**LIMIT Melanoma: (Lysosomal Inhibition + Melanoma ImmunoTherapy)**
**A phase 1/2 open label trial of nivolumab and hydroxychloroquine, nivolumab-relatlimab or nivolumab/ipilimumab and hydroxychloroquine in patients with advanced Melanoma**

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Protocol Number: IRB: 835033
UPCC: 01620
BMS: CA209-7H6

Investigational Agents: Nivolumab: commercial supply
Ipilimumab: commercial supply
Nivolumab-relatlimab: commercial supply
Hydroxychloroquine: commercial supply

IND Exempt

ClinicalTrials.gov Identifier: NCT04464759

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Amendment 4 06.13.2023

## PRINCIPAL INVESTIGATOR SIGNATURE

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|---|---|
| SPONSOR-INVESTIGATOR: | RAVI AMARAVADI, MD |
| STUDY TITLE: | LIMIT Melanoma: (Lysosomal Inhibition + Melanoma ImmunoTherapy) A phase 1/2 open label trial of nivolumab and hydroxychloroquine, nivolumab-relatlimab, or nivolumab/ipilimumab and hydroxychloroquine in patients with advanced melanoma |
| STUDY ID | IRB #835033 / UPCC 01620 |
| PROTOCOL VERSION | 6.13.2023 |

I have read the referenced protocol. I agree to conduct the study in accordance to this protocol, in compliance with the Declaration of Helsinki, Good Clinical Practices (GCP), and all applicable regulatory requirements and guidelines.

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| _____ | _____ |
| Principal Investigator Name | Signature |
| _____ | _____ |
| Affiliation | Date |

**Abbreviations:**

Ab: antibody
AE: adverse event
ALT: alanine aminotransferase
ANC: absolute neutrophil count
ASCO: American Society of Clinical Oncology
AST: aspartate aminotransferases
BAMM: Braf, Autophagy and MEK for Melanoma
BID: twice daily
BMS: Bristol-Myers Squibb
BP: blood pressure
BSA: body surface area
CNS: central nervous system
CR: complete response
CT: computed tomography
CTCAE: Common Terminology Criteria for Adverse Events
DLT: dose-limiting toxicity
DSMC: Data Safety and Monitoring Committee
ECOG: Eastern Cooperative Oncology Group
eCRF: electronic case report form
EKG: electrocardiogram
ESR: expedited safety report
FDA: Food and Drug Administration
FFPE: formalin fixed-paraffin embedded
HBV: hepatitis B virus
HCQ: hydroxychloroquine
HCV: hepatitis C virus
HUP: Hospital of the University of Pennsylvania
IB: investigators brochure
IHC: immunohistochemistry
IND: investigational new drug
INR: international normalization ratio
I-O: immune-oncology
IRB: institutional review board
IV: intravenous
LIMIT: Lysosomal Inhibition + Melanoma Immunotherapy
LLN: lower limit of normal
LVEF: left ventricular ejection fraction
MDSC: myeloid derived suppressor cells mg: milligrams
MRI: magnetic resonance imaging
MTD: maximum tolerated dose
NCI: National Cancer Institute
ORR: overall response rate
OS: overall survival
PBMC: peripheral blood mononuclear cells
PD: progressive disease
PD-1: programmed death-1
PFS: progression-free survival
PK: pharmacokinetic
PMN; polymorphonuclear
PPT1: palmitoyl-protein thioesterase 1
PR: partial response
PT: prothrombin time
PTT: partial thromboplastin time
RECIST: The Response Evaluation Criteria in Solid Tumors
SAE: serious adverse event
SD: stable disease
SUSAR: Suspected, Unexpected Serious Adverse Reaction
TAM (tumor associated macrophage)
TIL: tumor infiltrating lymphocytes
TNF: tumor necrosis factor
TSH: thyroid stimulating hormone
ULN: upper limit of normal
WBC: white blood cell

| **STUDY SUMMARY** | |
|---|---|
| **Title** | **LIMIT Melanoma: (Lysosomal Inhibition + Melanoma ImmunoTherapy) A phase 1/2 open label trial of nivolumab and hydroxychloroquine, nivolumab/ipilimumab and hydroxychloroquine, or nivolumab-relatlimab and hydroxychloroquine in patients with advanced melanoma** |
| **Protocol Number** | UPCC: 01620; IRB #835033; BMS: CA209-7H6 |
| **Phase** | Phase 1/2 |
| **Methodology** | Non-randomized open label 4-part phase 1/2<br><br>Part 1: Phase 1a dose escalation study with nivolumab + HCQ. After the maximum tolerated dose of HCQ has been identified, patients will be recruited for one of the following:<br>Part 2: Phase 2 expansion cohort of nivolumab + HCQ; OR<br>Part 3: Phase 1 dose escalation with nivolumab, ipilimumab and HCQ (3-drug combination)<br><br>Part 4: Phase 2 nivolumab-relatlimab + HCQ |
| **Study Duration** | 5 years |
| **Study Center(s)** | Single Institution |
| **Objectives** | **PRIMARY OBJECTIVES:**<br><br>**Phase 1a nivolumab + HCQ:** To evaluate the safety and tolerability of HCQ administered in combination with nivolumab in subjects with advanced/metastatic melanoma, and to select the maximum tolerated dose (MTD) of HCQ for further evaluation<br><br>**Phase 2 (expansion cohorts for nivolumab + HCQ)**: To assess the objective response rate (ORR) as measured by RECIST v1.1. in subjects with advanced melanoma that are treatment-refractory to anti-PD-1 antibody therapy (Cohort 2a) or treatment naïve to anti-PD-1 antibody-based therapy (Cohort 2b)<br><br>**Phase 1b Ipilimumab + Nivolumab + HCQ (3-drug combination):** To evaluate the safety and tolerability of HCQ administered in combination with nivolumab and ipilimumab followed by maintenance nivolumab in subjects with advanced/metastatic melanoma, and to select the maximum tolerated dose (MTD) of HCQ for further evaluation<br><br>**Phase 2c Nivolumab- relatlimab + HCQ:** To assess the objective response rate (ORR) as measured by RECIST v1.1. in subjects with advanced melanoma that are treatment-refractory to anti-PD-1 antibody therapy |

| **STUDY SUMMARY** | |
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| | **SECONDARY OBJECTIVES:**<br>To estimate other measures of anti-tumor activity, including progression-free survival (PFS), and duration of response, following combination therapy, 1-year survival rate<br><br>**CORRELATIVE OBJECTIVES:**<br>1) To measure changes in tumor microenvironment by IHC in serial tumor biopsies including MART1 (melanoma), CD8+ (cytotoxic T cells), F4/80 (TAM), Ly6G/C (MDSC). Using multiplexed IHC we will be able to determine if the addition of HCQ reduces PMN-MDSC, or converts M2 to M1 TAMs, and results in increased infiltration of T cells into the tumor<br>2) To evaluate the association between baseline tumor tissue expression of beclin1, LC3, p62, HLTF, and ALDH1A1, PPT1 and response to combination therapy<br>3) To characterize the pharmacodynamic impact of combination therapy on markers of immune modulation in the hypoxic tumor microenvironment, including tumor infiltrating lymphocytes, M2 to M1 macrophages, MDSC, and tumor specific T cells in the periphery<br>4) To determine if exosomal PDL1 can predict response to nivolumab + HCQ or nivolumab-relatlimab + HCQ |
| **Number of Subjects** | Original Planned Sample Size<br>Phase 1a nivolumab and HCQ Dose Escalation: 6-12 patients<br><br>Phase 2 nivolumab and HCQ<br>-- Cohort 2a (anti-PD1 Ab refractory): 27 patients (closed early)<br>-- Cohort 2b (anti-PD1 Ab naive): 43 patients (closed early)<br><br>Phase 1b nivolumab, ipilimumab and HCQ (3-drug combination)<br>Dose Escalation: 6-12 patients<br><br>**Total: up to 94 patients**<br>**2023 Amendment Sample Size**<br>**Phase 1a: 7**<br>**Phase 1b: 6-12**<br>**Phase 2a-2b: 9**<br>**Phase 2c** Phase 2 nivolumab-relatlimab+HCQ<br>Cohort 2c anti-PD-1 Ab refractory): 35 patients<br><br>**Total Sample Size: Up to 63** |

| **STUDY SUMMARY** | |
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| **Diagnosis and Main Inclusion/Exclusion Criteria** | 1. Histologic or cytologic evidence of melanoma Stage III unresectable or Stage IV, any genotype, and any PD-L1 IHC status<br>2. Phase 1a nivolumab + HCQ: any prior treatment, or treatment naïve Phase 2 nivolumab + HCQ:<br>-- Cohort 2a: prior immunotherapy in the adjuvant or metastatic setting is required<br>-- Cohort 2b: patients must be anti-PD-1 Ab-naïve<br>Phase 2 nivolumab-relatlimab + HCQ:<br>Cohort 2c: prior anti-PD-1 antibody in the adjuvant or metastatic setting is required<br>Phase 1b nivolumab + ipilimumab + HCQ: patients must be anti-PD-1 refractory<br>3. ECOG performance status of 0-1<br>4. At least one measurable site of disease by RECIST 1.1 criteria that has not been previously irradiated<br>5. Fresh or archived primary or metastatic tissue available for submission for correlative analyses<br>6. Patients with history of treated brain metastases are eligible if off systemic corticosteroids for at least 1 week<br>7. Patients must have normal organ function acceptable for combination therapy |
| **Study Product, Dose, Route, Regimen** | All study products obtained commercially<br>**Phase 1a nivolumab + hydroxychloroquine**<br>Hydroxychloroquine 400-600 mg po q12 h and nivolumab 480 mg IV every 4 weeks<br><br>**Phase 2 nivolumab + hydroxychloroquine**<br>Hydroxychloroquine 400-600 mg po q12 h (MTD from Phase I) and nivolumab 480 mg IV every 4 weeks<br><br>Phase 2c: nivolumab-relatlimab + hydroxychloroquine<br>Hydroxychloroquine 600 bid mg po and nivolumab 480 mg + relatlimab 160 mg IV every 4 weeks<br><br>**Phase 1b nivolumab + ipilimumab + hydroxychloroquine (3-drug combination):**<br>Hydroxychloroquine 400-600 mg po q12 h + nivolumab 1mg/kg IV plus ipilimumab 3 mg/kg IV every 3 weeks x4 cycles<br>then 6 weeks after the last dose of ipi/nivo begin maintenance nivolumab 480 mg IV every 4 weeks |
| **Duration of administration** | Until progression or up to 24 months of therapy if partial or complete response. |

| **STUDY SUMMARY** | |
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| **Study design** | Phase 1 (both 2- and 3-drug combinations) include a 3+3 dose escalation to determine MTD. Phase 2 cohorts each have a 2-stage design |
| **Duration of trial** | Approximately 5 years<br><br>Follow-up:<br>o All subjects will be evaluated per study calendar for 100 days after discontinuation of study treatment.<br>o All subjects will be followed for a minimum of one year from the start of treatment,unless disease progression is reported prior to one year. |

