Phase II study of front line therapy with nivolumab and salvage nivolumab + ipilimumab in patients with advanced renal cell carcinoma HCRN: GU16-260

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PROTOCOL SIGNATURE PAGE

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

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SYNOPSIS

TITLE	Study Title: Phase II study of front line therapy with nivolumab and salvage nivolumab + ipilimumab in patients with advanced renal cell carcinoma
PHASE	II
BACKGROUND AND SIGNIFICANCE	Renal Cell Carcinoma (RCC) is the most common malignant lesion of the kidney, with an estimated annual incidence in the U.S. of over 50,000 new cases and 13,000 deaths per year. With currently available treatments, the median overall survival is around 28 months. First-line treatment for clear cell RCC typically involves vascular endothelial growth factor receptor (VEGFR) tyrosine kinase inhibitors (TKIs) sunitinib or pazopanib. Additional agents for second-line therapy have included alternative VEGFR TKIs (e.g. axitinib), the mTOR inhibitor everolimus as well as agents active against multiple tyrosine kinases. Recently both nivolumab an anti-PD1 based immunotherapy (see below) and cabozantinib a TKI targeting both VEGFR and Met, have shown OS benefits relative to mTOR inhibition in patients with prior VEGRF TKI therapy resulting in their FDA approval. Nivolumab in the treatment of RCC. Preclincal and clinical studies have identified critical mechanisms by which cancers subvert normal immunomodulatory mechanisms (referred to as "checkpoints") to block the initiation, delivery, or activation of effective anti-tumor surveillance. These insights have led to the development of a new class of immunotherapeutics referred to as "checkpoint inhibitors," which include nivolumab, a human IgG4 monoclonal antibody that binds to the programmed death receptor-1 (PD-1) and blocks its interaction with PD-L1 and PD-L2, releasing PD-1 pathway-mediated inhibition of the immune response, including the anti-tumor immune response. Nivolumab was approved in November 2015 by the US FDA for use in patients with advanced RCC who have received prior VEGFR TKI therapy. The approval was based on a randomized (1:1) open-label study of nivolumab or everolimus. Nivolumab demonstrated a statistically significant improvement in overall survival (OS) compared to everolimus, with a median survival of 25.0 vs. 19.6 months (hazard ratio 0.73, $p<0.002$) and confirmed objective response rates of 21.5% vs 3.9%, respectively. In addition, grade 3 toxicity was significantly less and quality of life

	significantly improved in patients treated with nivolumab relative to those receiving everolimus. The OS benefit, history of durable responses and tolerability of nivolumab suggest that it be a preferred treatment for a subset of treatment naïve patients. However, currently there is very limited single agent data on the activity of nivolumab in treatment naïve patients with ccRCC. The BMS-sponsored study CA 209009 included 23 subjects with treatment-naïve RCC. In these patients, treatment with nivolumab 10 mg/kg every 3 weeks resulted in an overall response rate of 13% (3/23). Further anti-PD1/PDL1 therapies have shown anti-tumor activity in patients with over 20 different types of malignancy; however only anecdotal reports exist for this treatment approach in patients with non-clear cell (ncc)RCC. Finally, the combination of nivolumab and the CTLA4 checkpoint inhibitor ipilimumab has produced greater antitumor activity than nivolumab alone in patients with advanced melanoma and encouraging response rates in patients with ccRCC. It remains uncertain whether the addition of ipilimumab to nivolumab in patients who have failed to respond to nivolumab monotherapy could produce antitumor activity. To address all these concerns, we propose to conduct a study of nivolumab monotherapy in treatment naïve patients with advanced cc or nccRCC (Part A), with the addition of ipilimumab in patients with disease progression or who have failed to respond by 48 weeks into therapy (Part B) that will focus on uncovering biomarkers of response and resistance to PD-1 blockade, as well as possible predictors of subsequent response to CTLA-4 blockade. <u>In this trial, we examine the following questions:</u> 1) How active is PD-1 blockade in treatment naïve kidney cancer? 2) What factors, in addition to PD-L1 expression, can reliably predict durable response? 3) Do gene expression signatures which are likely capturing the presence, magnitude, and activity of adaptive immune infiltrates reliably predict durable response? 4) Is there a threshold for PD-L1 expression in either the tumor or immune cell infiltrate that accurately predicts durable response? 5) Is PD-L1 expression uniform and stable in an individual tumor and between a primary tumor and its metastases? 6) What are the mechanisms of innate resistance to PD-1 pathway blockade?

	7) Can the addition of ipilimumab overcome resistance and/or enhance response it patients with prolonged stable disease? 8) Do gene expression analyses of biopsies in PD1 resistant tumors predict the response to ipilimumab/nivolumab combination therapy?
OBJECTIVES	<u>Primary Objective:</u> Determine the PFS rate at 1 year of nivolumab in patients with treatment naïve ccRCC based on tumor PD-L1 expression. <u>Secondary Objectives:</u> • Determine the PFS rate at 1 year- by both RECIST and irRECIST of nivolumab in patients with treatment naïve ccRCC based on the PD1- Blockade Durable Response Predictive (PRP) biomarker model developed in the DFHCC Kidney Cancer SPORE • Determine the objective response rate (CR/PR=ORR), the ORR based on PDL1 expression and the PRP model, and duration of response for nivolumab in patients with treatment naïve ccRCC • Determine the response rate of combined nivolumab and ipilimumab therapy at the time of nivolumab failure (or lack of response at 1 year) • Determine the clinical activity (CR, PR and SD) and PFS rate at 1 year of nivolumab in patients with treatment naive nccRCC • Assess the toxicity of nivolumab monotherapy in patients with treatment naïve cc or nccRCC <u>Correlative Objectives:</u> • Explore novel potential predictive biomarkers for patients with treatment naïve ccRCC and nccRCC • Explore the mechanisms of innate and acquired resistance to nivolumab therapy in treatment naïve patients with ccRCC • Identify biomarkers associated with response to nivolumab + ipilimumab in patients whose disease has not responded or progressed on nivolumab monotherapy
STUDY DESIGN	The study will be a prospective Phase II trial of nivolumab in 134 treatment naïve patients with clear cell (cc)RCC and 40 treatment naïve patients with non-clear cell (ncc)RCC. Eligible patients with tumor tissue available will receive nivolumab 240 mg flat dose IV q 2 weeks for 12 weeks then every 3 weeks at a dose of 360 mg IV for 12 weeks followed by nivolumab 480 mg IV every 4 weeks. Tumor response will be assessed at weeks 12, 18 and 24 and then every 12 weeks. For patients who experience RECIST defined PD, but remain clinically asymptomatic with maintenance of performance status, a confirmatory scan after 4-6 weeks of

	additional therapy is suggested. Patients with persistent PD at confirmatory scan will be evaluated for enrollment on Part B of this study. Patients without confirmed PD can continue nivolumab therapy. Patients with symptomatic and radiographic PD will not require a confirmatory scan before proceeding to Part B of this study. Adverse events will be recorded continuously. Patients who experience symptomatic or confirmed PD (or have best response of SD at 48 weeks) on nivolumab monotherapy will be eligible for consideration for Part B (ipilimumab/nivolumab re-induction). Part B involves the addition of ipilimumab for up to 4 doses while maintaining nivolumab therapy. Dose of ipilimumab will be 1 mg/kg every 3 weeks together with nivolumab changed to 3 mg/kg every 3 weeks for up to 4 doses (must complete by week 16). Nivolumab will revert to 360 mg every 3 weeks for 12 weeks followed by nivolumab 480 mg IV every 4 weeks after the completion of treatment with ipilimumab (beginning week 13-19 of Part B). Patients will continue to be followed with serial imaging assessments 12, 18 and 24 weeks after the initiation of ipilimumab (Part B) and then every 12 weeks. The tumor measurements at the time of ipilimumab institution will be the new baseline. If unequivocal symptomatic or confirmed new PD (as defined above) develops, treatment will be discontinued. Patients for Part B must still meet the eligibility criteria for initial study enrollment. Patients with Grade 3 toxicity on nivolumab monotherapy, (excluding endocrine toxicity), serious symptomatic disease that in the opinion of the site investigator requires immediate use of an alternative treatment approach or continued PR/CR will be excluded from enrolling in Part B. It is estimated that roughly half of the patients accrued to the first line treatment will go on to enroll in Part B.
KEY ELIGIBILITY CRITERIA (See Section 3 for full eligibility criteria)	**Part A** 1. Patients must have advanced RCC (any histology) Collecting duct tumors and tumors originating from the renal pelvis or upper urinary tract are considered of urothelial origin and are excluded from this protocol. 2. Patients must have at least one measurable site of disease that has not been previously irradiated. If the patient has had previous radiation to the marker lesion(s), there must be evidence of progression since the radiation 3. Archival tissue of a metastatic lesion obtained within 1 year prior to study registration (within 4 weeks preferred) and tumor tissue from nephrectomy is required if available. In addition to archival tissue of a metastatic lesion and nephrectomy, patients must have at least one site of disease

	(not including bone metastases) accessible for biopsy. If biopsy/resection of a new lesion or primary tumor and slow freezing of fresh tissue for single cell RNAseq study (as specified in the CLM) is not feasible, the subject is not eligible for the study. All biopsies must be core needle or excisional. Fine needle aspirate is not acceptable. **NOTE:** The tissue collected from a surgical resection or multiple core biopsies of either a metastatic lesion or primary tumor for the slow freezing of fresh tissue after the patient has signed consent for the study could also be used for collecting the FFPE specimens. 4. ECOG performance status 0-2 5. Age ≥ 18 years 6. Patients must have adequate organ and marrow function within 14 days prior to study entry 7. Patients with controlled brain metastases are allowed on protocol if they have had brain metastases that were surgically resected without recurrence and/or up to 3 metastases treated with SRS without progression on MRI obtained at least 2 weeks after completion of radiation and within 28 days prior to initiation of nivolumab. **Part B** 1. Meet eligibility for Part A with exception that prior anti-PD1 therapy (must be with nivolumab in Part A of this study) is allowed 2. No grade 3 IrAE on nivolumab (excluding endocrine toxicity managed with replacement therapy) 3. Tumor biopsy prior to combination treatment is mandatory. If a biopsy/resection of a new lesion or primary tumor and slow freezing of fresh tissue for single cell RNAseq study (as specified in the CLM) is not feasible, the subject is not eligible for the study. All biopsies must be core needle or excisional. Fine needle aspirate is not acceptable. **Exclusion** 1. Untreated brain metastases 2. Major surgery or radiation therapy within 14 days of starting study treatment 3. Subjects with active autoimmune disease (see protocol for exceptions) 4. Concurrent medical condition requiring use of systemic corticosteroids with prednisone >10 mg per day. 5. Prior systemic therapy for Stage IV RCC (except for nivolumab as part of part A of this protocol).

STATISTICAL CONSIDERATIONS	The primary focus of this trial will be in the evaluation of PD-L1 to predict outcome and, also, secondarily to use the PRP model as a basis for outcome prediction. We expect that about 45% (or 54 expected) patients will have 0% PD-L1 and roughly 15% (or 18 expected) patients will express PD-L1 > 20%, which, given the sample size of 120 ccRCC patients provides 90% power using a 0.05 level two-sided test to test the hypothesis that PD-L1 >20% will be associated with significantly improved 1-year PFS rate for patients treated with PD-1 blockade (55% vs 13% taken as a binomial quantity). Considering the four groups of PD-L1 expression (0%, 1-5%, 5-20%, >20%), and with an expected patient allocation within groups of (45%, 25%, 15%, 15%), the Cochran-Armitage test gives at least 90% power to detect a trend in 1-year PFS of (13%, 19%, 35%, 55%) using a two-sided 0.05 level test for trend. Modest changes in the proportions of patients in the various PD-L1 categories will not substantially affect the power. For example, if the distribution across PD-L1 categories is [25%, 25%, 25%, 25%] rather than the anticipated [45%, 25%, 15%, 15%] the power for the trend test remains greater than 90%. As a secondary objective, we will apply the PRP model, developed in the DF/HCC Kidney SPORE Project 4, to assign each patient a probability of having PFS > 1 year. This will be assessed by both RECIST and irRECIST. The validity of the model will be assessed by the Brier score as well as via evaluation of the AUC. The exact distribution is unknown, but assuming an overall 1-year PFS of 28% and that 40% of patients are expected to be “positive” by the PRP model, 120 ccRCC patients give 90% power to detect a 29% higher 1-year PFS between 48 positive patients and 72 negative patients (via Fisher’s exact test and assuming a difference in PFS of 46% vs 17%). All testing above will be done at 1-sided $\alpha = 0.025$. The specific distributional breakdowns are unknown but the power is relatively robust. For example, if the split between positive and negative is more even (50/50) then power for the above difference in 1-yr PFS remains over 90%. If the actual difference in 1-yr PFS is smaller than anticipated for example 25% (44% vs 19%) then the test will still have at least 80% power. At this time, 10 fresh frozen baseline tumor specimens and 3 pairs of specimens have been collected with an additional patient likely to be collected before accrual of the original 120 patients is complete. Therefore, fourteen additional patients are to be enrolled to collect fresh frozen specimens necessary to provide 25 baseline and 15 paired baseline and resistant fresh frozen samples to be used for single cell RNAseq experiments aimed at identifying factors associated to resistance as outlined in the DOD Translational

	Research Partnership grant to Drs. Atkins and Wu. Assuming 15 patients non-responsive to nivolumab and up to 10 patients responding, comparisons of any quantitative expression level using a 0.05 level two-sided two-group t-test will have 80% power to detect at least a 1.2 SD shift in means using baseline samples. With 15 paired samples at baseline and at confirmed radiologic progression among the non-responders, there is at least 80% power to detect a change in expression of 0.78 SD in any markers using a two-sided 0.05 level paired t-test. For two binary markers that are at least 50% discordant there is at least 80% power to detect a +/- versus -/+ discordance of at least 47% using a two-sided 0.05 level McNemar's test. Non-clear cell (ncc) RCC patients (comprising a heterogeneous group of histologies) will also be enrolled in the trial, at an accrual rate that is expected to be 33% that of clear cell patients. Therefore, an expected cohort of 40 nccRCC patients will be enrolled in the trial and analyzed separately for evidence of anti-tumor activity, principally focusing on overall response rate (CR or PR). With 40 patients, the 95% confidence interval on the true objective response proportion will be no wider than 32.4 percentage points and the probability of observing five or more responses under the hypothesis that the true response rate is 0.20 is at least 92%, hence there is high power to detect responses in this cohort should the true response rate be interesting. Other endpoints of interest in this group of patients will include rates of SD and PFS at 1-year. Clear cell patients who reach best response of SD at 48 weeks or who experience progression will be offered the opportunity to enroll in the second component of the study, Part B, continued treatment with the addition of ipilimumab. The primary endpoint of this portion of the trial will be objective response (CR or PR) taking as baseline tumor measurements at the patient's start of Part B. It is expected that roughly one-half of the Part A patients will be eligible for and choose to enroll in Part B, or roughly 60 total patients. A two-stage design will be used for this portion of the study where in the first cohort 29 patients will be accrued, treated and evaluated for response in order to provide guidance on whether to continue to enroll the entire cohort of 60 patients. The first stage analysis will be conducted without pausing accrual to Part B. If among the first 29 Part B patients there are at least 2 responses, there will be sufficient evidence to continue recruitment to Part B. With a true uninteresting response rate of 5%, there is a 57% chance of declaring the regimen uninteresting; however, with an interesting response rate of 20%, there is greater than 95% chance of continuing. If after enrollment of 60 patients there are at least 11

	total responses, the lower limit on the 90% confidence interval for the true response rate will exclude 10% (ie, the 95% confidence interval will range from 10.6% to 28.5%, adjusted for the two-stage design). With 18 total responses, the 90% confidence interval on the true objective response proportion will exclude 20% (it, the confidence interval will range from 20.4% to 41.2%, adjusted for the two-stage design). Ncc RCC patients will also be available to enroll in Part B of the study. An estimated 20 patients will be enrolled and response rates in this resistant population will be reported. If no responses are seen in the first 14 patients that response rate will be deemed uninteresting. If any confirmed responses are seen, accrual of nccRCC patients will continue for all eligible patients completing Part A.
ACCRUAL GOALS	With multicenter accrual of 6-7 patients per month, the study should accrue 134 ccRCC patients (original 120 plus an additional 14 to provide paired baseline and resistant samples) to Part A in 2 years with an additional 1 year of follow-up time for Part A (1.5 years for Part B). The cohort of 40 nccRCC patients will be enrolled and analyzed separately for evidence of anti-tumor activity (CR, PR and SD and PFS at 1 year of nivolumab). These patients will also be eligible for participation in Part B. We anticipate that the accrual of these 40 patients will be able to be completed within the 1.5 years needed for accrual of the ccRCC patients.

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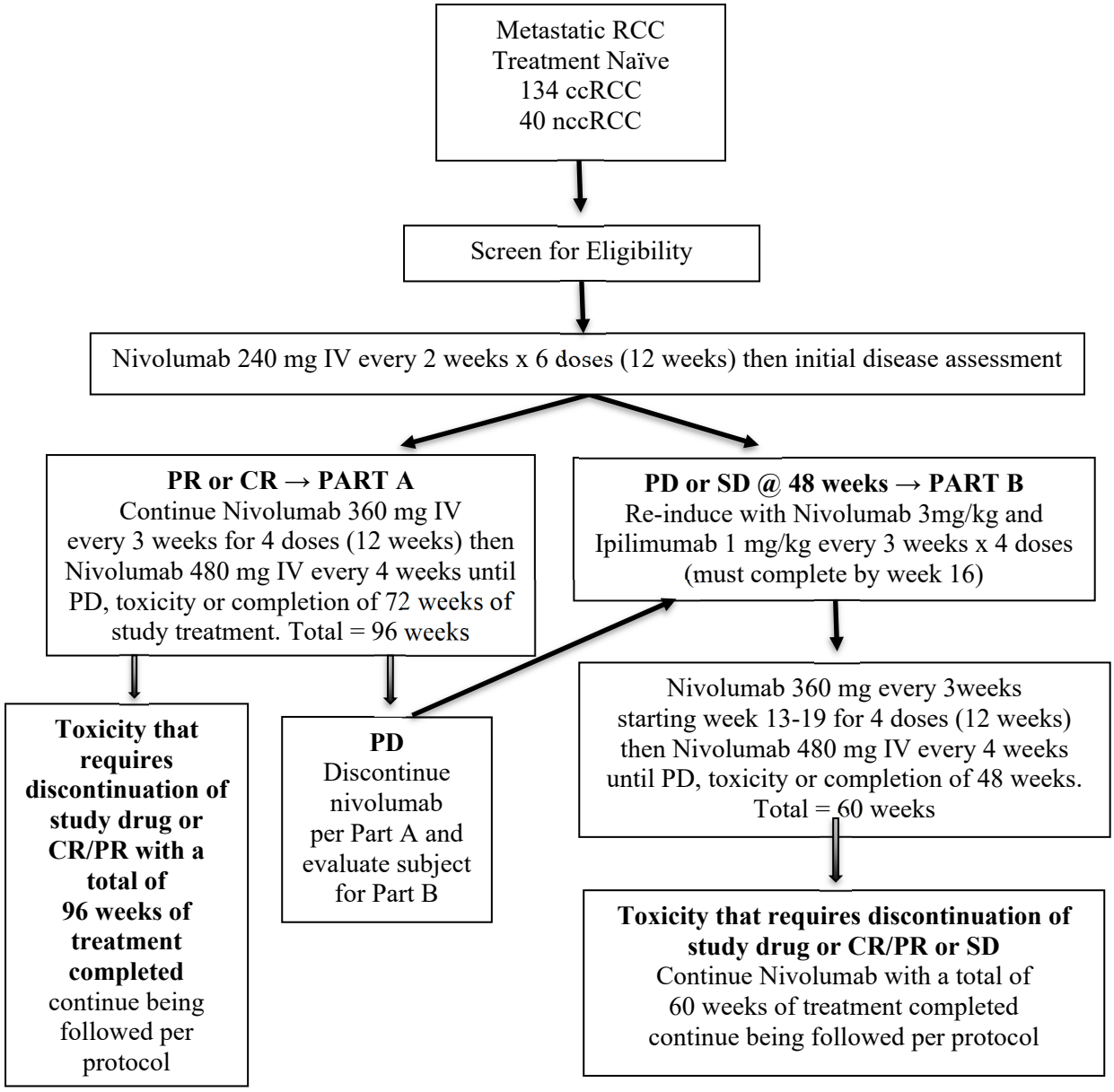
SCHEMA



Tumor Assessments weeks 12, 18 and 24 then every 12 weeks on both Part A and Part B.
Baseline reset for Part B.

