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EA8143

This is an FDA Registration Study

A **P**hase 3 **R**and**O**mized **S**tudy Comparing **PER**ioperative Nivolumab vs. Observation in Patients with **R**enal **C**ell **C**arcinoma Undergoing Nephrectomy (PROSPER RCC)

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ACTIVATION DATE

February 2, 2017

Addendum #1 – Incorporated Prior to Activation
Update #1 – Incorporated Prior to Activation
Addendum #2
Addendum #3
Addendum #4
Addendum #5
Addendum #6
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Agents	IND#	NSC#	Supply
Nivolumab		748726	DCTD, NCI-Supplied

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Rev. Add2

CANCER TRIALS SUPPORT UNIT (CTSU) ADDRESS AND CONTACT INFORMATION

To submit site registration documents:	**For patient enrollments:**	**Submit study data**
Regulatory documentation must be submitted to the CTSU via the Regulatory Submission Portal: Regulatory Submission Portal (Sign in at www.ctsu.org, and select the Regulatory Submission sub-tab under the Regulatory tab.) Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 to receive further instruction and support. Contact the CTSU Regulatory Help Desk at 1-866-651-2878 for regulatory assistance.	Please refer to the patient enrollment section of the protocol for instructions on using the Oncology Patient Enrollment Network (OPEN) which can be accessed at https://www.ctsu.org/OPEN_SYSTEM/ or https://OPEN.ctsu.org. Contact the CTSU Help Desk with any OPEN-related questions at ctsucontact@westat.com.	Data collection for this study will be done through Medidata Rave. Please see the data submission section of the protocol for further instructions. Do not submit study data or forms to CTSU Data Operations. Do not copy the CTSU on data submissions.
The most current version of the **study protocol and all supporting documents** must be downloaded from the protocol-specific Web page of the CTSU Member Web site located at https://www.ctsu.org. Access to the CTSU members' website is managed through the Cancer Therapy and Evaluation Program - Identity and Access Management (CTEP-IAM) registration system and requires user log on with CTEP-IAM username and password.		
For clinical questions (i.e., patient eligibility or treatment-related) Contact the Study PI of the Coordinating Group.		
For non-clinical questions (i.e., unrelated to patient eligibility, treatment, or data submission) contact the CTSU Help Desk by phone or e-mail: CTSU General Information Line – 1-888-823-5923, or ctsucontact@westat.com. All calls and correspondence will be triaged to the appropriate CTSU representative.		
The CTSU Web site is located at https://www.ctsu.org		

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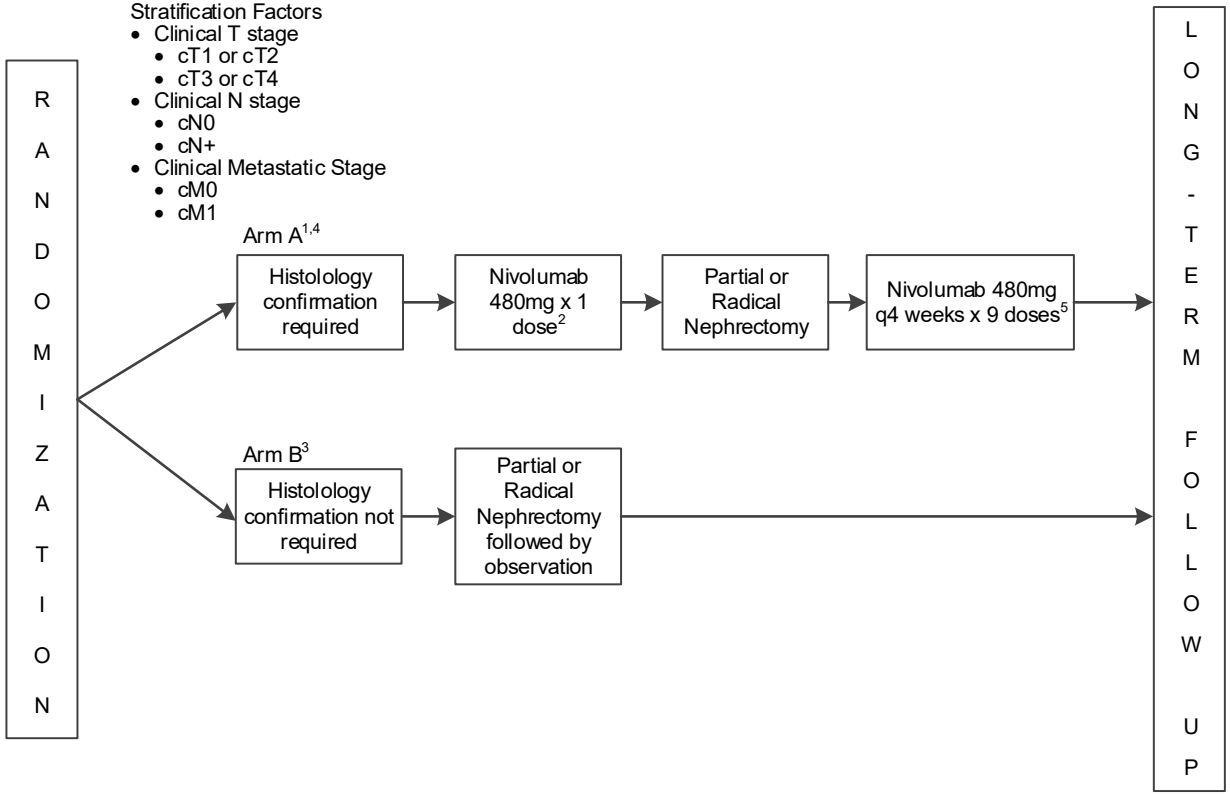
Schema



Accrual Goal = 805 patients

1. Patients randomized to Arm A must either have RCC confirmed by a standard of care biopsy in 12 months prior to randomization or if a biopsy was not already performed, a research biopsy must be performed after randomization to Arm A. RCC confirmation by biopsy is required in order to avoid exposing patients to neoadjuvant nivolumab who clearly have benign lesion or another type of cancer.
2. The neoadjuvant dose of nivolumab must be administered within 4 weeks (w 28 days) from randomization and prior to partial or radical nephrectomy.
3. Nephrectomy required in arm B within 8 weeks after randomization.
4. Nephrectomy required in arm A within 7 and 28 days from neoadjuvant dose.
5. First dose of adjuvant nivolumab should be within 4 to 10 weeks post-nephrectomy or last local treatment. See section 3.1.2 and 5.1.1.2.2.

1. Introduction

Rev. Add4

1.1 Background

More than 60,000 patients will be diagnosed with renal cell carcinoma (RCC) in 2015, and approximately 13,000 people will die from advanced disease.[1] With the increased use of imaging over the last several decades, the incidence of earlier stage and potentially more curable disease is rising. However despite nephrectomy, many patients recur and for them, subsequent cure is unlikely. Treatment options for advanced disease have significantly improved since 2005 with seven FDA approved targeted therapies that in sequence can improve overall survival (OS) compared to the interferon era. However, these multi-tyrosine kinase and mTOR inhibitors are generally cytostatic and durable complete responses are rare. Given the lack of cure with the available agents in the advanced setting, targeting microscopic disease in the perioperative setting is rational.

Despite resection of the primary tumor, approximately 30% of patients with localized disease and 65% of patients with nodal involvement relapse.[2] Currently there is no known effective adjuvant therapy for localized RCC after nephrectomy. At least eight trials have shown no benefit to adjuvant interferon- or interleukin-2-based therapies, or a CA-IX antibody. Trials are assessing the efficacy of the approved multi-targeted tyrosine kinase inhibitors in the adjuvant setting including sunitinib, sorafenib, pazopanib, and axitinib (accrual complete: S-Trac, SORCE, PROTECT; accrual ongoing: ATLAS) and the mTOR inhibitor everolimus (accrual ongoing: S0931, EVEREST). However, the first trial to report results, E2805 (ASSURE) revealed no benefit to 1 year of adjuvant sunitinib or sorafenib (Haas et al, Lancet, 2016).[45] The negative results of ASSURE provide even greater impetus to test an effective agent with a different mechanism of action in the perioperative setting.

Programmed Death-1 (PD-1) is a receptor expressed on activated T cells and a negative regulator of the immune response. Aberrant expression of PD-L1 by RCC tumor cells and tumor-infiltrating lymphocytes (TILs) may impair T cell function resulting in impaired host antitumor immunity.[3,4] PD-L1 may act by inhibiting both the cytokine production and the cytotoxic activity of PD-1+, tumor-infiltrating CD4+ and CD8+ T cells.[5] Higher expression of PD-L1 by tumor cells and TILs portends more aggressive pathology and tumor behavior as well as poorer survival.[3,6-8]

Multiple blocking antibodies against either the PD receptor or its ligands are being developed to interrupt this pathway. Nivolumab is a fully human IgG4 monoclonal antibody against PD-1 which has received FDA approval for advanced treatment refractory RCC.

Multiple phase 1 and 2 studies in patients with treatment refractory RCC revealed nivolumab's potential efficacy.[9,10] Its use in RCC was initially explored in two phase I refractory solid tumor trials, a phase 1 biomarker study, and a phase 2 dose ranging study. In the largest phase 1 experience, 34 RCC patients were treated with nivolumab at 1 or 10 mg/kg.[9,11] Median progression-free survival (PFS) was 7.3 months. Nearly 30% of patients experienced an objective response with median duration of response being 12.9 months. Another 27%

experienced disease stabilization > 24 weeks. Responses were generally rapid with a median time to response of 16 weeks. Notably, responses could occur after treatment cessation and persist off treatment. Median overall survival was encouraging at 22.4 months. These results were especially promising given that many of the patients were heavily pre-treated with 71% having had 2 or more lines of therapy.

Given the lack of a dose/toxicity relationship seen in the phase 1 studies, Motzer and investigators investigated the effect of dose on PFS in a randomized phase 2 study of 168 VEGF targeted therapy treatment refractory patients (NCT01354431).[10] No dose/PFS relationship was found, with median PFS ranging from 2.7 to 4.2 months at the 1, 3, and 10mg/kg doses (p=0.9). Nivolumab elicited objective responses in 20-22% and disease stabilization in 37-44%. Median time to achievement of an objective response was 2.8-3.0 months. Again the durability of responses was notable with a median duration of response of 22.3 months (4.8, NR) in the 10mg/kg arm and not yet reached in the two lower dose cohorts. Only 7% of patients required treatment discontinuation for drug related toxicity supporting the tolerability of this strategy. Median overall survival was favorable at 18.2-25.5 months. Fatigue was the most frequent toxicity (22-35%). No new toxicities were identified with 11% experiencing Grade 3-4 treatment-related events; none of which were due to pneumonitis. A parallel biomarker-focused trial using the same 3 dose levels was executed to explore predictors of response and identify mechanisms of resistance. (NCT01358721).[12] The results mirrored the efficacy and toxicity profile of the phase 2 study with an overall ORR of 17% (9-23%) and disease stabilization in another 32% in a mix of treatment refractory and naïve patients. At 24 weeks, 36% of patients were free from progression.

Recently, the phase 3 randomized study that compared nivolumab to everolimus in 821 patients who had VEGFR inhibitor-refractory disease demonstrated an overall survival advantage with nivolumab.[13] Nivolumab achieved a median OS of 25 months (95% CI: 21.8-NE) compared to 19.6 months (17.6-23.1) with everolimus; HR 0.73 (0.57-0.93, p=0.002). It also elicited a significantly higher objective response at 25% vs. 5% (p<0.001). There were 4 complete responses with nivolumab (1%) and 2 with everolimus. Median time to response was 3.5 months (range: 1.4-24.8 mo) and median duration of response was 12 months (0-27.6 mo). In the nivolumab responders, 48% had an ongoing response for 12 months or longer. Median PFS was similar between the two arms at 4.6 vs. 4.4 months (HR 0.88, p=0.11) highlighting that it may take more time for immunotherapy to engage the immune system and elicit tumor shrinkage or disease control. Post-hoc analysis of patients who had not progressed or died by 6 months: median PFS was 15.6 vs. 11.7 mo for everolimus (HR 0.64, 95% CI: 0.47-0.88). Notably, this is the first agent to demonstrate a clear survival benefit in patients with metastatic RCC across patients with MSKCC good, intermediate, and poor risk groups.

1.2 Rationale

Transitioning agents that have been previously found to be effective in advanced disease to an earlier disease setting is a common pathway for oncology drug development, and has been applied to multiple agents in several tumor types. This approach is especially relevant for higher risk RCC, where the relapse rate

is high and there are no drugs that have been shown to enhance the cure rate over surgery alone.

Nivolumab's efficacy is thought due to activation of anti-tumor PD-1 expressing T cells. Preliminary data from animal models suggest that the anti-tumor CD8 T cells that infiltrate tumors after PD-1 blockade may arise in the primary tumor, the surrounding microenvironment, and tumor-draining lymph nodes (Charles Drake, personal communication).[14] If true, nephrectomy may remove the antigen and the majority of these effector cells and cytokines that drive PD-1 and PD-L1 expression resulting in a less potent response to subsequent PD-1 blockade. The Campbell Laboratory has shown that RCC patients have elevated levels of peripheral circulating PD-1 cells prior to surgery which significant decreases after nephrectomy.[15] These observations provide our rationale to administer two priming doses of nivolumab prior to surgery with the goal of expanding the patient's endogenous anti-tumor PD-1 expressing cells. These effector cells can then traffic to other distant sites (e.g., spleen, lung, distant lymph nodes), escape surgical removal, and go on to potentially eradicate micrometastatic disease.

Further administration of nivolumab in the adjuvant setting will continue to reverse the inhibition of the anti-PD-1 immune cells and potentially kill residual microscopic disease. Administration in the setting of lower tumor burden may also enhance the efficacy of nivolumab. Recently, Joseph et al observed that smaller baseline tumor volume was prognostic for improved survival with the anti-PD-1 antibody pembrolizumab in melanoma (ASCO 2014, abstract 2015). Further, multiple immunosuppressive populations, such as MDSCs and Tregs, are likely to be decreased post-nephrectomy resulting in diminished inhibitory immune cell populations to counteract nivolumab-activated CD8 effector cells. In support of these general principles in RCC, two large phase 3 studies demonstrated a survival benefit to adding cytoreductive nephrectomy to interferon-a in the metastatic setting.[16]

Given the low rates of cure with the available agents once patients have evidence of metastatic disease, targeting micro-metastatic disease in the perioperative setting where the disease burden is smaller is logical. Using a survival-improving agent like nivolumab with a different mechanism of action than that of previously tested agents is clearly needed to improve outcomes. As the current standard of care after nephrectomy is surveillance, we will compare perioperative PD-1 blockade with nivolumab to an observation arm.

1.3 Safety of Nivolumab

As early stage patients are potentially cured with nephrectomy alone and are often asymptomatic, it is important that the adjuvant systemic therapy be tolerable, safe and directed to an appropriately high-risk population. In the phase I trials in refractory solid tumors, nivolumab was quite tolerable, and no maximally tolerated dose (MTD) was identified.[11,17] Only 5% of patients had to discontinue treatment due to an adverse event (AE). AEs were generally low grade and reversible. Fatigue, rash, diarrhea, and pruritus were the most frequently reported AEs. Grade 3 or 4 treatment-related AEs occurred in 14% of patients and 11% were serious AEs (SAEs). No relationship between dose and the spectrum, frequency, or severity of treatment-related AEs was observed. "Select" treatment-related AEs that were thought likely due to immune or inflammatory phenomenon have been reported and generally consisted of skin toxicity (e.g.,

rash, vitiligo), GI disorders (e.g., diarrhea, colitis, transaminitis), endocrinopathies (e.g, hypothyroidism), and pneumonitis. These events have generally been reversible with drug discontinuation, hormone replacement, steroids, or immunosuppressive agents. While pneumonitis is a concerning toxicity with this agent, it is also seen with relatively high incidence of up to 15-39% with the mTOR inhibitors.[18-21] With enhanced recognition, drug withdrawal, and early use of steroids, the incidence of fatal pneumonitis appears to have decreased since the initial phase 1 studies.

In the phase 3 study comparing nivolumab to everolimus, nivolumab again proved tolerable and safe in over 400 patients.[10] There were fewer treatment related AEs that led to treatment discontinuation (8 vs. 13%) and no treatment related deaths compared to 2 in the everolimus arm. The most common any grade nivolumab treatment-related AEs were fatigue (33%), nausea (14%), pruritus (14%), diarrhea (12%), decreased appetite (12%), and rash (10%). Grade 3 related events occurred in 18%. Only 1% of patients treated with nivolumab had Grade 4 events, which included fatigue (2%), nausea (< 1%), diarrhea (1%), decreased appetite (< 1%), rash (< 1%), anemia (2%), dyspnea (1%), pneumonitis (1%), and hyperglycemia (1%). More serious autoimmune toxicities such as pneumonitis, colitis, and hepatitis are rare and generally manageable with early recognition and multidisciplinary care but should be monitored.

Importantly, mean change in quality of life in the nivolumab group increased over time, which was significantly different than observed in the everolimus cohort ($p< 0.05$). Overall, the toxicity profile of nivolumab compares favorably to the approved TKIs and mTOR inhibitors, which could provide patients with a tolerability and quality of life advantage.

Nivolumab has been studied in over 8,600 subjects and is widely approved in multiple indications. Extensive details on the safety profile of nivolumab are available in the Investigator Brochure, and will not be repeated herein (ref IB).

Overall, the safety profile of nivolumab monotherapy as well as combination therapy is manageable and generally consistent across completed and ongoing clinical trials with no MTD reached at any dose tested up to 10 mg/kg. Most AEs were low-grade (Grade 1 to 2) with relatively few related high-grade (Grade 3 to 4) AEs. There was no pattern in the incidence, severity, or causality of AEs with respect to nivolumab dose level.

A pattern of immune-related adverse events has been defined, for which management algorithms have been developed; these are provided in Section 5.7. Most high-grade events were manageable with the use of corticosteroids or hormone replacement therapy (endocrinopathies) as instructed in these algorithms.

Safety concerns regarding delays to surgery or increased perioperative complications must be considered. Across the nivolumab trials in multiple solid tumors including a neoadjuvant lung study, there were no reported issues with wound healing or increased surgical complications in patients who underwent a variety of unplanned surgeries (BMS personal communication). Treatment-related toxicities can also adversely affect function and quality of life. Nivolumab is generally quite well-tolerated, however, fatigue, rash, pruritus and diarrhea can occur and should be quantified with respect to their impact on quality of life.

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1.4 Biomarker Exploration

Currently, there are no predictive biomarkers in RCC that can direct therapy selection. The neoadjuvant approach with a pre-therapy biopsy and serial serum collection will permit further characterization of the effects of nivolumab's PD-1 blockade on tumor tissue, T cell infiltrate, and other measures of the host immune response in patients that have pre-treatment biopsies.

Preoperative renal mass biopsy is increasingly being utilized and has a great safety record in contemporary analyses. Recent systematic review by the AHRQ reveals a low rate of major complications (< 1%, M. Allaf personal communication). A positive biopsy will increase the confidence of diagnosis and justifies the potential risks of neoadjuvant treatment in a potentially surgically curable patient population. Many institutions already have renal biopsy protocols in place where sampling is performed prior to surgery, and the practice is well accepted.

To minimize the risk of exposing patients with other cancers or clearly benign conditions to nivolumab and its potential adverse side effects, a biopsy ruling out these findings will be required in patients who are randomized to receive preoperative nivolumab (Arm A).

Additionally, the trial will permit further characterization of PD-L1 expression in RCC. The use of PD-L1 as a predictive biomarker is an area of active investigation requiring validation and development of accurate expression assays. As the phase 3 study of nivolumab in treatment-refractory RCC did not demonstrate that PD-L1 positivity correlated with overall survival, we will not stratify or require PD-L1 positivity for eligibility. We plan an exploratory analysis to evaluate whether primary tumor PD-L1 expression and a patient's immune profile correlates with better outcome. If proven, predictive biomarkers could be identified that will allow future individualization of treatment and spare patients futile toxicity.

1.5 Quality of Life Component

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1.5.1 Background and Rationale for Quality of Life (QOL) Study

Patients with no evidence of disease following nephrectomy are likely to be symptom free, after recovery from surgery. Symptomatic nivolumab induced adverse events identified in prior studies include fatigue, rash, diarrhea, pruritus, decreased appetite and nausea. An assessment of patient-reported symptom burden will aid in interpretation of results if a significant improvement in recurrence-free survival (RFS) is observed, particularly if the overall survival results are equivocal. These analyses will also provide information that can guide supportive care strategies if the treatment is found to be effective.

1.5.2 QOL Study Design

The approach to studying QOL is guided by a desire to capture treatment-related symptoms with fairly frequent assessments during the first year, followed by an assessment of what is expected to be emerging disease-related symptoms and functional burden at recurrence and 1 year post-treatment. Importantly, discontinuing study

drug for any reason (toxicity, progression, etc.) should NOT be a reason for terminating the QOL assessment. That is, regardless of treatment status, and including patients who go on to second-line therapy, scheduled assessments should be conducted to avoid bias induced by non-ignorable missing data over time. Scheduled assessments are noted in Section 5.9. Almost half of the patients are expected to have progressed by 5-years post baseline, providing a reasonable estimate of the impact of progression on QOL, which can in turn help inform the value of prolonging progression, if nivolumab is shown to do so.

1.5.3 Rationale for Quality of Life Measure Selection

The QOL assessments will be comprised of three instruments: The NCCN/FACT Kidney Symptom Index-19 (NFKSI-19), the PROMIS Physical Function 10-item short form (PROMIS PF-10) and a carefully selected subset of 9 questions from the PRO-CTCAE pool of items measuring patient-reported toxicity. Since this study involves patients entering treatment with few or no symptoms of disease, it will provide a unique opportunity to identify quality of life concerns attributable to treatment with nivolumab, among patients who start therapy without disease-related symptoms. In order to examine treatment-related symptoms during the first year, we will use selected items from the PRO-CTCAE (Basch et al 2014) and the NFKSI-19 (Rothrock et al, 2013).[44,40] The PRO-CTCAE items were selected based upon prior reports of the most common side effects of nivolumab. Selection of side effects from the PRO-CTCAE was guided by a procedure for selecting the more commonly expected side effects, as recommended Cella and Wagner (2015).[43] Drawing from the more than 100 PRO-CTCAE items available, we identified 9 questions, covering the following adverse events: Fatigue (item 1; 2 questions), decreased appetite (item 2; 2 questions), diarrhea (item 4; 1 question), nausea (item 5; 2 questions), itch (item 11; 1 question); rash (item 12; 1 question). In addition, we will use the 19-item NFKSI-19. This index provides a score for disease-related symptoms, bother with treatment side effects, and function/well-being. In addition to the selected PRO-CTCAE items, we note that the NFKSI-19 includes assessment of musculoskeletal pain (2 items), fatigue (2 items), dyspnea (1 item), cough (1 item) and fever (1 item). Similar to the descriptive approach proposed with the PRO-CTCAE items, these items can be reported individually as to their severity during the perioperative time period for nephrectomy on the experimental arm, and compared to the control arm, to aid evaluation of the possible additive effects of surgery and nivolumab. Finally, as a measure of overall burden of the combination of adverse events and disease response over time, we propose to use the PROMIS PF-10, which is a 10-item measure of physical function that has been validated for use in cancer. The PF-10 is scored on a T score metric, with mean=50 and SD=10; differences in the 4-6 point range are meaningful to patients (Yost et al).[41]

Because of its composition and demonstrated performance in prior research, we propose the NFKSI-19 as the basis for first-level PRO

comparisons, and the PROMIS PF-10 as a secondary evaluation of overall impact of disease and treatment upon functioning throughout the period of study. The single question on overall side effect bother, and the subscale measuring function/well-being, are expected to favor the nephrectomy-only arm in the first year. In the second year; however, we anticipate an advantage to the nivolumab arm with regard to the emergence of disease-related symptoms. Given the overlap of symptoms like fatigue as both a toxicity and a symptom of disease, attribution of its cause will be aided by noting the timing of onset relative to clinical events such as tumor response while on treatment, versus disease progression.

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1.6 Dose Justification

PPK analyses have shown that the PK of nivolumab is linear with proportional exposure over a dose range of 0.1 to 10 mg/kg, and no differences in PK across ethnicities and tumor types were observed. Nivolumab clearance and volume of distribution were found to increase as the body weight increases, but less than the proportional with increasing weight, indicating that mg/kg dosing represents an over-adjustment for the effect of body weight on nivolumab PK.

Nivolumab is safe and well tolerated up to 10 mg/kg Q2W. Adverse events have been broadly consistent across tumor types following monotherapy and have not demonstrated clear dose-response or exposure-response relationships. Additionally, the simulated median and 95th prediction interval of nivolumab summary exposures across body weight range (35 - 160 kg) are predicted to be maintained below the corresponding observed highest exposure experienced in nivolumab ie, 95th percentile following nivolumab 10 mg/kg Q2W from clinical study CA209003. Thus, while subjects in the lower body weight ranges would have slightly greater exposures than 80 kg subjects, the exposures are predicted to be within the range of observed exposures at doses (up to 10 mg/kg Q2W) used in the nivolumab clinical program, and are not considered to put subjects at increased risk.

The FDA recently approved nivolumab 480 mg to be given every 4 weeks (Q4W), which provides a more convenient dosing regimen for subjects. Based on PK modeling and simulations, administration of nivolumab 480 mg is predicted to provide Cavgss similar to 240 mg Q2W. While 480 mg Q4W is predicted to provide greater (approximately 20%) maximum steady state concentrations and lower (approximately 10%) steady state trough concentrations, these exposures are predicted to be within the exposure ranges observed at doses up to 10 mg/kg Q2W used in the nivolumab clinical program, and are not considered to put subjects at increased risk. Similar to the nivolumab 240 mg Q2W dosing regimen, the exposures predicted following administration of nivolumab 480 mg Q4W, are on the flat part of the exposure-response curves for previously investigated tumors, melanoma and NSCLC, and are not predicted to affect efficacy. Based on these exposure data, nivolumab 480 mg Q4W is expected to have similar efficacy and safety profiles to nivolumab 240 mg Q2W.

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1.7 Specific Hypothesis and Overarching Goal of the Study

When given in conjunction with radical or partial nephrectomy, patients who receive perioperative nivolumab (Arm A) will have increased recurrence-free

survival compared to those who undergo nephrectomy alone (Arm B). Ultimately, if proven successful in increasing recurrence-free survival or overall survival, the addition of an effective perioperative treatment for RCC would dramatically alter our current treatment paradigm from surveillance (+/- 1 year of adjuvant sunitinib in high risk clear cell patients) to an active and generally tolerable treatment that improves clinical outcomes.

2. Objectives

2.1 Primary Objectives

2.1.1 To compare recurrence-free survival (RFS) between patients with renal cell carcinoma randomly assigned to perioperative nivolumab in conjunction with radical or partial nephrectomy with patients randomized to surgery alone. (Rev Add5)

2.2 Secondary Objectives (Rev. Add4)

2.2.1 To evaluate for differences in recurrence-free survival associated with perioperative nivolumab compared to surgery alone among the subset of patients with clear cell histology.

2.2.2 To compare the overall survival between the two arms.

2.2.3 To describe the safety and tolerability of perioperative nivolumab

2.3 Correlative Objectives

2.3.1 To correlate the primary tumor's expression of PD-L1 with outcome.

2.3.2 To correlate the expression of PD-L1 on tumor tissue at nephrectomy and recurrence with outcome. (Rev. Add6)

2.3.3 To archive images for potential central confirmation of recurrence and for future correlative work with ACRIN, including markers predicting outcome or response.

2.3.4 To prospectively collect tumor and biologic specimens (e.g., serum, PBMCs) for future correlative studies.

2.3.5 To characterize the pharmacokinetics of nivolumab and explore exposure response relationships with respect to safety and efficacy.

2.3.6 To characterize the immunogenicity of nivolumab.

2.4 Quality of Life Objective

2.4.1 To evaluate differences in change from baseline in patient-reported symptoms and toxicities among patients randomized to treatment with nivolumab compared to surgery alone.

2.5 Other Exploratory Objectives (Rev. Add4)

2.5.1 To explore descriptively the efficacy of treatment with nivolumab in patients with non-clear cell (including unclassified) histologies.

2.5.2 To characterize the effects of nivolumab on bone metabolism and bone density. (Rev. Add6)

Rev. Add4

3. Selection of Patients

Each of the criteria in the checklist that follows must be met in order for a patient to be considered eligible for this study. Use the checklist to confirm a patient's eligibility. For each patient, this checklist must be photocopied, completed and maintained in the patient's chart.

In calculating days of tests and measurements, the day a test or measurement is done is considered Day 0. Therefore, if a test is done on a Monday, the Monday four weeks later would be considered Day 28.

ECOG-ACRIN Patient No. _____

Patient's Initials (L, F, M) _____

Physician Signature and Date _____

NOTE: CTEP Policy does not allow for the issuance of waivers to any protocol specified criteria (http://ctep.cancer.gov/protocolDevelopment/policies_deviations.htm). Therefore, all eligibility criteria listed in Section 3 must be met, without exception. The registration of individuals who do not meet all criteria listed in Section 3 can result in the participant being censored from the analysis of the study, and the citation of a major protocol violation during an audit. All questions regarding clarification of eligibility criteria must be directed to the Group's Executive Officer (EA.ExecOfficer@jimmy.harvard.edu) or the Group's Regulatory Officer (EA.RegOfficer@jimmy.harvard.edu).

NOTE: Institutions may use the eligibility checklist as source documentation if it has been reviewed, signed, and dated prior to randomization by the treating physician.

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NOTE: Renal Mass Biopsy in Arm A (Histologic proof required).

To avoid exposing patients to neoadjuvant nivolumab who do not have RCC, all patients randomized to Arm A must have histological confirmation of RCC.

Rev Add7

A standard of care diagnostic biopsy must have been completed within 12 months prior to randomization or the patient must agree to undergo a core biopsy if randomized to Arm A. If the biopsy clearly demonstrates a benign condition, oncocytoma, or a different type of cancer that is not RCC, the patient will come off study. All other outcomes, including results that are ambiguous or inconclusive ("non-diagnostic"), will be considered a good faith effort and the patient can continue on the study without a repeat biopsy if both the patient and treating investigator agree. Important: Patients randomized to Arm A must have their histology confirmed at the site level prior to receiving the neoadjuvant dose of nivolumab.

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3.1 Eligibility Criteria for Randomization

Rev. Add6

_____ 3.1.1 Patient must have a renal mass consistent with a clinical stage ≥ T2Nx RCC or TanyN+ RCC for which radical or partial nephrectomy is planned.

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Stage: T_____ N_____ M _____

Rev Add5

_____ 3.1.2 Patient must have no clinical or radiological evidence of distant metastases (M0) unless the presumed M1 disease is planned to be resected/definitively treated (e.g., thermal ablation, stereotactic radiation) at the same time or up to 12 weeks after the date of the initial procedure such that the patient is considered "no evidence of disease" (M1 NED).

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_____ 3.1.2.1 Liver, bone, or brain metastases are <u>not</u> permitted.

_____ 3.1.2.2 No more than 3 metastases are permitted, and all must be able to be removed or definitively treated within 12 weeks of the primary tumor resection.

Rev. Add8

_____ 3.1.3 If histological confirmation of RCC has not been done within 12 months prior to randomization, patient must be willing to undergo a core biopsy for this purpose if randomized to Arm A.

NOTE: This histologic confirmation can be a (1) standard of care diagnostic biopsy or (2) a research biopsy or a planned metastasectomy. Tissue must be obtained with results available prior to the neoadjuvant dose.

Patients randomized to Arm A: core tumor biopsy must have demonstrated RCC of any histology, including sarcomatoid, unclassified, or "unknown histology" (if preoperative biopsy was uninformative) with exception below for non-diagnostic biopsies.

If the biopsy performed following randomization clearly demonstrates a benign condition, oncocytoma or a different type of cancer that is not RCC, the patient is not eligible and must come off study.

A non-diagnostic biopsy is considered a good faith effort and does not need to be repeated unless deemed clinically necessary by the treating investigator.

NOTE: Refer to Section 10.5 for biopsy reimbursement guidelines.

Rev Add5
Rev Add8

_____ 3.1.4 Patient must not have any prior systemic or local anti-cancer therapy for the current RCC.

Rev Add6

_____ 3.1.4.1 Patient must not have undergone a partial nephrectomy for the current RCC.

_____ 3.1.4.2 Patient must not have had a metastasectomy for the current RCC diagnosis unless performed to render patient NED (in addition to the planned nephrectomy) within 6 months prior to the current diagnosis.

Rev. Add10

_____ 3.1.4.3 Patient must not have received current or past antineoplastic systemic therapies for RCC: i.e., chemotherapy, hormonal therapy, immunotherapy, or standard or investigational agents for treatment of RCC.

_____ 3.1.4.4 Patient must not have received prior treatment with an anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, or anti-CTLA-4 antibody, or any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways.

NOTE: Please see Sections 5.2 and 5.3 for the lists of therapies that are prohibited and permitted for patients during treatment.

_____ 3.1.5 Patient must be ≥ 18 years of age. Because no dosing or adverse event data are currently available on the use of nivolumab therapy in patients < 18 years of age, children are excluded from this study.

_____ 3.1.6 Patient must have an ECOG Performance Status of 0 or 1

_____ 3.1.7 Patient must not have a prior history of RCC that was treated with curative intent within the past 5 years.

_____ 3.1.7.1 Patients with a prior RCC that was treated >5 years before, are eligible if the current tumor is consistent with a new primary in the opinion of the treating investigator.

_____ 3.1.7.2 Patients with bilateral synchronous RCCs are eligible if they can be resected or definitively treated at the same time or within a 12 week window from time of initial nephrectomy (partial or radical) or procedure and maintain adequate residual renal function. The patient is not eligible if both kidneys are to be completely removed and subsequent hemodialysis will be required.

- Permitted forms of local therapy for second tumor:
  - Partial or radical nephrectomy
  - If kidney tumor is ≤3cm: thermal ablation (e.g., radiofrequency ablation, cryoablation or stereotactic radiosurgery)

_____ 3.1.8 Patient cannot have concurrent malignancies, with the following exceptions:

- Adequately treated basal cell or squamous cell skin cancer
- In situ cervical cancer
- A history of superficial Ta urothelial cancer is permitted (as long as not currently undergoing treatment) whereas T1 or greater disease of any stage is excluded if <3 years from diagnosis. Concurrent persistent disease is not permitted.

Rev Add7

- Adequately treated Stage I or II cancer from which the patient is currently in complete remission
- Any other cancer and stage from which the patient has been disease-free for at least 3 years prior to the time of randomization and as long as they are not receiving any current treatment (e.g. adjuvant or maintenance systemic or local therapy).
- Concurrent low risk prostate cancer on active surveillance.

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_____ 3.1.9 Patient must not have active known or suspected autoimmune disease. The following autoimmune disorders are permitted: patients with vitiligo, type I diabetes mellitus, controlled/stable hypo or hyperthyroidism due to autoimmune or non-autoimmune conditions (hormone replacement is allowed), psoriasis not requiring systemic treatment, or other conditions not expected to recur.

_____ 3.1.10 Patient must not have any ongoing condition requiring systemic treatment with either corticosteroids (> 10 mg daily prednisone equivalent) or other immunosuppressive medications with the exceptions outlined below. Patient must not have received any treatment with other immunosuppressive agents within 14 days prior to the first dose of study drug with the following exceptions:

- Topical, ocular, intra-articular, intranasal, inhaled steroids and adrenal replacement steroid doses > 10 mg daily prednisone or the equivalent are permitted in the absence of active autoimmune disease.
- A brief (less than 3 weeks) course of corticosteroids (any amount) for prophylaxis (for example: contrast dye allergy) or for treatment of non-autoimmune conditions (for example: nausea, delayed-type hypersensitivity reaction caused by a contact allergen) is permitted.

_____ 3.1.11 Patient must not have uncontrolled adrenal insufficiency.

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_____ 3.1.12 Patient must not have known evidence of chronic active liver disease or evidence of acute or chronic Hepatitis B Virus (HBV) or Hepatitis C (HCV). HBV and HCV testing must be completed within 8 weeks prior to randomization (See sections 3.1.22.8 and 3.1.22.9).

NOTE: If the patient has been treated and cured, and the HCV RNA is undetectable, the patient is eligible for this study.

_____ 3.1.13 Patient must not have any serious intercurrent illness, including ongoing or active infection requiring parenteral antibiotics.

_____ 3.1.14 Patient must not have known evidence of HIV infection, since the effects of nivolumab on anti-retroviral therapy have not been studied. HIV testing is only required if past or current history is suspected.

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_____ 3.1.15 Patient must not have any known medical condition (e.g. a condition associated with uncontrolled diarrhea such as ulcerative colitis or acute diverticulitis) that, in the investigator's opinion, would increase the risk associated with study participation or interfere with the interpretation of safety results.

_____ 3.1.16 Patient must not have had any major surgery within 28 days prior to randomization.

_____ 3.1.17 Patient must not be currently enrolled in other clinical trials testing a therapeutic intervention.

_____ 3.1.18 Patient must not have any history of severe hypersensitivity to a monoclonal antibody.

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_____ 3.1.19 Patient must have the ability to understand and the willingness to sign a written informed consent document.

Rev. Add10 _____ 3.1.20 Patients must not be pregnant or breast-feeding, as the effects of nivolumab on the developing human fetus or in the nursing infant are unknown.

All patients of childbearing potential must have a blood test or urine study within 2 weeks prior to randomization to rule out pregnancy.

A patient of childbearing potential is defined as anyone, regardless of sexual orientation or whether they have undergone tubal ligation, who meets the following criteria: 1) has achieved menarche at somepoint 2) has not undergone a hysterectomy or bilateral oophorectomy; or 3) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).

Patient of child bearing potential? _____ (Yes or No)

Date of blood test or urine study: _____

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Rev. Add10 _____ 3.1.21 Patients must not expect to conceive or father children by using accepted and effective method(s) of contraception, as described in the Informed Consent Form (ICF) and in Appendix VIII, or by abstaining from sexual intercourse for the duration of their participation in the study. Patients of childbearing potential must use adequate methods to avoid pregnancy for 5 months after the last dose of nivolumab.

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_____ 3.1.22 Patient must have the following baseline laboratory values within 8 weeks prior to randomization:

_____ 3.1.22.1 White blood cells ≥ 2000/uL

WBC:_____ Date of test:_____

_____ 3.1.22.2 Absolute Neutrophil Count (ANC) ≥ 1,500/mm^3

ANC:_____ Date of test:_____

_____ 3.1.22.3 Platelet Count ≥ 100,000/mm^3

Platelet count:_____ Date of test:_____

_____ 3.1.22.4 Hemoglobin ≥ 9.0g/dL

Hemoglobin:_____ Date of test:_____

Rev Add7 Transfusions permitted.

_____ 3.1.22.5 Serum creatinine ≤ 1.5 x upper limit of normal (ULN) or calculated creatinine clearance (CrCl) ≥ 40mL/min (CrCl= Wt (kg) x (140-age)*/72 x Cr. level, *female x 0.85)

Serum creatinine: _____ Date of test:_____
ULN:_____

CrCL:_____ Date of test:_____

_____ 3.1.22.6 Total Bilirubin ≤ 1.5 x ULN (except subjects with Gilbert Syndrome, who can have total bilirubin < 3.0 x ULN)

Total bilirubin:_____ Date of test:_____ ULN:_____

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Patient with Gilbert Syndrome?_____(Yes or No)

_____ 3.1.22.7 AST and ALT ≤ 2.5 x ULN

AST:_____ Date of test:_____ ULN:_____

ALT:_____ Date of test:_____ ULN:_____

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_____ 3.1.22.8 Hepatitis B Virus (HBV)*

Result: _____ Date of test: _____

_____3.1.22.9 Hepatitis C Virus (HCV)*

Result: _____ Date of test: _____

NOTE*: For patients that are positive for Hep B core antibody, hepatitis B surface antigen (HBsAg) must be negative. For patients that are positive for Hep C antibody, polymerase chain reaction (PCR) must be negative.

_____ _____

Physician Signature Date

OPTIONAL: This signature line is provided for use by institutions wishing to use the eligibility checklist as source documentation.

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4. Registration and Randomization Procedures

CTEP Investigator Registration Procedures

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account (https://ctepcore.nci.nih.gov/iam). In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) (i.e., clinical site staff requiring write access to OPEN, RAVE, or TRIAD or acting as a primary site contact) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) (https://ctepcore.nci.nih.gov/rcr).

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RCR utilizes five person registration types.

- IVR — MD, DO, or international equivalent;
- NPIVR — advanced practice providers (e.g., NP or PA) or graduate level researchers (e.g., PhD);
- AP — clinical site staff (e.g., RN or CRA) with data entry access to CTSU applications (e.g., Roster Update Management System (RUMS), OPEN, Rave,);
- Associate (A) — other clinical site staff involved in the conduct of NCI-sponsored trials; and
- Associate Basic (AB) — individuals (e.g., pharmaceutical company employees) with limited access to NCI-supported systems.

Documentation requirements per registration type are outlined in the table below.

Documentation Required	IVR	NPIVR	AP	A	AB
FDA Form 1572	✔	✔			
Financial Disclosure Form	✔	✔	✔		
NCI Biosketch (education, training, employment, license, and certification)	✔	✔	✔		
HSP/GCP training	✔	✔	✔		
Agent Shipment Form (if applicable)	✔				
CV (optional)	✔	✔	✔		

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and IRBs covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Added to a site roster
- Assigned the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN)
- Act as the site-protocol PI on the IRB approval
- Assigned the Clinical Investigator (CI) role on the Delegation of Tasks Log (DTL).

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In addition, all investigators act as the Site-Protocol PI, consenting/treating/drug shipment, or as the CI on the DTL must be rostered at the enrolling site with a participating organization (i.e., Alliance). Additional information can be found at the CTEP website at <https://ctep.cancer.gov/investigatorResources/default.htm>. For questions, please contact the RCR Help Desk by email at <RCRHelpDesk@nih.gov>.

CTSU Registration Procedures

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

IRB Approval:

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For CTEP and Division of Cancer Prevention (DCP) studies open to the National Clinical Trials Network (NCTN) and NCI Community Oncology Research Program (NCORP) Research Bases after March 1, 2019, all U.S.-based sites must be members of the NCI Central Institutional Review Board (NCI CIRB). In addition, U.S.-based sites must accept the NCI CIRB review to activate new studies at the site after March 1, 2019. Local IRB review will continue to be accepted for studies that are not reviewed by the CIRB, or if the study was previously open at the site under the local IRB. International sites should continue to submit Research Ethics Board (REB) approval to the CTSU Regulatory Office following country-specific regulations.

Sites participating on the NCI CIRB initiative that are approved by the CIRB for this study are not required to submit IRB approval documentation to the CTSU Regulatory Office. For sites using the CIRB, IRB approval information is received from the CIRB and applied to the RSS in an automated process. Signatory Institutions must submit a Study Specific Worksheet for Local Context (SSW) to the CIRB via IRB Manager to indicate their intent to open the study locally. The CIRB's approval of the SSW is then communicated to the CTSU Regulatory Office. In order for the SSW approval to be processed, the Signatory Institution must inform the CTSU which CIRB-approved institutions aligned with the Signatory Institution are participating in the study

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Sites using their local IRB or REB, must submit their approval to the CTSU Regulatory Office using the Regulatory Submission Portal located in the Regulatory section of the CTSU website. Acceptable documentation of local IRB/REB approval includes:

- Local IRB documentation;
- IRB-signed CTSU IRB Certification Form; and/or
- Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form.

In addition, the Site-Protocol Principal Investigator (PI) (i.e. the investigator on the IRB/REB approval) must meet the following criteria to complete processing of the IRB/REB approval record:

- Holds an Active CTEP status;
- Rostered at the site on the IRB/REB approval (applies to US and Canadian sites only) and on at least one participating roster;
- If using NCI CIRB, rostered on the NCI CIRB Signatory record;
- Includes the IRB number of the IRB providing approval in the Form FDA 1572 in the RCR profile; and
- Holds the appropriate CTEP registration type for the protocol.

Additional Requirements

Additional requirements to obtain an approved site registration status include:

- An active Federal Wide Assurance (FWA) number;
- An active roster affiliation with the Lead Protocol Organization (LPO) or a Participating Organization (PO); and
- Compliance with all protocol-specific requirements (PSRs).

Downloading Site Registration Documents:

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Download the site registration forms from the protocol-specific page located on the CTSU members' website. Permission to view and download this protocol and its supporting documents is restricted based on person and site roster assignment. To participate, the institution and its associated investigators and staff must be associated with the LPO or a PO on the protocol.