## 1.0 OBJECTIVES

### 1.1 Primary Objective

Phase 1a nivolumab and hydroxychloroquine (HCQ): To determine the maximum tolerated dose (MTD) and preliminary safety of HCQ when administered in conjunction with nivolumab in patients with advanced melanoma.

Phase 1b nivolumab and ipilimumab and HCQ (following Phase I nivolumab and HCQ): To determine the maximum tolerated dose (MTD) and preliminary safety of hydroxychloroquine (HCQ) when administered in conjunction with nivolumab and ipilimumab in patients with advanced melanoma.

Phase 2 (Cohorts 2a , 2b, 2c following Phase 1a nivolumab and HCQ): To assess the clinical efficacy as measured by response rate.

### 1.2 Secondary Objectives

1.2.1 To estimate the toxicity rates

1.2.2 To estimate progression-free survival

1.2.3 To estimate 1 year survival rates

1.2.4 Duration of therapy

### 1.3 Correlative Objectives

1.3.1 To measure changes in tumor microenvironment by IHC in serial tumor biopsies including MART1 (melanoma), CD8+ (cytotoxic T cells), F4/80 (TAM), Ly6G/C (MDSC). Using multiplexed IHC we will be able to determine if the addition of HCQ reduces PMN-MDSC, or converts M2 to M1 TAMs, and results in increased infiltration of T cells into the tumor.

1.3.2 To evaluate the association between baseline tumor tissue expression of beclin1, LC3, p62, HLTF, and ALDH1A1, PPT1 and clinical outcome on combination therapy.

1.3.3 To characterize the pharmacodynamic impact of combination therapy on markers of immune modulation in the hypoxic tumor microenvironment, including tumor infiltrating lymphocytes, M2 to M1 macrophages, MDSC, and tumor specific T cells in the periphery.

1.3.4 To determine if exosomal PDL1 can predict response to nivolumab + HCQ or nivolumab-relatlimab + HCQ

## 2.0 BACKGROUND AND RATIONALE

### 2.1 Clinical Unmet Need

**Novel combination approaches are needed to improve clinical response rates with anti-PD1 therapy.** Anti-PD1 immunotherapies have achieved unprecedented clinical benefit for melanoma with response rates of 33-40% as single agents [1]. Efforts to enhance this response rate have focused on combination with other immune checkpoints but besides the anti-PD1 Ab and anti-CTLA4 antibody strategy (58% response rate), which is associated with much higher rates of serious toxicity [2], no clearly superior combination has emerged. **As such, novel combinatorial treatment strategies that are tolerable and increase the response rate represent a major unmet need.** Our group has focused on targeting autophagy in cancer with the antimalarial compound hydroxychloroquine (HCQ) [3]. Previously we have found that autophagy is increased in melanoma [4]. We then found that BRAF inhibition induces cytoprotective autophagy, and HCQ can augment BRAF inhibitor therapy in animal studies [5]. More recently we have found that MAPK reactivation, which is the more commonly accepted mechanism of resistance to targeted therapy in melanoma drives autophagy induction (Ojha *Cancer Discovery* 2019). Therefore in targeted therapy for melanoma autophagy seems to be a major common resistance mechanism. These findings have led to a clinical trial of dabrafenib + trametinib + HCQ in BRAF mutant melanoma that has found striking response rates (see below for details). In parallel, our focus on targeting autophagy in cancer has led to the new discovery of the lysosomal enzyme PPT1 as an attractive new target in melanoma and other cancers [6]. New data indicates the molecular target of the lysosomal inhibitor HCQ is PPT1 (Rebecca et al *Cancer Discovery* 2019), and targeting PPT1 in the tumor microenvironment concurrently impairs tumor cell growth and results in M2 to M1 macrophage phenotype switching that enhances tumor immunity [7]. Based on these findings we performed a preclinical study in the B16 mouse model and found that HCQ significantly augmented anti-PD1 Ab in this model as well as a genetically engineered mouse model of BRAF mutant melanoma with an intact immune system (see below). These findings provide the rationale for pursuing the combination of PD1 Ab and HCQ. If this combination appears safe and effective in single arms study this paves the way for a 3 drug combination, or a randomized study to be conducted through ECOG.

### 2.2 Clinical development of PD-1 inhibitors in melanoma.

PD-1 is a negative regulator of T-cell activity that limits the activity of T cells at a variety of stages of the immune response when it interacts with its two ligands PD-L1 and PD-L2 [8]. PD-1 is primarily believed to inhibit effector T-cell activity in the effector phase within tissues. This pathway is likely important in the tumor microenvironment where PD-L1 expressed by tumors interacts with PD-1 on T cells to suppress T-cell effector function by limiting T-cell receptor localization to the immunologic synapse at the site of target engagement. The FDA approved anti-PD-1 antibodies pembrolizumab and nivolumab have robust clinical activity. In multiple clinical trials in patients with advanced melanoma nivolumab 2 mg/kg given every 2 weeks produced a response rate of 31%-44% depending on the median length of followup [1, 2]. Updated results from Keynote-006 which randomized patients with advanced melanoma to 10 mg/kg pembrolizumab every 2 versus 3 weeks versus ipilimumab 3 mg/kg every 3 weeks showed a similar response rate in 2 versus 3 week dosing schedules of 36-37% [9]. Longer term followup shows that depending on the makeup of the patient population response rates with 3-4 year followup shows that pembrolizumab can produce up to a 42% response rate (Long et al. ASCO abstract 2018). The sum of these data lead to the FDA approval of both pembrolizumab and nivolumab for the treatment of metastatic melanoma. Nivolumab given every 4 weeks at 480 mg showed equivalent PK properties to nivolumab 240 mg given every 2 weeks, and due to the longer schedule has become the de facto most used PD-1 Antibody regimen [10]. PD-L1 IHC is not required for PD1 inhibitor therapy in melanoma. The combination of ipilimumab and nivolumab produces a response rate of 58% but there has not been a significant difference

in overall survival demonstrated when comparing ipilimumab and nivolumab compared to nivolumab alone [11]. In exchange for these differences in efficacy ipilimumab and nivolumab produced a 48% grade 3 toxicity and 11% grade 4 toxicity in patients compared to nivolumab which produced a 17% grade 3, and 5% grade 4 toxicity rate.

### 2.3 Autophagy in Cancer

**Autophagy is an intrinsic cellular resistance mechanism induced by therapeutic stress from anti-cancer agents, and serves a key role in immune resistance within the hypoxic tumor microenvironment.** Autophagy is an intracellular mechanism in which toxic byproducts of cellular metabolism are engulfed by autophagic vesicles (AV) and disposed via lysosomal degradation [12]. Thus, autophagy prevents the accumulation of toxic products and maintains metabolic homeostasis through the recycling of cellular components. This highly regulated process is an emerging hallmark of cancer, in which autophagy supports the survival of established malignancy under challenging metabolic conditions, such as nutrient/growth factor deprivation or hypoxia. As such, upregulation of autophagy has been identified as an intrinsic tumor resistance mechanism to therapeutic stress induced by systemic anti-cancer therapies, including both traditional cytotoxic and targeted agents. For example, the induction of cytoprotective autophagy has been demonstrated in response to alkylating agents, as well as to BRAF inhibition in *BRAF*-mutant melanoma. These findings have laid the mechanistic rationale for several clinical investigations of combination chemotherapy or targeted therapy with inhibitors of cancer cell autophagy that have already been conducted by our group. [3]

Importantly, autophagy is highly upregulated in the hypoxic tumor microenvironment, and serves a key role in the cancer cell- tumor microenvironment interaction [13]. In this context, recent preclinical evidence demonstrates that autophagy helps define the anti-tumor immune response and immune effector cell resistance. For example, activation of autophagy in the hypoxic tumor microenvironment leads to the upregulation of phosphorylated STAT3, resulting in impaired cytotoxic T cell-mediated tumor lysis [14]. In addition, autophagy contributes to increased degradation of natural killer cell-derived granzyme B in hypoxic tumor cells. A very recent report demonstrates that knockout of the essential autophagy gene Beclin leads to augmented immune infiltration into the tumor microenvironment [15]. However, our own preliminary data demonstrates that *Atg7* knockout in the tumor cell alone does not augment PD1 Ab efficacy (see below), whereas targeting the lysosome with HCQ does augment PD1 Ab efficacy (see below). This data likely reflects the fact that ATG7 is required for secretion programs, which may be critical for the efficacy of anti-PD1 therapy. Targeting the lysosome is the only clinically feasible way of inhibiting autophagy to enhance PD1 Ab efficacy.

