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Biomarker-Driven Phase-2 Study of Combined Immune Checkpoint Blockade for AR-V7–Expressing Metastatic Castration-Resistant Prostate Cancer (STARVE-PC)

Johns Hopkins Kimmel Cancer Center

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IND Sponsor-Investigator: Emmanuel Antonarakis

IND number: 126838

Principal Investigator
Emmanuel Antonarakis, MD
Johns Hopkins Kimmel Cancer Center
Email: eantona1@jhmi.edu

Drew Pardoll, MD PhD
Johns Hopkins Kimmel Cancer Center
email: dpardoll1@jhmi.edu

Eugene Shenderov, MD PhD
Johns Hopkins Kimmel Cancer Center
Email: Eugene.Shenderov@jhmi.edu

Valsamo Anagnostou, MD PhD
Johns Hopkins Kimmel Cancer Center
Email: vanagno1@jhmi.edu

Statistician
Brandon Luber, MS
Hao Wang, PhD
Johns Hopkins Kimmel Cancer Center
Email:
bluber1@jhmi.eduhwang76@jhmi.edu

Project Manager
Ting Wang, MS, MHS
Johns Hopkins Kimmel Cancer Center
Email: twang2@jhmi.edu

Lead Study Coordinator:
Rana Harb, MS
Johns Hopkins Kimmel Cancer Center
Email: rharb1@jhmi.edu

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SYNOPSIS

Title	**Biomarker-Driven Phase-2 Study of Combined Immune Checkpoint Blockade for AR-V7–Expressing Metastatic Castration-Resistant Prostate Cancer (STARVE-PC)**
Lead site	Johns Hopkins Sidney Kimmel Comprehensive Cancer Center
Collaborating sites	Single-center study
IRB #	IRB00070748
Investigational agents	Ipilimumab Nivolumab
Phase	II
Target population	Men with progressive metastatic castration-resistant prostate cancer (CRPC) with detectable AR-V7 in CTCs
Study centers	Johns Hopkins Sidney Kimmel Comprehensive Cancer Center
Start date/Duration	The start date for the study (first patient first visit) is anticipated to be September 2015. The total duration of the study is expected to be approximately 24 months. All subjects will be followed up for 12 months after start of study treatment.
Expected enrollment	The study will enroll 15 evaluable patients, i.e. those positive for CTCs with detectable AR-V7. In cohort 2 (Amendment 1): The study will enroll 15 evaluable patients whose most recent therapy must be enzalutamide with progressive disease and enzalutamide will be continued.
Rationale	Escape from immune surveillance is a recognized feature of cancer growth and progression, and the clinical success of immune checkpoint blockade using monoclonal antibodies against CTLA-4 and PD-1 has been demonstrated in patients with melanoma, kidney cancer and lung cancer. It is now understood that CTLA-4 and PD-1 have complementary and non-overlapping roles in regulating adaptive immune responses. While PD-1 contributes mainly to T-cell exhaustion at peripheral sites, CTLA-4 inhibits T-cells at an earlier point in their maturation cycle. In preclinical models, combined blockade of both CTLA-4 and PD-1 resulted in more pronounced antitumor activity than blockade of either pathway alone. This principal has been exemplified in a clinical study of advanced melanoma patients, where the combination of ipilimumab (anti–CTLA-4) plus nivolumab (anti–PD-1) resulted in rapid and deep tumor regressions above and beyond those reported in prior trials of ipilimumab or nivolumab monotherapy. Promising results have also been seen with this combination

in kidney cancer. Importantly, the combination of ipilimumab and nivolumab is relatively safe, and immune-related adverse events are qualitatively similar to monotherapy treatments, and are generally reversible with steroid use.

Biomarker-driven trials are sorely lacking in prostate cancer. AR-V7 is an androgen receptor splice variant that is missing the ligand-binding domain of AR (the target of androgens and antiandrogens) and remains constitutively active as a transcription factor in a ligand-independent fashion. We have recently shown that AR-V7 can be reliably detected at the mRNA level from circulating tumor cells (CTCs) in patients with metastatic CRPC, that AR-V7 is present in ~30% of men with mCRPC, and that AR-V7 expression is associated with primary and acquired resistance to both abiraterone and enzalutamide (Antonarakis *et al.* NEJM 2014). To this end, men with AR-V7–expressing mCRPC did not achieve biochemical or radiographic responses to abiraterone/enzalutamide, and had markedly inferior PFS and OS with abiraterone/enzalutamide compared to AR-V7–negative patients. In addition, emerging data suggest that expression of AR-V7 is also associated with resistance to taxane-based chemotherapy (Thadani-Mulero *et al.* Cancer Res 2014). Therefore, developing effective treatments for AR-V7–associated mCRPC represents an urgent unmet medical need. In this regard, combined immune checkpoint blockade may be one such therapeutic approach for these patients.

The potential clinical utility of immune checkpoint blockade in prostate cancer is exemplified by the use of ipilimumab in men with mCRPC (Kwon *et al.* Lancet Oncol 2014). The collective aggregate of phase-2 and -3 data with ipilimumab monotherapy suggest a PSA response rate (≥50% PSA reduction) of approximately 15%, and a radiographic response rate of about 5% among men with measurable soft-tissue disease. These results are distinct from those seen with active immunotherapies (*e.g.* sipuleucel-T, ProstVac-VF) in men with mCRPC, where PSA or tumor responses are only observed in <1% of patients. Furthermore, the clinical application of PD-1/PD-L1–blocking antibodies in men with mCRPC is supported by data from our group and others showing that 1) prostate cancer cell lines can significantly up-regulate PD-L1 expression when treated with IFN-γ *in vitro*; 2) treatment of the same cell lines with anti-androgens (including enzalutamide) can induce or increase PD-L1 expression; and 3) that enzalutamide-resistant prostate cancer cell lines demonstrate striking expression of PD-L1 (Bishop *et al.* Oncotarget 2004). Taken together, these data support a clinical paradigm in which combination immune checkpoint blockade with ipilimumab plus nivolumab is utilized in men with AR-V7–positive mCRPC. This study is driven by our novel data on AR-V7: we can use expression of this splice variant as evidence that these patients will not have any meaningful response to enzalutamide or abiraterone (*i.e.* PSA response rates approaching 0%).

The **primary hypothesis** of the current study is that the combination of ipilimumab plus nivolumab will have a manageable safety profile, and will produce PSA responses in a significant proportion of patients with AR-V7–expressing mCRPC. In addition, we propose to serially examine AR-V7 status during the course of treatment with ipilimumab plus nivolumab, to determine how AR-V7 expression changes and correlates with PSA response. If the current study shows preliminary evidence of clinical activity, our ultimate goal is to compare the clinical efficacy of combined

checkpoint blockade *versus* abiraterone or enzalutamide in patients with AR-V7–positive mCRPC.

Objectives

Primary efficacy objective:

To evaluate PSA responses (>50% PSA decline) in mCRPC patients *with detectable AR-V7 transcript in CTCs*

In Cohort 2 (amendment 1):

To evaluate PSA responses (>50% PSA decline) and/or Objective Response in mCRPC patients *with detectable AR-V7 transcript in CTCs.*

Secondary safety objective:

To evaluate the safety and tolerability of ipilimumab + nivolumab in AR-V7–positive patients

Secondary correlative/biomarker objective:

To evaluate changes in AR-V7 detection (or expression levels) before and after treatment with ipilimumab + nivolumab and correlate with PSA responses.

Endpoints

Primary Endpoint:

PSA response rate (proportion of men with >50% PSA decline)

In Cohort 2 (amendment 1):

PSA response rate (proportion of men with >50% PSA decline) and/or Objective response rates (ORR) in patients with measurable disease. This is a composite endpoint.

Key Secondary Endpoints:

1. Frequency and intensity of adverse events (CTCAE v. 4)

2. Proportion of patients converting from AR-V7–positive to –negative (or changes in AR-V7 expression) during treatment

Additional Secondary Endpoints:

3. PSA progression-free survival (PSA-PFS) [PCWG2 criteria]

4. Clinical/radiographic progression-free survival (PFS) [PCWG2 criteria]

5. "Durable PFS", defined as lack of clinical/radiographic progression for ≥24 weeks

6. Objective response rates (ORR) in patients with measurable disease

7. Response duration in patients who develop an objective response
8. Overall survival (OS)

Additional Exploratory/Correlative Endpoints:

1. To explore the predictive value of baseline PD-L1 tumor expression in men with biopsy-amenable disease (or in CTCs)
2. To explore the relationship between baseline tumor mRNA immune profiling panel and clinical outcomes.
3. To explore the relationship between peripheral-blood absolute lymphocyte count (ALC) and clinical outcomes
4. To explore the relationship between serum cytokine profiles and clinical outcomes
5. To explore the relationship between serum antibody profiles and clinical outcomes
6. To explore the relationship between DNA damage repair mutations (including mutational load, hypermutation, and loss of heterozygosity) and clinical outcomes
7. To explore the relationship between MANAFEST assay and clinical outcomes

Study Drug, Dosage, and Route of Administration

Ipilimumab **1 mg/kg** IV + Nivolumab **3 mg/kg** IV, according to the schedule shown below

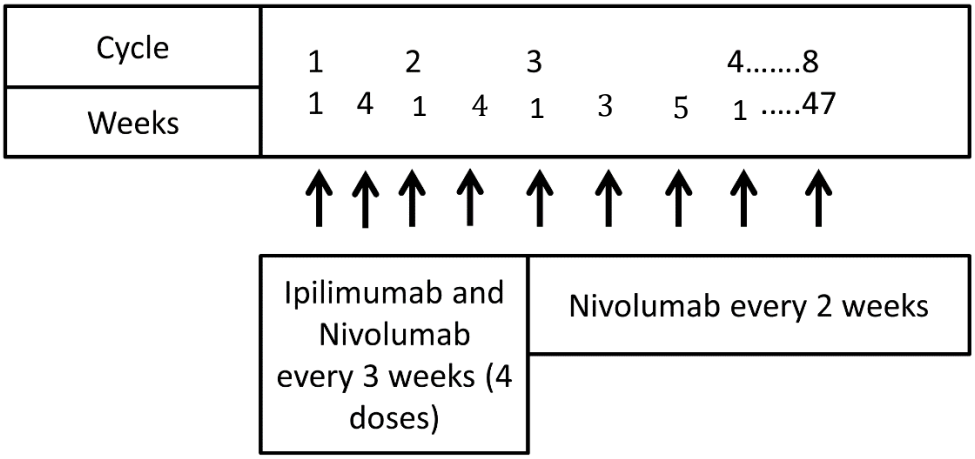
Study design

This will be a single-arm, open-label phase II trial.

Administration of ipilimumab + nivolumab will generally follow the treatment protocol used in the phase 3 trials of concurrent ipilimumab/nivolumab: concurrent administration every 3 weeks for fourDoses, followed by nivolumab alone every 2 weeks. The dosing regimen of nivolumab 3 mg/kg combined with ipilimumab 1 mg/kg was chosen because it exhibits similar clinical activity compared with

nivolumab 1mg/kg combined with ipilimumab 3 mg/kg along with a more favorable safety profile.

Treatment will continue until week 48 or until radiographic progression or unmanageable toxicity.



Correlative Research

CTCs will be collected from participants at 3 time points:

- Baseline
- 12 weeks
- Progression

At each time point, CTCs will be interrogated using qRT-PCR for the following transcripts:

- AR-FL (full-length AR, *i.e.* wild type)
- AR-V7 (AR splice variant-7)
- PSA
- PSMA
- PD-L1

Our primary correlative analysis will be to investigate the proportion of men who convert from AR-V7–positive at baseline to AR-V7–negative during/after therapy with ipilimumab/nivolumab (as well as changes in AR-V7 expression with treatment). We further plan to correlate changes in AR-V7 with PSA responses to ipilimumab + nivolumab.

	We also plan to explore the predictive value of baseline PD-L1 expression at the mRNA level in CTCs and at the protein level in metastatic biopsies (from patients with biopsy-amenable disease) in the context of ipilimumab + nivolumab treatment. We will also perform exploratory analyses of tumor immune-related mRNA expression, whole-exome tumor DNA sequencing, tumor RNA sequencing, and T-cell receptor (TCR) sequencing. Lastly, we will explore the circulating cytokine and antibody profiles in serum.
Statistical method	Our primary efficacy hypothesis is that we will observe PSA responses (>50% PSA declines) in a significant proportion of patients treated with ipilimumab plus nivolumab. Because there are currently no effective AR-directed therapies for men with AR-V7–associated CRPC (and PSA response rates with enzalutamide/ abiraterone approach 0%), we would consider ipilimumab + nivolumab promising if the PSA response rate is higher than 5%. We plan to enroll 15 patients. If we observe ≥3 PSA responses in 15 patients, the lower bound of 90% confidence interval of response rate would be above 5% (90% CI: 5.6% - 44%), and future study would be warranted. If we observe **≥3 PSA responses in 15 patients**, we will proceed with subsequent larger trials. If we see ≤2 out of 15 PSA responses, we will consider this therapy unworthy of further study in AR-V7-positive mCRPC. For cohort #2 (amendment 1) Our primary efficacy hypothesis is that we will observe PSA responses (>50% PSA declines) and/or Objective response rates (ORR) in patients with measurable disease in a significant proportion of patients treated with ipilimumab plus nivolumab. Because there are currently no effective AR-directed therapies for men with AR-V7–associated CRPC (and PSA response rates with enzalutamide/ abiraterone approach 0%), we would consider ipilimumab + nivolumab promising if the composite endpoint of PSA response rate and/or ORR is higher than 5%. We plan to enroll 15 patients. If we observe ≥3 PSA responses and/or ORR in 15 patients, the lower bound of 90% confidence interval of response rate would be above 5% (90% CI: 5.6% - 44%), and future study would be warranted. If we observe ≥3 PSA responses and/or ORR in 15 patients, we will proceed with subsequent larger trials. If we see ≤2 out of 15 PSA responses and/or ORR, we will consider this therapy unworthy of further study in AR-V7-positive mCRPC.
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 second most common cause of cancer deaths in men in the US. Approximately one in every six American men will be diagnosed with the disease during his lifetime [Siegel et al. Cancer Statistics 2012]. The disease continuum has been modeled as a series of states defined by the presence or absence of detectable metastases and whether testosterone levels are in the castrate or non-castrate range (figure 1). Each state represents a significant milestone in the illness that forms the basis for clinical research and for medical decision making in the context of routine clinical practice. The standard treatment for patients with metastatic disease is to block testosterone action with a gonadotropin releasing hormone (GnRH) analog and an anti-androgen. The results are predictable, with a decline in PSA followed by tumor regression, a period of stability in which the tumor does not proliferate and PSA remains stable, followed by rising PSA and regrowth as a castration resistant lesion. Prostate cancer progression despite castrate levels of testosterone represents a transition to a lethal disease phenotype referred to as castration-resistant prostate cancer (CRPC).

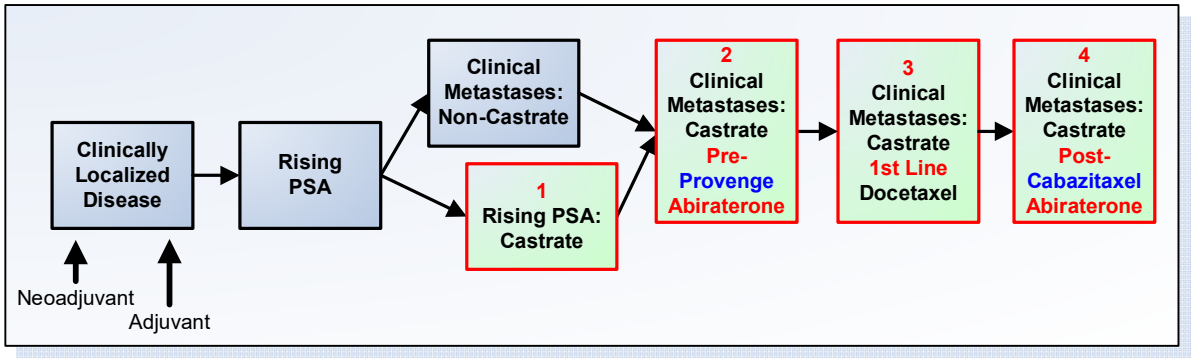


Figure 1. Clinical states of prostate cancer

It is now accepted that castration-resistant prostate cancer is not androgen-independent and continues to rely on androgen signaling (Longo NEJM 2010). Owing to this new understanding, several drugs have recently emerged for the treatment of castration-resistant prostate cancer; these agents either suppress the synthesis of extragonadal androgens or target the androgen receptor directly.2Enzalutamide is an inhibitor of androgen-receptor signaling that exerts its activity by binding avidly to the ligand-binding domain of the androgen receptor, competing with and displacing the natural ligands of this receptor (testosterone and dihydrotestosterone) while also inhibiting translocation of the androgen receptor into the nucleus and impairing transcriptional activation of androgen-responsive target genes (Tran *et al.* Science 2009; Scher *et al.* Lancet 2010). Abiraterone is an inhibitor of cytochrome P450 17A1 (CYP17A1) that impairs androgen-receptor signaling by depleting adrenal and intratumoral androgens (O'Donnell *et al.* Br J Cancer 2004; Attard *et al.* JCO 2008). After studies showed improved survival with these drugs, (Scher *et al.*

NEJM 2012; de Bono *et al.* NEJM 2011; Ryan *et al.* NEJM 2013) both agents were approved by the Food and Drug Administration for the treatment of metastatic castration-resistant prostate cancer.

Although enzalutamide and abiraterone represent breakthroughs in the treatment of metastatic castration-resistant prostate cancer, approximately 20 to 40% of patients have no response to these agents with respect to prostate-specific antigen (PSA) levels (i.e., they have primary resistance) Among patients who initially have a response to enzalutamide or abiraterone, virtually all eventually acquire secondary resistance. One plausible explanation for the resistance to both agents may involve the presence of androgen-receptor splice variants (Nadiminty *et al* Mol Cancer Ther 2013; Mostaghel *et al*. Clin Canc Res 2011). These alternatively spliced variants encode a truncated androgen-receptor protein that lacks the C-terminal ligand-binding domain but retains the transactivating N-terminal domain (Dehm *et al.*Cancer Res 2008; Hu *et al.*Cancer Res 2009). Although the resultant truncated proteins are unable to bind ligand, they are constitutively active as transcription factors and capable of promoting activation of target genes.

The presence of the androgen-receptor splice variant 7 (AR-V7) messenger RNA in circulating tumor cells (CTCs) strongly predicted for PSA response rate (PSA decline ≥50%), progression-free survival (PFS) and overall survival (OS) in a prospective study of men with CRPC who were beginning standard of care treatment with either enzalutamide or abiraterone. Overall, 62 of 71 men about to begin treatment with either abiraterone or enzalutamide had detectable CTCs, an 87% yield. 39% (12 of 31 patients) of men about to begin treatment with enzalutamide were AR-V7+, as were 19% (6 of 31) about to begin treatment with abiraterone (Antonarakis *et al.* NEJM 2014).

In the cohort of men who received enzalutamide, 0 of 12 men (0%) who carried detectable levels of AR-V7 in CTCs had a PSA response compared with 10 of 19 men (53%) who did not. Likewise, 0 of 6 men (0%) who were AR-V7+ and received abiraterone had a PSA response compared with 17 of 25 men (68%) who were AR-V7-. AR-V7 can be reliably detected from circulating tumor cells and appears to be associated with resistance to both enzalutamide and abiraterone, with essentially no clinical benefit seen in the largest prospective study.

Men with CRPC who are AR-V7+ therefore represent a large unmet medical need. Treatment options are limited and may include chemotherapy (docetaxel and/or cabazitaxel), vaccine therapy (sipuleucel-T) for those with asymptomatic disease, or radiopharmaceutical therapy (radium-223 dichloride) for those with symptomatic bone-only metastatic disease. However, these therapies have not been evaluated prospectively in this specific population and there is evidence to suggest that expression of AR-V7 may be associated with resistance to taxane-based chemotherapy (Thadani-Mulero *et al.* Cancer Res 2014).