Archival tumor tissue as described in eligibility and Section 8 is required if available prior to treatment on Part A. A fresh biopsy is required after the patient signs consent. Tumor tissue should be slow frozen for single cell RNAseq as described in the CLM. Such biopsy collection and processing is required for protocol eligibility for patients 121-134 with ccRCC accrued to this study. Tumor Biopsy required at crossover to Part B with slow frozen tissue processing for scRNAseq

1. BACKGROUND AND RATIONALE

1.1 Renal Cancer

The global incidence of RCC exceeds 300,000 worldwide with nearly 150,000 deaths annually with worsening mortality trends in the developing world compared to advanced countries in Europe and the United States.[1] In the US, the American Cancer Society's most recent estimates for kidney cancer for 2016 include approximately 62,700 new cases (39,650 in men and 23,050 in women) and about 14,240 deaths (9,240 men and 5,000 women) from the disease [2]. In the US, RCC is the 10th most common cancer with a 1.6% (1 in 63) lifetime risk of developing the disease. Metastatic disease is the proximate cause of death for most patients and can present equally as either de novo advanced RCC or as relapse following surgery for early stage disease. Decrements in life expectancy due to RCC have been estimated to be about 15 years for patients in several Western countries and there is a substantial health related quality of life impact [3]. Kidney cancer has a propensity to hematogeneously metastasize to any possible location, propagate tumor thrombus through the inferior vena cava into the heart, and due to the hypervascularity of the tumor is susceptible to tumor hemorrhage, causing additional morbidity and extended hospitalizations for management. Several distinct subtypes of RCC have been identified, including: clear cell cancer, comprising the majority of tumors and papillary, chromophobe collecting duct and unclassified tumors representing 15-25% of all tumors.

1.1.1 Clear Cell Renal Cell Carcinoma

Clear cell renal cell carcinomas (ccRCC) represent 75-85% of all RCC. They typically arise from the proximal renal tubule. Macroscopically, they may be solid or less commonly, cystic. In addition to occurring sporadically, ccRCC is specifically associated with von Hippel-Lindau (VHL) disease (a tumor suppressor gene). Both sporadic and VHL associated RCC typically have a deletion in 3p the site of the VHL gene [4] which leads to upregulation of hypoxia-inducible factor (HIF), even in the absence of hypoxia and promotes VEGF related tumor angiogenesis. A poor prognosis is associated with higher nuclear grade or the presence of a sarcomatoid pattern.[5]

1.1.2 Non Clear Cell

Papillary RCC accounts for approximately 15% of all kidney cancers, and these can be divided into type 1 and type 2 lesions based upon histopathologic features. Type 1 and type 2 papillary carcinomas differ in both clinical features and in their underlying genetic abnormalities. As with ccRCCs, papillary RCC originates from the proximal tubule, but these tumors are morphologically and genetically distinct malignancies. Type 1 papillary RCC typically presents with stage I or II disease, and these patients have a relatively favorable prognosis. Although type 1 lesions occur in patients with hereditary papillary RCC, the majority of these are sporadic. In the hereditary form of this disease, activating germline mutations are seen in MET. In nonhereditary forms of the disease, somatic mutations in MET have been identified in approximately 10 to 20 percent of cases [6]. In total, altered MET status (defined as mutation, splice variant, or gene fusion) or increased chromosome 7 copy number (which encodes MET but may also involve other genes) was identified in 81% of type 1 papillary renal-cell carcinoma.

Type 2 papillary RCC is frequently associated with aggressive tumors that are stage III or IV at presentation and are associated with a poor prognosis. These tumors have also been seen in the hereditary leiomyomatosis and renal cell cancer syndrome, which is caused by germline mutation in the gene for fumarate hydratase (FH). Very few patients had alterations in the MET pathway.

Chromophobe carcinomas make up approximately 5% of all RCCs. Histologically, they are composed of sheets of cells that are darker than ccRCC (Thoenes). They lack the abundant lipid and glycogen that is characteristic of most RCCs, and originate from the intercalated cells of the collecting system. Chromophobe carcinomas may have a lower risk of disease progression and death compared with ccRCC which likely relates to both their presentation at earlier stage and overall slower growth rate.

1.2 Renal Cancer Therapy

First-line treatment for clear cell RCC typically involves vascular endothelial growth factor receptor (VEGFR) tyrosine kinase inhibitors (TKIs) sunitinib or pazopanib. Additional agents for second-line therapy have included alternative VEGFR TKIs (e.g. axitinib) the mTOR inhibitor everolimus. as well as agents active against multiple tyrosine kinases. Recently both nivolumab an anti- programmed death-1 (PD1) based immunotherapy (see below) and cabozantinib a TKI targeting both VEGFR and Met, have shown overall survival (OS) benefits relative to mTOR inhibition in patients with prior VEGRF TKI therapy resulting in their FDA approval.

1.3 Nivolumab +/- Ipilimumab Summary

Nivolumab (also referred to as BMS-936558 or MDX1106) is a human monoclonal antibody (HuMAb; immunoglobulin G4 [IgG4]-S228P) that targets the programmed death-1 (PD-1) cluster of differentiation 279 (CD279) cell surface membrane receptor. PD-1 is a negative regulatory molecule expressed by activated T and B lymphocytes [7]. Binding of PD-1 to its ligands, programmed death–ligands 1 (PD-L1) and 2 (PD-L2), results in the down-regulation of lymphocyte activation. Inhibition of the interaction between PD-1 and its ligands promotes immune responses and antigen-specific T-cell responses to both foreign antigens as well as self-antigens. Nivolumab is expressed in Chinese hamster ovary (CHO) cells and is produced using standard mammalian cell cultivation and chromatographic purification technologies. The clinical study product is a sterile solution for parenteral administration. OPDIVO (nivolumab) is approved for use in multiple countries including the United States (US, Dec-2014), the European Union (EU, Jun-2015), and Japan (Jul-2014).

1.3.1 Non-Clinical Studies

Nivolumab has been shown to bind specifically to the human PD-1 receptor and not to related members of the CD28 family [8, 9]. 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 [9-11]. Nivolumab binds with high affinity to activated human T-cells expressing cell surface PD-1 and to cynomolgus monkey PD-1. In a mixed lymphocyte reaction (MLR), nivolumab promoted a reproducible concentration-dependent enhancement of IFN- γ release [12] In intravenous (IV) repeat-dose toxicology studies in cynomolgus monkeys, nivolumab was well tolerated at doses up to 50 mg/kg, administered weekly for 5 weeks, and at doses up to 50 mg/kg, administered twice weekly for 27 doses. While nivolumab alone was well tolerated in

cynomolgus monkeys, combination studies have highlighted the potential for enhanced toxicity when combined with other immunostimulatory agents [13]. In addition, an enhanced pre- and postnatal development (ePPND) study in pregnant cynomolgus monkeys with nivolumab was conducted [14]. Administration of nivolumab at up to 50 mg/kg 2QW was well tolerated by pregnant monkeys; however, nivolumab was determined to be a selective developmental toxicant when administered from the period of organogenesis to parturition at > 10 mg/kg). Specifically, increased developmental mortality (including late gestational fetal losses and extreme prematurity with associated neonatal mortality) was noted in the absence of overt maternal toxicity. There were no nivolumab-related changes in surviving infants tested throughout the 6-month postnatal period. Although the cause of these pregnancy failures was undetermined, nivolumab-related effects on pregnancy maintenance are consistent with the established role of PD-L1 in maintaining fetomaternal tolerance in mice [15].

1.3.2 Effects in Humans

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

1.3.2.1 Clinical Pharmacokinetics

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

1.3.2.2 Clinical Efficacy

Nivolumab has demonstrated durable responses exceeding 6 months as monotherapy and in combination with ipilimumab in several tumor types, including NSCLC, melanoma, RCC, and some lymphomas. In confirmatory trials, nivolumab as monotherapy demonstrated a statistically significant improvement in OS as compared with the current standard of care in subjects with advanced or metastatic NSCLC and in subjects with unresectable or metastatic melanoma. Nivolumab in combination with ipilimumab improved PFS and ORR over ipilimumab alone in subjects with unresectable or metastatic melanoma.

1.3.2.3 Clinical Safety

The overall safety experience with nivolumab, as a monotherapy or in combination with other therapeutics, is based on experience in approximately 8,600 subjects treated to date. For monotherapy, the safety profile is similar across tumor types. There is no pattern in the incidence, severity, or causality of AEs to nivolumab dose level. In Phase 3 controlled studies, the safety profile of nivolumab monotherapy is acceptable in the context of the observed clinical efficacy, and manageable using established safety guidelines. Clinically relevant AEs typical of stimulation of the immune system were infrequent and manageable by delaying or stopping nivolumab treatment and timely immunosuppressive therapy or other supportive care. 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. The most advanced combination under development is nivolumab + ipilimumab in subjects with unresectable or metastatic melanoma. Results to date suggest that the safety profile of nivolumab + ipilimumab combination therapy is consistent with the mechanisms of action of nivolumab and ipilimumab. The nature of the AEs is similar to that observed with either agent used as monotherapy; however, both frequency and severity of most AEs are increased with the combination.

1.4 Nivolumab in RCC

A first-in-human Phase I study of nivolumab was performed in patients with treatment refractory solid tumors [16]. Nivolumab was administered as a single dose ranging from 0.3 to 10mg/kg and objective responses were noted including in a patient with RCC and there were no dose limiting toxicities observed within 4 weeks of the initial dose. The serum half-life of nivolumab ranged from 12 to 20 days. A multi-dose Phase I dose escalation trial extended the above findings in 296 patients with advanced solid tumors given nivolumab at doses from 0.1 to 10 mg/kg every 2 weeks for up to 2 years [17]. Objective tumor responses were noted in 9 of 33 patients with RCC. Common treatment related side effects were fatigue, anorexia, nausea, rash, and diarrhea. Grade 3 or 4 toxicities were reported in 14% of patients and evident at all dose levels without obvious dose dependency but only 15 patients (5%) discontinued treatment for toxicity reasons. Immune related adverse events of special interest were observed including pneumonitis, colitis, hepatitis, hypophysitis and thyroiditis but were reversible. Extended follow up of patients on this study showed a median response duration of 12.9 months and median overall survival in this heavily pretreated population was 22.4 months [18]. Three of 5 patients who stopped treatment while responding continued to respond for at least 45 weeks. Among 168 RCC patients randomized in blinded fashion to nivolumab dosed at 0.3, 2 and 10mg/kg, there was no observable dose-response relationship with objective responses in 20-22% of patients and

median survival ranging from 18.2 to 25.5 months [19]. In the Checkmate 025 Phase III trial, 821 patients with advanced RCC with a clear cell component who had received previous antiangiogenic therapy, were randomly assigned to receive 3 mg/kg of nivolumab every 2 weeks or everolimus [19, 20]. The primary outcome, overall survival was significantly improved for nivolumab (median 25.0 versus 19.6 months) with HR 0.73 (98.5% CI, 0.57 to 0.93; P=0.002). The objective response rate was higher with nivolumab (25% vs. 5%; P<0.001) but the median progression-free survival was similar at 4.6 months versus 4.4 months. Grade 3 or 4 treatment-related adverse events were lower at 19% for nivolumab versus 37% for everolimus. Quality of life improved significantly more in the nivolumab group over 2 years relative to the everolimus group. These results led to the approval of nivolumab as second line therapy for patients with advanced RCC in the US. The absence of improvement in PFS with nivolumab suggested that many patients did not have benefit from nivolumab, highlighting the importance of predictive biomarker research.

In a biomarker-based trial of 91 patients with advanced RCC, 67 pretreated patients were randomized to receive 0.3, 2 or 10mg/kg of nivolumab every 3 weeks and 24 treatment-naïve patients received the 10mg/kg dose [21]. The primary objective was to study the immunomodulatory activity of nivolumab on tumor biopsies and serum chemokines. Among 56 evaluable specimens, 18 (32%) were PD-L1 positive defined as > 5% tumor cells with any tumor membrane staining by the Dako assay. Four of 18 (22%) of PD-L1(+) and 3/38 (8%) PD-L1(-) patients had objective responses and the overall response rate in the entire cohort was 16% with expected grade 3 immune related adverse events in 15%.

1.5 Nivolumab + Ipilimumab in RCC

The combination of nivolumab and ipilimumab has been extensively tested in patients with metastatic melanoma and found to have superior clinical outcomes to ipilumumab and likely nivolumab alone but at the cost of a higher frequency of serious adverse events [22, 23]. In a Phase I trial (CheckMate 016) patients with advanced RCC were randomized to receive nivolumab 3 mg/kg plus ipilimumab 1 mg/kg (N3 + I1, 47 patients) or nivolumab 1 mg/kg plus ipilimumab 3 mg/kg (N1 + I3, 47 patients) intravenously every 3 weeks for 4 doses followed by nivolumab every 2 weeks [24]. An exploratory nivolumab 3 mg/kg plus ipilimumab 3 mg/kg arm (N3 + I3) showed unacceptable early toxicity after 6 patients and did not proceed to the expansion phase. Approximately half the patients were previously treated. Treatment-related toxicities were seen in 88% of patients and 16% discontinued therapy for toxicity reasons. Serious Grade 3 or 4 treatment-related adverse events occurred in 34% and 64% of patients in N3 + I1 and N1 + I3, respectively, most commonly manifest as elevated lipase or amylase, elevated liver function tests, diarrhea and colitis. After a median 8 months of follow up, the objective response rate was 38% and 43% and median progression free survival was 30.3 and 36 weeks respectively in N3 + I1 and N1 + I3 arms. Among responders, the median duration of response was 67.7 weeks (range 4.1+ to 91.1+) in the N3 + I1 arm and 81.1 weeks (range 6.1+ to 81.1+) in the N1 + I3 arm. Overall both treatment arms appeared to confer similar anti-tumor activity but the N1 + I3 arm was associated with higher gastrointestinal and hepatic toxicities. The promising activity and tolerability of the N3 + I1 schedule has led to an ongoing randomized Phase III registrational trial comparing this combination to sunitinib monotherapy in the approved schedule, in patients with advanced clear cell RCC. This trial reached its accrual goal of 1070 patients in the fall of 2015. The co-primary endpoints are progression free and overall

survival. Trial results were reported in 2017 and published in 2018 [25]. The combination of nivolumab with ipilimumab improved PFS, ORR and OS over sunitnib in patients with intermediate and poor risk treatment naïve RCC and was FDA approved in April of 2018 for these patients. The nivo/ipi combination also prolonged OS relative to sunitinib when the good risk group was included in the analysis. The treatment was reasonably well tolerated with only a 3rd of patients having grade 3 IrAes and Quality of Life analyses showed the combination to be superior to sunitinib [25].

1.6 Biomarkers for Response and Resistance to Anti-PD1 Based Immunotherapy

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

In the phase I trial of nivolumab in patients with advanced solid tumors, 9 of 25 patients with PD-L1 positive tumors responded compared with 0 of 17 patients with PD-L1 negative tumors (p=0.006); only 5 of these patients had mRCC.[17] In the Checkmate 025 registration Phase III trial of nivolumab versus everolimus, tumor PD-L1 membrane expression (≥1% vs. <1% and ≥5% vs. <5%) was evaluated centrally using the Dako PD-L1 immunohistochemical stain [20]. Among 756 patients with quantifiable PD-L1 expression, 181 (24%) had > 1% PD-L1 expression in at least 100 tumor cells. However, the benefit of nivolumab relative to everolimus on overall survival did not differ in these two PDL1 defined subgroups at either this cutoff or when a 5% cut off was used. Interestingly, patients bearing PD-L1 positive tumors appeared to have shorter overall survival on either treatment relative to those with PD-L1 negative tumors, consistent with previous reports of PD-L1 expression being a marker of poor prognosis. The lack of predictive value for PD-L1 expression in RCC diverges from data for nivolumab in lung cancer and melanoma. However, it should be noted that those data primarily focused on ORR rather than overall survival and ORR data for the Checkmate 025 study has yet to be reported. In the aforementioned Phase I study of atezolizumab in RCC, there was no suggestion of a correlation between tumor cell PD-L1 status and antitumor activity. However immune cell PD-L1 expression measured using a diagnostic Spring Biosciences PD-L1 antibody SP142, suggested a trend towards improved ORR, PFS and OS with > 1% immune cell PD-L1 expression which was observed in 63% of the informative patients.

These data highlight the limitations of using PD-L1 expression to select patients for PD-1 axis targeting antibodies. The assays for PD-L1 expression are difficult to standardize, use distinct proprietary antibodies that have not been compared with each other, use different cutoff points and measure expression on different cells within the tumor microenvironment (tumor cells or an aggregate reading of a variety of immune cells). Furthermore, PD-L1 is an inducible molecule and tumors are frequently heterogeneous, affecting assay reproducibility especially at the low end of the range. Discordance between primary tumor and metastases for PD-L1 positivity in both directions was observed in kidney cancer in 11 of 53 cases (20.8%) using a validated anti-PD-L1 antibody (405.9A11) and formally quantified membranous expression in tumor cells [26].

The predictive utility of PD-L1 expression is likely to be more challenging in combination regimens. Lastly, while PD-L1 positivity is associated with poorer survival in non-clear cell RCC, its predictive value for immunotherapy is unknown [27].

At this time therefore, PD-L1 assay results are inadequate to be used to select or otherwise manage patients with advanced RCC receiving PD-1 targeting agents. Continued prospective biomarker validation studies, particularly looking at factors such as CD8 cell infiltrate and IFN-related gene expression signatures alone or in combination with PD-L1 expression which have shown more predictive power in other tumor sets, are clearly necessary in RCC.