### 2.4 Hydroxychloroquine

**Hydroxychloroquine is a well -tolerated lysosomal autophagy inhibitor used in humans for > 50 years.** Hydroxychloroquine (HCQ) is a commercially available oral medication that is commonly used for the treatment of rheumatoid arthritis at doses of 400 mg daily. Chloroquine derivatives, such as HCQ, are known to modulate autophagy by blocking the delivery of autophagic contents to the lysosome for degradation. Thus, by blocking the final step of autophagy, HCQ can lead to the accumulation of cytoplasmic AVs. Several early phase clinical trials were published by our group involving HCQ as a mechanism for autophagy inhibition in combination with a variety of targeted agents in cancer patients. In these studies, HCQ was evaluated in combination with vorinostat, bortezomib, temozolomide/radiation therapy, and temsirolimus in a variety of solid tumors [16-20]. In general, these studies reported an overall acceptable toxicity profile with these combination therapies, with primary toxicities of low-grade nausea, diarrhea, and myelosuppression. The recommended phase II dose of HCQ in combination with targeted agents has ranged from 600 mg daily to 1200 mg daily in divided doses.

### 2.5 Phase II studies involving HCQ in melanoma and other cancers

Indeed, more recent phase II studies by our group involving HCQ have yielded striking results. A phase I/II study of FOLFOX + Avastin + HCQ has produced a high response rate (70% ORR, 11% CR) with no additional observed toxicity (NCT01206530; manuscript under revision). Similarly, in *BRAF* mutant melanoma, the phase I/II trial of dabrafenib, trametinib, and HCQ has produced a **85% response rate** and **41% complete response rate** (NCT02257424;Mehnert et al Clinical Cancer Research 2022). Most excitingly, a neoadjuvant study of gemcitabine + abraxane +/- HCQ found that the arm that received HCQ had a significantly better pathological response rate, reduced lymph node metastases, and reduction in serum CA19-9 (Zeh et al. *Clinical Cancer Research* 2021).. Finally, in clear cell renal cell carcinoma, a phase I/II combination of HCQ with everolimus led to an increased PFS when compared to the historical control of everolimus alone (NCT01510119; Hass *Clinical Cancer Research 2019*). In most of these trials the addition of high dose HCQ (600 mg bid) did not produce significant adverse events. Additional clinical trials that demonstrated activity of HCQ regimens were in 3rd line colon cancer [21], and multiple myeloma.

### 2.6 HCQ, Lys05 and DQ661 are targeted therapies

The only clinically available autophagy inhibitors are the CQ derivatives. We have also generated a series of more potent dimeric aminoquinolines, Lys05 and DQ661 [6, 22]. We recently showed that DQ661 binds and inhibits the lysosome enzyme palmitoyl-protein thioesterase 1 (PPT1), which regulates intracellular trafficking of receptors and secreted proteins through palmitoylation. Many of the autophagy, mTOR, metabolic enzyme, solute carriers, and interferon signaling proteins are regulated by palmitoylation. We now have data that HCQ and Lys05 also bind to and inhibit this enzyme (Rebecca et al *Cancer Discovery* 2019). Therefore HCQ and more potent derivatives can be considered targeted therapies and not simply weak bases. The newer derivatives are being developed through a biotech company, but are still not clinically available. **Therefore this proposal will utilize HCQ as the proof-of-principle PPT1 inhibitor that is ready for immediate clinical translation.**

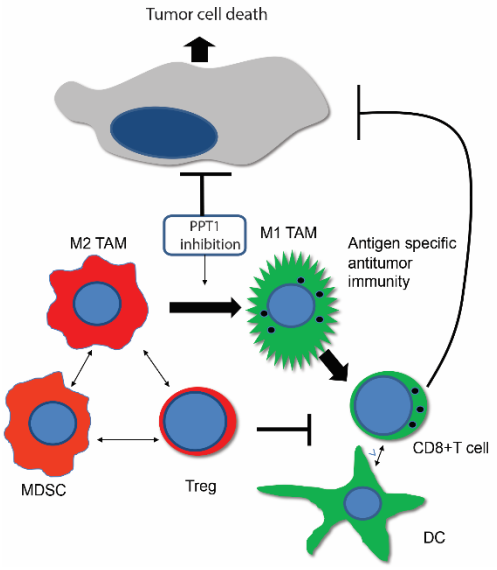



**Fig 2. HCQ treatment simultaneously impairs tumor cell growth while inducing M2-M1 macrophage phenotype switching that enables T-cell mediated killing.** TAM: tumor associated macrophage, MDSC: myeloid derived suppressor cell; DC: dendritic cell

### 2.7 Rationale for amendment 4.

The increased progression-free survival observed with relatlimab and nivolumab (nivo-rela) compared to nivolumab has reinvigorated the melanoma community. With less toxicity than nivolumab and ipilimumab, the nivo-rela combination is very promising, but efficacy in second-line PD-1 antibody refractory patients remains poor with a 12% response rate in a heavily pre-treated population[24]. There is substantial evidence that targeting autophagy can enhance the efficacy of immune checkpoint inhibition through multiple

mechanisms (detailed below). There may be an especially important interaction between autophagy/lysosome and the LAG3 receptor. The proposed amendment to the LIMIT melanoma trial will establish the preliminary safety and antitumor activity of nivo-rela with the well-tolerated autophagy inhibitor HCQ.

**2.8 Rationale for combined immune checkpoint and autophagy inhibition.**

Recently autophagy inhibition has been identified by 6 separate groups as a strategy to improve the efficacy of immune checkpoint inhibition [25-30]. A number of mechanisms have been proposed to explain these observations. These include the fact that MHC Class I molecules expressed by tumor cells are specific autophagy cargo. Autophagy inhibition especially using lysosomal inhibitors enhances surface expression and MHC Class I-mediated antigen presentation [25]. Autophagy inhibition with chloroquine derivatives also induces macrophage repolarization from an M2 to M1 phenotype enabling CD8+ T cell-mediated killing. Key to this mechanism is that STING is a specific lysosomal substrate, so lysosomal inhibition increases STING levels [26].

**2.9 Rationale for blocking the lysosome to enhance LAG3 receptor engagement**.

It has been long known that LAG3 is lysosomal substrate. Much of the LAG3 in cells is in the endocytic-lysosomal compartment [31]. LAG-3 trafficking from lysosomal compartments to the cell surface is dependent on the cytoplasmic domain through protein kinase C signaling in activated T cells [32]. There is some evidence that endo/lysosomal recycling following ligand interactions are required to maintain immunosuppressive signaling in activated T cells [33]. Lysosomal inhibition likely disrupts physiological receptor recycling leading to enhanced but dysfunctional cell surface expression of LAG3. It remains unknown if the roughly 10 μM concentrations of HCQ achieved in the blood of cancer patients is sufficient to enhance cell surface expression of LAG3, but it is enough to enhance MHC Class I expression in tumor cells, which may ultimately be the mechanism by which HCQ can augment nivo-rela. Therefore, there is sufficient rationale and an opportunity to safely test the combination of HCQ with niv-rela in advanced melanoma patients who have progressed on PD-1 based therapy.

**2.10 Extensive clinical characterization of anti-PD1 Ab immune effects at Penn.**

Our group has worked closely with Drs. Alex Huang and John Wherry at Penn to understand the effects of pembrolizumab both in the tumor microenvironment and in peripheral blood samples. Using advanced immunophenotyping techniques, we have performed comprehensive immune profiling of tissue and peripheral blood, with clinical correlation [34].

**2.11 Blood- and Tissue-based Pharmacodynamic Biomarkers Enable the Assessment of Therapeutic Modulation of Autophagy.**

The successful modulation of autophagy can be evaluated via a variety of peripheral blood and tissue-based pharmacodynamic biomarkers. In the aforementioned initial published clinical trials, the on-target effect of HCQ in blocking the final step of autophagy was demonstrated by the accumulation of AVs in peripheral blood mononuclear cells and tumor cells, as determined by electron microscopy. Further, as the ubiquitin-like protein LC3 is integrated into the autophagosome lipid bilayer during normal cellular autophagy, changes in LC3 expression by immunoblotting or immunohistochemistry can indicate successful modulation of the autophagy program. Finally, recent evidence has identified candidate secreted proteins that are associated with tumor autophagy levels in melanoma [35]. Thus, quantitative measurement of these secreted proteins may serve as a pharmacodynamic marker for changes in intracellular, tumor-based autophagic activity.

In addition, our group has recently evaluated potential predictors of sensitivity to chloroquine derivatives in cancer. We have performed differential gene expression profiles in both HCQ-sensitive and HCQ-resistant cancer cells. We have determined that combined expression patterns of aldehyde dehydrogenase1A1

(ALDH1A1) and helicase like transcription factor (HLTF) identify and differentiate HCQ-sensitive and HCQ-resistant cancer cells. Furthermore, using mechanistic studies, we have shown that ALDH1A1 overexpression enhances entry and cytotoxicity of lysosomal autophagy inhibitors (i.e. enhanced drug influx), and that HLTF expression allows for repair of DNA damage caused by lysosomal autophagy inhibitor-induced reactive oxygen species, therefore leading to HCQ resistance. As a result, the $ALDH1A1_{HIGH}/HLTF_{LOW}$ expression profile is associated with HCQ-sensitivity, whereas $ALDH1A1_{LOW}/HLTF_{HIGH}$ is associated with HCQ-resistance. Importantly, tumor RNA sequencing data from >700 patients with advanced solid tumors from the TCGA was queried, and notably RCC and melanoma were tumor types with the most prevalent HCQ-sensitive gene expression profiles [36].

Finally we have recently identified the molecular target of HCQ as palmitoyl-protein thioesterase 1 (PPT1). This is a depalmitoyalase in the lysosomal that regulates the localization of vacuolar type ATPase [6]. V-ATPase mislocalization following PPT1 inhibition results in lysosomal deacidification and autophagy inhibition. High concentrations of HCQ are required for PPT1 inhibition. We have developed a PPT1 IHC assay and this will be used to determine if PPT1 expression is associated with PFS in patients treated with nivolumab and HCQ.

