Nivolumab and Axitinib Phase II Study

TITLE: A Phase II Trial of Nivolumab plus Axitinib in Patients with Anti-PD1 Refractory Advanced Melanoma (HCC 20-101, CA209-7UE).

Protocol Version 7.0, 01/03/2024

IND NUMBER: 154662

Principal Investigator: Yana Najjar, MD

UPMC Hillman Cancer Center
5115 Centre Ave
Suite 1.32C
Pittsburgh, PA 15232

Statistician: Hong Wang, PhD

CONTENTS

1.0 TRIAL SUMMARY

Abbreviated Title	Nivolumab Plus Axitinib in Anti-PD1 Refractory Advanced Melanoma
Trial Phase	Phase II
Clinical Indication	Unresectable Stage III or IV Melanoma
Trial Type	Interventional, Clinical Translational
Type of control	Single arm study with historical control
Route of administration	Nivolumab IV, Axitinib PO
Trial Blinding	No
Treatment Groups	1 Arm: Nivolumab plus Axitinib
Number of trial subjects	31
Estimated enrollment period	2.5 years
Estimated duration of trial	4.5 years (2.5 years accrual, up to 2 years on treatment).
Duration of Participation	Up to 2 years
Estimated average length of treatment per patient	Up to 2 years

2.0 TRIAL DESIGN

2.1 Trial Design

This is an investigator-initiated, phase II trial of nivolumab plus axitinib for patients with unresectable stage III or IV melanoma who have progressed on prior anti-PD1 therapy with or without concomitant anti-CTLA4 therapy. Patients will receive treatment with nivolumab 480 mg IV every 4 weeks and axitinib PO 5 mg twice daily. Patients may continue both agents for up to two years if they do not experience progression or dose-limiting toxicity. Biopsies will be taken at baseline and at 12 weeks for an in-depth evaluation of the impact of the combination on the tumor and tumor microenvironment (TME), with an optional biopsy at the time of progression (**Figure 1**). We have shown that tumor oxidative metabolism and resultant hypoxia are associated with resistance to immunotherapy (IMT).[1] We have shown that in mice, a low dose of axitinib decreases intra-tumoral hypoxia and synergizes with checkpoint inhibitors (ICB) to eradicate melanoma tumors. Thus, we hypothesize that decreasing hypoxia in the TME will re-sensitize melanoma tumors to anti-PD1 immunotherapy and overcome resistance. Blood for research studies will be drawn at baseline (up to 7 days prior to initiation of therapy), at week 12, and at the time of progression. Patients will receive an oral dose of pimonidazole (0.5 g/m^2)[2] before each biopsy to permit in vivo evaluation of intra-tumoral hypoxia. This drug is safe, well-tolerated, and has been previously used in cancer patients to evaluate hypoxia without reported toxicity.[3-5] Pimonidazole hydrochloride (Natural Pharmacia International, Belmont, MA) has IND status for use for the clinical evaluation of hypoxia.[3]

2.2 Trial Diagram

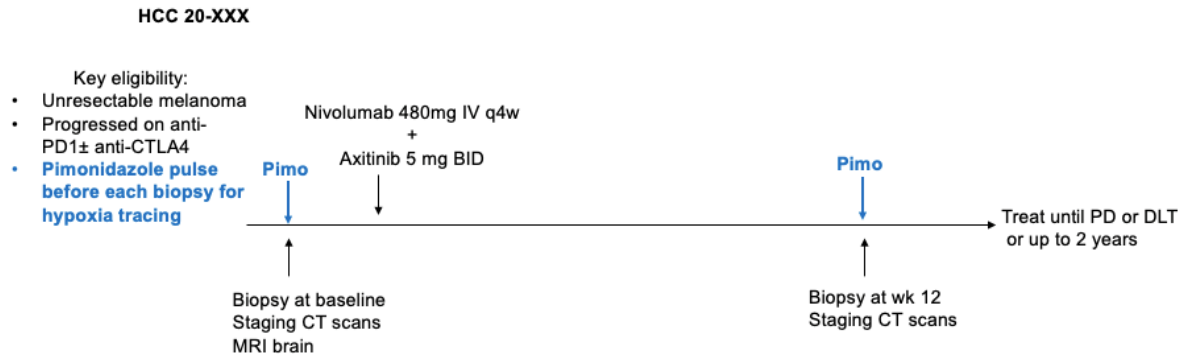

Figure 1. Study Schema

OBJECTIVE(S) & HYPOTHESIS(ES)

2.3 Primary Objective(s) & Hypothesis(es)

(1) Objective: To assess the overall response rate (ORR) of nivolumab plus axitinib in patients with anti-PD1 refractory melanoma as measured by clinical tumor measurement and radiologic tumor measurement by CT scans at baseline and after 12 weeks of treatment.

Hypothesis: Patients treated with nivolumab and axitinib will have a response rate greater than the estimated response rate in anti-PD1 refractory patients, which is approximately 10%. We propose this improved response will be due to a reduction of intra-tumoral hypoxia and resultant T cell dysfunction.

2.4 Secondary Objective(s) & Hypothesis(es)

(1) Objective: To evaluate the safety of the combination of nivolumab and axitinib.

Hypothesis: The combination of nivolumab and axitinib will be safe and well-tolerated.

(2) Objective: To determine the progression-free survival (PFS) and overall survival (OS) for patients treated with nivolumab plus axitinib.

Hypothesis: Treatment with nivolumab plus axitinib will prolong both PFS and OS.

Exploratory Objectives: Correlative analyses will include and are not limited to evaluation of hypoxia in the TME, TIL function, immune phenotype and tumor cell metabolism.

Hypothesis: Combination treatment will decrease intra-tumoral hypoxia, improve TIL function and yield a more favorable immune infiltrate.

3.0 BACKGROUND & RATIONALE

3.1 Background

With the regulatory approval of multiple effective new agents, the treatment paradigm for metastatic melanoma has changed drastically over the past several years. IMT has emerged as a viable and effective treatment for melanoma. However, despite the fact that response rates have increased in patients with advanced disease, only 40% of patients receive benefit from anti-PD1 monotherapy.[6] While response to the combination therapy (i.e., ipilimumab plus nivolumab) is closer to 60%, it is important to note that over half of these patients experience grade III/IV toxicities.[7] Furthermore, many patients who initially respond to treatment, later acquire resistance and ultimately progress. The mechanism behind this heterogenous response to IMT remains unclear, and to date, no validated biomarkers can reliably predict response in patients with advanced melanoma. PD-L1 expression, tumor mutational burden, immune gene signatures, and various other predictive biomarkers are under active investigation.[8,9]

Multiple mechanisms of resistance to IMT may be at play in melanoma. However, one key mechanism is the generation of an immunosuppressive and metabolically harsh TME.[10-12] The TME is generally considered to be nutrient poor, with decreased essential nutrients for T cell function such as glucose and amino acids, along with concurrent buildup of toxic byproducts such as lactic acid.[11] This altered metabolic landscape is induced by two main drivers: an altered angiogenic pattern as well as the intrinsic deregulated metabolism of the tumor itself. Unrestrained oxidative metabolism in the context of inadequate vasculature results in hypoxia, and increased glycolysis generates an acidic environment.[13] T cell effector functions are bio-energetically demanding and require fuel from the local environment.[10] Thus, despite adequate recruitment, the immune response may be blunted due to T cell dysfunction in part due to a failure to generate the energy required to meet their metabolic needs.

It is important to note that to date, there is no single standard of care to salvage patients who have progressed on IMT. Furthermore, metabolism represents a key, primordial mechanism by which T cells can be regulated, as we propose here.

We have shown that TIL harvested from melanoma-bearing mice have low mitochondrial mass and depressed glucose uptake. Metabolic profiling of T cells from tumors using Seahorse cell analysis reveals dramatically repressed oxidative function.[14] We have also shown that co-inhibitory molecules (PD-1, TIM-3, LAG-3) correlate with both T cell dysfunction and metabolic insufficiency. Thus, as T cells become increasingly 'exhausted', they also display increased metabolic insufficiency.

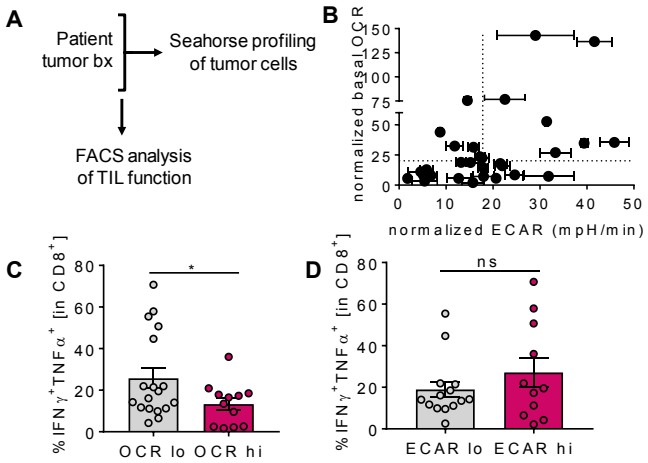

Figure 2. Functionality of melanoma TILs is related to oxidative metabolism of tumor cells. A, Scheme for immunometabolic profiling. **B**, OCR and ECAR of tumor cells from individual melanoma patients before immunotherapy. **C**,**D**, Polyfunctionality (IFNγ and TNFα co-production) in PMA/ionomycin re-stimulated TILs as a function of tumor cell (**C**) OCR or (**D**) ECAR.

We have utilized an immunometabolic profiling platform (**Figure 2A**) that allows for comprehensive analysis of the patients' tumor metabolism, immune infiltrate and T cell function, and hypoxia.[1] We have recently shown that tumor cells from a cohort of 30 melanoma patients obtained prior to treatment with IMT exist across a wide metabolic spectrum (**Figure 2B**), with high oxidative metabolism (OCR) and glycolytic metabolism (ECAR), hypermetabolic tumors that deregulate both pathways, or more indolent tumors with low metabolic activity.[1] Further, we found that CD8+ TILs isolated from tumors with high OCR (irrespective of tumor cell glycolysis) have significantly decreased polyfunctionality [IFN-γ and TNF-α co-production (**Figure 2C**)], whereas glycolytic capacity of tumors as measured by ECAR did not significantly correlate with T cell functionality (**Figure 2D**).[1]

To evaluate the impact that deregulated oxidative metabolism has on treatment response to anti-PD1, we characterized a cohort of 19 patients with metastatic melanoma just prior to anti-PD1 treatment. Interestingly, patients who did not respond to anti–PD1 checkpoint blockade

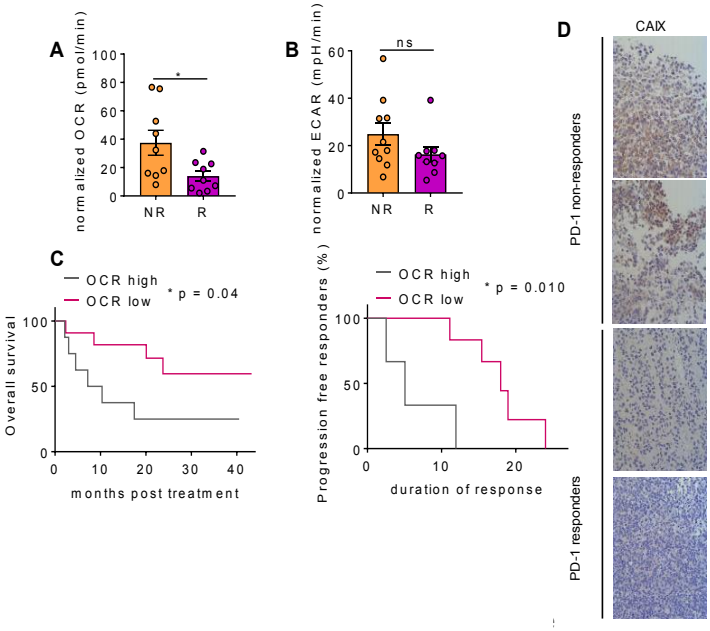

Figure 3. Tumor cells OXPHOS and consequent hypoxia is correlated with resistance to PD1 blockade in melanoma. **A,B** OCR (**A**) and ECAR (**B**) of tumor cells from patients with progressive disease (NR) or stable disease (partial or complete response, R). **C**, OR and PFS after anti-PD1 immunotherapy, as a function of tumor cell OCR. **D**, Carbonic anhydrase IX (CAIX; upregulated by hypoxia) IHC staining in pre-treatment biopsies from PD1 non-responders or responders.