1.7 Rationale for the Current Trial

Agents that target the VEGF and mTOR pathways significantly prolong progression free and likely overall survival for patients with metastatic ccRCC, but resistance invariably develops, often within the first year [28]. The clinical experience with high dose (HD) IL-2 has provided proof of principle that immunotherapy can produce durable responses in a small percentage of patients with ccRCC and obviate the need for subsequent therapy [29]. However, its toxicity and limited efficacy has severely narrowed its application. Agents that induce a high proportion of durable tumor responses with acceptable toxicity remain a critical unmet need for RCC patients. Tumor-induced immune suppression may explain why immunotherapies often fail to demonstrate clinical activity. The programmed death-1 (PD-1) pathway is one of the most critical mediators of tumor-induced immune suppression [30]. Many human solid tumors, including cc and non-ccRCC, express PD-L1, one of the ligands for PD-1, as a consequence of oncogene expression or as an adaptive response to exposure from IFN-γ produced by cytotoxic T lymphocytes [31]. PD-1 engagement by its ligands (PD-L1, PD-L2) inhibits T-cell proliferation, cytokine production, cytolytic function and survival [32]. Tumor-infiltrating lymphocytes (TIL) from patients typically express PD-1 and subsequently have impaired anti-tumor functionality [33, 34]. PD-L1 expression on renal tumors portends worse prognosis highlighting the clinical impact of this interaction [35].

Ongoing clinical trials evaluating PD-1/PD-L1 antibodies have demonstrated promising clinical efficacy with durable benefit in a subset of ccRCC patients who have progressed after prior therapy [17, 36-38]. Preliminary correlative studies have demonstrated that while tumor PD-L1 expression may increase the likelihood of benefit with PD-1 blockade, it fails to identify all responders. [17, 37, 39, 40]. Given the robust antitumor activity of VEGF pathway inhibitors, the application of single agent PD-1 blockade in the treatment naïve setting will require the development of biomarkers with greater positive predictive value. Combination approaches are actively being investigated (e.g. combined inhibition of both CTLA-4 and PD-1 and need to be rationally designed and implemented, given their additive toxicity and cost. In particular, tumors may escape PD-1 or PD-L1 blockade due to suboptimal T-cell activation, and CTLA-4 blockade could potentially overcome this escape mechanism [41]. Prior to PD-1 or PD-L1 blockade, CTLA-4 blockade could facilitate naïve T-cell activation and boost tumor reactive T-cells, producing interferon and resulting in upregulation of PD-L1 expression in the tumor microenvironment [42]. When used after PD-1 or PD-L1 blockade, CTLA-4 blockade could deplete T-regulatory cells that impair CD8-positive T-cell function [43]. Further work is needed to investigate the hypothesis that patients who do not respond to PD-1 or PD-L1 blockade may be salvaged by the addition of CTLA-4 blockade.

To optimize the therapeutic potential of single agent, PD-1 blockade in patients with kidney cancer, we propose a translational clinical trial that integrates clinicopathologic assessment, genomics and immunology to gain clearer mechanistic understanding of the effects of PD-1 blockade.

Our goals are to address several critical unanswered questions including: 1) How active is PD-1 blockade in treatment naïve kidney cancer? 2) What factors, in addition to PD-L1 expression, can reliably predict durable response? 3) Do gene expression signatures which are likely capturing the presence, magnitude, and activity of adaptive immune infiltrates reliably predict durable response 4) Is there a threshold for PD-L1 expression in either the tumor or immune cell infiltrate that accurately predicts durable response? 5) Is PD-L1 expression uniform and stable in an individual tumor and between a primary tumor and its metastases? 6) What are the mechanisms of innate resistance to PD-1 pathway blockade? 7) Can the addition of ipilimumab overcome resistance and/or enhance response it patients with prolonged stable disease?

Both the PD-1 blocking antibody nivolumab and PD-L1 blocking antibody atezolizumab are well tolerated and have shown efficacy in mRCC as monotherapy [34]. Nivolumab is a high affinity humanized monoclonal IgG4 antibody directed against human PD-1. It blocks its interaction with its ligands PD-L1 and PD-L2. It has shown encouraging efficacy in patients with melanoma and lung cancer [44, 45] and is currently FDA approved for patients with VEGFR TKI resistant RCC. The application of PD-1 pathway blockade in the treatment naïve setting may yield even better results and delay the need for subsequent lines of therapy in selected patients. Patients with melanoma who were previously untreated had greater response rates to nivolumab than ipilimumab-treated patients (40% vs 28% respectively). While combination therapies will likely play a large role in the future for many patients, there is a distinct subset of patients with RCC that will likely benefit with durable responses and increased PFS rate at 1 year to single immune checkpoint blockade [24, 37, 38, 46]. This hypothesis will be explored in Part A. The ability of the addition of ipilimumab to overcome resistance or improve response in patients following nivolumab will be explored in Part B.

Approximately 20% of all patients with kidney cancer have nccRCC. A recent analysis of tumor specimens revealed that while nccRCC expresses less PD-L1 on average, a substantial portion of nccRCC tumors express >5% PD-L1 on their surface, including: 43% of translocation-positive (3/7), 50% of oncocytoma (2/4), and 10% of papillary RCC (5/50) [47]. In the Phase 1 trial of atezolizumab, 1/7 non-clear cell mRCC patients had a partial response to therapy [37]. Given the current lack of effective therapies for sarcomatoid and non-clear cell kidney cancer and the preliminary evidence of clinical activity, the exploration of PD-1 blockade seems rational in this substantial group of patients.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Objectives

2.1.1 Primary Objective

Determine the PFS rate at 1 year of nivolumab in patients with treatment naïve ccRCC based on tumor PD-L1 expression.

2.1.2 Secondary Objectives

- Determine the PFS rate at 1 year- by both RECIST and irRECIST of nivolumab in patients with treatment naïve ccRCC based on the PD1- Blockade Durable Response Predictive (PRP) biomarker model developed in the DFHCC Kidney Cancer SPORE
- Determine the objective response rate (CR/PR=ORR), the ORR based on PDL1 expression and the PRP model, and duration of response for nivolumab in patients with treatment naïve ccRCC
- Determine the response rate of combined nivolumab and ipilimumab therapy at the time of nivolumab failure (or lack of response at 1 year)
- Determine the clinical activity (CR, PR and SD) and PFS at 1 year of nivolumab in patients with treatment naive nccRCC
- Assess the toxicity of nivolumab monotherapy in patients with treatment naïve cc or nccRCC

2.1.3 Correlative/Exploratory Objectives

- Explore novel potential predictive biomarkers for patients with treatment naïve ccRCC and nccRCC
- Explore the mechanisms of innate and acquired resistance to nivolumab therapy in treatment naïve patients with ccRCC
- Identify biomarkers associated with response to nivolumab + ipilimumab in patients whose disease has not responded or progressed on nivolumab monotherapy

2.2 Endpoints

2.2.1 Primary Endpoint

- PFS is defined as the time from Day 1 of treatment until the criteria for disease progression is met as defined by RECIST 1.1 or death as a result of any cause.
- The primary focus of this trial will be in the evaluation of PD-L1 expression levels to predict outcome. We expect that about 45% (or 54 expected) patients will have 0% PD-L1 and roughly 15% (or 18 expected) patients will express PD-L1 > 20%, which, given the sample size of 120 ccRCC patients provides 90% power using a 0.05 level two-sided test to test the hypothesis that PD-L1 >20% will be associated with significantly improved 1-year PFS rate for patients treated with PD-1 blockade (55% vs 13% taken as a binomial quantity).

2.2.2 Secondary Endpoints

- Apply the PRP model, developed in the DF/HCC Kidney SPORE Project 4, to assign each patient a probability of having PFS > 1 year.
- The endpoint for Part B (addition of ipilimumab) will be objective response (CR or PR) taking as baseline tumor measurements at the patient's start of Part B.
- An expected cohort of 40 nccRCC patients will be enrolled in the trial and analyzed separately for evidence of anti-tumor activity, principally focusing on objective response rate (CR or PR).
- Toxicity by CTCAE criteria of nivolumab monotherapy in treatment naïve patients with cc and nccRCC.

2.2.3 Correlative Endpoint

- Additional correlative studies will be performed in order to identify predictors of response and resistance.

3. ELIGIBILITY CRITERIA

3.1 Inclusion Criteria-Part A

Subject must meet all of the following applicable inclusion criteria to participate in this study:

1. Patients must have histologically confirmed advanced RCC (any histology). Collecting duct tumors and tumors originating from the renal pelvis or upper urinary tract are considered of urothelial origin and are excluded from this protocol.

2. Patients must have at least one measurable site of disease, per RECIST 1.1, that has not been previously irradiated. If the patient has had previous radiation to the marker lesion(s), there must be evidence of progression since the radiation.

3. Archival tissue of a metastatic lesion obtained within 1 year prior to study registration (within 4 weeks preferred) and tumor tissue from nephrectomy is required if available. In addition to archival tissue of a metastatic lesion and nephrectomy, patients must have at least one site of disease (not including bone metastases) accessible for biopsy. If biopsy/resection of a new lesion or primary tumor and slow freezing of fresh tissue for single cell RNAseq study (as specified in the CLM) is not feasible, the subject is not eligible for the study. All biopsies must be core needle or excisional. Fine needle aspirate is not acceptable. **NOTE:** The tissue collected from a surgical resection or multiple core biopsies of either a metastatic lesion or primary tumor for the slow freezing of fresh tissue after the patient has signed consent for the study could also be used for collecting the FFPE specimens.

4. ECOG performance status 0-2.

5. Age ≥ 18 years.

6. Have signed the current approved informed consent form.

7. Patients must have adequate organ function within 14 days prior to study entry as evidenced by screening laboratory values that must meet the following criteria:

System	Laboratory Value
Hematological	
White blood cell (WBC)	≥ 2000/μL
Absolute Neutrophil Count (ANC)	≥ 1500/μL
Platelets (Plt)	≥ 100 x10^3/μL
Hemoglobin (Hgb)	> 9.0 g/dL (with or without transfusion)
Renal	
Serum Creatinine OR	≤ 1.5 x ULN; if creatinine > 1.5, subject must demonstrate CrCl outlined below.
Calculated or measured creatinine clearance[1]	≥ 40 mL/min using Cockcroft-Gault formula
Hepatic	
Bilirubin[2]	≤ 1.5× upper limit of normal (ULN)
Aspartate aminotransferase (AST)	≤ 3 × ULN
Alanine aminotransferase (ALT)	≤ 3 × ULN

1: Female CrCl = (140 - age in years) x weight in kg x 0.85
72 x serum creatinine in mg/dL
Male CrCl = (140 - age in years) x weight in kg x 1.00
72 x serum creatinine in mg/dL
2: Except subjects with Gilbert Syndrome, who can have total bilirubin < 3.0 mg/dL

8. Patients should not have received prior systemic therapy for metastatic RCC. Prior radiotherapy must have been completed at least 2 weeks prior to the administration of study drug. Patients must be 2 weeks from prior major surgery and 1 week from pre-treatment biopsy. Prior systemic adjuvant therapy (excluding with PD1 or CTLA4 pathway blockers) is allowed if treatment completed > 12 months previously.

9. Women of childbearing potential (WOCBP) must use appropriate method(s) of contraception. WOCBP should use an adequate method to avoid pregnancy for 5 months after the last dose of study drug. **NOTE:** Contraception is not required for male participants.

10. Women of childbearing potential must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) during screening for registration purposes. This pregnancy test should be repeated within 24 hours prior to the start of nivolumab. **NOTE:** "Women of childbearing potential" is defined as any female who has experienced menarche and who 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.

11. Women must not be breastfeeding.

12. Be willing and able to comply with this protocol.

3.2 Exclusion Criteria

1. 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 2 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.

2. Patients with controlled brain metastases are allowed on protocol if they had brain metastases that were surgically resected without recurrence and/or up to 3 metastases treated with SRS without progression on MRI obtained at least 2 weeks after completion of radiation and within 28 days prior to initiation of nivolumab.

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

4. 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.

5. 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.

6. Active infection requiring systemic therapy

7. Has any other medical or personal condition that, in the opinion of the site investigator, may potentially compromise the safety or compliance of the patient, or may preclude the patient's successful completion of the clinical trial.

8. Patients should be excluded if they are positive test for hepatitis B virus surface antigen (HBV sAg) or hepatitis C virus ribonucleic acid (HCV antibody) indicating acute or chronic infection
9. Patients should be excluded if they have known history of testing positive for human immunodeficiency virus (HIV) or known acquired immunodeficiency syndrome (AIDS)

10. Allergies and Adverse Drug Reaction
    - History of allergy to study drug components
    - History of severe hypersensitivity reaction to any monoclonal antibody

11. Known additional malignancies within the past 3 years (excluding basal of squamous cell skin cancers, CIS or localized prostate cancer that has been treated or is being observed)

12. Prior solid organ or stem cell transplant

3.3 Inclusion/Exclusion Criteria- Part B

1. Must meet eligibility criteria for initiation of Part A with the exception of being allowed to have prior nivolumab in Part A of this protocol.

2. Must have evidence of either RECIST 1.1 defined Disease Progression or Stable Disease 1 year after initiating nivolumab therapy

3. Tumor biopsy prior to combination treatment is mandatory. If a biopsy/resection of a new lesion or primary tumor and slow freezing of fresh tissue for single cell RNAseq study (as specified in the CLM) is not feasible, the subject is not eligible for the study. All biopsies must be core needle or excisional. Fine needle aspirate is not acceptable.

4. Must not have had a Grade ≥ 3 irAE on nivolumab monotherapy (excluding endocrine toxicity managed with replacement therapy).

5. Must not have untreated brain metastases.

6 Must not have had major surgery or radiation therapy within 14 days of starting study treatment.

7. Must not have active autoimmune disease (see protocol for exceptions).

8. Must not have a concurrent medical condition requiring use of systemic corticosteroids with prednisone >10 mg per day.

9. Must not have had prior systemic therapy for Stage IV RCC (except for nivolumab as part of part A of this protocol).

4. SUBJECT REGISTRATION

All subjects must be registered through HCRN's electronic data capture (EDC) system. A subject is considered registered when an "On Study" date is entered into the EDC system.

Subjects must be registered prior to starting protocol therapy and begin therapy **within 5 business days** of registration.

5. TREATMENT PLAN

We will conduct a prospective Phase II trial of nivolumab in 134 treatment naïve patients with ccRCC. Eligible patients with biopsiable (biopsied) disease will receive nivolumab 240 mg IV every 2 weeks x 6 doses (2 cycles) then nivolumab every 3 weeks at a dose of 360 mg IV for 4 doses (2 cycles) followed by nivolumab 480 mg IV every 4 weeks. Tumor response will be assessed at weeks 12, 18 and 24 and then every 12 weeks. For patients who experience RECIST 1.1 defined PD, but remain clinically stable (and asymptomatic), a confirmatory scan after 4-6 weeks of additional therapy is suggested. Patients with persistent PD at confirmatory scan will be evaluated for enrollment on Part B of this study. Symptomatic patients may be evaluated for Part B immediately. Patients without confirmed PD can continue on nivolumab therapy.

Patients who experience symptomatic or confirmed PD (or have best response of SD at 48 weeks/end of Cycle 6) on nivolumab monotherapy will be eligible for consideration for Part B. Part B involves the addition of ipilimumab for up to 4 doses while maintaining nivolumab therapy. Dose of ipilimumab will be 1 mg/kg every 3 weeks together with nivolumab changed to 3 mg/kg every 3 weeks for up to 4 doses (must complete by week 16 of Part B). Nivolumab will revert to 360 mg every 3 weeks after the completion of treatment with ipilimumab for 4 doses (2 cycles) followed by nivolumab 480 mg IV every 4 weeks (must be started between week 13-19). Patients will be followed with serial imaging assessments weeks 12, 18 and 24 and then every 12 weeks after the initiation of ipilimumab. The tumor measurements at the time of (or within 4 weeks of) ipilimumab institution will be the new baseline.

Patients for Part B must still meet the eligibility criteria for initial study enrollment. Patients with Grade 3 toxicity on nivolumab monotherapy, serious symptomatic disease that in the opinion of the site investigator requires immediate use of an alternative treatment approach or continued PR/CR will be excluded from enrolling in Part B. It is estimated that roughly half of the patients accrued to the first-line treatment will go on to enroll in Part B.

An additional biopsy will be performed of a metastatic lesion at time of confirmed PD in all patients enrolling in Part B. Confirmation of tumor in the biopsy specimen must occur prior to initiation of treatment on Part B. Subjects unable to undergo a new tumor biopsy and have tissue slow frozen for scRNAseq are not eligible for Part B.

An additional cohort of 40 non-ccRCC patients will be enrolled and analyzed separately for evidence of anti-tumor activity (CR, PR and SD and PFS at 1 year of nivolumab). These patients will also be eligible for participation in Part B (as described for patients with ccRCC). We anticipate that the accrual of these 40 patients will be able to be completed within the 1.5 years needed for accrual of the ccRCC patients.

5.1 Part A: Nivolumab Administration

Nivolumab will be given every 2 weeks x 6 doses (2 cycles) at a dose of 240 mg Intravenously (IV) then every 3 weeks at a dose of 360 mg IV for 4 doses (2 cycles) followed by nivolumab 480 mg IV every 4 weeks until toxicity, disease progression, SD at 48 weeks (end of Cycle 6) or complete response with a maximum of 96 total weeks (12 weeks induction, 84 weeks maintenance) Patients with disease progression (at any time) or SD at 48 weeks (end of Cycle 6) will be eligible to be considered for participation in Part B of the study.

Part A

Drug	Dose	Route[3]	Schedule[1]	Cycle Length	Cycles/Weeks
Nivolumab	240 mg	IV over 30 minutes	Day 1, 15, and 29	6 weeks	C1 and C2 Weeks 1-12
Nivolumab	360 mg	IV over 30 minutes	Day 1 and 22	6 weeks	C3 and C4 Weeks 13-24
Nivolumab	480 mg	IV over 30 minutes	Day 1, 29 and 57	12 weeks	C5-C10 Weeks 25-96

[1] A window of ± 3 days may be applied to all study visits to accommodate observed holidays, inclement weather, scheduling conflicts etc. Date and time of each drug administration should be clearly documented in subject's chart and electronic case report forms (eCRFs).
[2] Treatment will continue until toxicity, PD, SD at 48 weeks (end of Cycle 6) or CR with a maximum of 96 weeks
[3] Infusion window of ± 10 minutes

5.2 Part B: Nivolumab + Ipilimumab

Patients with Grade 3 toxicity on nivolumab monotherapy, (excluding endocrine toxicity), serious symptomatic disease that in the opinion of the site investigator requires immediate use of an alternative treatment approach or continued PR/CR will be excluded from enrolling in Part B. It is estimated that roughly half of the patients accrued to the first line treatment will go on to enroll in Part B. Part B treatment is encouraged to start within 7 weeks of the last dose of Part A study drug, although there is no limit to the interval so long as there is no intervening systemic treatment for the metastatic RCC.

Ipilimumab will be given 1 mg/kg every 3 weeks together with nivolumab 3 mg/kg every 3 weeks for up to 4 doses. **NOTE:** ipilimumab/nivolumab combination treatment must be completed by week 16 even if < 4 doses are administered. Nivolumab will revert to 360 mg every 3 weeks after the completion of combination treatment with ipilimumab for 4 doses (2 cycles) followed by nivolumab 480 mg IV every 4 weeks until toxicity, disease progression or a maximum of 60 total weeks (12 weeks induction, 48 weeks maintenance) beginning week 13 to 19 of Part B depending on how long it takes to recover from any side effects related to combination therapy. Patients may be dosed no less than 18 days from the previous dose of drug; and dosed up to 3 days after the scheduled date, if necessary.