### 2.12 Rationale for correlative studies

To test the hypothesis that HCQ could enhance the therapeutic efficacy of nivolumab by inhibiting the lysosome, this clinical trial will incorporate several correlative studies. First, we will characterize the PK properties of HCQ when administered to patients receiving nivolumab. We anticipate that one third of the phase II portion of this trial will include biopsies before and after nivolumab + HCQ,nivolumab and ipilimumab + HCQ, or nivolumab-relatlimab + HCQ. This will allow pathological analysis including electron microscopy by which we can assess the degree of autophagy. This is feasible since the inhibition of the last step of autophagy by HCQ should cause an accumulation of autophagic vesicles that can be detected by electron microscopy and immunoblotting. We will also perform immunophenotyping on tumors to characterize the makeup of their immune infiltrates before and on therapy. Third, through serial blood draws, we can investigate non-invasive markers by which autophagic dynamics may be detected.

Tumor based assays:

The correlative studies will generate preliminary data to address the following questions regarding immune checkpoint inhibitor and HCQ treatment:

1) Is autophagy induced (immune checkpoint inhibitor and blocked (HCQ) effectively?
2) Are there viable biomarkers by which autophagy dynamics can be assessed noninvasively?
3) What are the immune consequences of combined anit PD1 Ab and autophagy blockade? Specifically what happens to the M2 and M1 macrophage populations, CD8+ and Treg populations and NK cell populations during treatment?
4) What are therapy associated changes in gene expression during response and at progression?

To answer these questions we will use the following technology:

Immunophenotyping of one core: Dr. Alexander Huang in John Wherry's lab at University of Pennsylvania has pioneered immunophenotyping of peripheral blood (measuring re-invigoration of exhausted phenotype CD8 T cells) in melanoma patients treated with anti-PD1 antibody pembrolizumab [34] . It is currently unknown whether a similar immunological response can be identified in patients on anitPD1 Ab and autophagy targeted therapies. We will collect CD45 cells infiltrating the tumor and use multi-parameter flow cytometry to characterize immune subpopulations.

Blood based assays

*Plasma based biomarkers of autophagy modulation.* Recent published data suggests that autophagy is not only involved in degradation of dysfunctional proteins but also plays a role in the maturation and secretion of functional proteins as well. By profiling the tumor secretome in cell culture and mouse xenograft model, we have identified a number of candidate proteins. These have been validated in human samples, and in vitro data demonstrate elevations in IL-8, Interleukin-1 Beta, LIF, DKK3, and FAM3C in high autophagy states compared to low autophagy states [35]. We therefore propose to analyze serum concentrations of these proteins in the presence and absence of HCQ and compare them to the gold standard of electron microscopy. We can then determine whether these proteins would be useful as an non-invasive biomarker of autophagy dynamics.

*Plasma exosomal PDL1 can predict response to anti-PD1 antibody*. Exosomes are lipid-encapsulated small vesicles secreted by cells to the extracellular milieu. Recently Wei Guo's lab at PENN found that metastatic melanoma cells secrete high levels of exosomes that carry a high level of PD-L1 proteins, which potently suppresses the function of $CD8^+$ T cells in circulation and facilitates tumor growth [37]. In patients with metastatic melanoma, the pre-treatment level of circulating exosomal PD-L1 stratifies responders from non-responders to anti-PD1 therapy. We also found that the level of exosomal PD-L1 changes during the course of anti-PD1 therapy. The magnitude of the early on-treatment changes in exosomal PD-L1 follows T cell re-invigoration, and is associated with patient response to therapy. Our research unveils a novel mechanism by which tumor cells suppress the immune system, and provides a rationale for a personalized approach to monitor patient response to anti-PD1 therapies.

## 3.0 PATIENT SELECTION

### 3.1 Inclusion Criteria

3.1.1 Histological or cytological evidence of melanoma, unresectable Stage III or Stage IV, any genotype, and any PD-L1 IHC status

3.1.2 Phase 1a nivolumab + HCQ: any prior treatment, or treatment naïve

Phase 2 nivolumab + HCQ:

Cohort 2a: prior immunotherapy in the adjuvant or metastatic setting is required
Cohort 2b: patients must be anti-PD-1 Ab-naïve, but may have received any prior other therapy

Phase 2c (Nivolumab+relatlimab + HCQ): prior PD-1 based immunotherapy in the adjuvant or metastatic setting is required; prior anti LAG3 antibody is not allowed.

Phase 1b nivolumab + ipilimumab + HCQ: patients must be anti-PD-1 refractory

3.1.3 ECOG performance status of 0-1

3.1.4 Age 18 years of age or older

3.1.5 Patients must have at least one measurable site of disease by RECIST 1.1 criteria that has not been previously irradiated. If there is only one RECIST 1.1 measurable lesion, serial research biopsies will not be pursued, but the patient is eligible

3.1.6 Patients must have fresh or archived primary or metastatic tissue available for submission for correlative analyses

3.1.7 Patients must be able to provide written informed consent

3.1.8 Negative serum pregnancy test within 28 days prior to commencement of dosing in premenopausal women. Negative urine pregnancy test within 24 hours of starting treatment. Women of non-childbearing potential may be included without serum pregnancy test if they are either surgically sterile or have been postmenopausal for ≥ 1 year. Women and men must use an effective method of contraception from 14 days prior to start of treatment, throughout the treatment period, and for at

least 6 months after the last dose of study treatment as directed by their physician. Effective methods of contraception are defined as those which result in a low failure rate (i.e., less than 1% per year) when used consistently and correctly (for example implants, injectables, or intra-uterine devices). At the discretion of the investigator, acceptable methods of contraception may include total abstinence in cases where the lifestyle of the patient ensures compliance. (Periodic abstinence [e.g., calendar, ovulation, symptothermal, postovulation methods] and withdrawal are not acceptable methods of contraception.) Hormonal-based methods (e.g., oral contraceptives) are permitted.

3.1.9 Patients must be able to swallow and retain oral medication and must not have any clinically significant gastrointestinal abnormalities that may alter absorption such as malabsorption syndrome or major resection of the stomach or bowels.

3.1.10 Patients must have adequate baseline organ function as determined by **Table 1**.

**Table 1. Definitions for adequate baseline organ function**

| System | Laboratory Values |
|---|---|
| | |
| **Hematologic** | |
| Absolute neutrophil count (ANC) | $\geq 1.0 \times 10^9$/L |
| Hemoglobin | $\geq$ 9 g/dL |
| Platelet count | $\geq 100 \times 10^9$/L |
| PT/INR[a] and PTT | $\leq$ 1.3 x ULN |
| | |
| **Hepatic** | |
| Total bilirubin[b] | $\leq$ 1.5 x ULN |
| AST and ALT | $\leq$ 2.5 x ULN |
| | |
| **Renal** | |
| Serum creatinine[c] | $\leq$ 1.5 mg/dL |
| | |

Abbreviations: ALT = alanine transaminase; ANC = absolute neutrophil count; AST = aspartate aminotransferase; INR = international normalized ratio; LLN = lower limit of normal; PT = prothrombin time; PTT = partial thromboplastin time; ULN = upper limit of normal.

[a] Subjects receiving anticoagulation treatment may be allowed to participate with INR established within the therapeutic range prior to enrollment.

[b] Subjects with known Gilbert's syndrome must have a total bilirubin < 3.0 x ULN).

[c] If serum creatinine is > 1.5 mg/dL, calculate creatinine clearance using standard Cockcroft-Gault formula. Creatinine clearance must be $\geq$ 50 mL/min to be eligible.

### 3.2 Exclusion Criteria:

**3.2.1** Patients with known serious concurrent infection or medical illness, including psychiatric disorders, which would jeopardize the ability of the patient to receive the treatment outlined in this protocol with reasonable safety.

3.2.2 Patients who are pregnant or breast-feeding.

3.2.3 Patients who received prior anti LAG3 antibody are excluded

3.2.4 Patients with brain metastases treated with whole brain radiation that have been stable for 2 months are eligible; patients with brain metastases treated with gamma knife or surgery are allowed to participate after 2 weeks have elapsed since their procedure. Subjects are excluded if

they have leptomeningeal disease or metastases causing spinal cord compression that are symptomatic or untreated or not stable for ³ 3 months (must be documented by imaging) or requiring corticosteroids> 20 mg prednisone equivalent daily. Subjects on a stable dose of corticosteroids > 1 month or who are tapering off steroids and have reached an equivalent of 20 mg prednisone can be enrolled with approval of the Study PI.

3.2.5 Patients must have discontinued active immunotherapy (IL-2, interferon, CTLA-4, etc.), chemotherapy, or investigational anticancer therapy at least 4 weeks prior to entering the study and oral targeted therapy at least 2 weeks prior to entering the study.

3.2.6 All prior anti-cancer treatment-related toxicities (except alopecia and laboratory values as listed in 3.1.10) must be ≤ Grade 1 or irreversible (hypophysitis) according to the Common Terminology Criteria for Adverse Events version 5 at the time of starting treatment. Patients that are asymptomatic on low dose maintenance hormone replacement delivered at a stable dose for prior toxicities are eligible.

3.2.7 Patients who are known to be experiencing an objective partial response or stable disease to immunotherapy at the time of study enrollment.

3.2.8 History of malignancy other than disease under study within 3 years of study enrollment with exceptions below: Subjects with a history of completely resected non-melanoma skin cancer, or subjects with indolent second malignancies are eligible.

3.2.9 Diagnosis of severe autoimmune disease requiring immunosuppressive medications. Patients with adrenal insufficiency on replacement dose steroids are eligible.