(n = 10) had tumor cells characterized by increased oxidative metabolism (**Figure 3A**) while glycolytic metabolism had no strong predictive value (**Figure 3B**). High oxidative tumor cell metabolism was associated with decreased PFS, DOR, and OS (**Figure 3C**) with anti–PD-1 ICB. Of note, anti-PD1 non-responders had increased staining with CAIX, a marker of intra-tumoral hypoxia. (**Figure 3D**).

<u>Preliminary studies:</u>

We have shown that patients whose tumors have high oxidative metabolism possess high intra-tumoral hypoxia, and that this phenotype is linked with T cell exhaustion and dysfunction.[1] Further, oxidative metabolism is associated with decreased PFS, DOR, and OS in patients with advanced melanoma. We have additionally found that tumor cells are more metabolically active after progression on IMT (**Figure 4**). Thus, strategies to remodel the TME's metabolic landscape are especially important in patients progressing on IMT.

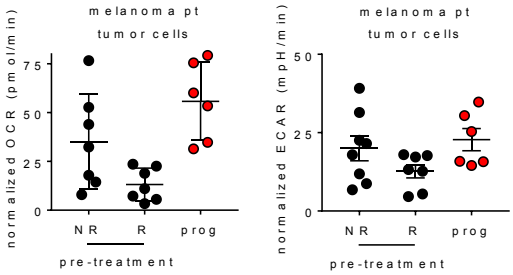

Figure 4. Tumor cells from melanoma patients who progress on ICB are uniformly hypermetabolic. OCR and ECAR of patient tumor cells pre-treatment (subdivided by response, as previously published) or obtained at

However, as discussed above, patients progressing on anti-PD1 therapy present a new problem—their TME has changed to become more aggressive, more immunosuppressive, and even more metabolically deregulated, with altered angiogenic patterns to support this increased metabolic state. As previously described, tumors create a tortuous blood supply through increased expression of VEGF, which creates both zones of hypoxia and highly perfused areas. In a cohort of 10 paired melanoma patient samples (at baseline and at progression on anti-PD1), we found that VEGFR1 is variably expressed, VEGFR2 is consistently strongly expressed (consistent with previous studies), and VEGFR3 is upregulated in the TME at progression on anti-PD1 (**Figure 5**). Here, we seek to target the VEGF pathway with axitinib to

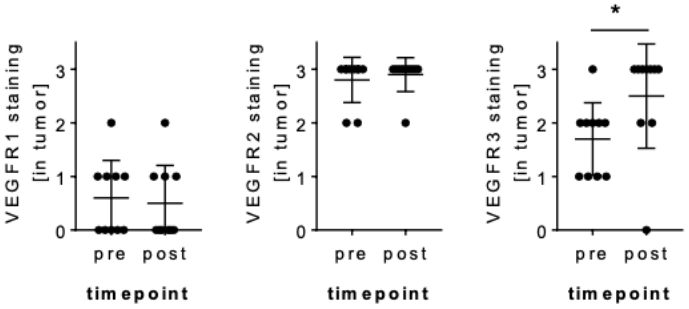

Figure 5: VEGFR expression in tumor biopsies from patients prior to anti-PD1 and at progression. Paired

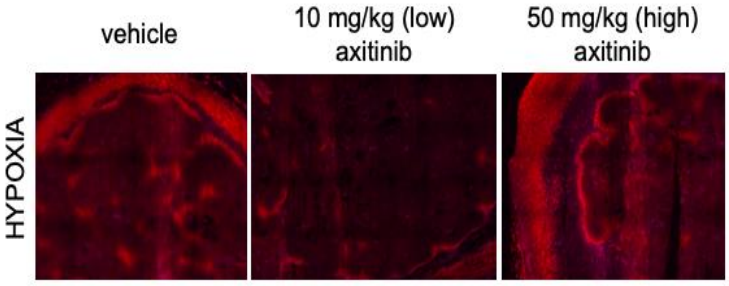

Figure 6. Low-dose axitinib reduces intratumoral hypoxia. Hypoxia staining by anti-pimo antibodies of B16 melanoma. Tumor-bearing mice received vehicle or axitinib at high or low dose for 4 days. Mice were pulsed with pimo before being euthanized. While high-dose axitinib exacerbates hypoxia, low-dose axitinib lowers intertumoral hypoxia.

correct the vasculature and restore metabolic balance to promote a better immune response. Axitinib is a VEGF receptor small molecule tyrosine kinase inhibitor with demonstrated safety and tolerability when combined with anti-PD1.[15,16] Further, axitinib has high inhibitory activity for VEGFR1, 2, and 3, which are each involved in altered angiogenesis. **We hypothesize that by modulating/correcting angiogenesis, axitinib will decrease intra-tumoral hypoxia and thus reduce T cell dysfunction.** We have shown that a low dose of axitinib is superior to a higher dose of axitinib in reducing intra-tumoral hypoxia in B16 melanoma (**Figure 6**). This finding is significant because we previously demonstrated that intra-tumoral hypoxia is associated with T cell exhaustion and decreased T cell effector function.[1] We have also performed pre-clinical experiments with anti-PD1 plus axitinib in murine models. Here, axitinib was given concurrently in B16 melanoma (generally insensitive to ICB) or in MC38 adenocarcinoma (partially sensitive to anti-PD1). The combination of low dose axitinib and ICB (CTLA4+PD1 in B16, PD1 monotherapy in MC38) provided an improved and durable response in both models (**Figure 7**).

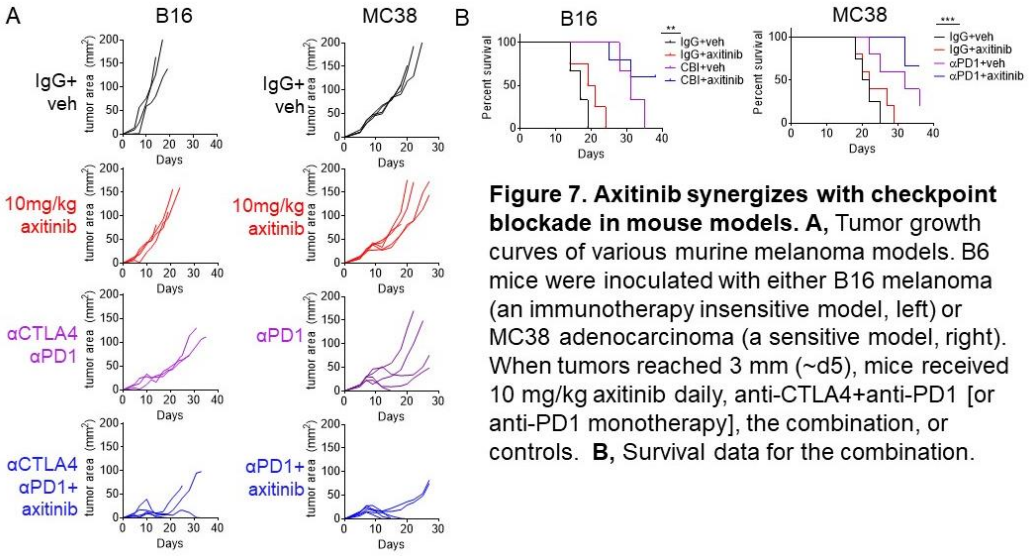

Figure 7. Axitinib synergizes with checkpoint blockade in mouse models. A, Tumor growth curves of various murine melanoma models. B6 mice were inoculated with either B16 melanoma (an immunotherapy insensitive model, left) or MC38 adenocarcinoma (a sensitive model, right). When tumors reached 3 mm (~d5), mice received 10 mg/kg axitinib daily, anti-CTLA4+anti-PD1 [or anti-PD1 monotherapy], the combination, or controls. **B,** Survival data for the combination.

We hypothesize that decreasing hypoxia in the TME will re-sensitize melanoma tumors to anti-PD1 therapy. We have chosen to use axitinib because it has already been safely combined with anti-PD1 therapy and was overall well-tolerated[15,16]. Importantly, the standard dose of axitinib (5 mg BID) corresponds to the low dose used in our murine models. The combination of pembrolizumab plus axitinib was superior to sunitinib in patients with advanced renal cell carcinoma, leading to FDA approval for combination treatment.[15] In addition, while axitinib has been combined with anti-PD1 in mucosal melanoma patients with promising results, this study was done in the front-line setting and did not include the extensive correlative analyses we propose.[16] Taken together, based on previously published work and data from our laboratories, **we hypothesize that axitinib can metabolically remodel the TME to render it**

more sensitive to ICB, specifically by reducing intra-tumoral hypoxia, increasing T cell infiltration, and increasing polyfunctional T cells.

3.1.1 Pharmaceutical and Therapeutic Background

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades. The PD-1 receptor-ligand interaction is a major pathway utilized by tumors to suppress the anti-tumor immune response. PD-1 is expressed on the cell surface of activated T-cells under healthy conditions, and it serves to down-modulate unwanted to excessive immune responses, such as autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade. The mechanism by which PD-1 down modulates T-cell responses is similar to, yet distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma. This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Nivolumab is a human IgG4 monoclonal antibody that binds to the PD-1 receptor and blocks the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Nivolumab is approved in the United Stated for the treatment of patients with unresectable or metastatic melanoma. It is

also indicated in the setting of disease progression following ipilimumab and/or a BRAF inhibitor (if BRAF V600E mutation positive).