Part B

Drug[1]	Dose	Route[4]	Schedule[3]	Cycle Length	Cycles/Weeks
Nivolumab	3 mg/kg	IV over 30 minutes	Day 1 and 22	6 weeks	C1 and C2 Weeks 1-12
Ipilimumab	1mg/kg	IV over 30 minutes			
Nivolumab	360 mg	IV over 30 minutes	Day 1 and 22	6 weeks	C3 and C4 Weeks 13-24
Nivolumab	480 mg	IV over 30 minutes	Day 1, 29 and 57	12 weeks	C5-C7 Weeks 25-60

[1] When study drugs (ipilimumab and nivolumab) are to be administered on the same day, separate infusion bags and filters must be used for each infusion. It is recommended that nivolumab be administered first. The second infusion will always be ipilimumab, and will start approximately 30 minutes after completion of the nivolumab infusion
[2] The dosing calculations should be based on the body weight where applicable. Institutional standards for recalculating the dose based on weight changes should be used. All doses should be rounded up or to the nearest milligram per institutional standard.
[3] A window of ± 3 days may be applied to all study visits to accommodate observed holidays, inclement weather, scheduling conflicts etc. Date and time of each drug administration should be clearly documented in subject's chart and electronic case report forms (eCRFs).
[4] Infusion window of ± 10 minutes

5.2 Concomitant Medications

5.2.1 Allowed Concomitant Medications

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

5.2.2 Prohibited Concomitant Medications

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

Patients may not use any of the following therapies during the study:

- Any non-study anti-cancer agent (investigational or non-investigational)
- Any other investigational agents
- Immunosuppressive agents (except as needed to treat toxicities that develop on therapy)
- Physiologic replacement doses of corticosteroids are permitted if required
- Any non-oncology vaccine therapies used for the prevention of infectious diseases (for up to 30 days prior to or after any dose of study drug)
  NOTE: Patients are permitted to receive the seasonal influenza vaccine. If seasonal influenza vaccine is considered, killed vaccines are mandatory.

6. TOXICITIES AND DOSE DELAYS

The NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.03 will be used to grade adverse events.

Subjects enrolled in this study will be evaluated clinically and with standard laboratory tests before and at regular intervals during their participation in this study as specified in Study Calendar & Evaluations.

Subjects will be evaluated for adverse events (all grades), serious adverse events, and adverse events requiring study drug interruption or discontinuation as specified in Study Calendar & Evaluations.

6.1 Dose Delays/Dose Modifications

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

6.1.1 Dose Delay Criteria

Dose delay criteria apply for all drug-related adverse events (regardless of whether or not the event is attributed to nivolumab, ipilimumab or both). Study drugs (both agents if on induction phase of Part B) must be held/omitted until treatment can resume. Please see Appendix A for AE management guidelines.

Nivolumab and ipilimumab administration should be omitted for the following:

- Any Grade ≥ 2 non-skin, drug-related adverse event, with the following exceptions:
  - Grade 2 drug-related fatigue or laboratory abnormalities that do not require a treatment delay (with the exception of Grade 3 AST, ALT or total bilirubin in patients with normal baseline values-see below).
- Any Grade 3 skin, drug-related adverse event
- Any Grade 3 drug-related laboratory abnormality, with the following exceptions:
  - Grade 3 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis do not require a dose delay. It is recommended to consult with the principal investigator for Grade 3 amylase or lipase abnormalities.
  - 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
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the site investigator, warrants delaying the dose of study medication.

6.1.2 Criteria to Resume Treatment

Subjects may resume treatment with study drugs when the drug-related AE(s) resolve to Grade ≤ 1 or baseline value, with the following exceptions:

- Subjects may resume treatment in the presence of Grade 2 fatigue
- Subjects who have not experienced a Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin toxicity

- 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 (Section 6.3.3) 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 the criteria to resume treatment are met, the subject should restart treatment at the next scheduled timepoint per protocol (i.e., missed doses are NOT made up).

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

6.1.3 Discontinuation Criteria

Treatment should be permanently discontinued for the following:

- Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment
- Any Grade 3 non-skin, drug-related adverse event lasting > 7 days, with the following exceptions for drug-related laboratory abnormalities, uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic adverse event, hypersensitivity reactions, and infusion reactions
    - Grade 3 drug-related uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic adverse event, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation (**NOTE:** Subjects with Grade 3 diarrhea or colitis while on Part B ipilimumab/nivolumab combination therapy could resume nivolumab alone if toxicity resolves to at least Grade 1 off of immunosuppressive drugs by end of week 16. Nivolumab monotherapy cannot start until toxicity is resolved to at least Grade 1 off immunosuppression x 2 weeks and cannot begin before week 13 or after week 19.
- Grade 3 drug-related laboratory abnormalities do not require permanent treatment discontinuation except those noted below
    - Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding
    - Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:
        - AST or ALT > 8 x ULN
        - Total bilirubin > 5 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:
    - Isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis and:
        - Decrease to < Grade 4 within 2 weeks of onset in the absence of immunosuppressive therapy.
        - Decrease to < Grade 4 and maintain < Grade 4 for a minimum of 1 week after stopping immunosuppressive therapy.
    - 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 (from scheduled dose) with the following exceptions:
    - Dosing interruptions to allow for prolonged steroid tapers to manage drug-related adverse events are allowed. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the sponsor-investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted
    - Dosing interruptions > 6 weeks that occur for non-drug-related reasons may be allowed if approved by the sponsor-investigator. **Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the Principal Investigator must be consulted.** Tumor assessments should continue as per protocol even if dosing is interrupted.
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the site investigator, presents a substantial clinical risk to the subject with continued nivolumab or ipilimumab dosing

6.2 Treatment of Nivolumab or Ipilimumab Related Infusion Reactions

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

All Grade 3 or 4 infusion reactions should be reported as an SAE if criteria are met. Infusion reactions should be graded according to NCI CTCAE v4.03 guidelines.

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

- Grade 1 symptoms: (Mild reaction; infusion interruption not indicated; intervention not indicated). Remain at bedside and monitor subject until recovery from symptoms. The following prophylactic premedications are recommended for future infusions:
    - Diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) at least 30 minutes before additional nivolumab administrations.
- Grade 2 symptoms: (Moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [eg, antihistamines, non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; prophylactic

medications indicated for 24 hours). Stop the nivolumab 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 pre-medications are recommended for future infusions:

  - Diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) should be administered at least 30 minutes before additional nivolumab or ipilimumab administrations. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.

- Grade 3 or Grade 4 symptoms: (Severe reaction, Grade 3: prolonged [ie, not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae [eg, renal impairment, pulmonary infiltrates]). Grade 4: (life threatening; pressor or ventilatory support indicated). Immediately discontinue infusion of nivolumab 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.*** Site investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery from symptoms. In the case of late-occurring hypersensitivity symptoms (eg, appearance of a localized or generalized pruritis within 1 week after treatment), symptomatic treatment may be given (eg, oral antihistamine, or corticosteroids).

6.3 Protocol Therapy Discontinuation

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

- Documented disease progression **NOTE:** Repeat scan should be performed in 4-6 weeks to confirm PD. A subject may be granted an exception to continue on treatment with confirmed radiographic progression if clinically stable or clinically improved. If PD confirmed patient can move to Part B and or go off study treatment.

- Completed 96 weeks of treatment with nivolumab (Part A) or 60 weeks of (nivolumab + ipilimumab for 12 weeks followed by nivolumab monotherapy for up to 48 weeks) (Part B). **NOTE:** 96 or 60 weeks of study medication are calculated from the date of first dose of each Part.
- Site investigator determines a change of therapy would be in the best interest of the subject
- Subject or legal representative (such as a parent or legal guardian) withdraws consent and requests to discontinue protocol therapy, whether due to unacceptable toxicity or for other reasons
    - In a subject decides to prematurely discontinue protocol therapy ("refuses treatment"), the subject should be asked if he or she may still be contacted for further scheduled study assessments. The outcome of that discussion should be documented in both the medical records and in the eCRF.
- Female subject becomes pregnant
- Unacceptable adverse experiences
- Intercurrent illness that prevents further administration of treatment
- Protocol therapy is interrupted for > 6 weeks (calculated from the date of the first missed dose). Any potential exceptions to this criterion must be discussed with and approved by the study chair.
- Noncompliance with protocol treatment or procedure requirements
- The subject is lost to follow-up
- Administrative reasons

6.2 Protocol Discontinuation

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

7. STUDY CALENDAR & EVALUATIONS

Study Evaluation	Screening		Part A (± 3 days)						Part B (± 3 days)							End of Treatment (EOT)		Follow Up[12]
			Induction		Maintenance				Prior to Part B	Induction		Maintenance						
			Cycle = 6 weeks				Cycle = 12 weeks			Cycle = 6 weeks				Cycle = 12 weeks		Safety Visits ± 7 days[11]		
			C1-C2		C3-C4		C5-C10			C1[10]-C2		C3-C4		C5-C7				
	-28 days	-14 days	D 1	D 15/29	D 1	D 22	D 1	D 29/57	-28 days	D 1	D 22	D 1	D 22	D 1	D 29/57	D 30	D 100	± 14 days
REQUIRED ASSESSMENTS																		
Informed Consent	X																	
Medical History/Smoking history	X																	
Diagnosis and Staging	X																	
Physical Exam[1]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Vital signs/ECOG PS Status[1]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
AEs & concomitant medications	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
LABORATORY ASSESSMENTS																		
Complete Blood Cell Count with diff (CBC)[2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Comprehensive Metabolic Profile (CMP)[2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Amylase, Lipase, LDH, CPK[3]	X		X		X		X		X	X		X		X		X		
HBV, HCV[3]	X																	
Thyroid Function (TSH)[3]	X		X		X		X		X	X		X		X		X		
Pregnancy test (serum or urine)[4]			X						X									
DISEASE ASSESSMENT																		
CT of chest[5]	X				X[5]		X[5]		X			X[5]		X[5]				X[5]
CT or MRI of abdomen and pelvis[5]	X				X[5]		X[5]		X			X[5]		X[5]				X[5]
TREATMENT EXPOSURE																		
Nivolumab[6]			X	X	X	X	X	X		X	X	X	X	X	X			
Ipilimumab[6]										X	X							
SPECIMEN COLLECTION																		
Whole Blood for Somatic Testing[7]			C1															
PBMCs and plasma [7]			X[7]		X[7]		X[7]		X[7]									
Archival Tumor Tissue[8]	X[8]																	
Fresh Tissue[8]		X[8]							X[8]									
SPECIMEN BANKING																		
Whole Blood, Plasma and Serum[9]			C1													X		
FOLLOW UP																		
Survival Status,Therapy																		X

Key to Footnotes

1. If the screening physical exam is conducted within 24 hours of dosing on Day 1, then a single examination may count as both the screening and pre-dose evaluation. Vitals required are temperature, pulse, respiratory rate, blood pressure, and oxygen saturation.
2. Hematology Labs should include CBC with differential (hemoglobin, hematocrit, white blood cells, platelets, neutrophils, lymphocytes, eosinophils and monocytes). Additional draws must be incorporated when monitoring recovery from any hematologic AE. Chemistry Labs should include albumin, BUN, creatinine, ALT, AST, bilirubin serum alkaline phosphatase, glucose, total protein, sodium, potassium, chloride, HCO3, calcium. Labs should be performed on the day of treatment but may be drawn up to 72 hours before to ensure return of results prior to dosing.
3. Other labs to be drawn: amylase, lipase, LDH, direct bilirubin (only if total bilirubin is elevated), uric acid and CPK Day 1 of every Cycle during treatment. Thyroid function will be monitored Day 1 of each cycle during treatment. TSH will be monitored and T3 and T4 (free versus total) should be drawn at site investigators discretion. It is suggested that if TSH is elevated T3 and T4 values may be done. At screening, testing should be performed for hepatitis C antibody and HBs Ag utilizing local standard informed consent procedures prior to this laboratory collection. These tests could be repeated later during the course of the study if clinically indicated.
4. Women of child-bearing potential must have a negative serum or urine pregnancy test within 24 hours prior to treatment with nivolumab and again at crossover to Part B for screening purposes.
5. Non-CNS Imaging should include a chest, abdomen and pelvis CT. Imaging of other sites should be obtained when clinically indicated. If follow up scans indicate partial or complete response, a repeat confirmatory scan should be obtained within 12 weeks (but no sooner than 6 weeks). Subjects with equivocal disease progression (e.g. a new lesion in the setting of major disease regression and the absence of or improvement in disease related symptoms) should have a scan confirming disease progression 4-6 weeks following the initial scan showing RECIST defined PD. Part A imaging will occur at baseline within 4 weeks of C1D1 of Part A, then at week 12 (after C1 and C2 of Part A Induction), week 18 (after C3 and before C4 of Part A Maintenance), week 24 (after C4 of Part A maintenance) and then every 12 weeks (± 1 week). Patients with symptomatic and radiographic PD will not require a confirmatory scan before proceeding to Part B of this study. Following crossover, imaging and calendar will be reset at Cycle 1. Part B imaging will occur at baseline within 4 weeks prior to crossover, then at week 12 (after C1 and C2 of Part B Induction), week 18 (after C3 and before C4 Part B Maintenance), week 24 (after C4 Part B Maintenance) and then every 12 weeks (± 1 week). Every 12 weeks radiology imaging will occur if the patient is < 2 years from study entry (or < 2 years from crossover), then radiology imaging will occur every 6 months (± 4 weeks) until 5 years from study entry. If tumor assessments are available for subjects who have not yet experienced progressive disease (PD) at the time treatment is discontinued, the follow-up tumor evaluations will be documented in the eCRF until PD or death is confirmed, or until another treatment is initiated.
6. 1 Cycle= (6 weeks) for Part A (induction and maintenance C3 and C4) and Part B (induction and maintenance C3 and C4). 1 Cycle = 12 weeks for Part A maintenance C 5-10 and Part B maintenance C5-C7. Treatment and follow-up schedule is the same for either nivolumab monotherapy or ipilimumab/nivolumab combination. Patients responding in Part A will receive treatment at most through Cycle 10 (week 96). Patients crossing over to Part B will receive treatment at most through Cycle 7 (week 60) from start of Part B. They will then have treatment stopped and be followed until disease progression or study closure. Once confirmed progression is confirmed or stable disease for 48 weeks (end of Cycle 6), nivolumab monotherapy will be discontinued for Part A and the patient will undergo evaluation for Part B treatment.
7. Mandatory Samples: Peripheral blood for somatic baseline testing will be collected prior to treatment on C1D1 Part A. PBMCs and plasma are preferred (or whole blood) for TCR assay and will be collected prior to treatment (C1D1), C3D1, C5D1 (Part A) and at time of progression (Prior to Part B). See Correlative Laboratory Manual (CLM) for additional details.

8. Archival tissue of a metastatic lesion obtained within 1 year prior to study registration (within 4 weeks preferred) and tumor tissue from nephrectomy is required if available. In addition to archival tissue of a metastatic lesion and nephrectomy, patients must have at least one site of disease (not including bone metastases) accessible for biopsy. If biopsy/resection of a new lesion or primary tumor and slow freezing of fresh tissue for single cell RNAseq study (as specified in the CLM) is not feasible, the subject is not eligible for the study. All biopsies must be core needle or excisional. Fine needle aspirate is not acceptable. NOTE: The tissue collected from a surgical resection or multiple core biopsies of either a metastatic lesion or primary tumor for the slow freezing of fresh tissue after the patient has signed consent for the study could also be used for collecting the FFPE specimens. See Correlative Laboratory Manual for complete details.
9. For Part A only: Whole blood will be collected prior to treatment C1D1. Whole blood for plasma and serum prior to treatment on C1D1and at the D30 end of treatment visit. All samples will be banked for unspecified cancer related research (optional). See Correlative Laboratory Manual for complete details.
10. Patients on Part B should be contacted weekly during Cycles 1 and 2. If a patient reports symptoms at any time during these cycles or abnormal labs are noted at the regularly scheduled visits (C1 D22, C2 D1 or D22) then more frequent laboratory testing and clinic visits are encouraged.. This decision is at the discretion of the site investigator.
11. An End of Treatment (EOT) visit should occur 30 and 100 days (± 7 days) from last dose of study drug. For patients experiencing toxicities, additional visits may be necessary and may occur occur every 2 weeks (± 3 days) until toxicities resolve, are stable, or start of new systemic therapy. Depending upon the toxicity, the every 2-week assessment may be accomplished via phone call, email or other avenues as appropriate. Mode of assessment will be at site investigator's discretion. If patients have progressive disease as the reason for discontinuation from study, the D100 may be a phone call.
12. Follow up is for subjects that have discontinued study treatment. Subjects should be followed every 3 months if < 3 years from study registration and every 6 months if 3-5 years from study registration. Patients who develop disease progression on Part B or on Part A (if ineligible for Part B) will be followed for survival only. Follow up may be accomplished via clinic visit, phone call, or other avenues as appropriate. A window of ± 14 days will be applied to follow up.

8. BIOSPECIMEN STUDIES AND PROCEDURES

A variety of factors that may impact the immunomodulatory properties and efficacy of nivolumab or nivolumab-ipilimumab combination will be investigated in tumor tissue and from all registered subjects prior to Part A and in those patients going onto to Part B as outlined in Section 7.

8.1 Analyses

8.1.1 Assessment of PD-L1 Expression on Tumor and Infiltrating Mononuclear Cells

Tumor cell surface PD-L1 expression on primary tumor specimens serves as a potential predictive biomarker of durable response to PD-1 antibody therapy [17]. However, data from several recent studies suggest that IHC staining for PD-L1 alone fails to identify all responders to PD-1 blocking Abs [39, 40]. Some patients with PD-L1 negative tumors respond to PD-1 pathway blockade, while many patients with PD-L1 positive tumors fail to do so. Interestingly, in melanoma, the benefit of combined PD-1 and CTLA-4 blockade versus PD-1 alone appeared to be greatest in patients with negative PD-L1 tumor expression [22]. We will test whether PD-L1 status is indeed predictive of higher response to combined checkpoint blockade.

8.1.2 Assessment of Tumor IFN Gamma Signature, Mutational Load, and Maximum Fuhrman Grade

In patients with metastatic RCC, treatment with nivolumab resulted in increased expression of genes indicative of Th1-type inflammatory response and cytotoxic T cell activation, such as ICOS, interferon-gamma, granzymes, and perforin [48]. In addition, CXCL9 and CXCL10, two interferon-gamma-regulated chemokines that in turn regulate trafficking of T cells, and are both prognostic [49] and predictive of response to ipilimumab [50]. We propose to study tumor interferon-gamma signature using RNA sequencing as a marker of response to PD-1 and PD-1/CTLA-4 blockade.

Accumulating evidence shows that anti-tumor immune responses focus on neoantigens generated by tumor mutation, and responsiveness to immunotherapy correlates with neoantigen burden. [51, 52]. Mutational load has been correlated with response to CLTA-4 and PD-1 blockade in melanoma [53, 54] and multiple other tumor types [55-57]. These data motivate us to perform a systematic analysis of mutation burden and neoantigen load using Whole Exome Sequencing (WES). We will explore potential neoantigens in subsets of responding and non-responding patients. We will also evaluate changes in neoantigen load at the time of resistance compared with baseline samples.

Tumor cell PD-L1 expression has been correlated with higher Fuhrman nuclear grade [26]. In a cohort of mRCC patients treated with PD-L1 antibody, overall response rate was slightly higher in patients with Fuhrman grade 4 and/or sarcomatoid histology (ORR 22% versus 15% in the overall cohort) [58]. Therefore, we will assess Fuhrman grade and sarcomatoid histology as predictors of response.