3.2.10 History of interstitial lung disease or chronic pneumonitis unrelated to prior immunotherapy. Prior interstitial pneumonitis related to immunotherapy that was completely treated with no need for ongoing clinical management is allowed.

3.2.11 Due to risk of disease exacerbation patients with porphyria or psoriasis are ineligible unless the disease is well controlled and they are under the care of a specialist for the disorder who agrees to monitor the patient for exacerbations.

3.2.12 Have a known immediate or delayed hypersensitivity reaction or idiosyncrasy to drugs chemically related to study drug, or excipients or to dimethyl sulfoxide.

3.2.13 Patients receiving cytochrome P450 enzyme-inducing anticonvulsant drugs (i.e. phenytoin, carbamazepine, Phenobarbital, primidone or oxcarbazepine) within 4 weeks of the start of the study treatment

3.2.14 Current use of a prohibited medication as described in section on Potential for Drug-Drug Interaction.

3.2.15 History or evidence of increased cardiovascular risk including any of the following:

- Patients with a baseline troponin > 2XULN
- Left ventricular ejection fraction (LVEF) < institutional lower limit of normal. Baseline echocardiogram is not required.
- A QT interval corrected for heart rate using the Bazett‘s formula > 500 msec;
- Current clinically significant uncontrolled arrhythmias. Exception: Subjects with controlled atrial fibrillation

- History of acute coronary syndromes (including myocardial infarction and unstable angina), coronary angioplasty, or stenting within 6 months prior to enrollment
- Current ≥ Class II congestive heart failure as defined by New York Heart Association
- History of myocarditis
-

## 4.0 TREATMENT PLAN

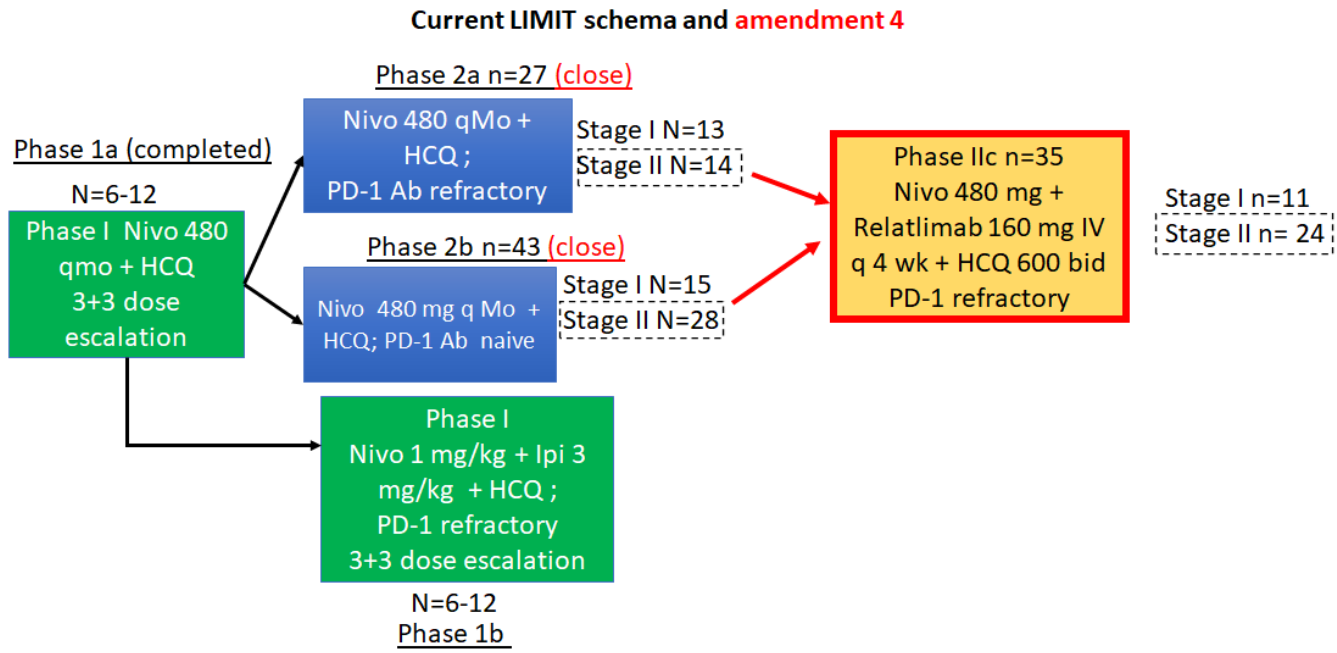



**Fig. 3 Modified LIMIT Melanoma Trial Schema.** Dotted lines indicate Stage II of Simon 2-stage design Stage II cohort that would be activated if there is sufficient activity in Stage I.

This is a Phase 1/2 study (Fig. 3), in which the Phase 1a portion will identify the MTD of combination HCQ + nivolumab therapy in a cohort of subjects with anti-PD-1 Ab naïve or refractory melanoma. This will be followed by an open-label expansion in Cohort 2a: anti-PD-1 Ab refractory patients and Cohort 2b: anti-PD-1 Ab naïve stage III unresectable or stage IV melanoma patients. Cohort 2c: anti-PD-1 refractory Stage III unresectable or Stage IV melanoma.

Once the phase 1a HCQ + nivolumab portion is completed and phase 2 is launched, the phase 1b HCQ + nivolumab + ipilimumab portion of the study will begin accrual to identify the MTD of HCQ + nivolumab + ipilimumab. **Phase 1a HCQ + Nivolumab.** The phase 1a dose-escalation will proceed via a standard 3+3 dose-escalation design evaluating combination therapy at 2 dose levels of HCQ (400 mg BID and 600 mg BID).

Dose Level 1: HCQ 400 mg PO twice daily + nivolumab 480 mg flat-dose IV every 4 weeks

Dose Level 2: HCQ 600 mg PO twice daily + nivolumab 480 mg flat-dose IV every 4 weeks

Safety will be assessed beginning on day 1 of therapy and will continue until the completion of study follow-up. Assessment for dose-limiting toxicity (DLT) in the Phase I portion will continue through the completion of 1 cycle of combination therapy (28 days). A minimum of 3 subjects will be enrolled and treated in the first dose-level cohort, and all 3 subjects will be observed for a minimum of 28 days before subsequent cohort enrollment begins. We anticipate proceeding rapidly through the dose-escalation portion of the study, given our prior experience with combination HCQ and multiple anti-cancer therapies

in similar settings. Following determination of the MTD, enrollment will proceed to the Phase II portion of the study. The phase II cohort expansions will further explore the safety, preliminary efficacy, and important pharmacodynamic correlative information at the MTD combination regimen.

**Phase 2 Expansion Cohort Dosing**: MTD HCQ (400 or 600 mg PO twice daily) + nivolumab 480 mg flat-dose IV every 4 weeks

Phase 2 Cohort a: Previously treated with anti-PD-1 Ab. In 2023 this cohort will be closed early due to developments in the Stage IV melanoma management that make this combination unlikely to be developed further.

Phase 2 Cohort b: Anti-PD-1 Ab naïve melanoma. In 2023 this cohort will be closed early due to developments in the Stage IV melanoma management that make this combination unlikely to be developed further.

**Phase 2c cohort: Nivolumab-relatlimab and HCQ. HCQ 600 mg PO twice a daily + nivolumab 480 mg + relatlimab 160 mg IV every 4 weeks**

Previously treated with Anti-PD-1 Ab.

All trial treatments will be administered on an outpatient basis. Nivolumab, ipilimumab, and nivolumab-relatlimab will be administered as 30-minute IV infusions. However given the variability of the infusion pumps, a window of -5 minutes to +10 minutes is allowed.

CT scans will be obtained every 3 cycles (84 days or 12 weeks +/- 1 week) for assessment of disease response, as per RECIST 1.1 criteria. Toxicity will be assessed using the NCI CTCAE criteria, version 5.0. Serial blood samples will be obtained for pharmacodynamic correlative assessments. Radiology-guided pre-treatment and on-treatment (cycle 1 day 12 +/- 2 days) metastatic core tissue sampling will be obtained for correlative pharmacodynamic and immunophenotyping studies.

The treatment period for combination therapy will continue every 28 days for up to 24 months. Safety follow-up will occur for approximately 100 days following the last administration of study treatment.

Once the Phase 1a nivolumab + HCQ study is completed and the phase 2 cohorts are launched, in parallel the phase 1b nivolumab + ipilimumab + HCQ study will begin accrual (Fig. 3). The Phase I (3-drug combination) trial will identify the MTD of combination HCQ + nivolumab 1 mg/kg i.v. every 3 weeks x4 in combination with ipilimumab 3 mg/kg i.v. every 3 weeks X 4 cycles followed by maintenance nivolumab therapy in patients with anti-PD-1 Ab refractory melanoma.

**Phase 1b nivolumab + ipilimumab + HCQ.** In the phase 1b nivolumab + ipilimumab + HCQ (3-drug combination) trial, dose-escalation will proceed via a standard 3+3 dose-escalation design evaluating combination therapy at 2 dose levels of HCQ (400 mg BID and 600 mg BID).

Dose Level 1: HCQ 400 mg PO BID + nivolumab 1 mg/kg IV given over 30 minutes , followed by ipilimumab 3 mg/kg IV given over 30 minutes. The nivolumab + ipilimumab infusions will be given every 3 weeks X 4 administrations followed by maintenance nivolumab 480 mg IV every 4 weeks until progression or 24 months. Maintenance nivolumab will be started 6 weeks after the last dose of ipilimumab and nivolumab. Patients who discontinue combination ipilimumab and nivolumab for Grade 3-4 toxicity will not be treated with maintenance nivolumab or HCQ, unless the grade 3-4 toxicity is easily managed and completely resolved. These patients will be evaluable for response, PFS and toxicity on study.