Therapies targeted at blocking escape from immune surveillance may remain effective in the setting of AR-V7 positivity. This strategy has been clinically successful in melanoma, kidney cancer, and lung cancer, using monoclonal antibodies against CTLA-4 and the PD-1/PD-L1 pathway. These pathways have complementary and non-overlapping roles in regulating adaptive immune responses. While PD-1 contributes mainly to T-cell exhaustion at peripheral sites, CTLA-4 inhibits T-cells at an earlier point in their maturation cycle. In CRPC, ipilimumab monotherapy has demonstrated PSA responses of approximately 15% with radiographic responses of approximately 5%. The addition of PD-1 blockade via nivolumab is hypothesized to improve this response rate in a population of men with AR-V7+ CRPC.

1.2 Nivolumab

1.2.1 Introduction

Nivolumab (also referred to as BMS-936558 or MDX1106) is a fully 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.

1.2.2 Nonclinical Studies

Nivolumab has been shown to bind specifically to the human PD-1 receptor and not to related members of the CD28 family, such as CD28 inducible co-stimulator (ICOS), cytotoxic T lymphocyte antigen-4 (CTLA-4), and B and T lymphocyte attenuator (BTLA). Nivolumab

inhibits the interaction of PD-1 with its ligands, PD-L1 and PD-L2, resulting in enhanced T-cell proliferation and interferon-gamma (IFN-γ) release in vitro.4,5,6 Fluorescent-activated cell sorter (FACS) analysis confirmed that nivolumab binds to transfected CHO and activated human

T-cells expressing cell surface PD-1 and to cynomolgus monkey PD-1, but not to rat or rabbit PD-1 molecules.2 Nivolumab has also been shown to bind to PD-1 on virus-specific CD8+ T-cells from chronically infected hepatitis C virus patients.

PD-1 inhibition in a mixed lymphocyte reaction (MLR) resulted in a reproducible concentration-dependent enhancement of IFN-γ release in the MLR up to 50 µg/mL. No

effect was observed with a human IgG4 isotype control or CD4+ T cells and dendritic cell (DC) controls.9

In intravenous (IV) repeat-dose toxicology studies in cynomolgus monkeys, nivolumab was well tolerated at doses up to 50 mg/kg, administered weekly for 5 weeks, and at doses up to 50 mg/kg, administered twice weekly for 27 doses. Nivolumab-related findings were limited to a reversible decrease of 28% in triiodothyronine (T3) among the females administered 27 doses of 50 mg/kg. No corresponding changes in the level of thyroxin (T4), thyroid-stimulating hormone (TSH), or histologic changes in the thyroid were observed.10,11 While nivolumab alone was well tolerated in cynomolgus monkeys, combination studies have highlighted the potential for enhanced toxicity when combined with other immunostimulatory agents.

Ipilimumab (BMS-734016), an anti-CTLA-4 monoclonal antibody (mAb) that blocks the down-regulation of T-cell activation, was used in combination with nivolumab to investigate the effects of concurrent inhibition of the PD-1 and CTLA-4 receptors in nonhuman primates. Although gastrointestinal (GI) toxicity has not been observed in cynomolgus monkeys treated with nivolumab alone, dose-dependent GI toxicity was evident in cynomolgus monkeys treated weekly for 4 weeks with a combination of nivolumab + ipilimumab at combinations of 10 and 3 mg/kg and 50 and 10 mg/kg, respectively. GI effects have also been observed at a low incidence after ipilimumab administration.

Two 4-week toxicity studies in cynomolgus monkeys were conducted with 2 anti-lymphocyte-activation gene 3 (LAG-3) mAbs that inhibit the function of LAG-3 on the surface of activated CD4+ and CD8+ T cells. The combination of nivolumab + MDX1408 was clinically well tolerated up to 50 mg/kg MDX1408 and 50 mg/kg nivolumab with findings limited to nonadverse and pharmacologically-mediated changes in the peripheral blood and lymphoid tissues, including increases in CD4+ T lymphocytes expressing CD25. However, the combination of 50 mg/kg nivolumab + 100 mg/kg BMS-986016, administered once weekly, was associated with moribundity (1 of 10 animals) attributed to central nervous system (CNS) vasculitis. Findings in surviving monkeys administered nivolumab alone or in combination with BMS-986016 were limited to minimal inflammation of the choroid plexus or vasculature of the brain parenchyma (1 male in the combination group), without evidence of other degenerative changes. These findings, which were observed at nivolumab exposures that were approximately 13x greater than those observed in humans at 3 mg/kg, every 2 weeks (Q2W), are consistent with an expected immunostimulatory pharmacologic effect of nivolumab and highlight the potential synergistic roles of PD-1 and LAG-3 in maintaining self-tolerance.14

In addition, an enhanced pre- and postnatal development (ePPND) study in pregnant cynomolgus monkeys with nivolumab was conducted. Administration of nivolumab at up to 50 mg/kg 2QW was well tolerated by pregnant monkeys; however, nivolumab was determined to be a selective developmental toxicant when administered from the period of organogenesis to parturition at10 mg/kg (area under the concentration-time curve [AUC] from time zero to 168 hours [AUC(0-168 h)] 117,000 μg•h/mL). Specifically, increased developmental mortality (including late gestational fetal losses and extreme

prematurity with associated neonatal mortality) was noted in the absence of overt maternal toxicity. There were no nivolumab-related changes in surviving infants tested throughout the 6-month postnatal period. Although the cause of these pregnancy failures was undetermined, nivolumab-related effects on pregnancy maintenance are consistent with the established role of PD-L1 in maintaining fetomaternal tolerance in mice.16

1.2.3 Effects in Humans

The PK, clinical activity, and safety of nivolumab have been assessed in completed Phase 1 and ongoing Phase 2 and 3 studies sponsored by Bristol-Myers Squibb Company (BMS) in subjects with non-small cell lung cancer (NSCLC), melanoma, and clear-cell renal cell carcinoma (RCC) in addition to other tumor types. The current Phase 3 clinical program focuses on squamous and nonsquamous NSCLC, malignant melanoma, and RCC. Clinical activity and safety information presented here focuses primarily on that obtained from CA209063 (Phase 2 study in refractory squamous NSCLC), CA209037 (Phase 3 study in melanoma), MDX1106-03 (also known as CA209003; Phase 1 study in metastatic NSCLC, colorectal cancer (CRC), melanoma, RCC, or metastatic castrate-resistant prostate cancer [mCRPC]), CA209010 (Phase 2 study in advanced/metastatic clear-cell RCC). Data are also provided from Phase 1 studies CA209004 (also known as MDX1106-04), CA209012, CA209016, CA209038, and CA209039. Nivolumab is being investigated both as monotherapy and in combination with chemotherapy, targeted therapies, and other immunotherapies.

1.2.4. Clinical Pharmacokinetics

The single-dose pharmacokinetics (PK) of nivolumab was linear and dose-proportional in the range of 0.3 mg/kg to 10 mg/kg. The multiple-dose PK of nivolumab was linear with dose-proportional increases in maximum serum concentration (Cmax) and area under the concentration-time curve over the dosing interval (AUC[TAU]) in the range of 0.1 mg/kg to 10 mg/kg. Both elimination and distribution of nivolumab in the dose range studied appear to be independent of dose in the dose-ranging studies, while the end of infusion and minimum serum concentration (Cmin) after the first dose were approximately dose proportional. Based on population PK (PPK) results (preliminary data), clearance of nivolumab is independent of dose in the dose range (0.1 mg/kg to 10 mg/kg) and tumor types studied. Body weight normalized dosing showed approximately constant trough concentrations over a wide range of body weights and, therefore, is appropriate for future clinical trials with nivolumab.

1.2.5. Clinical Efficacy

Nivolumab has demonstrated clinical activity as monotherapy and as combination therapy with ipilimumab in several tumor types, including RCC, melanoma, NSCLC, and some lymphomas. The majority of responses were durable and exceeded 6 months. Nivolumab has now been FDA-approved for the treatment of metastatic melanoma and metastatic squamous non-small cell lung cancer.

1.2.6. Clinical Safety

The overall safety experience with nivolumab, as a monotherapy or in combination with other therapeutics, is based on experience in approximately 4,000 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.

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 Ipilimumab

Ipilimumab is a recombinant, human monoclonal antibody that binds to the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4). Ipilimumab is an IgG1 kappa immunoglobulin with an approximate molecular weight of 148 kDa. Ipilimumab is produced in mammalian (Chinese hamster ovary) cell culture. CTLA-4 is a negative regulator of T-cell activation. Ipilimumab binds to CTLA-4 and blocks the interaction of CTLA-4 with its ligands, CD80/CD86. Blockade of CTLA-4 has been shown to augment T-cell activation and proliferation. The mechanism of action of ipilimumab's effect in patients with melanoma is indirect, possibly through T-cell mediated anti-tumor immune responses.

Ipilimumab 3 mg/kg is globally approved for advanced melanoma based on the results of a phase study III, MDX010-20. In MDX010-20, the ipilimumab monotherapy arm was administered 3 mg/kg ipilimumab every 3 weeks for four doses. In this arm, there were 79% drug related adverse events, with 21% being Grade 3/4 and 3/131 (2%) Grade 5. The most frequent adverse events of interest were rash (30%), pruritis (33%), diarrhea (33%), colitis (8%), endocrine disorders (9%), AST/ALT increased (2%), and hepatitis (1%). Any grade immune related adverse events were 60% and the Grade 3/4 immune related adverse events for the same cohort was 13% with the most frequent adverse events being diarrhea (5%), colitis (5%), rash (2%), and endocrine disorders (3%). Additional details on the safety profile of ipilimumab, including results from other clinical studies, are also available in the ipilimumab IB.

1.4 Benefits and Risks

The combination of nivolumab and ipilimumab is an experimental treatment for patients with AR-V7+ CRPC, for whom there is a significant unmet need given the lack of efficacy of further hormonal treatments. Ipilimumab has demonstrated evidence of clinical activity as a monotherapy in CRPC. The addition of nivolumab to ipilimumab has increased the response rate and PFS in other cancers while exhibiting an acceptable safety profile. These

responses are expected to be more durable than those induced by other agents in CRPC and may translate into improvements in PFS and OS.

Anticipated *benefits* of the treatment include the following:

- Anti-tumor efficacy as measured by radiographic and PSA criteria
- Manageable toxicity (favorable safety profile)
- Prolongation of overall survival

Nivolumab monotherapy has demonstrated clinical activity across several tumor types, including advanced prior treated melanoma, with objective response rates of 20 - 41% in 106 melanoma subjects treated at various dose levels in CA209003. Nivolumab has also demonstrated a manageable safety profile. The most common AEs included fatigue, rash, pruritis, diarrhea, and nausea (Nivolumab IB).

In the setting of post-chemotherapy CRPC (CA184-043), ipilimumab monotherapy has demonstrated statistically significant improvement in PFS over placebo, although an improvement in OS has not yet been demonstrated in an intent-to-treat population. The most common AEs included diarrhea, fatigue, nausea, anorexia, and pruritis. Other notable immune-related events occurring more frequently than placebo included hepatitis, hypothyroidism, and adrenal insufficiency (Kwon *et al* Lancet Oncology 2014).

The combination of nivolumab and ipilimumab has the potential for increased benefit compared to both ipilimumab monotherapy and nivolumab monotherapy. However, the combination of nivolumab and ipilimumab also has the potential for increased frequencies of adverse events. In phase I trials of the combination, the most common (reported at > 10% incidence) treatment related AEs are fatigue, rash, pruritus, diarrhea, lipase increased, pyrexia, ALT increase, AST increased, amylase increased and vitiligo. Although the preliminary data suggests an increase in adverse event frequency of nivolumab combined with ipilimumab compared to ipilimumab monotherapy or nivolumab monotherapy, there were no unexpected adverse events noted in the combination of nivolumab and ipilimumab.

In addition, many of the Grade 3-4 adverse events associated with the nivolumab combined with ipilimumab were laboratory in nature, without clinical sequalae and adverse events have been manageable and reversible following intervention dose delays or with systemic steroid treatment.

- Anticipated risks of the combination:

While a synergistic clinical benefit is hypothesized, a theoretical risk exists that nivolumab may, on the contrary, exert a deleterious effect on the efficacy of ipilimumab in these patients.

Additive or synergistic immune-related adverse events are expected as per prior nivolumab/ipilimumab combination studies in other tumor types, however there may be a risk of unexpected adverse events specific to this patient population.

1.5 Rationale and Dose Selection

1.5.1 Rationale for conducting the study

Escape from immune surveillance is a recognized feature of cancer growth and progression, and the clinical success of immune checkpoint blockade using monoclonal antibodies against CTLA-4 and PD-1 has been demonstrated in patients with melanoma, kidney cancer and lung cancer. It is now understood that CTLA-4 and PD-1 have complementary and non-overlapping roles in regulating adaptive immune responses. While PD-1 contributes mainly to T-cell exhaustion at peripheral sites, CTLA-4 inhibits T-cells at an earlier point in their maturation cycle. In preclinical models, combined blockade of both CTLA-4 and PD-1 resulted in more pronounced antitumor activity than blockade of either pathway alone. This principal has been exemplified in a clinical study of advanced melanoma patients, where the combination of ipilimumab (anti–CTLA-4) plus nivolumab (anti–PD-1) resulted in rapid and deep tumor regressions above and beyond those reported in prior trials of ipilimumab or nivolumab monotherapy. Promising results have also been seen with this combination in kidney cancer. Importantly, the combination of ipilimumab and nivolumab is relatively safe, and immune-related adverse events are qualitatively similar to monotherapy treatments, and are generally reversible with steroid use.

Biomarker-driven trials are sorely lacking in prostate cancer. AR-V7 is an androgen receptor splice variant that is missing the ligand-binding domain of AR (the target of androgens and antiandrogens) and remains constitutively active as a transcription factor in a ligand-independent fashion. We have recently shown that AR-V7 can be reliably detected at the mRNA level from circulating tumor cells (CTCs) in patients with metastatic CRPC, that AR-V7 is present in ~30% of men with mCRPC, and that AR-V7 expression is associated with primary and acquired resistance to both abiraterone and enzalutamide (Antonarakis *et al.* NEJM 2014). To this end, men with AR-V7–expressing mCRPC did not achieve biochemical or radiographic responses to abiraterone/enzalutamide, and had markedly inferior PFS and OS with abiraterone/enzalutamide compared to AR-V7–negative patients. In addition, emerging data suggest that expression of AR-V7 is also associated with resistance to taxane-based chemotherapy (Thadani-Mulero *et al.* Cancer Res 2014). Therefore, developing effective treatments for AR-V7–associated mCRPC represents an urgent unmet medical need. In this regard, combined immune checkpoint blockade may be one such therapeutic approach for these patients.

The potential clinical utility of immune checkpoint blockade in prostate cancer is exemplified by the use of ipilimumab in men with mCRPC (Kwon *et al.* Lancet Oncol 2014). The collective aggregate of phase-2 and -3 data with ipilimumab monotherapy suggest a PSA response rate (≥50% PSA reduction) of approximately 15%, and a radiographic response rate of about 5% among men with measurable soft-tissue disease. These results are distinct from those seen with active immunotherapies (*e.g.* sipuleucel-T, ProstVac-VF) in men with mCRPC, where PSA or tumor responses are only observed in <1% of patients. Furthermore, the clinical application of PD-1/PD-L1–blocking antibodies in men with mCRPC is supported by data from our group and others showing that 1) prostate cancer cell lines can significantly up-regulate PD-L1 expression when treated with IFN-γ *in vitro*; 2) treatment of the same cell lines with anti-androgens (including enzalutamide) can induce or increase PD-L1 expression; and 3) that enzalutamide-resistant prostate cancer cell lines demonstrate striking expression of PD-L1 (Bishop *et al.* Oncotarget 2004). Taken together,

these data support a clinical paradigm in which combination immune checkpoint blockade with ipilimumab plus nivolumab is utilized in men with AR-V7–positive mCRPC. This study is driven by our novel data on AR-V7: we can use expression of this splice variant as evidence that these patients will not have any meaningful response to enzalutamide or abiraterone (*i.e.* PSA response rates approaching 0%).

1.5.2 Rationale for dosage selection

Ipilimumab is currently approved for metastatic melanoma at a dose of 3mg/kg every 3 weeks for four doses. In CRPC, doses of 3 mg/kg, 5 mg/kg, and 10 mg/kg have been evaluated without a clear dose response for efficacy, though toxicity appeared more prominent at the 10 mg/kg dose, which served as the basis for the phase 3 CA184-043 trial.

Nivolumab has been evaluated in combination with ipilimumab in melanoma, renal cell carcinoma, and non-small cell lung carcinoma. In CA209012, there was similar efficacy between nivolumab 1mg/kg with ipilimumab 3mg/kg compared with nivolumab 3mg/kg with ipilimumab 1mg/kg with respect to overall response rate in NSCLC and RCC. However, toxicity appeared greater with ipilimumab 3mg/kg, particularly with respect to autoimmune hepatitis (Nivolumab IB).

1.5.3 Rationale for correlative studies

1.5.3.1 Rationale for evaluation of baseline PD-L1 tumor expression

PD-L1 is expressed by many tumor types and its expression has been noted to correlate with decreased immune system function and worse clinical prognosis. In enzalutamide-resistant CRPC, PD-L1 expression is highly expressed relative to enzalutamide-naïve tumors (Bishop et al. Oncotarget 2014). It is hypothesized that PD-L1 expression within the tumor microenvironment, either on tumor cells, macrophages or lymphocytes is a means of evading immune system detection and destruction. Still others postulate that PD-L1 expression on tumor cells is a surrogate for interferon-gamma release from neighboring activated T cells and thus portends a good prognosis for immunotherapy agents, and in particular, agents targeting the PD-1/PD-L1 axis.

The limited preliminary evidence suggests that PD-L1 expression may be a prognostic marker that may also predict for nivolumab clinical activity, although there was minimal difference in response seen in PD-L1-positive vs negative tumors in a trial of the combination of nivolumab and ipilimumab (Wolchok et al NEJM 2014). These observations bear exploration in the setting of CRPC. Therefore, baseline PD-L1 expression status will be prospectively assessed via an optional metastatic tumor biopsy in those men with accessible lesions as well as via CTCs.

1.5.3.2 Rationale for exploring the correlation between peripheral-blood absolute lymphocyte count (ALC) and clinical outcomes

The absolute lymphocyte count (ALC) is a potential pharmacodynamic biomarker for ipilimumab, with the ALC at 7 weeks (≥1000 cells/uL) and magnitude of ALC increases with

therapy demonstrating correlations with outcome in melanoma (Ku et al 2010, Postow and Panageas 2012). Baseline eosinophil and relative eosinophil count have also been associated with improved survival in these patients (Delyon et al 2013). However, in the nivolumab-ipilimumab trial in melanoma, there was no correlation noted between ALC and response. Nevertheless, ALC should be assessed as a potential biomarker in this trial.

1.5.3.3 Rationale for evaluation of changes in AR-V7 detection (or expression levels) before and after treatment with ipilimumab + nivolumab

AR-V7 appears to be a dynamic marker of resistance to androgen-receptor-targeted therapies. In a prospective trial of AR-V7 assessment, 6 of 42 men with undetectable AR-V7 prior to treatment with either enzalutamide or abiraterone converted to AR-V7-positive status during treatment (Antonarakis et al 2014). Clinical outcomes were intermediate between those that were negative vs positive at baseline. To further evaluate the utility of AR-V7 status as a biomarker, it will be important to determine whether immunotherapy may cause AR-V7-positive patients to lose AR-V7 detectability and whether this may correlate with clinical outcome.

CTCs will be collected for evaluation of AR-V7 status at baseline, week 12, and at progression/off-study (end-of-study visit). These time-points will allow dynamic evaluation of AR-V7 status following completion of the intensive treatment cycles (i.e. following concurrent therapy) and at time of potential development of resistance.