8.1.3 Investigation of Mechanisms of Innate and Acquired Resistance to Nivolumab Therapy

Resistance to PD-1 blockade may occur as as primary or secondary event. In order to elicit a successful anti-tumor immune response, tumor neoantigens must be presented by antigen-presenting cells to CD8+ T cells via major histocompatibility complex class I (MHC-I) molecules. Then, lymphocytes must be primed and activated. Lymphocytes must be trafficked to the tumor and subsequently infiltrate the tumor, then they must recognize the tumor cells, and finally they must produce cytotoxic effect. "Non-inflamed" tumors, those that lack tumor-infiltrating lymphocytes, may have a defect in early steps of the generation of this immune response and may have primary resistance to PD-1 blockade. "Inflamed" tumors, those that do contain tumor-infiltrating lymphocytes, may have defects in later steps such as loss or alteration of MHC-I [59-61]. Multiple other checkpoints may become activated [62-65] Finally other immunosuppressive cells in the tumor microenvironment may blunt the T-cell response. We will perform exploratory analyses to evaluate these mechanisms further, including exploratory analyses of immune cells using immunosequencing.

8.2. Tissue Collections

SEE CLM FOR ADDITIONAL DETAILS.

8.2.1 Archival Tumor Tissue

Archival tissue of a metastatic lesion (from core needle or excisional biopsy) obtained within 1 year prior to study registration (within 4 weeks preferred) and tumor tissue from nephrectomy is required if available. Confirmation of sufficient archival tissue must be obtained prior to C1D1 and shipped to the appropriate lab by end of Cycle 2. Biopsies must be core needle or excisional biopsies of primary tumor or visceral or lymph node metastases. Fine needle aspirates of any metastatic site, and biopsies of bone lesions are not acceptable.

8.2.2 Pre-Treatment Tumor Tissue

Patients must have at least one site of disease (not including bone metastases) accessible for biopsy after confirmation of eligibility/consent and prior to initiation of treatment. If biopsy/resection of a new lesion or primary tumor and slow freezing of fresh tissue for single cell RNAseq study (as specified in the CLM) is not feasible, the subject is not eligible for the study. All biopsies must be core needle or excisional. Fine needle aspirate biopsies are not acceptable, nor are biopsies of non soft tissue components of bone metastases. **NOTE:** The tissue collected from a surgical resection or multiple core biopsies of either a metastatic lesion or primary tumor for the slow freezing of fresh tissue after the patient has signed consent for the study could also be used for collecting the FFPE specimens

8.2.3 Tumor Tissue- At Progression

Tumor biopsy prior to combination treatment is mandatory. If a biopsy/resection of a new lesion or primary tumor and slow freezing of fresh tissue for single cell RNAseq study (as specified in the CLM) is not feasible, the subject is not eligible for the study. All biopsies must be core needle or excisional. Fine needle aspirate biopsies are not acceptable, nor are biopsies of non soft tissue components of bone metastases.

8.2.4 Tumor Tissue for RNAseq

Part of the tissue collected prior to C1D1 and at progression will be used for single cell RNAseq analysis. This analysis will be funded through the Department of Defense (DoD) via a proposal entitled, "Using Single Cell Transcriptomics to Understand Tumor and Immune Heterogeneity Driving Resistance and to Rationally Select Immune Therapy in Advanced RCC." In addition to the data from the sample analysis, the DoD will have access to de-identified subject data such as response outcomes.

8.3 Peripheral Blood Samples

8.3.1 Mandatory Whole Blood for Somatic Baseline Testing

Whole blood will be collected prior to treatment **Cycle 1 Day 1** for somatic testing.

8.3.2 Mandatory PBMCs and Plasma (preferred) or Whole Blood for TCR analysis

Whole blood for PBMCs and plasma isolation for TCR analysis should be collected at baseline (C1D1), C3D1, C5D1 and at time of disease progression. If PBMCs cannot be processed on site, whole blood may be collected. See CLM for collection and processing instructions.

8.3.3 Optional Whole Blood, Plasma and Serum for Banking

Subject consent will be obtained for additional samples to be collected for future unspecified cancer related research studies. HCRN will manage the banked samples. Samples will be banked indefinitely in the HCRN Biorepository.

This includes:

- Pre whole blood: Whole blood for banking will be collected for Part A only; prior to treatment Cycle 1 Day 1.
- Pre and Post Treatment plasma and serum: Whole blood for plasma and serum will be collected for Part A only; prior to treatment on Cycle 1 Day and at the D30 end of treatment visit.

8.4 Storage of Biospecimens

All specimens not exhausted in planned correlative studies for this trial will be banked for future unspecified cancer related research. Patient consent will be obtained for banking of these samples.

8.5 Confidentiality of Biospecimens

Samples that are collected will be identified by a subject's sequence ID at the time of registration to the trial. Any material issued to collaborating researchers will be anonymized and only identified by the subject's sequence ID.

9. CRITERIA FOR DISEASE EVALUATION

For the purposes of this study, subjects should be evaluated for response every 12 weeks. Non-CNS Imaging should include a chest, abdomen and pelvis CT. Imaging of other sites should be obtained when clinically indicated. If follow up scans indicate partial or complete response, a repeat confirmatory scan should be obtained within 12 weeks (but no sooner than 6 weeks). Subjects with equivocal disease progression (e.g. a new lesion in the setting of major disease

regression and the absence of or improvement in disease related symptoms) should have a scan confirming disease progression 4-6 weeks following the initial scan showing RECIST defined PD. Patients with symptomatic and radiographic PD will not require a confirmatory scan before proceeding to Part B of this study. The date of the first scan will be the documented date of progression.

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

The following general principles must be followed:

1. To assess objective response, it is necessary to estimate the overall tumor burden at baseline to which subsequent measurements will be compared. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than four weeks before registration.
2. Measurable disease is defined by the presence of at least one measurable lesion.
3. All measurements should be recorded in metric notation by use of a ruler or calipers.
4. The same method of assessment and the same technique must be used to characterize each identified lesion at baseline and during follow-up.

9.1 Definitions

9.1.1 Evaluable for Objective Response

Only those subjects who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These subjects will have their response classified according to the definitions stated below. Those subjects that are inevaluable will not be replaced.

(**NOTE:** Subjects who exhibit objective disease progression prior to the end of Cycle 1 will also be considered evaluable.)

9.1.2 Evaluable Non-Target Disease Response

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

9.2 Disease Parameters

9.2.1 Measurable Disease

Measurable disease is defined as the presence of at least one measurable lesion. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as $\geq$20 mm by chest x-ray, as $\geq$10 mm with CT scan, or $\geq$10 mm with

calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

NOTE: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. If the site investigator thinks it appropriate to include them, the conditions under which such lesions should be considered must be defined in the protocol.

9.2.2 Malignant Lymph Nodes

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

9.2.3 Non-measurable Lesions

All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥ 10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable. Non-measurable also includes lesions that are < 20 mm by chest x-ray.

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

9.2.4 Target Lesions

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

9.2.5 Non-target Lesions

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

9.3 Evaluation of Target Lesions

NOTE: In addition to the information below, also see section 4.3.2 in the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee, version 1.1 (Eur J Cancer 45;2009:228-247) for special notes on the assessment of target lesions.

Complete Response (CR)	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm. To be assigned a status of complete response, changes in tumor measurements must be confirmed by a repeat assessment performed no less than six weeks after the criteria for response is met.
Partial Response (PR)	At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters. To be assigned a status of partial response, changes in tumor measurements must be confirmed by a repeat assessment performed no less than six weeks after the criteria for response is met.
Progressive Disease (PD)	At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (**NOTE:** the appearance of one or more new lesions is also considered progressions). Subjects with equivocal disease progression (e.g. a new lesion in the setting of major disease regression and the absence of or improvement in disease related symptoms) should have a scan confirming disease progression 4-6 weeks following the initial scan showing RECIST defined PD. Subjects may continue on protocol therapy until repeat scan confirms PD. If repeat scan does not confirm PD, subjects should proceed with treatment and evaluation as directed by the protocol with the confirmatory scan (if between 4 and 6 weeks from last scan) being counted as the 6 week interval scan.
Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study. **NOTE:** a change of 20% or more that does not increase the sum of the diameters by 5 mm or more is coded as stable disease). To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval of 24 weeks.

9.4 Evaluation of Non-Target Lesions

Complete Response (CR)	Disappearance of all non-target lesions. All lymph nodes must be non-pathological in size (<10 mm short axis)
Non-CR/ Non-PD	Persistence of one or more non-target lesion(s)
Progressive Disease (PD)	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase. When the subject also has measurable disease, there must be an overall level of substantial worsening in non-target disease such that, even in the presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest "increase" in the size of one or more non-target lesions is usually not sufficient to qualify for unequivocal progression status. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare. When the subject only has non-measurable disease, the increase in overall disease burden should be comparable in magnitude to the increase that would be required to declare PD for measurable disease: i.e., an increase in tumor burden from "trace" to "large", an increase in nodal disease from "localized" to "widespread", or an increase sufficient to require a change in therapy. Although a clear progression of "non-target" lesions only is exceptional, the opinion of the site investigator should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or sponsor-investigator).

9.5 Evaluation of New Lesions

The appearance of new lesions constitutes Progressive Disease (PD). A growing lymph node that did not meet the criteria for reporting as a measurable or non-measurable lymph node at baseline should only be reported as a new lesion (and therefore progressive disease) if it:

- Increases in size to ≥ 15 mm in the short axis, or
- There is new pathological confirmation that it is disease (regardless of size). New effusion or ascites that appears during treatment should only be reported as a new lesion (and therefore progressive disease) if it has cytological confirmation of malignancy.

9.6 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence or non-protocol therapy (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

9.7 Evaluation of Best Overall Response

Target Lesions	Non-Target Lesions	New Lesions*	Overall Response	Remarks
CR	CR	No	CR	****
CR	Non-CR/ Non-PD***	No	PR	****
CR	Not evaluated	No	PR	
PR	Non-PD*** or not all evaluated	No	PR	
SD	Non-PD*** or not all evaluated	No	SD	Documented at least once ≥ 24 weeks from study entry
PD	Any	Yes or No	PD	No prior SD, PR or CR
Any	PD**	Yes or No	PD***	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.

** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

*** PD in non-target lesions should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase. Please refer to the Evaluation of Non-Target Lesions – Progressive Disease section for further explanation.

**** To be assigned a status of partial or complete response, changes in tumor measurements must be confirmed by a repeat assessment performed no less than six weeks after the criteria for response is met.

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

9.8 Definitions for Response Evaluation – RECIST 1.1

9.8.1 Duration of Overall Response

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

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

9.8.2 Duration of Stable Disease

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

To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval of 24 weeks.

9.8.3 Additional Criteria to Continue Treatment after Progression

Patients may continue on protocol therapy until repeat scan confirms PD. If repeat scan does not confirm PD, patients should proceed with treatment and evaluation as directed by the protocol with the confirmatory scan (if between 4 and 6 weeks from last scan) being counted as the 6 week interval scan.

9.8.4 Overall Survival

Overall survival is defined by the date of registration to date of death from any cause.

9.9 Immune Related RECIST (irRECIST)

PFS, ORR and duration of response per irRECIST are defined as specified for the respective endpoints using RECIST 1.1 above, with the exception that a confirmation assessment of PD (4-6 weeks after the initial PD assessment) is required for subjects who remain on treatment following a documented PD per RECIST 1.1. The first date of progression, confirmed on the subsequent imaging study, will be considered the date of progression. Subjects who discontinue treatment following a documented PD assessment per RECIST 1.1 will be counted as having disease progression on the date of the documented PD assessment.

10. DRUG INFORMATION

10.1 Nivolumab

Nivolumab was selected for dosage form development and is also referred to as BMS-936558-01 or BMS-936558. Nivolumab is a soluble protein consisting of 4 polypeptide chains, which include 2 identical heavy chains and 2 identical light chains.
Other Names Nivolumab, BMS-936558, MDX1106, anti-PD-1
Molecular Wt 146,221 daltons (143,619.17 daltons, protein portion)
Appearance Clear to opalescent, colorless to pale yellow liquid, few particulates may be present

Solution pH 5.5 to 6.5

10.1.1 Supplier/How Supplied

Nivolumab Injection, 100 mg/10 mL (10 mg/mL)
BMS will supply nivolumab at no charge to subjects participating in this clinical trial.

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

10.1.2 Preparation

Nivolumab Injection, 100 mg/10 mL (10 mg/mL). Nivolumab injection is to be administered as an IV infusion through a 0.2-micron to 1.2-micro pore size, low-protein binding in-line filter at the protocol-specified doses and infusion times. It is not to be administered as an IV push or bolus injection. Nivolumab injection can be infused undiluted (10 mg/mL) or diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to protein concentrations as low as 0.35 mg/mL. Total infusion volume not to exceed 120 mL. During drug product preparation and handling, vigorous mixing or shaking is to be avoided. Instructions for dilution and infusion of nivolumab injection may be provided in the clinical protocol or investigator's brochure. Care must be taken to assure sterility of the prepared solution as the product does not contain any antimicrobial preservative or bacteriostatic agent. No incompatibilities between nivolumab and polyvinyl chloride (PVC) and non-PVC/non-DEHP (di(2-ethylhexyl)phthalate) containers/IV components or glass bottles have been observed.

10.1.3 Storage and Stability

Nivolumab Injection, 100 mg/10 mL (10 mg/mL). Vials of nivolumab injection must be stored at 2° to 8°C (36° to 46°F) and protected from light and freezing.

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

After final drug reconciliation, unused nivolumab vials should be disposed at the site following procedures for the disposal of anticancer drugs. Certificate of destruction for unused supply should be retained for BMS

10.1.4 Handling and Disposal

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

10.1.5 Dispensing

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

10.1.6 Adverse Events

Please see the current Investigator's Brochure for complete details regarding AEs of nivolumab. The most common side effects of nivolumab are:

- Fatigue
- Skin reactions: including rash, itching, hives, redness, and dry skin. Toxic epidermal necrolysis, a potentially life threatening disease characterized by blistering and peeling of the top layer of skin resembling that of a severe burn, has occurred in one subject who received ipilimumab after nivolumab treatment.
- Diarrhea
- Nausea
- Abdominal pain
- Decreased appetite
- Low red blood cells
- Fever
- Joint pain or stiffness

10.2 Ipilimumab

Ipilimumab is a recombinant, human monoclonal antibody that binds to the 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.

Bristol-Myers Squibb (BMS) will supply ipilimumab. Ordering of ipilimumab will take place through the BMS vendor.

10.2.1 Pharmacokinetics (PK)

Ipilimumab as a single agent: The pharmacokinetics of ipilimumab was studied in 499 subjects with unresectable or metastatic melanoma who received doses of 0.3, 3, or 10 mg/kg administered once every 3 weeks for four doses. Peak concentration (Cmax), trough concentration (Cmin), and area under the curve (AUC) of ipilimumab were found to be dose proportional within the dose range examined. Upon repeated dosing of ipilimumab administered every 3 weeks, ipilimumab clearance was found to be time invariant, and minimal systemic accumulation was observed as evident by an accumulation index of 1.5-fold or less. Ipilimumab steady-state concentration was reached by the third dose. The following mean (percent coefficient of variation) parameters were generated through population PK analysis: terminal half-life of 14.7 days (30.1%); systemic clearance (CL) of 15.3 mL/h (38.5%); and volume of distribution at steady-state (Vss) of 7.21 L (10.5%). The mean (±SD) ipilimumab Cmin achieved at steady-state with the 3-mg/kg regimen was 21.8 mcg/mL (±11.2).

Nivolumab in combination with ipilimumab: The geometric mean (%CV) CL, Vss, and terminal half-life of nivolumab were 10.0 mL/h (50.3%), 7.92 L (30.1%), and 24.8 days (94.3%), respectively. When administered in combination, the CL of nivolumab was increased by 24%, whereas there was no effect on the clearance of ipilimumab. When administered in combination, the clearance of nivolumab increased by 42% in the presence of anti-nivolumab antibodies. There was no effect of anti-ipilimumab antibodies on the clearance of ipilimumab.

Specific Populations: Cross-study analyses were performed on data from subjects with a variety of conditions, including 420 subjects with melanoma who received single or multiple infusions of ipilimumab at doses of 0.3, 3, or 10 mg/kg. The effects of various covariates on ipilimumab PKs were assessed in population pharmacokinetic analyses.

Ipilimumab CL increased with increasing body weight; however, no dose adjustment of Ipilimumab is required for body weight after administration on a mg/kg basis. The following factors had no clinically meaningful effect on the CL of ipilimumab: age (range 26 to 86 years), gender, concomitant use of budesonide, performance status, HLA-A2*0201 status, positive anti-ipilimumab antibody status, prior use of systemic anticancer therapy, or baseline lactate dehydrogenase (LDH) levels. The effect of race was not examined as there were insufficient numbers of subjects in non-Caucasian ethnic groups.

Renal Impairment: Creatinine clearance at baseline did not have a clinically important effect on ipilimumab pharmacokinetics in subjects with calculated creatinine clearance values of 29 mL/min or greater.

Hepatic Impairment: Baseline AST, total bilirubin, and ALT levels did not have a clinically important effect on ipilimumab pharmacokinetics in subjects with various degrees of hepatic impairment.

10.2.2 Preparation

Ipilimumab is a sterile, preservative-free, clear to slightly opalescent, colorless to pale yellow solution for intravenous infusion, which may contain a small amount of visible translucent-to-white, amorphous ipilimumab particulates. It is supplied in single-use vials of 200 mg/40 mL Each milliliter contains 5 mg of ipilimumab and the following inactive ingredients: diethylene triamine penta acetic acid (DTPA) (0.04 mg), mannitol (10 mg), polysorbate 80 (vegetable origin) (0.1 mg), sodium chloride (5.85 mg), tris hydrochloride (3.15 mg), and Water for Injection, USP at a pH of 7.

Do not shake product.
Inspect parenteral drug products visually for particulate matter and discoloration prior to administration. Discard vial if solution is cloudy, there is pronounced discoloration (solution may have pale yellow color), or there is foreign particulate matter other than translucent-to white, amorphous particles.

Preparation of Solution:

- Allow the vials to stand at room temperature for approximately 5 minutes prior to preparation of infusion.
- Withdraw the required volume of ipilimumab and transfer into an intravenous bag.
- Dilute with 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to prepare a diluted solution with a final concentration ranging from 1 mg/mL to 2 mg/mL. Mix diluted solution by gentle inversion.
- Store the diluted solution for no more than 24 hours under refrigeration (2°C to 8°C, 36°F to 46°F) or at room temperature (20°C to 25°C, 68°F to 77°F).
- Discard partially used vials or empty vials of ipilimumab.

10.2.3 Storage and Stability
Store ipilimumab under refrigeration at 2°C to 8°C (36°F to 46°F). Do not freeze. Protect vials from light.

10.2.4 Administration
Do not mix ipilimumab with, or administer as an infusion with, other medicinal products. Flush the intravenous line with 0.9% Sodium Chloride Injection, USP or 0.5% Dextrose Injection, USP after each dose. Administer diluted solution over 30 minutes (+/- 10 minutes) through an intravenous line containing a sterile, non-pyrogenic, low-protein-binding in-line filter.

10.2.5 Handling and Disposal
Accountability for investigational product is the responsibility of the investigator. The research pharmacy will maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form.