VEGF, produced by endothelial cells, is critical for the regulation of both physiologic and pathologic angiogenesis.[17] This process plays a key role in tumor growth and metastasis. VEGF (also known as VEGF-A) is one of the members of the VEGF family also comprised of VEGF-B, VEGF-C, VEGF-D, and placenta growth factor (PLGF). Each member is uniquely expressed with varying receptor specificity and function.[18] VEGF is encoded by a transcript composed of 8 exons and 7 introns. Through alternative splicing, several different isoforms are generated with each isoform having differing degrees of pro-angiogenic properties.[19] VEGF expression is further regulated by the tumor environment, predominantly by hypoxia, which induces the transcription factor HIF1a, leading to increased VEGF expression.[20] The members of the VEGF family bind to varying combinations of VEGF receptors; VEGF binds only to VEGFR-1 and VEGFR-2, while VEGFR-3 binds VEGF-C and VEGF-D. VEGFR-1 through 3 are tyrosine kinase receptors with similar structures consisting of an extracellular domain of 7 IgG folds, a single transmembrane domain, and split tyrosine kinase domain. Binding of the ligand to the receptor induces dimerization of the receptor which is necessary for downstream signaling. Of note, the VEGFR-2 has been identified as the major mediator of VEGF induced angiogenesis, VEGFR-3 is involved in angiolymphogenesis, and the exact role of VEGFR-1 is not fully understood.

Axitinib is a second-generation tyrosine kinase inhibitor of VEGFR-1, VEGFR-2, and VEGFR-3. Axitinib is approved by the FDA for the treatment of metastatic renal cell carcinoma alone and more recently in conjunction with either pembrolizumab or avelumab in the first line setting.[21,22]

3.1.2 Preclinical and Clinical Trial Data

Nivolumab: Refer to the Investigator's Brochure for Preclinical and Clinical data of Nivolumab.

Axitinib: Refer to the Investigator's Brochure for Preclinical and Clinical data of Axitinib.

3.2 Rationale

3.2.1 Rationale for the Trial and Selected Subject Population

The critical role of angiogenesis in cancer biology has long been appreciated, and anti-angiogenic therapies have been successfully utilized in several cancer types, including renal cell carcinoma and colorectal cancer. However, while the significance of anti-angiogenic therapy was first thought to be related to decreasing tumor blood supply, it is now appreciated that its effect on oxygen tension and immune infiltration are critical in maintaining antitumor activity, and that normalization of the blood supply may help the immune response. Melanoma is understood to be an angiogenic tumor, with new vessel formation implicated in the

progression from atypical melanocyte, to horizontal growth phase, and subsequently to the more aggressive vertical growth phase.[23,24] Increased VEGF expression is associated with a greater depth of invasion. [25] Bevacizumab is an anti-VEGF monoclonal antibody that has been evaluated as monotherapy in the adjuvant setting, as well as in combination with chemotherapy or anti-CTLA4 in the advanced setting, with mostly disappointing results.[26-30] Axitinib, on the other hand, is a small molecule inhibitor of the VEGF receptor and binds to VEGFR-1, VEGFR-2, and VEGFR-3. Axitinib monotherapy has been evaluated in patients with advanced melanoma, with an ORR of 18.8% in the first line setting.[31] A phase II trial of axitinib with carboplatin and paclitaxel yielded an ORR of 22% and median PFS of 8.7 months.[32] A more recent phase IB trial evaluated toripalimab (anti-PD1) and axitinib in 33 patients with treatment naive metastatic melanoma. No dose limiting toxicities were observed, and by the cut-off date, 14/29 (48.3%) patients achieved an objective response, which is higher than would be expected with anti-PD1 monotherapy.[16] However, it must be noted that 88% of these patients had mucosal melanoma. Our preclinical data suggests that although high dose axitinib exacerbates hypoxia, low dose axitinib improves hypoxia. We hypothesize this is through normalization of aberrant tumor vasculature, which allows for improved tumor perfusion and immune cell infiltration. We have previously shown that T cells in a hypoxic TME are exhausted and dysfunctional.[10,33-35] Thus, combining axitinib with anti-PD1 may salvage a portion of patients with anti-PD1 refractory disease, which is supportive of other previously published findings utilizing immunotherapy with VEGF targeting.[36-38] We therefore propose a phase II investigator-initiated study of nivolumab plus axitinib in patients with unresectable stage III or IV melanoma who have progressed on anti-PD1 therapy.

3.2.2 Rationale for Dose Selection/Regimen/Modification

The choice for 480 mg every 4 weeks of nivolumab was made based on standard clinical practice in patients with melanoma. Initial pharmacokinetics assessed using the population PK model of nivolumab found that nivolumab had a wide therapeutic index from 0.1 to 10 mg/kg every 2 weeks.[8] The 3 mg/kg dose was found to be effective in phase III studies across tumor types and was approved by the FDA. Subsequently, a fixed dose of 240 mg every 2 weeks was approved based on comparable safety and efficacy data.[39] A fixed dose simplifies the dosing regimen, which is both more convenient for physicians and reduces potential for dosing errors. More recently, the FDA has expanded the approval to include 480 mg every 4 weeks based on safety and efficacy data that suggested this dosing was comparable to 3 mg/kg every 2 weeks.[40] This dosing regimen provides additional convenience for patients, particularly those who will be treated over a long timeframe, such as our cohort.

Regarding axitinib, 5 mg twice daily is the standard FDA approved dose. In a murine B16 melanoma model, we administered vehicle, axitinib at a low dose (10 mg/kg), or axitinib at a high dose (50 mg/kg) for 4 days. We found that high dose axitinib exacerbates hypoxia, while low dose axitinib lowers intra-tumoral hypoxia (**Figure 6**). Importantly, the standard dose of axitinib (5 mg BID) corresponds to the low dose used in our murine models.

3.2.3 Rationale for Endpoints

3.2.3.1 Efficacy Endpoints

The primary endpoint is the overall response rate after treatment with nivolumab plus axitinib. We hypothesize that patients treated with nivolumab plus axitinib will have a greater response rate compared to historical responses in anti-PD1 refractory patients, which is estimated to be 10%, based on responses to ipilimumab after anti-PD1 therapy.[41] An ORR of 25% or higher would be of clinical interest. We hypothesize that the mechanism by which there will be an improved response rate is that the addition of low dose axitinib will renormalize tumoral vasculature, improve tumor perfusion, immune infiltration, and reduce hypoxia. Our preclinical model was notable for a reduction in hypoxia seen in the low-dose group. Secondary efficacy endpoints include safety, PFS, and OS. The combination of pembrolizumab plus axitinib has been approved by the FDA for advanced renal cell carcinoma, and its toxicity profile is well-established. Here, we will use nivolumab in place of pembrolizumab. Nivolumab and pembrolizumab are virtually identical in terms of cellular target and known toxicity, thus we expect toxicity rate of the current combination therapy and dose to be acceptable. We hypothesize that treatment with nivolumab plus axitinib will prolong both PFS and OS.

3.2.3.2 Biomarker Research

The secondary endpoints of this study seek to identify the functional consequences of axitinib-induced remodeling of the tumor microenvironment with concomitant anti-PD1 blockade. We will analyze biopsy samples prior to treatment with axitinib plus nivolumab as well as 12 weeks after treatment. First, we will immunophenotype the TIL from patient tumor samples using antibodies to T cell markers (CD4, CD8, PD1, TIM-3, CD45RO, CD45RA, FOXP3, among others) via 14-color flow cytometry. Second, we will profile intra-tumoral hypoxia and immune architecture of patient samples, assessed via staining for CAIX, HIF1a, and VEGF utilizing highly multiplexed immunofluorescent imaging (CODEX) and pimonidazole CITE-seq to evaluate transcriptional changes. Third, we will assess proliferation and functional analysis with Ki-67 and cytokine production of TIL. Lastly, we will also metabolically profile the biopsies utilizing Seahorse.

4.0 METHODOLOGY

4.1.1 Subject Inclusion Criteria

In order to be eligible for participation in this trial, the subject must:

1. Be willing and able to provide written informed consent for the trial.

2. Be ≥ 18 years of age on day of signing informed consent.

3. Have unresectable (stage III) or advanced (stage IV) cutaneous or mucosal melanoma. Patients with uveal melanoma are not eligible.

4. Progressed on prior anti-PD1 therapy with or without anti-CTLA4 therapy. Patients may have progressed in the adjuvant setting if treated within the last 6 months. Prior treatment with BRAF/MEK inhibitors permitted, however, not required. Progression must be radiographic, and progression of disease will be confirmed by a radiologist. Patients must have progressed during anti-PD-1 therapy, defined as unequivocal progression on or within 3 months of the last dose of anti-PD-1 therapy if treated in the metastatic setting, or within 6 months if treated in the adjuvant setting.

5. Have measurable disease based on RECIST 1.1.

6. Patients do not have to have biopsiable disease to be eligible. However, patients with biopsiable disease must undergo biopsy at study entry and at week 12.

7. Have a performance status of 0 or 1 on the ECOG Performance Scale.

8. Demonstrate adequate organ function as defined in Table 1. All screening labs should be performed within 14 days of treatment initiation.

Table 1: Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,000 /mcL
Platelets	≥50,000 / mcL
Hemoglobin	≥7 g/dL without transfusion or EPO dependency (within 7 days of assessment)
Renal	
Serum creatinine **OR** Measured or calculated creatinine clearance (GFR can also be used in place of creatinine or CrCl*)	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants ≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
*Creatinine clearance should be calculated per institutional standard.	

9. Patients with brain metastases are permitted if they are asymptomatic or previously treated with CNS directed therapy with stable CNS disease for at least 2 weeks. Stable is defined as asymptomatic or not progressing on imaging.

10. Female patients of childbearing potential must have a negative urine or serum pregnancy test within 7 days from the time of registration. Also, within 24 hours prior to receiving the first dose of study medication then every 4 weeks while on treatment.

11. Female subjects of childbearing potential should be willing to use 2 methods of birth control or be surgically sterile or abstain from heterosexual activity for the course of the study and for 5 months after the last dose of study medication. Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.

12. Male subjects should agree to use an adequate method of contraception starting with the first dose of study therapy through 7 months after the last dose of study therapy.

Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

4.1.2 Subject Exclusion Criteria

The subject will be excluded from the trial if the subject:

1. Prior history of Grade 3 or 4 immune-related adverse events or immune-related adverse events requiring discontinuation of prior therapies.

2. History of hypertensive crisis or hypertensive encephalopathy

3. Significant thrombotic (e.g, deep vein thrombosis or pulmonary embolism) or hemorrhagic event within 6 months prior to enrollment).

4. Has a history of prior immune-related adverse event due to an anti-PD1 or anti-CTLA4 that has not resolved to grade 1 on a steroid dose of prednisone 10 mg or less at the time of study entry (excluding vitiligo and endocrine toxicity).

    - Patients with prior myocarditis or other immune-mediated cardiac adverse events are excluded regardless of grade.

    - Patients with prior Guillain-Barre syndrome, encephalitis, meningitis, or transverse myelitis are excluded regardless of grade.

- Patients with prior Stevens-Johnson syndrome or toxic epidermal necrolysis are excluded regardless of grade.

5. Has poorly controlled hypertension defined as systolic blood pressure (SBP) > 160 and/or diastolic blood pressure (DBP) > 100 despite antihypertensives. If subject is above this goal, treatment with anti-hypertensives to achieve better blood pressure control is permitted. Ambulatory blood pressure assessment is permitted if there is concern for discrepant blood pressure readings while patients are in clinic. Note: measurement of screening blood pressure (BP) reading is based on an average of 3 readings at least 2 minutes apart if the subject has an initial BP ≥150/90. Subjects with initial screening BP ≥150/90 mmHg can be treated with anti-hypertensive medication to achieve a well-controlled status and are eligible with reassessed SBP/DBP of < 160/100 mm Hg.