- Log on to the CTSU members' website https://www.ctsu.org using your CTEP-IAM username and password
- Click on the Protocols in the upper left of your screen
- Either enter EA8143 in the search field at the top of the protocol tree, or
- Click on the By Lead Organization folder to expand
- Click on the ECOG-ACRIN name link to expand, then select trial protocol EA8143
- Click on Documents, select the Site Registration and download and complete the forms provided. (Note: For sites under the CIRB initiative, IRB data will load automatically to the CTSU as described above.)

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Requirements for EA8143 Site Registration:

- EA8143 Protocol Training (please see Section 4.3 for more details).

Submitting Regulatory Documents

Submit required forms and documents to the CTSU Regulatory Office via the Regulatory Submission Portal on the CTSU website,

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To access the Regulatory Submission Portal log on to the CTSU members' website.

Regulatory → Regulatory Submission

Institutions with patients waiting that are unable to use the Regulatory Submission Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support.

Required Protocol Specific Regulatory Documents

1. Copy of IRB Informed Consent Document.

NOTE: Any deletion or substantive modification of information concerning risks or alternative procedures contained in the sample informed consent document must be justified in writing by the investigator and approved by the IRB.

2. A. CTSU IRB Certification Form.
   Or
   B. Signed HHS OMB No. 0990-0263 (replaces Form 310).
   Or
   C. IRB Approval Letter

3. Copies of the EA8143 specific regulatory documents, as outlined in the table below, are required for site registration. Please submit these documents per the frequency, regulatory reference, and instructions outlined in the table below.

NOTE: The above submissions must include the following details:

- Indicate all sites approved for the protocol under an assurance number.
  OHRP assurance number of reviewing IRB
  Full protocol title and number
  Version Date
  Type of review (full board vs. expedited)
  Date of review.
  Signature of IRB official

In addition to the annual CTEP Investigator Registration requirements, the following study specific documents are required to be submitted to CTSU Regulatory Office via the Regulatory Submissions Portal, where they will be entered and tracked in the CTSU RSS.

Regulatory Submission Portal: www.ctsu.org (members' area) → Regulatory Tab → Regulatory Submission.

Document	Frequency	Regulatory Reference	Instructions
Curriculum Vitae (CV) for principal investigator and all sub-investigators participating in the trial	• Study start–up	21 CFR 312.53(2) GCP: 4.1.1; 8.2.10; 8.3.5	• Label CV with EA8143
Study Specific Form FDA 1572	• Study start-up • Any time there is a change to any of the information originally provided	21 CFR 312.53(c)	• Instructions for completing form: http://www.fda.gov/downloads/aboutfda/reportsmanualsforms/forms/ucm223432.pdf • Blank form: https://www.fda.gov/media/71816/download
EA8143 Financial Disclosure Form** – documents Financial Interests and Arrangements of Clinical Investigators for principal investigator and all sub-investigators participating in the trial and listed on the FDA Form 1572	• Study start-up • Any time there is a financial status change • Any time a new investigator is added	21 CFR 54.4(b)).	• Blank form in Appendix IX:
EA8143 Institutional Review Board Chairman Collection Form	• Study start-up • Any time there is a change Chair oversight for the IRB of Record during the course of the trial* • Any time there is a change to the IRB of Record during the course of the trial*		• ***Not Required from sites utilizing the NCI CIRB as the IRB of Record for EA8143*** • Blank form located on the CTSU website at www.ctsu.org under 'Site Registration Documents' for EA8143

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* "During the course of the study" refers to the time from the date the clinical investigator enters a patient onto the study until the completion of the study. For the purposes of financial disclosure under part 54, completion of the study means that all study subjects have been enrolled and follow-up of primary endpoint data on all subjects has been completed in accordance with the protocol.

** The EA8143 Financial Disclosure Form is a required protocol specific regulatory document. This form is required independent of the Financial Disclosure Form that is required as part of the CTEP Investigator Registration Procedures.

Delegation of Task Log (DTL)

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Each site must complete a protocol-specific Delegation of Tasks Log (DTL) using the DTL application in the Delegation Log section on the CTSU members' website. The Clinical Investigator (CI) is required to review and electronically sign the DTL prior to the site receiving an Approved site registration status and enrolling patients to the study. To maintain an approved site registration status the CI must re-sign the DTL at least annually and when a new version of the DTL is released; and activate new task assignments requiring CI sign-off. Any individual at the enrolling site on a participating roster may initiate the site DTL. Once the DTL is submitted for CI approval, only the designated DTL Administrators or the CI may update the DTL. Instructions on completing the DTL are available in the Help Topics button in the DTL application and include a Master Task List, which describes DTL task assignments, CI signature, and CTEP registration requirements.

Checking Your Site's Registration Status:

You can verify your site registration status on the members' section of the CTSU website.

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- Go to https://www.ctsu.org Log on to the CTSU members' website; Click on the Regulatory at the top of your screen
- Click on the Site Registration
- Enter your 5-character CTEP Institution Code and click on Go

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NOTE: The status shown only reflects institutional compliance with site registration requirements as outlined above. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with the NCI or their affiliated networks.

Patient Enrollment

Patients must not start protocol treatment prior to randomization.

The Oncology Patient Enrollment Network (OPEN) is a web-based registration system available on a 24/7 basis. OPEN is integrated with CTSU regulatory and roster data and with the Lead Protocol Organization (LPOs) registration/randomization systems or Theradex Interactive Web Response System (IWRS) for retrieval of patient registration/randomization assignment. OPEN will populate the patient enrollment data in NCI's clinical data management system, Medidata Rave.

Requirements for OPEN access:

- A valid CTEP-IAM account;
- To perform enrollments or request slot reservations: Be on a LPO roster, ETCTN Corresponding roster, or PO roster with the role of Registrar. Registrars must hold a minimum of an AP registration type;
- If a Delegation of Tasks Log (DTL) is required for the study, the registrar(s) must hold the OPEN Registrar task on the DTL for the site; and
- Have an approved site registration for a protocol prior to patient enrollment.

To assign an Investigator (IVR) or Non-Physician Investigator (NPIVR) as the treating, crediting, consenting, drug shipment (IVR only), or receiving investigator for a patient transfer in OPEN, the IVR or NPIVR must list the IRB number used on the site's IRB approval on their Form FDA 1572 in RCR.

Prior to accessing OPEN, site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes.
- All patients have signed an appropriate consent form and HIPAA authorization form (if applicable).

NOTE: The OPEN system will provide the site with a printable confirmation of registration and treatment information. Please print this confirmation for your records.

Rev. Add7 Access OPEN at https://open.ctsu.org or from the OPEN link on the CTSU members' website. Further instructional information is provided on the OPEN tab of the CTSU members' side of the CTSU website at https://www.ctsu.org or at https://open.ctsu.org. For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

Rev. Add4 ## 4.1 Randomization

4.1.1 The following information will be captured at time of randomization:

4.1.1.1 Protocol Number

4.1.1.2 Investigator Identification

- Institution and affiliate name
- Investigator's name

4.1.1.3 Patient Identification

- Patient's initials (first and last)
- Patient's Hospital ID and/or Social Security number
- Patient demographics
  - Gender
  - Birth date (mm/yyyy)
  - Race
  - Ethnicity
  - Nine-digit ZIP code
  - Method of payment
  - Country of residence

Rev. Add7 ### 4.1.2 Eligibility Verification

Patients must meet all of the eligibility requirements listed in Section 3.1.

Rev. Add2 ### 4.1.3 Stratification Factors

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- cT1, cT2 or cT3, cT4
- cN0 or cN+
- cM0 or cM1

NOTE: Only oligometastatic disease is permitted and is defined as ≤3 metastases (see exclusions in the eligibility criteria) that can be resected or treated at the same time of nephrectomy or within a 12 week window.

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4.1.4 Additional Requirements

4.1.4.1 Patients must provide a signed and dated, written informed consent form.

NOTE: Copies of the consent are **not** collected by the ECOG-ACRIN Operations Office - Boston

RESEARCH BIOPSY: The establishment of research rates within the institution's financial office must be in place prior to the performance of the biopsy for confirmation of disease if considered non-standard of care. See Section 10.5 for guidelines.

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4.1.4.2 Pathological specimens must be submitted from all patients on both arms for central diagnostic review and classification and defined and/or undefined laboratory research studies as outlined in Section 10.

4.1.4.3 Peripheral blood, serum, and plasma are to be submitted for defined and/or undefined laboratory research studies per patient consent as outlined in Section 10.

4.1.4.4 Serum specimens must be submitted for pharmacokinetic and immunogenicity studies for Arm A patients only as outlined in Section 10.

4.1.4.5 Medidata Rave is a clinical data management system being used for data collection for this trial/study. Access to the trial in Rave is controlled through the CTEP-IAM system and role assignments. To access Rave via iMedidata, site staff will need to be registered with CTEP and have a valid and active CTEP-IAM account; and assigned one of the following Rave roles on the relevant Lead Protocol Organization (LPO) or Participating Organization roster at the enrolling site: Rave CRA, Rave Read Only, Rave CRA (LabAdmin), Rave SLA, or Rave Investigator. Refer to https://ctep.cancer.gov/investigatorResources/default.htm for registration types and documentation required. To the hold Rave CRA role or Rave CRA (Lab Admin) role, the user must hold a minimum of an AP registration type. To hold the Rave Site Investigator role, the individual must be registered as an NPIVR or IVR. Associates can hold read-only roles in Rave.

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If the study has a Delegation of Tasks Log (DTL), individuals requiring write access to Rave must also be assigned the appropriate Rave tasks on the DTL.

Upon initial site registration approval for the study in RSS, all persons with Rave roles assigned on the appropriate

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roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site users must log into the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM user name and password, and click on the "accept" link in the upper right-corner of the iMedidata page. Please note, site users will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen. If an eLearning is required and has not yet been taken, the link to the eLearning will appear under the study name in iMedidata instead of the Rave EDC link; once the successful completion of the eLearning has been recorded, access to the study in Rave will be granted, and a Rave EDC link will display under the study name.

Users that have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in RSS will also receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website, Rave tab under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website under the Rave tab at www.ctsu.org/RAVE/ or by contacting the CTSU Help Desk at 1-888-823-5923 or by e-mail at ctsucontact@westat.com.

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4.1.4.6 Digital Imaging Data Submission Using TRIAD

Copies of imaging studies are to be submitted via TRIAD.

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TRIAD submission schedule of CT or MRI scan of chest, abdomen and pelvis with IV and +/- oral contrast (until recurrence):

- Screening (Baseline) scans
- Year 1: 4.5 and 9 months (approximately weeks 20 and 40 from randomization)
- Year 2: every 6 months: 15 and 21 months from randomization
- Year 3: every 6 months: 27 and 33 months from randomization
- Years 4-5: annually: 39 and 51 months from randomization
- Any scan/s used to evaluate recurrence

Outside of the above time points please refer to the study parameters table and below for additional submissions into TRIAD.

- Pelvis imaging is only required at baseline and at recurrence per protocol. Pelvis imaging outside of these time-points should be performed as clinically indicated and these images should be uploaded to TRIAD.
- CT or MRI scans of chest and abdomen are to be performed as clinically indicated in follow-up 6-10 years from randomization and these images should be uploaded into TRIAD.
- Bone Imaging (when it meets protocol criteria)
- Optional bone mineral density test at baseline and week 40 (for patients that consent). Areal bone mineral density will be quantified by dual energy X-ray absorptiometry (DXA). Note that if patient consented, 40 week DXA should be completed only if baseline DXA completed. Images should be uploaded to TRIAD.

TRIAD is ACR's proprietary image exchange application that will be used as the sole method of data transfer to the ACR Clinical Research Center Core Laboratory for this trial. TRIAD can be installed on one or several computers of choice within the institutional "firewall" and on the institutional network; internet access is required. The TRIAD application can then be configured as a DICOM destination on either scanner(s) and/or PACS system for direct network transfer of study related images into the TRIAD directory. When properly configured, the TRIAD software de-identifies, encrypts, and performs a lossless compression of the images before they are transferred to the ACR Imaging Core Laboratory image archive in Philadelphia.

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<u>TRIAD Access Requirements:</u>

- Site radiology staff who will submit images through TRIAD will need to be registered with the Cancer Therapy Evaluation Program (CTEP) and have a valid and active CTEP Identity and Access Management (IAM) account, and be registered as an A, AP, NPIVR or IVR. Please refer to the CTEP Registration Procedures section for instructions on how to request a CTEP-IAM account and complete registration in RCR.

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To submit images, site staff must hold the TRIAD Site User role on an NCTN or ETCTN roster. Individuals requiring a TRIAD Site User role should contact the person holding a primary role at the site for their affiliated NCTN or ETCTN roster.

All individuals on the Imaging and Radiation Oncology Core provider roster have access to TRIAD, and may

submit images for credentialing purposes, or for enrollments to which the provider is linked in OPEN.

TRIAD Installations:

When a user applies for a CTEP-IAM account with the proper user role, he/she will need to have the TRIAD application installed on his/her workstation to be able to submit images. TRIAD installation documentation can be found by following this link https://triadinstall.acr.org/triadclient/

This process can be done in parallel to obtaining your CTEP-IAM account username and password and RCR registration.

If you have any questions regarding this information, please send an e-mail to the TRIAD Support mailbox at TRIAD-Support@acr.org or 1-703-390-9858.

4.1.4.7 Monitoring

ECOG-ACRIN has contracted with Alpha Oncology, a contract research organization (CRO) to conduct on-site and remote monitoring activities. The goal of these monitoring activities is to provide source document verification (SDV). Alpha Oncology will be in contact with sites as patients reach the pre-defined milestones for initiation of monitoring activities.

Alpha Oncology will also be conducting data sweeps. The goal of these data sweeps is to assist institutions with timely data entry and query resolution. Alpha Oncology will utilize phone requests and email notifications to request entry of outstanding data and/or completion of open queries. The objective of these sweeps is to collect data; they are not designed as an audit, nor are they intended to generate corrections to previously submitted data.

4.1.5 Data Quality Portal

The Data Quality Portal (DQP) provides a central location for site staff to manage unanswered queries and form delinquencies, monitor data quality and timeliness, generate reports, and review metrics.

The DQP is located on the CTSU members' website under Data Management. The Rave Home section displays a table providing summary counts of Total Delinquencies and Total Queries. DQP Queries, DQP Delinquent Forms and the DQP Reports modules are available to access details and reports of unanswered queries, delinquent forms, and timeliness reports. Review the DQP modules on a regular basis to manage specified queries and delinquent forms.

The DQP is accessible by site staff that are rostered to a site and have access to the CTSU website. Staff that have Rave study access can access the Rave study data using a direct link on the DQP.

To learn more about DQP use and access, click on the Help icon displayed on the Rave Home, DQP Queries, and DQP Delinquent Forms modules.

NOTE: Some Rave protocols may not have delinquent form details or reports specified on the DQP. A protocol must have the Calendar functionality implemented in Rave by the Lead Protocol Organization (LPO) for delinquent form details and reports to be available on the DQP. Site staff should contact the LPO Data Manager for their protocol regarding questions about Rave Calendaring functionality.

4.2 Instructions for Patients Who Do Not Start Assigned Protocol Treatment

If a patient does not receive any assigned protocol treatment, baseline and follow-up data will still be collected and must be submitted through Medidata Rave according to the schedule in the EA8143 Forms Completion Guidelines.

4.3 Additional Registration Training Requirement

ECOG-ACRIN has developed a training course to provide an overview of the EA8143 trial to site research staff and investigators. This training includes a general protocol overview and information regarding data collection and data management.

Prior to the first patient enrollment at a participating site, one investigator must review and complete the EA8143 training course. Patient enrollments will be blocked via the OPEN system until this training requirement is completed.

Additionally, ECOG-ACRIN recommends all research staff (CRAs, Data Managers, Imaging Staff, Investigators, Pharmacy Staff, etc.) involved in EA8143 complete the training.

Investigators and site staff are able to self-enroll in the course by accessing the training URL:
https://coccg813.mindflash.com/PublicCoursePage.aspx?c=1710661420

Rev. Add7

Upon completion of the training course continue through to the end of the module until you are directed to the electronic copy of your completion certificate. You will also receive an email notification at the email address registered with Mindflash that the training has been completed. Download and save the certificate, as you will need to upload this to the CTSU Regulatory Submission Portal, following the process listed below. Please note that only investigators need to submit the training completion certificate to the CTSU. Once submitted, the training record will be stored in the regulatory database as a person attribute and the corresponding site regulatory requirement will be update.

If you have questions regarding training, please contact the CTSU Regulatory Office at 1-866-651-2878 or the ECOG-ACRIN Clinical Education and Awareness Team at EAClinEd@ecog-acrin.org. Additional information can be accessed on the CTSU: Search EA8143 → Supplemental Documents → 12Dec2019 post date

Training Requirement Approval Process:

1. Complete the required protocol training, using the link provided in the protocol

2. Save a copy of the training completion certificate
3. Submit certificate to the CTSU Regulatory Office:
   - 3.1. Regulatory Submission Portal: https://www.ctsu.org
   - 3.2. Log in to the Members' area using your CTEP-IAM username and password
   - 3.3. Select the Regulatory Tab → Regulatory Submission → Add New Submission
   - 3.4. Under the "Documents being submitted are for:" drop-down menu, select "Specific Person(s) Only"
   - 3.5. In the "Pick Person(s)" field either select the applicable name(s) using the drop-down menu, or type the applicable name(s) into the search field to narrow down the choices in the drop-down menu
   - 3.6. Select "Add to Cart"
   - 3.7. Select "Next"
   - 3.8. Upload and submit a copy of your training certificate

5. Treatment Plan

Rev. Add4

5.1 Administration Schedule

Rev. Add8

5.1.1 Arm A: Perioperative Nivolumab

5.1.1.1 **Patients randomized to arm A PRIOR to the Activation of Amendment #4**

Prior to the activation of amendment 4, patients were treated with 2 neoadjuvant doses (240 mg each dose) prior to nephrectomy and up to 9 months of adjuvant therapy given every 2 weeks (240 mg each dose) for 3 months (6 cycles) then every 4 weeks (480 mg each dose) for 6 months (6 cycles). Patients who are unable to receive their second dose of neoadjuvant nivolumab within the protocol specified time frame of 14 days (+/-3 days) of the first dose were permitted to proceed to surgery as scheduled per the protocol and omit the 2nd dose. Nephrectomy was required to be performed ≥7 and ≤ 28 days from last neoadjuvant dose.

5.1.1.2 **Patients randomized to arm A AFTER the Activation of Amendment #4**

Rev Add5

5.1.1.2.1 **Dose and Schedule**

Nivolumab 480mg intravenously, given per the following guidance:

- After randomization, the neoadjuvant (pre-operative) dose of nivolumab must be administered within 4 weeks (within 28 days) from randomization and prior to partial or radical nephrectomy.
- Adjuvant (post-operative) dosing: 9 doses; 1 dose every 4 weeks for 9 months.

5.1.1.2.2 Timeframes for Perioperative Nivolumab Administration

Nephrectomy required to be performed ≥7 and ≤ 28 days from neoadjuvant dose.

NOTE: In bilateral RCC or metastectomy cases where there is more than one nephrectomy or local treatment planned, this window will pertain to the date of the initial local treatment.

Rev Add6

NOTE: Nephrectomy can be done at any qualified hospital, surgery is not required to be performed at an ECOG-ACRIN or NCTN affiliated center.

Rev Add7

- **First dose of adjuvant nivolumab should be within 4 to 10 weeks post-nephrectomy or last local treatment**. If longer timeframes are needed, it should be approved by study chair. No delay beyond 15 weeks from last local treatment will be permitted.

  NOTE: In bilateral RCC or metastectomy cases where there is more than one nephrectomy or local treatment planned, this window will pertain to the date of the last expected local treatment.

- **While it is not optional, patients who were unable to get the neoadjuvant dose for unforeseen reasons are still eligible for adjuvant dosing if they meet the other protocol treatment criteria.**

5.1.2 Arm B: Observation

Partial or radical nephrectomy followed by observation.

5.1.2.1 Timeframes for Nephrectomy

- Should be within 8 weeks after randomization in the observation arm (Arm B).

  NOTE: In bilateral RCC or metastectomy cases where there is more than one nephrectomy or local treatment planned, this window will pertain to the date of the initial local treatment.

Rev Add6

NOTE: Nephrectomy can be done at any qualified hospital, surgery is not required to be performed at an ECOG-ACRIN or NCTN affiliated center.

5.2 Prohibited and/or Restricted Treatments

The following medications are prohibited during the treatment phase of the study (unless utilized to treat a drug-related adverse event) and encouraged to avoid during the follow-up phase prior to recurrence:

- Immunosuppressive agents
- Immunosuppressive doses of systemic corticosteroids (except as stated below)
- Any concurrent anti-neoplastic therapy (including, but not limited to chemotherapy, hormonal therapy, immunotherapy, radiation therapy, or

standard or investigational agents for treatment of RCC) that would affect the primary endpoint of this study.

Rev. Add4

5.3 Permitted Therapy

Subjects are permitted the use of topical, ocular, intra-articular, intranasal and inhalational corticosteroids (with minimal systemic absorption).

Physiologic replacement doses of systemic corticosteroids are permitted even if > 10 mg daily prednisone (or equivalent) as long as no active autoimmune disease.

A brief course of corticosteroids for prophylaxis (e.g., for contrast dye allergy) or for treatment of non-autoimmune conditions (e.g., nausea, delayed-type hypersensitivity reaction caused by a contact allergen) is permitted.

Intravitreal injections of vascular endothelial growth factor (VEGF) inhibitors are permitted if used according to the approved ocular indication, such as macular degeneration.

Patients with bilateral synchronous RCCs or resectable M1 disease as detailed in the eligibility criteria are permitted to undergo resection or other definitive local treatments such as thermal ablation or radiosurgery as long as both procedures can be done within a 12 week time frame from the date of initial nephrectomy (partial or radical) or procedure (see eligibility). In the unanticipated event that both kidneys are completely removed after randomization, and the patient requires subsequent hemodialysis, the patient may continue on adjuvant nivolumab at the investigator's discretion.

5.4 Adverse Event Reporting Requirements

NOTE: The timeframes for reporting SAEs on this FDA registration trial are longer than the standard (all SAEs that occur within 100 days of the last dose of investigational treatment or surgery must be reported). Please review this section carefully to ensure all reporting requirements are met.

Rev Add5

NOTE: Starting April 1, 2018, CTEP-AERS reporting will use CTCAE v5. At this time, CTCAE v4 should continue to be used for both dose modifications and routine adverse event reporting.

5.4.1 Purpose

Adverse event (AE) data collection and reporting, which are a required part of every clinical trial, are done so investigators and regulatory agencies can detect and analyze adverse events and risk situations to ensure the safety of the patients enrolled, as well as those who will enroll in future studies using similar agents.

5.4.2 Terminology

- **Adverse Event (AE):** Any untoward medical occurrence associated with the use of an agent or surgery in humans, whether or not considered agent drug or surgery related. Therefore, an AE can be **ANY** unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product or

procedure, whether or not considered related to the medicinal product or procedure.

- **Attribution:** An assessment of the relationship between the adverse event and the protocol treatment, using the following categories.

Rev. Add10

ATTRIBUTION	DESCRIPTION
Unrelated	The AE is *clearly NOT related* to protocol treatment.
Unlikely	The AE is *doubtfully related* to protocol treatment.
Possible	The AE *may be related* to protocol treatment.
Probable	The AE is *likely related* to protocol treatment.
Definite	The AE is *clearly related* to protocol treatment.

- **CAEPR (Comprehensive Adverse Events and Potential Risks List):** An NCI generated list of reported and/or potential AEs associated with an agent currently under an NCI IND. Information contained in the CAEPR is compiled from the Investigator's Brochure, the Package Insert, as well as company safety reports.
- **CTCAE:** The NCI Common Terminology Criteria for Adverse Events provides a descriptive terminology that is to be utilized for AE reporting. A grade (severity) is provided for each AE term.
- **Hospitalization (or prolongation of hospitalization):** For AE reporting purposes, a hospitalization is defined as an inpatient hospital stay equal to or greater than 24 hours.

Rev Add5

  - **Exception:** Hospitalization for the planned partial or radical nephrectomy or any procedure (e.g. thermal ablation, radiation) to manage bilateral RCC or metastases will not be considered a SAE.
- **Life Threatening Adverse Event:** Any AE that places the subject at immediate risk of death from the AE as it occurred.
- **Serious Adverse Event (SAE):** Any adverse event occurring at any dose that results in **ANY** of the following outcomes:
  - Death
  - A life-threatening adverse event
  - Inpatient hospitalization or prolongation of existing hospitalization (for ≥ 24 hours).
    - **Exception:** Hospitalization for the planned partial or radical nephrectomy or any procedure (e.g. thermal ablation, radiation) to manage bilateral RCC or metastases will not be considered a SAE.
  - A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
  - A congenital anomaly/birth defect.

- Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

- **SPEER (Specific Protocol Exceptions to Expedited Reporting):** A subset of AEs within the CAEPR that contains list of events that are protocol specific exceptions to expedited reporting. If an AE meets the reporting requirements of the protocol, and it is listed on the SPEER, it should ***ONLY*** be reported via CTEP-AERS if the grade being reported exceeds the grade listed in the parentheses next to the event.

Rev. Add4
Rev Add5

5.4.3 Mechanisms for Adverse Event Reporting

Routine reporting: Adverse events are reported in a routine manner at scheduled times during a trial using the Medidata Rave clinical data management system. Please refer to Section 4 of the protocol for more information on how to access the Medidata Rave system and the EA8143 forms packet for instructions on what, where and when adverse events are to be reported routinely.

Rev. Add10

Expedited reporting: In addition to routine reporting, certain adverse events must be reported in an expedited manner for timelier monitoring of patient safety and care. The remainder of this section provides information and instructions regarding expedited adverse event reporting.

5.4.4 Expedited Adverse Event Reporting Procedures

Adverse events requiring expedited reporting will use CTEP's Adverse Event Reporting System (CTEP-AERS). CTEP's guidelines for CTEP-AERS can be found at http://ctep.cancer.gov.

For this study, a CTEP-AERS report must be submitted electronically via the CTEP-AERS Web-based application located at http://ctep.cancer.gov. so that ECOG-ACRIN, the NCI, and all appropriate regulatory agencies will be notified of the event in an expeditious manner.

In the rare event when Internet connectivity is disrupted a 24-hour notification is to be made by telephone to

- the AE Team at ECOG-ACRIN (857-504-2900)
- the NCI (301-897-7497)

An electronic report MUST be submitted via CTEP-AERS immediately upon re-establishment of internet connection.

Supporting and follow up data: Any supporting or follow up documentation must be uploaded to the Supplemental Data Folder in Medidata Rave within 48-72 hours. In addition, supporting or follow up documentation must be faxed to the NCI (301-897-7404) the same timeframe.

CTEP Technical Help Desk: For any technical questions or system problems regarding the use of the CTEP-AERS application, please contact the NCI Technical Help Desk at ncictephelp@ctep.nci.nih.gov or by phone at 1-888-283-7457.

5.4.5 Determination of Reporting Requirements

Many factors determine the reporting requirements of each individual protocol, and which events are reportable in an expeditious manner, including:

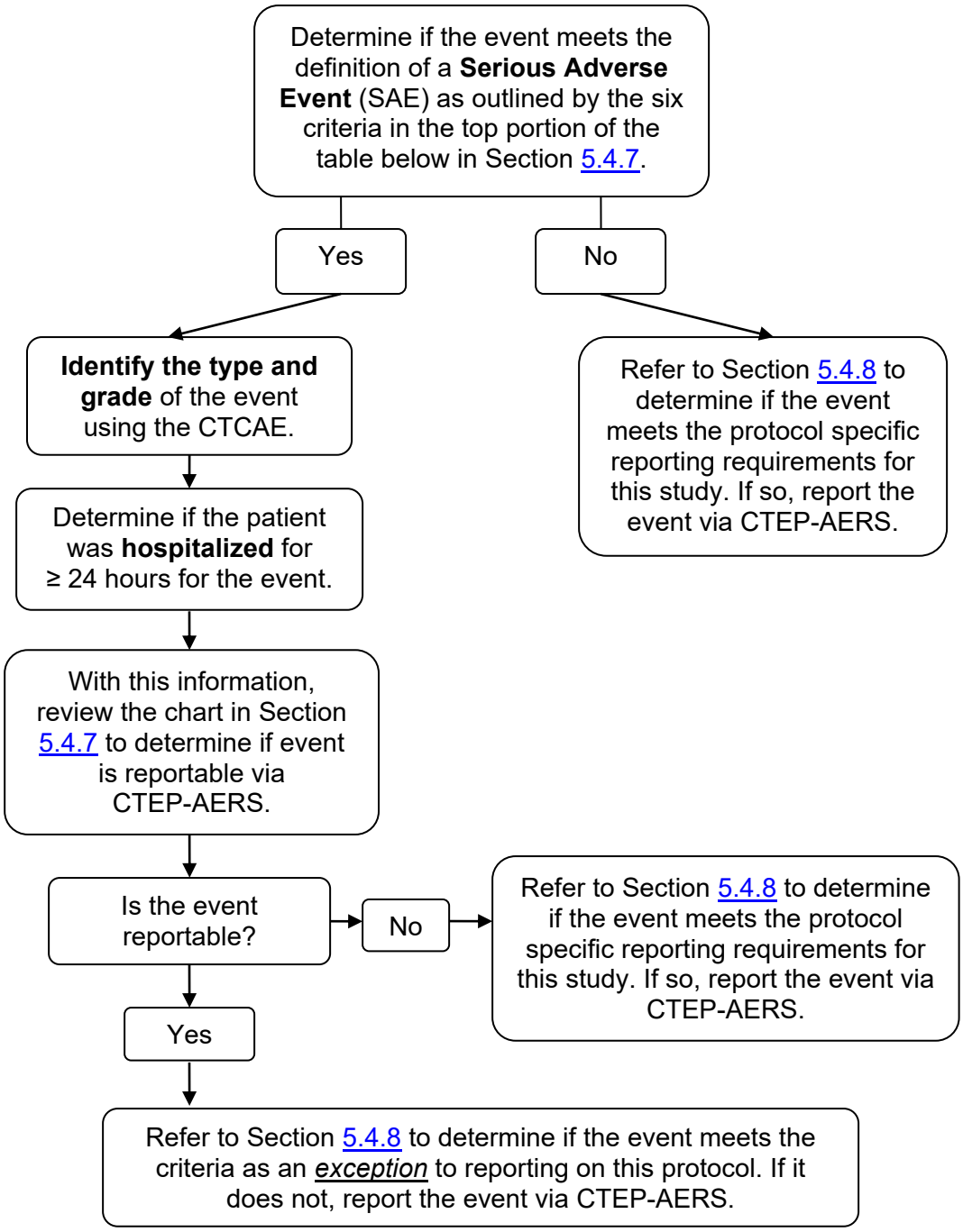
- the phase (0, 1, 2, or 3) of the trial
- whether the patient has received an investigational or commercial agent or both
- the seriousness of the event
- the Common Terminology Criteria for Adverse Events (CTCAE) grade
- whether or not hospitalization or prolongation of hospitalization was associated with the event
- when the adverse event occurred (within 100 days of the last administration of investigational agent or surgery vs. ≥ 100 days after the last administration of investigational agent or surgery)
- the relationship to the study treatment (attribution)

Using these factors, the instructions and tables in the following sections have been customized for protocol EA8143 and outline the specific expedited adverse event reporting requirements for study EA8143.

5.4.6 Steps to determine if an adverse event is to be reported in an expedited manner – Arms A and B

Rev. Add3
Rev. Add10

5.4.6.1 Guidelines for reporting adverse events **OCCURRING WHILE ON PROTOCOL TREATMENT AND WITHIN 100 DAYS** of the last administration of the investigational agent(s) or surgery.



Rev. Add4
Rev. Add10

5.4.6.2 Guidelines for reporting adverse events **OCCURRING GREATER THAN 100 DAYS** after the last administration of the investigational agent(s) or surgery.

Rev Add5
Rev Add7

If the adverse event meets the definition of a **Serious Adverse Event (SAE)** as outlined by the six criteria in the top portion of the table below in Section 5.4.7, OR the protocol specific requirements in Section 5.4.8 the following events require reporting as follows AND has an attribution of possible, probably or definite:

Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 4 and Grade 5 AEs

NOTE: Any death occurring greater than 100 days after the last dose of investigational agent or surgery with an attribution of possible, probable or definite must be reported via CTEP-AERS even if the patient is off study.

Expedited 10 calendar day reports for:

- Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization. Hospitalization for nephrectomy is not considered an adverse event.
  - **Exception:** Hospitalization for the planned partial or radical nephrectomy will not be considered a SAE.
- Grade 3 adverse events

5.4.7 Expedited Reporting Requirements for Arms A and B on protocol EA8143

Investigational Agents: Nivolumab

Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND *within 100 Days of the Last Administration of the Investigational Agent/Intervention or Surgery.*[1]

NOTE: Footnote 1 instructs how to report serious adverse events that occur more than 100 days after the last administration of investigational agent/intervention or surgery.

Rev. Add4

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)

NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)

An adverse event is considered serious if it results in **ANY** of the following outcomes:

1. Death
2. A life-threatening adverse event
3. An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4. A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. A congenital anomaly/birth defect.
6. Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria **MUST** be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

<table>
<tr><th>Hospitalization</th><th>Grade 1 Timeframes</th><th>Grade 2 Timeframes</th><th>Grade 3 Timeframes</th><th>Grade 4 & 5 Timeframes</th></tr>
<tr><td>Resulting in Hospitalization
≥ 24 hrs</td><td colspan="3">10 Calendar Days</td><td rowspan="2">24-Hour 5 Calendar Days</td></tr>
<tr><td>Not resulting in Hospitalization
≥ 24 hrs</td><td colspan="2">Not required</td><td>10 Calendar Days</td></tr>
</table>

NOTE: Protocol-specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR.

Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" – The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" – A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE.

[1] Serious adverse events that occur more than 100 days after the last administration of investigational agent/intervention or surgery and have an attribution of possible, probable, or definite require reporting as follows:

Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization
- Grade 3 adverse events

Rev. Add3

5.4.8 Additional instructions, requirements and exceptions for protocol EA8143

Additional Instructions

- For instructions on how to specifically report events that result in persistent or significant disability/incapacity, congenital anomaly, or birth defect events via CTEP-AERS, please contact the AEMD Help Desk at aemd@tech-res.com or 301-897-7497. This will need to be discussed on a case-by-case basis.

Rev. Add10

- **Reporting a death on study:** A death occurring while on study treatment or within 100 days of the last dose of study treatment requires both routine and expedited reporting, regardless of causality. Attribution to treatment or other cause must be provided

  NOTE: A death due to progressive disease should be reported as a Grade 5 "*Disease progression*" under the System Organ Class (SOC) "*General disorder and administration site conditions*". Evidence that the death was a manifestation of underlying disease (e.g. radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted

EA8143 specific expedited reporting requirements:

Rev Add5

- **Pregnancies (Arm A requirement only)**: Pregnancies and suspected pregnancies (including a positive or inconclusive pregnancy test, regardless of age or disease state) occurring while the female patient is on nivolumab, or within **100 days** of the female patient's last dose of nivolumab, are considered immediately reportable events. The pregnancy, suspected pregnancy, or positive/ inconclusive pregnancy test must be reported via CTEP-AERS within 24 hours of the Investigator's knowledge. Please refer to Appendix V for detailed instructions on how to report the occurrence of a pregnancy as well as the outcome of all pregnancies.
- **Infusion Reactions:** All Grade 3 and 4 infusion reactions that meet the definition of SAE must be reported via CTEP-AERS according to the timeframes outlined in the AE table in Section 5.4.7.
- Hospitalization for nephrectomy is NOT considered a serious adverse event. However, if the patient's hospitalization at the time of surgery is prolonged due potentially to the effect of neoadjuvant nivolumab, this would be considered a reportable SAE and must be reported via CTEP-AERS according to the timeframes outlined in the AE table in Section 5.4.7.

EA8143 specific expedited reporting exceptions:

For study arm A, the adverse events listed below **do not** require expedited reporting via CTEP-AERS:

- If an AE meets the reporting requirements of the protocol, and it is listed on the SPEER, it should ***ONLY*** be reported via CTEP-AERS if the grade being reported exceeds the grade listed in the parentheses next to the event.

5.4.9 Other recipients of adverse event reports and supplemental data

DCTD/NCI will notify ECOG-ACRIN/pharmaceutical collaborator(s) of all AEs reported to the FDA. Any additional written AE information requested MUST be submitted to BOTH the NCI and ECOG-ACRIN.

Adverse events determined to be reportable via CTEP-AERS must also be reported by the institution, according to the local policy and procedures, to the Institutional Review Board responsible for oversight of the patient.

5.4.10 Second Primary Cancer Reporting Requirements

Rev. Add10

All cases of second primary cancers, including acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), that occur following treatment on NCI-sponsored trials must be reported as follows:

- **A second malignancy is a cancer that is UNRELATED to any prior anti-cancer treatment (including the treatment on this protocol). Second malignancies require ONLY routine reporting as follows:**
  1. Complete a Second Primary Form in Medidata Rave within 14 days.
  2. Upload a copy of the pathology report to ECOG-ACRIN via Medidata Rave confirming the diagnosis.
  3. If the patient has been diagnosed with AML/MDS, upload a copy of the cytogenetics report (if available) to ECOG-ACRIN via Medidata Rave.
- **A secondary malignancy is a cancer CAUSED BY any prior anti-cancer treatment (including the treatment on this protocol). Secondary malignancies require both routine and expedited reporting as follows:**
  1. Complete a Second Primary Form in Medidata Rave within 14 days.
  2. Report the diagnosis on the Adverse Event Form or Late Adverse Event Form in the appropriate Treatment Cycle or Post Registration folder in Medidata Rave

     Report under a.) leukemia secondary to oncology chemotherapy, b.) myelodysplastic syndrome, or c.) treatment related secondary malignancy

NOTE: When reporting attribution on the AE Form, assess the relationship between the secondary malignancy and the current protocol treatment ONLY (and NOT relationship to any anti-cancer treatment received either before or after protocol treatment).

Rev Add5

3. Report the diagnosis via CTEP-AERS at http://ctep.cancer.gov. Report under a.) Leukemia secondary to oncology chemotherapy, b.) myelodysplastic syndrome, or c.) treatment related secondary malignancy
4. Upload a copy of the pathology report to ECOG-ACRIN via Medidata Rave and submit a copy to NCI/CTEP confirming the diagnosis.
5. If the patient has been diagnosed with AML/MDS, upload a copy of the cytogenetics report (if available) to ECOG-ACRIN via Medidata Rave and submit a copy to NCI/CTEP.

NOTE: The ECOG-ACRIN Second Primary Form and the CTEP-AERS report should not be used to report recurrence or development of metastatic disease.

NOTE: If a patient has been enrolled in more than one NCI-sponsored study, the ECOG-ACRIN Second Primary Form must be submitted for the most recent trial. ECOG-ACRIN must be provided with a copy of the form and the associated pathology report and cytogenetics report (if available) even if ECOG-ACRIN was not the patient's most recent trial.

NOTE: Once data regarding survival and remission status are no longer required by the protocol, no follow-up data should be submitted via CTEP-AERS or by the ECOG-ACRIN Second Primary Form.

Rev. Add3

5.5 Comprehensive Adverse Events and Potential Risks list (CAEPR) for BMS-936558 (Nivolumab, MDX-1106, NSC 748726)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI via CTEP-AERS (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. Frequency is provided based on 2069 patients. Below is the CAEPR for BMS-936558 (Nivolumab, MDX-1106).

NOTE: If an AE meets the reporting requirements of the protocol, and it is listed on the SPEER, it should ONLY be reported via CTEP-AERS if the grade being reported exceeds the grade listed in the parentheses next to the event in the SPEER.

Version 2.4, December 2, 2020[1]

Adverse Events with Possible Relationship to Nivolumab (CTCAE 5.0 Term) [n= 2069]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr 3)***
CARDIAC DISORDERS			
		Cardiac disorders - Other (cardiomyopathy)	
		Myocarditis	
		Pericardial tamponade[2]	
		Pericarditis	
ENDOCRINE DISORDERS			
	Adrenal insufficiency[3]		
	Hyperthyroidism[3]		
	Hypophysitis[3]		
	Hypothyroidism[3]		
EYE DISORDERS			
		Blurred vision	
		Dry eye	
		Eye disorders - Other (diplopia)[3]	
		Eye disorders - Other (Graves ophthalmopathy)[3]	
		Eye disorders - Other (optic neuritis retrobulbar)[3]	
		Eye disorders - Other (Vogt-Koyanagi-Harada)	
	Uveitis		

Adverse Events with Possible Relationship to Nivolumab (CTCAE 5.0 Term) [n= 2069]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 2)***
	Colitis[3]		
		Colonic perforation[3]	
	Diarrhea		***Diarrhea (Gr 3)***
	Dry mouth		***Dry mouth (Gr 2)***
		Enterocolitis	
		Gastritis	
		Mucositis oral	
	Nausea		***Nausea (Gr 2)***
	Pancreatitis[4]		
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr 3)***
	Fever		***Fever (Gr 2)***
	Injection site reaction		***Injection site reaction (Gr 2)***
HEPATOBILIARY DISORDERS			
		Hepatobiliary disorders - Other (immune-mediated hepatitis)	
IMMUNE SYSTEM DISORDERS			
		Allergic reaction[3]	
		Autoimmune disorder[3]	
		Cytokine release syndrome[5]	
		Immune system disorders - Other (GVHD in the setting of allotransplant)[3,6]	
		Immune system disorders - Other (sarcoidosis)[3]	
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Infusion related reaction[7]		
INVESTIGATIONS			
	Alanine aminotransferase increased[3]		***Alanine aminotransferase increased[3] (Gr 3)***
	Aspartate aminotransferase increased[3]		***Aspartate aminotransferase increased[3] (Gr 3)***
	Blood bilirubin increased[3]		***Blood bilirubin increased[3] (Gr 2)***
	CD4 lymphocytes decreased		***CD4 lymphocyte decreased (Gr 4)***
	Creatinine increased		
	Lipase increased		
	Lymphocyte count decreased		***Lymphocyte count decreased (Gr 4)***
	Neutrophil count decreased		
	Platelet count decreased		
	Serum amylase increased		

Adverse Events with Possible Relationship to Nivolumab (CTCAE 5.0 Term) [n= 2069]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		
		Hyperglycemia	***Hyperglycemia (Gr 2)***
		Metabolism and nutrition disorders - Other (diabetes mellitus with ketoacidosis)[3]	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		
		Musculoskeletal and connective tissue disorder - Other (polymyositis)	
		Myositis	
		Rhabdomyolysis	
NERVOUS SYSTEM DISORDERS			
		Encephalopathy[3]	
		Facial nerve disorder[3]	
		Guillain-Barre syndrome[3]	
		Myasthenia gravis[3]	
		Nervous system disorders - Other (demyelination myasthenic syndrome)	
		Nervous system disorders - Other (encephalitis)[3]	
		Nervous system disorders - Other (meningoencephalitis)	
		Nervous system disorders - Other (meningoradiculitis)[3]	
		Nervous system disorders - Other (myasthenic syndrome)	
		Peripheral motor neuropathy	
		Peripheral sensory neuropathy	
		Reversible posterior leukoencephalopathy syndrome[3]	
RENAL AND URINARY DISORDERS			
		Acute kidney injury[3]	
		Renal and urinary disorders - Other (immune-mediated nephritis)	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Pleural effusion[3]		
	Pneumonitis[3]		
		Respiratory, thoracic and mediastinal disorders - Other (bronchiolitis obliterans with organizing pneumonia)[3]	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
		Erythema multiforme[3]	

Adverse Events with Possible Relationship to Nivolumab (CTCAE 5.0 Term) [n= 2069]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Pruritus[3]		***Pruritus[3] (Gr 2)***
	Rash maculo-papular[3]		***Rash maculo-papular[3] (Gr 2)***
		Skin and subcutaneous tissue disorders - Other (bullous pemphigoid)	
	Skin and subcutaneous tissue disorders - Other (Sweet's Syndrome)[3]		
	Skin hypopigmentation[3]		
		Stevens-Johnson syndrome	
		Toxic epidermal necrolysis	

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Pericardial tamponade may be related to possible inflammatory reaction at tumor site.

[3]Nivolumab being a member of class of agents involved in the inhibition of “immune checkpoints”, may result in severe and possibly fatal immune-mediated adverse events probably due to T-cell activation and proliferation. This may result in autoimmune disorders that can include (but are not limited to) autoimmune hemolytic anemia, acquired anti-factor VIII immune response, autoimmune aseptic meningitis, autoimmune hepatitis, autoimmune nephritis, autoimmune neuropathy, autoimmune thyroiditis, bullous pemphigoid, exacerbation of Churg-Strauss Syndrome, drug rash with eosinophilia systemic symptoms [DRESS] syndrome, facial nerve disorder (facial nerve paralysis), limbic encephalitis, hepatic failure, pure red cell aplasia, pancreatitis, ulcerative and hemorrhagic colitis, endocrine disorders (e.g., autoimmune thyroiditis, hyperthyroidism, hypothyroidism, autoimmune hypophysitis/hypopituitarism, thyrotoxicosis, and adrenal insufficiency), sarcoid granuloma, myasthenia gravis, polymyositis, and Guillain-Barre syndrome.