1.5.3.4 Rationale for Intratumoral and Peripheral Immunologic Profiling

The Nanostring nCounter PanCancer Immune Profiling Panel allows multiplexed quantitative profiling of immune-related mRNAs present in the tumor biopsy sample. Nanostring includes 109 genes that define 24 immune cell types and populations. A broadly-based evaluation of intratumoral immune parameters will be useful to identify biomarkers that may predict response to checkpoint inhibitors.

Similarly, broad antibody and cytokine profiling using pre- and post-treatment sera can be used to screen for novel anti-prostate antibody responses and determine patterns of cytokine and chemokine modulation that may predict clinical benefit with ipilimumab and nivolumab therapy. The Life Technologies (Carlsbad, CA) ProtoArray technology will be used to evaluate over 8000 proteins to determine antibody response and the Life Technologies Luminex 25-Plex cytokine panel will be used to evaluate cytokine profiles. These data will be visualized per patient for both groups using heatmaps. Additionally, 100mL of serum will be cryopreserved for future antigen response assays.

2. OBJECTIVES

2.1 Objectives

Primary objective:

To evaluate the effect of administration of nivolumab and ipilimumab on the proportion of PSA responses (>50% PSA decline) in mCRPC patients *with detectable AR-V7 transcript in CTCs*

In Cohort 2 (amendment 1):

To evaluate the effect of administration of nivolumab and ipilimumab on the proportion of PSA responses (>50% PSA decline) and/or ORR in mCRPC patients *with detectable AR-V7 transcript in CTCs*

Secondary safety objective:

To evaluate the safety and tolerability of ipilimumab + nivolumab in AR-V7–positive patients

Secondary efficacy objectives:

1. To determine the progression free survival (PFS)
2. To determine the PSA-PFS
3. To determine the proportion of "durable" responses
4. To determine the overall response rate (ORR)
5. To determine the overall survival (OS)

Secondary correlative/biomarker objectives:

1. *To evaluate changes in AR-V7 detection (or expression levels) before and after treatment with* ipilimumab + nivolumab and correlate with PSA responses.
2. To explore potential biomarkers associated with clinical efficacy (ORR, PFS, and OS) of nivolumab and ipilimumab by analyzing absolute lymphocyte count in peripheral blood as well as PD-L1 expression in CTCs and/or in tumor biopsies, and immune profiling of sera and tumor tissue.
3. To explore the relationship between DNA damage repair mutations (including mutational load, hypermutation, and loss of heterozygosity) and clinical outcomes
4.
5. To explore the relationship between MANAFEST assay and clinical outcomes
6.

2.2 Endpoints

2.2.1 Efficacy Endpoints:

1. The proportion of subjects with PSA response (>50% PSA decline)

- PSA decline will be reported on all patients. Using PCWG2 guidelines, the percentage of change in PSA from baseline to 12 weeks, as well as the maximum decline in PSA will be reported for each patient using a waterfall plot.
- 2.2.1.1 In Cohort 2 (amendment 1):
- Primary efficacy endpoint will be a composite endpoint of PSA response rate (proportion of men with >50% PSA decline) and/or Objective response rates (ORR) in patients with measurable disease.

2. PSA progression-free survival (PSA-PFS) [PCWG2 criteria]

- PSA progression (PSA progression free survival; PSA-PFS) will be defined per the PCWG2 guidelines
- For those subjects showing an initial decline in PSA from baseline, is defined as an increase in PSA that is ≥25% and ≥2 ng/mL above the nadir, and which is confirmed by a second value 3 or more weeks later (i.e., a confirmed rising trend).
- For those subjects with no decline in PSA from baseline, is defined as an increase in PSA that is ≥25% and ≥2 ng/mL after 12 weeks.

Note: PSA progression as such will not lead to study discontinuation

3. Time to radiographic or clinical progression or death, whichever comes first (progression free survival; PFS). Based on RECIST version 1.1 and Prostate Cancer Working Group 2 (PCWG2) definitions including:

- Progression of soft tissue lesions according to RECIST 1.1
- Progression of bone lesions detected with bone scan according to PCWG2 criteria
- Radiologically-confirmed spinal cord compression or pathological fracture due to malignant progression, or other clinical event deemed to be cancer-related

4. "Durable PFS", defined as lack of clinical/radiographic progression for ≥24 weeks
5. Objective response rate (ORR) by RECIST 1.1 criteria in patients with measurable disease
6. Response duration in patients with objective response, defined as the time from treatment initiation until time of disease progression by PCWG2 definitions.
7. Time to death after start of study treatment (overall survival; OS)

2.2.2 Safety Endpoints:

1. Incidence and severity of adverse events and serious adverse events graded according to the National Cancer Institute – Common Terminology Criteria for adverse events (CTCAE) version 4.0
2. Changes in laboratory variables: hematology and serum biochemistry

3. Changes in vital signs (systolic/diastolic blood pressure, respiratory rate, and heart rate) during the treatment period
4. Changes in physical examination during the treatment period

3. STUDY DESIGN

3.1 Study Design

This study is a phase 2 single-arm, single center, open-label study of nivolumab 3mg/kg combined with ipilimumab 1mg/kg every 3 weeks for 4 doses, followed by nivolumab 3mg/kg alone every 2 weeks. . Treatment will continue until week 48, or until radiographic progression or unmanageable toxicity.

The target population is men with metastatic CRPC and detectable AR-V7 splice-variant in CTCs. The study is expected to enroll 15 subjects. Detectable AR-V7 from CTCs must be present in order to enroll. Prior vaccine therapy is allowed (e.g. sipuleucel-T) if completed at least 6 weeks prior to enrollment. Prior therapy with CTLA-4 or PD-1/PD-L1 inhibitors is not allowed. No dose increases or reductions will be allowed for nivolumab or ipilimumab.

Subjects will be assessed for PSA response beginning 6 weeks (±3 days) from enrollment and continuing every 6 weeks (±3 days for cycle 2, subsequently (±7 days). Subjects will be assessed for radiologic responses (RECIST 1.1) by CT beginning 12 weeks (±1 week) after enrollment and continuing every 12 weeks (±1 week). Bone scan progression (PCWG2 criteria) will be assessed beginning 12 weeks (±1 week) after enrollment and continuing every 12 weeks (±1 week).

Subjects will be allowed to continue study therapy after initial investigator-assessed PCWG2-defined progression if assessed by the investigator to be deriving clinical benefit and tolerating study drug. Such subjects should discontinue study therapy when further progression is documented.

The key endpoints of the study are:

- **Primary efficacy objective:**

  To evaluate PSA responses (>50% PSA decline) in mCRPC patients *with detectable AR-V7 transcript in CTCs*

  In Cohort 2 (amendment 1):

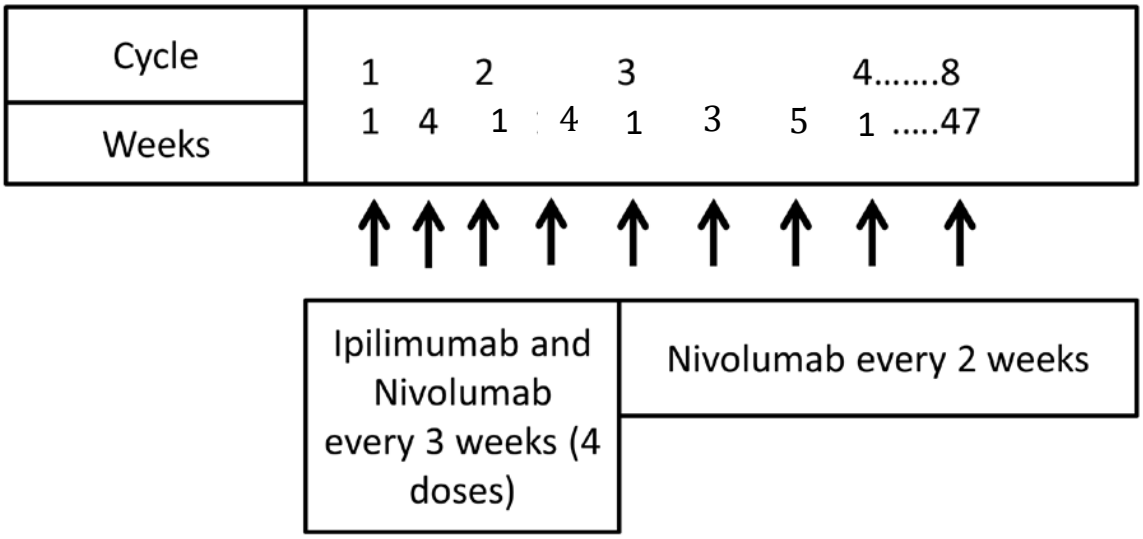
  To evaluate PSA response rate (proportion of men with >50% PSA decline) and/or Objective response rates (ORR) in patients with measurable disease

- **Secondary safety objective:**

  To evaluate the safety and tolerability of ipilimumab + nivolumab in AR-V7–positive patients, with attention to irAEs

  The **key correlative/biomarker objective** is to evaluate changes in AR-V7 detection (or expression levels) before and after treatment with ipilimumab + nivolumab

The treatment design schematic is presented below:



This study will consist of three phases: screening, treatment, and follow-up.

ScreeningPhase:

- Begins by establishing the subject's initial eligibility and signing of the informed consent form (ICF).
- Patients must have detectable CTCs with detectable AR-V7 splice-variant.

TreatmentPhase:

- Within 7 days from enrollment, the subject must receive the first dose of study medications (Day 1 of Cycle 1)
- On-study laboratory assessments (Cycle 1 Day 22 and beyond) should be drawn within 72 hours prior to dosing.
- Adverse event assessments should be documented at each clinic visit.
- Study drug dosing may be delayed for toxicity.

- Each cycle during the treatment phase is expected to last 6 weeks, unless there are delays.
- Treated subjects will be evaluated for response according to the RECIST 1.1 guidelines beginning 12 weeks (± 1 week) after enrollment and continuing every 12 weeks (± 1 week) as well as PCWG2 criteria (including PSA, bone scan, and symptoms) until disease progression or treatment discontinuation, whichever occurs later.
- This treatment phase ends when the subject is discontinued from study therapy.

Follow-UpPhase

- Begins when the decision to discontinue a subject from study therapy is made (no further treatment with study therapy).
- Subjects who discontinue treatment for reasons other than tumor progression will continue to have radiographic and PSA assessments beginning 12 weeks (± 1week) after enrollment and continuing every 12 weeks (± 1 week) thereafter until documented progression.
- Subjects will be followed for drug-related toxicities until these toxicities resolve, return to baseline or are deemed irreversible. All adverse events will be documented for a minimum of 100 days after last dose.
- After completion of the first two follow-up visits, subjects will be followed every 3 months for survival. Follow-up visits can be done by telephone.

The total duration of the study from start of randomization to final analysis of PSA response is expected to be 24 months (12 months of accrual + 12 months of follow-up), assuming a fixed accrual rate of 1.5 subjects per month (given approximately 4 subjects with AR-V7+ metastatic CRPC seen at Hopkins per month).

3.2 Schedule of Assessments

The following table (Table 1) reflects the assessments that will occur during the study, i.e. screening period, treatment period, and end-of-study visit.

Each cycle is 6 weeks and treatment will continue up to W48.

Visits should occur within a window of ±3 days during the first 2 cycles. Visits may occur within a window of ±7 days during subsequent cycles.

For radiologic evaluations, a window of ±1 week is acceptable.

The Investigator may perform more frequent examinations than shown in Table 1 if clinically indicated. Data from such additional examinations are also to be reported in the eCRF.

Table 1 Study Calendar

	Pre-study	Treatment/Intervention Period (1 cycle = 6 weeks)									
		Cycle 1 ±3 days		Cycle 2 ±3 days		Cycle 3-8 ± 7 days			End of treatment	Follow-up 1[m]	Follow-up 2[n]
	Day -28 to 0[a]	Wk 1 (±3 d)	Wk 4 (±3 d)	Wk 1 (±3 d)	Wk 4 (±3 d)	Wk 1 (±7 d)	Wk 3 (±7 d)	Wk 5 (±7 d)	Wk 49		
Informed consent	X										
Nivolumab[b]		X	X	X	X	X	X	X			
Ipilimumab		X	X	X	X						
Demographics	X										
Medical history	X	X[c]	X	X	X	X			X	X	X
Physical assessment	X	X[c]		X		X	X	X	X	X	X
Vital signs[d]	X	X	X	X	X	X	X	X	X	X	X
Height	X										
Weight	X	X[c]		X		X			X	X	X
Performance status	X	X	X	X	X	X			X	X	X
Toxicity assessment	X	X	X	X	X	X			X	X	X
EKG	X										
Histologic confirmation	X										
Disease assessment (Radiologic tests)[e,f]	X					X[f]					
CBC with diff	X	X	X	X	X	X	X	X	X	X	X
Comp Panel	X	X	X	X	X	X	X	X	X	X	X
TSH[g]	X			X		X	X	X	X	X	X
Hepatitis B/C[h]	X										
PSA	X			X		X			X		
Serum amylase and lipase	X	X	X	X	X	X			X	X	X
Serum Testosterone	X										
Metastatic tumor biopsy[i]	X										
AR-V7 Screening[j]	X										
CTCs[o]	X					X			X[l]		
Sera for immunoassays [k]	X					X			X		
Blood (100 mL) for PBLs[p]	X					X			X		
Plasma for ctDNA[q]	X										

Abbreviations: CBC, complete blood count; CMP, complete metabolic panel; CTC, circulating tumor cells; CT, computerized tomography; EKG, electrocardiogram; MRI, magnetic resonance imaging; PSA, prostate-specific antigen

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

b Beginning cycle 3, subjects will return to clinic every 2 weeks and receive nivolumab every 2 weeks

c Only needed if pre-study visit was >14 days prior to C1D1

d Including heart rate, blood pressure, pulse oximetry, pain, respiratory rate and weight

e CT c/a/p and bone scan. Radiologic documentation will be provided for subjects removed from study for progressive disease.

f And every subsequent 12 weeks until progression

g With reflexive T3 and fT4

h HBV sAg, HCV Ab

i Optional image-guided core biopsy (CT/MRI-guided or ultrasound-guided biopsy) may be obtained at baseline for patients with metastases that are amenable to biopsy

j AR-V7 Screening test should be performed within 4 weeks from screening visit. Order will be placed by provider in EPIC. Samples must be drawn before 12pm.

k Serum samples will be collected at three time points (pre-treatment, cycle 3, and end of treatment). At each time point, 2 SST (tiger top, i.e. BD Vacutainer Cat #367985) tubes should be collected, each containing ≥5mL of blood. After centrifugation, serum should be transferred into plastic cryovials and frozen at –20°C or colder until the time of analysis (see Appendix C section 5).

l 3rd CTC sample to be collected either at end of study or at time of progression if patient is continuing on treatment despite progression

m Follow-up 1 should occur 30 days after last treatment dose.

n Follow-up 2 should occur 84 days (6 weeks) after end of treatment.

o See Appendix C for CTC collection instructions

p Peripheral blood lymphocytes (PBLs) will be obtained by collecting 100 mL of blood into 50-mL heparinized conical tubes. PBLs will be prepared by Ficoll-Hypaque density gradient centrifugation according to standard protocols, and will be cryopreserved in a liquid nitrogen freezer at –140°C for further batched analyses (see Appendix C section 6).

q. See Appendix C for ctDNA plasma collection instructions

Table 2 Study Calendar for Cohort 2 (Amendment 1)

	Pre-study	**Treatment/Intervention Period (1 cycle = 6 weeks)**									
		Cycle 1 ±3 days		**Cycle 2 ±3 days**		**Cycle 3-8 ± 7 days**			**End of treatment**	**Follow-up 1[m]**	**Follow-up 2[n]**
	Day -28 to 0[a]	Wk 1 (±3 d)	Wk 4 (±3 d)	Wk 1 (±3 d)	Wk 4 (±3 d)	Wk 1 (±7 d)	Wk 3 (±7 d)	Wk 5 (±7 d)	Wk 49		
Informed consent	X										
Nivolumab[b]		X	X	X	X	X	X	X			
Ipilimumab		X	X	X	X						
Enzalutamide daily	X	X	X	X	X	X	X	X			
Demographics	X										
Medical history	X	X[c]	X	X	X	X			X	X	X
Physical assessment	X	X[c]		X		X	X	X	X	X	X
Vital signs[d]	X	X	X	X	X	X	X	X	X	X	X
Height	X										
Weight	X	X[c]		X		X			X	X	X
Performance status	X	X	X	X	X	X			X	X	X
Toxicity assessment	X	X	X	X	X	X			X	X	X
EKG	X										
Histologic confirmation	X										
Disease assessment (Radiologic tests)[e,f]	X					X[f]					
CBC with diff	X	X	X	X	X	X	X	X	X	X	X
Comp Panel	X	X	X	X	X	X	X	X	X	X	X
TSH[g]	X			X		X	X	X	X	X	X
Hepatitis B/C[h]	X										
PSA	X			X		X			X		
Serum amylase and lipase	X	X	X	X	X	X			X	X	X
Serum Testosterone	X										
Metastatic tumor biopsy[i]	X										
AR-V7 Screening[j]	X										
CTCs[o]	X					X			X[l]		
Sera for immunoassays [k]	X					X			X		
Blood (100 mL) for PBLs/MANAFEST[p]	X					X			X		

Plasma for ctDNA[q] (PGDx)	X										
Plasma for ctDNA[r] (Hurley lab)	X					X			X		

Abbreviations: CBC, complete blood count; CMP, complete metabolic panel; CTC, circulating tumor cells; CT, computerized tomography; EKG, electrocardiogram; MRI, magnetic resonance imaging; PSA, prostate-specific antigen

a Informed consent and radiologic assessments should be obtained within 8 weeks of study start date.
b Beginning cycle 3, subjects will return to clinic every 2 weeks and receive nivolumab every 2 weeks
c Only needed if pre-study visit was >14 days prior to C1D1
d Including heart rate, blood pressure, pulse oximetry, pain, respiratory rate and weight
e CT c/a/p and bone scan. Radiologic documentation will be provided for subjects removed from study for progressive disease.
f And every subsequent 12 weeks until progression
g With reflexive T3 and fT4
h HBV sAg, HCV Ab
i Optional image-guided core biopsy (CT/MRI-guided or ultrasound-guided biopsy) may be obtained at baseline for patients with metastases that are amenable to biopsy
j AR-V7 Screening test should be performed within 4 weeks from screening visit. Order will be placed by provider in EPIC. Samples must be drawn before 12pm.
k Serum samples will be collected at three time points (pre-treatment, cycle 3, and end of treatment). At each time point please follow Appendix C section 5.
l 3rd CTC sample to be collected either at end of study or at time of progression if patient is continuing on treatment despite progression
m Follow-up 1 should occur 30 days after last treatment dose.
n Follow-up 2 should occur 84 days (6 weeks) after end of treatment.
o See Appendix C sections 2, 3, and 4 for CTC collection instructions
p Peripheral blood lymphocytes (PBLs) will be obtained by collecting 100 mL of blood (as per Appendix C section 6).
q. See Appendix C section 7 for ctDNA plasma collection instructions
r. Refer to Appendix C section 8 for ctDNA plasma collection instructions for Hurley lab

4. PATIENT SELECTION

4.1 Target Population

The target population is men with AR-V7-positive metastatic castration-resistant prostate cancer.