Dose Level 2: HCQ 600 mg PO BID + the same nivolumab + ipilimumab combination, dosing, schedule, and specifications as dose level 1

Dose Level 1.5 HCQ 400 mg AM/600 mg PM po BID + the same nivolumab + ipilimumab combination, dosing, schedule, and specifications as dose level 1

Safety will be assessed beginning on day 1 of therapy and will continue until the completion of study follow-up. Assessment for dose-limiting toxicity (DLT) in the Phase I portion will continue through the completion of 1 cycles of combination therapy (21 days). A minimum of 3 subjects will be enrolled and treated in the first dose-level cohort, and all 3 subjects will be observed for a minimum of 21 days before subsequent cohort enrollment begins. We anticipate proceeding rapidly through the dose-escalation portion of the study, given our prior experience with combination HCQ and multiple anti-cancer therapies in similar settings.

## 5.0 DETAILS OF STUDY TREATMENT

### 5.1 Hydroxychloroquine

Mechanism of Action: The mechanism of action is not fully understood. Previously it was thought that HCQ and other chloroquine derivatives are weak bases that deacidify lysosomes through purely chemical basis. Recently our group has identified the missing molecular target of HCQ as palmitoyl protein thioesterase 1 (PPT1).

Storage and formulation: HCQ tablets are manufactured by a number of generic drug companies. Each tablet contains 200 mg hydroxychloroquine sulfate (equivalent to 155 mg base). It is dispensed in a tight, light-resistant container as defined in the USP/NF.HCQ should be stored at room temperature up to 30° C (86° F).

Pharmacokinetics: The PK of HCQ is characterized by a large volume of distribution, binding to red blood cells, and long time to peak concentration and steady state. Population PK studies in cancer patients have demonstrated dose proportional change in exposure.

Administration: Hydroxychloroquine is an oral medication, requiring the patients on study to keep a study diary. Diary must be submitted at each clinic visit. Hydroxychloroquine should be obtained commercially. The starting dose for HCQ will be 400 mg po twice daily continuously. Tablets of HCQ are available in 200 mg strength. HCQ will be administered in divided doses (every 12 hours) Take hydroxychloroquine sulfate tablets with a meal or a glass of milk. **The two daily doses of HCQ should be taken 12 hours apart, for example, 9 am and 9 pm,** and documented clearly on the patient calendar. The HCQ schedule may be adjusted if necessary to minimize gastrointestinal side effects.

For complete information please refer to the package inserts at **http://dailymed.nlm.nih.gov/dailymed/**

### 5.2 Nivolumab

Mechanism of Action: Binding of the PD-1 ligands, PD-L1 and PD-L2, to the PD-1 receptor found on T-cells, inhibits T-cell proliferation and cytokine production. Upregulation of PD-1 ligands occur in some tumors and signaling through this pathway can contribute to inhibition of active T-cell immune surveillance of tumors. Nivolumab is a human immunoglobulin G4 monoclonal antibody that binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2, releasing the PD-1 pathway-mediated inhibition of the immune response, including the anti-tumor immune response.

Storage and formulation: Nivolumab currently comes packaged in a concentrated form in 100 mg (10 mg/mL) single use vial. This concentrated solution should be diluted with 0.9% Sodium Chloride or 5% Dextrose Injection to prepare a diluted solution with a final concentration ranging from 1 mg/mL to 10 mg/mL. Nivolumab must be stored at 2°-8°C. Vials should be protected from light and should not be frozen.

Pharmacokinetics: The pharmacokinetics of nivolumab was studied in 909 patients over a dose range of 0.1 to 20 mg/kg administered as a single dose or as multiple doses every 2-4 weeks. The exposure to nivolumab increased dose proportionally over the dose range of 0.1 to 10 mg/kg administered every 2 weeks. The mean CL is 9.5 mL/h and mean elimination half-life is 25.7 days. Steady-concentrations of

nivolumab were reached by 12 weeks when administered at 3 mg/kg every 2 weeks and systemic accumulation was approximately 3-fold.

Administration: Nivolumab will be mixed, stored, and administered intravenously as per investigators brochure.

**5.3 Ipilimumab**

Mechanism of Action: Ipilimumab is a monoclonal antibody, more specifically a fully humanized IgG1 antibody produced in mammalian cell culture, to the cytotoxic T lymphocyte antigen-4 (CTLA-4) which activates antitumor immunity by inhibiting this major checkpoint

Storage and formulation: Ipilimumab is supplied and stored as a 50-mg single-use 10 mL vial. Preparation of solution: Allow the vials to stand at room temperature for approximately 5 minutes prior to preparation of infusion. Withdraw the required volume of ipilimumab and transfer into an intravenous bag. Dilute with 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to prepare a diluted solution with a final concentration ranging from 1 mg/mL to 2 mg/mL. Mix diluted solution by gentle inversion. Store the diluted solution for no more than 24 hours under refrigeration (2°C to 8°C, 36°F to 46°F) or at room temperature (20°C to 25°C, 68°F to 77°F). Discard partially used vials or empty vials. Administration instructions. Do not mix YERVOY with, or administer as an infusion with, other medicinal products. Flush the intravenous line with 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP after each dose. Administer diluted solution over 30 minutes through an intravenous line containing a sterile, non-pyrogenic, low-protein–binding in-line filter.

Pharmacokinetics: The pharmacokinetics (PK) of ipilimumab were studied in 785 patients with unresectable or metastatic melanoma who received doses of 0.3, 3, or 10 mg/kg once every 3 weeks for 4 doses. The PK of ipilimumab is linear in the dose range of 0.3 to 10 mg/kg. Following administration of YERVOY every 3 weeks, the systemic accumulation was 1.5-fold or less. Steady-state concentrations of ipilimumab were reached by the third dose; the mean Cmin at steady state was 19.4 mcg/mL at 3 mg/kg and 58.1 mcg/mL at 10 mg/kg every 3 weeks. The mean value (percent coefficient of variation) based on population PK analysis for the terminal half-life (t1/2) was 15.4 days (34%) and for clearance (CL) was 16.8 mL/h (38%).YERVOY with nivolumab: When ipilimumab 1 mg/kg was administered in combination with nivolumab 3 mg/kg, the CL of ipilimumab and nivolumab were unchanged compared to when YERVOY was administered alone. When administered in combination, the CL of ipilimumab was unchanged in presence of anti-ipilimumab antibodies and the CL of nivolumab increased by 20% in the presence of anti-nivolumab antibodies.

Administration: Ipilimumab will be mixed, stored and administered intravenously as per package insert.

**5.4 Nivolumab-relatlimab:**

Mechanism of Action: Relatlimab is a human IgG4 monoclonal antibody that binds to the LAG-3 receptor, blocks interaction with its ligands, including MHC II, and reduces LAG-3 pathway-mediated inhibition of the immune response. Antagonism of this pathway promotes T cell proliferation and cytokine secretion.

Binding of the PD-1 ligands, PD-L1 and PD-L2, to the PD-1 receptor found on T cells, inhibits T-cell proliferation and cytokine production. Upregulation of PD-1 ligands occurs in some tumors, and signaling through this pathway can contribute to inhibition of active T-cell immune surveillance of tumors. Nivolumab is a human IgG4 monoclonal antibody that binds to the PD-1 receptor, blocks interaction with its ligands PD-L1 and PD-L2, and reduces PD-1 pathway-mediated inhibition of the immune response, including the anti-tumor immune response. In syngeneic mouse tumor models, blocking PD-1 activity resulted in decreased tumor growth. The combination of nivolumab (anti-PD-1) and relatlimab (anti-LAG-3) results in increased T-cell activation compared to the activity of either antibody alone.

In murine syngeneic tumor models,LAG-3 blockade potentiates the anti-tumor activity of PD-1 blockage, inhibiting tumor growth and promoting tumor regression.

Storage and formulation: OPDUALAG is a fixed-dose combination of nivolumab and relatlimab. Visually inspect the solution in the drug product vial for particulate matter and discoloration prior to administration. OPDUALAG is a clear to opalescent, colorless to slightly yellow solution. Discard the vial if the solution is cloudy, discolored, or contains extraneous particulate matter other than a few translucent-to-white particles.

Preparation:
• During preparation of the infusion solution, use aseptic technique to assure sterility, as the product does not contain a preservative.
• OPDUALAG can be administered diluted or undiluted and administered at a final concentration as specified in the package insert
• Withdraw the required volume of OPDUALAG and transfer into an intravenous container. OPDUALAG is compatible with di(2-ethylhexyl)phthalate (DEHP)-plasticized polyvinyl chloride (PVC), ethyl vinyl acetate (EVA), and polyolefin (PO) intravenous bags.
• If diluting OPDUALAG prior to administration:
− Dilute OPDUALAG solution with 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to prepare an infusion meeting the final concentration and maximum infusion volume parameters as specified in Table 2 below.
− Then mix the diluted solution by gentle inversion. Do not shake.
• Discard partially used vials or empty vials following infusion preparation.
* The concentration range in each group includes 12 mg/mL nivolumab and 4 mg/mL relatlimab as the upper limit,
which represents a scenario in which the drug product is infused without dilution.

Storage of Prepared Solution:
Store the prepared solution either:
• at room temperature and room light for no more than 8 hours from the time of preparation to the end of the infusion. Discard the prepared solution if not used within 8 hours from the time of preparation;
-or-
• under refrigeration at 2°C to 8°C (36°F to 46°F) with protection from light for no more than 24 hours from the time of preparation, which includes the time allowed for equilibration of the infusion bag to room temperature and the duration of the infusion. Discard the prepared solution if not used within 24 hours from the time of preparation. Do not freeze.

Administration:
• Administer the infusion over 30 minutes through an intravenous line containing a sterile, non-pyrogenic, low protein binding in-line polyethersulfone (PES), nylon, or polyvinylidene fluoride (PVDF) filter (pore size of 0.2 micrometer to 1.2 micrometer).
• Flush the intravenous line at the end of the infusion.
• Do not coadminister other drugs through the same intravenous line.