[4]Pancreatitis may result in increased serum amylase and/or more frequently lipase.

[5]Cytokine release syndrome may manifest as hemophagocytic lymphohistiocytosis with accompanying fever and pancytopenia.

[6]Complications including hyperacute graft-versus-host disease (GVHD), some fatal, have occurred in patients receiving allo stem cell transplant (SCT) after receiving Nivolumab. These complications may occur despite intervening therapy between receiving Nivolumab and allo-SCT.

[7]Infusion reactions, including high-grade hypersensitivity reactions which have been observed following administration of nivolumab, may manifest as fever, chills, shakes, itching, rash, hypertension or hypotension, or difficulty breathing during and immediately after administration of nivolumab.

Adverse events reported on Nivolumab trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that Nivolumab caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Leukocytosis

CARDIAC DISORDERS - Atrial fibrillation; Atrioventricular block complete; Heart failure; Ventricular arrhythmia

EAR AND LABYRINTH DISORDERS - Vestibular disorder

EYE DISORDERS - Eye disorders - Other (iridocyclitis); Optic nerve disorder; Periorbital edema

GASTROINTESTINAL DISORDERS - Constipation; Duodenal ulcer; Flatulence; Gastrointestinal disorders - Other (mouth sores); Vomiting

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Edema limbs; Malaise; Pain

HEPATOBILIARY DISORDERS - Bile duct stenosis

IMMUNE SYSTEM DISORDERS - Anaphylaxis; Immune system disorders - Other (autoimmune thrombotic microangiopathy); Immune system disorders - Other (limbic encephalitis)

INFECTIONS AND INFESTATIONS - Bronchial infection; Lung infection; Sepsis; Upper respiratory infection

INVESTIGATIONS - Blood lactate dehydrogenase increased; GGT increased; Investigations - Other (protein total decreased); Lymphocyte count increased; Weight loss

METABOLISM AND NUTRITION DISORDERS - Dehydration; Hyperuricemia; Hypoalbuminemia; Hypocalcemia; Hyponatremia; Hypophosphatemia

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Back pain; Musculoskeletal and connective tissue disorder - Other (musculoskeletal pain); Musculoskeletal and connective tissue disorder - Other (polymyalgia rheumatica); Myalgia; Pain in extremity

NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (Histiocytic necrotizing lymphadenitis)

NERVOUS SYSTEM DISORDERS - Dizziness; Headache; Intracranial hemorrhage

PSYCHIATRIC DISORDERS - Insomnia

RENAL AND URINARY DISORDERS - Hematuria; Renal and urinary disorders - Other (tubulointerstitial nephritis)

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Bronchospasm; Cough; Dyspnea; Hypoxia

SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Hyperhidrosis; Pain of skin; Photosensitivity; Rash acneiform; Skin and subcutaneous tissue disorders - Other (rosacea)

VASCULAR DISORDERS - Flushing; Hypertension; Hypotension; Vasculitis

NOTE: BMS-936558 (Nivolumab, MDX-1106) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

5.6 Dose Modifications

All toxicity grades below are described using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website (http://ctep.cancer.gov).

There are no dose reductions for nivolumab. In the event of toxicities that are possibly, probably or definitely related to nivolumab, the drug should be held, early evaluation performed and toxicities managed as below.

Treatments identified in the management algorithms (Section 5.7) and the nivolumab prescribing information are recommended unless there are specific circumstances in which the treating physicians identifies an alternative treatment strategy is necessary

Rev. Add4

5.6.1 Criteria to omit dose of nivolumab

Doses of nivolumab should be omitted (not delayed) if any of the following criteria are met:

- Grade ≥ 1 pneumonitis
- Any Grade ≥ 2 drug-related adverse event, with the exception of:
  - Grade 2 fatigue, asthenia, or malaise
  - Grade 2 skin/rash event
  - Grade 2 diabetes
  - Grade 2 – 4 asymptomatic thyroid abnormalities
- Grade ≥ 3 diabetes or other endocrinopathy (with exception of asymptomatic thyroid abnormalities)
- Any symptomatic endocrinopathy
- Suspicion of adrenal crisis (e.g., hypotension, early signs of shock)
- Any Grade 3 skin drug-related adverse event
- Grade ≥ 2 creatinine rise.
  - Barring clinically significant renal toxicity, the first post-nephrectomy creatinine assessment can be used as the new baseline as long as criteria outlined in Section 3.1.22.5 are met.
- Grade ≥ 2 AST, ALT, or total bilirubin abnormalities
  - Grade 2 = AST or ALT > 3xULN and < 5xULN
  - Grade 2 = Total bilirubin > 1.5- 3xULN
- Any Grade 3 drug-related laboratory abnormality (excluding AST, ALT or Total Bilirubin which requires discontinuation) with the following exceptions:
  - Grade 3 lymphopenia does not require dose delay

- Any Grade ≥ 3 drug-related amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis does not require dose delay.
  - Grade 3 or 4 elevations that persist after an additional cycle should prompt consideration of imaging or other evaluation per investigator discretion.
- For CPK elevations, hold if Grade 2 and symptomatic; otherwise hold for Grade 3 or greater.
- Any grade encephalitis.
- Any other Grade 4 event with the exception of asymptomatic laboratory abnormalities that can be managed by electrolyte or hormone treatment, or those noted above such as asymptomatic amylase, lipase, and asymptomatic thyroid dysfunction being managed by hormone replacement.

For held doses:

- If the criteria to resume treatment are met within the dosing window (Day 1, Week X ± 3 business days), then the dose may be given.

  NOTE: Per BMS standards, the term “interruption” is reserved for interruption of the actual IV infusion during administration. The terms omission and interruption should not be used synonymously when completing the CRF forms.

Tumor surveillance assessments, QOL questionnaires collection and biomarker sampling should continue as per protocol even if dose is omitted.

Rev. Add4

5.6.2 Criteria to resume treatment with nivolumab

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

- Can resume with Grade 2 fatigue, asthenia, or malaise
- Subjects who have not experienced a prior Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin toxicity
- Grade 2 drug-related diarrhea or colitis must have resolved to baseline before treatment is resumed and any steroid therapy started for prolonged Grade 2 toxicity must be complete.
- Subjects with asymptomatic drug-related endocrinopathies which are adequately controlled with only physiologic hormone replacement may resume treatment.
- **For patients who have been treated with steroids for other Grade 2 events, they must have been tapered down to 10mg of prednisone or its equivalent and preferably off steroids without recurrence of symptoms or new events for 2 weeks prior to restarting treatment.**

- Any patient who recurs to Grade 3 during this tapering period should discontinue study therapy.

5.6.2.1 Timing of restarting nivolumab

- If the criteria to resume treatment are met, the subject should restart treatment at the next scheduled timepoint per protocol.
- If the treatment is withheld past the window period of the next scheduled nivolumab administration in order to allow for adequate recovery from the adverse event or tapering of immunosuppression, the dosing should continue to be withheld until the next scheduled study drug administration.
- See criteria in 5.6.3 for considerations pertaining to cases where treatment is held > 12 weeks past the scheduled dosing

Rev. Add10

Rev. Add4

5.6.3 Criteria which require permanent discontinuation of nivolumab

- Any Grade 2 drug-related uveitis, eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment
- Grade ≥ 2 pneumonitis
- The following Grade ≥ 3 drug related events require immediate discontinuation: renal toxicity (including acute kidney injury and increased creatinine), hepatitis, bronchospasm, diarrhea, colitis, neurologic toxicity, hypersensitivity reactions, infusion reactions, myositis, myocarditis, and uveitis.
- Examples (but not limited to) of Grade 3 drug-related neurotoxicity requiring discontinuation include:
  - Inflammatory CNS event, Guillain-Barre, myasthenia, polymyopathy, seizure
- Any other Grade 3 or greater non-skin, drug-related adverse event lasting > 7 days or that recurs, with the following exceptions:
  - Grade 3 drug-related endocrinopathies such as diabetes or thyroid dysfunction adequately controlled with only physiologic hormone replacement do not require discontinuation
  - Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except:
    - Grade 3 drug-related thrombocytopenia or lower grade associated with significant bleeding requires discontinuation
    - Grade ≥ 3 drug-related AST, ALT or total bilirubin requires discontinuation
    - Concurrent AST or ALT > 3 x ULN and total bilirubin > 2x ULN requires discontinuation

    - For any other Grade 3 events not listed above, at the discretion of the investigator, the patient may be allowed to be re-treated when the event is resolved and patient is off steroids – for the first occurrence. A second similar event requires discontinuation of nivolumab.
- Any Grade 4 drug related skin or renal toxicity
- Any Grade 3 or 4 drug related adrenal insufficiency
- Any Grade 3 or 4 drug related cardiac dysfunction
- Any Grade 2 drug related cardiac dysfunction events that do not recover to baseline or that reoccur
- Any immune-related toxicity requiring immunosuppressive therapy beyond steroids
- Any severe or Grade 3 immune-mediated adverse reaction that recurs to Grade ≥ 3 on reintroduction of nivolumab
- Persistent Grade 2 immune-mediated adverse drug reactions that do not recover to Grade 1 or resolve within 6 weeks after the last dose of study drug (exceptions: rash, fatigue, amylase/lipase elevations, thyroid disorders or diabetes)

Rev. Add10

- Dosing that is withheld > 12 weeks from the last dose with the following exceptions:
    - Dosing omissions to allow for prolonged steroid tapers to manage drug-related adverse events are allowed (provided they do not meet other discontinuation criteria above). Prior to re-initiating treatment in a subject with a dosing omission period lasting > 12 weeks from the last dose, the PI must be consulted.
    - Dosing omissions > 12 weeks from the last dose that occur for non-drug-related reasons may be allowed if approved by the PI.
- Any adverse event, laboratory abnormality, or intercurrent illness, which in the judgment of the Investigator presents a substantial clinical risk to the subject with continued nivolumab dosing.

5.6.4 Management/Next Dose Guidelines for BMS-936558 (Nivolumab) Cardiac Toxicities

Cardiac*	**Management/Next Dose for BMS-936558 (Nivolumab) Cardiac Toxicities**
≤ Grade 1	Hold dose pending evaluation and observation.** Evaluate for signs and symptoms of CHF, ischemia, arrhythmia or myositis. Obtain history EKG, CK (for concomitant myositis), CK-MB. Repeat troponin, CK and EKG 2-3 days. If troponin and labs normalize may resume therapy. If labs worsen or symptoms develop then treat as below. Hold pending evaluation
Grade ≥ 2 with suspected myocarditis	Hold dose.** Admit to hospital. Cardiology consult. Rule out MI and other causes of cardiac disease. Cardiac Monitoring. Cardiac Echo. Consider cardiac MRI and cardiac biopsy. Initiate high dose methylprednisolone. If no improvement within 24 hours, add either infliximab, ATG or tacrolimus. Consult algorithm for more details. Resume therapy if there is a return to baseline and myocarditis is excluded or considered unlikely.
Grade ≥ 2 with confirmed myocarditis	Off protocol therapy. Admit to CCU (consider transfer to nearest Cardiac Transplant Unit). Treat as above. Consider high dose methylprednisolone Add ATG or tacrolimus if no improvement. Off treatment.
**Including CHF, LV systolic dysfunction, Myocarditis, CPK, and troponin* ***Patients with evidence of myositis without myocarditis may be treated according as "other event"* Note: The optimal treatment regimen for immune mediated myocarditis has not been established. Since this toxicity has caused patient deaths, an aggressive approach is recommended.	
Treatment with steroids as clinically indicated	

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5.7 Management Algorithms for Immune-related Adverse Events

- The following algorithms contain recommended management guidelines for nivolumab immune-related AEs. The criteria in Section 5.6 are mandatory and should be followed over the algorithms if discrepant.
- The nivolumab prescribing information can also be used for AEs not detailed below and for other suggested steroid or management regimens. For any questions where the two may be discrepant or if questions arise, contact the Principal Investigator.
- **Clarifications regarding steroid use:**
  - Please note that in some cases the management algorithms recommend steroids if symptoms do not resolve in 7 days. However, this recommendation is not meant to delay steroid treatment if it is clinically indicated sooner.

- Before starting steroids baseline values should be obtained for cortisol, ACTH, TSH, free T4.
- Any patient started on corticosteroids initially who is determined to not require steroid treatment for an autoimmune adverse event may resume therapy after a 2 week observation period without further symptoms.

GI Adverse Event Management Algorithm

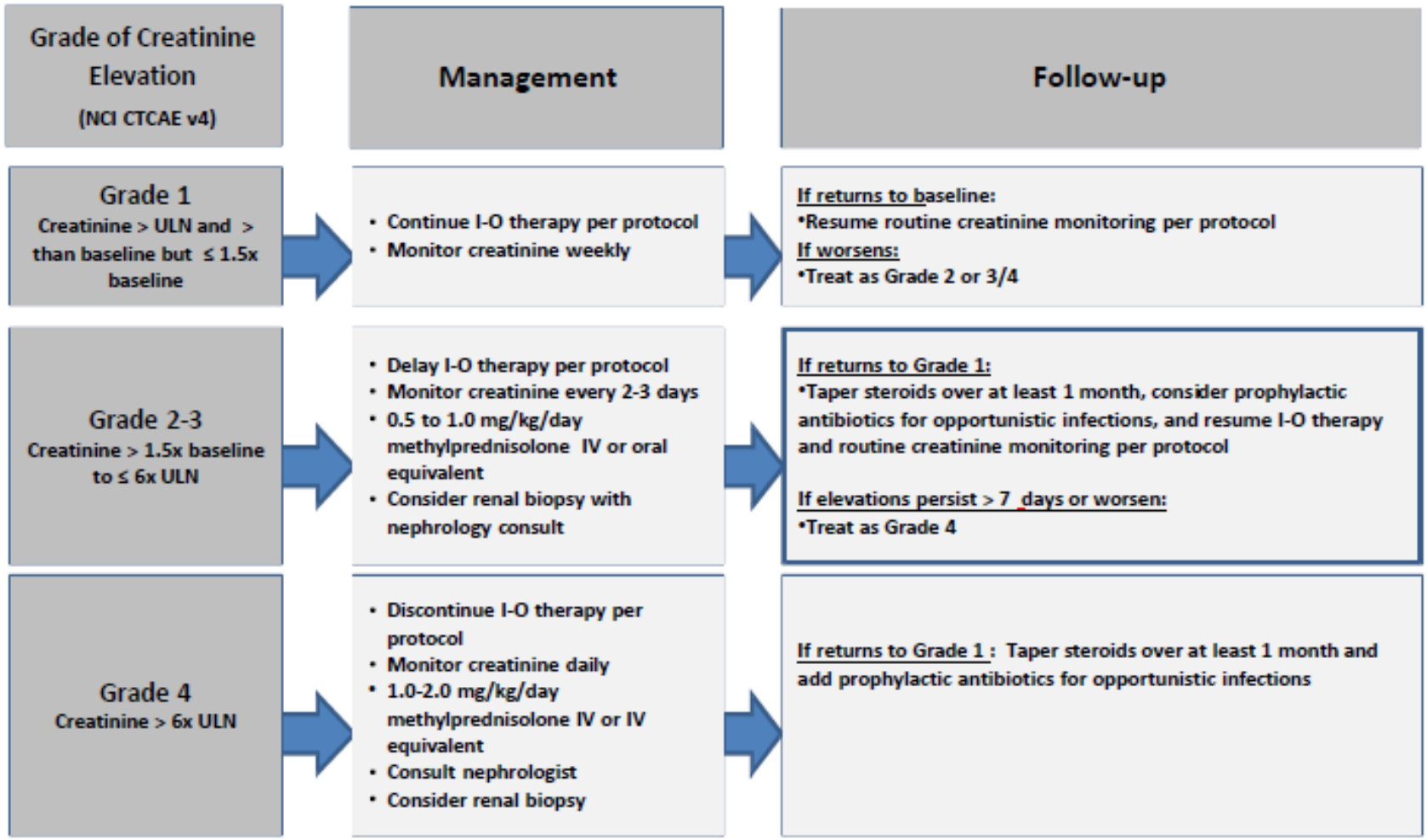
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.

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 2 or 3/4
Grade 2 Diarrhea: 4-6 stools per day over baseline; IV fluids indicated <24 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 ADL Colitis (G3): severe abdominal pain, medical intervention indicated, peritoneal signs G4: life-threatening, perforation	• Discontinue I-O therapy per protocol • 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections • Consider lower endoscopy	If improves: • Continue steroids until grade 1, then taper over at least 1 month If persists > 3-5 days, or recurs after improvement: • Add infliximab 5 mg/kg (if no contraindication). Note: Infliximab should not be used in cases of perforation or sepsis

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

Renal Adverse Event Management Algorithm

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



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

Pulmonary Adverse Event Management Algorithm

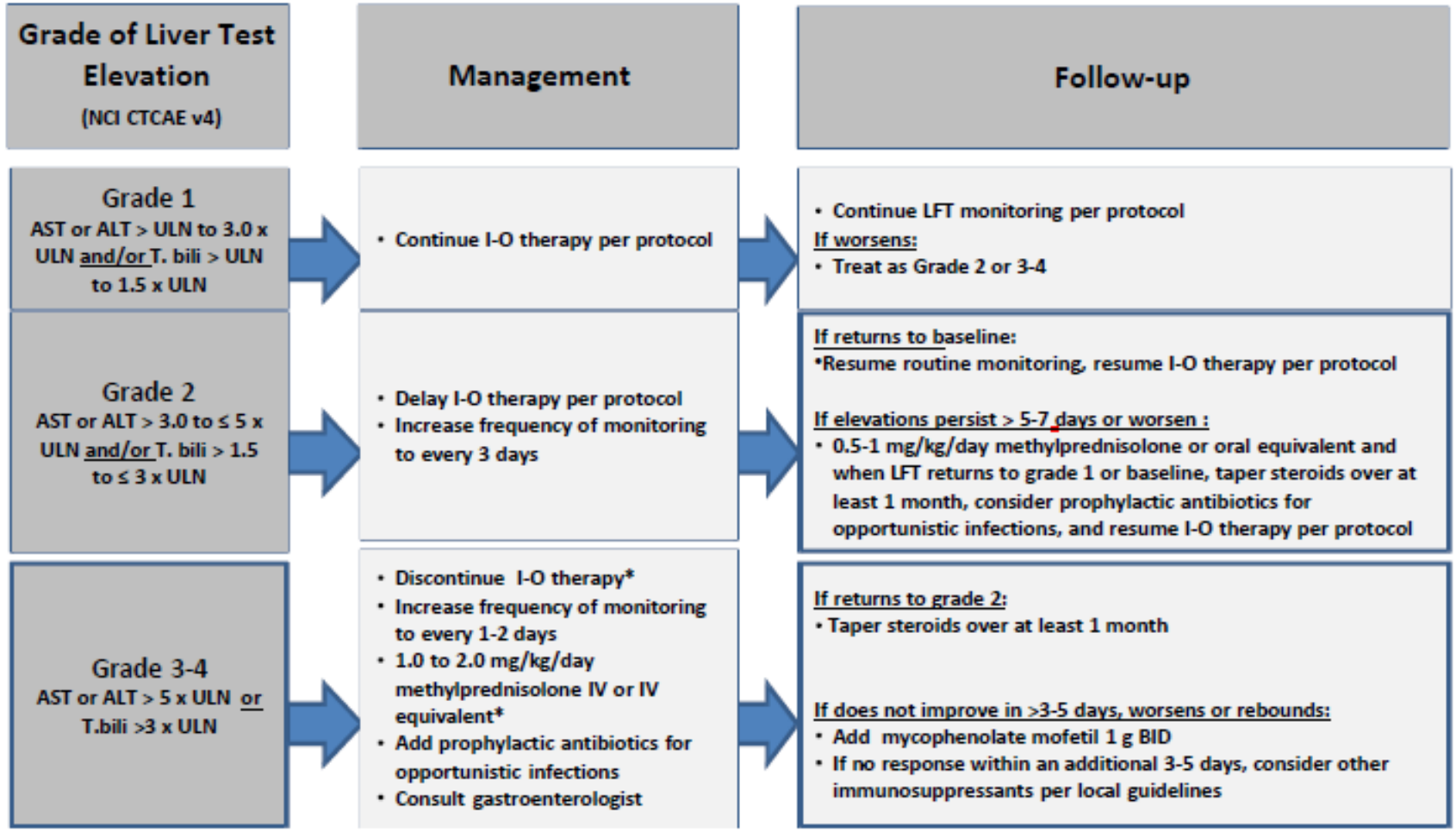
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.

Grade of Pneumonitis (NCI CTCAE v4)	Management	Follow-up
Grade 1 Radiographic changes only	• Consider delay of I-O therapy • Monitor for symptoms every 2-3 days • Consider Pulmonary and ID consults	• Re-image at least every 3 weeks If worsens: • Treat as Grade 2 or 3-4
Grade 2 Mild to moderate new symptoms	• Delay I-O therapy per protocol • Pulmonary and ID consults • Monitor symptoms daily, consider hospitalization • 1.0 mg/kg/day methyl-prednisolone IV or oral equivalent • Consider bronchoscopy , lung biopsy	• Re-image every 1-3 days If improves: • When symptoms return to near baseline, taper steroids over at least 1 month and then resume I-O therapy per protocol and consider prophylactic antibiotics If not improving after 2 weeks or worsening: • Treat as Grade 3-4
Grade 3-4 Severe new symptoms; New/worse Life-th	• Discontinue I-O therapy per protocol • Hospitalize • Pulmonary and ID consults • 2-4 mg/kg/day methylprednisolone IV or IV • Consider bronchoscopy, lung biopsy	If improves to baseline: • Taper steroids over at least 6 weeks worsening: sion

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.

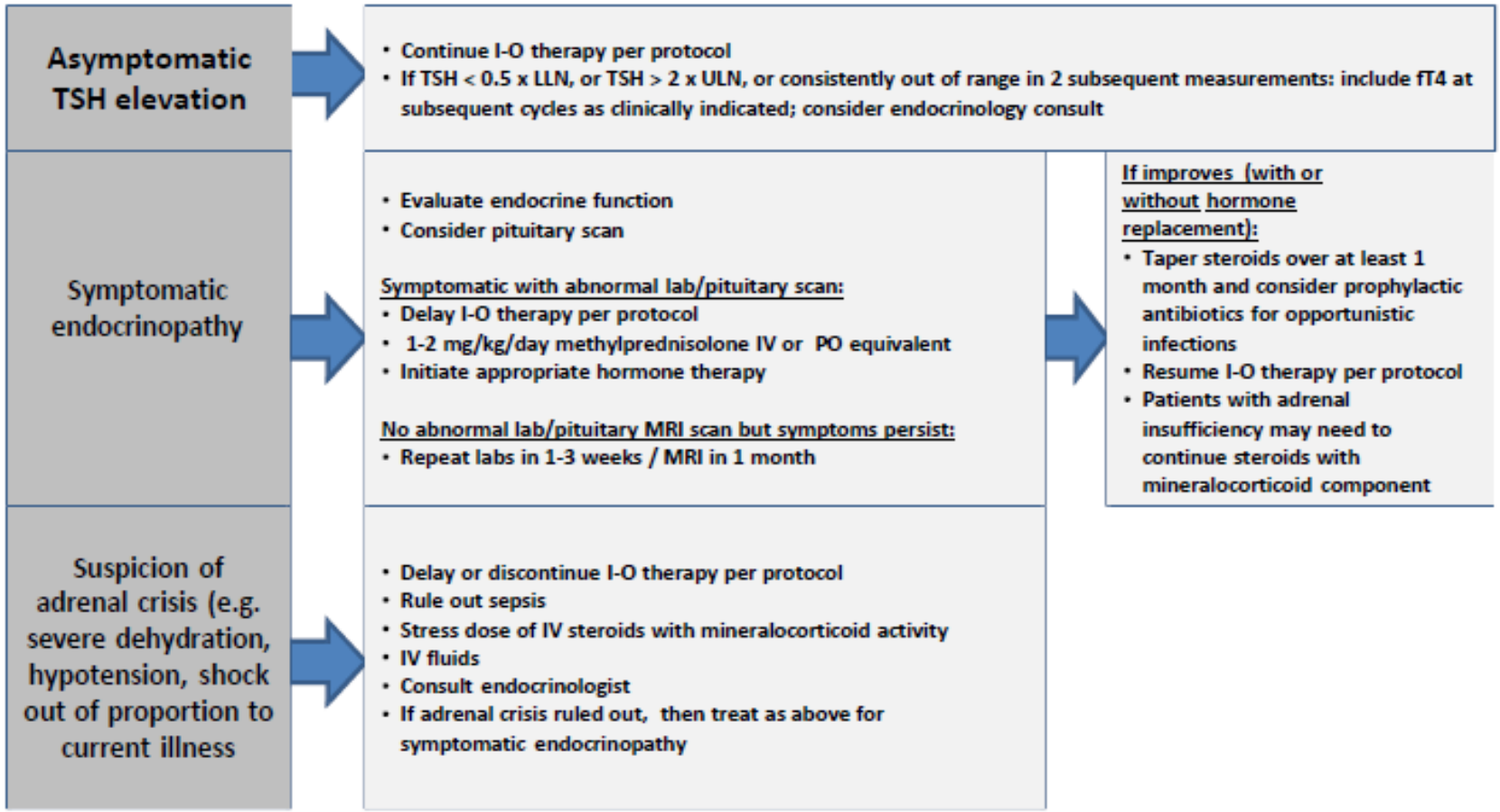


Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

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

Endocrinopathy Adverse Event Management Algorithm

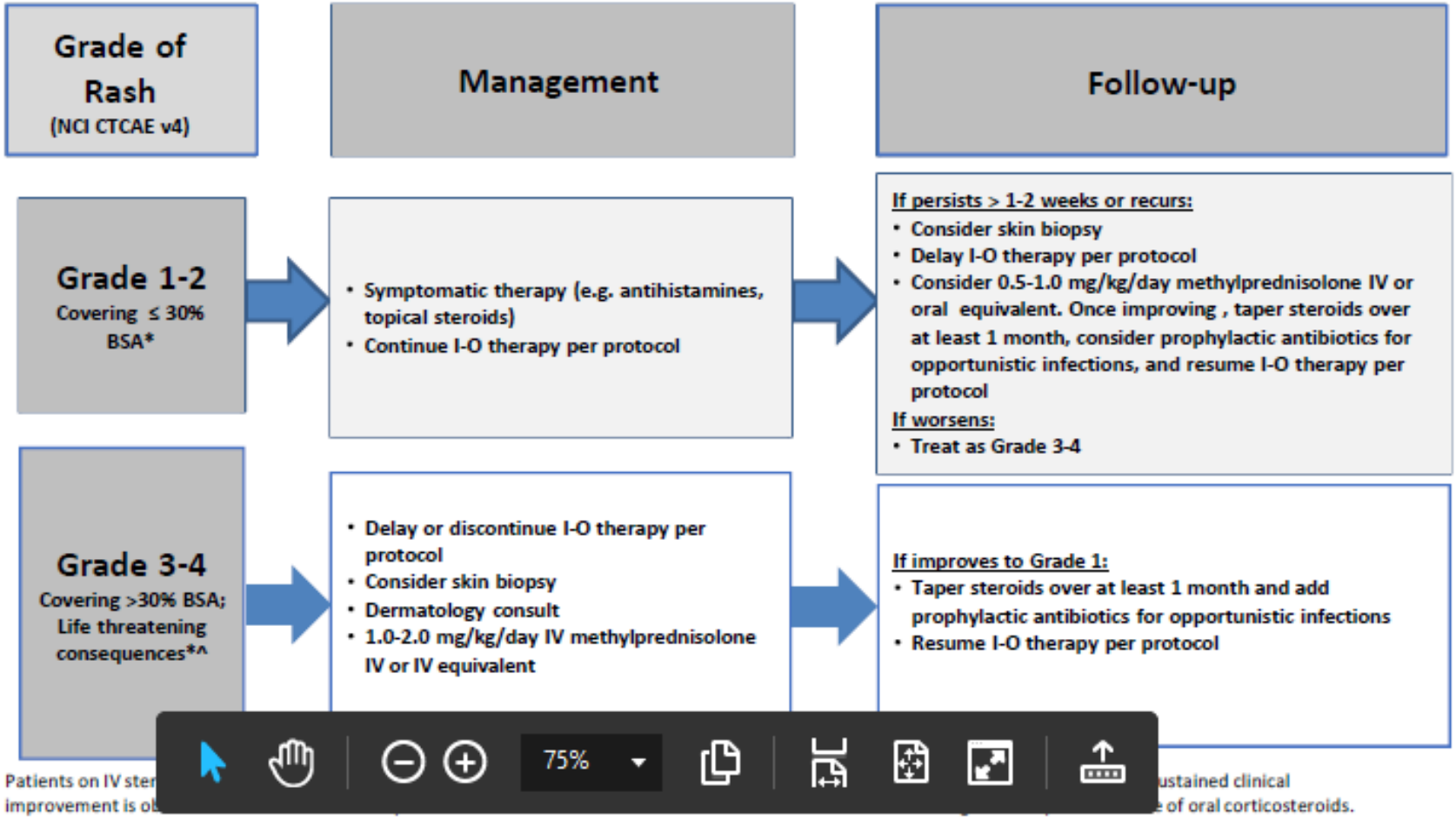
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider visual field testing, endocrinology consultation, and imaging.



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.



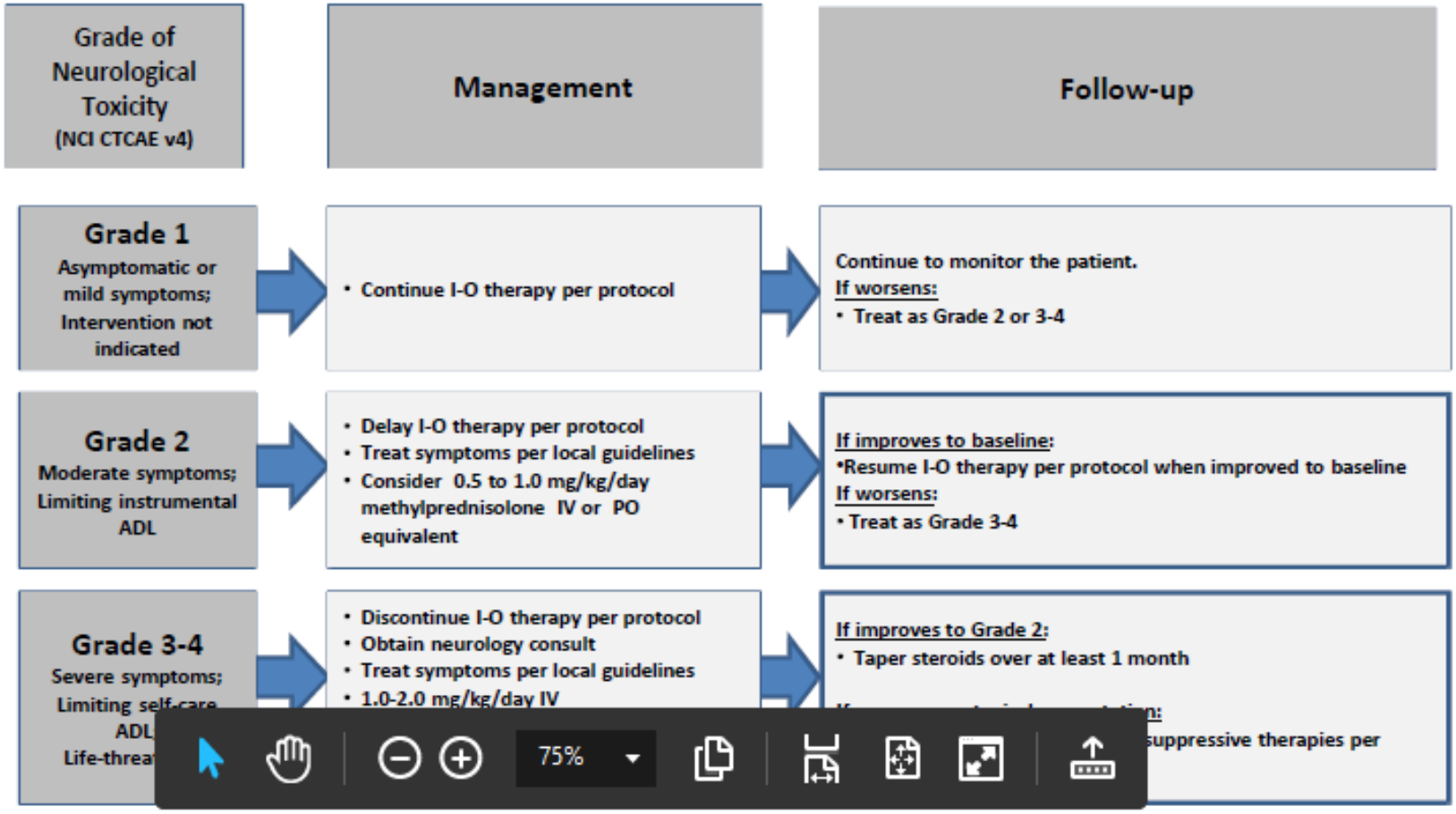
Patients on IV ster… ustained clinical improvement is ob… e of oral corticosteroids.

*Refer to NCI CTCAE v4 for term-specific grading criteria.

^If SJS/TEN is suspected, withhold I-O therapy and refer patient for specialized care for assessment and treatment. If SJS or TEN is diagnosed, permanently discontinue I-O therapy.

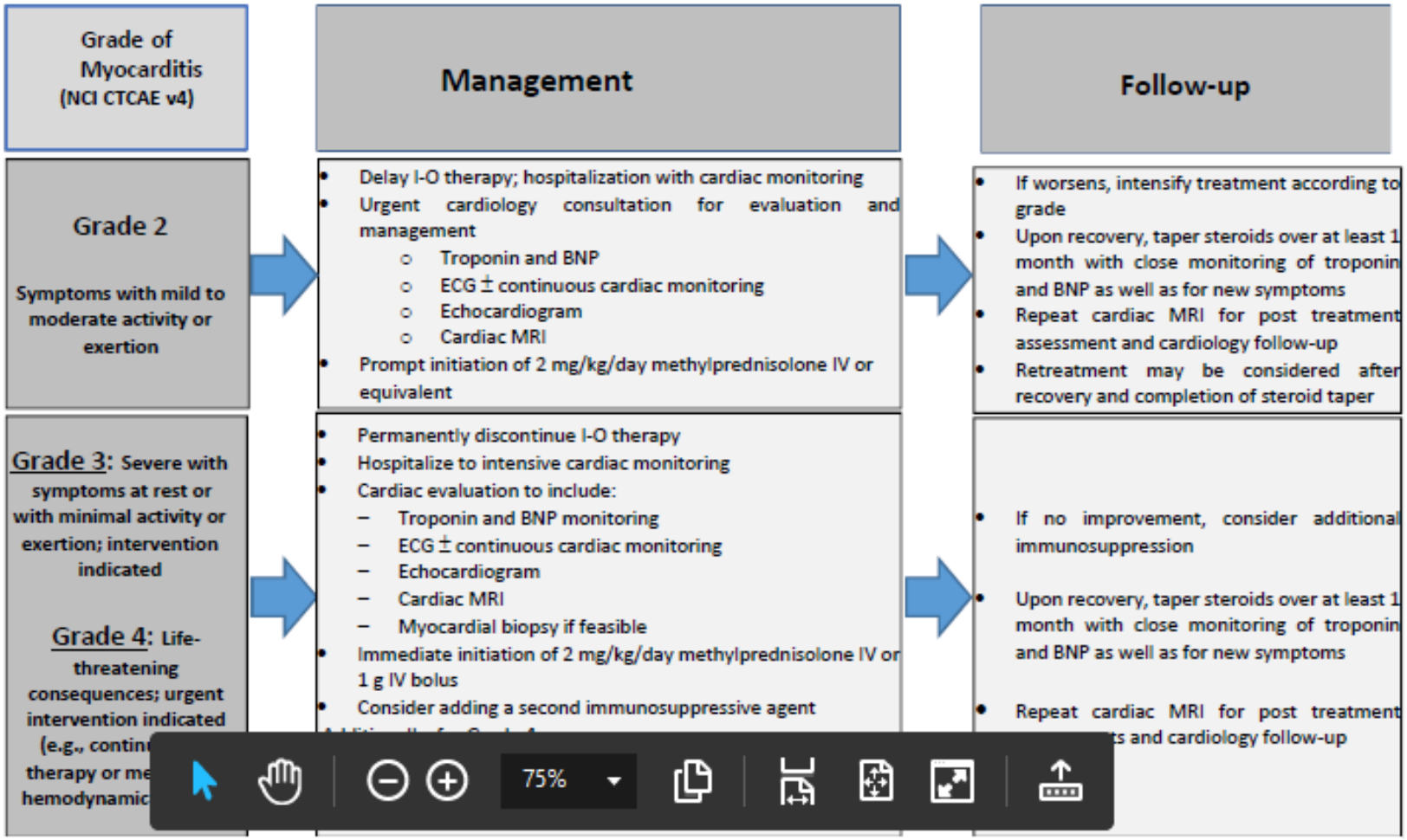
Neurological Adverse Event Management Algorithm

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



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

Rev. Add3
Rev. Add4

5.8 Supportive Care

- All supportive measures consistent with optimal patient care will be given throughout the study.
- Bisphosphonates and RANK ligand inhibitors used for osteopenia, osteoporosis, and hypercalcemia are permitted.
- **Treatment of Nivolumab Related Infusion Reactions**

  Since nivolumab contains only human immunoglobulin protein sequences, it is unlikely to be immunogenic and induce infusion or hypersensitivity reactions. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash, pruritus, arthralgias, hypotension, hypertension, bronchospasm, or other allergic-like reactions. All Grade 3 and 4 infusion reactions that meet the definition of SAE must be reported via CTEP-AERS according to the timeframes outlined in the AE table in Section 5.4.77. Infusion reactions reported via CTEP-AERS should be graded according to NCI CTCAE (Version 5.0) guidelines.

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

  - For **Grade 1** symptoms: (mild reaction; infusion interruption not indicated; intervention not indicated):
    - Study staff (e.g, RN, NP, PA or MD) should remain at bedside and monitor subject until recovery from symptoms. The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg IV or PO (or equivalent) and/or acetaminophen/paracetamol 325 to 1000 mg PO at least 30 minutes before additional nivolumab administrations.

Rev Add5

  - For **Grade 2** symptoms: (moderate reaction required therapy or infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids); prophylactic medications indicated for $\leq$ 24 hours):
    - Stop the nivolumab infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV or PO (or equivalent) and/or acetaminophen/paracetamol 325 to 1000 mg; remain at bedside and monitor subject until resolution of symptoms. Corticosteroid and/or bronchodilator therapy may also be administered as appropriate. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor subject closely. If symptoms recur, then no further nivolumab will be administered at that visit.
    - For future infusions, the following prophylactic premedications are recommended: diphenhydramine 50 mg IV or PO (or equivalent) and/or acetaminophen/paracetamol 325 to 1000 mg should be administered at least 30 minutes before nivolumab infusions. If necessary, corticosteroids (up to 25 mg of hydrocortisone or equivalent) may be used.

- For **Grade 3 or 4** symptoms: (severe reaction, Grade 3: prolonged [ie, not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates). Grade 4: Life-threatening; pressor or ventilator support indicated):
  - Immediately discontinue infusion of nivolumab. Begin an IV infusion of normal saline and treat the subject as follows: Recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV or PO with methylprednisolone 100 mg IV (or equivalent), as needed. Subject should be monitored until the Investigator is comfortable that the symptoms will not recur. Nivolumab will be permanently discontinued. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery of the symptoms.
  - In case of late-occurring hypersensitivity symptoms (e.g., appearance of a localized or generalized pruritus within 1 week after treatment), symptomatic treatment may be given (e.g., oral antihistamine or corticosteroids).

Rev. Add4

5.9 Quality of Life Instrument Administration

5.9.1 Quality of Life Instruments to be administered

The NCCN/FACT Kidney Symptom Index-19 (NFKSI-19), PROMIS PF-10 and PRO-CTCAE items will be administered to patients enrolled on EA8143. PRO-CTCAE items will be administered through the first year post-baseline, but not thereafter. The NFKSI-19 and PROMIS PF-10 will be administered at all scheduled time points.

5.9.2 Quality of Life Assessment Schedule

NOTE: All assessments are to be administered PRIOR to nivolumab infusion on Arm A.

Patients will be assessed according to the following schedule:

Baseline (pre-treatment and pre-nephrectomy)

Rev. Add10

- Post-randomization, prior to the initiation of protocol therapy (Arm A).
- Post-randomization, prior to date of nephrectomy (Arm B)

Post-baseline assessments

- Pre-nephrectomy (Arm A) but post neoadjuvant dose
- 8 weeks post-nephrectomy
- 20, 40 and 54 weeks post randomization
- At recurrence
- 2 years post randomization

5.9.3 Quality of Life Administration Instructions

- QOL Assessments should be administered at all scheduled assessments, regardless of whether the patient recurs or is removed from the protocol for any reason.

Rev. Add7

NOTE: The NFKSI-19, PROMIS, and PRO-CTCAE are available in Spanish translations. No other translations are available at this time. Sites should not translate their own versions of these documents. For the purposes of this study when a translated version is not available, document this reason in RAVE. For sites, this would not be considered a deficiency/ violation at the time of audit, as QOLs are not primary or secondary objective.

- The pre-treatment pre-nephrectomy baseline assessments of PRO-CTCAE items, PROMIS PF-10, and NFKSI-19 will be administered during the patient's clinic visit post-randomization, prior to the initiation of protocol therapy (Arm A) or nephrectomy (Arm B). Follow-up PRO-CTCAE items, PROMIS PF-10, and NFKSI-19 assessments are to be completed pre-nephrectomy but post neoadjuvant dose (Arm A), 8 weeks post-nephrectomy, 20, 40, and 54 weeks post randomization, and at recurrence. PROMIS PF-10, and NFKSI-19 assessments will also be administered 2 years post randomization. These assessments may not coincide with scheduled clinic visits. Patients will be given blank assessment forms and asked to complete assessment forms at home at these time points. Administration instructions for baseline (pre-treatment pre-nephrectomy) assessment and post-baseline assessments are detailed below.

Rev. Add10

- Baseline (pre-treatment and pre-nephrectomy) assessment
  - Whenever possible, the PRO-CTCAE, PROMIS PF-10, and NFKSI-19 assessments should be administered at the clinic visit before the patient is seen by the physician, before evaluations are performed and before test results are shared with the patient. In the event that the questionnaires are not administered at the clinic visit, the PRO-CTCAE, PROMIS PF-10, and NFKSI-19 data can be collected by telephone or mail as backup methods provided that PRO-CTCAE, PROMIS PF-10, and NFKSI-19 data is captured prior to initiation of treatment. For patients receiving nivolumab, assessments should be done prior to the start of the infusion.
  - The CRN/CRA should read the instructions printed on the questionnaire to the patient and ensure the patient understands the instructions. It is important to assure the patient that all material on the questionnaire is confidential and will not be shared with the health care team and that it will not become part of the medical record.

- Assistance in reading the questionnaire is permitted if the patient is unable to complete the questionnaire on his/her own (e.g. difficulty in reading, vision problems). It is important not to influence the response of the patient. Note why the patient required assistance and the type of assistance given on the Assessment Compliance Form.
- Patients should be instructed to answer all the questions regardless of whether the symptoms or conditions asked about are related to the cancer or cancer treatment. Discourage family members from being present during questionnaire completion or from influencing the patient's responses.
- Review the questionnaire for completeness before the patient leaves. If the patient has marked more than one answer per question, ask the patient which answer best reflects how they are feeling. If the patient has skipped a question or questions, assure that he or she intended to leave the question blank.
- If the patient refuses or cannot complete the questionnaire at any time point, he or she should be asked to do so at the next scheduled QOL assessment time point.
- The patient may decline to complete the QOL assessments for any reason. The reason must be documented on the Assessment Compliance Form.