4.2 Inclusion Criteria

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

1. Age at least 18 years at the time of signing the ICF
2. Histologically or cytologically confirmed adenocarcinoma of the prostate
3. Metastatic disease as defined by two or more bone metastases confirmed by bone scintigraphy or radiographic soft tissue metastasis
4. Detectable circulating tumor cells (CTCs) with detectable AR-V7 splice-variant by RT-PCR

5. Known castration-resistant disease, defined according to PCWG2 criteria as:

a) Castrate serum testosterone level: ≤50 ng/dL (≤1.7 nmol/L)

b) Subjects who have failed initial hormonal therapy, either by orchiectomy or by using a GnRH agonist in combination with an anti-androgen, must first progress through antiandrogen withdrawal prior to being eligible. The minimum timeframe to document failure of anti-androgen withdrawal will be four weeks

c) Serum PSA progression defined as two consecutive increases in PSA over a previous reference value within 6 months of first study treatment, each measurement at least one week apart. Serum PSA at screening ≥ 2 ng/mL

Or

Documented bone lesions by the appearance of two or more new lesions by bone scintigraphy

Or

Bidimensionally-measureable soft tissue metastatic lesion assessed by CT or MRI

6. Karnofsky Performance Status (KPS): ≥70% within 14 days before start of study treatment (ECOG ≤1)
7. Life expectancy: at least 6 months
8. Laboratory requirements:

Screening laboratory values must meet the following criteria *and should be obtained within 14 days prior to randomization/registration*

- WBC ≥ 2000/µL
- Neutrophils ≥ 1500/µL
- Platelets ≥ 100 x10^3/µL
- Hemoglobin > 9.0 g/dL
- Serum creatinine ≤ 1.5 x ULN or creatinine clearance (CrCl) ≥ 40 mL/min (if using the Cockcroft-Gault formula below):

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

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

- AST/ALT ≤ 3 x ULN
- Total Bilirubin ≤ 1.5 x ULN (except subjects with Gilbert Syndrome, who can have total bilirubin < 3.0 mg/dL)

9. Men who are sexually active with women of childbearing potential (WOCBP) must use any contraceptive method with a failure rate of less than 1% per year. *Men receiving nivolumab and who are sexually active with WOCBP will be instructed to adhere to contraception for a period of 31 weeks after the last dose of investigational product.*

a) WOCBP is defined as any female who has experienced menarch and has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy) or who is not postmenopausal. Menopause is defined clinically as 12 months of amenorrhea in a woman

over 45 in the absence of other biological or physiological causes. In addition, women under the age of 55 must have a documented serum follicle stimulating hormone (FSH) level less than 40 mIU/mL.

10. No evidence (within 5 years) of prior malignancies (except successfully treated basal cell or squamous cell carcinoma of the skin).
11. The subject is willing and able to comply with the protocol, and agrees to return to the hospital for follow-up visits and examination
12. The subject has been fully informed about the study and has signed the informed consent form and, where appropriate, HIPAA authorization for release of personal health information

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

4.2.1 For second cohort (amendment 1): 1 additional eligibility criteria will apply.

1. The most recent therapy must be enzalutamide and enzalutamide will be continued for study duration despite progressive disease (as defined in 4.2.5.c.) . **The minimum required dose of Enzalutamide at enrolment should be no less than 80 mg once daily.**

4.3 Exclusion Criteria

Subjects that meet any of the criteria listed below will not be eligible for study entry:

1. Has received an investigational therapeutic drug within the last 4 weeks prior to start of study treatment, or is scheduled to receive one during the treatment period.
2. Has received external radiotherapy within the last 4 weeks prior to start of study treatment.
3. Previous therapy with antiandrogens within 4 weeks
4. 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
5. Symptomatic metastatic disease with signs of rapid progression per investigator's clinical judgment
6. Concurrent use of other anticancer agents or treatments, with the following exceptions:

a) Ongoing treatment with LHRH agonists or antagonists, denosumab (Prolia) or bisphosphonate (eg, zoledronic acid) is allowed. Ongoing treatment should be kept at a stable schedule; however, if medically required, a change of dose, compound, or both is allowed.

7. Any treatment modalities involving major surgery within 4 weeks prior to the start of study treatment.
8. Symptomatic nodal disease, i.e. scrotal, penile or leg edema (≥CTCAE Grade 3)

9. Patients are excluded if they have active brain metastases or leptomeningeal metastases. Subjects with brain metastases are eligible if metastases have been treated and there is no magnetic resonance imaging (MRI) evidence of progression for at least 4 weeks after treatment is complete and within 28 days prior to the first dose of nivolumab administration. There must also be no requirement for immunosuppressive doses of systemic corticosteroids (> 10 mg/day prednisone equivalents) for at least 2 weeks prior to study drug administration.
10. 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 mellitus, residual hypothyroidism due to autoimmune condition only requiring hormone replacement, psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger
11. 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 > 10 mg daily prednisone equivalents are permitted in the absence of active autoimmune disease.
12. 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 > 10 mg/day prednisone equivalents. A brief course of corticosteroids for prophylaxis (eg, contrast dye allergy) or for treatment of non-autoimmune conditions (e.g. delayed-type hypersensitivity reaction caused by contact allergen) is permitted.
13. As there is potential for hepatic toxicity with nivolumab or nivolumab/ipilimumab combinations, drugs with a predisposition to hepatoxicity should be used with caution in patients treated with nivolumab-containing regimen.
14. Patients should be excluded if they have a positive test for hepatitis B virus surface antigen (HBV sAg) or hepatitis C virus ribonucleic acid (HCV antibody) indicating acute or chronic infection
15. Patients should be excluded if they have known history of testing positive for human immunodeficiency virus (HIV) or known acquired immunodeficiency syndrome (AIDS)
16. Allergies and Adverse Drug Reaction
17. History of allergy to study drug components
18. History of severe hypersensitivity reaction to any monoclonal antibody
19. Other primary tumor (other than CRPC) including hematological malignancy present within the last 5 years (except non-melanoma skin cancer or low-grade superficial bladder cancer).
20. Has imminent or established spinal cord compression based on clinical findings and/or MRI.
21. 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:
    a) Any uncontrolled infection
    b) Cardiac failure NYHA (New York Heart Association) III or IV

c) Crohn's disease or ulcerative colitis
d) Bone marrow dysplasia
e) Known allergy to any of the compounds under investigation
f) Unmanageable fecal incontinence

4.3.1 For second cohort (amendment 1): Concurrent therapy with enzalutamide will be permitted and is a requirement for enrollment.

4.4 Concomitant Medications

Because of potential drug-drug interactions, the eCRF must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. Any concomitant therapy, given to or taken by a subject from screening visit until 6 weeks after the last dose of study drugs will be recorded in the eCRF together with the indication. The generic or trade name, indication, dosage and, when applicable, the start and stop date will be recorded.

4.4.1 Blood transfusions/erythropoietin

Erythropoietin is not allowed during the study period or within the last 4 weeks prior to inclusion into the study.

Blood transfusions are allowed during the study period but not within the last 4 weeks prior to inclusion into the study.

4.4.2 Concomitant prostate cancer therapy

EBRT is not to be introduced until the follow-up period unless deemed absolutely necessary.

Patients will be allowed to continue prior LHRH agonist/antagonist therapy. Patients on bisphosphonates (eg, zoledronic acid) or denosumab (Prolia) will also be permitted to continue treatment. Preferably, ongoing treatment should be kept at a stable schedule; however, if medically required, a change of dose and/or compound will be allowed. If medically required, LHRH agonist/antagonist, denosumab, and bisphosphonates can be started during the study and preferably should be kept at a stable dose.

Systemic treatment with corticosteroids at doses corresponding to a prednisone dose above 10 mg/day must be avoided unless medically required..

4.4.3 Other concomitant therapies

Other medications, excluding those mentioned under "Disallowed Concomitant Therapy," may be given concomitantly as needed for the patient's welfare. Caution and, if possible monitoring, is advised with drugs with a predisposition to hepatoxicity given the risk for hepatotoxicity in ipilimumab- and nivolumab-containing regimens.

4.5 Disallowed Concomitant Therapy

Requirement for concomitant systemic treatment with any of the following therapies will lead to withdrawal from study treatment.

- Bicalutamide (eg, Casodex®) or other antiandrogens
- Use of all concomitant medications will be recorded in the patient's medical records and eCRF. This will include all prescription drugs, herbal products, vitamins, minerals, and over-the-counter medications. Any changes in concomitant medications also will be recorded in the patient's medical records and eCRF.

  Any concomitant medication deemed necessary for the welfare of the patient during the study may be given at the discretion of the investigator. However, it is the responsibility of the investigator to ensure that details regarding the medication are recorded in full in the patient's medical records and eCRF.

5. STUDY DRUGS

Study drug includes both Investigational [Medicinal] Product (IP/IMP) and Non-investigational [Medicinal] Product (Non-IP/Non-IMP) and can consist of the following:

- All products, active or placebo, being tested or used as a comparator in a clinical trial.
- Study-required premedication, and
- Other drugs administered as part of the study that are critical to claims of efficacy(eg, background therapy, rescue medications)

5.1 Investigational Product

An investigational products (nivolumab and ipilimumab), also known as investigational medicinal product in some regions, is defined a pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical study, including products already with a marketing authorization but used or assembled (formulated or packaged) differently than the authorized form, or used for an unauthorized indication, or when used to gain further information about the authorized form.

The investigational product should be stored in a secure area according to local regulations. It is the responsibility of the investigator to ensure that investigational product is only dispensed to study subjects. The investigational product must be dispensed only from official study sites by authorized personnel according to local regulations.

5.2 Non-investigational Product

Other medications used as support or escape medication for preventative, diagnostic, or therapeutic reasons, as components of the standard of care for a given diagnosis, may be considered as non-investigational products.

In this protocol, non-investigational product(s) is/are: medications used to treat nivolumab infusion-related reactions (eg, steroids, anti-emetics); these non-investigational products should be sourced by the investigator sites if available and permitted by local regulations.

5.3 Storage and Dispensing

The investigator should ensure that the study drug is stored in accordance with the environmental conditions (temperature, light, and humidity) as per product information and the Investigator Brochure and per local regulations. It is the responsibility of the investigator to ensure that investigational product is only dispensed to study subjects. The investigational product must be dispensed only from official study sites by authorized personnel according to local regulations. If concerns regarding the quality or appearance of the study drug arise, the study drug should not be dispensed and contact BMS immediately.

Please refer to the current version of the Investigator Brochure and/or shipment reference sheets for additional information on storage, handling, dispensing, and infusion information for nivolumab.

PRODUCT INFORMATION TABLE: Please also see respective Product Investigator Brochures

Table Product Description

Product Description and Dosage Form	Potency	Primary Packaging (Volume)/ Label Type	Secondary Packaging (Qty) /Label Type	Appearance	Storage Conditions (per label)
Nivolumab BMS-936558-01 Solution for Injection[a]	100 mg (10 mg/mL)	10 mL viall	5-10 vials per carton/ Open-label	Clear to opalescent colorless to pale yellow liquid. May contain particles	2 to 8□C. Protect form light and freezing
Ipilimumab Solution for Injection	200 mg (5 mg/mL)	40 mL vial	4 vials per carton/Open-label	Clear, colorless to pale yellow liquid. May contain particles	2 to 8°C. Protect from 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 and ipilimumab include laboratory coats and gloves.

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

5.4 Administration

Nivolumab and ipilimumab will be given together every 3 weeks for 4 doses.

Nivolumab dose = 3 mg/kg

Ipilimumab dose = 1 mg/kg

Following these 4 concurrent doses, nivolumab will be administered alone every 2 weeks until progression or end of study.

When study drugs (ipilimumab or nivolumab) are to be administered on the same day, separate infusion bags and filters must be used for each infusion. Nivolumab should be administered first. The second infusion will be ipilimumab, and will start approximately 30 minutes after completion of the nivolumab infusion.

BMS-936558 (nivolumab) is to be administered as a 60 minute IV infusion. Ipilimumab should be administered as a 90 minute infusion following.

Ipilimumab and nivolumab may be diluted in 0.9% Sodium Chloride Solution or 5% Dextrose solution.

The dosing calculations should be based on the body weight obtained at the visit immediately prior to infusion visit. If the subject's weight on the day of dosing differs by > 10% from the weight used to calculate the dose, the dose must be recalculated. All doses should be rounded up or to the nearest milligram per institutional standard.

Following the first 4 doses of the combination of nivolumab and ipilimumab, nivolumab will be given every two weeks at a dose of 3mg/kg.

Dose Modifications

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

During cycles 1 and 2, subjects may be dosed no less than 19 days from the previous dose of drug and dosed up to 7 days after the scheduled date if necessary.

Starting from cycle 3, subjects may be dosed no less than 12 days from the previous dose of drug and dosed up to 7 days after the scheduled date if necessary.

Treatment compliance will be monitored by drug accountability as well as the subject's medical record and eCRF.

6 STUDY PROCEDURES

6.1 Screening/Pretreatment Assessment

Before initiating any screening activities, the scope of the study should be explained to each subject. Subjects 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, subjects should be asked to sign and date a Notice of Privacy Practice research authorization/HIPAA form and 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 subject eligibility according to the inclusion/exclusion criteria (Sections 4.2 and 4.3). The following assessments will be performed during this period:

Imaging can be done before obtaining informed consent if done routinely. If appropriate bone scan, and CT/MRI exist within the required study timeline, these images will be used in the study and there will not be a requirement to obtain new images for the study protocol.

Within 8 weeks prior to treatment start:

1. Bone Technetium scan with careful identification of all disease related hotspots.

   Number of lesions related to metastatic disease should be recorded as follows (PCWG2 recommendation): 1, 2-4, 5-9, 10-20 or >20.

2. CT of chest/abdomen/pelvis.

Within 28 days prior to treatment start:

1. Record demographics and medical history (including detailed information about medical history of prostate carcinoma and bone metastases and previous treatments).
2. Conduct physical exam (abbreviated physical examination, vital signs, height/weight, etc).
3. ECG - a standard 12-lead ECG will be performed at screening and then as clinically indicated throughout the duration of the study. Results will be recorded as normal or abnormal with abnormal findings described in the eCRF. Clinically significant ECG changes observed at baseline should be recorded as symptoms, while clinically significant ECG changes observed after treatment should be recorded as adverse events.
4. Obtain histologic or cytologic confirmation of adenocarcinoma of the prostate (from patient records).
5. Perform laboratory tests:
   a) Circulating tumor cell (CTC) analysis for AR-V7 expression
   b) Hematology: Hematocrit, hemoglobin, platelet counts, red blood cell counts, white blood cell counts, whole blood cell differential
   c) Serum biochemistry: Sodium, potassium, chloride, calcium, phosphate, magnesium, AST, ALT, creatinine, urea, bilirubin (total), albumin, total protein, amylase, lipase
   d) PSA: The PSA value within 28 days prior to treatment start has to be at least 2 ng/mL. PSA progression (25% increase over reference not older than 6 months) has to be observed in two measurements, each measurement at least 1 week apart
   e) Testosterone (castrate serum testosterone levels of ≤ 50 ng/dL required)
   f) Thyroid stimulating hormone (TSH)
   g) Research labs (cytokine and antibody profiles, serum for cryopreservation)
6. Assess Karnofsky performance status (Table 3).
7. Record concomitant medications. Relevant information should be captured in the eCRF.

6.2 Treatment/Intervention Period

The Treatment Phase will start on the first day of treatment (Day 1) and last until any criteria for withdrawal from study treatment is reached and study treatment is withdrawn.

According the schedule of visits, subjects will attend the clinic every 3 weeks for the first 4 cycles, then subsequently every 2 weeks for nivolumab infusion and assessments. The visits will be primarily in place to judge any safety issues, both in terms of clinical adverse events but also for laboratory values. The laboratory values must be checked prior to each dosing of nivolumab and ipilimumab to ensure they are within the defined limits for dosing. Should the values not be within these limits, then dosing should be delayed to await recovery from toxicity prior to re-dosing.

The Treatment Phase will continue until week 48 or if any criteria for withdrawal from study treatment are reached a. The End-of-Study Treatment Visit will occur 100 days after the last dose of study drug for AE assessment.

6.2.1 Clinical and laboratory assessments

A schedule for study assessments is provided in section 3.2.

Subjects will have the following assessments **PRIOR** to administration of nivolumab and/or ipilimumab on day 1 of cycles

- Medical history (May use pre-treatment assessment for day 1, cycle 1)
- Physical Examination (abbreviated physical examination, Karnofsky performance status, vital signs (including pulse oximetry), weight, etc)
- Serum biochemistry (sodium, potassium, chloride, calcium, phosphate, magnesium, AST, ALT, creatinine, bilirubin (total), albumin, total protein, urea), amylase, lipase, LDH
- Hematology/CBC (hematocrit, hemoglobin, platelets, red blood cells, white blood cells, white blood cell differential)
- TSH with reflex T3/fT4
- PSA
- Recording of Adverse Events
- Recording of all Concomitant Medication/Treatment

The following will be obtained at the mid-cycle visit (3 weeks after administration of study drug(s) during cycles 1 and 2

- Serum biochemistry (sodium, potassium, chloride, calcium, phosphate, magnesium, AST, ALT, creatinine, bilirubin (total), albumin, total protein, urea), amylase, lipase
- Hematology/CBC (hematocrit, hemoglobin, platelets, red blood cells, white blood cells, white blood cell differential)
- Medical history
- Physical Examination
- Recording of Adverse Events
- Recording of all Concomitant Medication/Treatment

During cycles 3-8 at Week 3:

- Medical history
- Physical examination
- Recording of Adverse Events
- Recording of all concomitant medication/treatment
- CBC with diff
- Comp Panel
- TSH

During cycles 3-8 at Week 5:

- Serum biochemistry, amylase, lipase
- CBC with diff
- Medical history
- Physical examination
- Recording of Adverse Events
- Recording of all concomitant medication/treatment
- At week 13 and end-of-study visit CTCs and research labs

At week 13 and every subsequent 12 weeks:

- Radiologic evaluation (bone scan, CT/MRI of chest/abdomen/pelvis) to assess progression of disease – any progression noted prior to week 12 should not result in treatment discontinuation (as per PCWG2 Guidelines)

6.2.2 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: Common Terminology Criteria for Adverse Events (CTCAE version 4.0. In addition, safety will be assessed by the following parameters:

- Laboratory values: Laboratory values with CTCAE grade 3 or higher that are considered clinically significant by the treating physician have to be reported as an adverse event
- ECG: Clinically relevant changes from baseline, as judged by the Investigator, will be recorded as adverse events. A copy of the ECG page, signed and dated, including diagnosis should be stored in the patient file.
- Physical Examination: Any physical examination finding that is classified by the Investigator as a clinically significant change (compared to previous examination) will be considered an adverse event and documented on the eCRF and followed until the outcome is known.

6.2.3 Safety follow-up visit (6 weeks after End of Treatment)

An initial safety follow-up visit must occur 6 weeks after the completion or discontinuation of treatment.). Subjects withdrawn from the study because of AEs will be followed until the adverse event has either resolved or stabilized. Reasons for premature withdrawal should be determined and noted on the eCRF. The visit will include the following assessments:

- Physical Examination (abbreviated physical examination, Karnofsky performance status, vital signs etc.)
- Hematology/CBC (hematocrit, hemoglobin, platelets, red blood cells, white blood cells, white blood cell differential)
- Serum biochemistry (sodium, potassium, chloride, calcium, phosphate, magnesium, AST, ALT, creatinine, urea, bilirubin (total), albumin, total protein), amylase, lipase
- PSA
- TSH with reflex T3/fT4
- Recording of Adverse Events
- Recording of all concomitant medication/treatment

6.3 Correlative/Special Studies

6.3.1 Circulating tumor cells

At baseline, at the start of cycle 3, and at the end-of-treatment visit, blood samples will be collected by a Cancer Center phlebotomist from peripheral veins for analysis of circulating tumor cells (CTCs). This will serve as a "liquid" biopsy.