Pharmacokinetics: The pharmacokinetics (PK) of relatlimab following the administration of OPDUALAG were characterized in patients with cancer who received relatlimab 20 to 800 mg every 2 weeks (0.25 to 10 times the approved recommended dosage) or 160 to 1440 mg every 4 weeks (1 to 9 times the approved recommended dosage) either as a monotherapy or in combination with nivolumab dosages of 80 or 240 mg every 2 weeks or 480 mg every 4 weeks.

Steady-state concentrations of relatlimab were reached by 16 weeks with an every 4-week regimen and the systemic accumulation was 1.9-fold. The average concentration (C avg) of relatlimab after the first dose increased dose proportionally at doses ≥160 mg every 4 weeks.
Following the recommended dosage, the geometric mean [coefficient of variation (CV%)] maximum and average concentrations (Cmax and Cavg) of relatlimab at steady state were 62.2 (30%), and 28.8 (45%) µg/mL, respectively; and the mean Cmax and Cavg of nivolumab at steady state were 187 (33%) and 94.4 (43%) µg/mL, respectively.
In RELATIVITY-047, the nivolumab geometric mean minimum concentration (Cmin ) at steady state in the OPDUALAG arm was comparable to the nivolumab arm.

Distribution:
The geometric mean (CV%) volume of distribution at steady state of relatlimab is 6.6 L (20%) and 6.6 L (19%) of nivolumab.

Elimination:
The geometric mean (CV%) clearance of relatlimab is 5.5 mL/h (41%) at steady state, 10% lower than after the first dose [6 mL/h (39%)]. Following OPDUALAG (nivolumab 480 mg and relatlimab 160 mg administered every 4 weeks) administration, the geometric mean (CV%) effective half-life (t1/2 ) of relatlimab is 26.2 days (37%). The geometric mean (CV%) clearance of nivolumab is 7.6 mL/h (40%) at steady state, 21% lower
than after the first dose [9.6 mL/h (40%)] and the terminal t1/2 is 26.5 days (36%).

Specific Populations:
The following factors had no clinically important effect on the clearance of nivolumab and relatlimab: age (17 to 92 years), sex, race (White, Asian, and Black/African American), mild or moderate renal impairment (eGFR 30 to 89 mL/min/1.73 m 2 ), mild hepatic impairment (total bilirubin [TB] less than or equal to upper limit of normal [ULN] and AST greater than ULN or TB greater than 1 to 1.5 times ULN and any AST) or moderate hepatic impairment (TB greater than 1.5 to 3 times ULN and any AST). The effects of severe renal impairment, or severe hepatic impairment on the pharmacokinetics of nivolumab and relatlimab are unknown.

**5.5 Concomitant Medication, Drug-Drug interactions and Procedures**

Subjects must be instructed not to take any medications, including over the counter products, without first consulting with the investigator.

Because HCQ has known effects on P450 enzymes, patients requiring anti-convulsants may be treated with any of the non-enzyme inducing anti-convulsants which include: felbamate, valproic acid, gabapentin, lamotrigine, tiagibine, topiramate, or levetiracetam. Due to the fact that both zonisamide and HCQ accumulate in red blood cells, zonisamide should be avoided if possible. For nausea, aprepitant should be avoided. Radiation therapy to the surgical bed with gamma knife radiotherapy while on treatment is allowed for surgically resected brain metastases. Gamma knife for new CNS lesions may be performed while on study, but further of treatment of these patients will be considered treatment beyond progression. All other concomitant medications are permitted.

The following medications are not allowed during the study. The sponsor must be notified if the subject receives any of these during the study:

1. Any investigational anticancer therapy

2. Any concurrent chemotherapy, radiotherapy (except radiotherapy as designated in this study or radiotherapy indicated for CNS metastasis), immunotherapy, biologic or hormonal therapy for cancer treatment, except as noted in the exclusion criteria. Concurrent use of hormones for noncancer-related conditions (e.g. insulin for diabetes, hormone replacement therapy) is acceptable.

3. Immunosuppressive medications, including, corticosteroids at doses exceeding 10mg/day of prednisone or equivalent, methotrexate, azathioprine, and TNF-alpha blockers. Use of immunosuppressive medication for the management of study treatment-related AEs or in subjects with contrast allergies is acceptable. In addition, use of topical, inhaled and intranasal corticosteroids is permitted

4. Live attenuated vaccines during the study through 180 days after the last dose of both drugs

5. Herbal and natural remedies should be avoided

### 5.6 Duration of Protocol Treatment and Follow-up

Treatment will continue until disease progression or unacceptable toxicity for a maximum of 24 months for patients with partial or complete response. For patients experiencing stable disease treatment may continue at the discretion of the treating physician in consultation with the Study Chair and Medical Monitor. Treatment may also be continued for isolated progression that can be treated with local therapy. For patients that experience a complete response treatment (including HCQ) can be discontinued after 9 months of combined therapy. Subjects will be evaluated for at least 100 days after discontinuation of study treatment and will be followed for a minimum of one year from the start of treatment, unless disease progression is reported prior to one year. One-year survival information will be extracted from the medical record or by phone call for subjects who have discontinued protocol treatment and completed the 100 day follow-up evaluations prior to one year from the start of treatment.

## 6.0 DOSE LIMITING TOXICITY AND DOSE ESCALATION RULES

### 6.1 Definition of Dose Limiting Toxicity (DLT)

For phase 1a nivolumab and HCQ, if occurring in the first 4 weeks of combined therapy the following will be considered DLTs:

1. grade 4 neutropenia with fever
2. grade 4 thrombocytopenia
3. Any non-hematologic toxicity of grade 3 or higher that is at least possibly treatment-related and that is refractory to supportive measures. These include but are not limited to fatigue, nausea, diarrhea, visual disturbance. This excludes nausea and vomiting which have not been treated with optimal anti-emetic therapy. Grade 3 rash can also occur with nivolumab, and therefore will not be considered a DLT, unless the quality of the rash is more severe than what would be expected with nivolumab alone.

For Phase I nivolumab, ipilimumab and HCQ, rash, fatigue and gastrointestinal adverse events are common to nivolumab, Ipilimumab and high dose HCQ. Since grade 3 rash, fatigue and diarrhea can occur with nivolumab, ipilimumab or the combination in the first 3 weeks a grade 3 will be considered a DLT in this trial only if maximal supportive measures are refractory and rash does not improve with steroids. Grade 3 non-hematological toxicities not common to all of the three drugs that are refractory to maximal supportive care will be considered DLTs.

Any DLT that causes a patient to miss > 28 consecutive days of HCQ will result in the patient being taken off HCQ treatment. Patients will continue on nivolumab or nivolumab and ipilimumab if that is appropriate at that point and remain evaluable for toxicity and response as long as they received at least 4 weeks of combined nivolumab + HCQ prior to discontinuation of HCQ.

### 6.2 Dose Escalation Rules

The target dose limiting toxicity (DLT) rate is ≤ 33%. The MTD is defined as a) the dose producing DLT in 2 out of 6 patients, or b) the dose level below the dose which produced DLT in ≥ 2 out of 3 patients, or in ≥ 3 out of 6 patients. No intra-patient dose escalation will be permitted. Patients will be evaluable for a Phase I cohort if they completed 75% of their expected dose of HCQ for the first cycle.

The first three patients will be enrolled on dose level 1 as described in Table 3. If a DLT is observed in 1 patient out of 3 the cohort will be expanded to 6. If a DLT occurs in 2 out of 3 or more patients per cohort, then the cohort one dose below will be the declared MTD provided that at least 6 patients have been treated at that level with no more than 2 having DLTs. The rules for dose escalation and cohort size are outlined in Table 2. No intra-patient dose escalation is planned. Patients will be evaluable for DLT if they have completed 75% of expected HCQ dose for combined HCQ and nivolumab or HCQ + nivolumab + ipilimumab during the first cycle of therapy.

**Table 2: Criteria for Dose Escalation and Cohort Size**

| **# with DLT** | **At dose level 1** | **At dose level 2** | **At dose level 1.5** |
|---|---|---|---|
| **0/3** | Escalate to dose level 2 | Declare dose level 2 the RP2D | Declare dose level 1.5 the RP2D |
| **1/3** | Add 3 patients | Add 3 patients | Add 3 patients |
| **≥2/3** | Close trial | De-escalate to dose level 1.5 | Declare dose level 1 MTD |
| | ***If 6 are enrolled:*** | | |
| **1/6** | Escalate to dose level 2 | Declare dose level 2 MTD | Declare dose level 1.5 MTD |
| **2/6** | Close trial | De-escalate to dose level 1.5 | Declare Dose level 1 MTD |

RP2D: Recommended phase II dose

### 6.3 Dose Levels

**Table 3: Hydroxychloroquine plus Nivolumab Dose Escalation Schema**

| **Dose level** | **Nivolumab** | **HCQ** |
|---|---|---|
| 1 | 480 mg q4 weeks | 400 mg bid |
| 1.5 | 480 mg q4 weeks | 400 mg am / 600 mg pm |
| 2 | 480 mg q4 weeks | 600 mg bid |

**Table 4: Hydroxychloroquine plus Nivolumab and Ipilimumab Dose Escalation Schema**

| **Dose level** | **Nivolumab** | **Ipilimumab** | **HCQ** |
|---|---|---|---|
| 1 | 1 mg/kg IV q 3weeks x4 | 3 mg/kg IV q 3 weeks x4 | 400 mg bid |
| 1.5 | 1 mg/kg IV q 3 weeks x4 | 3 mg/kg IV q 3 weeks x4 | 400 mg am / 600 mg pm |
| 2 | 1 mg/kg IV q 3 weeks x4 | 3 mg/kg IV q 3 weeks x4 | 600 mg bid |

### 6.4 Single arm Phase 2 trial with 2 cohorts

Once the MTD or recommended phase II dose for HCQ when given in combination with nivolumab is determined in the phase I portion of this trial, a non- randomized 2 cohort single arm open label phase II trial will be conducted. The trial is designed using a Simon 2 stage phase II trial approach (see Section 12.2), however, accrual will not be halted until enough patients in the first stage are evaluable for the primary endpoint (response) to trigger the stopping rule.