- Post-Baseline Assessments (pre-nephrectomy but post neoadjuvant dose on Arm A, post-nephrectomy on both Arms, at weeks 20, 40, and 54, at 2 years, and at recurrence.)
  - Assessments at 20, 40, and 54 weeks should be done when patient returns to clinic for scans and labs, prior to receiving scan.
  - The CRN/CRA should contact the patient by telephone, e-mail or postal mail 1-3 days prior to the target assessment date to remind the patient to complete their assessments. If the returned questionnaires are not received at the site, a reminder contact phone call should occur no longer than 5 days following the target completion date. Patients should complete the HRQL assessment forms on or as close as possible to the target assessment date. If the patient misses the target assessment date they can complete the assessment as long as they have not started the subsequent cycle of protocol therapy.
  - The CRN/CRA should confirm that the patient has a stamped, addressed envelope to return his/her completed QOL assessment forms. The CRN/CRA may offer to complete the QOL assessment by telephone. The mode of administration (mail, telephone) should be noted on the Assessment Compliance Form. If the patient has

misplaced the forms, the site can send new blank forms by mail, fax or email.

- For telephone administration of QOL assessment, encourage the patient to have the written form in front of them to read along during the assessment. Read the instructions printed on the questionnaires to the patient and ensure the patient understands the instructions. If the patient is unable to complete the questionnaires on his/her own (e.g. difficulty in reading, vision problems) follow-up QOL assessments should be conducted by telephone. Telephone administration of QOL assessment by the CRN/CRA is preferable to having the patient ask a family member or someone in the household for assistance with completing the forms. It is important not to influence the response of the patient. Note why the patient required assistance and the type of assistance given on the Assessment Compliance Form.
- The CRN/CRA should instruct the patient to record the actual date the patient completed the QOL assessment on each form, reminding the patient that the date recorded on the forms is their target date of assessment completion. It is important to assure the patient that all material on the questionnaire is confidential and will not be shared with the health care team and that it will not become part of the medical record.
- Patients should be instructed to answer all the questions regardless of whether the symptoms or conditions asked about are related to the cancer or cancer treatment. Discourage family members from being present during questionnaire completion or from influencing the patient's responses.

Rev. Add4

5.10 Duration of Therapy

Patients will receive protocol therapy unless:

- Extraordinary Medical Circumstances: If at any time the constraints of this protocol are detrimental to the patient's health, protocol treatment should be discontinued. In this event submit forms according to the instructions in the EA8143 Forms Packet.
- Patient withdraws consent.
- Patient experiences unacceptable toxicity.
- Non-protocol therapies are administered.
- Pregnancy.

Rev. Add7

- Patient is found not to have RCC at biopsy or nephrectomy (Off-treatment date should be the date of biopsy or surgery, when it is found the patient does not have RCC).

After Amendment #4, patients will not receive more than 1 dose of neoadjuvant nivolumab and 9 doses of adjuvant nivolumab (4-week intervals) during the

course of the study. If a patient started on trial prior to Amendment #4, they may receive more doses given the 2 week treatment intervals, but no more than 2 doses of neoadjuvant therapy and 9 months of adjuvant therapy.

Patients will not receive nivolumab at recurrence as part of the study. Subsequent therapy at recurrence is at the treating physician's discretion.

5.11 Second Primary Cancers

Rev Add5

Patients who develop second (but not secondary) primary cancers may continue protocol treatment at the discretion of their physician as long as they do not require systemic therapy for the treatment of that secondary cancer. Surgical resection, radiation (with the exception of to the renal bed), and other local therapies may be considered.

5.12 Duration of Follow-up

Rev Add6

For this protocol, all patients (except those found not to have RCC at biopsy or nephrectomy), including those who discontinue protocol therapy early, will be followed for response until recurrence, even if non-protocol therapy is initiated and for survival for 10 years from the date of randomization. All patients must also be followed through completion of all protocol therapy.

6. Measurement of Effect

Rev. Add4

6.1 Diagnosis of Renal Cell Cancer Recurrence

The diagnosis of a first renal cell carcinoma recurrence can be made only when the clinical and pathology findings meet the criteria acceptable for recurrence as defined below. Anything not listed as acceptable should be considered unacceptable for evidence of renal cell cancer recurrence and should not be an indication to alter protocol therapy if still on therapy at time of possible recurrence. Any recurrence of malignant disease should be proven by core needle biopsy or resection whenever possible. At the time of tumor recurrence the investigator should clearly indicate the site of tumor recurrence and whether multiple sites are involved.

Supporting documentation must be submitted following diagnosis of renal cell carcinoma recurrence or second primary cancer. Refer to the EA8143 Forms Packet for data to be collected. Copies of the pathology, radiology and, if relevant, surgery reports should be uploaded via Medidata Rave. If biopsy material is available, a copy of the pathology report should also accompany the materials submission to the CBPF.

The following criteria for recurrence are required. Supporting documentation includes a copy of radiology and pathology reports.

- Positive cytology or biopsy
- If biopsy is not possible of a solitary new lung, soft tissue, or visceral lesion that is ≥1 cm, confirmation of growth by at least 5mm or appearance of other new lesions on subsequent scans at least 4 weeks later.
- If biopsy is not possible of a solitary new lung, soft tissue, or visceral lesion that is <1 cm, confirmation of growth to ≥1cm or appearance of new lesions on subsequent scans at least 4 weeks later.
- If biopsy is not possible of a lymph node ≥1.5cm in short axis, confirmation of growth by at least 5mm or appearance of other new lesions on subsequent scans at least 4 weeks later.
- For bone lesions:
  - A positive radiographic study such as bone scan with 2 or more new lesions that are confirmed with MRI or CT
  - For a solitary lesion or equivocal finding on a scan, biopsy is required or subsequent scans demonstrating growth or at least one new lesion at least 4 weeks later.
- For brain lesion(s), a positive brain CT or MRI is acceptable without a biopsy or confirmation.

NOTE: Patients should continue treatment until recurrence has been proven per these guidelines.

Rev. Add4

6.2 Survival

Survival will be measured from the date of randomization.

Rev. Add4

6.3 Time to Recurrence

This interval will be measured from the date of randomization to the appearance of new lesion(s). Date of recurrence will be backdated to the time the lesion was first observed.

Rev. Add4
Rev Add5

6.4 Patients with Residual Disease at the time of Surgery or who do not undergo nephrectomy

Patients who are found to have residual local, regional or distant disease at the time of surgery or who do not undergo surgery will be included as recurrence events using the date of randomization. Patients will not continue on to the adjuvant portion of nivolumab if randomized to that arm. The following should be done as detailed in the study calendar under “recurrence.”

- History and physical exam
- Waist circumference

Rev Add7

NOTE: waist circumference is being collected to investigate the relationship of BMI to clinical outcome.

- ECOG performance status
- CT/MRI scan chest abdomen /pelvis with IV +/- oral contrast (if none within 8 weeks)
- Bone imaging (if none within 8 wks and only if clinically indicated)
- Tumor Biopsy (if nephrectomy specimen not available)
- EKG if clinically indicated

Rev. Add4

7. Study Parameters

Rev Add5

7.1 Therapeutic Parameters for both arms

1. Baseline imaging should be done ≤ 8 weeks prior to randomization.
2. Eligibility and baseline laboratories outlined in Sections 3, 7.2, and 1.1 should be done and resulted ≤ 8 weeks before randomization.

7.2 Arm A: Perioperative Nivolumab

Therapeutic Parameters Table

Procedures	Baseline[2]	Day 1, Neoadjuvant Cycle(s)[1,8]	Pre-Surgery	Surgery	Day 1, Adjuvant Cycles[1,8]	Month 4.5 (Week 20)[1]	Month 9 (Week 40)[1]	Within 6 Weeks after Final Treatment	Follow-up[1,11]	Recurrence
History and Physical examination	X	X			X			X	X[15]	X
Waist Circumference[13]	X									X
Medication review	X	X		X	X			X		X
ECOG performance status	X	X			X			X		X
Height	X									
Vital signs (including BP, HR, temperature) and weight	X	X			X			X		
HCV, HBV[3]	X									
Laboratory parameters[4]	X	X	X		X			X	X	
Toxicity assessment	X	X		X	X			X	X	
CT or MRI scan of chest, abdomen and pelvis with IV and +/- oral contrast[5]	X					X	X		X[5]	X
Bone imaging (bone scan or PET/CT)	X[6]									X[6]
Rev Add6 OPTIONAL Bone imaging (dual energy x-ray absorptiometry)[16]	X						X			
Tumor Biopsy	X[12]									X[17]
Partial or radical nephrectomy[9]				X						

Therapeutic Parameters Table										
Procedures	Baseline[2]	Day 1, Neoadjuvant Cycle(s)[1,8]	Pre-Surgery	Surgery	Day 1, Adjuvant Cycles[1,8]	Month 4.5 (Week 20)[1]	Month 9 (Week 40)[1]	Within 6 Weeks after Final Treatment	Follow-up[1,11]	Recurrence
Pregnancy Testing[7]	X	X[7]			X[7]					
Nivolumab administration[8]		X			X					
QOL	X[10]									
Survival									X	
EKG, echocardiogram	X[14]									

1. Cycles are 4 weeks long (28 days). Dosing window is +/-3 business days. Months 4.5 (Week 20), 9 (Week 40), and all follow-up time-points are to be calculated as weeks/months from the date of randomization.
   - Prior to Amendment 4, there will be 2 neoadjuvant cycles (doses) and up to 12 adjuvant cycles (doses)
   - After Amendment 4, there will be only 1 neoadjuvant cycle (dose) and up to 9 adjuvant cycles (doses)

Rev Add8 2. Eligibility parameters must be done and resulted within 8 weeks prior to randomization. Biopsy results must be available prior to neoadjuvant nivolumab dose. Hepatitis test results must be available prior to randomization. Baseline QOL will be administered per Footnote 10.

Rev Add5
Rev. Add8 3. Patients will require HBV and HCV testing utilizing local consent procedures for this collection. Results must be available prior to randomization. For patients that are positive for Hep B core antibody, hepatitis B surface antigen (HBsAg) should be negative. For patients that are positive for Hep C antibody, polymerase chain reaction (PCR) should be negative. These tests could be repeated later during the course of the study if clinically indicated.

4. On site (Local) Laboratories include:
   - **Baseline labs within 8 weeks prior to randomization:** CBC (wbc with differential, hemoglobin and platelet count), CMP (Na, K, Cl, CO2 or HCO3, BUN, creatinine, glucose), LDH, liver function tests (AST, ALT, total bilirubin, alkaline phosphatase), serum calcium, albumin, total protein; cortisol, TSH, free T4 (only required if TSH is abnormal), amylase, lipase, PT/INR, PTT*
     - *PTT is only required for patients on heparin.
   - **Every on-study visit after randomization (within 72 hours before dose) and within 6 weeks after final treatment:** CBC (wbc with differential, hemoglobin and platelet count), CMP (Na, K, Cl, CO2 or HCO3, BUN, creatinine, glucose), LDH*, liver function tests (AST, ALT, total bilirubin, alkaline phosphatase), serum calcium, albumin, total protein.
     - *LDH is only required at imaging time-points during on-study time-points.
     - CBC and CMP labs must be reviewed prior to treatment on Day 1 of each cycle. If other labs are drawn, they need to be reviewed when resulted but do not need to hold dosing for their result.
     - Amylase and lipase are to be performed only as clinically indicated.
     - PT/INR and PTT are to be performed only as clinically indicated.

- **Cycle 1 Day 1 (neoadjuvant dose), Cycle 2 Day 1 (first adjuvant dose), Cycle 5 Day 1 (fourth adjuvant dose), Cycle 8 Day 1 (seventh adjuvant dose) (within 72 hours before dose):** TSH, free T4 (only required if TSH is abnormal)

Rev Add7

- **Pre-surgery labs (after the neoadjuvant dose of nivolumab but within 5 business days prior to surgery):** CBC with diff, CMP (Na, K, Cl, CO2 or HCO3, BUN, creatinine, glucose), TSH, free T4 (only required if TSH is abnormal), PT/INR, PTT*, cortisol**
  - *PTT is to be performed for patients on heparin.
  - **Cortisol is to be performed as clinically indicated.
- **Follow-up[11] Labs:** LDH*, CMP**, CBC***
  - *LDH is to be performed at follow-up time-points prior to year 6, then as clinically indicated during year 6-10 follow-up visits.
  - **CMP is to be performed at follow-up time-points prior to year 6, then as clinically indicated during year 6-10 follow-up visits.
  - ***CBC is to be performed as clinically indicated.
- **Laboratory windows:** if treatment is delayed, they must be repeated within the noted window
  - On-therapy visits: within 72 hours prior to nivolumab dose
  - Pre-surgical labs: within 5 business days before surgery
  - Follow-up[11] labs (the first follow-up visit should be within 6 weeks after the final dose of nivolumab. Long- term follow-up labs +/-35 days of protocol dictated date).

Rev Add8

5. IV contrast is required unless contraindicated. The use of oral contrast is optional. Scan schedule should remain the same even if doses have been omitted. Scans can be obtained +/- 7 business days, with the exception of baseline scans, which must be done within 8 weeks prior to randomization.

   Scan schedule: (Until recurrence)

   Year 1: 4.5 and 9 months (approximately weeks 20 and 40 from randomization)

   Year 2: q 6 months: 15 and 21 months from randomization

   Year 3: q 6 months: 27 and 33 months from randomization

   Years 4-5: annually: 39 and 51 months from randomization

   NOTE: Pelvis imaging is only required at baseline and at recurrence per protocol. Pelvis imaging outside of these time-points should be performed as clinically indicated. Images should be uploaded into TRIAD. For images demonstrating recurrence or progression, scans should be uploaded to the “Recurrent/progression” time point.

   NOTE: CT or MRI scans of chest and abdomen are to be performed as clinically indicated in follow-up 6-10 years from randomization. Images should be uploaded into TRIAD.

   Digital copies of all imaging studies are to be submitted via upload to TRIAD™ per Section 4.1.4.6. If you have any technical questions, please send an e-mail to the TRIAD Support mailbox at TRIAD-Support@acr.org. If you have questions related to what imaging is to be submitted and under what time point you can email EA8143@acr.org.

6. Bone imaging (e.g. bone scan or PET/CT) is only required if elevated alkaline phosphatase or if clinician believes it is warranted due to bone pain/symptoms. Images should be uploaded into TRIAD under “unscheduled”.

Rev. Add10 7. Beta HCG pregnancy testing can be done by blood or urine and is required only for patients of childbearing potential. Pregnancy testing is to be done within 2 weeks prior to randomization. Pregnancy testing is to be done within 24 hours prior to the first dose of nivolumab. Pregnancy testing by blood or urine must also be performed at least every 4 weeks while on nivolumab therapy but the dose that day does not need to be held prior to result.

Rev Add5 8. Nivolumab administration:

- Neoadjuvant dose: nivolumab must be administered within 28 days after randomization.
- Nivolumab x 1 dose intravenously within 7-28 days prior to partial or radical nephrectomy
- Adjuvant dosing: The first dose of nivolumab in adjuvant setting must be within 4-10 weeks of the last resection or local treatment
- Nivolumab every 4 weeks intravenously post partial or radical nephrectomy
- Doses must be given within +/- 3 business day window (holidays and weekends are exempted). Otherwise they should be omitted. Patient should stay on schedule as dictated by the study calendar as if omitted dose was received.

NOTE: Please refer to Section 5.1.1.1 for the administration schedule of perioperative nivolumab for patients enrolled prior to the activation of Amendment #4.

9. Nephrectomy (partial or radical, or initial local treatment of metastatic disease) must be done between 7-28 days after neoadjuvant dose of nivolumab.
10. Please refer to Section 5.9 for detailed instructions on the administration of the QOL forms.
11. Patients will be followed for 10 years past their date of randomization. Once the patient is in long-term follow-up after 6 weeks post treatment visit, except those found not to have RCC at biopsy or nephrectomy, these procedures/assessments must be performed every 3 months for patients < 2 years from randomization; every 6 months for patients 2-5 years from randomization; and every 12 months for patients 6-10 years from randomization. Please note that survival information may be obtained by any means allowable by site's IRB (e.g., can be obtained by phone call if this is allowed by IRB). There are no specific requirements for follow-up if patient is >10 years from study entry. At the time of important analyses, additional updates to survival status may be requested. Continue to follow footnote 5 and 6 for additional CT or MRI scan of chest, abdomen, and pelvis with IV +/- oral contrast time-points. Continue to follow footnote 15 for History and Physical Examination guidelines. Follow Section 4.1.4.6 in relation to imaging for follow-up.
    - These procedures are not required after a patient has demonstrated recurrent disease per Section 6; however, submission of forms per the forms submission schedule is still mandatory. Telephone confirmation of survival post-recurrence is sufficient. QOL measures are required post-recurrence at time-points per Section 5.9 and footnote 10. Continue to follow footnote 5 for CT or MRI scan of chest, abdomen and pelvis time-points.

Rev. Add8 12. Core needle biopsy for histology confirmation of RCC is mandatory for patients randomized to Arm A with results available prior to the neoadjuvant dose. If patient has had biopsy (core or other type) within twelve (12) months prior to randomization that confirms RCC, they do not need to undergo a repeat biopsy. Non-diagnostic biopsies are considered a good faith effort and patients do not need to repeat biopsy unless deemed clinically necessary by treating physician. If the biopsy performed following randomization clearly demonstrates a benign condition, oncocytoma or a different type of cancer that is not RCC, the patient is not eligible and must come off study.
13. Please refer to Appendix X for instructions regarding how to measure waist circumference. This parameter is to be completed at baseline and at recurrence or off-study.

14. EKG and echocardiograms should be conducted as clinically indicated per the investigator's discretion for any patients with a history of CHF or at risk because of underlying cardiovascular disease or exposure to cardiotoxic drugs. For patients with evidence of CHF, MI, cardiomyopathy, or myocarditis, further cardiac evaluation, lab tests and cardiology consultations, including EKG, CPK, troponin, and echocardiogram, should be conducted as clinically indicated.

Rev Add8
Rev Add6

15. History and physical examinations are to be conducted every 3 months if less than 2 years from randomization, every 6 months if 2-5 years from randomization, then every 12 months during year during 6-10 years post- randomization. Physical examinations are conducted as clinically indicated once the patient reaches year 6.

Rev Add7

16. Optional test: Areal bone mineral density will be quantified by dual energy X-ray absorptiometry (DXA) at the spine, hip, femoral neck and distal 1/3 radius in research participants receiving nivolumab as well as observation arm at baseline and week 40. Note that if patient consented, 40 week DXA should be completed only if baseline DXA completed. Images should be uploaded to TRIAD.

17. Any recurrence of malignant disease should be proven by core needle biopsy or resection whenever possible. If there is evidence of recurrence see Section 6.1 for guidelines.

7.3 Arm B: Observation

Therapeutic Parameters Table												
Procedures	Baseline[1]	Pre-surgery	Surgery	Within 6 Weeks after Surgery	Week 12[13, 14]	Week 15[13, 14]	Month 4.5 (Week 20)[13, 14]	Week 30[13, 14]	Month 9 (Week 40[13, 14])	Week 54[13, 14]	Follow-up[9, 13]	Recurrence
History and Physical examination	X			X							X[14]	X
Waist Circumference[10]	X											X
Medication review	X			X								X
ECOG performance status	X			X								X
Height	X											
Vital signs including BP, HR, temperature, weight	X			X								
HCV, HBV[3]	X											
Laboratory parameters[2]	X	X		X	X		X		X	X	X	
Toxicity assessment	X	X		X	X		X		X	X		
Telephone call for symptom assessment[12]						X		X				
CT or MRI scan of chest, abdomen and pelvis with IV and +/- oral contrast[4]	X						X		X		X	X
Bone Imaging (bone scan or PET/CT)	X[5]											X[5]
Rev Add6 OPTIONAL Bone imaging (dual energy x-ray absorptiometry)[16]	X								X			
Tumor Biopsy	X[15]											X[17]

Therapeutic Parameters Table												
Procedures	Baseline[1]	Pre-surgery	Surgery	Within 6 Weeks after Surgery	Week 12[13, 14]	Week 15[13, 14]	Month 4.5 (Week 20)[13, 14]	Week 30[13, 14]	Month 9 (Week 40[13, 14])	Week 54[13, 14]	Follow-up[9, 13]	Recurrence
Partial or radical nephrectomy[7]			X									
Pregnancy Testing[6]	X											
QOL	X[8]											
Survival											X	
EKG, echocardiogram	X[11]											

Rev Add5

1. Eligibility parameters must be done within ≤ 8 weeks prior to randomization. Baseline QOL will be administered per Footnote 8.
2. On site (Local) laboratories at baseline, pre-surgery, 6 weeks after surgery and from date of randomization 12, 20, 40, 54 weeks include (+/-7 business days of planned visit):
   - **Baseline labs:** CBC (wbc with differential, hemoglobin and platelet count), Chemistries (Na, K, Cl, CO_2 or HCO3, BUN, creatinine, glucose), LDH, Liver function tests (AST, ALT, total bilirubin, alkaline phosphatase), serum calcium, albumin, total protein; cortisol, TSH, free T4 (only required if TSH is abnormal), amylase, lipase, PT/INR, PTT*
     - *PTT is to be performed for patients on heparin.
   - **Pre-surgery labs:** CBC with diff, CMP (Na, K, Cl, CO_2 or HCO3, BUN, creatinine, glucose), TSH*, free T4 (only required if TSH is abnormal), PT/INR, PTT**, cortisol***. The preoperative labs should be done within 5 business days prior to surgery. Baseline labs can be used if they meet this 5 day window.
     - *TSH is to be performed as clinically indicated.
     - **PTT is to be performed for patients on heparin.
     - ***Cortisol is to be performed as clinically indicated.
   - **Within 6 weeks after surgery, and from the date of randomization: weeks 12, 20, 40, 54:** CBC (wbc with differential, hemoglobin and platelet count), Chemistries (Na, K, Cl, CO_2 or HCO3, BUN, creatinine, glucose), LDH*, Liver function tests (AST, ALT, total bilirubin, alkaline phosphatase), serum calcium, albumin, total protein. TSH**, free T4 (only required if TSH is abnormal), amylase***, lipase***.
     - *LDH is only required at imaging time-points
     - **TSH is to be performed as clinically indicated.
     - ***Amylase and lipase are to be performed as clinically indicated.
     - If the “Within 6 Weeks after Surgery” and “Week 12” time-points overlap, then one lab blood draw is sufficient to cover both.
   - **Follow-up[9] labs** [(after 54 weeks): +/-35 days of protocol dictated date]: LDH*, CMP**, CBC***
     - *LDH is to be performed at follow-up time-points prior to year 6, then only as clinically indicated during year 6-10 follow-up visits.

- **CMP is to be performed at follow-up time-points prior to year 6, then only as clinically indicated during year 6-10 follow-up visits.
- ***CBC is to be performed only as clinically indicated.

Rev. Add8 3. Patients will require HBV and HCV testing utilizing local consent procedures for this collection. Results must be available prior to randomization. For patients that are positive for Hep B core antibody, hepatitis B surface antigen (HBsAg) should be negative. For patients that are positive for Hep C antibody, polymerase chain reaction (PCR) should be negative. These tests could be repeated later during the course of the study if clinically indicated.

4. IV contrast is required unless contraindicated. The use of oral contrast is optional. Scans can be obtained +/- 7 business days, with the exception of baseline scans, which must be done within 8 weeks prior to randomization.

   Scan schedule: (Until recurrence)

   Year 1: 4.5 and 9 months (approximately weeks 20 and 40 post- randomization)

   Year 2: q 6 months: 15 and 21 months from randomization

   Year 3: q 6 months: 27 and 33 months from randomization

   Years 4-5: annually: 39 and 51 months from randomization

   NOTE: Pelvis imaging is only required at baseline randomization at recurrence per protocol. Pelvis imaging outside of these time-points should be performed as clinically indicated. Any imaging performed should be uploaded into TRIAD. Recurrent imaging should be uploaded under “unscheduled visit.” For images demonstrating recurrence or progression, scans should be uploaded to the “Recurrent/progression” time point.

   NOTE: CT or MRI scans of chest and abdomen are to be performed as clinically indicated in follow-up 6-10 years post-randomization. Images should be uploaded into TRIAD.

   Digital copies of all imaging studies are to be submitted via upload to TRIAD™ per Section 4.1.4.6. If you have any technical questions, please send an e-mail to the TRIAD Support mailbox at TRIAD-Support@acr.org. If you have questions related to what imaging is to be submitted and under what time point you can email EA8143@acr.org

Rev Add5 5. Bone imaging (e.g. bone scan or PET/CT) is only required if elevated alkaline phosphatase (if >ULN local range) or if clinician believes it is warranted due to bone pain/symptoms and all images should be uploaded into TRIAD under “unscheduled.”

Rev. Add10 6. Beta HCG pregnancy testing by blood or urine is required for patients of childbearing potential. Pregnancy testing is to be done within 2 weeks prior to randomization.

7. Nephrectomy (partial or radical) or initial local treatment of metastasis must be done within 8 weeks after randomization.

8. Please refer to Section 5.9 for detailed instructions on the administration of the QOL forms.

9. Patients will be followed for 10 years past their date of randomization. Once the patient is in long-term follow-up, which is defined as after week 54 (post randomization), these procedures/assessments must be performed every 3 months for patients < 2 years from randomization; every 6 months for patients 2-5 years from randomization; and every 12 months for patients 6-10 years from randomization. Please note that survival information may be obtained by any means allowable by site’s IRB (i.e., can be obtained by phone call if this is allowed by IRB). There are no specific requirements for follow-up if patient is >10 years from study entry. At the time of important analyses, additional updates to survival status may be requested. Continue to follow footnote 4 for additional CT or MRI scan of chest, abdomen, and pelvis with IV +/- oral contrast time-

points. Continue to follow footnote 14 for History and Physical Examination requirements. Follow Section 4.1.4.6 in relation to imaging for follow-up.

- These procedures are not required after a patient has demonstrated recurrent disease per Section 6; however, submission of forms per the forms submission schedule is still mandatory. Telephone confirmation of survival post-recurrence is sufficient. QOL measures are required post-recurrence at time-points per Section 5.9 and Footnote 8. Continue to follow footnote 4 for CT or MRI scan of chest, abdomen and pelvis time-points.
- These procedures are not required for the patients who are found to not have RCC at biopsy or nephrectomy.

10. Please refer to Appendix X for instructions regarding how to measure waist circumference. This parameter is to be completed at baseline, and at recurrence or off-study.
11. EKG and echocardiograms should be conducted as clinically indicated for any patients with a history of CHF or at risk because of underlying cardiovascular disease or exposure to cardiotoxic drugs. For patients with evidence of CHF, MI, cardiomyopathy, or myocarditis, further cardiac evaluation, lab tests and cardiology consultations, including EKG, CPK, troponin, and echocardiogram, should be conducted as clinically indicated.
12. Telephone assessments can be done +/- 7 business days from scheduled time-point.
13. Week 12, Week 15, Month 4.5 (Week 20), Week 30, Month 9 (Week 40), Week 54, and all additional follow-up time-points are to be calculated as weeks/months from the date of randomization.
14. History and physical examinations are to be conducted every 3 months if less than 2 years from randomization, every 6 months if 2-5 years from randomization, then every 12 months during 6-10 years post- randomization. Physical exams are to be conducted as clinically indicated once the patient reaches year 6.
15. Baseline core needle biopsy is encouraged but not mandatory for patients who have not had a biopsy (core or other type) within twelve (12) months prior to randomization that confirms RCC.
16. Rev Add5 Rev Add7 Optional test: Areal bone mineral density will be quantified by dual energy X-ray absorptiometry (DXA) at the spine, hip, femoral neck and distal 1/3 radius in research participants receiving nivolumab as well as observation arm at baseline and week 40. Note that if patient consented, 40 week DXA should be completed only if baseline DXA completed images should be uploaded to TRIAD.
17. Any recurrence of malignant disease should be proven by core needle biopsy or resection whenever possible. See guidelines section 6.1. Submission of biopsy is strongly encouraged but not mandatory.

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7.4 Biological Specimen Submissions

Specimens are to be submitted as outlined in Section 10.

All specimens submitted must be entered and tracked via the online ECOG-ACRIN Sample Tracking System (STS).

Biological Materials	Prior to Start of Treatment	Prior to Nephrectomy[9]	Nephrectomy	Month 9 (Week 40 Post Randomization)	Recurrence	Submit to:
MANDATORY for Central Diagnostic Review and Defined Laboratory Research Studies:						
Diagnostic Primary Tumor Tissue	X[8] (Arm A required and Arm B if collected)		X (Both Arms)[12]			CBPF
From patients who answer "Yes" to "I agree to have my samples collected and I agree that my samples and related information may be used for laboratory studies."						
Tumor Tissue[2]			X (Both Arms)		X (Both Arms)	CBPF
Rev. Add6 Serum (two 8.5mL SST red top tubes)[1]	X (Arm A Only)	X (Both Arms)		X (Both Arms)	X (Both Arms)	
From patients who answer "Yes" to "I agree to provide additional specimens for research."						
Tumor Tissue[11]	X (Both Arms)					CBPF
Peripheral Blood (ten 10mL green top heparin tubes)[1]	X (Arm A Only)	X (Both Arms)		X (Both Arms)	X (Both Arms)	
Plasma (two 10mL EDTA purple top tubes)[1]	X (Arm A Only)	X (Both Arms)		X (Both Arms)	X (Both Arms)	

Biological Materials	Cycle 1, Day 1 (pre-dose)[4,]	Cycle 2, Day 1 (pre-dose)[4]	Cycle 2, Day 1 (EOI-nivo)[5]	Cycle 3, Day 1 (pre-dose)[4]	Cycle 5, Day 1 (pre-dose)[4]	Cycle 5, Day 1 (EOI-nivo)[5]	Cycle 7, Day 1 (pre-dose) [4]	Cycle 7, Day 1 (EOI-nivo)[5]	First 2 Follow-Up Visits[7]
MANDATORY (Arm A Only) for Pharmacokinetic and Immunogenicity Studies: Serum SST vacutainer tubes)[3,6, 10]	1 – 8.5mL SST	1 – 8.5mL SST	1 – 4mL SST	1 – 8.5mL SST	1 – 8.5mL SST	1 – 4mL SST	1 – 8.5mL SST	1 – 4mL SST	1 – 8.5mL SST

CBPF = Central Biorepository and Pathology Facility

1. Kits are being provided for the collection and shipment of the blood specimens. See Appendix VI for instructions. Kit orders will on average be delivered within three (3) business days from the time the order is placed.

Rev Add5 2. Additional surgical FFPE primary tumor and normal tissue blocks, and recurrence FFPE primary tumor tissue blocks, if available, are requested from consenting patients who answer "Yes" to "*I agree to have my samples collected and I agree that my samples and related information may be used for laboratory studies.*"

3. If patient discontinues study drug treatment during the sampling period, they will move to sampling at the follow-up visits.

4. All pre-dose blood specimens should be collected prior to the start of nivolumab infusion (anytime within 24 hours prior to first dose).

5. EOI-nivo = End of Nivolumab Infusion. These serum specimens should be collected prior to stopping the nivolumab infusion (preferably within 2 minutes prior to end of infusion but within 15 minutes of the end of infusion). If the infusion takes longer than expected, the collection at the end of infusion should be adjusted accordingly.

6. Kits are being provided for the collection and shipment of the serum specimens for the pharmacokinetic and immunogenicity studies. See Section 10.3 for instructions.

Rev. Add8 7. First (1st) follow-up visit approximately six (6) weeks post discontinuation of nivolumab. Second (2nd) follow-up visit approximately three months from first follow-up visit. Note that specimens should be collected even if the visit is delayed.

Rev Add6
Rev. Add8 8. In patients randomized to Arm A, diagnosis of RCC must be confirmed by core biopsy after randomization unless the patient had a prior biopsy confirming RCC within twelve (12) months prior to randomization in order to avoid exposing patients to neoadjuvant nivolumab who clearly have a benign lesion or another type of cancer. A non-diagnostic biopsy is considered good faith effort and patient may proceed with treatment at investigator and site discretion. Pre-surgery biopsy tumor tissue (primary tissue is preferred if metastasis is also present) and related pathology reports must be submitted for central diagnostic review and defined laboratory research studies within one (1) month following randomization or collection as outlined in Section 10. Patients randomized to Arm B are encouraged, but not required, to have a core biopsy after randomization if they have not had a biopsy within twelve (12) months of randomization that confirms diagnosis of RCC. Biopsy tumor tissue (primary tissue is preferred if metastasis is also present) and related pathology reports must be submitted if collected for central diagnostic review and defined laboratory research studies within one (1) month following randomization or collection as outlined in Section 10.

9. For Arm B patients- serum, peripheral blood, and plasma are to be collected Prior to Nephrectomy. For Arm A patients- serum, peripheral blood and plasma are to be collected after Cycle one (1) but Prior to Nephrectomy.

10. Patients on treatment prior to amendment #4 should follow new PK schedule.

11. Additional representative formalin-fixed paraffin embedded (FFPE) primary tumor tissue blocks from pre-trial diagnostic biopsy is requested from patients who answer "Yes" to "*I agree to provide additional specimens for research*."

Rev. Add8 12. Surgical FFPE primary tumor tissue blocks must be submitted from patients on BOTH ARMS A and B for central diagnostic review along with related pathology reports within one (1) month following collection.

8. Drug Formulation and Procurement

This information has been prepared by the ECOG-ACRIN Pharmacy and Nursing Committees.

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Availability

NCI -supplied agents may be requested by eligible participating investigators (or their authorized designee) at each participating institution. The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The eligible participating investigators at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), NCI Biosketch, Agent Shipment Form, and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead participating investigator at that institution.

Submit agent requests through the PMB Online Agent Order Processing (OAOP) application. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account and the maintenance of an “active” account status, a “current” password, and active person registration status. For questions about drug orders, transfers, returns, or accountability, call or email PMB any time. Refer to the PMB’s website for specific policies and guidelines related to agent management.

NCI Supplied Agent(s) – General Information

NOTE: Under no circumstances can commercially supplied nivolumab (OPDIVO) be used or substituted for the NCI-supplied **nivolumab**.

NOTE Sites may order initial agent supplies when a subject is being screened for enrollment onto the study.

Questions about drug orders, transfers, returns, or accountability should be addressed to the PMB by calling 240-276-6575 Monday through Friday between 8:30 AM and 4:30 PM Eastern Time or email PMBAfterHours@mail.nih.gov anytime.

Drug Returns: All unused drug supplies must be returned to the PMB. When it is necessary to return study drug (e.g., sealed vials remaining when a patient permanently discontinues protocol treatment, expired vials recalled by the PMB), investigators must return the study drug to the PMB using the NCI Return Agent Form available on the NCI home page (http://ctep.cancer.gov) or by calling the PMB at 240-276-6575.

Drug Accountability: The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, disposition, and return of all drugs received from the PMB using the NCI Investigational Agent Accountability Record available on the NCI home page (http://ctep.cancer.gov) or by calling the PMB at 240-276-6575.

Useful Links and Contacts

- CTEP Forms, Templates, Documents: http://ctep.cancer.gov/forms/
- NCI CTEP Investigator Registration: RCRHelpDesk@nih.gov
- PMB policies and guidelines: http://ctep.cancer.gov/branches/pmb/agent_management.htm

- PMB Online Agent Order Processing (OAOP) application: https://ctepcore.nci.nih.gov/OAOP
- CTEP Identity and Access Management (IAM) account: https://ctepcore.nci.nih.gov/iam/
- CTEP IAM account help: ctepreghelp@ctep.nci.nih.gov
- IB Coordinator: IBCoordinator@mail.nih.gov
- PMB email: PMBAfterHours@mail.nih.gov

PMB phone and hours of service: (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET)

Investigator Brochure Availability

The current versions of the IBs for the agents will be accessible to site investigators and research staff through the PMB OAOP application. Access to OAOP requires the establishment of a CTEP IAM account and the maintenance of an "active" account status, a "current" password and active person registration status. Questions about IB access may be directed to the PMB IB Coordinator via email.

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8.1 Nivolumab

8.1.1 Amino Acid Sequence:

4 polypeptide chains, which include 2 identical heavy chains with 440 amino acids and 2 identical light chains.

8.1.2 Other Names:

BMS-936558, MDX1106

8.1.3 Classification:

Anti-PD-1MAb

8.1.4 M.W.:

146,221 Daltons

8.1.5 Mode of Action:

Nivolumab targets the programmed death–1 (PD-1, cluster of differentiation 279 [CD279]) cell surface membrane receptor. PD-1 is a negative regulatory receptor expressed by activated T and B lymphocytes. Binding of PD-1 to its ligands, programmed death–ligand 1 (PD-L1) and 2 (PD-L2), results in the down-regulation of lymphocyte activation. Nivolumab inhibits the binding of PD-1 to PD-L1 and PD-L2. 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.

8.1.6 Description:

Nivolumab Injection is a clear to opalescent, colorless to pale yellow liquid; light (few) particulates may be present. The drug product is a sterile, nonpyrogenic, single-use, isotonic aqueous solution formulated in sodium citrate dihydrate, sodium chloride, mannitol, diethylenetriaminepentacetic acid (pentetic acid), polysorbate 80 (Tween® 80), and water for injection. Dilute solutions of hydrochloric

acid and/or sodium hydroxide may be used for pH adjustment (pH 5.5-6.5).

8.1.7 How Supplied:

Nivolumab is supplied by Bristol-Myers Squibb and distributed by the Pharmaceutical Management Branch, CTEP/DCTD/NCI as 100 mg vials (10 mg/mL) with a 0.7mL overfill. It is supplied in 10 mL type I flint glass vials, with fluoropolymer film-laminated rubber stoppers and aluminum seals.

8.1.8 Preparation:

Nivolumab injection can be infused undiluted (10 mg/mL) or diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose. When the dose is based on patient weight (i.e., mg/kg), nivolumab injection can be infused undiluted or diluted to protein concentrations as low as 0.35 mg/mL. When the dose is fixed (eg, 240 mg, 360 mg, or 480 mg flat dose), nivolumab injection can be infused undiluted or diluted so as not to exceed a total infusion volume of 160 mL. For patients weighing less than 40 kilograms (kg), the total volume of infusion must not exceed 4 mL per kg of patient weight. During drug product preparation and handling, vigorous mixing or shaking is to be avoided.

Nivolumab infusions are compatible with polyvinyl chloride (PVC) or polyolefin containers and infusion sets, and glass bottles.

8.1.9 Storage:

Vials of Nivolumab injection must be stored at 2°- 8°C (36°- 46°F) and protected from light and freezing. The unopened vials can be stored at room temperature (up to 25°C, 77°F) and room light for up to 48 hours.

If a storage temperature excursion is identified, promptly return Nivolumab to 2°C-8°C and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to PMBAfterHours@mail.nih.gov for determination of suitability.

8.1.10 Stability:

Shelf-life surveillance of the intact vials is ongoing.

The administration of undiluted and diluted solutions of Nivolumab must be completed within 24 hours of preparation. If not used immediately, the infusion solution may be stored up to 24 hours in a refrigerator at 2°-8°C (36°-46°F) and a maximum of 8 hours of the total 24 hours can be at room temperature (up to 25°C, 77°F) and room light. The maximum 8-hour period under room temperature and room light conditions includes the product administration period.

CAUTION: The single-use dosage form contains no antibacterial preservative or bacteriostatic agent. Therefore, it is advised that the product be discarded 8 hours after initial entry.

8.1.11 Route of Administration

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Intravenous infusion over 30 minutes +/- 5 minutes. Do not administer as an IV push or bolus injection.

8.1.12 Method of Administration

Administer through a 0.2 micron to 1.2 micron pore size, low-protein binding (polyethersulfone membrane) in-line filter.

8.1.13 Potential Drug Interactions:

The indirect drug-drug interaction potential of nivolumab was assessed using systemic cytokine modulation data for cytokines known to modulate CYP enzymes. There were no meaningful changes in cytokines known to have indirect effects on CYP enzymes across all dose levels of nivolumab. This lack of cytokine modulation suggests that nivolumab has no or low potential for modulating CYP enzymes, thereby indicating a low risk of therapeutic protein-drug interaction.

8.1.14 Patient Care Implications:

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Patients of childbearing potential receiving nivolumab must continue contraception for a period of 5 months after the last dose of nivolumab.

8.1.15 Side Effects

See Section 5.5 for the Comprehensive Adverse Event and Potential Risks (CAEPR) list.

8.1.16 References

LexiComp (2014), AHFS (2014)

9. Statistical Considerations

9.1 Design and Objectives

This study is a randomized comparison of neoadjuvant and adjuvant nivolumab given perioperatively to surgery alone.

9.1.1 Primary objective

To compare recurrence-free survival (RFS) between patients with renal cell carcinoma randomly assigned to perioperative nivolumab in conjunction with radical or partial nephrectomy with patients randomized to surgery alone.

9.1.2 Major secondary objectives

- To evaluate for differences in recurrence-free survival associated with perioperative nivolumab compared to surgery alone among the subset of patients with clear cell histology
- To compare the overall survival between the two arms
- To describe the safety and tolerability of the two arms

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9.1.3 Correlative objectives

- To correlate the primary tumor's expression of PD-L1 with outcome
- To correlate the expression of PD-L1 on tumor tissue at recurrence with outcome
- To archive images for central confirmation of recurrence and for future correlative work with ACRIN, including markers predicting outcome or response.
- To prospectively collect tumor and biologic specimens (e.g., serum, PBMCs) for future correlative studies.
- To characterize the pharmacokinetics of nivolumab and explore exposure response relationships with respect to safety and efficacy.
- To characterize the immunogenicity of nivolumab.

9.1.4 Quality of Life objective

- To evaluate differences in change from baseline in patient-reported symptoms and toxicities among patients treated with nivolumab compared to surgery alone.

9.1.5 Other exploratory objectives

- To explore descriptively the efficacy of treatment with nivolumab in patients with non-clear cell (including unclassified) histologies.

9.2 Endpoints

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9.2.1 Primary Endpoint

The primary endpoint is recurrence-free survival (RFS). RFS is defined as the time from randomization to disease recurrence or death from any cause. Patients who do not undergo surgery or whose

surgery does not render them disease-free or who have other evidence of disease after surgery will be included in the RFS analysis as events on Day 1. Patients without recurrence will be censored at the date of last disease evaluation. Patients are expected to discontinue protocol therapy and receive standard of care treatment (e.g., targeted therapy) or other clinical trial at the time residual disease is discovered. Patients who develop second primary cancers (but not secondary cancers thought due to nivolumab if on that arm) may continue protocol treatment at the discretion of their physician as long as they do not require systemic therapy for the treatment of that secondary cancer. Surgical resection, radiation (with the exception of to the renal bed), and other local therapies may be considered. Whether or not they remain on treatment, they must continue to be followed according to the schedule in the protocol to evaluate for recurrence of their renal cell cancer.

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9.2.2 Secondary endpoints

9.2.2.1 Overall survival, defined as time from randomization to death from any cause. Patients who have not died will be censored at date of last contact.

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9.2.2.2 RFS among patients with clear cell cancer

9.3 Statistical Analysis Plan

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A hierarchical testing plan will be used to evaluate the primary and major secondary endpoints. First RFS as defined above will be assessed among all randomized patients using a one-sided 2.5% test. If that test is significant, then the second test comparing RFS in the subset of clear cell histology will be performed at a one-sided 2.5% level. If both the first two tests are significant, then OS will be compared among all randomized patients using a one-sided 2.5% test. Finally, if the first 3 tests are significant, then OS will be compared in the clear cell subset, again at the one-sided 2.5% level. Details of the tests are given in the following subsections. If any comparison in the hierarchical sequence is not significant, the subsequent comparisons will still be performed, but will be regarded as exploratory.

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9.3.1 Primary Endpoint

The primary endpoint of RFS will be assessed among all randomized patients with RCC using a stratified log rank test, with nominal one-sided Type I error of 2.5%. The Type I error will be adjusted for interim analyses as described below.

Patients who are confirmed to not have RCC histology via biopsy or nephrectomy will be excluded from the primary analysis. The local site pathologist's determination will be used for the primary analysis. In the case of contradictory biopsy and nephrectomy results the nephrectomy histology will be used to determine inclusion. If the nephrectomy histology is poorly differentiated and the biopsy result can define the histology, then the biopsy result will be used. In the event that a patient does not have histology determination either by biopsy or nephrectomy, the case will be counted as unclassified RCC in the primary analysis. Blinded central review of histology will also be

Rev Add5

performed. The rates of agreement will be reported, and a sensitivity analysis based on central review results will be performed.

The removal of the biopsy requirement in Arm B introduces potential for bias in the identification of non-RCC patients between arms as patients may be randomized prior to pathologic diagnosis. Thus the rate of nephrectomy submission on Arm B will be closely monitored. Additional monitoring will occur to ensure an acceptable rate of non-diagnostic biopsies on Arm A (see Section 9.8.5).

The primary RFS endpoint definition here includes patients who do not have surgery or who have residual disease after surgery as events on day 1 (this is not a standard definition of 'RFS', since these patients have not been rendered free of disease). The potential impact of these early events on the results will be assessed by a sensitivity analysis where follow-up is censored on day 1 for these patients, instead of counting them as RFS events. Separate analyses will be done 1) censoring the patients who do not have surgery, 2) censoring the patients who have residual disease after surgery, and 3) censoring both groups.