6.3.1.1 AR-V7 evaluation

CTC analyses will be conducted using the commercially-available AlereTM CTC AdnaTest platform (AdnaGen, Langenhagen, Germany). This assay does not enable CTC enumeration. Isolation and enrichment of CTCs will be performed using the ProstateCancerSelect kit, and mRNA expression analyses will be performed using the ProstateCancerDetect kit with multiplexed reverse-transcription polymerase-chain-reaction (qRT-PCR) primers to detect the presence of CTCs, and custom primers will be designed to detect the full-length-AR (AR-FL) and AR splice variant-7 (AR-V7). The relative AR-V7 transcript abundance will be determined by calculating the ratio of AR-V7 to AR-FL

Blood samples will be collected using standard BD Vacutainer® lavender top blood collection tubes (Becton Dickinson, Franklin Lakes, NJ) (Product #: 367862) by venipuncture, and carried to the lab on ice. Laboratory processing will be carried out within 2 hours of collection, according to instructions provided by the AlereTM CTC AdnaTest (Alere Inc., San Diego, CA). The AdnaTest is a CE-marked, RNA-based CTC enrichment and detection test with two components/kits. Briefly, the ProstateCancerSelect (Product No. T-1-520) kit will be used to enrich CTC from 5ml blood using magnetic particles coated with a combination of antibodies recognizing prostate cancer cells, while the ProstateCancerDetect (Product No. T-1-521) kit will be used to make cDNA for detection of prostate cancer-associated RNA transcripts using multiplexed polymerase chain reaction (PCR). On the basis of detection of PCR signals for PSA, PSMA, or EGFR (very rarely detected) by the Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA), CTC calls will be made for each sample tested.

The test was previously adapted for detection and quantification of AR-FL and AR-V7 by quantitative real-time PCR using custom primers specific for AR-FL (Antonarakis et al, NEJM 2014): (forward: 5'- CAGCCTATTGCGAGAGAGCTG-3', reverse: 5'-GAAAGGATCTTGGGCACTTGC-3') and AR-V7 (forward: 5'-CCATCTTGTCGTCTTCGGAAATGTTA-3', reverse: 5'-TTTGAATGAGGCAAGTCAGCCTTTCT-3'). Briefly, PCR reactions were carried out under optimized conditions at 95°C x 10s, 58°C x 30s, and 72°C x 30s for 39 cycles followed by melting curve analysis.

6.3.1.2 PD-L1 evaluation
Standard qRT-PCR analysis of PD-L1 expression in CTCs will be carried out using cDNA derived from CTCs along with negative and positive controls.

Platform: Qiagen RotorGene (Qiagen, Milan, Italy) will be used and the level of PD-L1 mRNA will be calculated as the ratio of PD-L1 mRNA level and 18S housekeeping gene level for each sample. Primers will be designed using Primer Blast (http://www.ncbi.nlm.nih.gov/tools/primer-blast/): set 1, 18S -FW (forward) 5' AAACGGCTACCACATCCAAG -3', 18S-RE (reverse) 5'-CCTCCAATGGATCCTCGTTA-3', and set 2, PD-L1-FW (forward) 5'-TACTGGCATTTGCTGAACGC 3', PD-L1-RE (reverse) 5'-CTTGTAGTCGGCACCACC.

6.3.1.3 Epic Sciences PD-L1 evaluation

Blood samples from lab draws concurrent with CTC analysis as discussed above will be sent to Epic Sciences (San Diego, CA) for PD-L1 evaluation on both CTCs and circulating leukocytes.

6.3.2 Tumor Biopsy
For patients with soft-tissue (lymph node and visceral) metastases that are amenable to biopsy, an optional core biopsy may be obtained at baseline.

Sample collection: Patients will be referred to the Johns Hopkins Biopsy Service for an image-guided core biopsy (CT/MRI-guided or ultrasound-guided biopsy).

Four or more standard core biopsies will be obtained from a single metastatic site (soft tissue, lymph node). Half of the collected tissue (i.e. at least 2 cores will undergo standard formalin-fixation and paraffin-embedding (FFPE) in the Department of GU Pathology (Dr George Netto), while the other half (i.e. at least 2 cores) will be frozen fresh in liquid nitrogen. To minimize tissue autolysis during fresh tissue collection, a dedicated tissue technician will be paged to the biopsy suite to initiate processing steps including flash freezing in liquid nitrogen and FFPE preparation, within 30 minutes of the biopsy procedure. Tissue must be gently pat-dried with a sterile gauze prior to freezing to avoid formation of crystals. The frozen specimens will be stored in a–80o C freezer in the laboratory of Dr Jun Luo. Upon accumulation of core biopsies from 5 - 10 patients, the frozen biopsies will be sectioned using a cryostat and stained slides will delivered to the Dept. of GU Pathology for histological annotation along with the matched FFPE specimens.

Amendment 1: Somatic DNA sequencing for DNA-repair and other mutations will be performed from pre-treatment (fresh or archival) metastatic or primary biopsies. Additional testing will include whole-exome sequencing, mutational load analysis, hypermutation analysis, and loss of heterozygosity analysis. Tumor RNA sequencing, and T-cell receptor (TCR) sequencing will also be performed.

6.3.2.1 PD-L1 evaluation

FFPE samples will be sent for IHC-based analyses aimed at quantifying the expression of proteins involved in PD-1 signaling such as PD-1, PD-L1, and PD-L2. Additional IHC analyses

may be completed to determine the relative abundance of other protein markers associated with tumor-infiltrating immune cells (eg, CD4, CD8). The abundance of each protein monitored (or combinations of proteins) will be correlated with clinical endpoints.

FFPE tissue will also be evaluated for in situ PD-L1 mRNA expression via in situ hybridization (RISH). This will performed to detect PD-L1 using the ACD (Advanced cell Diagnostics, Hayward, CA) RNAscope 2.0 Brown kit. Briefly, formalin-fixed paraffin-embedded (FFPE) tissue or cell pellet blocks will be sectioned and the slides baked for one hour at 600C. The slide were subsequently de-paraffinized with xylene for 20 min at room temperature, and allowed to air dry following two rinses using 100% ethanol. Following a series of pretreatment steps, the cells were permeablized using protease to allow probe access to the RNA target. ACD target probes, a series of paired oligonucleotides forming a binding site for a preamplifier, were custom designed to detect RNA corresponding to PD-L1 mRNA. Hybridization of the probes to the RNA targets will be performed by incubation in the oven for 2 hours at 40C. Following two washes, the slides will be processed for standard signal amplification steps per manufacturer's instructions.

The FFPE tissue may also be evaluated using genetic mutation detection methods, and/or by qRT-PCR as part of additional exploratory analyses seeking biomarker associations with clinical endpoints.

6.3.2.2 AR-V7

Analysis of FFPE tissues: We plan to carry out immunohistochemical (IHC) staining using validated antibodies against markers of the AR axis, including ARFL, AR-V7, the cell cycle protein UBE2C (induced by AR-V7), and PSA (induced by AR-FL). IHC analysis will be performed by Dr. George Netto at the Department of GU Pathology. Specific antibodies have been evaluated in Dr. Luo's laboratory (Hu et al 2012), and will be provided to Dr. Netto.

Analysis of fresh frozen tissues: Following histological analysis of frozen sections, tumor lesions will be identified and marked by Dr. George Netto at the Dept. of GU Pathology. This will be followed by manual trimming of the frozen blocks to enrich marked lesions for downstream molecular analyses. Following trimming and sectioning, tumor sections will be subject to RNA extraction. These RNA samples will undergo quality control using the Agilent Bioanalyzer in Dr. Jun Luo's laboratory. Dr. Luo's laboratory will perform standard qRT-PCR analysis of AR axis genes (namely AR-FL, AR-V, PSA, TMPRSS2, PSMA, UBE2C, and CYP17A1) along with negative and positive controls, as previously reported (Hu et al 2012).

6.3.2.3 Tumor mRNA Profile

RNA samples obtained from fresh frozen tissue as per section 6.3.2.2 will be analyzed via the Nanostring nCounter PanCancer Immune Profiling Panel, to allow multiplexed quantitative profiling of immune-related mRNAs present in the tumor biopsy sample.

6.3.3 Circulating Immune Biomarker Profiling

Serum will be obtained from patients at baseline, cycle 3, and end-of-study. Cytokine panel will be assessed via the Luminex 25-Plex panel (Life Techonologies; Carlsbad, CA) and antibody profile will be assessed via ProtoArray (Life Technologies; Carlsbad, CA).

6.3.4 Circulating tumor DNA (ctDNA)

Plasma will be obtained from patients at baseline to determine DNA mismatch repair (MMR) markers from ctDNA. The ctDNA panel will be assessed via the PlasmaSelect assay (PGDx; Baltimore, MD) and Hurley lab protocol (appendix C, section 8).

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

7.1 Dose adjustments

Dose reductions or dose escalations of nivolumab or ipilimumab are not permitted.

7.1.1 For second cohort (amendment 1):

If ≥grade 3 toxicity or intolerable side effects thought to be related to enzalutamide occur, enzalutamide should be withheld for 1 week or until symptom(s) improve to ≤grade 2, then resumed at same dose, or reduced to a dose of 120mg or 80 mg once daily at the discretion of the principal investigator.

7.2 Dose delay and treatment discontinuation

Dose delay criteria apply for all drug-related adverse events (regardless of whether or not the event is attributed to nivolumab, ipilimumab, or both). All study drugs must be delayed until treatment can resume.

7.2.1 For second cohort (amendment 1):

Enzalutamide may be held for adverse events thought to be related to enzalutamide. If a subject is off of enzalutamide for longer than 30 consecutive days, either directly prior to starting the study or after enrollment, the principal investigator needs to be alerted.

7.3 Dose Delay Criteria

Nivolumab and ipilimumab 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:
- If a subject has a baseline AST, ALT, or total bilirubin that is within normal limits, delay dosing for drug-related grade ≥ 2 toxicity
- If a subject has baseline AST, ALT, or total bilirubin within the Grade 1 toxicity range, delay dosing for drug-related Grade ≥ 3 toxicity
- Grade 3 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis do 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.

During cycles 1 and 2, both nivolumab and ipilimumab must be delayed at the same time.

Because of the potential for clinically meaningful nivolumab or ipilimumab-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.

In order to standardize the management across subjects for the overlapping adverse event management algorithms present in both the nivolumab and ipilimumab IB (**GI, hepatic, and endocrine** algorithms), the recommendations are to follow the nivolumab IB adverse event algorithms as opposed to the ipilimumab IB algorithms (see nivolumab IB).

7.4 Criteria to Resume Treatment

Missed doses of nivolumab and/or ipilimumab should be administered as soon as the subject meets criteria to resume treatment. If a dose has been missed, the subject should not 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.
- Subjects with baseline Grade 1 AST/ALT or total bilirubin who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of Grade 2 AST/ALT OR total bilirubin.
- Subjects with combined Grade 2 AST/ALT AND total bilirubin values meeting discontinuation parameters should have treatment permanently discontinued.
- Drug-related pulmonary toxicity, diarrhea, or colitis, must have resolved to baseline before treatment is resumed.
- Drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment.

If treatment is delayed > 6 weeks, the subject must be permanently discontinued from study therapy.

During cycles 1 and 2, both nivolumab and ipilimumab must be resumed on the same day. All four doses of nivolumab combined with ipilimumab must be given prior to beginning nivolumab monotherapy (cycle 5 and beyond).

If the subject is unable to resume both nivolumab and ipilimumab, permanent discontinuation is required.

7.5 Discontinuation Criteria

Treatment with nivolumab and ipilimumab 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 laboratory abnormalities do not require treatment discontinuation except:
    - Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding requires discontinuation
    - Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:
      - AST or ALT > 5 x ULN
      - Total bilirubin > 3 x ULN
      - Concurrent AST or ALT > 3 x ULN and total bilirubin > 2 x ULN
- 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
- Any dosing interruption lasting > 6 weeks unless the investigator is consulted and agrees with the rationale for resuming therapy after a delay > 6 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 or ipilimumab dosing

During cycles 1 and 2, both nivolumab and ipilimumab must be discontinued at the same time.

7.6 Treatment of Nivolumab or Ipilimumab-Related Infusion Reactions

Since nivolumab and ipilimumab 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 (version 4.0) 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 [eg, antihistamines, non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; prophylactic medications indicated for up to 24 hours).

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

Immediately discontinue infusion of nivolumab or ipilimumab. 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 or ipilimumab 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 (eg, appearance of a localized or generalized pruritis within 1 week after treatment), symptomatic treatment may be given (eg, oral antihistamine, or corticosteroids).

7.7 Treatment Beyond Disease Progression

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

Subjects will be permitted to continue treatment beyond initial investigator assessed progression without reconsent as long as they meet the following criteria:

- Investigator-assessed clinical benefit and Subject is tolerating study drug.
- The assessment of clinical benefit should take into account whether the subject is clinically deteriorating and unlikely to receive further benefit from continued treatment.

- Subjects should discontinue study therapy upon evidence of further progression, defined as an additional 10% or greater increase in tumor burden volume from time of initial progression (including all target lesions and new measurable lesions).
- New lesions are considered measurable at the time of initial progression if the longest diameter is at least 10 mm (except for pathological lymph nodes, which must have a short axis of at least 15 mm). Any new lesion considered non-measurable at the time of initial progression may become measurable and therefore included in the tumor burden measurement if the longest diameter increases to at least 10 mm (except for pathological lymph nodes, which must have an increase in short axis to at least 15 mm).

7.8 Immunotherapy Adverse Event Management

Because of the potential for clinically meaningful nivolumab or ipilimumab-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.

7.9 Removal of Subjects from the Study, Therapy Assessment

7.9.1 Subject withdrawal

Single subject termination is by definition when the patient is withdrawn or when the patient has died or completed his 12 month follow-up visit (from start of study treatment). 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 PCWG2 criteria for radiographic progression and not for PSA)
- Lack of subject compliance
- Protocol violation

7.10 Treatment Compliance

Trained medical personnel will administer nivolumab and ipilimumab and dispense other study medication. 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.

7.11 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 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.

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.

7.12 Return of Study Drug

If study drug will not be destroyed upon completion or termination of the study, all unused and/or partially used study drug that was supplied by BMS must be returned to BMS. The return of study drug will be arranged by the investigator.

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.

Arrangements for the return of study drug will be made by the investigator.

8 ENROLLMENT PLAN

The start date for the study (first patient first visit) is anticipated to be June 2015.

The total duration of the study is expected to be approximately 24 months including an enrollment period of 12 months. All subjects will be followed up for 12 months after start of study treatment.

9 SAFETY

The Investigator will review the safety data throughout the course of the study. The following safety variables will be evaluated: AEs, serum hematology values, serum biochemistry variables and abbreviated physical examination and changes in vital signs (systolic/diastolic blood pressure, respiratory rate, and heart rate).

A baseline recording of any symptoms of illness will be performed before the first administration of nivolumab and ipilimumab.

9.1 Adverse Event and Serious Adverse Event

9.1.1 Definitions

Adverse Event

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

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

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

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

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

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

Serious Adverse Event or Serious Adverse Reaction

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

- results in death
- is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or causes prolongation of existing hospitalization (see **NOTE** below)
- results in persistent or significant disability/incapacity

- is a congenital anomaly/birth defect
- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [eg, medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)
- Potential drug induced liver injury (DILI) is also considered an important medical event.
- Suspected transmission of an infectious agent (eg, pathogenic or nonpathogenic) via the study drug is an SAE.
- Although pregnancy, overdose, and cancer are not always serious by regulatory definition, these events must be handled as SAEs.

The following hospitalizations are not considered SAEs:

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

<u>Potential Drug Induced Liver Injury (DILI)</u>

Wherever possible, timely confirmation of initial liver-related laboratory abnormalities should occur prior to the reporting of a potential DILI event. All occurrences of potential DILIs, meeting the defined criteria, must be reported as SAEs. Potential drug induced liver injury is defined as:

1) ALT or AST elevation > 3 times upper limit of normal (ULN)
   AND
2) Total bilirubin > 2 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase)
   AND

3) No other immediately apparent possible causes of AST/ALT elevation and hyperbilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drug(s) known to be hepatotoxic.

Unexpected Adverse Reaction

An unexpected AR is an AR of which the nature or severity is not consistent with the applicable product information (e.g., Investigator's brochure for an unapproved investigational product or summary of product characteristics (SPC) for an authorized product). When the outcome of the AR is not consistent with the applicable product information this AR should be considered as unexpected.

Treatment-Emergent Adverse Event

Treatment-emergent AEs (TEAEs) are defined as events which occur following the first injection of study treatment, or that started prior to the first injection and worsened during treatment.

9.1.2 Responsibility

Investigator's Responsibilities

The Investigator shall report all SAEs independent of causality to BMS as per sections 9.3 and 9.4.

AEs and/or laboratory abnormalities identified in the protocol as critical to safety evaluations shall be reported to the Sponsor according to the reporting requirements and within the time periods specified in the protocol.

For reporting death of a subject, the Investigator shall supply the Institutional Review Board (IRB) with any additional information requested.

Each individual AE should be evaluated by the Investigator with regard to date of onset, its seriousness, severity, and duration, causal relationship to the investigational medicinal product and/or concomitant therapy and outcome.

IND Holder's Responsibilities

The IND Holder is responsible for the ongoing safety evaluation of the investigational product.

The IND Holder is responsible for the prompt notification to all concerned Investigators, the ECs/IRBs and regulatory authorities where nivolumab and ipilimumab studies are ongoing, of findings that affect the health of the subjects, impact on the conduct of the study or alter the regulatory authority's authorization to continue the study.

The IND Holder has to keep detailed records of all AEs reported to him by the Investigators and to perform an evaluation with respect to seriousness, causality and expectedness. These records shall be submitted to the Competent Authorities in the countries where the clinical study is being conducted, if they so request.

Each individual AE should be evaluated by the IND Holder, with regard to its seriousness and causal relationship to the investigational medicinal product and/or concomitant therapy. The IND Holder will assess whether or not the AE is unexpected.

9.2 Assessment of AE; Seriousness, Causality, Severity and Expectedness

Seriousness will be determined according to the definition, see Section 9.1.

Causality will be determined based on the definition in Section 9.1. All AEs judged by the Investigator as having a reasonable suspected causal relationship to an investigational medicinal product qualify as ARs.

All toxicities/AEs will be graded according to CTCAE version 4.0. In the eCRF the Investigator's opinion of the relationship of the AE(s) to the investigational drug, will be categorized as unrelated, possibly or probably related, as defined below.

Unrelated: An AE which after careful examination at the time of evaluation, is judged to be clearly and incontrovertibly due to extraneous causes (disease, environment, etc) and do not meet the criteria for drug relationship listed under possible or probable.

Possible: An AE which after careful examination at the time of evaluation, the connection with the test drug administration, or placebo, cannot be ruled out.

Probable: An AE which after careful examination at the time of evaluation, the connection to the test drug administration, or placebo, appears, with a high degree of certainty, to be related to test drug.

Severity: The term "severe" is used to describe the intensity (severity) of a specific event. Note that it is not the same as "serious", which is based on subject /event outcome or action criteria.

The severity of all events will be graded according to the CTCAE version 4.0 by the Investigator. For events not listed in the toxicity table, severity should be recorded as:

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental ADL5[1]
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL
- Grade 4: Life-threatening consequences; urgent intervention indicated.
- Grade 5: Death related to AE

Where:

[1] Activities of Daily Living (ADL). Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc. Self care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

Mild: The event causes discomfort without disruption of normal daily activities.

Moderate: The event causes discomfort that affects normal daily activities.

Severe: The event makes the subject unable to perform normal daily activities or significantly affects his/her clinical status.

Life-threatening: The subject was at risk of death at the time of the event

Disabling: The event causes a substantial disruption of a person's ability to conduct normal life functions.

Expectedness is defined in Section 8.1. Reports have to be considered as unexpected if they add significant information on the specificity or severity of an expected adverse reaction. The expectedness of an adverse event/reaction will be determined by the investigator.

The event is unexpected if it is not consistent with the applicable product information, i.e. the Investigator's Brochure for nivolumab and ipilimumab.

9.3 Reporting of Adverse Events

NONSERIOUS ADVERSE EVENT

- Nonserious Adverse Events are to be provided to BMS in aggregate via interim or final study reports as specified in the agreement or, if a regulatory requirement [e.g. IND US trial] as part of an annual reporting requirement.
- The collection of nonserious AE information should begin at initiation of study drug. All nonserious adverse events (not only those deemed to be treatment-related) should be collected continuously during the treatment period and for a minimum of 100 days following the last dose of study treatment.
- Nonserious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up is also required for nonserious AEs that cause interruption or discontinuation of study drug and for those present at the end of study treatment as appropriate.