6. Has Class III or IV heart failure based on the New York Heart Association

7. Has had major surgery within 4 weeks of randomization. This does not include outpatient surgeries that do not require post-operative admission.

8. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy (greater than the equivalent of prednisone 10 mg daily, unless for prior endocrine toxicity) or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment. Premedication with steroids for contrast imaging studies is permitted.

9. Has a known history of active TB (Bacillus Tuberculosis).

10. Hypersensitivity to nivolumab or axitinib, or any of their excipients.

11. Has had prior chemotherapy or targeted small molecule therapy within 1 week prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.

    - Note: Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study.

    - Note: If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

12. Has had radiation within 2 weeks of randomization

13. Has current use or anticipated need for treatment with drugs or foods that are known strong cytochrome P450 (CYP34A4/5) inhibitors including but not limited to atazanavir, clarithromycin, indinavir, itraconazole, ketoconazole, nefazodone,

nelfinavir, ritonavir, saquinavir, telithromycin, troleandomycin, voriconazole, and grapefruit or grapefruit juice. NOTE: The topical use of these medications, such as 2% ketoconazole cream is allowed.

14. Has current use or anticipated need for treatment with drugs known to be strong CYP3A4/5 inducers, including but not limited to carbamazepine, phenobarbital, phenytoin, rifabutin, rifampin, and St. John's wort.

15. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy, in situ cervical cancer, in situ colon cancer, or nonmetastatic prostate cancer not on systemic therapy.

16. Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least 2 weeks prior to the first dose of trial treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability.

17. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (e.g., thyroxine or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

18. Has a history of (non-infectious) pneumonitis that required steroids or current pneumonitis.

19. Has an active infection requiring systemic IV antibiotic therapy.

20. Has had any of the following within the past 6 months

    - Myocardial infarction or unstable angina
    - Ventricular arrythmia
    - Acute decompensated heart failure
    - Cerebrovascular accident
    - Hypertensive emergency requiring ICU admission

21. Presence of a disorder that may impact absorption of axitinib, such as inability to take oral medication, requirement for IV alimentation, prior gastric resection, treatment for active peptic ulcer confirmed by endoscopy within the past 3 months, active GI bleed, malabsorption syndrome.

22. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

23. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

24. Is pregnant or breastfeeding or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 5 months after the last dose of trial treatment for females and 7 months after the last dose of trial treatment for males.

25. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies) if the CD4 count is less than 350 mm3 or serum HIV viral load is < 25,000 IU/mL.

26. Has a known history of or is positive for hepatitis B (hepatitis B surface antigen [HBsAg] reactive) or hepatitis C (hepatitis C virus [HCV] RNA [qualitative] is detected). Note: Without known history, testing only needs to be performed if there is clinical suspicion for Hepatitis B or C.

27. Is currently incarcerated or otherwise detained.

28. Has received a live vaccine within 30 days of planned start of study therapy.

Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal iNinfluenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.

4.2 Trial Treatments

The treatment plan is outlined in Table 2

Table 2: Treatment Schedule. Treatment with nivolumab and axitinib begins on C1D1.

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period
Nivolumab	480 mg	Q4W	IV infusion	Day 1 of each 4-week cycle

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period
Axitinib	5 mg	Twice daily	Oral	Daily

4.2.2 Patient Compliance

Patients will be required to return all bottles of axitinib at the beginning of each 4-week period. The number of tablets remaining will be documented and recorded.

4.2.3 Duration of Therapy

Treatment will continue for up to 2 years or until progression of disease or unacceptable toxicities.

4.2.4 Starting Dose and Modifications

4.2.4.1 Nivolumab

The Nivolumab dose if 480 mg IV every 4 weeks.

Adverse events (both non-serious and serious) associated with nivolumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Nivolumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per table 3 below. See Section 4.3.1 for supportive care guidelines, including use of corticosteroids.

At the end of the study period, Bristol-Myers Squibb Company will not continue to supply study drug to subjects/investigators unless the Sponsor-Investigator chooses to extend the study. The treating physician is responsible to ensure that the subject receives appropriate standard of care or other appropriate treatment.

No modifications in the dose of nivolumab are permitted. Dose delays are permitted, as outlined below.

Table 3: Nivolumab Dose Delay and Discontinuation Guidelines for Drug-Related Adverse Events

Toxicity	Hold Treatment for Grade	Timing for Restarting Treatment	Treatment Discontinuation
Diarrhea/Colitis	2-3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks

Toxicity	Hold Treatment for Grade	Timing for Restarting Treatment	Treatment Discontinuation
	4	Permanently discontinue	Permanently discontinue
AST, ALT, or Increased Bilirubin	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose
	3-4	Permanently discontinue (see exception below)[a]	Permanently discontinue
Type 1 diabetes mellitus (if new onset) or Hyperglycemia	T1DM or 3-4	Hold for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure	Resume when patients are clinically and metabolically stable
Hypophysitis	2-4	Toxicity resolves to Grade 0-1. Therapy with can be continued while endocrine replacement therapy is instituted	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue
Hypothyroidism		Therapy with can be continued while thyroid replacement therapy is instituted	Therapy with can be continued while thyroid replacement therapy is instituted
Infusion Reaction	2[b]	Toxicity resolves to Grade 0-1	Permanently discontinue if toxicity develops despite adequate premedication
	3-4	Permanently discontinue	Permanently discontinue
Pneumonitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4	Permanently discontinue	Permanently discontinue
Renal Failure or Nephritis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4	Permanently discontinue	Permanently discontinue
All Other Drug-Related Toxicity[c]	3 or Severe	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue

Note: Permanently discontinue for any severe or Grade 3 drug-related AE that recurs or any life-threatening event.

[a] For patients with liver metastasis who begin treatment with Grade 2 AST or ALT, if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week then patients should be discontinued.

[b] If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose; Refer to Table 5– Infusion Treatment Guidelines for further management details.

[c] Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.

4.2.4.2 Axitinib

Axitinib will be administered at the standard dosing of 5mg po twice daily.

Table 4. Axitinib Dose Modification Guidelines for Drug-Related Adverse Events

Toxicity	Action
Grade 4 non-hematologic toxicity considered related to axitinib per investigator judgment (including persistent nausea, vomiting, diarrhea, hypertension despite optimal medical therapy), excluding clinically manageable laboratory abnormalities (e.g. electrolytes).	Discontinue axitinib.
Grade 3 non-hematologic toxicity considered related to axitinib per investigator judgment (including persistent nausea, vomiting, diarrhea, hypertension, proteinuria despite optimal medical therapy), excluding clinically manageable laboratory abnormalities (e.g. electrolytes).	Hold axitinib until recovery to Grade 0-1 or baseline and resume at the same dose level. If grade 3 toxicity reoccurs despite reduction, patient may be dose reduced by 1 dose level upon recovery to Grade 0-1 or baseline. Prompt palliative and supportive measures mandated per local standard of care (eg, antiemetic).
Grade 3 Thrombocytopenia, ie, PLTS <50,000 mm3 (50.0 x 109/L) with bleeding.	Discontinue Axitinib
Grade 3 hematologic toxicity considered related to axitinib per investigator judgment	-Hold axitinib until recovery to Grade ≤ 2 or baseline and resume axitinib at the same dose level. -If grade 3 toxicity reoccurs, patient may either be held until recovery and continued at same dose, or undergo dose reduction by 1 dose level.
Grade 4 toxicity considered related to axitinib per investigator judgment	Discontinue Axitinib
No recovery of toxicities within 6 weeks to grade 0 or grade 1	Discontinue Axitinib

Table 5. Axitinib Dose Levels

Dose Level	Dose
Level 0 (starting dose)	5 mg po bid
Level -1	3 mg po bid
Level -2	2 mg po bid

4.2.5 Timing of Dose Administration

Nivolumab: Trial treatment should be administered on Day 1 of each cycle after all procedures/assessments have been completed as detailed on the Trial Flow Chart (Section 6.0). Trial treatment may be administered up to 3 days before or after the scheduled Day 1 of each cycle due to administrative reasons.

All trial treatments will be administered on an outpatient basis.

Nivolumab 480 mg will be administered as a 30 minute IV infusion every 4 weeks. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

Nivolumab will be prepared and infused per institutional standards

Axitinib: Axitinib 5 mg PO twice daily, 12 hours apart, will be taken for the entire duration of treatment. Patients will be given specific instructions on how to take axitinib. If a dose is missed, it may be taken up to 8 hours later that same day.

Dosing interruptions of nivolumab and axitinib are permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the patient's study record.

Should the combination of nivolumab and axitinib be further investigated for the treatment of anti-PD1 refractory melanoma, given the potential for late onset immune-related toxicities for nivolumab, any such late toxicity will be considered in the determination of the final dose and dose schedule. The number of required dose reductions of axitinib will also be taken into full consideration.

4.2.6 Trial Blinding/Masking

This is an open-label trial; therefore, the Sponsor, investigator and subject will know the treatment administered.

4.3 Rescue Medications & Supportive Care

4.3.1 Supportive Care Guidelines

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to study drug(s).

Note: if after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance (as outlined below). Refer to Section 4.2.4 for dose modification.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event.

<u>For toxicities where the attribution is Nivolumab (immune mediated toxicity)</u>

- **Pneumonitis**:
    - For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
    - For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
    - Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.
- **Diarrhea/Colitis**:

  Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).

    - All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
    - For **Grade 2 diarrhea/colitis,** administer oral corticosteroids.
    - For **Grade 3 or 4 diarrhea/colitis,** treat with intravenous steroids followed by high dose oral steroids.
    - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
- **Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or ≥ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)**
    - For **T1DM** or **Grade 3-4** Hyperglycemia
        - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.

        - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

- **Hypophysitis**:
    - For **Grade 2** events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
    - For **Grade 3-4** events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
- **Hyperthyroidism or Hypothyroidism**:

  Thyroid disorders can occur at any time during treatment. Monitor patients for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.

    - **Grade 2** hyperthyroidism events (and **Grade 2-4** hypothyroidism):
        - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
        - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.
    - **Grade 3-4** hyperthyroidism
        - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
- **Hepatic**:
    - For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).
        - Treat with IV or oral corticosteroids
    - For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours.
    - When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.
- **Renal Failure or Nephritis**:

- For **Grade 2** events, treat with corticosteroids.
- For **Grade 3-4** events, treat with systemic corticosteroids.
- When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Management of Infusion Reactions**: Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

  Table 6 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of nivolumab.

Table 6: Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated.	No subsequent dosing

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
	Subject is permanently discontinued from further trial treatment administration.	
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

For toxicities where the attribution is Axitinib (non-immune mediated toxicity)

Dose modifications as in Section 4.2.4.2. Management of hypertension and febrile neutropenia per investigator.

4.4 Diet/Activity/Other Considerations

4.4.1 Diet

Subjects should maintain a normal diet unless modifications are required to manage an AE such as diarrhea, nausea or vomiting. Diet should exclude grapefruit and grapefruit juice.

4.4.2 Contraception

Nivolumab and axitinib may have adverse effects on a fetus in utero. Furthermore, it is not known if these agents have transient adverse effects on the composition of sperm.