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

Drug should be destroyed at the site, after the investigator approves the drug destruction policy at the site. Drug will not be returned to BMS. Destruction will be documented in the Drug Accountability Record Form and certificate of destruction provided to BMS.

10.2.6 Compatibility
No incompatibilities between the nivolumab and ipilimumab have been observed. When both study drugs are to be administered on the same day, separate infusion bags and filters must be used for each infusion. Nivolumab is to be administered first. The nivolumab infusion must be promptly followed by a saline flush to clear the line of nivolumab before starting the ipilimumab infusion. The second infusion will always be ipilimumab, and will start at least 30 minutes after completion of the nivolumab infusion.

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

Nivolumab may be diluted in 0.9% Sodium Chloride Solution.

10.2.7 Adverse Events
Based on data from the Phase I study, nivolumab in combination with ipilimumab has an acceptable safety profile in RCC. In summary, AEs were reported in 97.7% of subjects treated with nivolumab in combination with ipilimumab. The most frequently reported drug-related AEs in subjects treated with 3-mg/kg nivolumab + 1-mg/kg ipilimumab included fatigue (11 subjects, 52.4%), rash (8 subjects, 38.1%), diarrhea (6 subjects, 28.6%), and pruritus (6 subjects, 28.6%); the majority were Grade 1-2. The most frequently reported drug-related AEs in subjects treated with 1-mg/kg nivolumab + 3-mg/kg ipilimumab included fatigue (16 subjects, 69.6%); nausea, ALT increased, AST increased (9 subjects each, 39.1%); and diarrhea (8 subjects, 34.8%). The majority were Grade 1-2. The majority of deaths were due to disease progression. No drug-

related deaths have been reported to date. Please refer to the most current IB for a comprehensive list of AEs.

11. ADVERSE EVENTS

The descriptions and grading scales found in the NCI CTCAE v4.03 will be utilized for AE assessment throughout the life of the protocol. A copy of the CTCAE v4.03 criteria can be downloaded from the CTEP website at http://ctep.cancer.gov. All forms for AE/SAE recording and reporting can be found in the Study Procedure Manual or in the EDC system (Documents and Information Tab).

11.1 Definitions

11.1.1 Adverse Event (AE)

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

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

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

Hospitalization for elective surgery or routine clinical procedures that are not the result of an AE (e.g., surgical insertion of central line) should not be recorded as an AE.

Disease progression should not be recorded as an AE, unless it is attributable to the study regimen by the site investigator.

11.1.2 Serious Adverse Event (SAE)

A SAE is an adverse event that:

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

medical, surgical) to prevent one of the other serious outcomes listed in the definition above). Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions not resulting in hospitalization; or the development of drug dependency or drug abuse.

11.1.3 Unexpected Adverse Event

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

11.1.4 Relatedness

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

Unrelated	Adverse Event is ***not related*** to the study drug(s)
Unlikely	Adverse Event is ***doubtfully related*** to the study drug(s)
Possible	Adverse Event ***may be related*** to the study drug(s)
Probable	Adverse Event is ***likely related*** to the study drug(s)
Definite	Adverse Event is ***clearly related*** to the study drug(s)

11.1.5 Pregnancy

If, following initiation of the study drugs, it is subsequently discovered that a subject is pregnant or may have been pregnant at the time of study drug exposure, including during at least 6 half lives after study drug administration, the study drug will be permanently discontinued in an appropriate manner (eg, dose tapering if necessary for subject safety).

The site investigator must immediately notify HCRN who will then notify Worldwide Safety @BMS of this event via the HCRN Pregnancy Form in accordance with SAE reporting procedures. Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the HCRN Pregnancy Form. Any pregnancy that occurs in a female partner of a male study participant should be reported to HCRN who will then report it to BMS. Information on this pregnancy will be collected on the HCRN Pregnancy Form.

11.1.6 Overdose

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

11.2 Reporting

11.2.1 Adverse Events

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

11.2.2 Serious Adverse Events (SAEs)

11.2.2.1 Site Requirements for Reporting SAEs to HCRN

- SAEs will be reported from time of signed informed consent until 100 days after discontinuation of study drug(s) or until a new anti-cancer treatment starts, whichever occurs first.
- SAEs will be reported on the SAE Submission Form **within 1 business day** of discovery of the event.
- SAEs include events related and unrelated to the study drug(s).
- All SAEs will be recorded in the subject's medical record and on the appropriate study specific eCRF form within EDC system.
- All SAEs regardless of relation to study drug will be followed until resolution to ≤ Grade 1 or baseline and/or deemed clinically insignificant and/or until a new anti-cancer treatment starts, whichever occurs first.

The site will submit the completed SAE Submission Form to HCRN **within 1 business day** of discovery of the event**.** The form may be submitted to HCRN electronically to safety@hoosiercancer.org. The site investigator is responsible for informing the IRB and/or other local regulatory bodies as per local requirements. The original copy of the SAE Submission Form and the email correspondence must be kept within the study file at the study site.

Once the SAE has resolved (see resolution guidelines listed in 11.2.2.1), sites must submit a follow-up SAE Submission Form within a reasonable timeframe to HCRN electronically to safety@hoosiercancer.org.

11.2.2.2 HCRN Requirements for Reporting SAEs to BMS

HCRN will report all SAEs to BMS **within 1 business day** of receipt of the SAE Submission Form from a site. Follow-up information will be provided to BMS as it is received from site. Contact information for sending SAE information to BMS below:

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

HCRN and the Sponsor-investigator will ensure that all SAEs in the clinical database are reported to BMS and any applicable health authority during the conduct of the study. This reconciliation will **occur at least quarterly** and be initiated by HCRN on behalf of the sponsor-investigator. HCRN will request on behalf of the sponsor-investigator a reconciliation report from: aepbusinessprocess@bms.com. During reconciliation, any events found to not be reported previously to BMS must be sent to Worldwide.Safety@BMS.com.

11.3 Sponsor-Investigator Responsibilities

HCRN will send a SAE summary to the sponsor-investigator **within 1 business day** of receipt of SAE Submission Form from a site. The sponsor-investigator will promptly review the SAE summary and assess for expectedness and relatedness.

11.4 HCRN Responsibilities to FDA

HCRN will manage the Investigational New Drug Application (IND) associated with this protocol on behalf of the sponsor-investigator. HCRN will cross-reference this submission to the BMS's parent IND at the time of submission. Additionally, HCRN will submit a copy of these documents to BMS at the time of submission to FDA.

HCRN will be responsible for all communication with the FDA in accordance with 21CFR312 including but not limited to the 7 and 15 Day Reports, as well as an Annual Progress Report. Additionally, HCRN will submit a copy of these reports to BMS at the time of submission to FDA.

11.5 IND Safety Reports Unrelated to this Trial

BMS will provide to HCRN IND safety reports from external studies that involve the study drug(s) per their guidelines. HCRN will forward safety reports to the sponsor-investigator who will review these reports and determine if revisions are needed to the protocol or consent. HCRN will forward these reports to participating sites **within 1 business day** of receiving the sponsor-investigator's review. Based on the sponsor-investigator's review, applicable changes will be made to the protocol and informed consent document (if required). All IND safety reports will also be made available to sites via the EDC system.

Upon receipt from HCRN, site investigators (or designees) are responsible for submitting these safety reports to their respective IRBs, as per their IRB policies.

12. STATISTICAL METHODS

12.1 Part A Primary Endpoint

The primary focus of this trial will be in the evaluation of PD-L1 to predict outcome and, also, secondarily to use the PRP model as a basis for outcome prediction. We expect that about 45% (or 54 expected) patients will have 0% PD-L1 and roughly 15% (or 18 expected) patients will express PD-L1 > 20%, which, given the sample size of 120 ccRCC patients provides 90% power using a 0.05 level two-sided test to test the hypothesis that PD-L1 >20% will be associated with significantly improved 1-year PFS rate for patients treated with PD-1 blockade (55% vs 13% taken as a binomial quantity). Considering the four groups of PD-L1 expression (0%, 1-5%, 5-20%, >20%), and with an expected patient allocation within groups of (45%, 25%, 15%, 15%),

the Cochran-Armitage test gives at least 90% power to detect a trend in 1-year PFS of (13%, 19%, 35%, 55%) using a two-sided 0.05 level test for trend. Modest changes in the proportions of patients in the variouse PD-L1 categories will not substantially affect the power. For example, if the distribution across PD-L1 categories is [25%, 25%, 25%, 25%] rather than the anticipated [45%, 25%, 15%, 15%] the power for the trend test remains greater than 90%.

As a secondary objective, we will apply the PRP model, developed in the DF/HCC Kidney SPORE Project 4, to assign each patient a probability of having PFS >1 year. The validity of the model will be assessed by the Brier score as well as via evaluation of the AUC. The exact distribution is unknown, but assuming an overall 1-year PFS of 28% and that 40% of patients are expected to be "positive" by the PRP model, 120 ccRCC patients give 90% power to detect a 29% higher 1-year PFS between 48 positive patients and 72 negative patients (via Fisher's exact test and assuming a difference in PFS of 46% vs 17%). All testing above will be done at 1-sided α=0.025. The specific distributional breakdowns are unknown but the power is relatively robust. For example, if the split between positive and negative is more even (50/50) then power for the above difference in 1-yr PFS remains over 90%. If the actual difference in 1-yr PFS is smaller than anticipated for example 25% (44% vs 19%) then the test will still have at least 80% power.

12.2 Part B

Clear cell patients who reach best response of SD at 48 weeks or who experience progression will be offered the opportunity to enroll in the second component of the study, Part B, continued treatment with the addition of ipilimumab. The primary endpoint of this portion of the trial will be objective response (CR or PR) taking as baseline tumor measurements at the patient's start of Part B. It is expected that roughly one-half of the Part A patients will be eligible for and choose to enroll in Part B, or roughly 60 total patients. A two-stage design will be used for this portion of the study where in the first cohort 29 patients will be accrued, treated and evaluated for response in order to provide guidance on whether to continue to enroll the entire cohort of 60 patients. The first stage analysis will be conducted without pausing accrual to Part B. If among the first 29 Part B patients there are at least 2 responses, there will be sufficient evidence to continue recruitment to Part B. With a true uninteresting response rate of 5%, there is a 57% chance of declaring the regimen uninteresting; however, with an interesting response rate of 20%, there is greater than 95% chance of continuing. If after enrollment of 60 patients there are at least 11 total responses, the lower limit on the 90% confidence interval for the true response rate will exclude 10% (ie, the 95% confidence interval will range from 10.6% to 28.5%, adjusted for the two-stage design). With 18 total responses, the 90% confidence interval on the true objective response proportion will exclude 20% (it, the confidence interval will range from 20.4% to 41.2%, adjusted for the two-stage design).

12.3 Non-clear cell (ncc) RCC: Part A

Subjects (comprising a heterogeneous group of histologies) will also be enrolled in the trial, at an accrual rate that is expected to be 33% that of clear cell patients. Therefore, an expected cohort of 40 nccRCC patients will be enrolled in the trial and analyzed separately for evidence of anti-tumor activity, principally focusing on overall response rate (CR or PR). With 40 patients, the 95% confidence interval on the true objective response proportion will be no wider than 32.4 percentage points and the probability of observing five or more responses under the hypothesis that the true response rate is 0.20 is at least 92%, hence there is high power to detect responses in

this cohort should the true response rate be interesting. Other endpoints of interest in this group of patients will include rates of SD and PFS at 1-year.

12.4 NccRCC patients: Part B

These subjects will also be available to enroll in Part B of the study. An estimated 20 patients will be enrolled and response rates in this resistant population will be reported. If no responses are seen in the first 14 patients that response rate will be deemed uninteresting. If any confirmed responses are seen accrual of ncc patients will continue for all eligible patients completing Part A.

12.5 Single Cell RNA seq Correlative (ccRCC)

At this time, 10 fresh frozen baseline tumor specimens and 3 pairs of specimens have been collected with an additional patient likely to be collected before accrual of the original 120 patients is complete. Therefore, fourteen additional patients are to be enrolled to collect fresh frozen specimens necessary to provide 25 baseline and 15 paired baseline and resistant fresh frozen samples to be used for single cell RNAseq experiments aimed at identifying factors associated to resistance as outlined in the DOD Translational Research Partnership grant to Drs. Atkins and Wu. Assuming 15 patients non-responsive to nivo and up to 10 patients responding, comparisons of any quantitative expression level using a 0.05 level two-sided two-group t-test will have 80% power to detect at least a 1.2 SD shift in means using baseline samples. With 15 paired samples at baseline and at confirmed radiologic progression among the non-responders, there is at least 80% power to detect a change in expression of 0.78 SD in any markers using a two-sided 0.05 level paired t-test. For two binary markers that are at least 50% discordant there is at least 80% power to detect a +/- versus -/+ discordance of at least 47% using a two-sided 0.05 level McNemar's test.

12.6 Study Design

This is a phase II open-labeled study or single agent nivolumab involving two distinct groups of systemic treatment naïve patients – those with either metastatic clear cell (cc) or non-clear cell (ncc) RCC. One hundred and twenty patients will be accrued to the ccRCC group and up to 40 to the nccRCC group. Eligible patients will be enrolled at time of documented progression or prolonged SD, into Part B of the study, which involves the addition of ipilimumab to nivolumab. Part B will constitute a separate open-labeled phase II study with baseline parameters being reset at time of subject enrollment. With multicenter accrual of 6-7 patients per month, the study should accrue in 2 years.

12.7 Endpoints

12.7.1 Definition of Primary Endpoint

- PFS is defined as the time from Day 1 of treatment until the criteria for disease progression is met as defined by RECIST 1.1 or death as a result of any cause.
- The primary focus of this trial will be in the evaluation of PD-L1 expression levels to predict outcome. We expect that about 45% (or 54 expected) patients will have 0% PD-L1 and roughly 15% (or 18 expected) patients will express PD-L1 > 20%, which, given the sample size of 120 ccRCC patients provides 90% power using a 0.05 level two-sided test to test the hypothesis that PD-L1 >20% will be associated with significantly

improved 1-year PFS rate for patients treated with PD-1 blockade (55% vs 13% taken as a binomial quantity).

12.7.2 Secondary Endpoints

- Apply the PRP model, developed in the DF/HCC Kidney SPORE Project 4, to assign each patient a probability of having PFS > 1 year.
- The endpoint for Part B (addition of ipilimumab) will be objective response (CR or PR) taking as baseline tumor measurements at the patient's start of Part B.
- An expected cohort of 40 nccRCC patients will be enrolled in the trial and analyzed separately for evidence of anti-tumor activity, principally focusing on overall response rate (CR or PR).
- Toxicity by CTCAE criteria of nivolumab monotherapy in treatment naïve patients with cc and nccRCC.

12.8 Analysis Datasets

The primary analysis for both Part A and Part B will be the enrolled population comprising all subjects who meet eligibility criteria and are registered onto the study.

12.9 Assessment of Safety

This will be performed in all subjects that contribute data to the safety analysis e.g any subject who receives at least one dose of treatment on Part A of this protocol. The safety analysis for Part B will be considered separately and will include any subject who receives at least one dose of treatment on Part B. Safety will be assessed using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), v4.03. In addition, Immune Related Adverse events IrAE will also be recorded.

12.10 Assessment of Efficacy

All subjects with ccRCC who are registered on study will be assessed for progression free survival in order to determine the PFS rate at 1 year. Those patients who cross over to Part B of the study and have measurable disease will be assessed for tumor response using RECIST 1.1 with the pre-Part B tumor measurements serving as baseline.

All subjects with nccRCC who are registered on study will be assessed for tumor response using traditional RECIST 1.1 criteria both for Part A and Part B (if they have measurable disease at time of crossover).

12.11 Interim Analysis/Criteria for Stopping Study

A two-stage design will be used for Part B for the ccRCC population. In this study the first cohort 29 patients will be accrued, treated and evaluated for response in order to provide guidance on whether to continue to enroll the entire cohort of 60 patients. The first stage analysis will be conducted without pausing accrual to Part B. If among the first 29 Part B patients there are at least 2 responses, there will be sufficient evidence to continue recruitment to Part B. With a true uninteresting response rate of 5%, there is a 57% chance of declaring the regimen uninteresting; however, with an interesting response rate of 20%, there is greater than 95% chance of continuing.

13. TRIAL MANAGEMENT

13.1 Data and Safety Monitoring Plan (DSMP)

The study will be conducted with guidance from the Georgetown-Lombardi Comprehensive Cancer Center's (Georgetown LCCC) DSMP.

HCRN oversight activities include:

- Review all adverse events requiring expedited reporting as defined in the protocol
- Provide trial accrual progress, safety information and data summary reports to the sponsor-investigator
- Submit data summary reports to the lead institution Data Safety Monitoring Committee per Georgetown LCCC's DSMP.
- Submit data summary reports to the DSMC for review per DSMC SOP

13.2 Georgetown-Lombardi Comprehensive Cancer Center's Data Safety Monitoring Committee

HCRN will provide the following for the Georgetown LCCC's DSMC to review:

- Adverse event summary report
- Monitoring reports and audit results if applicable
- Data related to stopping/decision rules described in study design
- Study accrual patterns
- Protocol deviations

The Georgetown LCCC DSMC will review study data every quarter. Documentation of DSMC reviews will be provided to sponsor-investigator and HCRN. Issues of immediate concern by the DSMC will be brought to the attention of the sponsor-investigator and other regulatory bodies as appropriate. The sponsor-investigator will work with HCRN to address the DSMC's concerns.

13.3 Data Quality Oversight Activities

Remote validation of the EDC system data will be completed on a continual basis throughout the life cycle of the study. Automated edit check listings will be used to generate queries in the EDC system and transmitted to the site to address in a timely fashion. Corrections will be made by the study site personnel.

Monitoring visits to the trial sites may be made periodically during the trial to ensure key aspects of the protocol are followed. For cause audits may be performed as necessary. During onsite monitoring visits, source documents will be reviewed for verification of agreement with data entered into the EDC system. It is important for the site investigator and their relevant personnel to be available for a sufficient amount of time during the monitoring visits or audit, if applicable. The site investigator and institution guarantee access to source documents by HCRN or its designee.

The trial site may also be subject to quality assurance audit, as well as inspection by appropriate regulatory agencies.

13.4 Compliance with Trial Registration and Results Posting Requirements

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

14. DATA HANDLING AND RECORD KEEPING

14.1 Data Management

HCRN will serve as the Clinical Research Organization for this trial. Data will be collected through a web based clinical research platform, a system compliant with Good Clinical Practices and Federal Rules and Regulations. HCRN personnel will coordinate and manage data for quality control assurance and integrity. All data will be collected and entered into the EDC system by study site personnel from participating institutions.

14.2 Case Report Forms and Submission

Generally, clinical data will be electronically captured in the EDC system and correlative results will be captured in the EDC system or other secure database(s). If procedures on the study calendar are performed for standard of care, at minimum, that data will be captured in the source document. Select standard of care data will also be captured in the EDC system, according to study-specific objectives.

The completed dataset is the sole property of the sponsor-investigator's institution and should not be exported to third parties, except for authorized representatives of appropriate Health/Regulatory Authorities, without permission from the sponsor-investigator and HCRN.

14.3 Record Retention

To enable evaluations and/or audits from Health Authorities/HCRN, the site investigator agrees to keep records, including the identity of all subjects (sufficient information to link records; e.g., hospital records), all original signed informed consent forms, copies of all source documents, and detailed records of drug disposition. All source documents are to remain in the subject's file and retained by the site investigator in compliance with the site contract with HCRN. No records will be destroyed until HCRN confirms destruction is permitted.