Laboratory Test Abnormalities

- All laboratory test results captured as part of the study should be recorded following institutional procedures. Test results that constitute SAEs should be documented and reported as such.
- The following laboratory abnormalities should be documented and reported appropriately:
- any laboratory test result that is clinically significant or meets the definition of an SAE
- any laboratory abnormality that required the subject to have study drug discontinued or interrupted
- any laboratory abnormality that required the subject to receive specific corrective therapy.

Pregnancy

- Any pregnancy that occurs in a female partner of a male study participant should be reported to BMS. Information on this pregnancy will be collected on the Pregnancy Surveillance Form.
- Overdose
- An overdose is defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. All occurrences of overdose must be reported as an SAE.

Other Safety Considerations

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

9.4 Reporting of 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 (eg, 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.

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, or

•A congenital anomaly or birth defect.

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

For the 7 days SAE submission, reports can be submitted by facsimile to DOP1/OHOP at **(301)-796-9845** and to the attention of Sakar Wahby, PharmD, the division's Regulatory Project Manager

9.5 Procedures to be followed in the Event of Abnormal Laboratory Tests

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

The following laboratory abnormalities should be documented and reported appropriately:

•Any laboratory test result that is clinically significant or meets the definition of an SAE

•Any laboratory abnormality that required the subject to have study drug discontinued or interrupted

•Any laboratory abnormality that required the subject to receive specific corrective therapy.

9.6 Other Safety Parameters Including Demographics

9.6.1 Medical and surgical history of prostate cancer

A summary of the subject's relevant medical and surgical history of prostate cancer (i.e. time period from diagnosis of underlying malignancy to enrollment in the study, time of occurrence of skeletal metastases) should be recorded on the appropriate eCRF page.

9.6.2 Vital signs

Vital signs include heart rate, systolic and diastolic blood pressures, pulse oximetry, and respiratory rate. Vital signs will be assessed pre administration as detailed in Section 6.1. Normal limits for vital signs are provided below in Table 2 below.

Table 2. Criteria for normal limits for vital signs

Vital signs parameter	Normal limits	
	Low	High
Systolic BP (mm Hg)	85	149
Diastolic BP (mm Hg)	60	95
Heart rate (bpm)	60	100
O2 saturation	95	100
Respiration rate (rpm)	12	22

Both "new" and "worsening" vital signs abnormalities are anticipated in subject's over the course of a clinical study. A "new" abnormality is defined as one that occurs when a subject's normal baseline vital signs develop clinically significant values ("notable") post baseline. A "worsening" abnormality is defined as one that occurs when a subject's "notable" baseline vital signs become worse post baseline by 25%.

Notable vital signs results should be interpreted in conjunction with the clinical situation of the subject. Once AE notification is decided upon, the Investigator is required to follow the procedure described for AE notification and document the clinically notable abnormality on the AE eCRF page. Any notable abnormal vital signs finding or related AE must be followed until the outcome is known.

Before vital signs are recorded, the subject should be resting for at least 5 minutes. The same position will be used each time vital signs are recorded for a given subject, and blood pressure will be measured from the arm contra lateral to the site of administration of nivolumab and ipilimumab.

9.6.3 Physical examination

An abbreviated physical examination consisting of general appearance, lungs, cardiovascular system and abdomen and other physical findings will be done at each hospital visit where the subject meets with the treating physician.

Any physical examination finding that is classified by the Investigator as a clinically significant change (worsening compared to previous examination) will be considered as an AE, documented on the subject's eCRF, and followed until the outcome is known.

The Investigator will also assess the Karnofsky performance status at time points outlined in Section 6.

Karnofsky performance status is defined as follows:

Table 3. Karnofsky performance status

Scale (%)	Description
100	Normal; no complaints (ECOG 0)
90	Able to carry on normal activities; minor signs or symptoms of disease (ECOG 0)
80	Normal activity with effort (ECOG 1)
70	Cares for self. Unable to carry out on normal activity or to do active work (ECOG 1)
60	Requires occasional assistance and frequent medical care (ECOG 2)
50	Requires considerable assistance and frequent medical care (ECOG 2)
40	Disabled; requires special care and assistance (ECOG 3)
30	Severely disabled; hospitalization indicated though death not imminent (ECOG 4)
20	Very sick. Hospitalization necessary. Active supportive treatment necessary (ECOG 4)
10	Moribund (ECOG 4)
0	Dead (ECOG 5)

It is of importance to evaluate the Karnofsky performance status thoroughly, since this is one of the efficacy parameters. Even if the subject has to withdraw from the study due to difficulties in returning to hospital visits, the Karnofsky performance status should be evaluated at withdrawal.

9.6.4 ECG

A standard 12–lead ECG will be performed at screening. Results will be recorded as normal or abnormal; abnormal findings will be described in the eCRF and when clinically relevant, findings should be recorded as symptoms before administration and should be followed up during study as needed per the Investigator's judgment. The ECG will be evaluated by the local Investigator. The signed and dated ECG page (including diagnosis) should be stored in the subject's medical record.

ECG does not need to be repeated during the study unless clinically indicated.

9.6.5 Clinical laboratory parameters

Safety serum biochemistry and hematology parameters will be analyzed at each local laboratory as for standard of care.

Blood samples for the determination of serum chemistry and hematology will be drawn at pre-specified time-points, see Section 7. Additional hematology, chemistry and/or urine assessment are under the Investigator's judgment.

The following laboratory tests will be performed (Table 4):

Table 4. Clinical laboratory parameters

Serum biochemistry	**Hematology**
Alanine aminotransferase (ALT)	Hematocrit
Albumin	Hemoglobin
Aspartate aminotransferase (AST)	Platelets
Calcium	Red blood cell count
Chloride	White blood cell count
Creatinine	Differential (percents and absolutes)
Lactate Dehydrogenase (LDH)	Neutrophils
Magnesium	Lymphocytes
Phosphate	Monocytes
Potassium	Eosinophils
Protein, total	Basophils
Sodium	

Reference ranges from each laboratory will be provided to the PCCTC. If during the study, ranges should be changed, the Investigator is requested to provide updated laboratory normal values.

Laboratory values with CTCAE Grade 3 or higher and considered clinically significant by the treating physician have to be reported as an AE during the treatment period. During the follow-up period only changes in laboratory values judged to be related to the study drug will be reported.

The Investigator will interpret all clinical laboratory test results outside the reference range, using the following criteria:

1 = Value out of reference range, but not a clinically significant worsening from previous examination

2 = Value out of reference range, and a clinically significant worsening from previous examination

9.7 Safety Monitoring

The study will be continuously monitored for adverse events, which we define here as Grade 3 or 4 events that do not improve to Grade 1 or better within two weeks of implementing the adverse event monitoring algorithm (see Appendix A). If this toxicity rate appears to be higher than 30%, we will temporarily halt the study pending dose modification. Specifically, we will apply a Bayesian toxicity monitoring rule that suspends the enrollment if the posterior probability of risk being larger than 0.3 is 75% or higher.

In a study of the combination of ipilimumab and nivolumab in melanoma with greater ipilimumab dosing, 21% of patients developed Grade 3 or 4 treatment-related events that were dose-limiting.

The monitoring rule uses beta(0.5, 3.5) as the prior distribution. This means that the prior guess for the proportion of toxicity is 12.5% and there is 90% probability that this proportion is between 0% and 44%.

The decision rule for safety stopping is as follows:

Stop if:

# AE	3	4	5	6	7
Out of	3	5	8	11	13
	4	6	9	12	14
		7	10		15

For example, if three out of the first 3 patients have adverse events, we will stop the accrual. If four or more out of the first 5-7 patients have adverse events, we will stop.

The operating characteristics of the stopping rule are shown below and are based on 5000 simulations:

True AE rate	% Simulated trials declaring unsafe	Average sample size (out of 15)
0.15	2.7	14.8
0.20	7.2	14.4
0.25	13.6	13.9
0.30	24.7	13.1
0.35	36.7	12.2
0.40	50.5	11.2

0.45	66.4	9.8

10 EFFICACY ASSESSMENT

The efficacy assessments will be based on changes in biochemical markers and radiological evaluations.

10.1 PSA

PSA will be measured at baseline, and according to the Schedule of Assessments,. The samples will be collected, shipped and analyzed according the laboratory manual.

10.1.1 PSA response

For PSA, response will be described as the proportion of patients achieving a >50% decrease at any time during therapy relative to baseline. Using PCWG2 guidelines, the percentage of change in PSA from baseline to 12 weeks, as well as the maximum decline in PSA will be reported for each subject using a waterfall plot.

10.1.2 PSA Progression-free Survival (PSA-PFS)

Progression, for those subjects showing an initial decline in PSA from baseline, is defined as an increase in PSA that is ≥25% and ≥2 ng/mL above the nadir, and which is confirmed by a second value 3 or more weeks later (i.e., a confirmed rising trend). Progression, for those subjects with no decline in PSA from baseline, is defined as an increase in PSA that is ≥25% and ≥2 ng/mL after 12 weeks.

PSA-PFS will be measured from the time of treatment initiation until the time of clinical progression, radiographic progression, or death (see Appendix B). For subjects who have not yet demonstrated progression by the follow-up visit, patients will be censored at the date of the last tumor assessment that shows a lack of progression. This outcome will be depicted via Kaplan-Meier analysis.

10.2 Secondary Endpoints

10.2.1 Conversion from AR-V7-positive to AR-V7-negative

Conversion of AR-V7 positivity will be defined as the proportion of patients with negative AR-V7 in CTCs at either the week 13 time-point or the end-of-study time-point. Patients for whom CTCs cannot be detected at either of these two time-points will be considered to not be evaluable for this endpoint.

10.2.2 Criteria for Response, Progression, and Relapse

RECIST criteria will only apply to soft tissue lesions. PCWG2 discourages the use of overall response criteria, and in this protocol, such criteria will only apply to soft tissue lesions, will not incorporate bone lesions, and will not incorporate changes in tumor markers.

Bone lesions: Post-treatment changes will be described as “new lesions” or “no new lesions.”

There will be no descriptions of post-treatment "responses" for bone metastases. As such, only progression will be defined in regards to bone lesions, using the following table as a descriptor.

Patients are defined as progressing when they meet bone or soft tissue progression (both are not required).

10.2.2.1 Progression-free survival (PFS)

PFS will be defined as the duration of time from start of treatment to time of progression (per PCWG2 criteria) or death, whichever occurs first.

For soft tissue lesions, this is based on RECIST 1.1; or for bone disease, based on PCWG2 definitions which require the appearance of at least 2 new lesions with a confirmatory bone scan at least 6 or more weeks later.

If an event was not observed for a subject, or the subject withdrew, the time to event will be censored at the date of last contact recorded on the eCRF.

If a subject progresses according to both radiological and clinical criteria, the earlier response will determine the time of response for the endpoint.

If the occurrence of an event requires subsequent confirmation, the time of the event will be taken as the time of the first observation rather than the confirmation. For example, for a subject with radiological progression, the time of the progression will be the date of the bone scan at which two or more new lesions were first observed, not the date of the follow-up scan at which their presence was confirmed.

A subject in whom clinical or radiographic progression has been observed but not confirmed and who then subsequently dies will be considered to have progressed at the time of the observation (that is, they will be treated as if a confirmatory observation had been made). A subject who dies before radiographic or clinical progression has been observed will be considered to have progressed at the date of death, regardless of cause.

10.2.2.2 Durable PFS

Durable PFS will be defined as the proportion of patients without clinical or radiographic progression or death at 24 weeks from start of treatment.

10.2.2.3 Objective Response Rate (ORR)

ORR will be defined as the proportion of patients with a complete response (CR) or partial response (PR) in measurable soft-tissue lesions as defined by RECIST 1.1 criteria (see Appendix B).

10.2.2.4 Overall Survival (OS)

Overall survival will be defined as the time from study enrollment to death. This will be analyzed as a Kaplan-Meier plot.

10.2.3 AR-V7

The primary correlative analysis will be to investigate the proportion of men who convert from AR-V7–positive at baseline to AR-V7–negative during/after therapy with ipilimumab/nivolumab. This will be assessed at the 12-week time point as well as at time of progression.

Additionally, the change in AR-V7 expression, expressed as a ratio of AR-V7 to full-length AR (AR-FL) will be assessed at each time point.

11 STATISTICAL CONSIDERATIONS

11.1 Statistical Hypothesis and Sample Size

Our primary efficacy hypothesis is that we will observe PSA responses (>50% PSA declines) in a significant proportion of patients treated with ipilimumab plus nivolumab. Because there are currently no effective AR-directed therapies for men with AR-V7–associated CRPC (and PSA response rates with enzalutamide/ abiraterone approach 0%), we would consider ipilimumab + nivolumab promising if the PSA response rate is higher than 5%. We plan to enroll 15 patients. If we observe ≥3 PSA responses in 15 patients, the lower bound of 90% confidence interval of response rate would be above 5% (90% CI: 5.6% - 44%), and future study would be warranted.

If we observe **≥3 PSA responses in 15 patients**, we will proceed with subsequent larger trials. If we see ≤2 out of 15 PSA responses, we will consider this therapy unworthy of further study in AR-V7–positive mCRPC.

For cohort #2 (amendment 1)
Our primary efficacy hypothesis is that we will observe PSA responses (>50% PSA declines) and/or Objective response rates (ORR) in patients with measurable disease in a significant proportion of patients treated with ipilimumab plus nivolumab. Because there are currently no effective AR-directed therapies for men with AR-V7–associated CRPC (and PSA response rates with enzalutamide/ abiraterone approach 0%), we would consider ipilimumab + nivolumab promising if the composite endpoint of PSA response rate and/or ORR is higher than 5%. We plan to enroll 15 patients. If we observe ≥3 PSA responses and/or ORR in 15 patients, the lower bound of 90% confidence interval of response rate would be above 5% (90% CI: 5.6% - 44%), and future study would be warranted.
If we observe ≥3 PSA responses and/or ORR in 15 patients, we will proceed with subsequent larger trials. If we see ≤2 out of 15 PSA responses and/or ORR, we will consider this therapy unworthy of further study in AR-V7-positive mCRPC.

11.2 Analysis Populations

Evaluable for safety: All subjects who are enrolled and receive at least one administration of nivolumab and/or ipilimumab will be considered the safety population.

Evaluable for PSA response: All patients who have received at least one cycle (6 weeks) of therapy and have their PSA re-evaluated will be considered eligible for PSA response.

Evaluable for objective response: All patients who have measureable disease present at baseline, have received at least one cycles (6 weeks) of therapy, and have had their disease re-evaluated will be considered evaluable for objective response. These patients will have

their response classified according to RECIST 1.1 criteria. (Note: patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable).

11.3 Statistical Method

11.3.1 Standard listings

All individual data collected in the eCRF will be presented in data listings. Subjects screened but not included in the study will not be presented in any tables or listings.

11.3.2 Analysis of demographic and baseline characteristics

Demographic data and other baseline characteristics (including medical and disease history) will be summarized using the standard summary statistics.

11.3.3 Analysis of primary objective

The proportion of PSA responses (>50% PSA decline) and the corresponding 95% binomial confidence intervals will be reported.

11.3.3.1 For second cohort (Amendment 1)

The proportion of PSA responses (>50% PSA decline) and/or Objective responses in patients with measurable disease and the corresponding 95% binomial confidence intervals will be reported.

11.3.4 Analysis of secondary safety objective

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 30 days after treatment discontinuation. The incidence of adverse events will be tabulated and reviewed for potential significance and clinical importance.

11.3.5 Analysis of secondary correlative/biomarker objectives

The proportion of patients converting from AR-V7-positive to AR-V7-negative during treatment will be reported, along with the corresponding 95% binomial confidence interval, and compared across time points using McNemar's test. The changes in AR-V7 expression, expressed as a ratio of AR-V7 to full-length AR (AR-FL) will be analyzed over time using linear mixed effects models..

The relationships between binary measures (e.g. response) and candidate biomarkers will be investigated using logistic regression, and using cox regression for time-to-event-endpoints (e.g. PFS). Associations will be summarized in terms of point and interval estimates of hazard ratios, odds ratios, or other statistics, as appropriate for the analyses completed.

11.3.6 Analysis of other secondary objectives

Time-to-event endpoints of PSA-PFS, PFS, and OS will be presented using Kaplan-Meier estimates and corresponding 95% confidence intervals.

The proportion of "Durable PFS", defined as lack of clinical/radiographic progression for ≥24 weeks, will be reported along with the corresponding 95% binomial CI.

ORR will be estimated as the proportion of subjects whose best overall response is either a CR or PR with corresponding 95% binomial CI.

11.3.7 Handling of drop-outs and/or missing data

Subjects that withdraw/are being withdrawn for other reasons than safety prior to receiving one injection of nivolumab or ipilimumab (i.e. non-evaluable subjects) will be replaced. In safety and exploratory analyses, reason of missing/drop out will be documented. Analyses based on completers and missing imputation using the best available knowledge (or use multiple imputation when missing is completely at random) will be performed and compared.

11.3.8 Sub-group analysis

No sub-group analyses are planned. However, sub-groups may be identified on a data-driven basis, and such analyses will be considered exploratory and hypothesis generating only.

12 DATA REPORTING AND REGULATORY REQUIREMENTS

12.1 Data Reporting and Regulatory Requirements

12.2.1 Data Entry

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

12.2.2 Case report forms

Case report forms (e-crf) will be generated by Staff at SKCCC for the collection of all study data. Investigators will be responsible for ensuring that the CRFs are kept up-to-date.

12.2.3 Source documents

Study personnel will record clinical data in each patient's source documents (ie, the patient's medical record). Source documentation will be made available to support the patient research record.

12.2.4 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 7 years after the completion of all study activities.

12.2 Data Management

12.2.1 Lead research program coordinators

A Lead Research Program Coordinator at SKCCC will be assigned to the study. A Lead Research Program Coordinator will manage the study activities. 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.

12.2.2 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.

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

12.2.3 Data and Safety Monitoring

This is a DSMP Level II study under the SKCCC Data and Safety Monitoring Plan (12/6/2012). Data Monitoring of this protocol will occur on a regular basis with the frequency dependent on the rate of subject accrual and the progress of the study. The protocol will be monitored internally at SKCCC by the Principal Investigator and externally by the SKCCC CRO in accordance with SKCCC guidelines. Trial monitoring and reporting will be done through the Safety Monitoring Committee (SMC) at SKCCC.

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.

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.

12.3 Good Clinical Practice

The study will be conducted in accordance with the International Conference on Harmonisation (ICH) for Good Clinical Practice (GCP) and the appropriate regulatory requirement(s). The investigator will be thoroughly familiar with the appropriate use of the study drug as described in the protocol and Investigator's Brochure. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to appropriate regulations.

12.3.1 Ethical Considerations

The study will be conducted in accordance with applicable regulatory requirement(s) and will adhere to GCP standards. The IRB/IEC will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the patients. The study will be conducted only at sites where IRB/IEC approval has been obtained. The protocol, Investigator's Brochure, informed consent form, advertisements (if applicable), written information given to the patients (including diary cards), safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB/IEC by the investigator. BMS requests that informed consent documents be reviewed by BMS or designee prior to IRB/IEC submission.

12.3.2 Patient Information and Informed Consent

After the study has been fully explained, written informed consent will be obtained from either the patient or his/her guardian or legal representative before study participation. The method of obtaining and documenting the informed consent and the contents of the consent must comply with the ICH-GCP and all applicable regulatory requirements.