Since this is not a placebo-controlled trial and the endpoint of RFS could also be subject to evaluation time bias,[24] several approaches will be employed to assess the robustness of the outcome. Every effort will be made to assure that patients undergo scans at scheduled time points, with scan intervals consistent between arms. Scans at baseline and progression will be archived for potential blinded future review. A sensitivity analysis will be done in which the dates of progression events detected at non-scan visits are carried forward in time to the next scheduled scan time. Another sensitivity analysis will compare the proportion of patients alive and recurrence-free at the 27-month post-randomization scan between the arms.

9.3.2 Secondary Endpoints

9.3.2.1 RFS among patients with clear cell histology

When 253 RFS events have been observed among patients with predominantly clear cell histology, the stratified logrank test will be used to evaluate the endpoint of RFS. We assume that 85% of patients will have clear cell histology and that the 5-year RFS among these patients is 55.8%, as for the general population. There will be adequate power (82%) to detect the same treatment difference in this subset as in the full population, using a one-sided stratified log rank test with Type I error of 2.5%.

The proportion of patients with clear cell histology will be monitored as the study is accruing to ensure balance between arms. Additionally, the proportion of patients randomized to each arm who do not have RCC will be monitored throughout. Patients proven to not have RCC will be discontinued from the study.

A sensitivity analysis of RFS among patients with recurrence documented by biopsy or cytology will be conducted.

9.3.2.2 Overall survival (OS)

Patients randomized to placebo on ASSURE had 5-year overall survival of 78.7%. Adequate power (80%) will exist to detect a 33% reduction in the survival hazard rate, among patients randomized to nivolumab. Power for this comparison using a one-sided 2.5% stratified log rank test will be available when 196 deaths have occurred, which is expected to be about 9 years after the start of the study.

At the time of the primary survival analysis, an analysis of disease-specific survival will be conducted.

9.3.2.3 Other Secondary Analyses

Adverse events will be described separately for patients treated on each arm to evaluate safety and tolerability.

- Analysis of Patient-Reported Outcomes

  Please see Section 12.

Rev. Add6

- Analyses of Correlative and exploratory Endpoints

  Please see Section 11.

Rev. Add4

9.4 Sample Size Considerations

The primary endpoint, as defined above, is recurrence-free survival (RFS). Although this endpoint will primarily be tested in the entire cohort, the study is designed to have adequate power to detect differences in RFS associated with treatment among the subset of patients with clear cell histology. The primary analysis population will include all patients with RCC as randomized. Patients who do not undergo surgery for any reason or whose primary tumor resection does not render them free of disease (e.g., residual tumor, positive margins) will be included in the RFS analysis as events on Day 1. Patients will be stratified on clinical stage (T2 vs. >T2), clinical nodal status (negative vs. positive), and clinical metastatic status (M0 or oligo M1).

Estimates of RFS among the eligible pool of patients for this study are based on ASSURE, the recently completed adjuvant study of sunitinib, sorafenib, or placebo. Patients randomized to placebo following curative resection had a 5-year DFS of 55.8%. The study is designed to demonstrate a 14.4% absolute improvement in 5-year RFS among patients disease-free post nephrectomy treated with nivolumab to 70.2%. We assume that the distribution is exponential with a constant failure (hazard) rate and that accrual is uniform following a brief start-up interval at study activation. We further assume that 10% of patients will either not undergo nephrectomy or will not be disease-free following nephrectomy; these patients will be included in the RFS analysis as events at Day 1 (“not NED” events). This rate is assumed to be the same on both arms, and the deleterious effect is assumed to be proportional in this subset. We also assume that, among the 90% of patients with adequate nephrectomy randomized to nivolumab,10% of patients will discontinue treatment early; the statistical effect

of early discontinuation will make the patient similar to patients on the observation arm--an effect similar to crossing over to observation. The 5-year RFS rates were weighted using proportions shown in the following table. After these adjustments, the effective 5-year RFS rates on the observation and nivolumab arms will be 50.2% and 61.9%, respectively.

Arm	Cohort	Proportion	Estimated 5-year RFS	Adjusted 5-year RFS
Nivolumab	Inadequate / no nephrectomy	10%	0 (all events on Day 1)	61.884%
	Adequate nephrectomy, discontinue treatment early	9%	55.8% (null rate)	
	Adequate nephrectomy and treatment	81%	70.2%	
Observation	Inadequate or no nephrectomy	10%	0 (all events on Day 1)	50.2%
	Adequate nephrectomy	90%	55.8%	

We plan a 1:1 randomization and accrual of 264 patients per year (22 per month) for 2.9 years, with 2.6 additional years of follow-up. This represents a total of 766 RCC patients of all histologies; 383 randomized to each arm. Assuming interim analyses as described below and using a stratified log-rank test with one-sided Type I error of 2.5%, there will be approximately 84.2% power to detect the stated reduction in risk.

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The protocol had defined full information to be 285 RFS events, including “not-NED” events. In August 2020, after monitoring of the rate of not-NED events had occurred, it was observed that the proportion of patients with these events was a random quantity that might differ from the assumed rate in the final data. Since the “not-NED” events will be included in the analysis but are not expected to contribute to the power, this could affect the power of the final comparison. Under the assumptions above, 76 of the 285 events were expected to be not-NED events in the final analysis. The expected number of follow-up RFS events (recurrences and deaths) for full power was 209. Simulations showed that even if the proportion of subjects with not-NED events is as high as 15%, an analysis with 209 follow-up events would still give > 80% power, while an analysis with 285 total events would not. It was therefore decided to change the definition of information time to be based on the number of follow-up events only, and full information will now exist when 209 “follow-up” RFS events have been observed. The total number of events (counting both follow-up and not-NED events) for full information will also be capped at 325, under the assumption that at most 15% of the cases would have not-NED events. The not-NED events will still be included in the primary analyses.

Without a required pre-randomization biopsy, some patients may enroll who have benign lesions or other non-RCC cancers. We assume that this group will comprise no more than 5% of enrolled patients. These patients will be removed

from the study and the total accrual goal will be 805 total patients for a target of 766 RCC patients.

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9.5 Projected Accrual

The total expected accrual is 805 patients, at a projected rate of 22 patients per month.

ECOG-ACRIN's most recent adjuvant renal trial, E2805, accrued 1943 patients in 52 months, an overall average rate of 37 patients per month. S0931, the adjuvant everolimus study in RCC, has accrued all of its target 1170 patients, with an average rate of 22 patients per month. There is significant and wide interest in the experimental agent, nivolumab, so we expect equivalent accrual rates once sites are activated.

Rev. Add2
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9.6 Randomization Scheme

Randomization will be done using permuted blocks within stratum, where strata are defined as follows:

Clinical T Stage (clinical T1 or T2 vs. greater than clinical T2)

Clinical Nodal Status (cN0 vs. cN+)

Clinical Metastatic Status (cM0 or cM1): definition of permitted oligometastatic M1 disease detailed in eligibility criteria.

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9.7 Gender and Ethnicity

Based on previous data from E2805, the anticipated accrual in subgroups by gender and race is:

Racial Categories	**Ethnic Categories**				**Total**
	Not Hispanic or Latino		**Hispanic or Latino**		
	Female	**Male**	**Female**	**Male**	
American Indian/ Alaska Native	1	3	0	0	**4**
Asian	3	14	0	0	**17**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	18	19	0	0	**37**
White	228	478	11	30	**747**
More Than One Race	0	0	0	0	**0**
Total	**250**	**514**	**11**	**30**	**805**

The accrual targets in individual cells are not large enough for definitive treatment comparisons to be made within these subgroups. Therefore, overall accrual to the study will not be extended to meet individual subgroup accrual targets.

9.8 Monitoring Procedures and Interim Analyses

9.8.1 DSMC

The ECOG-ACRIN Data Safety Monitoring Committee will review accumulating safety data at each semiannual meeting starting with the first enrollment and continuing through release of the study for analysis.

This study will be monitored by the ECOG-ACRIN Data Safety Monitoring Committee (DSMC). The DSMC meets twice each year. For each meeting, all monitored studies are reviewed for safety and progress toward completion. When appropriate, the DSMC will also review interim analyses of outcome data. Copies of the toxicity reports prepared for the DSMC meetings are included in the study reports prepared for the ECOG-ACRIN group meeting (except that for double blind studies, the DSMC may review unblinded toxicity data, while only pooled or blinded data will be made public). These group meeting reports are made available to the local investigators, who may provide them to their IRBs. Only the study statistician and the DSMC members will have access to interim analyses of outcome data. Prior to completion of this study, any use of outcome data will require approval of the DSMC. Any DSMC recommendations for changes to this study will be circulated to the local investigators in the form of addenda to this protocol document. A complete copy of the ECOG-ACRIN DSMC Policy can be obtained from the ECOG-ACRIN Operations Office.

9.8.2 Interim Analysis for Harm and Inefficacy

The study will be monitored for harm and inefficacy with early stopping rules. At 25% information, the DSMC may consider recommending that the study stop for harm if the lower bound of a 95% confidence interval on the hazard ratio (nivolumab/observation) is above 1.0. The inefficacy monitoring approach, starting at 44% information, is described by Freidlin et al.[24,39] The study will be stopped if there is not at least a small trend in favor of the alternative hypothesis starting at this time. The inefficacy analysis will be done at each subsequent semi-annual meeting of the ECOG ACRIN Data Safety Monitoring Committee (DSMC) if there has been at least a 10% increment in the information. The DSMC will also monitor for any unusual harm/inefficacy signals in the non-clear cell subgroup.

9.8.3 Interim Analyses for Efficacy

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Rev. Add10

The protocol had planned interim efficacy analyses beginning at 34% of the planned full information and continuing every 6 months after that. In Fall 2020, it was decided to drop the early efficacy analyses, since mature follow-up would be needed to adequately interpret the effects of treatment. Interim efficacy analysis schedule was therefore switched to plan analyses at only 65% and 85% of full information. To correspond to the semiannual ECOG-ACRIN DSMC meeting schedule, the analyses will be performed for the first meeting where this amount of information is available. Significance levels at each

analysis will be determined from the O'Brien-Fleming error spending rate function using the actual information times.

The error spending plan is summarized in the following table. Because interim analyses are timed to coincide with meetings of the DSMC, the exact boundary value and its associated nominal significance will be based on the number of events at the time of the analysis.

Analysis	Information Proportion	Follow-up Events	Upper Boundary	Nominal Significance
1	0.65	136	2.55	0.005
2	0.85	178	2.21	0.013
3	1.00	209	2.05	0.020

9.8.4 Rates of Incomplete Resection and Treatment Discontinuation

At each semiannual meeting of the DSMC, the rates of incomplete resection and treatment discontinuation will be reviewed. Specifically, the following will be reviewed:

- Proportion of patients on each arm with residual local disease or metastatic disease post-surgery
- Proportion of patients on each arm discontinuing treatment or observation immediately post-nephrectomy
- Proportion of patients not undergoing nephrectomy
- Proportion of patients with no cancer or cancer other than RCC at biopsy or nephrectomy.

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If at any interim analysis time as described above the lower bound of a 90% confidence interval on any of these rates is greater than 10%, the DSMC may recommend that the study's design be reviewed for continued feasibility. Any potential changes to the design (for example, increasing the study size to compensate for these patients) would occur prior to the first interim efficacy analysis.

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9.8.5 Rates of Nephrectomy Specimen Submission and Non-Diagnostic Biopsy

The lack of diagnostic biopsy requirement on Arm B creates a potential for imbalance between arms with respect to removal of non-RCC patients. Therefore, after 100 patients are enrolled on Arm B the rate of nephrectomy submission will be reported to CTEP. For every subsequent 100 patients enrolled to this arm, if the submission rate is below 95% the DSMC will be alerted and a corrective plan of action presented.

An analogous rule will apply to the rate of indeterminate biopsies on Arm A.

9.8.6 Adherence to Scan Schedule

Since the study is not placebo-controlled, it is possible that anxiety about possible recurrence could lead to more frequent scans on the

observation arm. To assess the study's robustness against potential observation time bias arising from inconsistencies in scan intervals between the two arms, the following sensitivity analyses will be considered. If the results of these analyses are consistent with those of the primary analysis, there will be more confidence that the results are not affected by observation time bias.

2-year RFS Proportion

An analysis of the proportion of patients alive and free of recurrence two years post-randomization will be conducted. Patients who are alive and have at least one scan on or after the 27-month scan (the closest scheduled scan to 2 years) will be considered event free; all other patients will be included as events. An appropriate stratified test will be used to compare the proportions. For example, if the assumption of a common odds ratio across strata can be supported, a Mantel Henszel test will be used. Otherwise a more conservative test, such as a stratified Fisher's exact test, may be necessary.

Carry Forward Unscheduled Scans

Another sensitivity analysis will establish windows around the protocol-specified scan time points (+/- 1 week). For scans done outside the acceptable window on which recurrence is detected, the date of recurrence will be carried forward to the next scheduled scan date. Then the stratified log-rank test will be used to compare RFS between arms.

9.8.7 Accrual Monitoring

Accrual will be monitored using NCI's Phase III Accrual guidelines. Specifically, if accrual is not occurring at 20% of the target rate by quarter 5, the study team may need to consider redesigning the study. If it is not accruing at 50% of the target rate by quarter 8, it may need to be closed.

9.8.8 Safety Monitoring

9.8.8.1 Surgical Complications

After the first 50 patients have been followed for 4 weeks post-surgery on both arms, an interim analysis of surgical complication rates will be conducted.

Based on previous data from E2805, 14% is the expected rate of surgical complications in the absence of an effect of neoadjuvant nivolumab. A true complication rate of 28% among the first 50 patients on an arm would be unacceptable. This trial will undergo a review if complications are reported for 9 or more patients among the first 50 patients on either arm. Potential outcomes of the review could be to modify the study, suspend accrual for further investigation, add a second review, continue as planned, or terminate the study.

9.8.8.2 Semiannual Reports

Comprehensive analyses of interim toxicity data are routinely performed at least twice yearly. These reports are posted on the CTSU website and on the ECOG-ACRIN members website to be available to local institutions.

9.8.8.3 Monthly Toxicity Monitoring

Serious adverse events reported using the CTEP-AERS system will be monitored using ECOG-ACRIN's standard monthly toxicity monitoring process. A study is flagged for monitoring if an arm of the protocol has a previously uncirculated Grade 5 event, 2 previous reports in the past month containing a Grade 3 or higher event, or 6 reports in the past 6 months containing a Grade 3 or higher event. The toxicity monitoring team, consisting of the study chair, statistician, and an independent reviewer, will assess the accumulating information and determine if further review is necessary.

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10. Submission of Biospecimens for Research

If procured, primary tumor tissue from diagnostic biopsies and surgical tumor tissue **from both arms** must be submitted for central diagnostic review and defined laboratory research studies. Additional tumor tissue from the diagnostic core biopsy, surgery and recurrence, if available, are to be submitted per patient consent. Peripheral blood is to be submitted from consenting patients for defined and undefined laboratory research studies. Serum must be submitted for pharmacokinetic and immunogenicity studies as defined in Section 10.3 for Arm A patients only.

It is required that all specimens submitted on this trial be entered and tracked using the ECOG-ACRIN Sample Tracking System (see Section 10.4). An STS shipping manifest form is to be included with every submission.

All specimens must be labeled clearly with the ECOG-ACRIN protocol number (EA8143), ECOG-ACRIN patient sequence number, patient's initials, date and time of specimen collection, time point and specimen type.

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10.1 Specimen Collection and Submission Schedule

Specimens are to be submitted as follows:

- MANDATORY (Arm A): Pre-surgery biopsy tumor tissue (primary tumor tissue is preferred if metastasis is also present) must be submitted within one (1) month following randomization or collection if submitting blocks or within one (1) week of sectioning fresh slides.

  NOTE: Patients randomized to Arm B are encouraged, but not required, to have a core biopsy after randomization if they have not had a biopsy within twelve (12) months of randomization that confirms diagnosis of RCC.

- MANDATORY (Both Arms): Surgical tumor tissue must be submitted within one (1) month of collection if submitting blocks or within one (1) week of sectioning fresh slides.
- OPTIONAL (Both Arms): Additional diagnostic core needle biopsy, nephrectomy tumor and normal tissue, and recurrence core needle biopsy or resection tissue, if available, should be submitted within one (1) month of collection if submitting blocks or within one (1) week of sectioning fresh slides per patient consent. See Section 10.2.1.
- OPTIONAL (Both Arms): Peripheral blood specimens are to be submitted per patient consent as outlined in Section 10.2.2. Blood specimens are to be collected at the following time points for each tube type:

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  - Prior to Start of Treatment, Arm A Only
  - Prior to Nephrectomy, Both Arms (For Arm B patients, collect Prior to Nephrectomy. For Arm A patients, collect after Cycle one (1) but Prior to Nephrectomy).
  - Week 40 Post Randomization, Both Arms
  - Recurrence, Both Arms

- MANDATORY (Arm A): Serum specimens **must** be submitted for pharmacokinetic and immunogenicity studies from Arm A patients as outlined in Section 10.3. Serum specimens must be collected at the following time

points (specimens collected at EOI-nivo time points are only for PK studies, and are not part of immunogenicity studies):

- Cycle 1, Day 1, Prior to Start of Nivolumab Infusion
- Cycle 2, Day 1, Prior to Start of Nivolumab Infusion
- Cycle 2, Day 1, **EOI-nivo:** End of Nivolumab Infusion
- Cycle 3, Day 1, Prior to Start of Nivolumab Infusion
- Cycle 5, Day 1, Prior to Start of Nivolumab Infusion
- Cycle 5, Day 1, **EOI-nivo:** End of Nivolumab Infusion
- Cycle 7, Day 1, Prior to Start of Nivolumab Infusion
- Cycle 7, Day 1, **EOI-nivo:** End of Nivolumab Infusion

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- First follow-up visit, approximately six (6) weeks post discontinuation of nivolumab
- Second follow-up visit, approximately three (3) months from first follow-up visit. Note that follow-up specimens should be collected even if the visit is delayed.

NOTE: All pre-dose serum specimens should be collected prior to the start of nivolumab infusion (anytime within 24 hours prior to first dose).

NOTE: EOI-nivo = End of Nivolumab Infusion. These serum specimens should be collected prior to stopping the nivolumab infusion (preferably within 2 minutes prior to end of infusion but within 15 minutes of the end of infusion is acceptable). If the infusion takes longer than expected, the collection at the end of infusion should be adjusted accordingly.

10.2 Submissions to the ECOG-ACRIN Central Biorepository and Pathology Facility (CBPF)

If you have any questions concerning specimen collection and shipment, please contact the ECOG-ACRIN CBPF at (844) 744-2420.

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10.2.1 Pathology Submissions

Representative formalin-fixed paraffin embedded (FFPE) tumor tissue blocks from pre-trial diagnostic pathology materials must be submitted for central diagnostic review and defined laboratory research studies.

Arm A Biopsy: Image guided percutaneous core needle biopsy of primary tumors (or metastasis) will be performed prior to starting treatment. Primary tumor is preferred over metastasis. Six non-fragmented biopsy cores should be obtained. One biopsy core will be sent to surgical pathology for standard of care histological analysis by the local pathologist and the five (5) other biopsy cores will be retained for research purposes. These cores will be placed in specimen containers and paraffin embedded. Of note, the paraffin embedded research cores will not be utilized until a histological diagnosis is reached. In the event that a histological diagnosis cannot be reached with the dedicated diagnostic tissue core, one or more of these research cores will be released to pathology for analysis.

NOTE: Patients randomized to Arm B are encouraged, but not required, to have a core biopsy after randomization if they have not had a biopsy within twelve (12) months of randomization that confirms diagnosis of RCC.

Submitting pathologist and clinical research associate may refer to Appendix I which outlines the Pathology Submission Guidelines.

ICON (who is distributing the PK kits) is also providing zipper lock bags and specimen ID/barcode accession labels and tissue requisition forms for the baseline tissue submissions. Place blocks and/or slides in the zipper lock bags.

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The pathology specimens and reports are to be labeled with the institutional Pathology ID# and ICON specimen ID/barcode/accession label on the zipper lock bags provided as well as the information above.

Required Materials

Forms: Must be submitted with all pathology submissions.

- STS generated shipping manifest form
- Copy of the institutional diagnostic and surgical pathology report
- Immunological study reports, if available
- ICON Tissue Requisition Form (baseline only)

Tumor Tissue Submissions:

- MANDATORY (Arm A): Representative formalin-fixed paraffin embedded FFPE blocks from any pre-trial diagnostic primary or metastatic tumor tissue biopsy (primary tumor tissue is preferred if metastasis is also present).
- MANDATORY (Both Arms): Representative formalin-fixed paraffin embedded FFPE primary tumor blocks from surgical tumor tissue biopsy
- MANDATORY (Both Arms): Representative H&E slides from diagnostic and surgical tumor tissue blocks
- From consenting patients (both arms) who answer “Yes” to *“I agree to provide additional specimens for research”*: Additional representative formalin-fixed paraffin embedded (FFPE) primary tumor tissue blocks from diagnostic biopsy
- From consenting patients (both arms) who answer “Yes” to *“I agree to have my samples collected and I agree that my samples and related information may be used for laboratory studies”*: Additional surgical FFPE primary tumor and normal tissue blocks
- From consenting patients (both arms) who answer “Yes” to *“I agree to have my samples collected and I agree that my samples and related information may be used for laboratory studies”*: Recurrence FFPE primary tumor tissue blocks (if available).

- Leftover tumor tissue (both arms) will be stored from patients who answer “Yes” to “My samples and related information may be kept in a bio-bank for use in future health research.”

NOTE: In order to address heterogeneity in expression of candidate biomarkers (including PD-L1) (Callea et al., Cancer Immunol Res 2015), for nephrectomy specimens, tumor tissue blocks containing predominant and highest Fuhrman grade areas should be selected. A block containing tissue from uninvolved kidney cortex should also be selected.

NOTE: During central diagnostic review, ECOG-ACRIN CBPF may request additional or alternative pathology materials selected by the review team to complete tumor tissue submission requirements.

NOTE: If blocks are unavailable for submission, cores and fresh cut slides are to be submitted. All cores and slides must be adequately labeled, with slides numbered sequentially in the order cut. Alternative submission requirements for each selected block:

- One (1) H&E slide from each source block, and
- Fresh Cut Slides: Twenty (20) 4 µm unstained, positively charged, air-dried plus slides from the thickest part of the tumor (Superfrost Plus recommended). After cutting, the slides should be kept refrigerated (2-5°C) until shipment.
- One (1) or more core punches (minimum of 4mm diameter). If core punch tool is unavailable, request core punch kit from the ECOG-ACRIN CBPF (844) 744-2420. Adequately label every slide and core submitted.

If these criteria cannot be met, please contact the ECOG-ACRIN CBPF (eacbpf@mdanderson.org) to obtain alternative submission requirements.

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10.2.2 Blood Submissions

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Blood is to be collected at the time points in Section 10.1 per patient consent for defined and/or undefined laboratory research studies.

Kits for the collection and shipment of the blood specimens are ordered on-line from Cenetron Central Laboratories. Instructions are provided in Appendix VI. Questions regarding kits can be directed to projectmanagement@cenetron.com or call the Cenetron Clinical Trials Group at (512) 439-2000. Kits must be ordered after the patient has been randomized to the trial and will generally arrive within three (3) business days from when the order was placed.

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10.2.2.1 Specimen Preparation Guidelines

SST Red Top Tubes

- Draw two (2) 8.5mL SST tubes of blood at each time point. Invert each tube gently five times.
- Allow to clot at room temperature for 20 minutes
- Centrifuge 15 minutes or longer at 1000-1500g (approximately 3000 rpm)
- Remove serum and aliquot into eight (8) cryovials
- Place in -70°C freezer and ship overnight on dry ice or batch for subsequent shipping. If a -70°C freezer is not available, store blood at -20°C until shipped. If dry ice is not available, please contact the ECOG-ACRIN CBPF at (844) 744-2420 for shipping alternatives.

Green Top Heparin Tubes

- Draw ten (10) 10mL green top heparin tubes of blood at each time point.
- Package and ship the day of collection at ambient temperature (no wet or dry ice and no cool packs) on the day of collection.

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Purple Top EDTA Tubes

- Draw two (2) 10mL EDTA tubes of blood at each time point. Invert each tube gently 8-10 times.
- Within 20 minutes of collection, centrifuge 15 minutes or longer at 1000-1500g (approximately 3000 rpm)

  NOTE: 20 minutes is preferred, but collection can be done within 2 hours.

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- Aliquot plasma into eight (8) cryovials
- Replace the stopper on the EDTA tube containing the cells
- Place plasma and residual cells in -70°C freezer and ship overnight on dry ice or batch for subsequent shipping. If a -70°C freezer is not available, store blood at -20°C until shipped. If dry ice is not available, please contact the ECOG-ACRIN CBPF at (844) 744-2420 for shipping alternatives.

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10.2.3 Shipping Procedures

Tissue blocks are to be shipped at ambient temperature within one (1) month following randomization or collection.

Fresh cut tissue slides must be shipped overnight within one (1) week of sectioning on refrigerated gel packs.

Ship serum, plasma, and remaining cells frozen on dry ice.

The green top heparin tubes must be shipped the same day they are drawn because they must be processed the day after collection. Ship

Rev. Add10 the green top heparin tubes at ambient temperature (no wet or dry ice and no cool packs).

Rev. Add10 Ship Monday-Thursday via overnight courier using the CBPF's FedEx Priority overnight account using the FedEx online ship manager.

Ship pathology materials, peripheral blood, serum, plasma, and residual cells to:

MD Anderson Cancer Center CBPF
Mike Balco
2130 West Holcombe Boulevard, LSP9.4227
Houston, TX 77030
Phone: Toll Free (844) 744-2420 (713-745-4440 Local or International Sites)
Fax: (713) 563-6506
Email: eacbpf@mdanderson.org

Access to the FedEx shipping account for shipments to the ECOG-ACRIN CBPF at MD Anderson Cancer Center can only be obtained by logging onto fedex.com with an account issued by the ECOG-ACRIN CBPF. For security reasons, the account number will no longer be given out in protocols, over the phone, or via email. If your site needs to have an account created, please contact the ECOG-ACRIN CBPF by email at eacbpf@mdanderson.org

Rev. Add6 An STS shipping manifest form and ICON Tissue Requisition Form (baseline only) must be generated and shipped with all specimen submissions.

Rev. Add4 10.3 Serum Submissions for Pharmacokinetic and Immunogenicity Studies (Arm A only)

Serum specimens must be submitted at all time points outlined in Section 10.1.

Collection and shipping kits are available. Each kit will contain the materials (vacutainers, Micronic transport tubes, labels, shippers, requisition forms, air bills) for the collection and submission of the serum.

Rev. Add7 Please email Appendix XII "EA8143 ICON Site Information/Initial Kit Order Form" in order to obtain three (3) starter kits and the Laboratory Instruction Manual. Please note ICON requires fifteen to twenty (15-20) business days to process this form and ship out the initial kits. To reorder kits sites must register with 'iSite', ICON Central Laboratories Secure Website Portal. Please see instructions outlined in Appendix XIII. Kit reorder shipments usually take about seven (7) to ten (10) business days. Updated Laboratory Instruction Manuals can also be obtained via iSite.

Questions regarding kits can be directed to ICON toll-free at (877) 797-4422 or LabSiteHelp@iconplc.com. Please reference protocol number: CA209-531 (EA8143) when contacting ICON.

Do not mix contents from one kit with another.

- Blood specimens will be collected by direct venipuncture or through an indwelling catheter. If catheter is used for blood collection, then approximately one (1) mL of blood should be withdrawn initially and discarded. Only saline

is permitted to keep catheters patent. If blood are obtained through heparin lock, sufficient blood (~1mL) must be withdrawn to remove the heparin solution. Blood specimens should not be collected from the same line as an infusion. Specifically, if the compound is infused through an IV line, the contralateral arm should be used to collect the blood specimens. If central line is used for dosing, then direct venipuncture in either arm would be appropriate. When the drug is infused through multi-lumen catheter, the alternate lumen should not be used for sampling due to the risk of drug contamination.

- Draw SST vacutainer tubes of blood at each time point as outlined in the Section 7.4 table.
- To ensure sufficient blood volume for the required tests is obtained, fill SST tubes until vacuum is exhausted and blood ceases to flow.
- Immediately after collection, gently invert each blood specimen five (5) times for complete mixing and allow blood to clot for 30-45 minutes at room temperature (tube standing upright).
- Centrifuge specimens at room temperature for 10 minutes (swing out) or 15 minutes (fixed) 1100-1300 x g until clot and serum are well separated.
- Aliquot according to the following:
  - Transfer all serum from the 4mL gold top SST collection tubes into one (1) appropriately labeled, screw capped, polypropylene Micronic transport tubes.
  - Transfer serum equally from the 8.5mL red/gray top SST collection tube into two (2) appropriately labeled, screw capped, polypropylene Micronic transport tubes.

    NOTE: Write the ECOG-ACRIN five-digit patient sequence on each bar code label before collecting specimens.

    NOTE: Aliquoted serum specimens must be kept in cryoblock (4-6°C) or in chipped wet ice at all times until stored in -20°C or colder freezer.
- Store the serum specimens immediately (within two (2) hours of collection) at -20°C or colder (-70°C freezer preferred) to ensure stability of the serum specimens until they are batch shipped overnight on dry ice via FedEx to ICON using the shipping supplies and mailer provided in the kit.
- Serum should be batch shipped monthly. Serum from multiple patients may be shipped together, but must be in separate biohazard bags with absorbent material.
- Ship serum Monday-Thursday only, do not ship on the day before a weekend or holiday.
- Serum must be submitted in pre-labeled bar-coded tubes with the appropriate completed laboratory requisition form containing the matching barcode. No unlabeled serum specimens are to be shipped.
- Complete all required information on the requisition forms or processing of the serum may be delayed. Place the requisition form on the outside of the biohazard bag.

For the Requisition Form:

- Place 'Requisition' bar code label on each copy of the requisition form
- Subject ID Number is the ECOG-ACRIN assigned EA8143 patient number

The laboratory is open to receive shipments Monday through Friday.

NOTE: Supplies leftover from used kits should be discarded.

Please refer to the Laboratory Instruction Manual for details on the Specimen Collection, Processing, and Shipping Guidelines.

If you have any questions concerning serum specimen collection and shipment please refer to the contact information provided in the Laboratory Instruction Manual.

An STS Shipping Manifest Form must be submitted with every shipment.

The exact date and time (to the minute – start and stop time, and duration of infusion must be noted) of nivolumab administration and of serum specimen collection must be entered into the ECOG-ACRIN Sample Tracking System (STS) and be provided with the documentation submitted with the serum.

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10.4 ECOG-ACRIN Sample Tracking System

It is **required** that all specimens submitted on this trial be entered and tracked using the ECOG-ACRIN STS. The software will allow the use of either 1) an ECOG-ACRIN user-name and password previously assigned (for those already using STS), or 2) a CTSU username and password.

When you are ready to log the collection and/or shipment of the specimens required for this study, please access the Sample Tracking System software by clicking https://webapps.ecog.org/Tst

Important: Please note that the STS software creates pop-up windows, so you will need to enable pop-ups within your web browser while using the software. A user manual and interactive demo are available by clicking this link: http://www.ecog.org/general/stsinfo.html

Please take a moment to familiarize yourself with the software prior to using the system.

An STS generated shipping manifest form should be shipped with all specimen submissions.

Please direct your questions or comments pertaining to the STS to ecog.tst@jimmy.harvard.edu

Study Specific Notes

The exact date and time (to the minute – start and stop time, and duration of infusion) of nivolumab administration and of serum specimen collection must be entered into the ECOG-ACRIN Sample Tracking System and provided with the documentation submitted with the serum.

Generic Specimen Submission Form (#2981v3) will be required only if STS is unavailable at time of specimen submission. Notify the laboratory of the shipment by faxing a copy of the completed form to the laboratory.

Retroactively enter all specimen collection and shipping information when STS is available.

10.5 Institutional Reimbursements

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10.5.1 Arm A Biopsy Reimbursements

Core biopsies performed following randomization to confirm RCC, are reimbursable at the research rate of $3,000 per biopsy, one biopsy per patient. Biopsies that were billed to insurance are not eligible for reimbursement. Biopsies obtained prior to randomization are not eligible for reimbursement. However, if the patient had a biopsy more than twelve (12) months from time of randomization, then they are eligible for a repeat biopsy that is eligible for reimbursement.

All sites are eligible for the reimbursement for applicable biopsies regardless of cooperative group. The site must set up the mechanism for the 'billing' of these biopsies. ECOG-ACRIN recommends billing the cooperative group Principal Investigator (PI) of the site. Contact your coordinating group's operations office and ask to whom this account should be named.

Please note that the completed EA8143 Biopsy Reimbursement Form (Appendix VII) and supporting documentation (if requested) **MUST** be submitted in order to receive the reimbursement.

Prior to recruiting patients to the biopsy portion, the following conditions must be met:

a. The research rate of $3,000 per biopsy is deemed acceptable by the IRB or central research office. Costs above $3,000 are not to be billed to the patient or patient's insurance.
b. The research rate is reported to the institution's financial office and an account established in the institution's cooperative group principal investigator's name.

Supporting documentation for the biopsies may be requested prior to the release of any funds. Expenses for biopsies will be paid only to participating institutions, not to any other persons or entities, at the stated research rate of $3,000 per biopsy.

NOTE: Reimbursements are not applicable for biopsies billed to patients or their insurance companies.

Distribution of the reimbursements requires:

- Submission and receipt of the biopsies via the ECOG-ACRIN Sample Tracking System (STS)
- Receipt of EA8143 Biopsy Reimbursement Form (Appendix VII) to the ECOG-ACRIN Operations Office - Boston.
- Supporting documentation indicating performance of biopsy was not billed to insurance.

Reimbursements will be paid from the ECOG-ACRIN Operations Office to the ECOG-ACRIN Principal Investigator (PI) of the submitting ECOG-ACRIN institution.

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10.5.2 Pharmacokinetic Reimbursements

To offset research-related costs associated with the collection and submission of the serum specimens at the time points outlined above in Section 10.1, institutions are eligible to receive reimbursements. Please see the Funding Sheet for further details. Serum specimens must be collected and submitted to trigger payment. Receipt of the serum specimens will be verified prior to the release of funds.

NOTE: Neither patients nor their insurance companies are to be billed for the collection or submission of the pharmacokinetic serum specimens.

Distribution of the reimbursements requires:

- Submission of the required pharmacokinetic serum specimens using the ECOG-ACRIN Sample Tracking System (STS) [Refer to Section 10.4 for STS requirements]
- Receipt and verification of the serum specimens by ICON Laboratory Services.

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10.6 Use of Specimens in Research

Pathological materials will be distributed to investigators for central diagnostic review and defined laboratory research studies. Serum specimens will be distributed to investigators for defined laboratory research studies.

Specimens from patients who consented to allow their specimens to be used for future undefined ECOG-ACRIN approved research studies will be retained in an ECOG-ACRIN designated central repository.

For this trial, specimens will be retained at the ECOG-ACRIN Central Biorepository and Pathology Facility.

Specimens submitted will be processed to maximize their utility for current and future research projects. Tissue processing may include, but not limited to, extraction of DNA and RNA and construction of tissue microarrays (TMAs). DNA, RNA, serum, and plasma (if appropriate) will be isolated from the submitted peripheral blood specimens.

Any residual blocks will be available for purposes of individual patient management on specific written request per submission of the ‘Specimen Return Request for Patient Care or Legal Issue’ form to the ECOG-ACRIN Central Biorepository and Pathology Facility.

Residuals/derivatives from the serum specimens submitted for the pharmacokinetic and immunogenicity studies will not be retained for future research studies.

If future use is denied or withdrawn by the patient, the specimens will be removed from consideration for use in any future research study. Pathology materials may be retained for documentation purposes or returned to the site. All other specimens will be destroyed per guidelines of the respective repository.

10.7 Specimen Inventory Submission Guidelines

Inventories of all specimens submitted from institutions will be tracked via the ECOG-ACRIN STS and receipt and usability verified by the receiving laboratory. Inventories of specimens forwarded and utilized for approved laboratory research studies will be submitted by the investigating laboratories to the ECOG-ACRIN Operations Office - Boston on a monthly basis in an electronic format defined by the ECOG-ACRIN Operations Office - Boston.

11. Laboratory Research Studies

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11.1 Pharmacokinetic and Immunogenicity Studies (Arm A Mandatory)

This study evaluates the effect of nivolumab in patients in the adjuvant setting with localized renal cell carcinoma undergoing nephrectomy and affords the opportunity to assess the long term pharmacokinetics and immunogenicity of nivolumab.

The pharmacokinetic analysis will be used to describe the pharmacokinetics of nivolumab in patients and to assess the effects of patient factors on the nivolumab pharmacokinetics. Serum specimens from the following time points will be used for this pharmacokinetic analysis: Cycle 1 Day 1 (pre-dose), Cycle 2, Day 1 (pre-dose and EOI-nivo), Cycle 3 Day 1 (pre-dose), Cycle 5 Day 1 (pre-dose and EOI-novi), Cycle 7 Day 1 (pre-dose and EOI-nivo), and First 2 Follow-Up Visits.

Serum concentration analyses for nivolumab will be performed by validated bio-analytical methods for nivolumab.

The immunogenicity specimen is a serum specimen collected to assess the presence of anti-drug antibodies to nivolumab. If ADA +, the samples would be assessed for Nab as well. This is a specimen collected in tandem with the PK specimen, at pre-dose on the indicated study days. Along with specimens to quantitate drug concentrations, given nivolumab is a Mab, specimens are included to assess incidence of immunogenicity. Serum specimens from the following time points will be used for this immunogenicity analysis: Cycle 1 Day (pre-dose), Cycle 2 Day 1 (pre-dose), Cycle 3 Day 1 (pre-dose), Cycle 5 Day 1 (pre-dose), Cycle 7 Day 1 (pre-dose), and First 2 Follow-Up Visits.

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11.2 Correlation of PD-L1 Tumor Tissue Expression with Outcome

In the metastatic setting, primary tumor expression of PD-L1 portends prognosis and may correlate with a higher chance of response to nivolumab but does not ensure response.[8,9] In the phase 3 study, PD-L1 positivity did not predict higher overall survival with nivolumab. (Motzer, 2015 #1861). Conversely, patients with PD-L1 negative tumors can still achieve response. This could be due to the dynamic nature of the marker, intratumoral heterogeneity, or differences in PD-L1 expression between the primary tumor and metastases. We will further characterize the association of the PD-L1 positivity of the tumor and tumor-immune infiltrating cells at the time of pretreatment biopsy, nephrectomy, and at recurrence with outcomes as exploratory aims with the goal of identifying better selection criteria for this strategy.

Immunohistochemical (IHC) analysis for PD-L1 will be performed on formalin-fixed, paraffin-embedded tumor tissue specimens using: the BMS assay, Dako PD-L1 28-8 IHC assay, which uses the Dako PD-L1 antibody will be performed on archival or newly obtained tissue. (Cogswell JP Cancer immunotherapy. http://www.freepatentsonline.com/).

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11.2.1 Statistical Considerations

An independent pathologist who is blinded to the outcomes will score the percentage of cells exhibiting cell-surface staining for PD-L1. PD-

L1 positivity will be defined per specimen by a 5% expression threshold.[22,23] Additional analyses will assess the 1% and 10% threshold levels. We will also capture information with respect to the relative abundance and location (peritumoral/intratumoral) of immune cell (macrophage and lymphocyte) PD-L1 staining. To be eligible for analyses, the section must have at least 100 tumor cells. We will test the hypothesis that patients whose tumors have a higher expression of PD-L1 have improved RFS on nivolumab (i.e., we will test the expression-by-treatment interaction using a proportional hazards model).

We assume that viable tissue from nephrectomy specimens will be available for 80% of patients, and that 30% of patients have tumors that express PD-L1. We further assume that the proportion of patients with tissue from each of the two treatment arms is equal, so the overall observed hazard ratio will be a mixture of that expected for the experimental and observation arms. There will be 80% power to detect a hazard ratio of about 1.63 comparing DFS between patients with high and low PD-L1 if those who express PD-L1 have better outcome.

Additionally, among patients with biopsy specimens available, we will evaluate for differences in PD-L1 expression and immune cell infiltrate levels between biopsy and nephrectomy specimens. If there are no differences between biopsy and surgery levels in the observation arm but we observe differences in the neoadjuvant nivolumab arm, the effect will be attributable to the neoadjuvant administration and not to differences due to the nature of the specimens (biopsy vs surgery).

McNemar's Test will be used to explore categorical changes within each treatment group, although changes are only expected in treated patients. The following table shows the sample size that would be required and the change that would be detectable with adequate power given a range of proportions positive for PD-L1 in the biopsy specimen. We assume about half of patients will have paired biopsy and tumor specimens with viable material (maximum 190 per arm). There are no published data about the direction of change, so power and sample sizes are shown for both increases and decreases. A 50% relative change in proportions would be considered clinically meaningful (e.g., from 30% positive pre-treatment to 45% positive post-treatment). Differences detectable with 190 patients per group are also shown in the table below.

% Positive pre-treatment (biopsy)	% Positive post-treatment (nephrectomy)	Power	Required sample size
30%	45%	83%	190
	15%	93%	190
40%	20%	92%	125
	60%	90%	140
	24%	90%	190

% Positive pre-treatment (biopsy)	% Positive post-treatment (nephrectomy)	Power	Required sample size
	57%	90%	190
50%	25%	89%	75
	75%	89%	75
	33%	91%	190
	67%	91%	190
60%	30%	91%	65
	90%	90%	47
	43%	90%	190
	76%	90%	190

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11.2.2 Specimen Submission

Tumor tissue specimens obtained at the time of the pre-surgical biopsy, nephrectomy, and biopsy at recurrence (if available) will be used in this analysis.

11.3 Additional Potential Laboratory Research Studies.

When sufficient information is available from the parent study a full correlative science proposal or amended protocol document detailing the scientific hypotheses, research plans, and assay methods for use of the bio-specimens, and a formal statistical analysis plan with adequate power justification will be submitted to and reviewed by CTEP.

Results of these studies are for the purposes of the trial only and will not be returned to the site or reported to the patient.

List of studies in order of priority:

Tissue:

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1. Immunohistochemistry (IHC)/immunofluorescence (IF) analysis of RCC tumors
   (A) Multiplex IF assays to be done at Dr. Signoretti's laboratory at Brigham and Women's Hospital using two established panels
2. NanoString discovery of immune gene signatures (RNA specimens)
3. Tumor sequencing for predictive genes: Whole exome/transcriptome sequencing. Alternatively, based on the field at the time of analysis, genome or targeted sequencing of panels of genes may be performed.
4. T cell receptor repertoire characterization

Blood:

1. T cell proliferation assays to detect neoantigen responses using cryopreserved lymphocytes
2. T cell receptor repertoire characterization

3. Circulating Cytokine Levels

Rev. Add8

11.4 Immunohistochemistry (IHC)/Immunofluorescence (IF) Analysis of RCC Tumors

RCC is characterized by complex interactions between the host immune response and a variety of immunosuppressive pathways operative in the tumor microenvironment.[26] Despite getting to the tumor, effector lymphocytes may encounter regulatory cells in the tumor microenvironment that can functionally inactivate their potential anti-cancer activities.[26] PD-L1 expressed by the tumor cell can inhibit the proliferation, cytokine production and cytotoxic activity of anti-tumor PD-1+, CD4+ and CD8+ T cells.[4] Its expression on tumor cells and tumor-infiltrating lymphocytes (TILs) may impair host T cell anti-tumor function. Baseline and on-treatment levels of helper and cytotoxic T lymphocyte infiltrate will be characterized. Tissue for biomarker analysis will be harvested at baseline from the primary tumor (biopsy), at nephrectomy (primary tumor and when available, any resected lymph nodes), and at recurrence. Normal kidney or nodal tissue will also be sampled concurrently. IHC will be used to quantify PD-L1 and PD-L2 expression on tumor cells as well as on infiltrating immune cells. Multiplex IF assays will be utilized to characterize the immune infiltrate. These assays will quantify CD8+ T cells, Tregs (FoxP3+ CD4+ cells), and total CD4 T cells (in both normal and tumor tissue). Co-expression of multiple immune checkpoint molecules (e.g. PD1, TIM3, LAG-3. VISTA, B7-H3) on tumor infiltrating immune cells will be also assessed by multiplex IF.

Staining levels expressed as percent positivity will be summarized using descriptive statistics. Staining levels expressed as scores will be tabulated categorically. Changes over time will be portrayed graphically by arm. Correlations between baseline immune infiltrate, changes after nivolumab, and efficacy outcomes will be explored.