14.4 Confidentiality

There is a slight risk of loss of confidentiality of subject information. All records identifying the subjects will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. Information collected will be maintained on secure, password protected electronic systems. Paper files that contain personal information will be kept in locked and secure locations only accessible to the study site personnel.

Subjects will be informed in writing that some organizations including the sponsor-investigator and his/her research associates, HCRN, BMS, IRB, or government agencies, like the FDA, may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

If the results of the study are published, the subject's identity will remain confidential.

15 ETHICS

15.1 Institutional Review Board (IRB) Approval

The final study protocol and the final version of the informed consent form must be approved in writing by an IRB. The site investigator must submit written approval by the IRB to HCRN before he or she can enroll subjects into the study.

The site investigator is responsible for informing the IRB of any amendment to the protocol in accordance with local requirements. In addition, the IRB must approve all advertising used to recruit subjects for the study. The protocol must be re-approved by the IRB, as local regulations require.

Progress reports and notifications of serious and unexpected adverse events will be provided to the IRB according to local regulations and guidelines.

15.2 Ethical Conduct of the Study

The study will be performed in accordance with ethical principles originating from the Declaration of Helsinki. Conduct of the study will be in compliance with ICH Good Clinical Practice, and with all applicable federal (including 21 CFR parts 56 & 50), state, or local laws.

15.3 Informed Consent Process

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

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

16 REFERENCES

1. Znaor, A., et al., International variations and trends in renal cell carcinoma incidence and mortality. Eur Urol, 2015. 67(3): p. 519-30.
2. Society, A.C. Kidney Cancer (Adult) - Renal Cell Carcinoma. 2016 February 28, 2016]; Available from: http://www.cancer.org/cancer/kidneycancer/detailedguide/kidney-cancer-adult-key-statistics.
3. Gupta, K., et al., Epidemiologic and socioeconomic burden of metastatic renal cell carcinoma (mRCC): a literature review. Cancer Treat Rev, 2008. 34(3): p. 193-205.
4. Yao, M., et al., VHL tumor suppressor gene alterations associated with good prognosis in sporadic clear-cell renal carcinoma. Journal of the National Cancer Institute, 2002. 94(20): p. 1569-1575.
5. Oda, H. and R. Machinami, Sarcomatoid renal cell carcinoma. A study of its proliferative activity. Cancer, 1993. 71(7): p. 2292-2298.
6. Cancer Genome Atlas Research, N., et al., Comprehensive Molecular Characterization of Papillary Renal-Cell Carcinoma. N Engl J Med, 2016. 374(2): p. 135-45.
7. Sharpe, A.H., et al., The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. Nat Immunol, 2007. 8(3): p. 239-45.
8. Bristol-Myers Squibb Company, Nonclinical Study Report: Medarex Study No. MDX1106-025-R. In vitro characterization of a fully human anti-PD-1 monoclonal antibody. Document Control No. 930046580, 2006.
9. Bristol-Myers Squibb Company, Nonclinical Study Report: Medarex Study No. MDX1106-028-R. Binding and blocking characteristics of chimeric anti-mouse PD-1 antibody, 4H2. Document Control No. 930046578., 2007.
10. Velu, V., et al., Enhancing SIV-specific immunity in vivo by PD-1 blockade. Nature, 2009. 458(7235): p. 206-10.
11. Bristol-Myers Squibb Company, Nonclinical Study Report: Medarex Study No. MDX1106-032-R. Effects of anti-PD-1 administration on staged MC38 tumors in mice. Document Control No. 930046566., 2006.
12. Wang, C., et al., In vitro characterization of the anti-PD-1 antibody nivolumab, BMS-936558, and in vivo toxicology in non-human primates. Cancer Immunol Res, 2014. 2(9): p. 846-56.
13. Bristol-Myers Squibb Company, Nonclinical Study Report: Study No. DN12123; BMS-986016 and BMS-936558: Four-week intravenous combination toxicity study in monkeys with a 6-week recovery. Document Control No. 930070016, 2013.
14. Bristol-Myers Squibb Company, BMS-936558: Intravenous Study of pre- and postnatal development in cynomolgus monkeys with a 6-month postnatal evaluation. Final report for Study DN12001. . Document Control No. 930073964.
15. Habicht, A., et al., A Link between PDL1 and T Regulatory Cells in Fetomaternal Tolerance. The Journal of Immunology, 2007. 179(8): p. 5211-5219.
16. Brahmer, J.R., et al., Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol, 2010. 28(19): p. 3167-75.
17. Topalian, S.L., et al., Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med, 2012. 366(26): p. 2443-54.

18. McDermott, D.F., et al., Survival, durable response, and long-term safety in patients with previously treated advanced renal cell carcinoma receiving nivolumab. Journal of Clinical Oncology, 2015: p. JCO. 2014.58. 1041.
19. Motzer, R.J., et al., Nivolumab for Metastatic Renal Cell Carcinoma: Results of a Randomized Phase II Trial. J Clin Oncol, 2015. 33(13): p. 1430-7.
20. Motzer, R.J., et al., Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma. N Engl J Med, 2015. 373(19): p. 1803-13.
21. Choueiri, T.K., et al. Immunomodulatory activity of nivolumab in previously treated and untreated metastatic renal cell carcinoma (mRCC): Biomarker-based results from a randomized clinical trial. in ASCO Annual Meeting Proceedings. 2014.
22. Larkin, J., et al., Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. N Engl J Med, 2015. 373(1): p. 23-34.
23. Postow, M.A., et al., Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. N Engl J Med, 2015. 372(21): p. 2006-17.
24. Hammers, H., et al., 1050OPhase I study of nivolumab in combination with ipilimumab in metastatic renal cell carcinoma (MRCC). Annals of Oncology, 2014. 25(suppl 4): p. iv361-iv362.
25. Motzer R. J., et al, Nivolumab plus Ipilimumab versus Sunitinib in Advanced Renal-Cell Carcinoma. N Engl J Med. 2018 Apr 5;378(14):1277-1290. doi: 10.1056/NEJMoa1712126. Epub 2018 Mar 21.
26. Callea, M., et al., Differential Expression of PD-L1 between Primary and Metastatic Sites in Clear-Cell Renal Cell Carcinoma. Cancer Immunol Res, 2015. 3(10): p. 1158-64.
27. Choueiri, T.K., et al., PD-L1 expression in nonclear-cell renal cell carcinoma. Ann Oncol, 2014. 25(11): p. 2178-84.
28. Motzer, R.J., et al., Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med, 2007. 356(2): p. 115-24.
29. McDermott, D.F., et al., Randomized phase III trial of high-dose interleukin-2 versus subcutaneous interleukin-2 and interferon in patients with metastatic renal cell carcinoma. J Clin Oncol, 2005. 23(1): p. 133-41.
30. Keir, M.E., et al., PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol, 2008. 26: p. 677-704.
31. Keir, M.E., G.J. Freeman, and A.H. Sharpe, PD-1 regulates self-reactive CD8+ T cell responses to antigen in lymph nodes and tissues. J Immunol, 2007. 179(8): p. 5064-70.
32. Dong, H., et al., Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med, 2002. 8(8): p. 793-800.
33. Blank, C., et al., Blockade of PD-L1 (B7-H1) augments human tumor-specific T cell responses in vitro. Int J Cancer, 2006. 119(2): p. 317-27.
34. Ahmadzadeh, M., et al., Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. Blood, 2009. 114(8): p. 1537-44.
35. Thompson, R.H., et al., Costimulatory B7-H1 in renal cell carcinoma patients: Indicator of tumor aggressiveness and potential therapeutic target. Proc Natl Acad Sci U S A, 2004. 101(49): p. 17174-9.
36. Brahmer, J.R., et al., Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med, 2012. 366(26): p. 2455-65.

37. Cho, D.C., et al. Clinical activity, safety, and biomarkers of MPDL3280A, an engineered PD-L1 antibody in patients with metastatic renal cell carcinoma (mRCC). in ASCO Annual Meeting Proceedings. 2013.
38. McDermott, D.F., et al., Clinical activity and safety of anti-PD-1 (BMS-936558, MDX-1106) in patients with previously treated metastatic renal cell carcinoma (mRCC). J Clin Oncol, 2012. 30(15 Suppl): p. 4505.
39. Callahan, M.K., et al. Peripheral and tumor immune correlates in patients with advanced melanoma treated with combination nivolumab (anti-PD-1, BMS-936558, ONO-4538) and ipilimumab. in ASCO Annual Meeting Proceedings. 2013.
40. Grosso, J., et al. Association of tumor PD-L1 expression and immune biomarkers with clinical activity in patients (pts) with advanced solid tumors treated with nivolumab (anti-PD-1; BMS-936558; ONO-4538). in ASCO Annual Meeting Proceedings. 2013.
41. Kim, J.M. and D.S. Chen, Immune escape to PD-L1/PD-1 blockade: seven steps to success (or failure). Ann Oncol, 2016.
42. Serra-Bellver, P., S. Valpione, and P. Lorigan, Sequential immunotherapy regimens—expect the unexpected. The Lancet Oncology, 2016.
43. Simpson, T.R., et al., Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma. J Exp Med, 2013. 210(9): p. 1695-710.
44. Hamid, O., et al., Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med, 2013. 369(2): p. 134-44.
45. Gandhi, L., et al., Abstract CT105: MK-3475 (anti-PD-1 monoclonal antibody) for non-small cell lung cancer (NSCLC): Antitumor activity and association with tumor PD-L1 expression. Cancer Research, 2014. 74(19 Supplement): p. CT105-CT105.
46. Wolchok, J.D., et al., Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med, 2013. 369(2): p. 122-33.
47. Callea, M., et al. PD-L1 expression in primary clear cell renal cell carcinomas (ccRCCs) and their metastases. in ASCO Annual Meeting Proceedings. 2014.
48. Choueiri, T.K., et al., Immunomodulatory Activity of Nivolumab in Metastatic Renal Cell Carcinoma. Clin Cancer Res, 2016.
49. Kondo, T., et al., Favorable prognosis of renal cell carcinoma with increased expression of chemokines associated with a Th1-type immune response. Cancer Sci, 2006. 97(8): p. 780-6.
50. Ji, R.R., et al., An immune-active tumor microenvironment favors clinical response to ipilimumab. Cancer Immunol Immunother, 2012. 61(7): p. 1019-31.
51. Fritsch, E.F., et al., HLA-binding properties of tumor neoepitopes in humans. Cancer Immunol Res, 2014. 2(6): p. 522-9.
52. Rajasagi, M., et al., Systematic identification of personal tumor-specific neoantigens in chronic lymphocytic leukemia. Blood, 2014. 124(3): p. 453-62.
53. Snyder, A., et al., Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med, 2014. 371(23): p. 2189-99.
54. Van Allen, E.M., et al., Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. Science, 2015. 350(6257): p. 207-11.
55. Le, D.T., et al., PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med, 2015. 372(26): p. 2509-20.
56. Rizvi, N.A., et al., Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science, 2015. 348(6230): p. 124-8.

57. Rosenberg, J.E., et al., Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet, 2016. 387(10031): p. 1909-20.
58. McDermott, D.F., et al., Atezolizumab, an Anti-Programmed Death-Ligand 1 Antibody, in Metastatic Renal Cell Carcinoma: Long-Term Safety, Clinical Activity, and Immune Correlates From a Phase Ia Study. J Clin Oncol, 2016. 34(8): p. 833-42.
59. Seliger, B., Molecular mechanisms of MHC class I abnormalities and APM components in human tumors. Cancer Immunol Immunother, 2008. 57(11): p. 1719-26.
60. Campoli, M. and S. Ferrone, HLA antigen changes in malignant cells: epigenetic mechanisms and biologic significance. Oncogene, 2008. 27(45): p. 5869-85.
61. Garrido, F., T. Cabrera, and N. Aptsiauri, "Hard" and "soft" lesions underlying the HLA class I alterations in cancer cells: implications for immunotherapy. Int J Cancer, 2010. 127(2): p. 249-56.
62. Sakuishi, K., et al., Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. J Exp Med, 2010. 207(10): p. 2187-94.
63. Woo, S.R., et al., Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. Cancer Res, 2012. 72(4): p. 917-27.
64. Goding, S.R., et al., Restoring immune function of tumor-specific CD4+ T cells during recurrence of melanoma. J Immunol, 2013. 190(9): p. 4899-909.
65. Jing, W., et al., Combined immune checkpoint protein blockade and low dose whole body irradiation as immunotherapy for myeloma. J Immunother Cancer, 2015. 3(1): p. 2.
66. Bu, X., K.M. Mahoney, and G.J. Freeman, Learning from PD-1 Resistance: New Combination Strategies. Trends Mol Med, 2016. 22(6): p. 448-51.
67. Gerlinger, M., et al., Genomic architecture and evolution of clear cell renal cell carcinomas defined by multiregion sequencing. Nat Genet, 2014. 46(3): p. 225-33.
68. Hugo, W., et al., Genomic and Transcriptomic Features of Response to Anti-PD-1 Therapy in Metastatic Melanoma. Cell, 2016. 165(1): p. 35-44.
69. Stadler, Z.K., et al., Reliable Detection of Mismatch Repair Deficiency in Colorectal Cancers Using Mutational Load in Next-Generation Sequencing Panels. J Clin Oncol, 2016.
70. Van Allen - Genomic correlates of CTLAi response in melanoma (Science 2015).pdf.
71. Cancer Genome Atlas Research, N., Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature, 2013. 499(7456): p. 43-9.

17. APPENDIX A

GI Adverse Event Management Algorithm

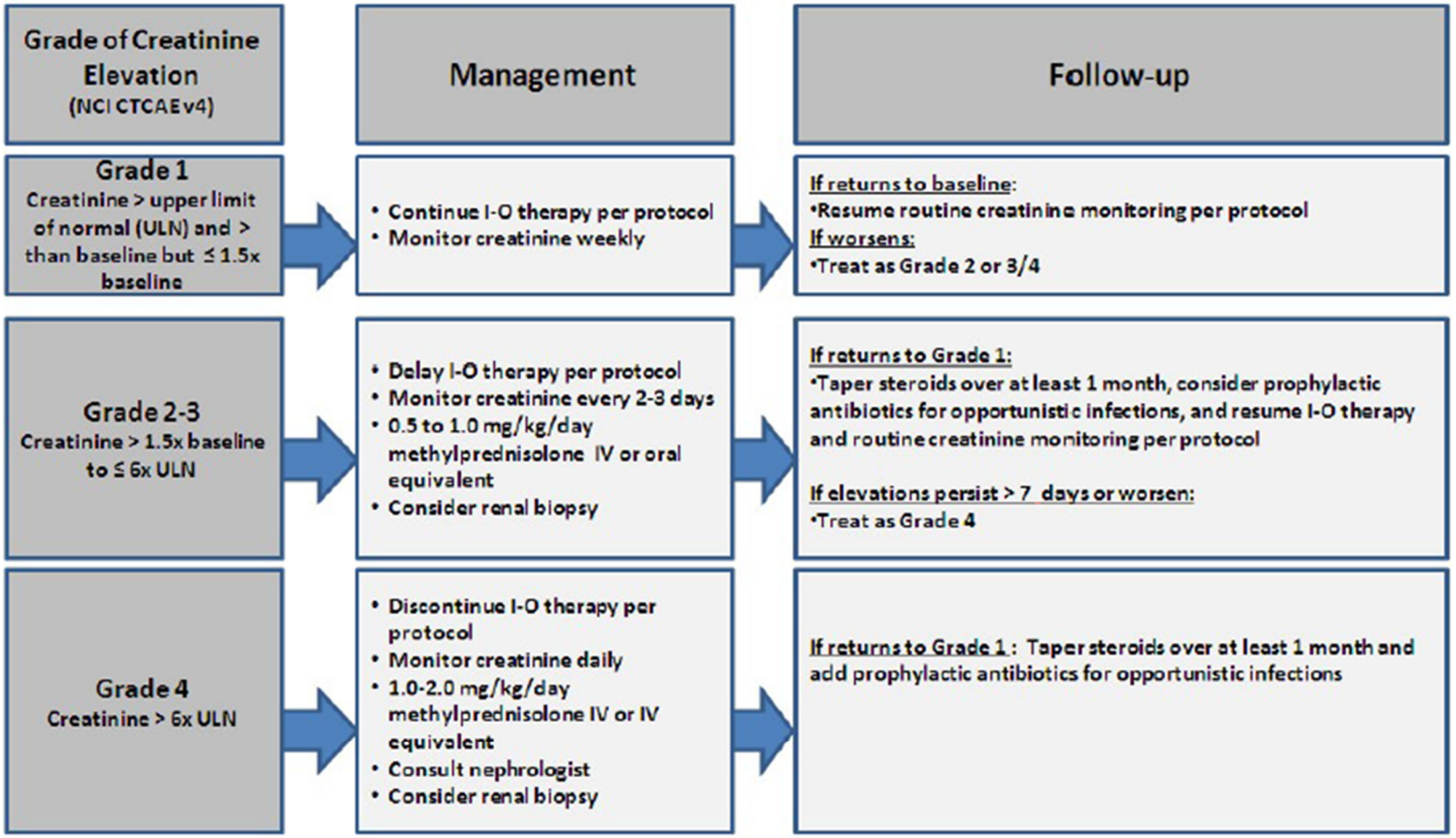
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.