For this trial, male subjects will be considered to be of non-reproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

Female subjects will be considered of non-reproductive potential if they are either:

(1) postmenopausal (defined as at least 12 months with no menses without an alternative medical cause; in women < 45 years of age a high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.);

OR

(2) have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion, at least 6 weeks prior to screening;

OR

(3) has a congenital or acquired condition that prevents childbearing.

Female and male subjects of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving study drug and for 5 months after the last dose of study drug for females and 7 months for males by complying with one of the following:

(1) practice abstinence† from heterosexual activity;

OR

(2) use (or have their partner use) acceptable contraception during heterosexual activity.

Acceptable methods of contraception are‡:

Single method (one of the following is acceptable):

- intrauterine device (IUD)
- vasectomy of a female subject's male partner
- contraceptive rod implanted into the skin

Combination method (requires use of two of the following):

- diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- cervical cap with spermicide (nulliparous women only)
- contraceptive sponge (nulliparous women only)
- male condom or female condom (cannot be used together)
- hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

†Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and ERCs/IRBs. Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 120 days after the last dose of trial therapy. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

4.4.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on study treatment, or may have been pregnant, including at least 5 half-lives after product administration, the subject will immediately be removed from the study and the investigational product will be permanently discontinued in an appropriate manner. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor and to BMS and Pfizer without delay and within 24 hours to the Sponsor and within 2 working days to BMS and Pfizer if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn). The investigator must immediately notify worldwide.safety@bms.com of this event and complete the appropriate form in accordance with SAE reporting procedures within 24 hours of awareness of the event. Protocol-required procedures for study discontinuation and follow-up must be performed on the participant. Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome, and where applicable, offspring information must be reported to BMS as well. Please note: if needed, a BMS pregnancy surveillance form can be provided.

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the Sponsor and to BMS and Pfizer. Information on this pregnancy will be collected on the Pregnancy Surveillance Form. In order for Sponsor or designee to collect any pregnancy surveillance information from the female partner, the female partner must sign an informed consent form for disclosure of this information.

4.4.4 Use in Nursing Women

It is unknown whether the study drugs are excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, subjects who are breast-feeding are not eligible for enrollment.

4.5 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator or the Sponsor if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons.

A subject must be discontinued from the trial for any of the following reasons:

- The subject or legal representative (such as a parent or legal guardian) withdraws consent.
- Confirmed radiographic disease progression

  Note: For unconfirmed radiographic disease progression, please see Section 5.2.2

  Note: A subject may be granted an exception to continue on treatment with confirmed radiographic progression if clinically stable or clinically improved, please see Section 7.1.2.7.1
- Unacceptable adverse experiences as described in Section 5.2.1.2
- Intercurrent illness that prevents further administration of treatment
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Completed 24 months of uninterrupted treatment with nivolumab or 24 administrations of study medication, whichever is later.

  Note: 24 months of study medication is calculated from the date of first dose.
- After trial discontinuation (whether due to subject withdrawal, progression, or dose limiting toxicity), patients will be seen at week 4 for follow up, and then followed once 100 days after the last day of treatment even if they start a new anticancer therapy. Patients will be contacted by phone for survival follow up for up to 5 years.

4.5.1 Discontinuation of Study Therapy after CR

Discontinuation of treatment may be considered for subjects who have attained a confirmed CR that have been treated for at least 24 weeks with nivolumab and axitinib and had at least two treatments with axitinib beyond the date when the initial CR was declared.

4.6 Clinical Criteria for Early Trial Termination

Early trial termination will be the result of the criteria specified below:

1. Quality or quantity of data recording is inaccurate or incomplete
2. Poor adherence to protocol and regulatory requirements
3. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects
4. Plans to modify or discontinue the development of the study drug

In the event of BMS or Pfizer deciding to no longer supply study drug(s), ample notification will be provided so that appropriate adjustments to subject treatment can be made.

5.0 TRIAL PROCEDURES

5.1 Study Flow Chart

	Baseline*	C1 +/-3 days	C2 +/-3 day	C3 +/-3 day	C4 +/-3 day	C5 +/-3 day	C6 +/-3 day	C7 +/-3 day	C8 +/-3 day	C9 +/-3 day	C10 +/-3 day	C11 +/-3 day	C12 + +/-3 day	EOT	Follow up[#]
Informed consent	X														
Demographics	X														
Medical history	X														
Concurrent meds	X	X----------X												X	X
Physical exam	X	X----------X												X	X
Vital signs	X	X----------X												X	X
Performance status (ECOG)	X	X----------X												X	X
CBC w/diff, plts	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serum chemistry [a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine dipstick	X			x		x		x		x		x		x	
Adverse event evaluation		X----------X												X	X
Radiologic evaluation & Tumor measurements	X	Tumor measurements will be evaluated with the first scans at week 12 (+/- 1 week). Subsequent scans will be repeated every 12 weeks (+/- 1 week). Patients with complete or partial response will have confirmation scans at least 4 and up to 12 weeks later. Documentation (radiologic) must be provided for patients removed from study for progressive disease. Brain MRI will be obtained at baseline, and will be repeated at 12 weeks if patients have known brain mets, or develop neurological symptoms** . CT scans of the brain can be utilized if MRI cannot be obtained in a timely manner per the treating physician.													X
B-HCG [b]	X	X---------- X													
TSH, free T4[c]	X		X		X		X		X		X		X	X	

Tumor bx[d]	X				X										
Research Blood draws [e]	X				X									X	
Axitinib administration [f]		X	X	X	X	X	X	X	X	X	X	X	X		
Nivolumab administration		X	X	X	X	X	X	X	X	X	X	X	X		
Microbiome sampling [g]	X				X							X		X	

*: Baseline is within 30 days of start of treatment, except for labs (14 days).

#: After trial discontinuation (whether due to subject withdrawal, progression, or dose limiting toxicity), patients will be seen at week 4 for follow up. Survival follow-up will be obtained at day 100 days after the last day of treatment (this can be done by phone), even if they start a new anticancer therapy. Patients will be contacted by phone for survival follow up for up to 5 years.

**: If known to have prior brain metastases, must not have evidence of active (enlarging and/or symptomatic lesions) brain disease on MRI evaluation within 2 weeks from SRS or WBRT treatment. CT scans of the brain can be utilized if MRI cannot be obtained in a timely manner per the treating physician at baseline and also at 2 week follow up, if asymptomatic.

a. albumin, alkaline phosphatase, total bilirubin, direct bilirubin (if total bilirubin is > ULN), bicarbonate, BUN, calcium, chloride, CPK, creatinine, fasting glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.

b: Urine or serum pregnancy test (women of childbearing potential) (+/- 3 days). To be done at screening, within 24 hours prior to receiving the first dose of study medication and then every 4 weeks while on treatment.

c: TSH and free T4 to be done at screening, cycle 2 and then q 8 weeks (even cycles) while on treatment (+/- 3 days).

d: Baseline biopsy will be obtained up to 30 days prior to initiation of treatment and at C4 week 12, (+/-7 days). Patients will be treated with pimonidazole 0.5g/m2 po 16-24h before each biopsy. Biopsy at progression of disease is optional. Patients must have eligibility completed and be registered on study prior to the baseline biopsy.

e: Research Blood draws are to be done at baseline (up to 30 days prior to initiation of therapy), cycle 4 week 12 and at progression (+/- 7 days). This includes 10 green top tubes, 2 red top tubes and an ACD yellow top tube (yellow top at baseline only).

f: Axitinib pill counts will be reviewed and axitinib dispensed at each study visit (every 4 weeks). Patients will in addition be provided an axitinib drug diary, which should be returned at each visit.

g. Microbiome sampling will be obtained at baseline (up to 30 days prior to initiation of therapy), cycle 4 week 12 and at progression (+/- 7 days). Samples may be brought into clinic.

The Trial Flow Chart – Section 5 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

5.1.1 Administrative Procedures

5.1.1.1 Informed Consent

The Investigator will make certain that an appropriate informed consent process is in place to ensure that potential research subjects, or their authorized representatives, are fully informed about the nature and objectives of the clinical study, the potential risks and benefits of study participation, and their rights as research subjects. The Investigator, or a sub-investigator(s) designated by the Investigator, will obtain the written, signed informed consent of each subject, prior to performing any study-specific procedures on the subject. The date and time that the subject, or the subject's authorized representative, signs the informed consent form and a narrative of the issues discussed during the informed consent process will be documented in the subject's case history. The Investigator will retain the original copy of the signed informed consent form, and a copy will be provided to the subject, or to the subject's authorized representative.

The Investigator will make certain that appropriate processes and procedures are in place to ensure that ongoing questions and concerns of enrolled subjects are adequately addressed and that the subjects are informed of any new information that may affect their decision to continue participation in the clinical study. In the event of substantial changes to the clinical study or the risk-to-benefit ratio of study participation, the Investigator will obtain the informed consent of enrolled subjects for continued participation in the clinical study.

5.1.1.2 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial.

5.1.1.3 Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the Investigator.

5.1.1.4 Concomitant Medications Review

The investigator or qualified designee will record medication, if any, taken by the subject during the trial. All medications related to reportable SAEs and ECIs should be recorded as defined in Section 7.2.

Concomitant medications will stop being collected at the time of starting a new anti-cancer therapy.

5.1.1.5 Disease Details and Treatments

5.1.1.5.1 Disease Details

The investigator or qualified designee will obtain prior and current details regarding disease status.

5.1.1.5.2 Prior Treatment Details

The investigator or qualified designee will review all prior cancer treatments including systemic treatments, radiation and surgeries.

5.1.1.6 Assignment of Screening Number

Screening numbers will be assigned by sequential numbering.

5.1.1.7 Assignment of Treatment Number

Treatment numbers will be assigned by sequential numbering.

5.1.1.8 Trial Compliance (Medication/Diet/Activity/Other)

Axitinib pills will be counted at each monthly visit.

5.1.2 Clinical Procedures/Assessments

Patients will undergo study treatments and evaluation at the UPMC-Hillman Cancer Center. Patients will continue to be evaluated every 12 weeks (+/- 1 week) with scans, labs and MD visit.

5.1.2.1 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 5.0 (see Section 11.2). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

Please refer to section 7.2 for detailed information regarding the assessment and recording of AEs.

5.1.2.2 Full Physical Exam

The investigator or qualified designee will perform a complete physical exam during the screening period. Clinically significant abnormal findings should be recorded as medical history.

5.1.2.3 Vital Signs

The investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment and at treatment discontinuation as specified in the Trial Flow Chart (Section 5.0). Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only.

5.1.2.4 Eastern Cooperative Oncology Group (ECOG) Performance Scale

The investigator or qualified designee will assess ECOG status (see Section 11.1) at screening, prior to the administration of each dose of trial treatment and discontinuation of trial treatment as specified in the Trial Flow Chart.

5.1.2.5 Tumor Imaging and Assessment of Disease

PET/CT and CT
-At baseline and week 12, extra-cranial tumor assessment will be conducted with contrast-enhanced PET/CT or CT and applied using RECIST 1.1 criteria.
-A screening scan will be performed prior to enrollment unless a recent (within 28 days of enrollment) scan is available. Target and non-target lesions must be identified at time of screening scan and the same lesions must be re-assessed at each restaging scan.
-Repeat PET/CT or CT imaging will be obtained at week 12, and then CT will be obtained at 12 week intervals.