12.3.3 Patient Confidentiality

In order to maintain patient privacy, all data capture records, drug accountability records, study reports and communications will identify the patient by initials and the assigned patient number. If requested, the investigator will grant auditor(s) from BMS or its designees and regulatory authority(ies) access to the patient's original medical records for verification of data gathered on the data capture records and to audit the data collection process. The patient's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

12.3.4 Investigator Compliance

The investigator will conduct the study in compliance with the protocol given approval/favorable opinion by the IRB/IEC and the appropriate regulatory authority(ies). Changes to the protocol will require approval from BMS and written IRB/IEC approval/favorable opinion prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to patients. The IRB/IEC may provide, if applicable regulatory authority(ies) permit, expedited review and approval/favorable opinion for minor change(s) in ongoing studies that have the approval /favorable opinion of the IRB/IEC. The investigator will submit all protocol modifications to BMS and the regulatory authority(ies) in accordance with the governing regulations.

Any departures from the protocol must be fully documented in the source documents.

12.3.5 On-site Audits

Regulatory authorities, the IEC/IRB and/or BMS may request access to all source documents, data capture records, and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the investigator, who must provide support at all times for these activities.

12.3.6 Investigator and Site Responsibility for Drug Accountability

Accountability for the study drug at all study sites is the responsibility of the principal investigator. The investigator will ensure that the drug is used only in accordance with this protocol. Drug accountability records indicating the drug's delivery date to the site, inventory at the site, use by each patient, and amount returned to BMS or a designee or disposal of the drug (if applicable and if approved by BMS) will be maintained by the clinical site. Accountability records will include dates, quantities, lot numbers, expiration dates (if applicable), and patient numbers.

12.4 Record Retention

The investigator will maintain all study records according to the ICH-GCP and applicable regulatory requirement(s).

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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 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.

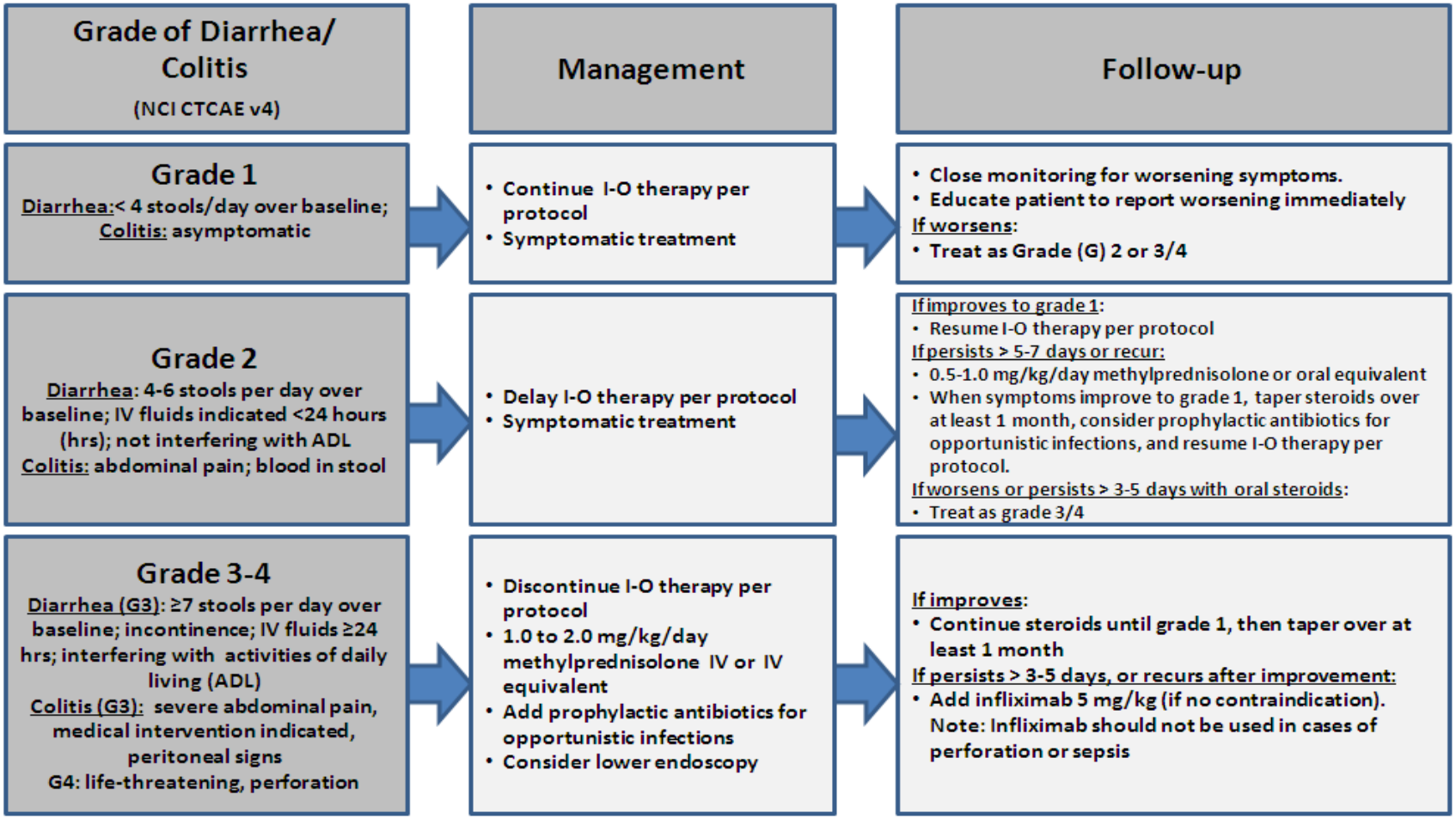
In patients who develop recurrent adverse events in the setting of ongoing or prior immunosuppressant use, an opportunistic infection should be considered in the differential diagnosis.

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

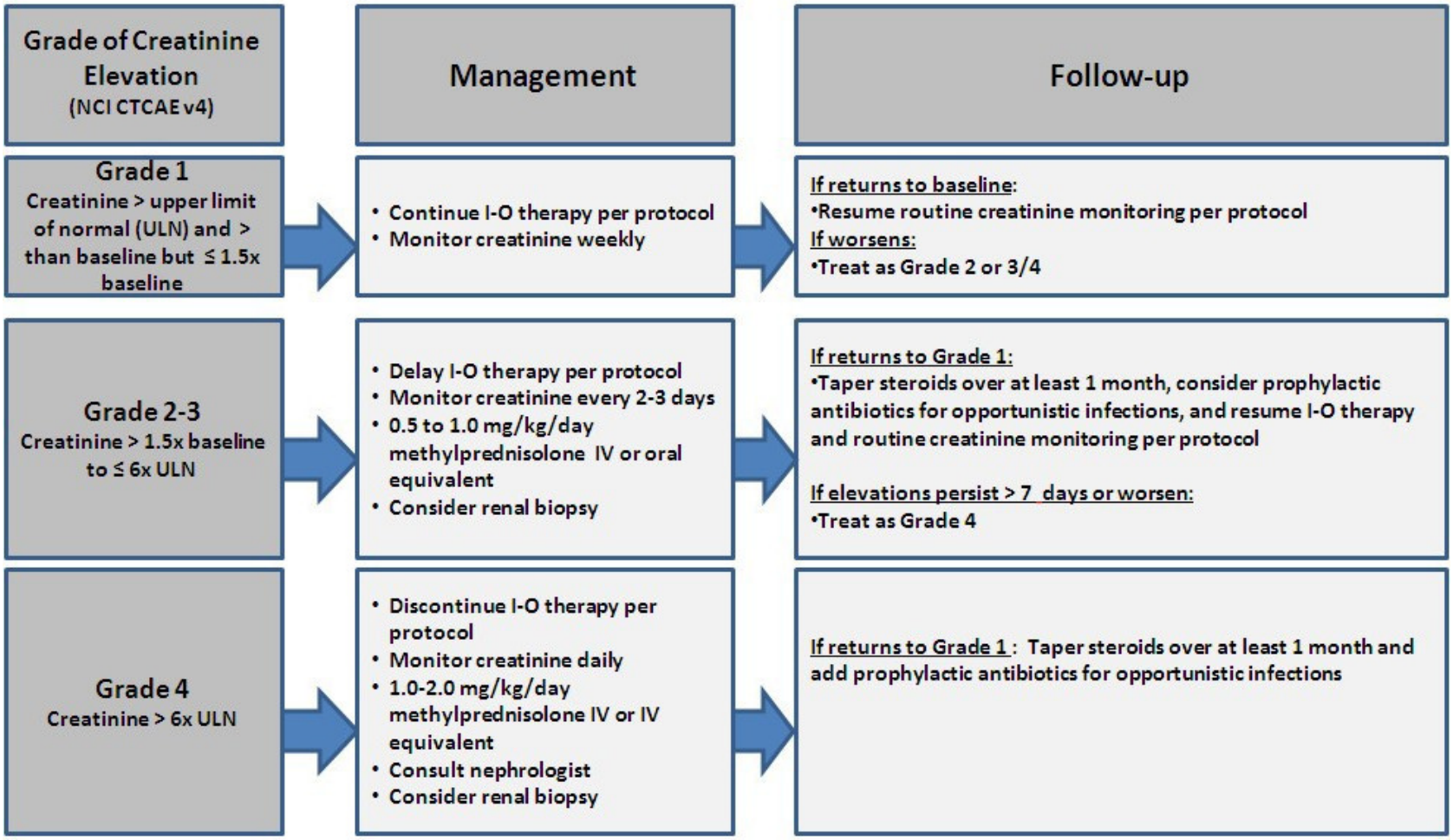
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.



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

Renal Adverse Event Management Algorithm

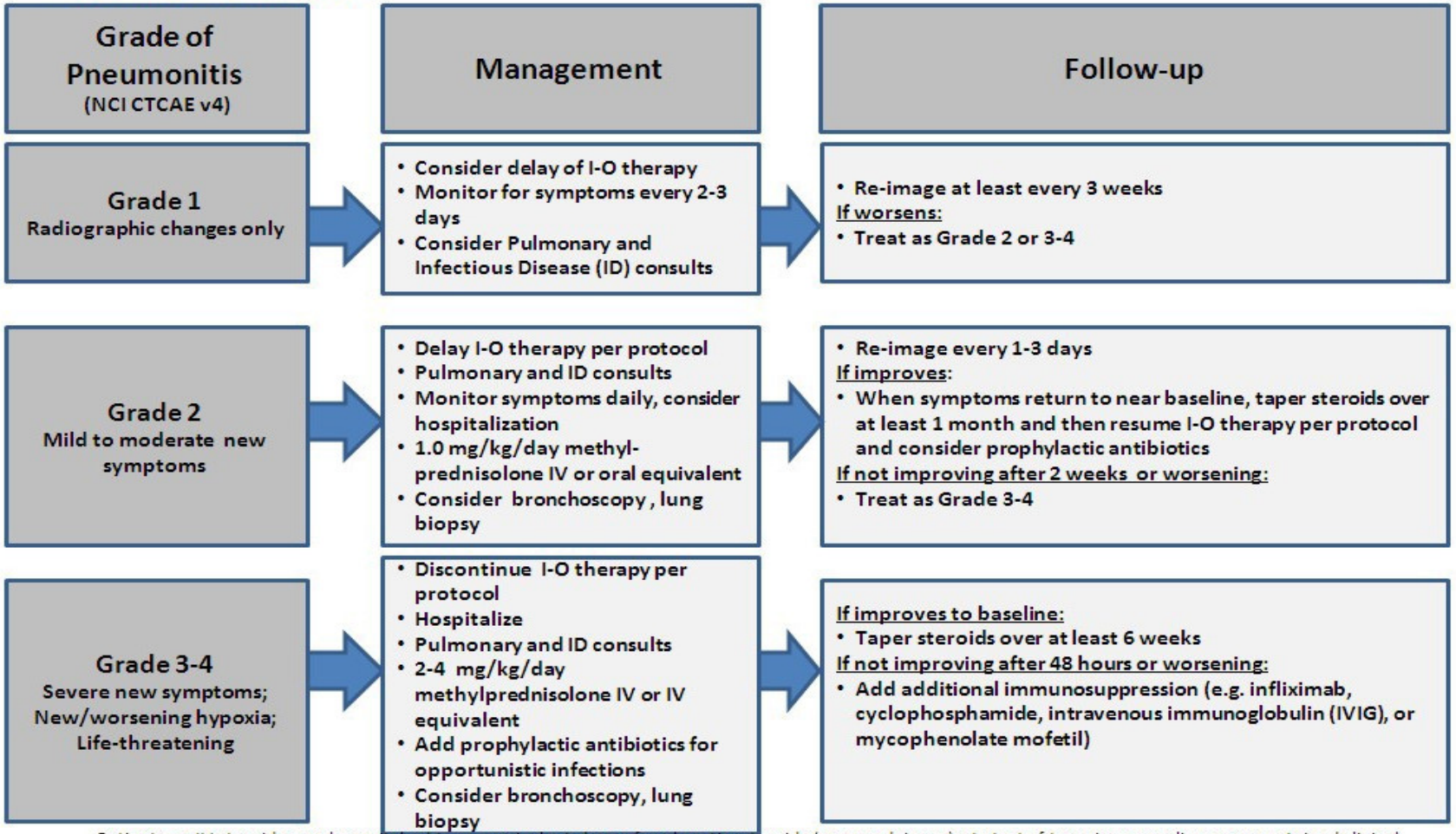
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy



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

Pulmonary Adverse Event Management Algorithm

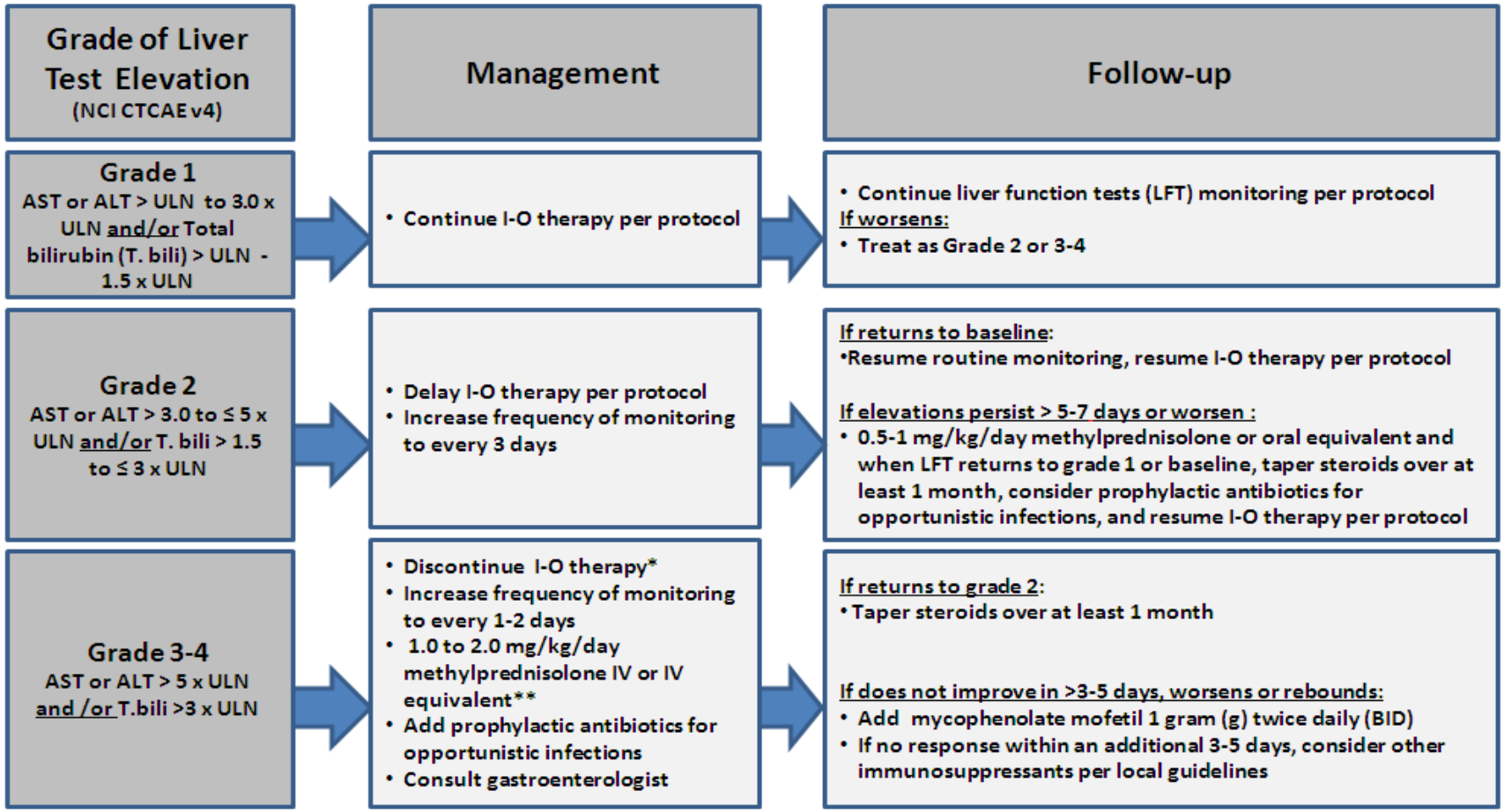
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.



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

Hepatic Adverse Event Management Algorithm

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



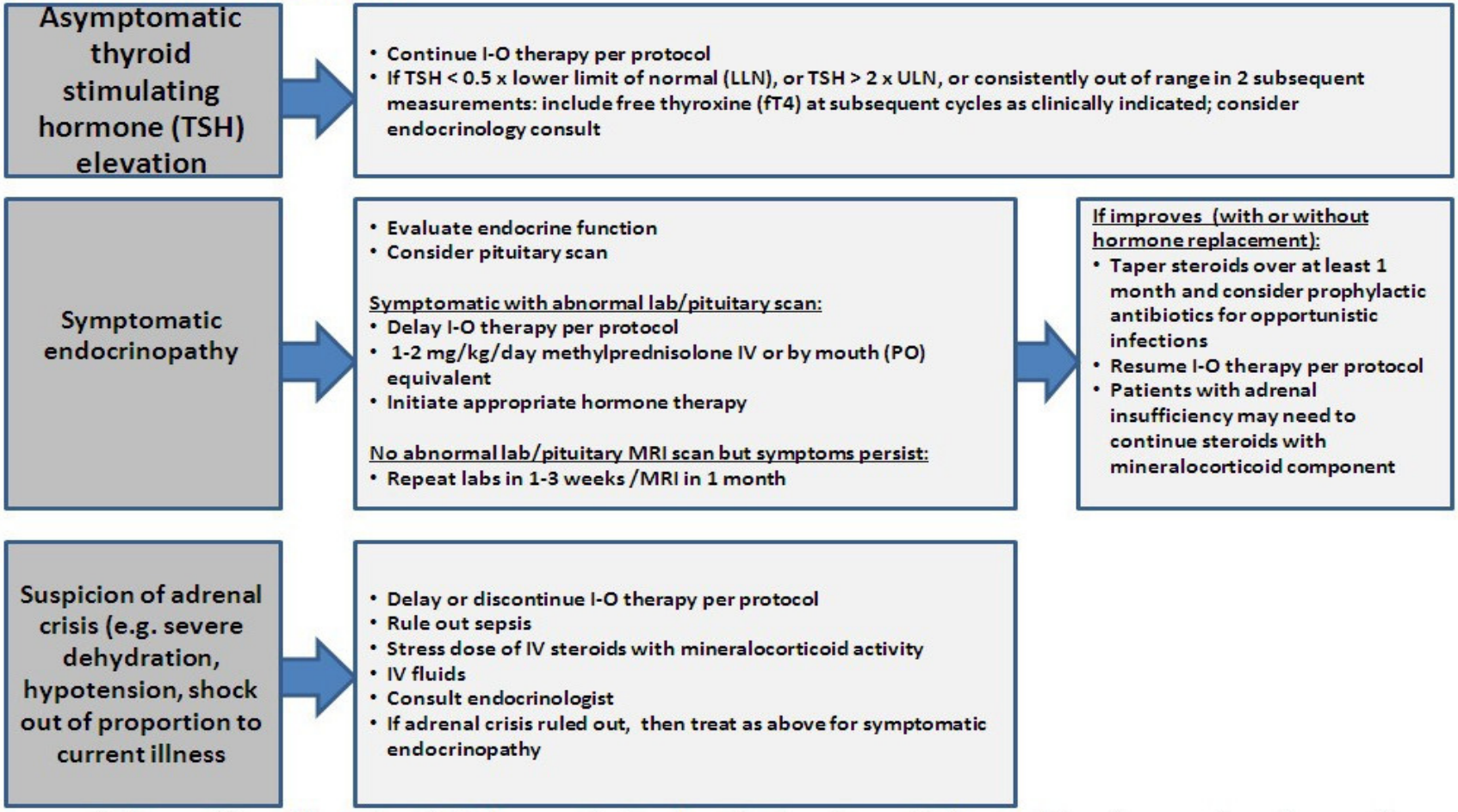
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