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

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

Renal Adverse Event Management Algorithm

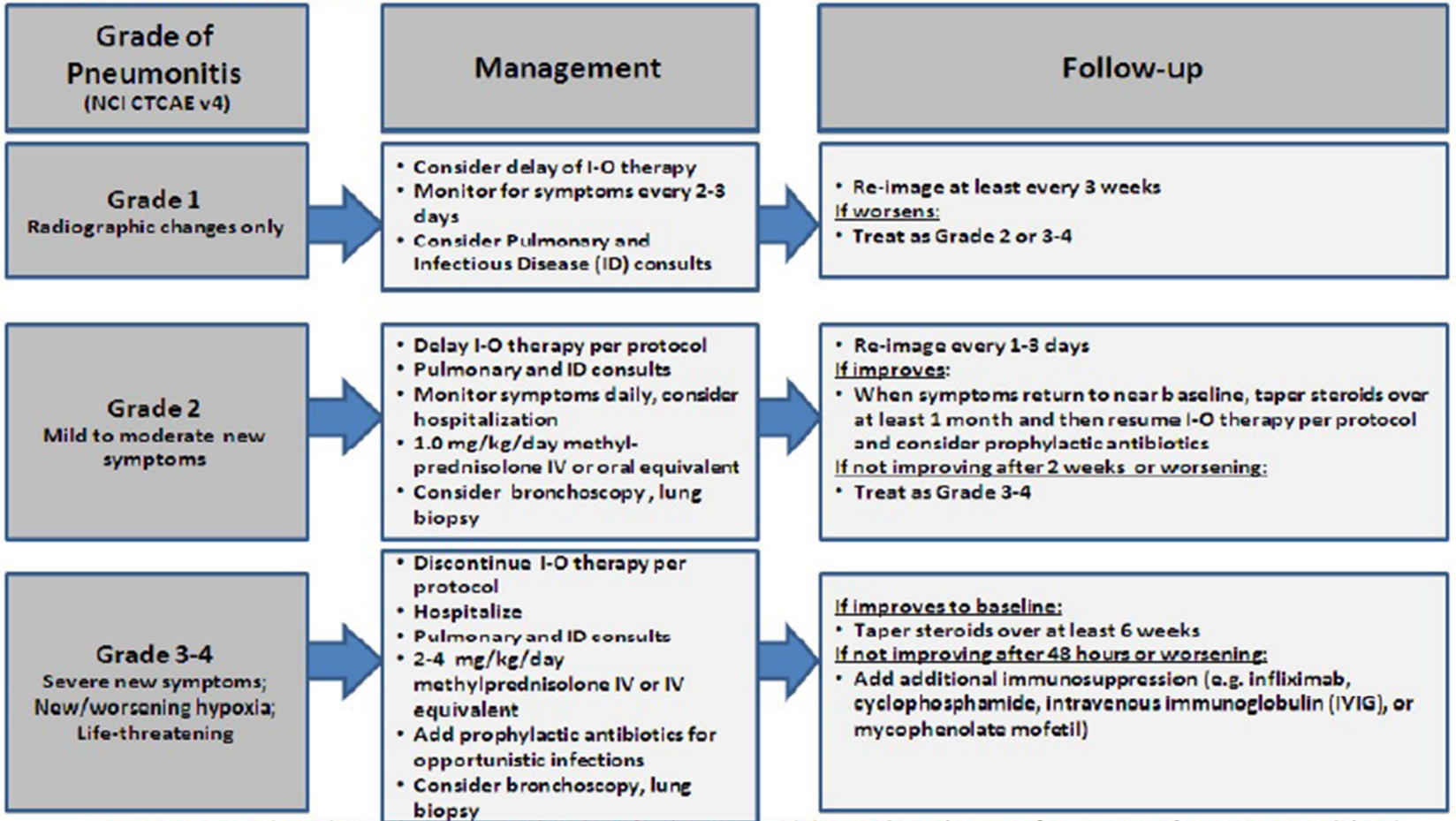
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy



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

Pulmonary Adverse Event Management Algorithm

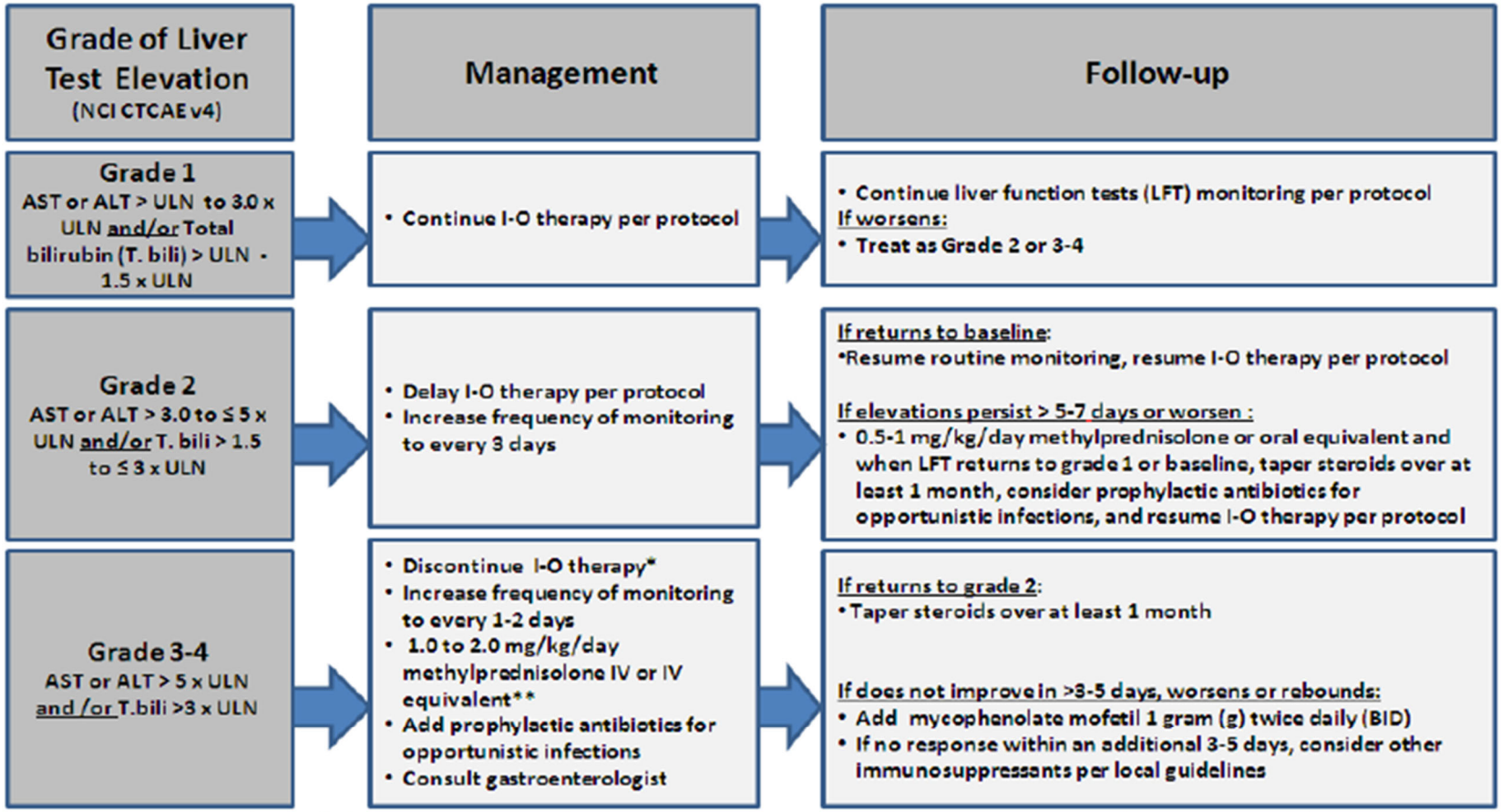
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.



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.



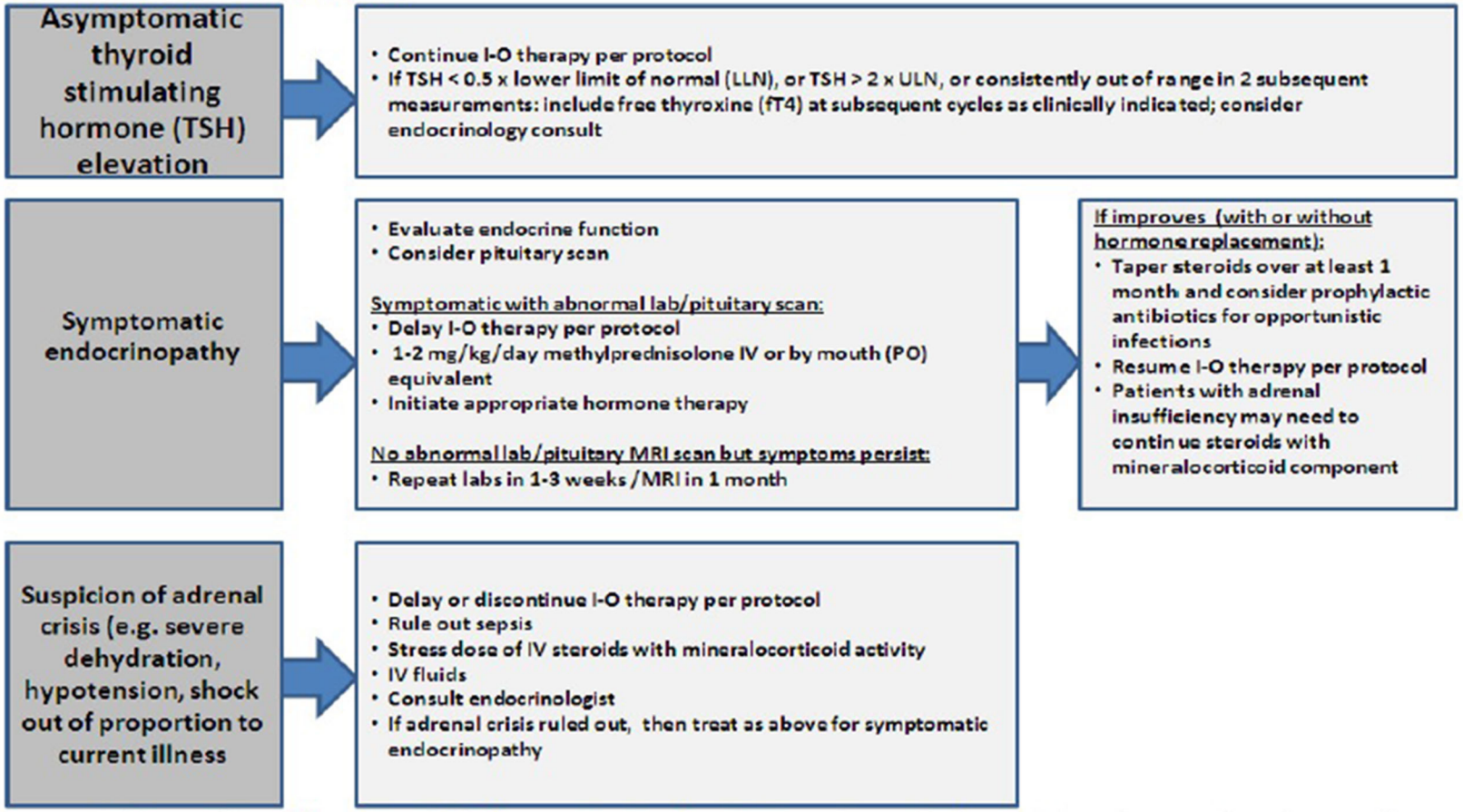
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

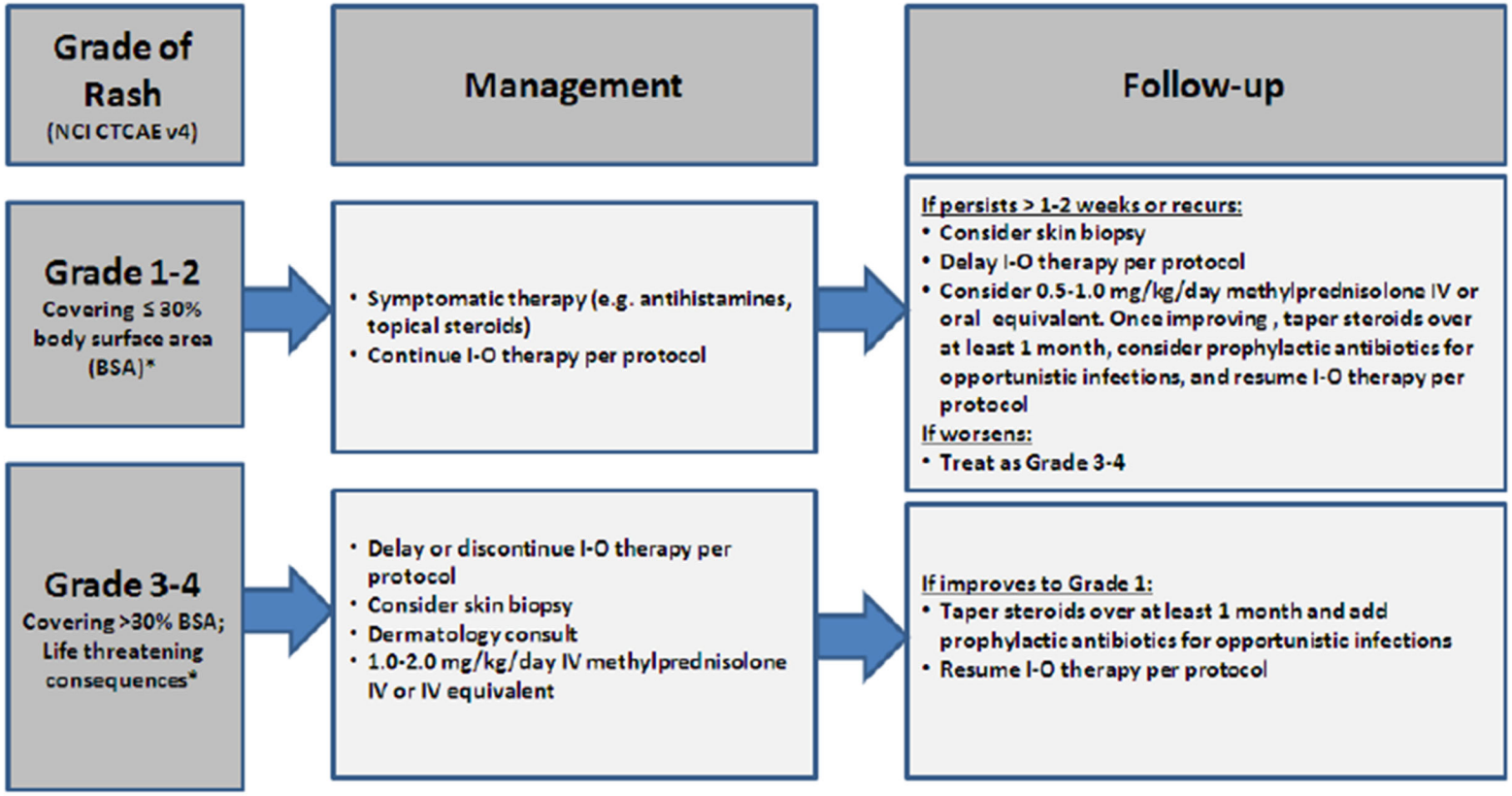
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider visual field testing, endocrinology consultation, and imaging.



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

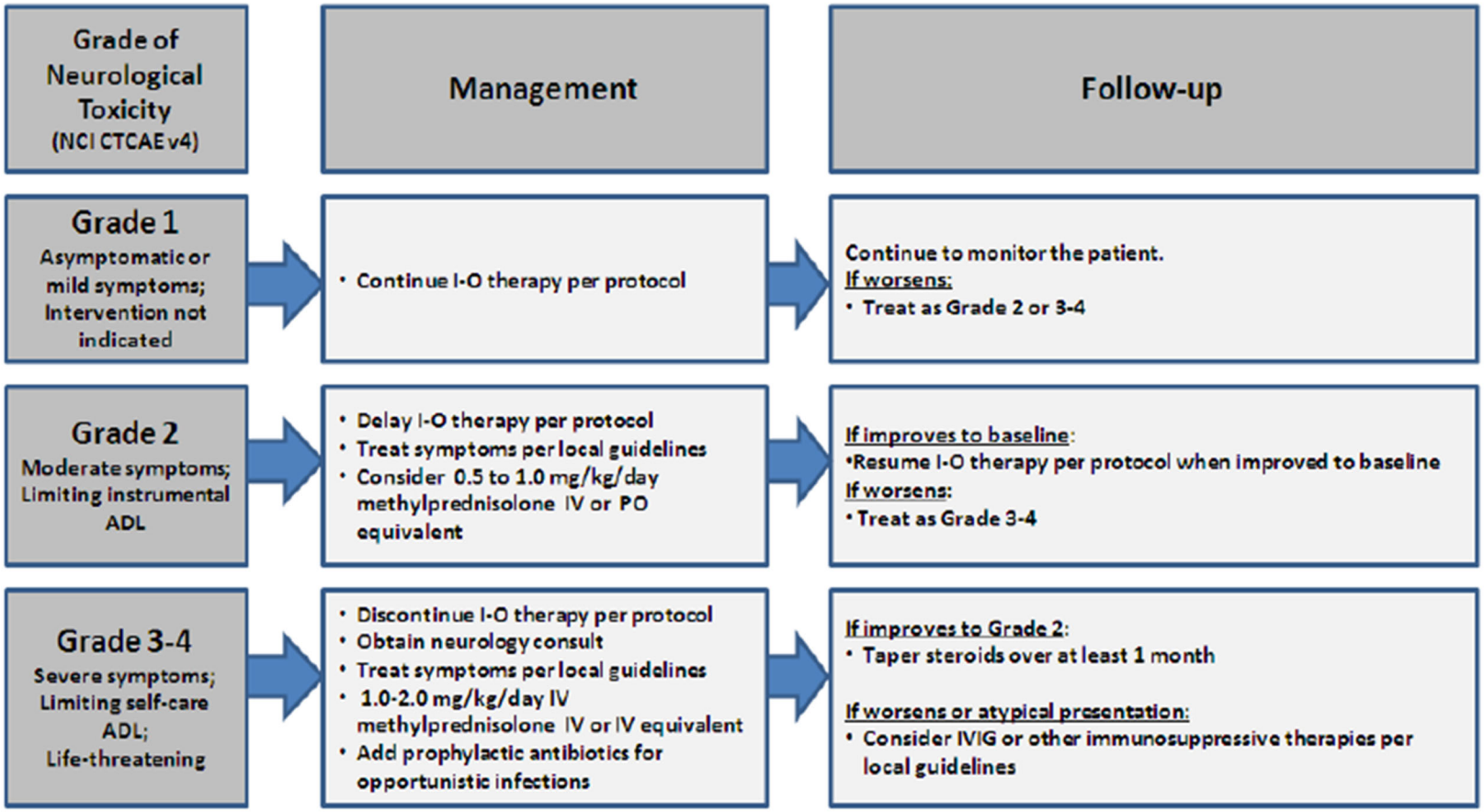
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



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.

Myocarditis Adverse Event Management Algorithm

Grade of Myocarditis (NCI CTCAE v4)	Management	Follow-up

Grade 2
Symptoms with mild to moderate activity or exertion
	• Delay I-O therapy; hospitalization with cardiac monitoring
	• Urgent cardiology consultation for evaluation and management
	  ○ Troponin and BNP
	  ○ ECG ± continuous cardiac monitoring
	  ○ Echocardiogram
	  ○ Cardiac MRI
	• Prompt initiation of 2 mg/kg/day methylprednisolone IV or equivalent
		• If worsens, intensify treatment according to grade
		• Upon recovery, taper steroids over at least 1 month with close monitoring of troponin and BNP as well as for new symptoms
		• Repeat cardiac MRI for post treatment assessment and cardiology follow-up
		• Retreatment may be considered after recovery and completion of steroid taper

Grade 3: Severe with symptoms at rest or with minimal activity or exertion; intervention indicated
 	
Grade 4: Life-threatening consequences; urgent intervention indicated (e.g., continuous IV therapy or mechanical hemodynamic support)
	• Permanently discontinue I-O therapy
	• Hospitalize to intensive cardiac monitoring
	• Cardiac evaluation to include:
	  – Troponin and BNP monitoring
	  – ECG ± continuous cardiac monitoring
	  – Echocardiogram
	  – Cardiac MRI
	  – Myocardial biopsy if feasible
	• Immediate initiation of 2 mg/kg/day methylprednisolone IV or 1 g IV bolus
	• Consider adding a second immunosuppressive agent
	Additionally, for Grade 4:
	• Hospitalize/transfer to institution with expertise in intensive cardiac monitoring
	• Consider ATG as second agent given its immediate effect
		• If no improvement, consider additional immunosuppression
		• Upon recovery, taper steroids over at least 1 month with close monitoring of troponin and BNP as well as for new symptoms
		• Repeat cardiac MRI for post treatment assessments and cardiology follow-up

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
Prophylactic antibiotics should be considered in the setting of ongoing immunosuppression.
ATG = anti-thymocyte globulin; BNP = B-type natriuretic peptide; ECG = electrocardiogram; IV = intravenous; MRI = magnetic resonance imaging