-Complete response/partial response confirmation assessments must take place at least 4 and up to 12 weeks after the documentation of initial response. If the last radiographic assessment for extra-cranial disease was more than 8 weeks prior to discontinuation from study and progressive disease has not been documented, disease assessment should be obtained at the time of study discontinuation.
-If patients have PD by RECIST 1.1 criteria, the Immune Related Response Criteria (irRC) will be used for assessment of tumor response for the purposes of managing patients on protocol treatment and decision making for discontinuation of study therapy due to disease progression. These disease assessments will be performed by the investigator with site radiology reading.
- For the purposes of the secondary efficacy endpoint of the study, response assessment based on RECIST 1.1 will be applied as the primary measure and irRC by investigators will be evaluated as a secondary assessment only if patient have PD by RECIST 1.1. If patients have PD by RECIST 1.1 but have SD or PR by irRC, they will be permitted to continue treatment. In order for a patient to continue to receive study treatment beyond progression by RECIST 1.1, in addition to having SD or PR by irRC, patients must not have symptoms or signs of disease progression, including significantly worse laboratory values or a decline in ECOG performance status.
- At the time of radiographic progression of disease, if treatment is to continue beyond PD, patients will be re-consented and adequately informed of all FDA-approved therapy, and potential clinical benefit, that the patient may be foregoing in order to continue receiving the investigational product.

MRI
Intra-cranial tumor assessments will be conducted with contrast-enhanced MRI.
A screening scan will be performed prior to enrollment unless a recent (within 4 weeks of enrollment) scan is available. If brain metastases are known or identified at screening, brain imaging should be repeated at 12 weeks or as it is standard, unless acute neurological symptoms develop. If no brain metastases are known or identified at pre-study imaging, no brain imaging need be performed following pre-study assessment unless acute neurological symptoms develop. CT scans of the brain can be utilized if MRI cannot be obtained in a timely manner per the treating physician.

5.1.2.6 Tumor Tissue Collection and Correlative Studies Blood Sampling

Patients will have blood drawn for correlative studies at baseline (up to 30 days prior to treatment initiation), at week 12 (+/- 3 days) study flow chart says +/- 7 days, which is correct?, and at progression. This includes 10 green top tubes, 2 red top tubes and an ACD yellow top tube (yellow top at baseline only). Refer to lab manual for full details.

Patients will undergo biopsy for tumor tissue collection at baseline (up to 30 days prior to treatment initiation) and at week 12 (+/- 7 days). A biopsy at time of progression is optional. Refer to lab manual for full details.

5.1.3 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below.
Laboratory Safety Evaluations (Hematology, Chemistry and Urinalysis): Laboratory tests for hematology, chemistry, urinalysis, and others are specified in Table 7.

Table 7: Laboratory Tests

Hematology	Chemistry	Other
Hematocrit	Albumin	Serum β-human chorionic gonadotropin†
Hemoglobin	Alkaline phosphatase	(β-hCG)†
Platelet count	Alanine aminotransferase (ALT)	Free thyroxine (T4)
WBC (total and differential)	Aspartate aminotransferase (AST)	Thyroid stimulating hormone (TSH)
Absolute Neutrophil Count	Lactate dehydrogenase (LDH)	
	Calcium	
	Chloride	Blood for correlative studies
	Glucose	
	Potassium	
	Sodium	
	Phosphorous	
	Total Bilirubin	
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*	
	Total protein	
	Blood Urea Nitrogen	
	CPK	
† Perform on women of childbearing potential only. If urine pregnancy results cannot be confirmed as negative, a serum pregnancy test will be required.		

5.1.4 Other Procedures

5.1.4.1 Withdrawal/Discontinuation

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 7.2 - Assessing and Recording Adverse Events. Subjects who a) attain a CR or b) complete 24 months of treatment with nivolumab may discontinue treatment.

5.1.5 Visit Requirements

Visit requirements are outlined in Section 5.0 - Trial Flow Chart. Specific procedure-related details are provided above in Section 6.0 - Trial Procedures.

5.2 Assessing and Recording Adverse Events

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the treatments agents, is also an adverse event.

All adverse events that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

From the time of treatment allocation/randomization through 100 days following cessation of treatment, or until the participant initiates new anticancer therapy, whichever is earlier, all adverse events must be reported by the investigator. Such events will be recorded at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in section 7.2.3.1. The investigator will make every attempt to follow all subjects with non-serious adverse events for outcome.

Adverse events will not be collected for subjects during the pre-screening period (for determination of archival tissue status) as long as that subject has not undergone any protocol-specified procedure or intervention. If the subject requires a blood draw, fresh tumor biopsy etc., the subject is first required to provide consent to the main study and AEs will be captured according to guidelines for standard AE reporting.

5.2.1 Reporting of Pregnancy and Lactation to the Sponsor

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them) that occurs during the trial.

Pregnancies and lactations that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Pregnancies and lactations that occur from the time of treatment allocation/randomization through 120 days following cessation of Sponsor's product, or 100 days following cessation of treatment if the subject initiates new anticancer therapy, including during at least 5 half-lives after product administration, whichever is earlier, must be reported by the investigator. All reported pregnancies must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as

serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor and within 24 hours (1 business day) to BMS and Pfizer Global Safety.

5.2.2 Immediate Reporting of Adverse Events to the Sponsor and to BMS and Pfizer

If any SAE meets FDA/IRB reporting guidelines, we would transfer the information onto a Medwatch form for submission to BMS and/or Pfizer.

5.2.2.1 Serious Adverse Events

A serious adverse event is any adverse event occurring at any dose or during any use of nivolumab and/or axitinib that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Suspected transmission of an infectious agent (e.g., pathogenic or nonpathogenic) via the study drug;
- Is another important medical event

- **Note:** In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable to Sponsor in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by the Sponsor for collection purposes.
    - Is a new cancer (that is not a condition of the study);
    - Is associated with an overdose.

THE FOLLOWING HOSPILIZATIONS ARE NOT CONSIDERED SAEs:

- a visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered an important medical or life-threatening event)
- elective surgery, planned prior to signing consent
- admissions as per protocol for a planned medical/surgical procedure
- routine health assessment requiring admission for baseline/trending of health status (eg, routine colonoscopy)
- Medical/surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation is required in these cases.

- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (eg, lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason).
- Admission for administration of anticancer therapy in the absence of any other SAEs (applies to oncology protocols)

The Sponsor will reconcile the clinical database SAE cases (case level only) transmitted to BMS Global Pharmacovigilance (Worldwide.Safety@bms.com). Frequency of reconciliation should be every 3 months and prior to the database lock or final data summary. BMS GPV&E will email, upon request from the Investigator, the GPV&E reconciliation report. Requests for reconciliation should be sent to aepbusinessprocess@bms.com. The data elements listed on the GPV&E reconciliation report will be used for case identification purposes. If the Investigator determines a case was not transmitted to BMS GPV&E, the case should be sent immediately to BMS.

Refer to Table 3 in Section 4.2.4 for additional details regarding each of the above criteria.

For the time period following the subject's written consent to participate in the study through 100 days of discontinuation of dosing, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study (reference Section 7.2.3.3 for additional details) that occurs to any subject must be reported within 24 hours to the Sponsor and within 24 hours or 1 business day to BMS and Pfizer Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 100 days following cessation of treatment with nivolumab, or until the participant initiates new anticancer therapy, whichever is earlier, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study (reference Section 7.2.3.3 for additional details), whether or not related to study treatment, must be reported within 24 hours to the Sponsor and within 24 hours or 1 business day to BMS and Pfizer Global Safety.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to treatment that is brought to the attention of the investigator at any time following consent through the end of the specified safety follow-up period specified in the paragraph above, or at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor and to BMS and Pfizer Global Safety.

REPORTING OF SERIOUS ADVERSE EVENTS

All events meeting the definition of a serious adverse event should be reported according to the departmental SAE checklist and SAE form. The initial SAE form should be sent to the following within 24 hours / 1 business day of the Principal Investigator becoming aware:

1. Yana Najjar, Principal Investigator (and Sponsor)
2. crssafetysubmissions@upmc.edu
3. Local Institutional Review Board when reporting requirements are met.
4. BMS Global Safety by email at Worldwide.Safety@BMS.com or by facsimile at +1 609-818-3804
5. Pfizer:
   USA.AEReporting@pfizer.com
   (CEPs, Non Clinical Trial reports)

   Fax:
   Toll-Free (local): 1 866 997-8322
   (Clinical Trial, Compassionate Use , IIR reports)

   Technical Support:
   Tel.: 800 752-9737 (toll-free)
   ONLY for technical support if fax number is not working

In addition to completing appropriate patient demographic and suspect medication information, the report should include as applicable the following information that is available at the time of report within the Sections B and C of the departmental SAE form:

- CTCAE term(s) and grade(s)
- current status of study drug
- all interventions to address the AE (testing and result, treatment and response)
- hospitalization and/or discharge dates
- event relationship to study drug

Follow-up reports:

Additional information may be added to a previously submitted report by adding to the original departmental SAE form and submitting it as follow-up or creating supplemental summary information and submitting it as follow-up with the original departmental SAE form.

All subjects with serious adverse events must be followed up for outcome.

5.2.2.2 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 24 hours to the Sponsor and within 2 working days to BMS Global Safety.

For the time period beginning when the consent form is signed until treatment allocation/randomization, any ECI, or follow up to an ECI, that occurs to any subject must be

reported within 24 hours to the Sponsor and within 24 hours/1 business day to BMS and Pfizer Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 100 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to treatment, must be reported within 24 hours to the Sponsor and within 24 hours to BMS and Pfizer Global Safety.

Events of clinical interest for this trial include:

1. An overdose of nivolumab or axitinib, as defined in Section 7.2.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.

2. An elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

> *Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology.

5.2.2.3 Protocol-Specific Exceptions to Serious Adverse Event Reporting

Efficacy endpoints as outlined in this section will not be reported to BMS and Pfizer as described in Section 7.2.3.- Immediate Reporting of Adverse Events to the Sponsor and to BMS and Pfizer, unless there is evidence suggesting a causal relationship between the drug and the event. Any such event will be submitted to the Sponsor within 24 hours and to BMS and Pfizer Global Safety within 2 working days either by electronic or paper media.

Specifically, the suspected/actual events covered in this exception include any event that is disease progression of the cancer under study.

The Sponsor will monitor unblinded aggregated efficacy endpoint events and safety data to ensure the safety of the subjects in the trial. Any suspected endpoint which upon review is not progression of the cancer under study will be forwarded to BMS and Pfizer Global Safety as a SAE within 2 working days of determination that the event is not progression of the cancer under study.

Hospitalization related to convenience (e.g., transportation issues etc.) will not be considered a SAE.

5.2.3 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 5.0. Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade recorded on the adverse event case report forms/worksheets.

All adverse events regardless of CTCAE grade must also be evaluated for seriousness.