11.4.1 General IHC Methods

All immunohistochemical staining will be performed utilizing the DAKO automated stainer. 5µm-thick paraffin-embedded tissue sections will be dewaxed, soaked in alcohol, and after microwave treatment in antigen unmasking solution for 10 minutes, incubated in 3% hydrogen peroxide for 15 minutes to inactivate endogenous peroxidase. Sections will then be incubated with the appropriate primary antibody and detection will be performed using the DAKO EnVision™+ System according to the manufacturer's instructions.

Immunohistochemically stained slides will be evaluated by a Pathologist. Scoring will be performed according to the staining intensity and the percentage of positive cells. This analysis will be performed at the DF/HCC Renal Cancer Program Pathology Core at Brigham and Women's Hospital under the direction of Dr. Sabina Signoretti.

11.4.2 Correlation of PD-L1 Tumor Tissue Expression with Outcome

Immunohistochemical (IHC) analysis for PD-L1 will be performed on formalin-fixed, paraffin-embedded tumor tissue specimens using two multiplex IF assays to be done at Dr. Signoretti's laboratory at Brigham and Women's Hospital: Tumor infiltrating activated cytolytic

CD8-positive T cell will be investigated by four-color IF using validated CD8, Ki67, Granzyme-A, and Perforin-1 antibodies. Co-expression of immune checkpoint molecules on immune cell subsets (CD8-positive or CD68-positive or DC-Lamp-positive) will be investigated by multiplex IF using validated antibodies against CD8, CD68, DC-Lamp, PD-L1, PD-L2, PD-1, TIM-3, LAG-3, VISTA and B7-H3. Tumor infiltrating Tregs will be quantified by IF using anti-CD4 and anti-FOXP3 antibodies.

Image acquisition will be performed using the Mantra platform (Perkin Elmer). Positive cells will be quantified using the Inform software program (Perkin Elmer). Results of image analysis will be validated visually by Dr. Signoretti.

11.4.3 Statistical Considerations

An independent pathologist who is blinded to the outcomes will score the percentage of cells exhibiting cell-surface staining for PD-L1. PD-L1 positivity will be defined per specimen by a 5% expression threshold.[22,23] Additional analyses will assess the 1% and 10% threshold levels. We will also capture information with respect to the relative abundance and location (peritumoral/intratumoral) of immune cell (macrophage and lymphocyte) PD-L1 staining. To be eligible for analyses, the section must have at least 100 tumor cells. We will test the hypothesis that patients whose tumors have a higher expression of PD-L1 have improved RFS on nivolumab (i.e., we will test the expression-by-treatment interaction using a proportional hazards model).

We assume that viable tissue from nephrectomy specimens will be available for 80% of patients, and that 30% of patients have tumors that express PD-L1. We further assume that the proportion of patients with tissue from each of the two treatment arms is equal, so the overall observed hazard ratio will be a mixture of that expected for the experimental and observation arms. There will be 80% power to detect a hazard ratio of about 1.63 comparing DFS between patients with high and low PD-L1 if those who express PD-L1 have better outcome.

Additionally, among patients with biopsy specimens available, we will evaluate for differences in PD-L1 expression and immune cell infiltrate levels between biopsy and nephrectomy specimens. If there are no differences between biopsy and surgery levels in the observation arm but we observe differences in the neoadjuvant nivolumab arm, the effect will be attributable to the neoadjuvant administration and not to differences due to the nature of the specimens (biopsy vs surgery).

McNemar's Test will be used to explore categorical changes within each treatment group, although changes are only expected in treated patients. The following table shows the sample size that would be required and the change that would be detectable with adequate power given a range of proportions positive for PD-L1 in the biopsy specimen. We assume about half of patients will have paired biopsy

and tumor specimens with viable material (maximum 190 per arm). There are no published data about the direction of change, so power and sample sizes are shown for both increases and decreases. A 50% relative change in proportions would be considered clinically meaningful (e.g., from 30% positive pre-treatment to 45% positive post-treatment). Differences detectable with 190 patients per group are also shown in the table below.

% Positive pre-treatment (biopsy)	% Positive post-treatment (nephrectomy)	Power	Required sample size
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	24%	90%	190
	57%	90%	190
50%	25%	89%	75
	75%	89%	75
	33%	91%	190
	67%	91%	190
60%	30%	91%	65
	90%	90%	47
	43%	90%	190
	76%	90%	190

11.4.4 Specimen Submission

Tumor tissue specimens obtained at the time of the pre-surgical biopsy, nephrectomy, and biopsy at recurrence will be used in this analysis. No additional specimens are requested.

11.5 NanoString Discovery of Immune Gene Signatures

RNA samples from archived FFPE tissue can often be low quality. Platforms that permit multiplex analysis of limited amounts of RNA such as the NanoString Technologies nCounter platform may be more efficient than traditional RT-PCR techniques. NanoString uses a fluorescent barcode technology to measure up to 800 targets per sample. The goal of these exploratory analyses will be to define signatures that correlate with an increased CD8 infiltrate post-nivolumab treatment, as well as signatures that correlate with a lack of infiltrate – i.e. a non-response signature. Novel transcripts discovered via NanoString will be verified and quantified at the protein level via IHC as described above.

Methods: Using sections identified by the study pathologist (Sabina Signoretti), total RNA will be isolated using Ambion RecoverAll Total Nucleic Acid Isolation Kit for FFPE Tissues (Applied Biosystems, Waltham, MA). RNA expression will be quantified using the 770 gene nCounter PanCancer Immune Profiling Panel

(NanoString Technologies, Seattle, WA) by counts. NanoString nCounter expression data will be analyzed using the "edgeR" and "limma" R/Bioconductor packages as follows: Each sample will first be rescaled using the "relative log expression" method proposed by Anders and Huber. Count data will then be transformed to log2-counts per million, the mean-variance relationship estimated, and the appropriate observational-level weights computed as described by Law et al (26). Finally, differential gene expression between therapy response groups will be analyzed using mixed effects linear modeling by robust regression, controlling for technical replication (27) and applying an empirical Bayes approach for moderating gene-wise variances (28). We will also perform Analysis of Functional Annotation (29) to identify relevant biological processes and signaling pathways associated with a therapy response (defined as CD8 T Cell infiltration quantified via IHC as above). Since the NanoString platform to be used in this study contains only immunological genes, we will apply a non-competitive method based on rotation gene set testing for linear models (30). Gene lists used in this analysis were retrieved from the Broad Institute Molecular Signature Database (MSigDB) (31). The Benjamini and Hochberg method will be used to control for multiple testing (32). The NanoString analyses will be performed in the Johns Hopkins University high-throughput genetics core, under the direction of Dr. Charles Drake, with bioinformatics analyses directed by Dr. Luigi Marchioni.

11.5.1 Specimen Submission

Tumor tissue specimens obtained at the time of the pre-surgical biopsy, nephrectomy, and biopsy at recurrence (if available) will be used in this analysis.

11.6 Tumor Sequencing for Predictive Genes

11.6.1 Whole Exome/Transcriptome Sequencing

Whole exome and transcriptome sequencing of the patient's RCC tissue specimens and matched germline samples may enable identification of predictive markers of resistance and clinical benefit that can lead to future personalization of therapy with this perioperative strategy or identify potential resistance mechanisms that may make this approach futile and suggest future combinatorial strategies. Several genes have been identified to be prognostic when mutated in RCC such as BAP1 and SEDT2.

11.6.2 Whole exome sequencing is a cancer genomic assay that can detect somatic mutations, copy number variations and structural variants in tumor DNA in all of the coding regions of the genome, and can be done from fresh, frozen or formalin-fixed paraffin-embedded samples (Van Allen, et al Nature Medicine 2014). Whole exome sequencing surveys exonic DNA sequences of all ~20,000 cancer genes. To be eligible for DNA isolation, tissue must contain at least 20% tumor nuclei. The DNA is then analyzed by massively parallel sequencing using a solution-phase Illumina hybrid capture kit and an Illumina HiSeq 2500 sequencer at the Broad Institute using established methodology. In parallel, capture based whole transcriptome sequencing, which can be performed from either frozen or formalin-fixed samples (e.g. Van Allen et al Science 2015), will be done on all

tumor samples to determine gene expression quantification through orthogonal methods, as well as identify fusion products and alternative splicing events. Dr. Eliezer Van Allen at DF/HCC will lead this substudy.

Alternative sequencing methods such as genomic and targeted panels of genes may also be utilized depending on the field at the time.

11.6.3 Specimen Submission

Tumor tissue specimens obtained at the time of the pre-surgical biopsy, nephrectomy, and biopsy at recurrence (if available) will be used in this analysis.

11.7 Characterization of T Cell Receptor Repertoire (TCR) and Change in Response to Nivolumab

The diversity of the T cell receptor repertoire is critical to the anti-tumor immune response and to combating tumor escape. Subsequent changes in a patient's T cell receptor (TCR-β) repertoire over time and in response to therapy likely have significant influence on survival. Early work has unveiled intriguing observations into the impact of TCRs on clinical outcomes. For example, a prospective T cell monitoring study revealed that baseline high frequency of CTLA-4+, PD-1+, or non-naive T cells was associated with improved clinical outcomes with the CTLA-4 antibody, ipilimumab when given in combination with the GVAX vaccine in patients with castration-resistant prostate cancer (CRPC).[1] In another study of patients with CRPC and melanoma, Fong and colleagues found significant increases in the size and diversity of the TCR-β repertoire after treatment with ipilimumab.[2] While the degree of change in TCR clonotype abundance did not necessarily correlate with improved outcomes, the patients who maintained a high frequency of TCR-β clonotypes during treatment had greater overall survival. Interestingly, the clones that expanded the most in response to treatment were non-naïve T cells suggesting that responders have an existing pre-primed T cell armamentarium that converts to an effector response during treatment. Another study of melanoma patients treated with the PD-1 antibody pembrolizumab observed that responders tended to have a more clonal TCR repertoire at baseline, which then expanded as much as 10 times more than those patients who progressed in response to therapy.[3] In RCC, Choueiri et al demonstrated that TCR clonality in blood and tumor may be associated with survival in a cohort of patients with metastatic RCC treated with single agent nivolumab suggesting that TCR rearrangement could be an important factor when evaluating response (Choueiri ASCO 2015).

Next generation sequencing (NGS) can detect millions of uniquely rearranged TCR-β clonotypes using primers that can recognize the variable (V), diversity (D), joining (J), and constant gene segments.[2] A clonotype represents a distinct VDJ readout. NGS platforms can monitor how these unique TCR-β sequences transform over time and in response to therapies that may modulate the immune response.[2]

Differences in baseline TCR diversity and abundance may impact response to therapy. Moreover, administration of immunotherapy may influence the TCR

repertoire. Using peripheral blood mononuclear cells and tumor tissue samples, we will characterize the TCR repertoire serially at baseline, after nivolumab (in arm A), and at the time of recurrence to assess for changes over time and in response to therapy. We will evaluate for any associations between clonality and risk of recurrence and survival.

The Adaptive platform is one of the most advanced high output sequencing technologies for sequencing TCRs and will be used to assess the effect of nivolumab on the T cell repertoire in peripheral blood.

The following is a condensed version of the methodology, which will be performed to evaluate TCR clonotype abundance.[2]

(1) Cryopreserved PBMCs from baseline, serially, and at the time of recurrence will be thawed. To amplify and sequence the TCR-β repertoire, RNA will be isolated using AllPrep DNA/RNA mini and/or micro kits (Qiagen).[2] Life Technology Vilo kits will be used to reverse transcribe the RNA to complementary DNA (cDNA). Locus-specific primer sets for TCR-β will be used to amplify the cDNA.[2]

(2) To identify and enumerate the different clonotypes, the reads will be mapped to V and J seqments. A clonotype will be defined as identical sequences of the mapped readouts. Frequency, a.k.a., clone abundance, will be assessed by dividing the number of samples with the same clonotype by the total number of reads sampled.

11.7.1 Statistical Analysis:

(1) Absolute T cell clonotype abundance will be determined by assessing the number of unique clonotypes identified. High frequency will be defined as the number of unique TCR clonotypes $>10^{-3}$ similar to that used by Cha et al.[2] The data will be summarized descriptively and graphically.

(2) To measure the degree of repertoire change from diagnosis to on therapy or to time of recurrence, we will use Morisita's distance as utilized by Cha and colleagues.[2] Morisita's distance ranges from 0-1. Zero indicates minimal change in TCR repertoire size (number of unique clonotypes) and 1 indicates maximal change after treatment. For example, in the Cha study, median travel distance for untreated patients was 0.039 vs. 0.197 for the ipilimumab treated patients (p=0.005, Mann-Whitney).[2] This finding suggested that CTLA-4 blockade elicited significant changes (either increases or decreases) in clonotype abundance consistent with increased turnover of the TCR repertoire. This method is useful as it is not as vulnerable to sample size fluctuations.[2]

(3) To evaluate TCR diversity, change in repertoire size per patient will be used. We will identify the top 25% of unique clonotypes by sorting for clone frequency. Fold change after recurrence will be measured and plotted on a logarithmic scale.

(4) Working with a computational biologist, the DESeq R package will be employed to identify which patients had significant changes in TCR repertoire size (aka, "differentially abundant" clonotypes) and

whether the changes were due more to clonotype loss or gain.[2,9] This established DESeq algorithm has been modified to adjust for normal variation in the repertoire.

11.8 Circulating Cytokine Levels

Serum levels of circulating pro- and anti-inflammatory cytokines such as TNF-alpha, IL-8, IL-1 and IL-6 will be quantified in all patients at baseline, time of nephrectomy, and ~3-4 months after nephrectomy on nivolumab or surveillance, and at recurrence. The list of cytokines to be explored will be curated based on the NanoString results and other relevant results at the time of analysis, but in general will utilize the luminex multiplexed platform (Austin TX). Briefly, this platform uses internal standards and a spline-fit curve method to accurately quantify panels of 17-35 cytokines from replicates of 25 microliters of patient sera. This analysis will be performed at the DF/HCC under the direction of Dr. Rupal Bhatt in the DF/HCC Immune Monitoring Core.

11.8.1 Statistical Analysis:

The following questions will be considered:

Among patients randomized to perioperative nivolumab:

- Do cytokine levels change in response to neoadjuvant nivolumab?
  - Changes between randomization and pre-nephrectomy, assuming paired samples available for 90% of patients randomized to nivolumab (345 pts).
- Do cytokine levels change in response to adjuvant nivolumab?
  - Changes between pre-nephrectomy and 36 weeks, assuming paired samples similarly available
- Do cytokine levels change in response to any nivolumab?
  - Changes between randomization and 36 weeks
- Is there an association between changes in cytokine levels and clinical outcome among patients treated with nivolumab or placebo?

As an example of power that will exist for the above analyses of the magnitude of change, we consider a preliminary analysis of IL-8, which was measured at baseline and 4 weeks among 59 patients treated with sorafenib on ASSURE. Mean levels among 59 patients measured at baseline and 4 weeks were 4.19 and 4.70 respectively, with standard errors of 0.32 and 0.41. We estimate that 90% of patients randomized to nivolumab will have paired serum samples available for this analysis.

Assuming a standard deviation of 3.15, there will be 83% power using a two-sided Wilcoxon rank sum test with 5% Type I error.

Cutpoints that best distinguish patients with robust CD8 infiltration at randomization from those without a robust infiltration will be estimated using recursive partitioning. If the number of available samples permits, patients will be randomly divided into test and validation datasets for assessing the robustness of the cutpoints, but an

exploratory analysis without internal validation is assumed in the following sample size estimates.

Once the study is completed, we will perform additional analyses to explore associations between cytokine levels and clinical outcome. For these studies, we estimate that 90% of patients will have blood samples at both baseline (randomization) and 3 months (approximately 345 patients per arm). At full information, we estimate that between 90 and 124 disease recurrences or deaths will have occurred among these patients on a given arm, depending on the efficacy of treatment. Within each arm, there will be 80% power to detect a hazard ratio of 1.67 to 1.84 using a two-sided log-rank test with two-sided Type I error of 5%, assuming percent change is split at the median.

If changes are observed, then among all patients:

- Are there differences in the magnitude of change between patients randomized to nivolumab and those randomized to observation?
- Is there an interaction between treatment and cytokine change with respect to clinical outcome?

Differences in RFS between patients with and without robust CD8 infiltration will be explored. A Cox proportional hazards model will be used to test the interaction between treatment and cytokine changes. These analyses are exploratory in nature.

11.8.2 Specimen Submission

Specimens from the SST tubes will be used in this analysis.

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11.9 T Cell Proliferation Assays to Detect Neoantigen Responses using Cryopreserved Lymphocytes

These studies will be performed as previously described (Sheikh, 2012). Briefly, mutation associated neo-antigens (MANA) will be predicted using the PDGx platform. For each patient, 4-8 peptides with optimal MHC binding that patient's HLA-A alleles will be synthesized under GMP conditions. T cell proliferation to neoantigens will be assayed using a standard tritiated thymidine (^{3}H-thymidine) incorporation assay. The degree of proliferation will be expressed as a stimulation index (SI), defined as ^{3}H-thymidine incorporation in the presence of antigen divided by ^{3}H-thymidine incorporation with media alone. For these studies, proliferation to the CEF (CMV, EBV, Flu) pool will be used as a positive control. Control, i.e. irrelevant peptides will be used as a negative control. It should be noted that we are well aware that alternative, more complex methods are available to measure antigen-specific T cell responses, including Elispot, Fluroispot, CFSE dilution and multiplexed tetramer staining. For the purposes of this study, however, we plan to initially conduct screening studies with standard, robust H3 proliferation assays. If interesting T cell reactivity is noted, we will follow-up with additional studies to further characterize the nature of the MANA response, those additional studies will utilize flow cytometry with intracellular staining to determine whether the MANA response is productive, i.e. whether it is characterized by secretion of effector cytokines like IFN-γ, TNF-α and IL-2.

These studies are exploratory, and descriptive summary statistics will be utilized, as above focusing on CD8 infiltrate “responders” versus “non-responders”, i.e. patients without a significant post-treatment infiltrate at the time of surgery.

11.9.1 Specimen Submission

Specimens from the cryopreserved lymphocytes will be used in this analysis.

11.10 Lab Data Transfer Guidelines

The data collected on the above mentioned laboratory research studies will be submitted electronically using a secure data transfer to the ECOG-ACRIN Operations Office – Boston by the investigating laboratories on a quarterly basis or per joint agreement between ECOG-ACRIN and the Investigator.

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12. Bone Metabolism Imaging & Serum Correlative Research Study (Optional)

For patients that consent to take part in the optional imaging research study, all dual energy X-ray absorptiometry (DXA) bone scans are to be submitted via TRIAD as outlined in Section 4.1.4.6.

12.1 Changes in Bone Metabolism and Density in Response to Nivolumab Administration

The skeletal toxicities of immune therapy (IT) are unknown. Bone loss and occult fracture may be unrecognized sequelae of IT threatening long-term cancer survival. Skeletal failure is associated with substantial morbidity and mortality but can be prevented with current osteoporosis drugs; therefore, it is critical to understand the adverse effects of IT on bone metabolism and the mechanisms by which unfavorable bone remodeling states may occur. Checkpoint inhibitors activate host T-cells, unleashing an anti-tumor immune response. There is preliminary data to indicate that IT accelerates skeletal deterioration. Pro-inflammatory states and immune reconstitution have established, adverse effects on the skeleton. Increased levels of cytokines including IL-6 and TNF-α in rheumatoid arthritis and postmenopausal osteoporosis drive bone-resorbing osteoclastogenesis while simultaneously stunting bone-building osteoblast maturation. These same cytokines are implicated in IT's anti-tumor effects, but with unknown skeletal sequelae. In HIV, antiretroviral initiation fosters T-cell secretion of Receptor Activator of Nuclear Factor-κB Ligand (RANK-L) and reduced osteoprotegerin (OPG), leading to bone loss and increased fracture risk. Checkpoint inhibition also mobilizes and reconstitutes the immune system, though the skeletal aftermath is unclear.

This correlative study will test the hypotheses that 1) PD-1 blockade with consequent T-cell activation used for the treatment of RCC has adverse downstream effects on bone metabolism and density, 2a) systemic inflammatory and bone regulatory markers will change following checkpoint inhibition, and 2b) that these changes will be related to unfavorable alterations in skeletal metabolism.

To test these hypotheses, serum bone turnover and inflammatory markers, bone density data will be measured in participants treated and untreated with nivolumab to investigate the following objectives:

To characterize the effect of nivolumab on measures of bone remodeling. We will follow participants and quantify baseline and post-treatment bone parameters to include the following: A) serum C-telopeptides (CTX, bone resorption) and B) procollagen type 1 N-terminal propeptide (P1NP, bone formation).

To characterize the effect of nivolumab on measures of bone density. We will follow participants and quantify baseline and post-treatment bone parameters to include the following: A) areal bone density using dual energy X-ray absorptiometry and B) volumetric bone density using quantitative computed tomography (derived from CT scans obtained from screening and surveillance visits).

To investigate inflammatory and immunogenic mechanisms by which T-cell activation with nivolumab administration mediates changes in parameters of

bone remodeling and density in patients with RCC. We will quantify baseline and post-treatment serum levels of 1) circulating pro- and anti-inflammatory cytokines including TNF-α, INF-gamma, IL-1 and IL-6, and 2) circulating RANK-L and OPG in all participants. Serum findings will be correlated with changes in parameters of bone strength and metabolism.

12.1.1 Study Procedures

The capacity of a bone to resist fracture is determined by bone remodeling and quantity. We have hypothesized that IT leads to deficiencies in these parameters of bone metabolism and strength. Serum markers of bone formation and resorption help quantify bone remodeling. Clinically, bone quantity is measured using dual energy X-ray absorptiometry (DXA) which provides areal bone mineral density (aBMD) parameters. Quantitative CT scan (QCT) provides volumetric bone mineral density (vBMD) Derangement to any component of skeletal homeostasis – quantity, quality or remodeling – will compromise bone strength and increase fracture risk. To characterize the effect of nivolumab on measures of bone remodeling and density, serum studies and bone density measurements will be collected on study participants, outlined below.

12.1.2 Serum Markers of Bone Metabolism

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At baseline, prior to nephrectomy and 40-weeks post-randomization, parameters of osteoclast-mediated bone resorption (C-telopeptides, CTX) and osteoblast-mediated bone formation (Procollagen 1 N-terminal peptide, P1NP) will be measured using serologic assays on serum collected from all research participants who consented to optional research receiving nivolumab as well as controls as a part of the existing parent study protocol. CTX (Serum Crosslaps ELISA; CV: 2.3%; Nordic Bioscience A/S, Herlev, Denmark) and P1NP (Elecsys 2010 Immunoassay System; CV: 6.5%; Orion Diagnostica UniQ P1NP RIA, Espoo, Finland) will be measured from archived serum (section 10.2.2) and then analyzed in batches at the JHU Advanced Chemistry Laboratory.

12.1.2.1 Statistical Analysis

We will determine if nivolumab therapy is associated with changes in bone turnover marker levels (CTX and P1NP) at 40 weeks post-randomization. The *dependent* variables are CTX and P1NP levels at 40 weeks post-randomization using serologic assays on serum collected from all consenting research participants. The primary outcome measure will be percent change from baseline to peak biomarker serum level (continuous). The *independent* variable is receipt of nivolumab for individuals undergoing nephrectomy. Initially, we will use multivariable linear or nonlinear regression (depending on the distribution of each bone turnover biomarker) to test whether nivolumab therapy prior to nephrectomy is associated with the percent change in CTX and P1NP levels from baseline to

maximum levels post-nephrectomy. However, since the bone turnover biomarker levels assessed over time from the same subject are likely to be correlated, we will also pursue a more efficient, albeit complicated analysis that will utilize all the bone turnover biomarker data collected over time in one model to assess whether nivolumab therapy is associated with the outcome longitudinal profiles of each bone turnover biomarker. ***Subgroup analyses*** will be performed for 1) sex; 2) age, 3) turnover state at baseline, 4) tumor response. Secondary outcomes of interest include modeling both the mean change in CTX and P1NP from baseline to 40 weeks post-randomization, as well as the mean change in CTX and P1NP from surgery to 40 weeks post-randomization. We will also explore the relationship of vitamin D, calcium, renal function and parathyroid hormone with possible changes in bone remodeling over time through correlation analyses, as well as, potential role as either a mediating or moderating in the association between nivolumab and biomarkers. *Power calculation***:** Change in CTX and P1NP levels from baseline to max. Based upon study population of 766 patients (383 receiving nivolumab therapy and 383 not receiving nivolumab therapy) with analyzable data while assuming 5% attrition in each group, we will have 80% power to detect a small effect size (Cohen's f^2) of 0.02 for CTX and P1NP based upon an F test from a mutivariable linear regression analyses conservatively assuming adjustment for 6 covariates (in addition to time and time x treatment interaction terms) with an alpha of 0.05 (two-sided). Power calculations were based on the effect size, as there is no preliminary data in this patient population.

12.1.3 Measures of Areal and Volumetric Bone Density

At baseline and 40-weeks post- randomization, aBMD at the spine, hip, femoral neck and distal 1/3 radius will be quantified using DXA using standard imaging protocols in research participants receiving nivolumab (n>50) as well as controls (n>50). There is no restriction on center participation. At baseline and 40-weeks post-nephrectomy, vBMD of the spine (non-contrast scans) and hip (with or without contrast) will be quantified using screening and surveillance CT imaging collected per parent study protocol in research participants at all participating sites. Parameters of vBMD will be calculated on CT scans collected under the existing protocol (opportunistic use) with validated software (MindWays, Austin, TX).

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NOTE: If a patient consented to participate in this sub-study, the 40 week DXA should be completed only if the baseline DXA completed.

12.1.3.1 Statistical Analysis

We will determine if nivolumab therapy is associated with change in aBMD or vBMD at 40 weeks post-randomization. The *dependent* variable is aBMD or vBMD. The *independent* variable is receipt of neoadjuvant nivolumab therapy in patients undergoing nephrectomy. We will determine the effect of neoadjuvant nivolumab therapy on changes in BMD measurements using multivariable linear or nonlinear (depending on the distribution of each bone quantity biomarker) regression. Subgroup analyses will be performed, as above. *Power calculation***:** Change in BMD measurements from baseline to 40 weeks post-randomization. A sample of 50 patients (25 receiving neoadjuvant nivolumab therapy and 25 not receiving neoadjuvant nivolumab therapy) with available BMD measurements at both baseline and 40 weeks post randomization will permit, at 80% power and an alpha of 0.05 (two-sided), the detection of a clinically significant large effect size (Cohen's f^2) of 0.35 from multivariable linear regression models conservatively assuming adjustment for 4 covariates.

12.1.4 Serum Measures of Circulating Cytokine Levels and Additional Regulators of Bone Metabolism

Serum levels of cytokines known to both stimulate and inhibit osteoclastic and osteoblastic activity will be quantified in serum collected from all participants who answer "yes" to additional research conducted on serum collections (see 10.2.2). Cytokines such as IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10 and TNF-α will be quantified in all patients at baseline, prior to nephrectomy, and at 40-weeks post randomization. The list of cytokines to be explored will be curated based on the NanoString results and other relevant results by correlatives at the time of analysis, but will utilize the Luminex Multiplex platform (Invitrogen /ThermoFischer Scientific, Austin, TX). This analysis will be performed in collaboration with Dr. Rupal Bhatt (Dana Farber / Harvard Cancer Center Immune Monitoring Core). Additional Regulators of Bone Metabolism: At baseline, prior to nephrectomy, and 40-weeks post-randomization, OPG and RANK-L (Alpco Diagnostics, Salem, NH) as well as TGF-B (R&D Systems, Minneapolis) will be measured using serologic assays on serum collected from all consenting research participants receiving nivolumab as well as controls as a part of the existing protocol. Serum will be frozen at -80C and then analyzed in batches at the JHU Advanced Chemistry Laboratory.

12.1.4.1 Statistical Analysis

We will determine if higher levels of pro and anti-inflammatory cytokines and circulating RANK-L/OPG are associated with elevations in bone strength and metabolism. The *dependent* variable are the biomarkers

measured at multiple time points with primary and secondary measures defined in Aim 1. The *independent* variables will T-cell secreted regulators of bone metabolism (Table 1). The data for this hypothesis have a nested structure (i.e., repeated measurement of cytokines, RANK-L and OPG levels and nivolumab derived inflammation over time (level one) within patients (level two). Our outcome is at the time level (level one). Multiple correlation analyses will be performed between the inflammatory markers and other continuous independent measures using Pearson correlation including exploratory stratified analyses by group assignment. In unadjusted and adjusted multilevel linear or nonlinear (depending on the distribution of each biomarker) regression, we will test whether nivolumab derived inflammation measures at each time point predict the current or using a temporal lag, the next time points measure of a biomarker. We will determine the effect of pro and anti-inflammatory cytokines on elevations in the biomarkers using generalized estimating equations (GEE) to adjust for the correlation within a subject of multiple measures of a biomarker over time. Subgroup analyses will be performed, as above.
Power calculation: Enrollment of approximately 600 patients, 80% power and an alpha of 0.05 (two-sided) will permit the detection of at least a 0.12 correlation between indicators of nivolumab derived inflammation and biomarkers using a Pearson correlation.

13. Quality of Life (QOL) Studies

Rev. Add4

13.1 Statistical Considerations

The primary objective is to evaluate differences in change from baseline in patient-reported outcomes and toxicities among patients randomized to treatment with nivolumab compared to surgery alone. Within this objective are several specific questions of interest:

1. How do quality of life and symptom/toxicity burden change following a short course of neoadjuvant nivolumab?
2. How does quality of life change from pre- to post-nephrectomy?
3. Are there differences in the change in quality of life and symptom burden following nephrectomy between patients randomized to nivolumab and those randomized to surgery alone?
4. Is there a difference in the change in quality of life over time between arms at 40 weeks (in the midst of treatment in patients on arm A)?
5. Is there a difference in the pattern of change in quality of life over time between arms after the end of treatment (up to 2 years)?
6. Is there a difference in the pattern of change in quality of life over time from baseline to recurrence?

The overarching goal is to characterize the impact of nivolumab on symptoms and quality of life. Therefore, question 4 above is of primary interest and will drive the sample size. The primary analysis will have 3 dimensions. Overall Type I error of 5% will be split among the 3 dimensions described below.

The QOL component of the study will be mandatory, and target accrual of 320 patients is planned in order to have adequate power to detect an effect size of 0.5 for the primary analysis, which consistent with a clinically meaningful change. As the target accrual approaches, the consent form will be amended to remove reference to the QOL component.

Rev. Add4

13.1.1 Symptoms - Items from NFKSI-19

Although it was originally developed and validated to assess disease-related symptoms in patients with advanced kidney cancer, this instrument will also be useful to capture many of the most common side effects associated with nivolumab. Six of the 8 symptoms occurring among patients enrolled in the nivolumab arm of the Checkmate trial (existing reference 13) at rates of 5% or greater are captured on the NFKSI-19: fatigue, nausea, diarrhea, decreased appetite, cough, and dyspnea. These, along with pain, weakness, and fever, will form the symptom assessment instrument for the study.

The null hypothesis is that there is no difference in the change from baseline to week 40 in this symptom score. The alternative hypothesis is that the change in symptom burden differs among patients randomized to observation. This hypothesis will be tested using a two-sided Wilcoxon rank sum test with Type I error of 3%. As shown in the table, there will be 90% power to detect an effect size of about 0.45 if adherence at 40 weeks is 80%, and an effect size of about 0.5 if

adherence is 65%. Estimates of 40-week adherence are projected based on ASSURE, which demonstrated that 85% of enrolled patients had quality of life information available at 22 weeks. Estimates of variability for the FKSI-19 have been previously published[40]; we assume similar variability on the selected subset of items.

Standard Deviation	Correlation	Standard Deviation of Change	Difference between Nivolumab and Observation arms in mean change score between randomization and Week 40	
			65% Adherence (104 per arm)	80% Adherence (128 per arm)
9.0	0.4	9.86	4.93	4.44
	0.6	8.05	4.02	3.62
10.5	0.4	11.50	5.75	5.18
	0.6	9.39	4.70	4.23
12.0	0.4	13.15	6.57	5.92
	0.6	10.73	5.37	4.83
13.5	0.4	14.79	7.39	6.65
	0.6	12.07	6.04	5.43

13.1.2 Bother from side effects of treatment

At 40 weeks, in the midst of nivolumab treatment on Arm A, we will compare the proportions of patients who respond to the NFKSI-19 item with “somewhat”, “quite a bit”, or “very much” between the two arms. The null hypothesis is that the rates will be the same. The alternative is that the rates will be higher among patients receiving nivolumab. Using Fisher’s exact test with 1-sided Type I error of 0.005 (0.5%), there will be good power (90% to 95%) to detect a 20% difference in proportions (for example, 25% on the nivolumab arm and 5% on the observation arm), assuming adherence is between 65% and 80%.

13.1.3 Quality of Life: Change in PROMIS-physical function

The null hypothesis is that there will be no difference between arms in the change in physical function as assessed by the PROMIS instrument from baseline to 40 weeks. The alternative is that there is a difference. This is a two-sided hypothesis, since it is unknown whether the freedom from treatment-related interference on the observation arm will be outweighed by declines in function related to disease recurrence. Minimally important differences (MIDs) have been estimated for the PROMIS Physical Function instrument in advanced-stage cancer patients[41], and are 4.0 – 6.0 points, corresponding to effect sizes between 0.45 and 0.67. This hypothesis will be tested using a two-sided Wilcoxon rank sum test with Type I error of 1%. There will be 82 to 99% power to detect an effect size in this range if adherence at 40 weeks is 80%, and 72 to 98% power if adherence is 65%.

13.2 Analysis Plan

The primary QOL analysis can be done once all patients have submitted 40-week quality of life forms, expected to be about 3 years after activation assuming accrual occurs as planned. At this time, analyses of questions 1 through 4 above can be conducted. Quality of life data are expected to be fully mature when all patients have been followed for 2 years, which is expected to be 4.9 years after study activation.

Instruments will be scored using standard procedures. Instrument scores (and subscale scores as appropriate) will be prorated by multiplying the sum of the scale by the number of items in the scale, then dividing by the number of items actually answered. When there are missing data, prorating in this way is acceptable as long as more than 50% of items are answered.

The distribution of scores on all instruments and subscales will be estimated at each time point and reported using standard tabular and graphic analyses. The impact of missing data will be characterized.

In addition to the analyses described above, changes in NFKSI-19 and PROMIS Physical Function scores from baseline to pre-nephrectomy among patients randomized to neoadjuvant nivolumab will be explored using a Wilcoxon signed rank test. The response proportions to the item, “I am bothered by side effects of treatment” will also be reported. Similarly, changes from pre-nephrectomy to 8 weeks post-nephrectomy will be analyzed using this test. Differences in scores post-nephrectomy between the arms will be described and tested using a Wilcoxon rank-sum test.

There is at least 80% power to detect a clinically meaningful ½-standard deviation change in each of these analyses, assuming 2-sided Type I error of 5%.

Total and subscale scores on NFKSI-19 at the time of recurrence will be described in order to characterize patient-perceived symptoms experienced at the time of scan-detected recurrence.

Differences in the pattern of change over time in NFKSI-19 and PROMIS Physical Function scores will be examined using mixed effects models. A significant time-by-treatment interaction from the model would indicate such a difference. The model will include stratification factors and could include baseline scores if they are found to be different at baseline.

The above secondary analyses will be done using two-sided tests with Type I error of 5%.

13.3 PRO-CTCAE

PRO-CTCAE items related to toxicities associated with nivolumab administration have been systematically chosen for collection, based on frequencies and severity observed on the Checkmate study in advanced renal cell cancer (13). These include fatigue, nausea, itch, diarrhea, decreased appetite, and rash. These items will be captured at baseline, pre-nephrectomy (Arm A), 8 weeks post-nephrectomy, week 20, week 40, and week 54, which are the times at which clinic visits for assessment of adverse events occur commonly on both arms. We

will describe the severity, interference, and (as appropriate) frequency rates, along with response rates at each time point.

13.4 Missing Data

Describing quality of life is challenging in the presence of missing data. Such missingness can occur at several levels and due to a variety of causes. Patients may not complete all items on an instrument. As previously indicated, we will score instruments if patients complete at least half of the items. Instrument administration can be omitted at a given time point if patients do not come to the clinic for scheduled visits, or if there is not time during a visit to administer QOL instruments. We have provided contingency instructions in the protocol for mailing in QOL forms if these circumstances occur. We have scheduled the PRO-CTCAE assessments to occur in alignment with physician-assessed AEs, during clinic visits. At each timepoint, if QOL is not completed as planned, sites are asked to provide the reason that QOL was not done. These forms are completed with a very high compliance rate (> 95%), so reasons can be examined and acted on where possible. Reasons for missing data will be reported as part of the analysis.

This study is a registration study, and as such will include an on-site monitoring plan as well as telephone sweeps for outstanding data. These can be used to prospectively alert sites to upcoming QOL timepoints for patients.

Nonetheless, missing data may occur. This may increase over time, possibly among patients who have had disease recurrence. It cannot be assumed to be missing completely at random (MCAR) or even missing at random (MAR), since patients who recur or die are likely to have higher rates of missing data. We are extremely interested in the symptom burden attributable to disease recurrence, however, and the later time points are essential to explore. To address this, we will use a model-based method proposed by Schluchter[42], jointly modeling the distribution of RFS and QOL with a multivariate normal model. The model will compare the area under the quality of life curves for the two arms, taking into account the missing data.

14. Electronic Data Capture

Please refer to the EA8143 Forms Completion Guidelines for the forms submission schedule. Data collection will be performed exclusively in Medidata Rave.

This study will be monitored by the Clinical Data Update System (CDUS) Version 3.0. Cumulative protocol and patient specific CDUS data will be submitted electronically to CTEP on a quarterly basis, either by FTP burst of data or via the CDS web application. Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting the data using the CDUS can be found on the CTEP website (http://ctep.cancer.gov/reporting/cdus.html).

Imaging studies are to be submitted via TRIAD (Section 4.1.4.6)

14.1 Records Retention

FDA regulations (21 CFR 312.62) require clinical investigators to retain all trial-related documentation, including source documents, long enough to allow the sponsor to use the data to support marketing applications.

This study will be used in support of a US marketing application (New Drug Application), all records pertaining to the trial (including source documents) must be maintained for:

- two years after the FDA approves the marketing application, or
- two years after the FDA disapproves the application for the indication being studied, or
- two years after the FDA is notified by the sponsor of the discontinuation of trials and that an application will not be submitted.

Please contact the ECOG-ACRIN Operations Office – Boston prior to destroying any source documents.

15. Patient Consent and Peer Judgment

Current FDA, NCI, state, federal and institutional regulations concerning informed consent will be followed.

16. References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA: A Cancer Journal for Clinicians 2015; 65(1): 5-29.
2. Lam JS, Shvarts O, Leppert JT, Pantuck AJ, Figlin RA, Belldegrun AS. Postoperative surveillance protocol for patients with localized and locally advanced renal cell carcinoma based on a validated prognostic nomogram and risk group stratification system. J Urol 2005; 174(2): 466-72; discussion 72; quiz 801.
3. Thompson RH, Gillett MD, Cheville JC, 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): 17174-9.
4. Latchman YE, Liang SC, Wu Y, et al. PD-L1-deficient mice show that PD-L1 on T cells, antigen-presenting cells, and host tissues negatively regulates T cells. Proc Natl Acad Sci U S A 2004; 101(29): 10691-6.

5. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and Activity of Anti-PD-L1 Antibody in Patients with Advanced Cancer. N Engl J Med 2012.
6. Krambeck AE, Dong H, Thompson RH, et al. Survivin and b7-h1 are collaborative predictors of survival and represent potential therapeutic targets for patients with renal cell carcinoma. Clin Cancer Res 2007; 13(6): 1749-56.
7. Thompson RH, Dong H, Kwon ED. Implications of B7-H1 expression in clear cell carcinoma of the kidney for prognostication and therapy. Clin Cancer Res 2007; 13(2 Pt 2): 709s-15s.
8. Thompson RH, Dong H, Lohse CM, et al. PD-1 is expressed by tumor-infiltrating immune cells and is associated with poor outcome for patients with renal cell carcinoma. Clin Cancer Res 2007; 13(6): 1757-61.
9. McDermott DF, Drake CG, Sznol M, et al. Survival, Durable Response, and Long-Term Safety in Patients With Previously Treated Advanced Renal Cell Carcinoma Receiving Nivolumab. J Clin Oncol 2015; 33(18): 2013-20.
10. Motzer RJ, Rini BI, McDermott DF, et al. Nivolumab for Metastatic Renal Cell Carcinoma: Results of a Randomized Phase II Trial. J Clin Oncol 2015; 33(13): 1430-7.
11. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, Activity, and Immune Correlates of Anti-PD-1 Antibody in Cancer. N Engl J Med 2012.
12. Choueiri TK FM, Escudier BJ, Kim JJ, Kluger HM, Stadler WM, Perez-Gracia JM, McNeel DG, Curti BD, Harrison MR, Plimack ER, Appleman LJ, Fong L, Drake CG, Cohen LJ, Srivastava S, Jure-Kunkel M, Hong Q, Kurland JF, Sznol M;. Immunomodulatory activity of nivolumab in previously treated and untreated metastatic renal cell carcinoma (mRCC): Biomarker-based results from a randomized clinical trial. J Clin Oncol 32:5s, 2014 (suppl; abstr 5012).
13. Motzer RJ, Escudier B, McDermott DF, et al. Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma. The New England Journal of Medicine 2015; 373(19): 1803-13.
14. Woo SR, Turnis ME, Goldberg MV, et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. Cancer Research 2012; 72(4): 917-27.
15. Macfarlane AWt, Jillab M, Plimack ER, et al. PD-1 Expression on Peripheral Blood Cells Increases with Stage in Renal Cell Carcinoma Patients and Is Rapidly Reduced after Surgical Tumor Resection. Cancer Immunology Research 2014; 2(4): 320-31.
16. Flanigan RC, Mickisch G, Sylvester R, Tangen C, Van Poppel H, Crawford ED. Cytoreductive nephrectomy in patients with metastatic renal cancer: a combined analysis. J Urol 2004; 171(3): 1071-6.
17. Brahmer JR, Drake CG, Wollner I, 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): 3167-75.
18. Hudes G, Carducci M, Tomczak P, et al. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. N Engl J Med 2007; 356(22): 2271-81.

19. Maroto JP, Hudes G, Dutcher JP, et al. Drug-related pneumonitis in patients with advanced renal cell carcinoma treated with temsirolimus. J Clin Oncol 2011; 29(13): 1750-6.
20. Motzer RJ, Escudier B, Oudard S, et al. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. Lancet 2008; 372(9637): 449-56.
21. White DA, Camus P, Endo M, et al. Noninfectious pneumonitis after everolimus therapy for advanced renal cell carcinoma. Am J Respir Crit Care Med 2010; 182(3): 396-403.
22. Frank I, Blute ML, Cheville JC, Lohse CM, Weaver AL, Zincke H. Solid renal tumors: an analysis of pathological features related to tumor size. J Urol 2003; 170(6 Pt 1): 2217-20.
23. Pierorazio PM, Hyams ES, Mullins JK, Allaf ME. Active surveillance for small renal masses. Reviews in Urology 2012; 14(1-2): 13-9.
24. Freidlin B, Korn EL, Hunsberger S, Gray R, Saxman S, Zujewski JA. Proposal for the use of progression-free survival in unblinded randomized trials. J Clin Oncol2007; 25(15): 2122-6.
25. Webster WS, Lohse CM, Thompson RH, et al. Mononuclear cell infiltration in clear-cell renal cell carcinoma independently predicts patient survival. Cancer 2006; 107(1): 46-53.
26. Taube JM, Anders RA, Young GD, et al. Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Sci Transl Med 2012; 4(127): 127ra37.
27. Thompson RH, Kuntz SM, Leibovich BC, et al. Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up. Cancer Res 2006; 66(7): 3381-5.
28. Law CW, Chen Y, Shi W, Smyth GK. Voom: precision weights unlock linear model analysis tools for RNA-seq read counts. Genome Biol 2014; 15(2): R29.
29. Smyth GK, Michaud J, Scott HS. Use of within-array replicate spots for assessing differential expression in microarray experiments. Bioinformatics 2005; 21(9): 2067-75.
30. Smyth G. Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. 2004. Statistical Applications in Genetics and Molecular Biology; 3.
31. Kortenhorst MS, Wissing MD, Rodriguez R, et al. Analysis of the genomic response of human prostate cancer cells to histone deacetylase inhibitors. Epigenetics 2013; 8(9): 907-20.
32. Wu D, Lim E, Vaillant F, Asselin-Labat M-L, Visvader JE, Smyth GK. ROAST: rotation gene set tests for complex microarray experiments. Bioinformatics 2010; 26(17): 2176-82.
33. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proceedings of the National Academy of Sciences of the United States of America 2005; 102(43): 15545-50.

34. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B (Methodological) 1995: 289-300.
35. Santegoets SJ, Stam AG, Lougheed SM, et al. T cell profiling reveals high CD4+CTLA-4 + T cell frequency as dominant predictor for survival after prostate GVAX/ipilimumab treatment. Cancer Immunology, Immunotherapy : CII 2013; 62(2): 245-56.
36. Cha E, Klinger M, Hou Y, et al. Improved survival with T cell clonotype stability after anti-CTLA-4 treatment in cancer patients. Science Translational Medicine 2014; 6(238): 238ra70.
37. Tumeh PC, Harview CL, Yearley JH, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 2014; 515(7528): 568-71.
38. Anders S, Huber W. Differential expression analysis for sequence count data. Genome Biology 2010; 11(10): R106.
39. Freidlin B, Korn EL, Gray R, A general inefficacy interim monitoring rule for randomized clinical trials, Clinical Trials 2010; 7: 197-208.
40. Rothrock NE, Jensen SE, Beaumont JL, et al. Development and initial validation of the NCCN/FACT Symptom Index for advanced kidney cancer. Value in Health 2013; 16: 789-796.
41. Yost KJ, Eton DT, Garcia SE, Cella D. Minimally important differences were estimated for six Patient-Reported Outcomes Measurement Information System-Cancer scales in advanced cancer patients. J Clin Epidemiol 2011; 64: 507-516.
42. Schluchter MD. Methods for the analysis of informatively censored longitudinal data. Stat Med 1992; 11: 1861-1870.
43. Cella D, Wagner L, Re-personalizing precision medicine: Is there a role for patient-reported outcomes? J Commun Support Oncol 2015; 13(7): 275-277.
44. Basch E, Reeve BB, Mitchell SA, et al. Development of the National Cancer Institute's Patient Reported Outcomes Version of the Common Terminology Criteria for Adverse Events (PRO_CTCAE). J Natl Cancer Inst 2014; 106.
45. Haas NB, Manola J, Uzzo RG, et al. Adjuvant sunitinib or sorafenib for high-risk, non-metastatic renal-cell carcinoma (ECOG-ACRIN E2805); a double-blind, placebo-controlled, randomized, phase 3 trial. Lancet 2016. Doi 10.1016/50140-6736(16) 00559-6.

A Phase 3 RandOmized Study Comparing PERioperative Nivolumab vs. Observation in Patients with Renal Cell Carcinoma Undergoing Nephrectomy (PROSPER RCC)

Appendix I

Rev. Add4
Rev. Add8

Pathology Submission Guidelines

The following items are included in Appendix I:

1. Guidelines for Submission of Pathology Materials (instructional sheet for Clinical Research Associates [CRAs])
2. Instructional memo to submitting pathologists
3. ECOG-ACRIN Generic Specimen Submission Form (#2981)

Guidelines for Submission of Pathology Materials

The following pathology materials are to be submitted within one (1) month of randomization or collection or within one (1) week of sectioning fresh slides:

1. Pathology Submissions:
   - MANDATORY (Arm A): Representative formalin-fixed paraffin embedded FFPE blocks from any diagnostic primary or metastatic tumor tissue biopsy (primary tumor tissue is preferred if metastasis is also present).
     - Arm A Biopsy: Image guided percutaneous core needle biopsy of primary tumors (or metastasis) will be performed prior to starting treatment. Primary tumor is preferred over metastasis. Six non-fragmented biopsy cores should be obtained. One biopsy core will be sent to surgical pathology for standard of care histological analysis by the local pathologist and the five (5) other biopsy cores will be retained for research purposes. These cores will be placed in specimen containers and paraffin embedded. Of note, the paraffin embedded research cores will not be utilized until a histological diagnosis is reached. If in the event that a histological diagnosis cannot be reached with the dedicated diagnostic tissue core, one or more of these research cores will be released to pathology for analysis

       NOTE: Patients randomized to Arm B are encouraged, but not required, to have a core biopsy after randomization for patients who have not had a biopsy within twelve (12) months prior to randomization that confirms RCC.
   - MANDATORY (Both Arms): Representative formalin-fixed paraffin embedded FFPE blocks from surgical tumor tissue must be submitted within one (1) month of collection.
   - MANDATORY (Both Arms): Representative H&E slides from diagnostic and surgical tumor tissue blocks
   - OPTIONAL (Both Arms): Additional representative formalin-fixed paraffin-embedded (FFPE) primary tumor tissue blocks from diagnostic core needle biopsy, surgical FFPE primary tumor and normal tissue blocks, and recurrence or resection FFPE primary tumor tissue blocks (if available)

   NOTE: If blocks are unavailable for submission, cores and fresh cut slides are to be submitted. All cores and slides must be adequately labeled, with slides numbered sequentially in the order cut. Alternative submission requirements:
     - One (1) H&E slide from each source block, and
     - Twenty (20) 4 µm unstained, positively charged air-dried plus slides from the thickest part of the tumor (Superfrost Plus recommended). After cutting, the slides should be kept refrigerated (2-5°C) until shipment. Fresh slides must be shipped overnight within one (1) week of sectioning on refrigerated gel packs.
     - One (1) or more core punches (minimum of 4mm diameter). If core punch tool is unavailable, request core punch kit from the ECOG-ACRIN CBPF (844) 744-2420

   If these criteria cannot be met, please contact the ECOG-ACRIN CBPF (eacbpf@mdanderson.org) to obtain alternative submission requirements.
2. Forms and Reports:

   NOTE: Adequate patient identifying information must be included with every submission. It is strongly recommended that full patient names be provided. The information

will be used only to identify patient materials and will help to expedite any required communications with the institution (including site pathologists).

ICON (who is distributing the PK kits) is also providing zipper lock bags and specimen ID/barcode accession labels and tissue requisition forms for the baseline tissue submissions. Place blocks and/or slides in the zipper lock bags.

The pathology specimens are to be labeled with the institutional Pathology ID# and ICON sample ID/barcode/accession label on the zipper lock bags provided.

The following items are to be included with the pathology materials:

- Institutional Diagnostic and Surgical Pathology Reports
- ECOG-ACRIN Generic Specimen Submission Form (#2981) [If STS is unavailable]
- Sample Tracking System (STS) Shipping Manifest Form
- Immunological study reports, if available
- ICON Tissue Requisition Form (baseline only)

3. Mail pathology materials to:

Rev. Add10

MD Anderson Cancer Center CBPF
Mike Balco
2130 West Holcombe Boulevard, LSP9.4227
Houston, TX 77030
Phone: Toll Free (844) 744-2420 (713-745-4440 Local or International Sites)
Fax: (713) 563-6506
Email: eacbpf@mdanderson.org

If you have any questions concerning the above instructions, contact the pathology coordinator at the ECOG-ACRIN Central Biorepository and Pathology Facility by telephone: (844) 744-2420 or email: eacbpf@mdanderson.org

Robert L. Comis, MD, and Mitchell D. Schnall, MD, PhD
Group Co-Chairs

MEMORANDUM

TO: _____
(Submitting Pathologist)

FROM: Stanley Hamilton, M.D., Chair
ECOG-ACRIN Laboratory Science and Pathology Committee

DATE: _____

SUBJECT: *Submission of Pathology Materials for EA8143: A Randomized Phase III Study Comparing Perioperative Nivolumab vs. Observation in Patients with Unfavorable Localized Renal Cell Carcinoma Undergoing Nephrectomy*

A patient has been entered onto an ECOG-ACRIN protocol by _____ (ECOG-ACRIN Investigator). This protocol requires the submission of pathology materials for central diagnostic review and defined and/or undefined laboratory research studies.

Please return the surgical pathology report(s), the slides and/or blocks and any other required materials to the Clinical Research Associate (CRA). The CRA will forward all required pathology materials to the ECOG-ACRIN Central Biorepository and Pathology Facility.

Blocks and/or slides submitted for this study will be retained at the ECOG-ACRIN Central Biorepository and Pathology Facility for undefined future research studies. Paraffin blocks will be returned upon written request for purposes of patient management.

If you have any questions regarding this request, please contact the ECOG-ACRIN Central Biorepository and Pathology Facility at Tel: (844) 744-2420, Fax: (713) 563-6506 or Email eacbpf@mdanderson.org.

The ECOG-ACRIN CRA at your institution is:

Name: _____

Address: _____

Phone: _____

Thank you.

ECOG-ACRIN Generic Specimen Submission Form

Form No. 2981v3

Institution Instructions: This form is to be completed and submitted with **all specimens** ONLY if the Sample Tracking System (STS) is not available. **Use one form per patient, per time- point.** All specimens shipped to the laboratory must be listed on this form. Enter all dates as MM/DD/YY. Keep a copy for your files. Retroactively log all specimens into STS once the system is available. **Contact the receiving lab to inform them of shipments that will be sent with this form.**

Protocol Number _____ **Patient ID** _____ **Patient Initials** Last _____ First _____

Date Shipped _____ **Courier** _____ **Courier Tracking Number** _____

Shipped To (Laboratory Name) _____ **Date CRA will log into STS** _____

FORMS AND REPORTS: Include all forms and reports as directed per protocol, e.g., pathology, cytogenetics, flow cytometry, patient consult, etc.

Required fields for all samples				**Additional fields for tissue submissions**				**Completed by Receiving Lab**
Protocol Specified Timepoint:								
Sample Type (fluid or fresh tissue, include collection tube type)	**Quantity**	**Collection Date and Time 24 HR**		**Surgical or Sample ID**	**Anatomic Site**	**Disease Status** (e.g., primary, mets, normal)	**Stain or Fixative**	**Lab ID**

Fields to be completed if requested per protocol. Refer to the protocol-specific sample submissions for additional fields that may be required.					
Leukemia/Myeloma Studies:	**Diagnosis**	**Intended Treatment Trial**	**Peripheral WBC Count (x1000)**	**Peripheral Blasts %**	**Lymphocytes %**
Study Drug Information:	**Therapy Drug Name**	**Date Drug Administered**	**Start Time 24 HR**	**Stop Time 24HR**	
Caloric Intake:	**Date of Last Caloric Intake**		**Time of Last Caloric Intake 24HR**		

CRA Name _____ **CRA Phone** _____ **CRA Email** _____

Comments

9/12/14

A Phase 3 RandOmized Study Comparing PERioperative Nivolumab vs. Observation in Patients with Renal Cell Carcinoma Undergoing Nephrectomy (PROSPER RCC)

Appendix II

Patient Thank You Letter

We ask that the physician use the template contained in this appendix to prepare a letter thanking the patient for enrolling in this trial. The template is intended as a guide and can be downloaded from the web site at http://www.ecog.org. As this is a personal letter, physicians may elect to further tailor the text to their situation.

This small gesture is a part of a broader program being undertaken by ECOG-ACRIN and the NCI to increase awareness of the importance of clinical trials and improve accrual and follow-through. We appreciate your help in this effort.

[PATIENT NAME] [DATE]

[PATIENT ADDRESS]

Dear [PATIENT SALUTATION],

Thank you for agreeing to take part in this important research study. Many questions remain unanswered in cancer. With the participation of people like you in clinical trials, we hope to improve treatment and quality of life for those with your type of cancer.

We believe you will receive high quality and comprehensive care. My research staff and I will maintain very close contact with you. This will allow me to provide you with the best care while learning as much as possible to help you and other patients with kidney cancer.

On behalf of ***[INSTITUTION]*** and ECOG-ACRIN, we thank you again and look forward to helping you.

Sincerely,

[PHYSICIAN NAME]

A Phase 3 RandOmized Study Comparing PERioperative Nivolumab vs. Observation in Patients with Renal Cell Carcinoma Undergoing Nephrectomy (PROSPER RCC)

Appendix III

CRADA/CTA

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.
2. For a clinical protocol where there is an investigational Agent used in combination with (an)other investigational Agent(s), each the subject of different collaborative agreements , the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data."):
    a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.
    b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own investigational Agent.
    c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational Agent.
3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm). Additionally, all Clinical Data and Results and Raw Data will be collected , used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.
5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.
6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

   ncicteppubs@mail.nih.gov

   The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

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Appendix IV

ECOG Performance Status

PS 0	Fully active, able to carry on all pre-disease performance without restriction
PS 1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature e.g., light house work, office work.
PS 2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
PS 3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
PS 4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.

A Phase 3 RandOmized Study Comparing PERioperative Nivolumab vs. Observation in Patients with Renal Cell Carcinoma Undergoing Nephrectomy (PROSPER RCC)

Appendix V

Rev. Add3

Instructions for Reporting Pregnancies on a Clinical Trial

What needs to be reported?

All pregnancies and suspected pregnancies (including a positive or inconclusive pregnancy test regardless of age or disease state) of a female patient while she is on nivolumab, or within 100 days of the female patient's last dose of nivolumab must be reported in an expeditious manner. The outcome of the pregnancy and neonatal status must also be reported.

How should the pregnancy be reported?

The pregnancy, suspected pregnancy, or positive/inconclusive pregnancy test must be reported via CTEP's Adverse Event Reporting System (CTEP-AERs)

(http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm)

When does a pregnancy, suspected pregnancy or positive/inconclusive pregnancy test need to be reported?

An initial report must be done within 24 hours of the Investigator's learning of the event, followed by a complete expedited CTEP-AERs report within 5 calendar days of the initial 24-hour report.

What other information do I need in order to complete the CTEP-AERs report for a pregnancy?

- The pregnancy (fetal exposure) must be reported as a Grade 3 "Pregnancy, puerperium and perinatal conditions – Other (pregnancy)" under the System Organ Class (SOC) "Pregnancy, puerperium and perinatal conditions"
- The pregnancy must be reported within the timeframe specified in the Adverse Event Reporting section of the protocol for a Grade 3 event.
- The start date of the pregnancy should be reported as the calculated date of conception.
- The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agent(s) should be documented in the "Description of Event" section of the CTEP-AERs report.

What else do I need to know when a pregnancy occurs to a patient?

- The Investigator must follow the female patient until completion of the pregnancy and must report the outcome of the pregnancy and neonatal status via CTEP-AERs.
- The decision on whether an individual female patient can continue protocol treatment will be made by the site physician in collaboration with the study chair and ECOG-ACRIN Operations Office – Boston. Please contact the ECOG-ACRIN Operations Office – Boston to ask for a conference call to be set up with the appropriate individuals.

Rev. Add10

- *It is recommended the female patient be referred to an obstetrician-gynecologist, preferably one experienced in reproductive toxicity for further evaluation and counseling.*

How should the outcome of a pregnancy be reported?

The outcome of a pregnancy should be reported as an *amendment* to the initial CTEP-AERs report if the outcome occurs on the same cycle of treatment as the pregnancy itself. However, if the outcome of the pregnancy occurred on a subsequent cycle, a *new* CTEP-AERs report should be initiated reporting the outcome of the pregnancy.

What constitutes an abnormal outcome?

An abnormal outcome is defined as any pregnancy that results in the birth of a child with persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions (formerly referred to as disabilities), congenital anomalies, or birth defects. For assistance in recording the grade or category of these events, please contact the CTEP AEMD Help Desk at 301-897-7497 or aemd@tech-res.com, for it will need to be discussed on a case by case basis.

Reporting a Pregnancy Loss

A pregnancy loss is defined in CTCAE as "*A death in utero.*"

It must be reported via CTEP-AERs as a Grade 4 "*Pregnancy loss*" under the System Organ Class (SOC) "*Pregnancy, puerperium and perinatal conditions".*

A pregnancy loss should **NOT** be reported as a Grade 5 event as currently CTEP-AERs recognizes this event as a patient's death.

Reporting a Neonatal Death

A neonatal death is defined in CTCAE as "*A newborn death occurring during the first 28 days after birth*" that is felt by the investigator to be at least possibly due to the investigational agent/intervention. However, for this protocol, any neonatal death that occurs within 28 days of birth, without regard to causality, must be reported via CTEP-AERs AND any infant death after 28 days that is suspected of being related to the *in utero* exposure to nivolumab must also be reported via CTEP-AERs.

It must be reported via CTEP-AERs as Grade 4 "Death neonatal" under the System Organ Class (SOC) "General disorder and administration site conditions".

A neonatal death should **NOT** be reported as a Grade 5 event as currently CTEP-AERs recognizes this event as a patient's death.

Additional Required Forms:

When submitting CTEP-AERs reports for pregnancy, pregnancy loss, or neonatal loss, the **CTEP *'Pregnancy Information Form'*** must be completed and faxed along with any additional medical information to CTEP (301-897-7404). This form is available on CTEP's website (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/PregnancyReportForm.pdf)

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Rev. Add4
Rev. Add8

Appendix VI

EA8143 Collection and Shipping Kit Order Instructions

Specimen Collection/Shipping Kits are being provided by CENETRON CENTRAL LABORATORIES and are to be ordered ONLINE.

Starter kits are not available. Kit requests are to be made AFTER patient randomization.

Questions regarding kits can be directed to projectmanagement@cenetron.com or call the Cenetron clinical trials group at (512) 439-2000.

Ordering Process:

- At time of patient randomization, provide the contact for kit ordering in OPEN.
- Following randomization of the patient to the trial, go to the website www.cenetron.com and click on the 'Order Kits' button at the top right. It is recommended that kits be ordered same day as patient randomization.
- The order form is not study specific and can be used for any study. Complete the online form as follows:
  - **Sponsor (REQUIRED):** ECOG-ACRIN
  - **Contact Name (REQUIRED):** Name of the site kit contact. Should match the name of the individual provided in OPEN as the kit contact
  - **Protocol Number (REQUIRED):** EA8143
  - **Phone Number (REQUIRED):** Phone number of the kit contact. Please ensure that this is a number that can be reached from an external caller
  - **Site Number (REQUIRED):** Institution NCI Site ID
  - **FAX Number:** Fax number of the kit contact
  - **Investigator:** Last name of the kit contact is adequate
  - **Email (REQUIRED):** Email of the site kit contact. Must be entered twice to confirm
  - **Date Supplies Needed (REQUIRED):** Add three (3) business days or more to order date. (Reminder that weekends and holidays must also be considered in this timeline)
  - **KIT NAME (REQUIRED):** EA8143 Collection Kit
  - **Quantity:** 1
  - **Comments:** Provide EA8143 Patient Case ID# and full shipping address
    - 'Patient Case ID =' #####
    - *Ship Kit to* name of the individual to whom the kit is being shipped. (May be different than the kit contact provided above)
    - Full street address, town, state and zip code
  - Answer the security question

Please complete this form correctly, including the valid ECOG-ACRIN patient case number and complete shipping address. If information is missing the kit processing will be delayed.

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Appendix VII

EA8143 Biopsy Reimbursement Form

This form is to be used to request reimbursements for the performance and submission of the **non-SOC** biopsies as outlined in Section 10. Reimbursements are NOT applicable for biopsies performed as part of standard of care, and thus billed to patients or their insurance.

If you have questions about the reimbursement process, please contact the EA funding team at ea.fundingsheet@jimmy.harvard.edu.

Please fax the completed form to the ECOG-ACRIN Translational Science Team (TST), FAX: (617) 589-0914

Institution CTEP ID: _____

Name of Investigator: _____

NCI Investigator ID #: _____

Payee Address

Payee/W-9 Name: _____

Payee Tax ID #: _____

Attention To: _____

Street Address: _____

City, State, Zip: _____

Any Requested Reference on Payment (i.e. Invoice #): _____

	ECOG-ACRIN Case ID	Time Point	Date of Service	Service Performed	Amount Requested
#1		Following randomization, prior to start of protocol therapy			$ 3000.00
#2					$ 3000.00
#3					$ 3000.00

I confirm that these patients are registered to the protocol referenced above, the patient numbers and procedure dates are correct, and the biopsies were performed for the purposes of the trial only, following randomization of the patient to the lowest step of the trial, and that the biopsy was NOT standard of care and was NOT billed to insurance or the patient

Signature: _____ **Date:** _____

If there are problems with this invoice, please contact:

Name	Phone	Fax	Email

ECOG-Operations Office Use Only: **TST Reviewer:** _____ **Date:** _____

Patient	#1	#2	#3
Date of Randomization			
Registering Institution			
Data in STS indicates "Not billed to insurance"	☐	☐	☐
Performed after randomization, prior to start of therapy.	☐	☐	☐
Specimen indicated as received by the receiving laboratory in STS	☐	☐	☐
Approved	☐	☐	☐

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Appendix VIII

Methods Of Contraception

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At a minimum, patients must agree to use one highly effective OR one less effective method of contraception as listed below:

HIGHLY EFFECTIVE METHODS OF CONTRACEPTION

Highly effective methods of contraception have a failure rate of < 1% when used consistently and correctly. Patients of child bearing potential and partners of male subjects, who are of childbearing potential, are expected to use one of the highly effective methods of contraception listed below. Male patients must inform their partners who are of childbearing potential of the contraceptive requirements of the protocol and are expected to adhere to using contraception with their partner. Contraception methods are as follows:

1. Progestogen only hormonal contraception associated with inhibition of ovulation.
2. Hormonal methods of contraception including oral contraceptive pills containing combined estrogen + progesterone, vaginal ring, injectables, implants and intrauterine devices (IUDs) such as Mirena.
3. Nonhormonal IUDs, such as ParaGard
4. Bilateral tubal occlusion
5. Vasectomised partner with documented azoospermia 90 days after procedure
    - Vasectomised partner is a highly effective birth control method provided that partner is the sole sexual partner of the WOCBP trial participant of childbearing potential and that the vasectomised partner has received medical assessment of the surgical success.
6. Intrauterine hormone-releasing system (IUS)
7. Complete abstinence
    - Complete abstinence is defined as the complete avoidance of heterosexual intercourse. (refer to Glossary of Terms)
    - Complete abstinence is an acceptable form of contraception for all study drugs and must be used throughout the duration of the study treatment (plus 5 half-lives of the investigational drug plus 30 days).
    - It is not necessary to use any other method of contraception when complete abstinence is elected.
    - Patients who choose complete abstinence must continue to have pregnancy tests, as specified in Section 7
    - Acceptable alternate methods of highly effective contraception must be discussed in the event that the patient chooses to forego complete abstinence.
    - The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient.

LESS EFFECTIVE METHODS OF CONTRACEPTION

8. Diaphragm with spermicide
9. Cervical cap with spermicide

10. Vaginal sponge with spermicide
11. Male or female condom with or without spermicide*
12. Progestogen-only oral hormonal contraception, where inhibition of ovulation is not the primary mode of action.

* A male and a female condom must not be used together.

UNACCEPTABLE METHODS OF CONTRACEPTION

13. Periodic abstinence (calendar, symptothermal, post-ovulation methods)
14. Withdrawal (coitus interruptus)
15. Spermicide only
16. Lactation amenorrhea method (LAM)

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Appendix IX

Financial Disclosure Information Form

The following items are included in Appendix IX:

1. EA8143 Financial Disclosure Information Form

EA8143 FINANCIAL DISCLOSURE INFORMATION FORM

Name:				
☐**Investigator**	☐**Sub Investigator**	☐**Independent Assessor**	☐**IRC Member**	☐**Other (specify):**
NOTE: Name and Role must match the information on Form FDA 1572 or Site Information Sheet (SIS) and Curriculum Vitae (CV), as applicable.				
Protocol Number:	**EA8143 - CA209**	**Site Number:**		

Please answer each of the questions below BY CHECKING THE APPROPRIATE BOX.
If you check YES to any question you MUST provide details of the disclosable information.

Please note that if there is any discrepancy between the financial disclosure information reported on this form (or updated versions of it) and internal financial records, an explanation will be required.

Do you, your spouse, or your dependent children participate in any financial arrangement with Bristol-Myers Squibb and/or Ono Pharmaceutical Co., LTD whereby the value of the compensation could be influenced by the outcome of the study (i.e. could be higher for a favorable outcome than for an unfavorable outcome)? If y**es**, please describe: *This could be compensation that is explicitly greater for a favorable result, compensation in the form of an equity interest in BMS and/or Ono or compensation tied to sales of the product, such as a royalty interest. [21 CRF 54.2 (a)]*	**YES**☐	**NO**☐
During the time you are conducting the study and for one (1) year following completion of the study, will you, your spouse or dependent children or your institution (to the extent used to support your activities), receive any significant payments of other sorts from Bristol-Myers Squibb and/or Ono Pharmaceutical Co., LTD that exceed $25,000 USD (exclusive of the costs of conducting the clinical study or other clinical studies)? If **yes**, please provide the USD amount and the nature of the payments: *Such payments might be a grant to you **or your institution** to fund ongoing research, compensation in the form of equipment, retainers for ongoing consultation, or honoraria. [21 CFR 54.2 (f)]*	**YES**☐	**NO**☐
Do you, your spouse or dependent children, hold any proprietary interest (property or other financial interest) in the product? If **yes**, please describe: *Proprietary interest would include, but not be limited to, a patent, trademark, copyright or licensing agreement [21 CRF 54.2 (c)]*	**YES**☐	**NO**☐
Do you, your spouse or dependent children, hold any significant equity interest in Bristol-Myers Squibb and/or Ono Pharmaceutical Co., LTD (any ownership interest [stock], stock options or other financial interest) that exceeds $50,000 USD? If **yes**, please provide the USD amount and the nature of the equity interest below: *Equity interest includes any options, puts, calls, straddles and other privileges in addition to an equity ownership position in BMS and/or Ono. This does not include ownership interest, stock options or other financial interests over which you have no direct control or input as to the quantities or amounts, e.g., a 401k, IRA, Mutual Fund. [21 CRF 54.2 (b)]*	**YES**☐	**NO**☐

In accordance with U.S. Code of Federal Regulations (21 CFR Part 54), I declare that the information provided on this form is, to the best of my knowledge and belief, true, correct and complete. Furthermore, if my financial interests and arrangements, or those of my spouse and dependent children, change from the information provided above during the course of the study or within one year following completion of the study as per site closure letter, I will promptly notify Bristol-Myers Squibb.			
SIGNATURE & DATE			
	DAY	**MONTH**	**YEAR**

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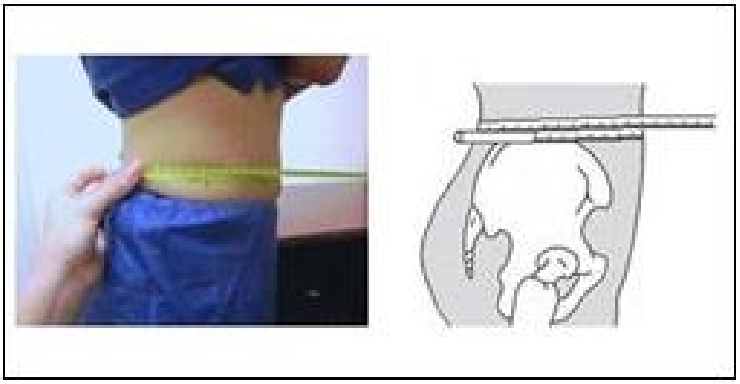
Appendix X

How To Measure Your Waist Circumference[2]

How To Measure Your Waist Circumference[2]

To correctly measure waist circumference:



- Stand and place a tape measure around your middle, just above your hipbones
- Make sure tape is horizontal around the waist
- Keep the tape snug around the waist, but not compressing the skin
- Measure your waist just after you breathe out

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Appendix XI

American Joint Committee on Cancer (AJCC) TNM Staging System for Kidney Cancer (7th Edition)

Rev. Add10

Primary tumors (T)

TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Tumor ≤ 7 cm in greatest dimension, limited to the kidney
T1a	Tumor ≤ 4 cm in greatest dimension, limited to the kidney
T1b	Tumor > 4 cm but ≤ 7 cm in greatest dimension, limited to the kidney
T2	Tumor > 7 cm in greatest dimension, limited to the kidney
T2a	Tumor > 7 cm but ≤10 cm in greatest dimension, limited to the kidney
T2b	Tumor > 10 cm, limited to the kidney
T3	Tumor extends into major veins or perinephric tissues but not into the ipsilateral adrenal gland and not beyond the Gerota fascia
T3a	Tumor grossly extends into the renal vein or its segmental (muscle-containing) branches, or tumor invades perirenal and/or renal sinus fat but not beyond the Gerota fascia
T3b	Tumor grossly extends into the vena cava below the diaphragm
T3c	Tumor grossly extends into the vena cava above the diaphragm or invades the wall of the vena cava
T4	Tumor invades beyond the Gerota fascia (including contiguous extension into the ipsilateral adrenal gland)

Regional lymph node (N)

NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in regional lymph node(s)

Distant metastasis (M)

M0	No distant metastasis
M1	Distant metastasis

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Rev. Add4

Appendix XII

Rev. Add2

EA8143 ICON Site Information/Initial Kit Order Form

Please complete this form for the initial shipment of three (3) kits and the Laboratory Instruction Manual. ICON requires fifteen to twenty (15-20) days to process this form and ship out the initial kits. Please complete and email to EA8143_initial_ICON_kit@ecog-acrin.org as soon as possible. Questions regarding kits can be directed to ICON toll-free at (877) 797-4422 or LabSiteHelp@iconplc.com. Please reference protocol number: CA209-531 (EA8143) when contacting ICON.

To reorder kits sites must register with iSite, ICON Central Laboratories Secure Website Portal. See Appendix XIII for instructions. Kit reorder shipments usually take about seven (7) to ten (10) business days.

Please write using block letters.

STUDY COORDINATOR LAST NAME	
STUDY COORDINATOR FIRST NAME	
STUDY COORDINATOR TELEPHONE NUMBER	
STUDY COORDINATOR FAX NUMBER	
CELL PHONE NUMBER/PAGER NUMBER	
EMAIL ADDRESS	
PROVIDE ADDRESS WHERE KITS ARE TO BE SHIPPED BELOW	
INSTITUTION	
DEPARTMENT	
BUILDING/FLOOR/ ROOM*	
STREET	
CITY/STATE/POSTAL CODE/COUNTRY	
ADDRESS WHERE LABORATORY SPECIMENS ARE COLLECTED	
INSTITUTION NCI CTEP ID#	
INVESTIGATOR NCI CTEP ID#	
INVESTIGATOR LAST NAME	

INVESTIGATOR FIRST NAME	
STREET ADDRESS	
CITY/STATE/POSTAL CODE/COUNTRY	
INVESTIGATOR TELEPHONE NUMBER	
INVESTIGATOR FAX NUMBER	
INVESTIGATOR EMAIL ADDRESS	

*** Mandatory information for hospital, clinic or large sites.**

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Rev. Add4

Appendix XIII

Rev. Add2

iSite User Guide for Kit Reordering

Getting Started

To ensure security, only email addresses on file with ICON Central Laboratories are able to register with iSite. You must submit the EA8143 ICON Site Information/Initial Kit Order Form before you can create an account in iSite. If you are unsure of the email address that ICON has for your site please contact ICON toll-free at (877) 797-4422 or LabSiteHelp@iconplc.com. Please reference protocol number: CA209-531 (EA8143) when contacting ICON.

Creating an Account in iSite

Step 1. To register, visit https://isite.iconplc.com

Step 2. Click **Create Account**. *The Registration screen displays.*

Step 3. Enter the **Email Address** that is on file with ICON Central Laboratories. Click **Submit**. *Registration screen will display automated message advising of email being sent to complete the registration process.*

Step 4. From your email account, open the message from iSite Support. Click the link. *The 'Create a New Account' screen will display.*

Step 5. Select your **Name***, enter in a **Password****, **Confirm password** and accept the **Terms and conditions**. Click Register. The 'Select Study' screen displays.

***NOTE:** If more than one name displays, this indicates our database has the email address entered associated to each contact listed. Choose the name you want associated with iSite.

****NOTE:** Follow the instructions on the Registration screen of iSite for password requirements.

Log In

Step 1. Go to https://isite.iconplc.com

Step 2. Enter **Email Address** used to create the account and **Password**.

Step 3. Click **Log In**. *The 'Select Study' screen displays*

IMPORTANT: After three unsuccessful attempts, your account will be locked. You will need to click the **Forgot password** link to change your password.

Selecting a Study

Step 1. From the **Home** screen, in the **To Start** section, select the applicable **Study** from the drop down list.

NOTE: Only active studies that you are participating in are listed in the drop down menu. If you are only participating in one study, it will automatically be selected for you.

To order kits select the tab at the top of the screen for **Supplies and Kit Ordering** and then select your site.

Changing Your Password

If you need to change your password, you may do so directly from iSite.

Step 1. From the menu bar, click **MY ACCOUNT**. *The 'My Account' screen displays.*

Step 2. Click **Change Password**. *The 'Change Password' screen displays.*

Step 3. Enter your **Current password, New password** and **Confirm new password**. Click **Change Password**. *When entered correctly, "Congratulations! Your password has been changed successfully" will display.*

NOTE: Follow the instructions on the Change Password screen for password requirements.

Logging Out of iSite

Step 1. From any tabbed window, click **Log Out**. *The window displays "You have successfully logged out.*

A Phase 3 RandOmized Study Comparing PERioperative Nivolumab vs. Observation in Patients with Renal Cell Carcinoma Undergoing Nephrectomy (PROSPER RCC)

Rev. Add10

Appendix XIV

Correlative Studies of the EA8143 Trial

Title of Study: Correlative Studies of the EA8143 Trial
Principal Investigator: Sabina Signoretti, MD
Co-Investigators: Mohamad Allaf, MD
Naomi Haas, MD
David McDermott, MD
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Abstract:

PROSPER RCC (EA8143, NCT03055013) is a Phase III, randomized trial that investigates the impact of perioperative nivolumab compared with observation on recurrence-free survival (RFS) in patients with high-risk Renal Cell Carcinoma (RCC) undergoing radical or partial nephrectomy. On April 8th, 2021, it was announced that *treatment with pembrolizumab led to improvement in disease-free survival in patients with RCC following nephrectomy, meeting the primary endpoint of the phase 3 KEYNOTE-564 trial.* These findings raise cautious optimism that the PROSPER trial will also meet its primary endpoint, demonstrating that anti-PD-1 therapy is also effective, and perhaps even more so, when administered perioperatively in patients with high-risk RCC.

While immune-checkpoint inhibitors are approved for patients with stage 3 resected melanoma, their use has been limited by their toxicity. Therefore, the development of biomarkers that can identify the subset of patients with RCC who are most likely to experience disease recurrence after surgery and benefit from neoadjuvant/adjuvant therapy is warranted. Here we plan to validate biomarkers for RCC that we have already developed in different settings and have been published in high impact journals.

Intratumor immune infiltration and expression of immune checkpoint molecules (e.g., PD-L1) are associated with poor prognosis in localized clear RCC (ccRCC) but improved clinical outcome to anti-PD-1/PD-L1 therapy in metastatic ccRCC. We have recently identified tissue-based immune biomarkers (including T cell immunophenotypes, gene expression signatures and endogenous retroviruses) associated with response or resistance to nivolumab therapy in metastatic ccRCC. **We hypothesize that immune biomarkers levels** assessed in tumor tissue specimens from the PROSPER trial **are associated with disease recurrence in patients not receiving nivolumab therapy (control arm). We expect that the immune biomarkers will be predictive of clinical outcome to perioperative nivolumab relative to control** (especially if the trial meets its primary endpoint).

Recently, our group has developed robust plasma-based assays for circulating biomarkers (i.e., cell-free methylated DNA and kidney injury molecule-1 (KIM-1)) that allow the detection of RCC in various clinical settings. **We hypothesize that biomarker levels in post-nephrectomy plasma samples** from the PROSPER trial **are associated with disease recurrence in the entire trial**

cohort. We are also interested in exploring whether biomarker levels in pre-treatment plasma specimens can predict clinical outcomes.

Finally, we anticipate that **the comparison of tumor intrinsic and tumor extrinsic features in paired pre- and post-treatment (i.e., nephrectomy) tissue specimens will provide novel insights into immune cell populations and/or signaling pathways that mediate response or resistance to PD-1 blockade.**

These hypotheses will be tested by performing multiplex immunofluorescence, RNA (transcriptome) sequencing and whole-exome sequencing on tissue specimens; a microbead-based assay (for KIM-1) and an assay for detection of cell-free methylated DNA sequencing on blood specimens. **Please note that all these correlative analyses (with the exception of the detection of cell-free methylated DNA, which is a newer method) are identical to those embedded in the clinical trial protocol (Section 11.3).** Of note, the **proposed studies will not exhaust the collected materials.**

As the field is moving forward quickly, it would be critical to start **correlative studies before the results of the trial are reported *to be maximally impactful as correlative science.*** If the trial meets its primary endpoint, the timely identification of predictive biomarkers for perioperative nivolumab in high-risk RCC would ensure the optimal utilization of this regimen in the clinic. However, even if the trial fails to meet its endpoints, the proposed studies are likely to identify prognostic (and possibly predictive) biomarkers as well as potential therapeutic targets for localized high risk RCC that could inform the design of future clinical investigations.

Specific Aims: Objectives and Hypotheses

a. Objectives: The main goal of this proposal is to develop tissue- and blood-based biomarkers that guide optimal treatment of patients with high-risk localized RCC.

b. Hypotheses:

1. TISSUE-BASED BIOMARKERS: On the basis of current knowledge of RCC biology and our preliminary data (see below), we hypothesize that tissue-based immune biomarkers identified in the metastatic setting also predict clinical outcome in high-risk RCC. More specifically, we hypothesize that biomarker levels assessed in pre- and/or post-treatment tumor tissue specimens from the PROSPER trial (a) are associated with disease recurrence in patients not receiving nivolumab therapy (control arm); (b) predict clinical outcome to perioperative nivolumab relative to control, especially if the trial meets its primary endpoint.

If, as anticipated, **multiple biomarkers are found to predict clinical outcome in high risk RCC**, in future efforts we will assess whether a combination of these biomarkers can improve the risk stratification, with the final goal of developing an **integrated model for prediction of RFS using a biomarker panel.**

The specific hypotheses that we will test in this proposal are listed below:

(i) ***High levels of mildly exhausted CD8$^+$ tumor infiltrating cells*** (determined by multiplex immunofluorescence) ***combined with tumor cell PD-L1 expression*** (Pignon et al. *Clin Cancer Res* 2019; Ficial et al, *Clin Cancer Res* 2021) are **(a)** associated with worse RFS in patients not receiving nivolumab therapy (control arm) and **(b)** predictive for benefit of perioperative nivolumab relative to control, especially if the trial meets its primary endpoint. Therefore, this biomarker could be useful to identify patients that are likely to relapse after nephrectomy and benefit from perioperative nivolumab.

(ii) ***A molecular RCC subtype characterized by high expression of T-effector and cell proliferation related genes*** (determined by RNAseq) (cluster #4 in Motzer et al, *Cancer Cell* 2020) is **(a)** associated with worse RFS in patients not receiving nivolumab therapy (control arm), and **(b)** predictive for benefit of perioperative nivolumab relative to control, especially if the trial meets its primary endpoint. Therefore, this biomarker could be useful to identify patients that are likely to relapse after nephrectomy and benefit from perioperative nivolumab.

(iii) ***Expression of human endogenous retroviruses (hERVs)*** (determined by RNAseq) (Ficial et al, *Clin Cancer Res* 2021) is **(a)** associated with worse RFS in patients not receiving nivolumab therapy (control arm) and **(b)** predictive for benefit of perioperative nivolumab relative to control, especially if the trial meets its primary endpoint. Therefore, this biomarker could be useful to identify patients that are likely to relapse after nephrectomy and benefit from perioperative nivolumab.

(iv) ***Mutations in SETD2 and potentially other genomic alterations*** (determined by whole exome sequencing) (McDermott et al, *J Clin Oncol* 2020) **(a)** are associated with worse RFS in patients not receiving nivolumab therapy (control arm), and **(b)** might be predictive for benefit of perioperative nivolumab relative to control, especially if the trial meets its primary endpoint. Therefore, this biomarker could be useful to identify patients that are likely to relapse after nephrectomy and benefit from perioperative nivolumab.

(v) ***High levels of a gene signature of interaction between terminally exhausted CD8 cells and tumor associated macrophages*** (determined by RNAseq) (Braun et al, *Cancer Cell* 2021) is **(a)** associated with worse RFS in both study arms (regardless of the outcome of the trial), and **(b)** predictive for lack of benefit of perioperative nivolumab relative to control, especially if the trial meets its primary endpoint. Therefore, this biomarker could be useful to identify patients that are likely to relapse after nephrectomy but don't benefit from perioperative nivolumab, and thus need other therapeutic approaches and/or intensified monitoring.

(vi) ***Exploratory hypothesis:*** High levels of intratumoral PD-1^{+} regulatory T cells (determined by multiplex immunofluorescence) are **(a)** associated with worse RFS in both study arms (regardless of the outcome of the trial) and **(b)** predictive for lack of benefit of perioperative nivolumab relative to control, especially if the trial meets its primary endpoint. Therefore, this biomarker might be useful to identify patients that are likely to relapse after nephrectomy but don't benefit from perioperative nivolumab, and thus need other therapeutic approaches and/or intensified monitoring.

(vii) ***Exploratory hypothesis:*** Comparison of tumor intrinsic and tumor extrinsic features in paired pre- and post-treatment (i.e., nephrectomy) tissue specimens will provide novel insights into immune cell populations and/or signaling pathways that mediate response or resistance to PD-1 blockade. This work will include analyses of data obtained from genomic, transcriptomic, multiplex IF and histopathologic (from H&E-stained slides) studies.

2. **BLOOD-BASED BIOMARKERS:** We developed robust plasma-based assays for circulating biomarkers (i.e., cell-free methylated DNA and kidney injury molecule-1 (KIM-1)) that allow the detection of RCC in various clinical settings. We hypothesize that biomarker levels measured in post-nephrectomy plasma specimens collected at week 40 within the PROSPER trial are associated with disease recurrence in the entire clinical trial cohort, regardless of the outcome of the trial.

We are also interested in exploring whether biomarker levels in pre-treatment plasma specimens can predict clinical outcomes.

The specific hypotheses that we will test in this proposal are listed below:

(i) ***Kidney injury molecule-1 (KIM-1) levels*** (Xu et al, *Clin Cancer Res* 2021) measured in post-nephrectomy plasma specimens are associated with disease recurrence in both study arms. Therefore, this non-invasive biomarker could be useful to identify patients that are likely to relapse after nephrectomy (with or without perioperative treatment).

(ii) ***Detection of cell-free methylated DNA*** (Nuzzo et al, *Nat Med* 2020) in post-nephrectomy plasma specimens are associated with disease recurrence in both study arms. Therefore, this non-invasive biomarker could be useful to identify patients that are likely to relapse after nephrectomy (with or without perioperative treatment).

(iii) ***Exploratory hypothesis:*** KIM-1 levels in pre-treatment plasma sps can predict clinical outcomes after nephrectomy.

Background and Significance

Bio-specimens Being Requested:

Currently, pretreatment tissue specimens have been collected from 335 patients and nephrectomy specimens have been collected from 401 patients. Pre-nephrectomy plasma specimens have been collected from 391 patients and post-nephrectomy plasma specimens have been collected from 179 patients. Specimen collection is still ongoing, and we anticipate that we will be able to collect specimens from all the patients who have provided consent for use of specimens for research (n=670).

From the nephrectomy specimen, we request ten (10) 4µm unstained slides from a representative tissue block and access to all available H&E slides/digital images (once pathology review is complete)

From the pretreatment biopsy specimen, we request fifteen (15) 4µm unstained slides and access to all available H&E slides/digital images (once pathology review is complete).

We have a specific interest in identifying biomarkers that can identify the subset of patients with RCC who are most likely to experience disease recurrence after surgery and can benefit from neoadjuvant anti-PD-1 therapy. The PROSPER trial is the only clinical trial investigating the efficacy of perioperative PD-1 blockade in high risk RCC.

From the post-nephrectomy specimen (week 40), we request one 10mL EDTA purple top tube.

From the pre-nephrectomy specimen, we request one 10mL EDTA purple top tube.

Preliminary Data and Study Justification:

TISSUE-BASED BIOMARKERS

We have used a multi-faceted approach to identify predictors of response and resistance to anti-PD-1 monotherapy in patients with metastatic RCC. These preliminary data constitute the basis for the development of biomarkers for nivolumab in patients with localized kidney cancer undergoing nephrectomy.

1. Candidate biomarkers of response to anti-PD-1 therapy

(i) High levels of mildly exhausted CD8⁺ tumor infiltrating cells combined with tumor cell PD-L1 expression are associated with improved clinical outcomes to anti-PD-1 monotherapy in metastatic RCC. Results from preclinical studies have demonstrated that CD8⁺ PD-1⁺ cells that do not express additional inhibitory receptors, are less dysfunctional than CD8⁺ PD-1⁺ cells expressing multiple inhibitory receptors (e.g., CD8⁺PD-1⁺TIM-3⁺ cells are more dysfunctional than CD8⁺PD-1⁺TIM-3⁻ cells (1, 2) Therefore, mildly dysfunctional CD8⁺ tumor infiltrating lymphocytes (TILs) may be more effectively re-invigorated by anti-PD-1 therapy. In line with these data, using tumor specimens from CheckMate 010 trial, we have shown that high levels of CD8⁺ TILs expressing PD-1 but not the inhibitory receptors TIM-3 and LAG-3 (hereafter referred to as IF Biomarker) are associated with response to anti-PD-1 therapy in ccRCC.(8) Moreover, we have also shown that the combination of the IF biomarker with tumor cell PD-L1 expression identifies three subgroups of patients with different clinical outcomes (see Pignon et al. *Clin Cancer Res* 2019 for details) (3).

