. Update the SAE report according to the outcome of the review.

6. ACTIONS REQUIRED FOR REPEAT EPISODES OF POTENTIAL HY'S LAW

This section is applicable when a patient meets PHL criteria on study treatment and has already met PHL criteria at a previous on study treatment visit.

The requirement to conduct follow-up, review and assessment of a repeat occurrence(s) of PHL is based on the nature of the alternative cause identified for the previous occurrence. The investigator should determine the cause for the previous occurrence of PHL criteria being met and answer the following question:

- Was the alternative cause for the previous occurrence of PHL criteria being met chronic or progressing malignant disease?
    - If No: follow the process described in Section 4 of this Appendix.
    - If Yes: Determine if there has been a significant change in the patient's condition# compared with when PHL criteria were previously met.
        - If there is no significant change no action is required.
        - If there is a significant change follow the process described in Section 4 of this Appendix.

A "significant" change in the patient's condition refers to a clinically relevant change in any of the individual liver biochemistry parameters (ALT, AST or total bilirubin) in isolation or in combination, or a clinically relevant change in associated symptoms. The determination of whether there has been a significant change will be at the discretion of the Investigator, this may be in consultation with the Study Physician if there is any uncertainty.

7. REFERENCES

FDA Guidance for Industry (issued July 2009) 'Drug-induced liver injury: Premarketing clinical evaluation':
http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM174090.pdf