Table 8: Evaluating Adverse Events

An investigator who is a qualified physician, will evaluate all adverse events as to:

V4.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during the course of study treatment that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization for an elective procedure to treat a pre-existing condition that has not worsened is not a serious adverse event. A pre-existing condition is a clinical condition that is diagnosed prior to the use of study treatment and is documented in the patient's medical history.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new **cancer** (that is not a condition of the study) **(**although not serious per ICH definition, is reportable); **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event for collection purposes. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours to the Sponsor and to BMS and Pfizer within 2 working days.	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	

5.2.4 Sponsor Responsibility for Reporting Adverse Events

All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations.

6.0 STATISTICAL CONSIDERATION

6.1 Study Design

This is a single arm phase II clinical trial to test the efficacy of nivolumab plus axitinib in patients with advanced melanoma who have progressed on anti-PD1 (+/- anti-CTLA4). The primary endpoint of this study is the object response rate (ORR). We use Simon's minimax two-stage design as detailed in Section 6.3.

6.2 Safety monitoring

The combination of pembrolizumab plus axitinib has been approved by FDA for advanced renal cell carcinoma, and its toxicity profile has been well established. In this study, we will use nivolumab in place of pembrolizumab, and we expect these two drugs are very similar, thus we expect the toxicity rate of the current combination therapy and dose is acceptable, so we don't think it necessary to conduct a phase I dose-finding study. However, to be cautious, we will 1) use a Bayesian monitoring scheme and 2) incorporate a trial stopping rule into the protocol. The Bayesian monitoring scheme will continuously monitor the rate of severe adverse events (SAE). The SAE is defined as grade 3 and higher toxicity that are attributable to the combination therapy. A non-informative prior of Beta(1, 1) for the SAE rate will be used. We will hold the recruit if the posterior probability Pr(SAE rate>30%)>=0.7 and the study committee and the PI will decide whether to modify or discontinue the study. The stopping boundary for toxicity is given in the following table:

Number of SAEs >=	in Number of Patients =
2	3-5
3	6-8
4	9-11
5	12-14
6	15-17
7	18-20
8	21-23
9	24-26

10	27-29
11	30-31

We simulate the operating characteristics under various assumed true toxicity rate (see the following table):

Scenario	True Prob. of SAE	Prob. of Early Stopping	Average Num. of Patients	Average Num. of SAE
1	0.1	0.10	28	3
2	0.2	0.37	22	4
3	0.3	0.71	14	4
4	0.4	0.93	8	3

Regarding the analysis of safety, we will consider a toxicity event to be an adverse event (evaluated by CTCAE 5.0) that is possibly, probably, or definitely related to treatment. The maximum grade of toxicity for each category of interest will be recorded for each patient and the summary results will be tabulated by category and grade.

If one Grade 5 event or two Grade 4 events for AEs considered at least possibly related to combination study therapy (possibly attributed to each drug) occur, the trial will be halted to accrual and a thorough safety analysis will be conducted prior to enrolling new patients.

6.3 Sample Size and Accrual

The null hypothesis that the true ORR is 10% will be tested versus a one-sided alternative of 25% or higher, using Simon's minimax two-stage design. In the first stage, 16 patients will be accrued. If there are <2 responses, the study will be stopped and alternative doses of axitinib considered. If at least 2 responses are observed, 15 additional patients will be enrolled in the 2nd stage. The combination therapy will be regarded as worthy of further study if ≥6 responses are observed in both stages. Total sample size is 31 patients. This design has a type I error rate of 0.08 with a power of 0.81 when the true response rate is 0.25. Secondary endpoints of this study are PFS, OS and safety. Survival endpoints (PFS and OS) will be estimated with the Kaplan-Meier method, and compared between marker value groups with the log-rank test. We will also perform extensive correlative analysis (flow, IHC, metabolic analyses).

The accrual rate of the trial is estimated to be 1-2 patients/month, therefore, we will be able to finish the accrual of the study in approximately 24 months.

6.4 Statistical Analysis Plan

6.4.1 Analysis Sets

DLT evaluable patients are study-eligible patients (i.e., those meeting all of the protocol inclusion/exclusion criteria) who have finished one cycle of the regimen without a DLT, and those who received at least one dose of the regimen and experienced DLT in the first cycle.

Response evaluable patients are study-eligible patients (i.e., those meeting all of the protocol inclusion/exclusion criteria) who have received the regimen and stayed on study long enough for at least one follow-up of response. Patients who didn't come back for scan but clinically progressed will also be considered as evaluable for response, and the best response for these patients is progressive disease.

Safety population - all study eligible patients who received any dose of the study drug will be included in the safety population.

Efficacy population - the data from all treated patients will be used in the intent to treat analyses of tumor response and survival (progression-free and overall). Patients who complete at least one cycle of the treatment will be included in the per-protocol analysis of bioactivity endpoint. Patients who are considered non-compliant are not evaluable for the per-protocol analysis of the primary efficacy endpoint, but still evaluable for safety analysis, so they will be replaced for the primary efficacy analysis.

6.4.2 Analysis of ORR (Primary)

Overall response rate (ORR) will be estimated by the percentage of patients who achieve CR, PR or SD by RECIST criteria (detailed in Sections 5.1.2.5 and 9.2), with corresponding exact 95% confidence limits being reported. The difference between marker value groups will be examined with Fisher's exact tests.

6.4.3 Safety Analysis (Secondary)

We will consider a toxicity event to be an adverse event (evaluated by CTCAE 5.0) that is possibly, probably, or definitely related to treatment. The maximum grade of toxicity for each category of interest will be recorded for each patient and the summary results will be tabulated by category and grade.

All adverse events occurring up to 28 days after the last intake of study medication will be recorded. These adverse events will be reported as listings and summarized as frequency tables by treatment group and the body system. Additional presentations will include summaries by severity and relationship to trial treatment.

Withdrawals of patients from study medication will be reported as listings and summary tables. All summaries and listings of adverse events and laboratory data will be based on the safety population.

6.4.4 Analysis of PFS and OS (Secondary)

PFS will be measured from the initial date of treatment to the date of documented progression, or the date of death (in the absence of progression), whichever occurs first. OS will be measured from the initial date of treatment to the recorded date of death.

PFS and OS will be estimated by the Kaplan-Meier method. The corresponding median survival times (with 95% confidence intervals) will be determined, as will the cumulative percentage of patients remaining progression-free (and the cumulative percentage-alive) at selected time points after initial treatment (e.g., 3, 6,12, 18 months). Log-rank test will be used to compare the PFS and OS between subgroups when event number permitted.

6.4.5 Correlative Translational Assays (Secondary)

Multiple lab outcomes will be compared between baseline and on treatment to examine the change in TME, metabolic profiling and cell hypoxia. These will also be compared between baseline and the value at the progression for progressors. All these pre vs post comparisons will be done with paired t-tests or Wilcoxon signed rank tests where appropriate. The change from pre- to post- treatment will be compared between subgroups (such as between responders and non-responders) with two sample t-test or Wilcoxon rank sum test where appropriate. The association of clinical response with tumor metabolism and with T cell function will be explored with logistic regressions. The association of survival endpoints (DOR, PFS, OS) with tumor metabolism and with T cell function will be explored with Cox proportional hazards models.

7.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

7.1 Investigational Product

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

Clinical Supplies will be provided by BMS and Pfizer as summarized in Table 8.

Table 9: Product Descriptions

Product Name & Potency	Dosage Form
Nivolumab 100 mg/10ml vial	Solution for Injection
Axitinib 5 mg	Tablets

Pimonidazole (FDA IND# 73,095) is not used with therapeutic intent, and has a non-hazardous designation. It has been widely used for *in-vivo* evaluation of intratumor hypoxia, and patients will take PO pimonidazole before each on-treatment biopsy: thus, patients undergoing biopsy will take 2 doses total, each dose 12 weeks apart. A single dose of 0.5 gm/m^2 of pimonidazole used for tumor hypoxia measurement in humans is equivalent to approximately 13 mg/kg and, therefore, far below the dose of 300 mg/kg/day for 10 days that is near the threshold for liver damage for primates. Pimonidazole is not toxic to hypoxic cells at the concentrations used for hypoxia marking in humans (See Material Safety Data Sheet).

Packaging and Labeling Information

Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

7.2 Clinical Supplies Disclosure

This trial is open-label; therefore, the subject, the trial site personnel, the Sponsor and/or designee are not blinded to treatment. Drug identity (name, strength) is included in the label text; random code/disclosure envelopes or lists are not provided.

7.3 Storage and Handling Requirements

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label.

Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site.

Clinical supplies may not be used for any purpose other than that stated in the protocol.

7.4 Returns and Reconciliation

The investigator is responsible for keeping accurate records of the clinical supplies received from BMS and Pfizer or designee, the amount dispensed to and returned by the subjects and the amount remaining at the conclusion of the trial. Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept

8.0 ADMINISTRATIVE AND REGULATORY DETAILS

8.1 DATA SAFETY AND MONITORING PLAN

Investigator/Sub-investigators, regulatory, CRS management, clinical research coordinators, clinical research associates, data managers, and clinic staff meet monthly in disease center

Data Safety Monitoring Boards (DSMB) to review and discuss study data to include, but not limited to, the following:

- Serious adverse events
- Subject safety issues
- Recruitment issues
- Accrual
- Protocol deviations
- Unanticipated problems
- Breaches of confidentiality

Minutes from the disease center DSMB meetings are available to those who are unable to attend in person.

All toxicities encountered during the study will be evaluated on an ongoing basis according to the NCI Common Toxicity Criteria version 5.0. All study treatment associated adverse events that are serious, at least possibly related and unexpected will be reported to the IRB. A set of Bayesian continuous monitoring stopping rules will be implemented to ensure that the true severe adverse event (SAE) rate is controlled under 30% (see section 7). Any modifications necessary to ensure subject safety and decisions to continue, or close the trial to accrual are also discussed during these meetings. If any literature becomes available which changes the risk/benefit ratio or suggests that conducting the trial is no longer ethical, the IRB will be notified in the form of an Unanticipated Problem submission and the study may be terminated.

All study data reviewed and discussed during these meetings will be kept confidential. Any breach in subject confidentiality will be reported to the IRB in the form of an Unanticipated Problem submission. The summaries of these meetings are forwarded to the UPCI DSMC which also meets monthly following a designated format.

For all research protocols, there will be a commitment to comply with the IRB's policies for reporting unanticipated problems involving risk to subjects or others (including adverse events). DSMC progress reports, to include a summary of all serious adverse events and modifications, and approval will be submitted to the IRB at the time of renewal.

Protocols with subjects in long-term (survival) follow-up or protocols in data analysis only, will be reviewed twice a year rather than monthly.

Both the UPCI DSMC as well as the individual disease center DSMB have the authority to suspend accrual or further investigate treatment on any trial based on information discussed at these meetings.

All records related to this research study will be stored in a locked environment. Only the researchers affiliated with the research study and their staff will have access to the research records.

8.2 Quality Control and Quality Assurance

The Sponsor-Investigator and the University of Pittsburgh and UPMC will permit direct access of the study monitors and appropriate regulatory authorities to the study data and to the corresponding source data and documents to verify the accuracy of this data.

8.3 Data Handling and Record Keeping

Data recording/case report forms/visit checklists

A Case Report Form will be completed for each subject enrolled into the clinical study.