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

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

Endocrinopathy Management Algorithm

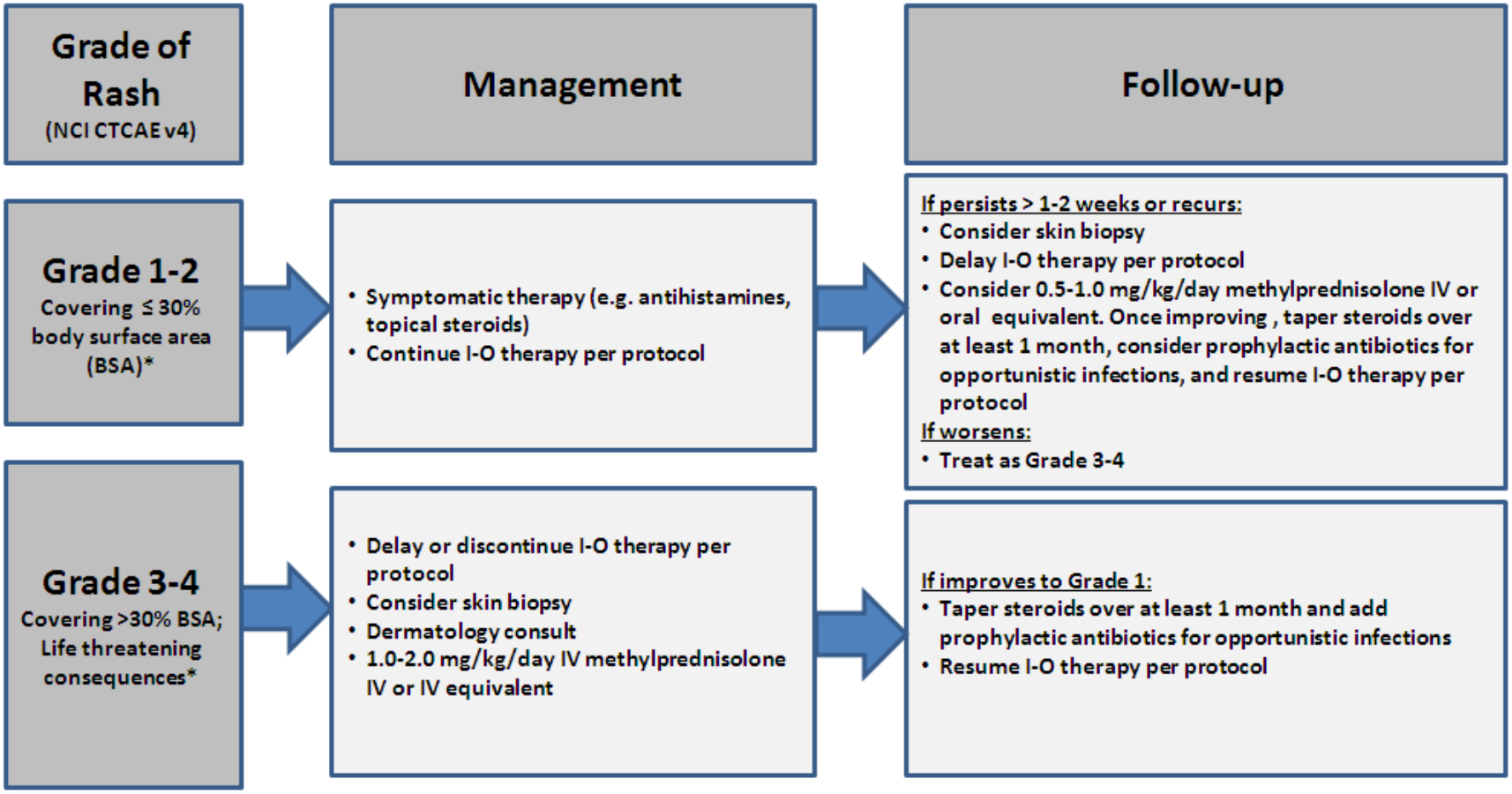
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider visual field testing, endocrinology consultation, and imaging.



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

Skin Adverse Event Management Algorithm

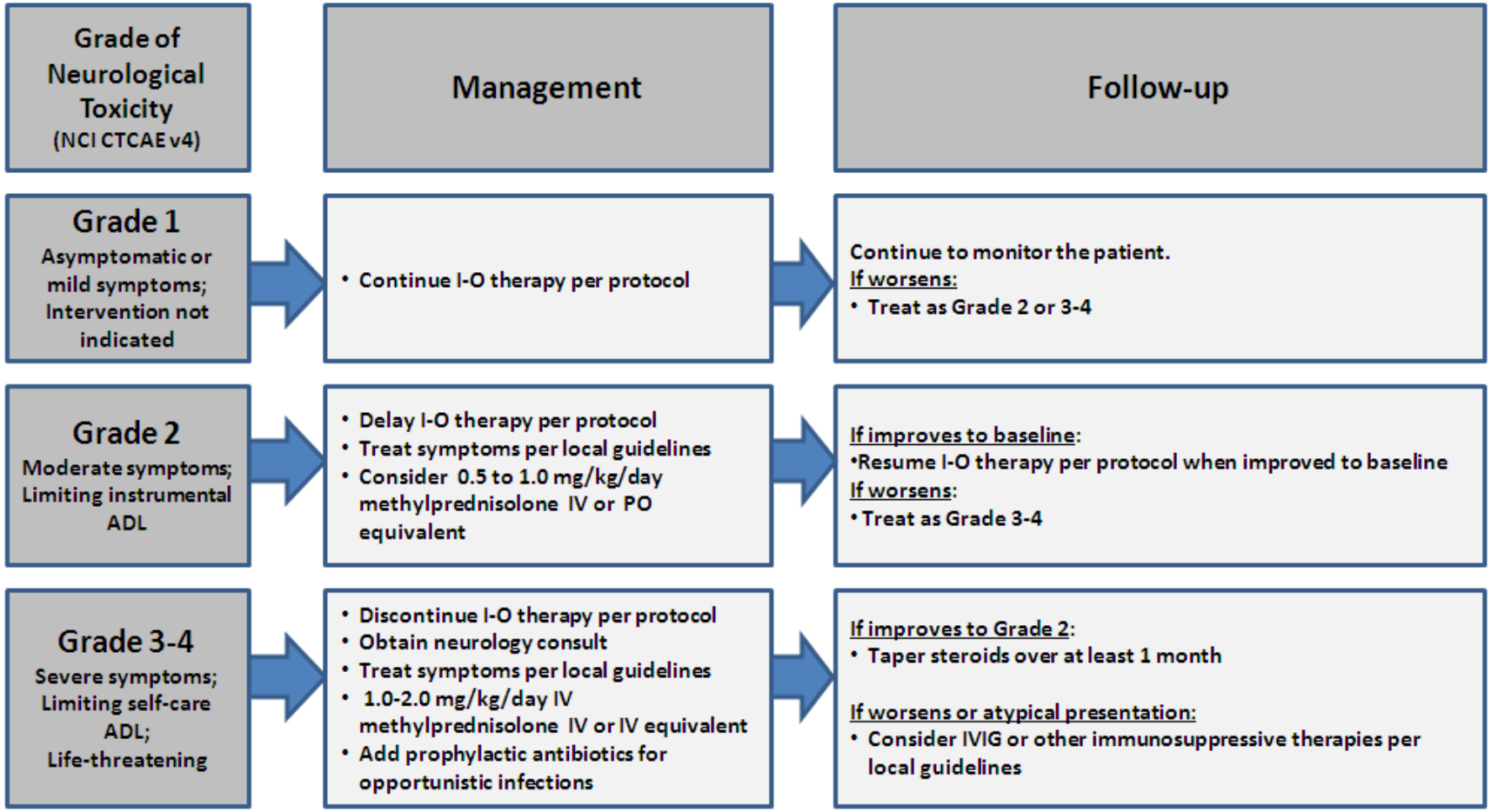
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



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

Neurological Adverse Event Management Algorithm

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



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

Appendix B: Prostate Cancer Clinical Trials Working Group (PCWG2) Criteria for Response

Pathology response criteria

When evaluating measurable soft-tissue target lesions, the RECIST definitions will apply. The first assessment must show an increase in the sum longest diameter (LD) of both preexisting and new lesions of ≥ 20% when compared with the smallest sum LD recorded since treatment started.

In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated further (*e.g.* by aspirate/biopsy) before confirming the complete response status.

Target Lesions

All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and will be recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, and should be chosen based on their suitability for accurate repetitive measurements. Lymph nodes need to be ≥ 20 mm in at least one dimension to be considered target or evaluable lesions to assess changes in size.

It may be the case that, on occasion, the largest lesion does not lend itself to reproducible repeated measurements in which case the next largest lesion that can be measured reproducibly should be selected. A sum of the diameters (longest for all lesions, including nodes) for all target lesions will be calculated and reported as the baseline sum diameters. The baseline sum LD will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

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

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

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new non-osseous lesions is also considered progression). Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules, palpable lymph nodes) and at

least 10 mm in diameter as assessed using calipers (e.g., skin nodules). Per PCWG2 and Cou302: Visceral (lung, liver adrenal) or extranodal lesions need to be ≥ 10mm in one dimension, using spiral CT. However, lymph nodes need to be ≥ 20 mm in at least one dimension to be considered new.

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

Non-target Lesions

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

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm long axis). Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

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

Evaluating best overall response

The best overall response is the best response recorded from the start of treatment until disease progression/recurrence. The investigator's determination of best overall response will be based on the response criteria and will not require confirmation scans. For defining disease progression, confirmation scans will only be required in the case where progression is seen on the first follow-up bone scan, but not if progression is shown on CT.

Patients with global deterioration of health status who require discontinuation of treatment without objective evidence of disease progression should be classified as having symptomatic deterioration. Every effort should be made to document their objective progression, even after discontinuation of treatment.

The table below summarized the recommendations of the PCWG2 for measuring response/progression outcomes in phase II clinical trials of prostate cancer. Although these guidelines have been largely followed in the design of the present trial, this table should not be used as a substitute for the clinical protocol.

Prostate Cancer Clinical Trials Working Group (PCWG2) Outcome Measures

Variable	Control/Relieve/Eliminate
PSA	Record the percent change from baseline (rise or fall) at 12 weeks, and separately, the maximal change (rise or fall) at any time using a waterfall plot
Soft-tissue lesions	Use RECIST with caveats Only report changes in lymph nodes that were ≥ 2 cm in diameter at baseline Record changes in nodal and visceral soft tissue sites separately Record complete elimination of disease at any site separately Confirm favorable change with second scan Record changes using waterfall plot
Bone	Record outcome as new lesions or no new lesions *First scheduled reassessment:* No new lesions: continue therapy New lesions: perform a confirmatory scan 6 or more weeks later Confirmatory scan: No new lesions: continue therapy Additional new lesions: progression *Subsequent re-assessments:*

	No new lesions: continue New lesions: progression
Symptoms	Consider independently of other outcome measures Document pain and analgesia at entry with a lead in period and measure repeatedly at 3- to 4-week intervals Perform serial assessments of global changes in HRQOL, urinary or bowel compromise, pain management, additional anticancer therapy Ignore early changes (< 12 weeks) in pain or HRQOL in absence of compelling evidence of disease progression Confirm response or progression of pain or HRQOL end points > 3 weeks later

Abbreviations: PSA, prostate-specific antigen; HRQOL, health-related quality of life

Appendix C: Specimen Collection and Shipping for STARVE-PC study

CTCs will be collected at screening visit, at the start of cycle 3, and at either the end of study visit or at time of progression if patient is continuing on treatment despite progression.

1. Screening AR-V7 Assay (for eligibility)

Please note: Specimens should be drawn in the morning and delivered by noon the same day.

1) AR-V7 testing will be required to determine eligibility. This will be ordered by the provider in EPIC (AR-V7 Prostate Cancer).
2) Specimens will be processed by the Molecular Diagnostics CLIA core lab.
3) Specimen should be drawn in the morning and hand-delivered to the lab in Park SB202 by noon the same day (samples must begin processing within 4 hours of collection). Alternately, specimens may be delivered to tube station 141 in Weinberg building by 11:30AM the same day.
4) Please note laboratory hours are Mon-Fri, 8:30-5:30.

2. CTC Adnatest for AR-V7

1) Blood draws should be scheduled on Monday-Friday.
2) Blood samples will be collected in one BD Vacutainer ACD Solution A tubes (BD ACD-A) (yellow top). Product # for the BD ACD-A tube is 364606 (US).
3) Ensure that at least 8.5 mL of blood is drawn into each tube. Avoid low volume to minimize agitation during shipping.
4) Invert the tubes gently 180 degrees and back 3-4 times.
5) After blood is drawn, **page Danilo Piana Pager 3-3027** IMMEDITELY who will carry blood samples to Jun Luo's lab in 415 Marburg Building (Johns Hopkins Hospital, 600 N Wolfe St, Baltimore, MD 21287).

3. CTC PD-L1 Evaluation

1) Collect Blood in 2 Streck Cell-Free DNA BCT tubes. Product number from Streck, Inc, Omaha NE is 218961.
2) Ensure that at least 10 mL of blood is drawn into each tube. Avoid low volume to minimize agitation during shipping.
3) Invert the tubes gently 180 degree and back 8-10 times

4) Maintain Blood at room temperature (6°C to 37°C) until pick-up
5) After blood is drawn, **page Danilo Piana Pager 3-3027** who will carry blood samples to Ken Pienta's lab in Marburg 116 (delivery contact for the STARVE-PC samples will be Stephanie Glavaris).

4. CTC for EPIC Sciences PD-L1 Evaluation

SHIP ALL SAMPLES OVERNIGHT!

Sample Collection:

IMPORTANT: The first 5 mL of blood collected from the fresh venous puncture cannot be used for the collection into the Streck tubes. Please ensure that at least one blood tube of 5 ml or more is collected prior to collection of blood in Streck tube to avoid adversely affecting the test results.

Prevention of Backflow:

Since Streck Cell-Free DNA BCT tubes contain chemical additives, it is important to avoid possible backflow from the tube. To guard against backflow, observe the following precautions:

- Keep patient's arm in the downward position during the collection procedure.
- Hold the tube with the stopper uppermost.
- Release tourniquet once the blood starts to flow into the tube, or within 2 minutes of application.
- Tube contents should not touch stopper or the end of the needle during the collection procedure.

Blood Collection Instructions:

**Schedule courier for same-day sample pic-up prior to collection

1. Confirm blood tube is not expired. Expired tubes should not be used for blood collection. Tubes will be provided by EPIC Sciences.
2. Draw whole blood sample into 10 mL Streck Cell-Free DNA BCT tube (*see note regarding prevention of backflow). Fill tube until blood flow stops. NOTE: Epic requires a minimum of 4mL blood per sample, but a full 10 mL tube of blood should be provided when possible.
3. Remove tube from adapter and immediately mix by gentle inversion 8 to 10 times. Tube inversion prevents clotting. Inadequate or delayed mixing may result in inaccurate test results.
4. Label the tube with subject's identification and date and time of blood draw.
5. Keep sample at room temperature and ship on day of collection.

Sample Shipment Instructions:

1. Place blood tube into foam insert in provided kit, and place in aluminum canister. Place canister in supplied cardboard box.
2. Open pre-assembled reusable specimen shipping container, remove insulation panel.
3. Place two liquid (red) E23 panels that have been stored at room temperature in the bottom of the shipping container rotated at 90 degrees from each other.
4. Place box containing canister with blood specimen directly on top of previously placed panel (see lab manual for figure).
5. Place one liquid (red) E23 panel that has been stored at room temperature on top of the box rotated at 90 degrees from the panel below it.
6. Place one solid (red) E23 panel that has been stored at 4 degrees Celsius on top of box.
7. Place locking lid on top of the 4th panel with the seam facing up to ensure insulation.
8. Close shipping box and tape appropriately.

Ship to:
Epic Sciences
c/o Dena Marrinucci
9381 Judicial Drive, STE 200
San Diego, CA 92121
Tel: 858-356-6610

Samples may be mailed to Epic Sciences for Monday–Saturday delivery

Send Email to:
partners@epicsciences.com
Include:
1. Tracking number
2. Number of samples being shipped
3. Date and time of each blood draw
4. Case report form including white blood cell count of patient(s)

5. Sera for Immunoassays

At each time point (Pre-treatment, cycle 3, and end of treatment visit), the following research blood samples should be collected and processed as outlined below:

• Draw approximately 10 mL of peripheral blood into 1 SST (tiger top, i.e. BD Vacutainer Cat #367985) tube.

- Allow blood to coagulate for 20 minutes, then centrifuge at 25oC, 1500 x g (2700-3000 rpm), for 15 minutes.
- Pipette the serum into 10 cryotubes/snap-top tubes (about 0.5 mL/tube).
- Store cryotubes/snap-top tubes frozen, below –20oC (–70oC preferred), until the time of analysis.

Please **Ping "Vaccine Team"** (pager 410-283-0693) and the Immune Core will be notified to pick up the sample after draw.

6. Peripheral blood lymphocytes (PBLs)

At each time point (Pre-treatment, cycle 3, and end of treatment visit), the following research blood samples should be collected and processed as outlined below:

- Draw approximately 100 mL of peripheral blood into ten 10-mL heparinized tubes.
- PBLs will be prepared by Ficoll-Hypaque density gradient centrifugation according to standard protocols
- Samples will be cryopreserved in a liquid nitrogen freezer at –140°C for further batched analyses.

Please **Ping "Vaccine Team"** (pager 410-283-0693) and the Immune Core will be notified to pick up the sample after draw.

7. Plasma for ctDNA for PGDx

At pre-treatment visit, the following research blood samples should be collected and processed as outlined below:

- Draw approximately 10 ml of peripheral blood into 2 Streck Cell-Free DNA BCT tubes.
- Invert tubes several times after collection.
-
- Please **Ping "Vaccine Team"** (pager 410-283-0693) and the Immune Core will be notified to pick up the sample after draw.The following steps should then be followed for plasma processing:
  - Transport the tube immediately to the laboratory.
  - Upon arrival in the laboratory, spin tubes at 814 g (2000 rpm, Sigma) for 20 min at 4°C or room temperature.

- Transfer supernatant to a 50 ml tube without disturbing the cellular layer using a 2 or 10 ml pipette.
- Aliquot 1.05 ml plasma from the large tube to 1.5 ml tubes. (Note: About 5 ml of the 10 ml of blood will be plasma so approximately 5 aliquots).
- Discard cell pellet.
- Spin tubes at 18,000 g (~14,000 rpm, Beckman) for 10 min at room temperature.
- Transfer 1 ml of plasma from the 1.5 ml tube to a fresh 2 ml tube without disturbing the pellet.
- Store plasma vials at -80°C

8. Plasma for ctDNA for Hurley Lab

At the pre-treatment, 12 week, and end of treatment (week 49) visits, the following research blood samples should be collected and processed as outlined below:

- Draw approximately 10 ml of peripheral blood into 2 Streck Cell-Free DNA BCT tubes.
- Gently invert tubes several times after collection, but do not shake.
- Leave tubes at room temperature.
- Leave tubes in the blue research bin in the phlebotomy suite of Weinberg.
- **Please immediately contact Paula Hurley at (cell) 410-271-8417 or (office) 410-614-9453 for pick up.**