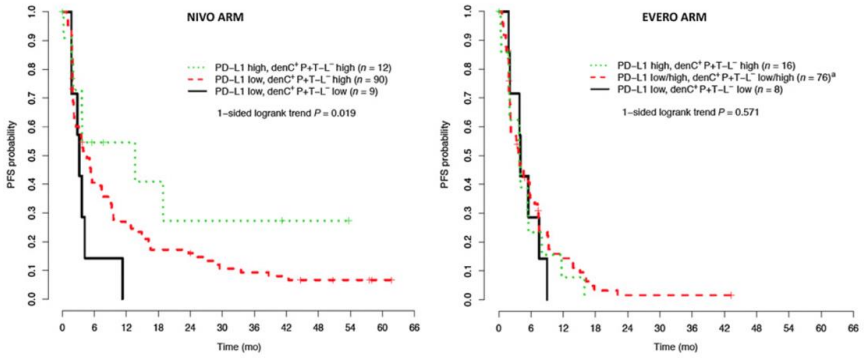


Figure 1. Stratification of PFS by tumor cell PD-L1 expression and immunophenotype of infiltrating T cells in CheckMate 025 trial. In the nivolumab arm (left) but not in the everolimus arm (right), patients with high expression of PD-1 (without TIM-3 and LAG-3) on T cells and high expression PD-L1 on tumor cells (TC) (green line) were the most likely to benefit from nivolumab; those with high expression of PD-1 (without TIM-3 and LAG-3) on T cells in absence of TC PD-L1 expression (red line) had an intermediate response; and those with neither high T cell PD-1 expression (without TIM-3 and LAG-3) nor TC PD-L1 expression (black line) had an overall poor response.

Subsequently, we have validated these findings in the randomized CheckMate 025 trial. At an optimized cut-off, nivolumab-treated patients with high IF biomarker (24/116, 21%) had higher overall response rate (ORR) (46% vs. 20%, *P*=0.01) and longer median PFS (9.6 vs. 3.7 months, *P*=0.03) than those with low IF biomarker (4).

In models considering a biomarker-by-treatment interaction term for both PFS and OS were significant (2-sided *P*=0.02 and 0.08, respectively, significance threshold set at 0.15 for the interaction term) (4). When combined with expression levels of the IF biomarker, TC PD-L1 expression of ≥1% further separated clinical outcomes (ORR and PFS) for those treated with nivolumab, but not for those treated with everolimus (**Figure 1 and Table 1**) (see Ficial et al. *Clin Cancer Res* 2021 for details) (4).

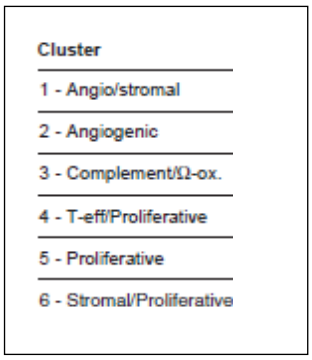
Marker Combination	**n**	**Nivolumab (n=111) ORR**
PD-L1 High / IF Biomarker High	12	50%
PD-L1 Low / IF Biomarker High	90	22%
PD-L1 Low / IF Biomarker Low	9	11%
Trend test 1-sided *P*-value	**0.027**	

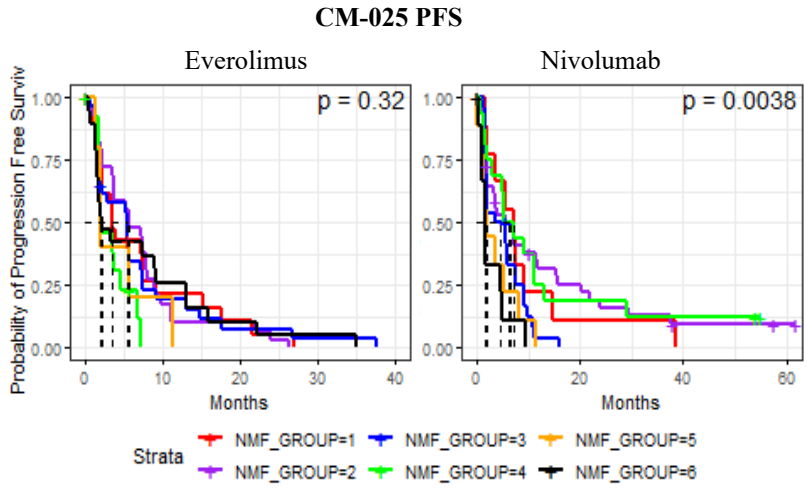
Table 1. ORR per tumor cell PD-L1 (cutoff ≥1%) and log density of CD8+PD-1+TIM-3-LAG-3-TIC levels (high vs. low) in the nivolumab arm of the CM-025 trial.

Overall, these results obtained from two independent cohorts indicate that combining CD8⁺ cell immunophenotyping with TC PD-L1 status is effective in identifying subsets of

patients with high likelihood of experiencing durable responses to anti-PD-1 therapy in previously treated patients with metastatic RCC.

(ii) A molecular RCC subtype characterized by high expression of T-effector and cell proliferation related genes is associated with improved clinical outcomes to anti-PD-1 monotherapy in metastatic RCC. A biomarker analysis was recently conducted on specimens from patients enrolled in the IMmotion151 clinical trial that compared the efficacy of atezolizumab (a PD-L1 ICI) plus bevacizumab (anti-VEGF), versus sunitinib in the first-line setting in advanced ccRCC.





The investigators (including Dr. McDermott) identified several molecular RCC subtypes and provided evidence that these molecular subgroups associate with differential clinical outcomes on atezolizumab plus bevacizumab versus sunitinib (5). Of note, atezolizumab + bevacizumab significantly improved clinical benefit in tumors with high T-effector and/or cell-cycle gene signatures, relative to sunitinib (see Motzer et al *Cancer Cell* 2020 for details) (5). To assess whether this molecular classification could be useful to also predict outcome to anti-PD-1 monotherapy, we recently applied the IMmotion 151 signatures to the patient specimens from the Checkmate 025 trial. In line with IMmotion 151 results, we observed that patients in cluster 4 (T effector/proliferative) benefited more from nivolumab compared to everolimus (HR 2.82, 95% CI 1.15-6.93) (**Figure 2**).

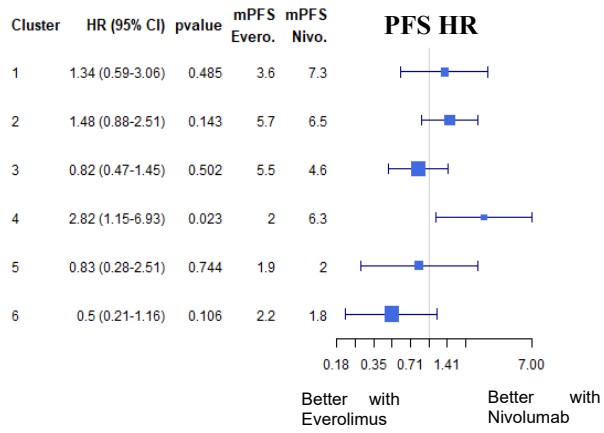


Figure 2. Association between transcriptomic clusters and clinical outcomes to nivolumab or everolimus (CheckMate-025 trial).

In terms of ORR, there was a strong trend toward better response in nivolumab treated patients compared to everolimus treated patients in cluster 4 (33.3% versus 0%; p = 0.07). This trend was confirmed when CR (complete response)/PR (partial response) were compared to progressive disease only (62.5% vs 0, p=0.04).

(iii) Expression of human endogenous retroviruses (hERVs) is associated with improved clinical outcomes to anti-PD-1 monotherapy in metastatic RCC. There is some evidence that expression of hERVs correlates with high levels of immune infiltration, increased cytolytic activity and immune checkpoint expression in ccRCC (6-8). hERV expression has also been shown to correlate with response to immune checkpoint inhibitor (ICI) therapy in small patient cohorts (6,7). Of note, it has been demonstrated that HERV-E/ERVE4 (henceforth referred to as ERVE4)-derived epitopes can elicit a tumor-restricted $CD8^+$ T cell-mediated immune response (7, 9, 10). Based on the published literature, we conducted our own discovery analyses using specimens obtained from patients treated with the anti-PD-1 antibody nivolumab and non-ICI (everolimus, control) as part of the CheckMate 025 trial(29). We performed RT-pPCR on formalin-fixed paraffin-embedded (FFPE) pre-treatment tumors to assess ERVE4 mRNA levels in 224 patients (nivolumab=112; everolimus=112). ERVE4 expression as a continuous measure was associated with PFS in the nivolumab arm (HR=0.42, *P*=0.020). At the optimized cutoff (Youden's Index), nivolumab-treated patients expressing ERVE4 had a significantly longer PFS (5.3 vs 3.6 months, *P*=0.017) than patients not expressing ERVE4. In the everolimus arm, ERVE4 status was not associated with clinical outcomes (**Figure 3**). We also demonstrated that immune-related gene signature scores including Tumor Inflammation Score (TIS), IMmotion150_Teff, Cytolytic Activity, and JAVELIN were upregulated in ERVE4-positive tumors (FDR q < 0.25) (**Figure 4**) (see Ficial et al. *Clin Cancer Res* 2021 for details) (4).

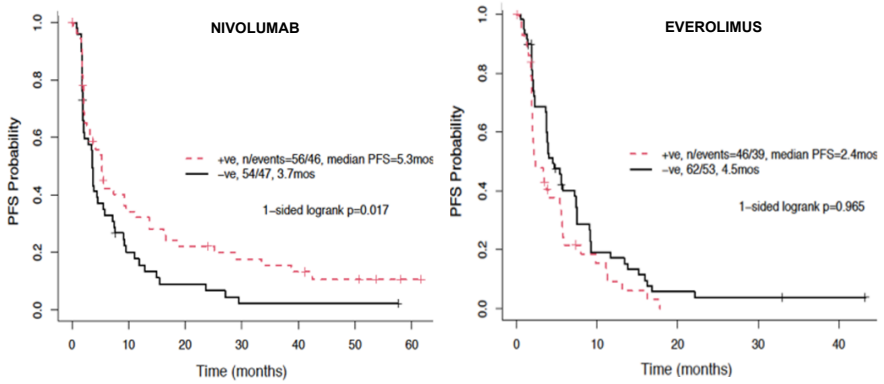


Figure 3. Kaplan-Meier curves for PFS per ERVE4 status (positive is red line, negative is black line) at the optimized cut-off (Youden's Index approach) in the nivolumab (left panel) and everolimus (right panel) arms (CheckMate-025 trial).

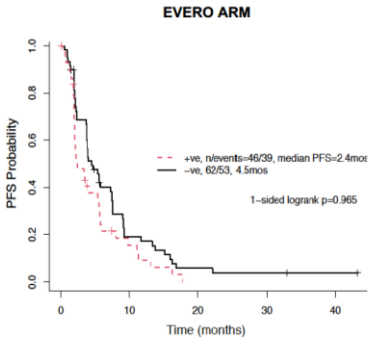


Figure 4. Immune-related gene signature scores upregulated in ERVE4-positive (orange) and ERVE4-negative (purple) tumors. Dotted lines indicate FDR q-value of 0.25 (light) and 0.05 (dark).

Our team has now optimized a computational pipeline for quantification of hERV expression at a transcriptome-wide level using RNA-seq, which we validated using qPCR measurements of individual hERV transcripts (see Braun et al. *Nat Med* 2020 for details) (11).

(iv) Mutations in SETD2 tend to be associated with improved clinical outcomes to anti-PD-1 monotherapy in metastatic RCC. The impact of genetic alterations on predicting clinical benefit from anti-PD-1 therapy was recently assessed in tumor specimens obtained from the clinical trial KEYNOTE-427 (led by David McDermott), which investigated the safety and efficacy of first-line pembrolizumab monotherapy in patients with metastatic RCC. We found a trend toward an association between mutations in SETD2 and higher ORR (44% (95% CI 25-66) in mutant cases versus 24% (95% CI 15-37) in non-mutant cases) (12). Of note, SETD2 mutations are clinically associated with worse kidney cancer specific survival in localized RCC. The impact of SETD2

mutations in predicting responses to anti-PD-1 therapy needs to be further investigated in larger patient cohorts.

2. Candidate biomarkers of resistance to anti PD-1 monotherapy

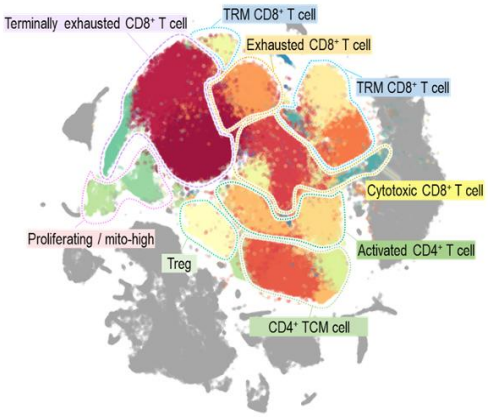


Figure 5. T-distributed stochastic neighbor embedding (tSNE) plot of single-cell RNA-seq data obtained from clear cell RCC cases showing sub-clustering of T cells with major groups of T cell populations labeled.

(v) Terminally exhausted CD8⁺ TILs are associated with resistance to anti-PD-1 monotherapy in metastatic RCC. As mentioned above, the expression of multiple inhibitory receptors on $CD8^+$ cells identifies highly dysfunctional, terminally exhausted T cells that might fail to be re-activated by PD-1 blockade alone.(30, 31) Recently, we performed additional analyses of CheckMate 025 data aimed at directly interrogating whether, among tumors harboring a $CD8^+$ infiltrate, a high proportion of terminally exhausted TILs correlated with lack of response to anti-PD-1 therapy. Our analysis revealed that high percentage of $CD8^+$ $PD\text{-}1^+$ TILs expressing TIM-3 was associated with poorer response to nivolumab monotherapy. Using a cut-off that maximizes sensitivity and specificity for ORR, we observed that ORR to nivolumab was 14% in the group of patients presenting with a high percentage of terminally exhausted $CD8^+$ $PD\text{-}1^+$ $TIM\text{-}3^+$ TILs, compared to 35% in the group patients presenting with a low percentage of terminally exhausted $CD8^+$ $PD\text{-}1^+$ $TIM\text{-}3^+$ TILs (P=0.009).

(vi) Intratumoral myeloid infiltration is associated with resistance to anti-PD-1/PD-L1 to therapy in metastatic RCC. An analysis led by David McDermott was conducted using specimens obtained from participants of the IMmotion150 clinical trial that compared the efficacy of atezolizumab monotherapy atezolizumab combined with bevacizumab, or sunitinib in the first-line setting in advanced ccRCC. RNA-seq data were utilized to assess whether levels of T cell effector function/IFNγ response (T-eff signature) or myeloid cell-associated gene activity (myeloid signature) might be predictive of treatment benefit. Tumors that were found to have high T-eff gene signature and high myeloid signature ($T\text{-}eff_{high}Myeloid_{high}$) showed significantly shorter PFS on atezolizumab monotherapy versus those with $T\text{-}eff_{high}Myeloid_{low}$ tumors (13). More recently, the effect of gene signatures was also investigated in tumor specimens obtained from the clinical trial KEYNOTE-427 (also led by David McDermott) testing the efficacy of pembrolizumab monotherapy for patients with metastatic RCC in the first-line setting (including both ccRCC and non-ccRCC). By analyzing bulk tumor RNA-seq data from 78 ccRCC, they found that a myeloid signature (MDSC signature) was inversely associated with response (12). Overall, these data support the hypothesis that intratumoral myeloid infiltration mediates resistance to first-line PD-1/PD-L1 monotherapy in RCC.

(vii) A dysfunction circuit between terminally exhausted T cells and tumor associated macrophages (TAMs) is associated with poor outcome to anti-PD-1 monotherapy in metastatic RCC. Because myeloid cells play a crucial role in the tumor microenvironment and seem to contribute to anti-PD1 treatment resistance, we sought to evaluate individual myeloid subsets in tumor and adjacent (normal) tissues. For that, we relied on **single-cell** transcriptomic profiling of immune cells in ccRCC and adjacent normal tissue from 13 patients at various clinical stages to evaluate how infiltrating immune cells change with advancing disease (**Figure 5**). Our preliminary analyses revealed increased $CD8^+$ T cell exhaustion (P=2.2×10^{-16}) and a higher proportion of immunosuppressive M2-like macrophages in advanced disease. Through computational inference of cell-cell interactions, we further identified both known and novel inhibitory interactions between terminally exhausted $CD8^+$ T cells and TAMs that are enriched in metastatic disease. (**Figure 6**). This terminal $CD8^+$ exhaustion and TAM interaction "signature" was enriched in advanced disease stages (P=8.2×10^{-08}). Furthermore, in an external dataset (TCGA KIRC cohort), high expression of this signature was significantly associated with worse OS across all disease stages (P=2.5×10^{-04}, **Figure 7**). This finding was validated in an additional external cohort of metastatic ccRCC patients treated with PD-1 blockade, where high expression of the terminal exhaustion/TAMs interaction gene signature was also associated with worse OS (P=0.043). These data were recently published in *Cancer Cell* (14).

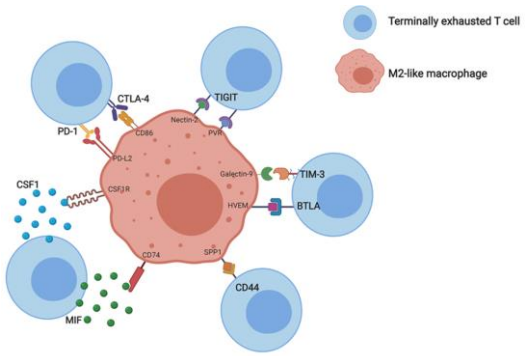


Figure 6. Schema of inhibitory interactions between terminally exhausted T cells and M2-like macrophages.

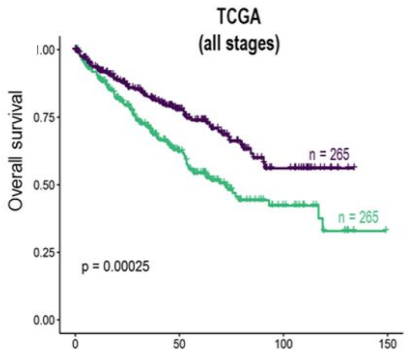


Figure 7. TCGA tumors with a high terminal exhaustion/TAM interaction signature (>median) (green line) have worse OS.

(viii) Intratumoral PD-1$^+$ regulatory T cells might mediate resistance to anti-PD-1 monotherapy. Beyond terminally exhausted T cells and myeloid cells, regulatory T (Treg) cells have also been linked to resistance to anti-PD-1 monotherapy. It has long been recognized that Treg cells can promote tumor development and progression by suppressing antitumor immunity. A study led by Arlene Sharpe (collaborator), focusing on PD-1 function in Treg cells, recently demonstrated that PD-1-deficient Treg cells exhibit an enhanced immunosuppressive function both in vitro and in vivo, indicating that PD-1 inhibits Treg cell activation and suppressive capacity (15). It is therefore conceivable that the efficacy of anti-PD-1 therapy might be limited by the activation of the highly immunosuppressive PD-1$^+$ Treg cells in the tumor microenvironment. This hypothesis is further supported by an independent study from other investigators showing that PD-1 blockade enhances PD-1$^+$ Treg cell-mediated suppressive function via activation of the TCR and CD28 signals, and that PD-1$^+$ Treg cells activated by PD-1 blockade are involved in resistance to anti-PD-1 therapies (16).

BLOOD-BASED BIOMARKERS

We developed robust plasma-based assays for circulating biomarkers (i.e. cell-free methylated DNA and kidney injury molecule-1 (KIM-1)) that allow the detection of RCC in various clinical settings. It would now be important to assess whether biomarker levels measured in post-

nephrectomy plasma specimens can predict disease recurrence in the neoadjuvant setting by analyzing specimens from the EA8143 trial.

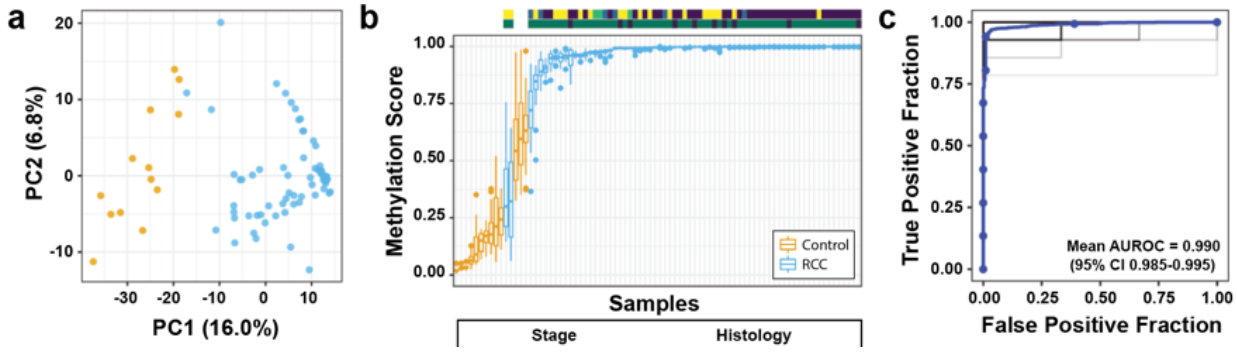
(i) Plasma KIM-1 is associated with recurrence risk after nephrectomy for localized Renal Cell Carcinoma in the ASSURE trial. There is currently no blood-based biomarker for renal cell carcinoma (RCC) onset or prognosis. We have identified kidney injury molecule-1 (KIM-1) / T cell immunoglobulin mucin domain-1 (TIM-1) as a potential RCC biomarker that is elevated in the plasma of RCC patients. KIM-1 is a transmembrane protein with known functions in immune regulation and kidney injury. Our work shows that KIM-1 levels correlate with clinical outcome in patients with metastatic RCC and that KIM-1 levels decrease after nephrectomy. We have also previously shown that plasma KIM-1 can be detected up to 5 years prior to RCC diagnosis. Despite recent advances in the treatment of metastatic RCC including targeted therapies and immune checkpoint therapies, there remain key unmet needs in RCC management: Can we identify a high-risk patient population who may be most likely to benefit from adjuvant therapy after nephrectomy for localized disease? We recently showed that plasma KIM-1 is a prognostic biomarker in patients with localized RCC after nephrectomy. The ECOG-ACRIN E2805 (ASSURE) trial evaluated adjuvant sunitinib, sorafenib, or placebo in resected high-risk RCC. KIM-1 levels were measured from banked plasma at trial enrollment 4-12 weeks post-nephrectomy (17). Lognormal accelerated failure time (AFT) models were used to test for association between KIM-1 and disease-free survival (DFS) as well as overall survival (OS). Plasma from 418 patients was analyzed. Higher post-nephrectomy KIM-1 was associated with worse DFS across all study arms after adjustment for Fuhrman grade, T-stage, N-stage, and tumor histology (survival time ratio 0.56 for 75^{th} vs 25^{th} percentile of KIM-1, 95% CI 0.42-0.73, $p < 0.001$). The association between KIM-1 and DFS was stronger among patients with pathologic nodal involvement (p-value for interaction 0.0086). The addition of post-nephrectomy KIM-1 improved the concordance of clinical prognostic models (SSIGN concordance 0.57 vs 0.43, $p = 0.05$; UISS concordance 0.60 vs 0.40, $p = 0.0005$). Higher post-nephrectomy KIM-1 was also associated with worse overall survival (OS) after multivariable adjustment (survival time ratio 0.71 for 75^{th} vs 25^{th} percentile of KIM-1, 95% CI 0.56-0.91, $p < 0.001$).
Thus, post-nephrectomy plasma KIM-1 is associated with DFS and OS in RCC, and may be a biomarker for microscopic residual disease. It will be important to validate these findings in other cohorts. We will assess KIM-1 in the EA8143 cohort to support the hypothesis that post nephrectomy KIM-1 could stratify patients most likely to benefit from neoadjuvant therapy.

(ii) Cell-free methylated DNA immunoprecipitation and high-throughput sequencing (cfMeDIP–seq) is a highly sensitive assay capable of detecting early-stage RCC. Identifying tumor-specific alterations in cell-free DNA (cfDNA) presents a powerful opportunity to potentially reduce morbidity and mortality through early and accurate cancer detection. Cell-free methylated DNA immunoprecipitation and high-throughput sequencing (cfMeDIP-seq) is a novel method for plasma cfDNA methylation profiling, demonstrated to be highly sensitive for detecting and classifying several tumor types, performing equally well in patients with localized and metastatic disease (18,19). Additional benefits include low DNA input (≤ 10 ng) and balance of genome coverage, resolution, and cost. We performed the first independent validation of cfMeDIP-seq for 1) cancer detection using plasma cfDNA, reporting an AUROC of 0.99 for accurate classification of patients with stage I-IV RCC versus cancer-free controls, and 2) the first application of cfMeDIP-seq on urine cfDNA for cancer detection, reporting an AUROC of 0.86 for accurate classification of patients with RCC versus cancer-free controls (20).
The excellent performance of this epigenetic approach to detect RCC-specific alterations in cfDNA may reflect underlying tumor biology. Molecular characterization of RCC tumors in TCGA identified 289 genes with aberrant DNA methylation compared to only 19 recurrently mutated genes (21). Consistent with this observation, we achieved significantly higher detection rates using cfMeDIP-seq than assays to detect somatically acquired DNA mutations: less than 80% in

patients with metastatic RCC (22,23). Further, these studies did not account for false positive results from clonal hematopoiesis. We performed a comparative analysis of cfMeDIP-seq versus massively parallel sequencing of cfDNA to detect tumor-specific genetic alterations in 34 patients with metastatic RCC (24). Clonal hematopoiesis was detected in 28% of specimens (consistent with published reports) (25), while tumor variants were detected in only 21% of specimens. In contrast, cfMeDIP-seq achieved 100% sensitivity at 88% specificity. The preponderance of data demonstrating superior sensitivity of cfMeDIP-seq for detecting RCC across all stages, and in comparison to other approaches, highlights the promise of this assay.

We have performed the first independent validation of cfMeDIP-seq for detection of RCC and the first application of cfMeDIP-seq on urine cfDNA for cancer detection. We performed cfMeDIP-seq on 148 specimens: N=99 stage I-IV RCC cases, N=21 stage IV urothelial bladder cancer (UBC) cases, and N=28 cancer-free controls. Notably, 33.3% of plasma specimens and 66.7% of urine specimens were collected from patients with TNM stage I/II disease.

cfMeDIP-seq accurately classifies plasma cfDNA RCC cases from cancer-free controls and other cancer types: The top 300 DMRs cleanly partitioned plasma RCC and control specimens **(Figure 8a)**. 67/69 (97.1%) of RCC specimens were assigned a higher median methylation score than all control specimens **(Figure 8b)**, culminating in a mean AUROC of 0.99 **(Figure 8c)**. There was no statistical association between RCC stage (p=0.09) or histology (p=0.38) with methylation score (see Nuzzo et al, *Nat Med* 2020 for details) (20).



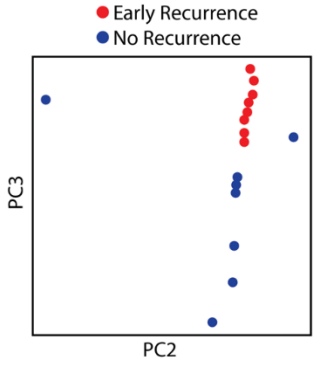


Figure 9. PCA plot showing that patients with early recurrence clustered separately from patients with no *recurrence in PC3.*

cfMeDIP-seq differentiates between patients with and without recurrence post-nephrectomy: We subsequently generated preliminary data evaluating the performance of cfMeDIP-seq in patients with localized RCC who had undergone nephrectomy with post-operative restaging imaging negative for metastases. We selected plasma collected following nephrectomy from patients with high-risk localized disease who either recurred within 6 months (early recurrence) or who did not recur after long-term follow-up. We performed cfMeDIP-seq on these 16 specimens and plotted a principal component (PC) analysis based on the cfDNA methylation profiles **(Figure 9)**. Although the analysis is influenced by two "outlier" specimens, the PCA plot clearly shows that patients with early recurrence clustered separately from patients with no recurrence in PC3. The two outlier specimens were a result of technical issues and would be excluded from subsequent analysis due to not passing quality control metrics. This data supports that cfDNA methylation profiling using cfMeDIP-seq is discriminating between patients at high risk of recurrence following nephrectomy for localized RCC versus those who are likely cured. We plan to further test this hypothesis by analyzing specimens from patients enrolled in the EA8143 trial.

Research Design and Methods

All laboratory methods listed here are identical to those embedded in the clinical trial protocol (Section 11.3), with the exception of the detection of cell-free methylated DNA, which is a newer methodology.

Appendix containing the relevant publications from our group that are cited below can be downloaded using the link below:
https://www.dropbox.com/s/e6fhgfsv6jzsi2x/appendix_relevant_publications.pdf?dl=0The Methods that will be utilized for tissue-based analyses include:

1) Multiplex immunofluorescence for measurement of intratumoral $CD8^+$ $PD\text{-}1^+$ $TIM\text{-}3^-$ $LAG\text{-}3^-$ lymphocytes, described in detail in Pignon et al, *Clin Cancer Res* 2019 and Ficial et al, *Clin Cancer Res* 2021 (see Appendix).
2) RNA (transcriptome) sequencing for assessment the transcriptomic clusters (i.e., molecular RCC subtypes), described in detail in Motzer et al, *Cancer Cell* 2020 (see Appendix).
3) RNA (transcriptome) sequencing for the measurement of ERV levels, described in detail in and Braun et al, *Nat Med* 2020 (see Appendix).
4) Whole-exome sequencing for assessment of SETD2 mutations and other genomic alterations described in detail in Braun et al, *Nat Med* 2020 (see Appendix).
5) RNA (transcriptome) sequencing for the assessment of a gene signature of interaction between terminally exhausted CD8 cells and tumor associated macrophages, described in detail in Braun et al, *Cancer Cell* 2021 (see Appendix).

The Methods that will be utilized for blood-based analyses include:

1) A microbead-based assay for the measurement of KIM-1 levels in plasma, described in Xu et al, *Clin Cancer Res* 2021 (see Appendix).
2) Cell-free methylated DNA immunoprecipitation and high-throughput sequencing (cfMeDIP-seq), described in detail in Nuzzo et al, *Nat Med* 2020 (see Appendix).

Statistical Considerations

a. Endpoints:

The primary endpoint will be recurrence-free survival, defined as time from randomization to recurrence or death from any cause. Patients who have not recurred or died will be censored at the date of last evaluation. All patients that were eligible for primary clinical analysis will be eligible for the correlative study. Also, those that either did not undergo nephrectomy or are not disease-free after nephrectomy will be included in the correlative analysis and will be considered as having event at Day 1 just like the primary clinical analysis.

b. Statistical Analysis Plan for Addressing the Primary Objectives:

Tissue and plasma-based hypotheses are listed above. Tissue-based hypotheses #1-4 focus on the association of the particular biomarker with RFS among the control arm patients only since these hypotheses stay valid regardless of the primary endpoint results. Tissue-based hypothesis #5 and plasma-based hypotheses focus on both the treatment and the control arms, because the hypothesis holds regardless of the treatment they receive. We have taken a more conservative approach, where the power calculations for each of the aims are clinically relevant regardless of the outcome of the trial, since the primary outcome is unknown.

To assess the tissue-based hypotheses, log-rank test will be used to test the differences in RFS between biomarker groups. For plasma-based hypothesis #1, analysis methods used in Xu study (Xu et al, *Clin Cancer Res* 2021) will be replicated: KIM-1 levels will be divided into quartiles and will be compared to the other quartiles in terms of RFS. Univariable, PH Cox model and AFT model will be used to evaluate the associations between KIM-1 and clinical outcomes and the power statement is based on the PH Cox model. Lastly, for plasma-based hypothesis #2, basic descriptive statistics will be reported, such as the sensitivity and the specificity of cfDNA detection in RCC recurrence.

c. Statistical Justification for Sample Size

Sample Size Estimate: 670 baseline and nephrectomy/post-nephrectomy specimens. However, a sample size of 536 was used for power calculation assuming that 80% of patients will have specimens available for analysis.

Rationale for the Sample Size Estimate: 670 patients have consented for use of specimens for research. Assuming 80% of the 670 participants' specimens will be available, our total sample size for power calculation is assumed to be 536. The prevalence for each biomarker of interest are based on past studies. Expected 5-year RFS rates in each biomarker group was estimated using the prevalence of the biomarker and the assumed 5-year RFS rates in both treatment arms of EA8143.

Tissue-based Aims:

(i) High levels of mildly exhausted CD8$^+$ tumor infiltrating cells combined with tumor cell PD-L1 expression: According to studies lead by Pignon and Ficial, about 11% of the RCC patients had both high PD-L1 and high CD8$^+$PD-1$^+$TIM-3$^-$LAG-3$^-$ TIC expressions. As a result, 5-year RFS rates in high PD-L1 & high CD8$^+$PD-1$^+$TIM-3$^-$LAG-3$^-$ TIC is assumed to be 30%, with about 25% increase in 5-year RFS for those not in that group. Since only the control group is of interest, with 268 patients' specimens available, there will be 77.7% power to detect a hazard ratio of 2.01 or higher with a two-sided type I error rate of 5% using log-rank test. Full information will occur at 109 events.

	PD-L1 high & CD8$^+$PD-1$^+$TIM-3$^-$LAG-3$^-$ TIC high	**Other**
Prevalence	11%	89%
5-yr RFS	30%	55%
Sample Size	29	239
HR	2.01	
Power	77.7%	

(ii) A molecular RCC subtype characterized by high expression of T-effector and cell proliferation related genes: According to the Rini study, about 14% of the RCC patients were included in the T-effector/proliferative cluster. With 268 patients' specimens, there will be about 84.9% power to detect a difference between 5-year RFS rates of 30% in T-effector/proliferative cluster and 55% in other group, corresponding to a hazard ratio of 2.01 or higher with a two-sided type I error rate of 5% using log-rank test. Full information will occur at 111 events.

	T-effector/proliferative cluster	**Other**
Prevalence	14%	86%
5-yr RFS	30%	55%
Sample Size	38	230
HR	2.01	
Power	84.9%	

(iii) Expression of human endogenous retroviruses (hERVs): According to the Ficial study, hERV4700-ENV was expressed in 40% of RCC patients. With 268 patients' available specimens, there will be about 86.4% power to detect a difference between 5-year RFS rates of 40% in those that express hERVs and 60% that don't, corresponding to hazard ratio of 1.79 or higher with a two-sided type I error rate of 5% using log-rank test. Full information will occur at 110 events.

	Express hERVs	**Other**
Prevalence	40%	60%
5-yr RFS	40%	60%
Sample Size	107	161
HR	1.79	
Power	86.4%	

(iv) Mutations in SETD2: According to preliminary studies, there are SETD2 mutations in about 25% of the RCC patients. Assuming that 268 patients' specimens are available, there will be 80.9% power to detect a difference between 5-year RFS of 35% for those with SETD2 mutation vs. 55% for others, which leads to hazard ratio of 1.76 or higher with two-sided type I error rate of 5% using log-rank test. Full information will occur at 114 events.

	SETD2 mutation	**Other**
Prevalence	25%	75%
5-yr RFS	35%	55%
Sample Size	67	201
HR	1.76	
Power	80.9%	

(v) High levels of a gene signature of interaction between terminally exhausted CD8 cells and tumor associated macrophages: Braun study utilized the median as the cutoff of gene signature, so we assumed that about 50% of patients express high levels of a gene signature of interaction between terminally exhausted CD8 cells and tumor associated macrophages. Since both the treatment and the control arms are of interest for this aim, assuming that 536 of the patients' specimens are available, there will be 90.1% power to detect a difference in 5-year RFS of 50% for those expressing high gene signatures vs. 65% for those that don't, corresponding to hazard ratio of 1.61 or higher with two-sided type I error rate of 5% using log-rank test. Full information will occur at 193 events.

	High gene signature of interaction between terminally exhausted CD8 call and tumor associated macrophages	**Other**
Prevalence	50%	50%
5-yr RFS	50%	65%
Sample Size	268	268
HR	1.61	
Power	90.1%	

Blood-based Aims:

(i) Kidney injury molecule-1 (KIM-1) levels: The main goal of this aim is to replicate the analysis that the Xu study conducted. In Xu study, the 5-year DFS was about 50% in KIM-1 levels greater than median and those that had KIM-1 level greater than median value had a 5-year DFS of about 65%, hence those rates were also used as our 5-year RFS rate assumptions. Since both the treatment and the control arms are of interest, assuming that 536 of the patients' specimens will be available, there will be about 90.1% power to detect the difference between those with KIM-1 levels less than median and greater than it. Full information will occur at 193 events. Similarly, 5-year RFS of 45% was used for those in 1st quartile KIM-1 level versus 60% for those that have higher than 1st quartile KIM-1 level, and with the same sample size, there will be about 83.5% power to detect the difference in these two groups. Full information will occur at 199 events. Two-sided type I error rate of 5% was used and log-rank test will be utilized for both comparisons. Comparisons for those that are in other quartiles will also be assessed against others.

	KIM-1 level less than median	**KIM-1 level greater than median**
Prevalence	50%	50%
5-yr RFS	50%	65%
Sample Size	268	268
HR	1.61	
Power	90.1%	

	1st quartile KIM-1 level patients	Other
Prevalence	25%	75%
5-yr RFS	45%	60%
Sample Size	134	402
HR	1.56	
Power	83.5%	

(ii) Detection of cell-free methylated DNA: Cell-free methylated DNA immunoprecipitation and high-throughput sequencing (cfMeDIP–seq) is a highly sensitive assay capable of detecting early-stage RCC, cfDNA demonstrated a sensitivity of 100% and specificity of 88%. However, to be more realistic, 97% was assumed as the sensitivity, since in Nuzzo study, 67 of 69 RCC specimens were assigned a higher median methylation score than all control specimens. Also, with the assumption that about 56% (average between 50.2% 5-year RFS for control arm and 61.9% 5-year RFS for treatment arm) of the patients will be recurrence-free at 5 years, we consequently assumed that 44% will have a recurrence at 5 years. On top of that, prevalence of 44% is assumed since only those that have a recurrence are known to have cfDNA detected. The confidence intervals for sensitivity and specificity assuming binomial distribution can be found in the table below.

cfDNA detection	**Recurrence**	
	Yes	**No**
Yes	229	36
No	7	264

	Estimate	**Two-sided 95% Confidence Interval**
Sensitivity	0.97	(0.94,0.99)
Specificity	0.88	(0.84,0.91)

References:

1. Blackburn SD, Shin H, Haining WN, *et al.* Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nat Immunol* 2009;10(1):29-37.
2. Sakuishi K, Apetoh L, Sullivan JM, *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):2187-2194.
3. Pignon JC, Jegede O, Shukla SA, *et al.* irRECIST for the evaluation of candidate biomarkers of response to nivolumab in metastatic clear cell renal cell carcinoma: analysis of a phase II prospective clinical trial. Clin Cancer Res. 2019:25(7):2174-2184.
4. Ficial M, Jegede OA, Sant'Angelo M, *et al*. Expression of T-cell Exhaustion Molecules and Human Endogenous Retroviruses as Predictive Biomarkers for response to Nivolumab in Metastatic Clear Cell Renal Cell Carcinoma. Clin Cancer Res. 2021 Mar 1;27(5):1371-1380.

5. Motzer RJ, Banchereau R, Hamidi H, *et al.* Molecular Subsets in Renal Cancer Determine Outcome to Checkpoint and Angiogenesis Blockade. Cancer Cell. 2020 Dec 14;38(6):803-817.
6. Panda A, de Cubas AA, Stein M, Riedlinger G, *et al.* Endogenous retrovirus expression is associated with response to immune checkpoint blockade in clear cell renal cell carcinoma. JCI insight. 2018;3(16).
7. Smith CC, Beckermann KE, Bortone DS, *et al.* Endogenous retroviral signatures predict immunotherapy response in clear cell renal cell carcinoma. The Journal of clinical investigation. 2018;128(11):4804-20.
8. Rooney MS, Shukla SA, Wu CJ, *et al.* Molecular and genetic properties of tumors associated with local immune cytolytic activity. Cell. 2015;160(1-2):48-61. Epub 2015/01/17. doi: 10.1016/j.cell.2014.12.033. PubMed PMID: 25594174; PMCID: PMC4856474.
9. Takahashi Y, Harashima N, Kajigaya S, *et al.* Regression of human kidney cancer following allogeneic stem cell transplantation is associated with recognition of an HERV-E antigen by T cells. J Clin Invest. 2008;118(3):1099-109.
10. Cherkasova E, Scrivani C, Doh S, *et al.* Detection of an Immunogenic HERV-E Envelope with Selective Expression in Clear Cell Kidney Cancer. Cancer Res. 2016;76(8):2177-85.
11. Braun DA, Hou Y, Bakouny Z, *et al.* Interplay of somatic alterations and immune infiltration modulates response to PD-1 blockade in advanced clear cell renal cell carcinoma. Nat Med. 2020;26(6):909-18.
12. McDermott DF, 1 Lee JL, Donskov F, *et al.* Association of gene expression with clinical outcomes in patients with renal cell carcinoma treated with pembrolizumab in KEYNOTE-427. J Clin Oncol 2020; 15 Suppl:5024.
13. McDermott DF, Huseni MA, Atkins MB, *et al.* Clinical activity and molecular correlates of response to atezolizumab alone or in combination with bevacizumab versus sunitinib in renal cell carcinoma. Nat Med 2018;24:749-57.
14. Braun DA, Street K, Burke KP, *et al.* Progressive immune dysfunction with advancing disease stage in renal cell carcinoma. Cancer Cell. 2021 May 10;39(5):632-648.
15. Tan CL, Kuchroo JR, Sage PT, *et al.* PD-1 restraint of regulatory T cell suppressive activity is critical for immune tolerance. J Exp Med. 2021;218.
16. Kumagai S, Togashi Y, Kamada T, *et al.* The PD-1 expression balance between effector and regulatory T cells predicts the clinical efficacy of PD-1 blockade therapies. Nat Immunol. 2020;21(11):1346-58.
17. Xu W, Puligandla M, Halbert B, *et al.* Plasma KIM-1 Is Associated with Recurrence Risk after Nephrectomy for Localized Renal Cell Carcinoma: A Trial of the ECOG-ACRIN Research Group (E2805). Clin Cancer Res. 2021 Apr 8. doi: 10.1158/1078-0432.CCR-21-0025. Online ahead of print.
18. Shen, S. Y. *et al.* Sensitive tumour detection and classification using plasma cell-free DNA methylomes. Nature. 2018; 563:579–583.
19. Shen, S. Y., Burgener, J. M., Bratman, S. V. & De Carvalho, D. D. Preparation of cfMeDIP-seq libraries for methylome profiling of plasma cell-free DNA. Nat. Protoc. 2019 Oct;14(10):2749-2780.
20. Nuzzo PV, Berchuck JE, Korthauer K,*et al.* Detection of renal cell carcinoma using plasma and urine cell-free DNA methylomes. Nat. Med. 2020 Oct;26(10):1663.

21. The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature. 2013 Jul 4;499(7456):43-9.

22. Zill OA, Banks KC, Fairclough SR, *et al*. The Landscape of Actionable Genomic Alterations in Cell-Free Circulating Tumor DNA from 21,807 Advanced Cancer Patients.Clin Cancer Res. 2018 Aug 1;24(15):3528-3538.

23. Pal SK, Sonpavde G, Agarwal N, *et al*. Evolution of Circulating Tumor DNA Profile from First-line to Subsequent Therapy in Metastatic Renal Cell Carcinoma. Eur. Urol. 2017 Oct;72(4):557-564.

24. Lasseter K, Nassar AH, Hamieh L, *et al.* Plasma cell-free DNA variant analysis compared with methylated DNA analysis in renal cell carcinoma. Genet. Med. 2020 Aug;22(8):1366-1373.

25. Ptashkin RN, Mandelker DL, Coombs CC, *et al.* Prevalence of Clonal Hematopoiesis Mutations in Tumor-Only Clinical Genomic Profiling of Solid Tumors. JAMA Oncol*. 2018* Nov 1;4(11):1589-1593.