Source Data are the clinical findings and observations, laboratory and test data, and other information contained in Source Documents. Source Documents are the original records including, but not limited to, hospital medical records, physician or office charts, physician or nursing notes, subject diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, x-rays, etc. When applicable, information recorded on the CRF shall match the Source Data recorded on the Source Documents.

8.4 Ethics

Institutional Review Board (IRB) approval

The Investigator will obtain, from the University of Pittsburgh Institutional Review Board (IRB), prospective approval of the clinical protocol and corresponding informed consent form(s); modifications to the clinical protocol and corresponding informed consent forms, and advertisements (i.e., directed at potential research subjects) for study recruitment.

The only circumstance in which a deviation from the current IRB-approved clinical protocol/consent form(s) may be initiated in the absence of prospective IRB approval is to eliminate an apparent immediate hazard to the research subject(s). In such circumstances, the -Investigator will promptly notify the University of Pittsburgh IRB of the deviation.

The University of Pittsburgh IRB operates in compliance with FDA regulations at 21 CFR Parts 50 and 21 CFR 56, and in conformance with applicable International Conference on Harmonization (ICH) Guidelines on Good Clinical Practice (CGP).

Ethical and scientific conduct of the clinical research study

The clinical research study will be conducted in accordance with the current IRB-approved clinical protocol; ICH GCP Guidelines adopted by the FDA; and relevant policies, requirements, and regulations of the University of Pittsburgh IRB, University of Pittsburgh and UPMC, Commonwealth of Pennsylvania, and applicable federal agencies.

9.0 APPENDICES

9.1 ECOG Performance Status

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.
* As published in Am. J. Clin. Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.* The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.	

9.2 Common Terminology Criteria for Adverse Events V5.0 (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for adverse event reporting. (http://ctep.cancer.gov/reporting/ctc.html)

9.3 Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 Criteria for Evaluating Response in Solid Tumors

RECIST version 1.1* will be used in this study for assessment of tumor response. While either CT or MRI may be utilized, as per RECIST 1.1, CT is the preferred imaging technique in this study.

*As published int eh European Journal of Cancer (2009)
E.A. Eisenhauer, P. Therasse, J. Bogaerts, L.H. Schwartz, D. Sargent, R. Ford, J. Dancey, S. Arbuck, S. Gwyther, M. Mooney, L. Rubinstein, L. Shankar, L. Dodd, R. Kaplan, D. Lacombe, J. Verweij. New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1). Eur J Cancer. 2009 Jan;45(2):228-47.

In addition, volumetric analysis will be explored by central review for response assessment.

1. Najjar YG, Menk AV, Sander C, et al. Tumor cell oxidative metabolism as a barrier to PD-1 blockade immunotherapy in melanoma. JCI Insight 2019.
2. Varia M. Oral Pimonidazole HCl as Tumor Hypoxia Marker: Report of First Clinical Use - Pharmacokinetics and Tolerance. International Journal of Radiation Oncology*Biology*Physics 2009;75:S539-S40.
3. Dhani NC, Serra S, Pintilie M, et al. Analysis of the intra- and intertumoral heterogeneity of hypoxia in pancreatic cancer patients receiving the nitroimidazole tracer pimonidazole. Br J Cancer 2015;113:864-71.
4. Durand RE, Aquino-Parsons C. The fate of hypoxic (pimonidazole-labelled) cells in human cervix tumours undergoing chemo-radiotherapy. Radiother Oncol 2006;80:138-42.
5. Ragnum HB, Vlatkovic L, Lie AK, et al. The tumour hypoxia marker pimonidazole reflects a transcriptional programme associated with aggressive prostate cancer. Br J Cancer 2015;112:382-90.
6. Callahan MK, Postow MA, Wolchok JD. Targeting T Cell Co-receptors for Cancer Therapy. Immunity 2016;44:1069-78.
7. Larkin J, Hodi FS, Wolchok JD. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. N Engl J Med 2015;373:1270-1.
8. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. The New England journal of medicine 2012;366:2443-54.
9. Ji RR, Chasalow SD, Wang L, et al. An immune-active tumor microenvironment favors clinical response to ipilimumab. Cancer Immunol Immunother 2012;61:1019-31.
10. Delgoffe GM, Powell JD. Feeding an army: The metabolism of T cells in activation, anergy, and exhaustion. Mol Immunol 2015;68:492-6.
11. Romero IL, Mukherjee A, Kenny HA, Litchfield LM, Lengyel E. Molecular pathways: trafficking of metabolic resources in the tumor microenvironment. Clin Cancer Res 2015;21:680-6.
12. Zhang Y, Ertl HC. Starved and Asphyxiated: How Can CD8(+) T Cells within a Tumor Microenvironment Prevent Tumor Progression. Front Immunol 2016;7:32.
13. Justus CR, Sanderlin EJ, Yang LV. Molecular Connections between Cancer Cell Metabolism and the Tumor Microenvironment. Int J Mol Sci 2015;16:11055-86.
14. Scharping NE, Menk AV, Moreci RS, et al. The Tumor Microenvironment Represses T Cell Mitochondrial Biogenesis to Drive Intratumoral T Cell Metabolic Insufficiency and Dysfunction. Immunity 2016;45:374-88.
15. Rini BI, Plimack ER, Stus V, et al. Pembrolizumab plus Axitinib versus Sunitinib for Advanced Renal-Cell Carcinoma. N Engl J Med 2019;380:1116-27.
16. Sheng X, Yan X, Chi Z, et al. Axitinib in Combination With Toripalimab, a Humanized Immunoglobulin G4 Monoclonal Antibody Against Programmed Cell Death-1, in Patients With Metastatic Mucosal Melanoma: An Open-Label Phase IB Trial. J Clin Oncol 2019:JCO1900210.
17. Apte RS, Chen DS, Ferrara N. VEGF in Signaling and Disease: Beyond Discovery and Development. Cell 2019;176:1248-64.
18. Koch S, Claesson-Welsh L. Signal transduction by vascular endothelial growth factor receptors. Cold Spring Harbor perspectives in medicine 2012;2:a006502.
19. Houck KA, Ferrara N, Winer J, Cachianes G, Li B, Leung DW. The vascular endothelial growth factor family: identification of a fourth molecular species and

characterization of alternative splicing of RNA. Molecular endocrinology (Baltimore, Md) 1991;5:1806-14.
20. Holmes K, Roberts OL, Thomas AM, Cross MJ. Vascular endothelial growth factor receptor-2: structure, function, intracellular signalling and therapeutic inhibition. Cellular signalling 2007;19:2003-12.
21. Motzer RJ, Penkov K, Haanen J, et al. Avelumab plus Axitinib versus Sunitinib for Advanced Renal-Cell Carcinoma. The New England journal of medicine 2019;380:1103-15.
22. Rini BI, Plimack ER, Stus V, et al. Pembrolizumab plus Axitinib versus Sunitinib for Advanced Renal-Cell Carcinoma. The New England journal of medicine 2019;380:1116-27.
23. Erhard H, Rietveld FJ, van Altena MC, Brocker EB, Ruiter DJ, de Waal RM. Transition of horizontal to vertical growth phase melanoma is accompanied by induction of vascular endothelial growth factor expression and angiogenesis. Melanoma Res 1997;7 Suppl 2:S19-
24. Streit M, Detmar M. Angiogenesis, lymphangiogenesis, and melanoma metastasis. Oncogene 2003;22:3172-9.
25. Rajabi P, Neshat A, Mokhtari M, Rajabi MA, Eftekhari M, Tavakoli P. The role of VEGF in melanoma progression. J Res Med Sci 2012;17:534-9.
26. Corrie PG, Marshall A, Nathan PD, et al. Adjuvant bevacizumab for melanoma patients at high risk of recurrence: survival analysis of the AVAST-M trial. Ann Oncol 2019.
27. Del Vecchio M, Mortarini R, Canova S, et al. Bevacizumab plus fotemustine as first-line treatment in metastatic melanoma patients: clinical activity and modulation of angiogenesis and lymphangiogenesis factors. Clin Cancer Res 2010;16:5862-72.
28. Vihinen PP, Hernberg M, Vuoristo MS, et al. A phase II trial of bevacizumab with dacarbazine and daily low-dose interferon-alpha2a as first line treatment in metastatic melanoma. Melanoma Res 2010;20:318-25.
29. von Moos R, Seifert B, Simcock M, et al. First-line temozolomide combined with bevacizumab in metastatic melanoma: a multicentre phase II trial (SAKK 50/07). Ann Oncol 2012;23:531-6.
30. Hodi FS, Lawrence D, Lezcano C, et al. Bevacizumab plus ipilimumab in patients with metastatic melanoma. Cancer Immunol Res 2014;2:632-42.
31. Fruehauf J, Lutzky J, McDermott D, et al. Multicenter, phase II study of axitinib, a selective second-generation inhibitor of vascular endothelial growth factor receptors 1, 2, and 3, in patients with metastatic melanoma. Clin Cancer Res 2011;17:7462-9.
32. O'Day SJ, Atkins MB, Boasberg P, et al. Phase II multicenter trial of maintenance biotherapy after induction concurrent Biochemotherapy for patients with metastatic melanoma. J Clin Oncol 2009;27:6207-12.
33. Najjar YG, Menk AV, Sander C, et al. Tumor cell oxidative metabolism as a barrier to PD-1 blockade immunotherapy in melanoma. JCI Insight 2019;4.
34. Scharping NE, Delgoffe GM. Tumor Microenvironment Metabolism: A New Checkpoint for Anti-Tumor Immunity. Vaccines 2016;4.
35. Scharping NE, Menk AV, Moreci RS, et al. The Tumor Microenvironment Represses T Cell Mitochondrial Biogenesis to Drive Intratumoral T Cell Metabolic Insufficiency and Dysfunction. Immunity 2016;45:701-3.
36. Shigeta K, Datta M, Hato T, et al. Dual PD-1 and VEGFR-2 blockade promotes vascular normalization and enhances anti-tumor immune responses in HCC. Hepatology 2019.

37. Yasuda S, Sho M, Yamato I, et al. Simultaneous blockade of programmed death 1 and vascular endothelial growth factor receptor 2 (VEGFR2) induces synergistic anti-tumour effect in vivo. Clin Exp Immunol 2013;172:500-6.
38. Yi M, Jiao D, Qin S, Chu Q, Wu K, Li A. Synergistic effect of immune checkpoint blockade and anti-angiogenesis in cancer treatment. Mol Cancer 2019;18:60.
39. Zhao X, Suryawanshi S, Hruska M, et al. Assessment of nivolumab benefit-risk profile of a 240-mg flat dose relative to a 3-mg/kg dosing regimen in patients with advanced tumors. Annals of oncology : official journal of the European Society for Medical Oncology 2017;28:2002-8.
40. Zhao X, Ivaturi V, Gopalakrishnan M, et al. Abstract CT101: A model-based exposure-response (E-R) assessment of a nivolumab (NIVO) 4-weekly (Q4W) dosing schedule across multiple tumor types. Cancer Research 2017;77:CT101-CT.
41. Bowyer S, Prithviraj P, Lorigan P, et al. Efficacy and toxicity of treatment with the anti-CTLA-4 antibody ipilimumab in patients with metastatic melanoma after prior anti-PD-1 therapy. British journal of cancer 2016;114:1084-9.