## 7.0 TOXICITY CRITERIA, MONITORING, DOSE DELAYS AND MODIFICATIONS

### 7.1 Toxicity Criteria

This study will utilize the CTCAE version 5.0 for toxicity and Adverse Event Reporting. A copy of the CTCAE version 5.0 can be downloaded from the CTEP home page (https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm). All appropriate treatment areas should have access to a copy of the CTCAE version 5.0.

### 7.2 Dose Delays

Major Events are grade 3 and 4 hematologic and non-hematologic toxicities that are not treatment - related. Treatment should be delayed for major events if HCQ may further complicate the non-treatment related event. If a major event requires a delay of treatment, treatment must be delayed until toxicity is resolved (≤ Grade 2 or ≤ Baseline). For treatment-related toxicities and major events, if toxicity (exceptions are endocrine and mild skin toxicities) is not resolved in ≤ 28 days, patient will be taken off treatment, unless there is an exception granted by the medical monitor.

### 7.3 Ocular toxicity

The only toxicity that requires discontinuation of HCQ is retinopathy. Published literature indicates HCQ retinopathy is idiosyncratic and is uncommon in patients receiving HCQ for less than a few years. Our ongoing study of dabrafenib, trametinib, and HCQ (BAMM; NCTNCT02257424) found no clinically meaningful ocular toxicity in 10 patients studied extensively with serial ocular exams [38]. Therefore, we have not included mandatory ocular exams in this protocol. However, if there is a visual field deficit, retinal vein occlusion, serous retinopathy, bullseye retinopathy, or retinal detachment, HCQ should be permanently discontinued.

### 7.4 Nivolumab Side Effects Overview

Common Side Effects: Diarrhea, inflammation of the colon (colitis), increase in liver enzymes, fatigue, swelling of the extremities, flu-like feeling, pain, skin itchiness, skin rash or xerosis (increased dryness), vitiligo (loss of pigment or color in the skin), nausea, abdominal pain, decreased appetite, fever, vomiting, constipation, flatulence, dry mouth, weight loss, cough, shortness of breath, headache, peripheral neuropathy (numbness or tingling of the fingers or toes), dizziness, change in sensation of taste, joint pain or stiffness, dehydration, increased blood sugar, low sodium levels, infections, inflammation of the optic disc, low blood pressure, and difficulty sleeping.

Less Likely Side Effects: inflammation of the pancreas, decreased movement of the intestines, inflammation of the thyroid, lung inflammation (pneumonitis), shortness of breath, joint swelling, muscle soreness, weakness, stiffness, or spasm, adrenal gland abnormalities, inflammation of the heart or lining of the heart, acute kidney injury or failure, and increase in inflammatory blood proteins.

Rare Side Effects:

- Myasthenia gravis, a nerve disease that may cause weakness of the eye, face, breathing and swallowing muscles. One death in a patient who received the combination was considered due to myasthenia gravis and severe infection (sepsis)
- A syndrome associated with fever, WBC activation and abnormal function (including destruction of other blood cells by WBCs), low blood cell counts, rash, and enlargement of the spleen.

### 7.5 Treatment of Nivolumab, Nivolumab +Ipilimumab, or nivolumab-relatlimb Related Infusion Reactions

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

All Grade 3 or 4 infusion reactions should be reported as serious adverse events (SAEs) if criteria are met. Infusion reactions should be graded according to National Cancer Institute (NCI) CTCAE 5.0 guidelines.

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

For Grade 1 symptoms: (Mild reaction; infusion interruption not indicated; intervention not indicated). Remain at bedside and monitor patient until recovery from symptoms. The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or acetaminophen 325 to 1000 mg at least 30 minutes before additional nivolumab administrations.

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

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

### 7.6 Ipilimumab Side Effects Overview

Common Side Effects: Diarrhea, inflammation of the colon (colitis), increase in liver enzymes, fatigue, skin itchiness, skin rash, nausea, abdominal pain, decreased appetite, fever, vomiting, headache, constipation, adrenal gland abnormalities, and thyroid gland abnormalities.

Less Likely Side Effects: Chills, weakness, muscle pain, and redness of skin.

Rare Side Effects: Decrease or total loss of hormones of pituitary gland, allergic reactions, inflammation of the liver, inflammation of the pituitary gland, decreased red blood cells, loss of color (pigment) from areas of skin, decreased or blurry vision, or inflammation of the eye, numbness or tingling in your fingers or toes, inflammation or loss of the lining of the brain or spinal cord, inflammation of the kidneys, and joint pain.

### 7.7 Ipilimumab and Nivolumab

Common Side Effects: Diarrhea, inflammation of the colon (colitis), increase in liver enzymes, fatigue, swelling of the extremities, flu-like feeling, pain, skin itchiness, skin rash or xerosis (increased dryness), vitiligo (loss of pigment or color in the skin), nausea, abdominal pain, decreased appetite, fever, vomiting, constipation, flatulence, dry mouth, weight loss, cough, shortness of breath, headache, peripheral neuropathy (numbness or tingling of the fingers or toes), dizziness, change in sensation of taste, joint pain or stiffness, dehydration, increased blood sugar, low sodium levels, infections, inflammation of the optic disc, low blood pressure, and difficulty sleeping.

Less Likely Side Effects: inflammation of the pancreas, decreased movement of the intestines, inflammation of the thyroid, lung inflammation (pneumonitis), shortness of breath, joint swelling, muscle soreness, weakness, stiffness, or spasm, adrenal gland abnormalities, inflammation of the heart or lining of the heart, acute kidney injury or failure, and increase in inflammatory blood proteins.

Rare Side Effects: Myasthenia gravis, a nerve disease that may cause weakness of the eye, face, breathing and swallowing muscles. One death in a patient who received the combination was considered due to myasthenia gravis and severe infection (sepsis)
A syndrome associated with fever, WBC activation and abnormal function (including destruction of other blood cells by WBCs), low blood cell counts, rash, and enlargement of the spleen.

### 7.8 Nivolumab and relatlimab

<u>Very common (may affect more than 1 in 10 people)</u>

- Infection of the urinary tract (the parts of the body that collect and pass out urine)
- Decreased number of red blood cells (which carry oxygen) and white blood cells (lymphocytes, neutrophils, leucocytes; which are important in fighting infection)
- Underactive thyroid gland (which can cause tiredness or weight gain)
- Decreased appetite
- Headache
- Difficulty breathing, cough
- Diarrhoea (watery, loose or soft stools), vomiting; nausea; stomach pain; constipation
- Skin rash (sometimes with blisters), skin colour change in patches (vitiligo), itching

- Pain in the muscles, bones and joints

- Feeling tired or weak, fever.

- Changes in the results of tests:
  abnormal liver function (increased amounts of the liver enzymes alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase in your blood)
  abnormal kidney function (increased amounts of creatinine in your blood)
  decrease of sodium and magnesium, and decrease or increase of calcium and potassium.

Common (may affect up to 1 in 10 people)

- Infections of the upper respiratory tract (nose and upper airways)

- Decreased number of platelets (cells which help the blood to clot), increase in some white blood cells

- Decreased secretion of hormones produced by adrenal glands (glands situated above the kidneys), inflammation of the pituitary gland situated at the base of the brain, overactive thyroid gland, inflammation of the thyroid gland

- Diabetes, low sugar levels in the blood; weight loss, high levels of the waste product uric acid in the blood, decreased levels of the protein albumin in the blood, dehydration
  state of confusion

- Inflammation of the nerves (causing numbness, weakness, tingling or burning pain of the arms and legs), dizziness, changes in the sense of taste

- Inflammation of the eye (which causes pain and redness, vision problems or blurry vision), vision problems, dry eyes, excessive tear production
- Inflammation of the heart muscle

- Inflammation of a vein, which can cause redness, tenderness and swelling
  Inflammation of the lungs (pneumonitis), characterised by coughing and difficulty breathing;

- Nasal congestion (blocked nose)

- Inflammation of the intestines (colitis), inflammation of the pancreas, inflammation of the stomach (gastritis), difficulty swallowing, mouth ulcers and cold sores; dry mouth
  inflammation of the liver (hepatitis)

- Unusual hair loss or thinning (alopecia), isolated area of skin growth that becomes red and itchy (lichenoid keratosis), sensitivity to light, dry skin

- Painful joints (arthritis), muscle spasms, muscle weakness

- Kidney failure (changes in amount or colour of urine, blood in urine, swelling ankles, loss of appetite), high levels of proteins in the urine

- Edema (swelling), flu-like symptoms, chills

- Reactions related to the administration of the medicine.

- Changes in the results of tests carried out by your doctor may show:
  - abnormal liver function (higher blood levels of the waste product bilirubin, higher blood levels of the liver enzyme gamma-glutamyl transferase)
  - increase in sodium and magnesium
  - increased level of troponin (a protein released into the blood when the heart is damaged)
  - increased level of the enzyme that breaks down glucose (sugar) (lactate dehydrogenase), the enzyme that breaks down fats (lipase), the enzyme that breaks down starch (amylase)

<u>Uncommon (may affect up to 1 in 100 people)</u>

- Inflammation and infection in the hair follicles
  disorder in which red blood cells are destroyed faster than they can be made (haemolytic anaemia)

- Underactive function of the pituitary gland situated at the base of the brain; underactive function of the glands producing sex hormones
  inflammation of the brain, which may include confusion, fever, memory problems or seizures (encephalitis), a temporary inflammation of the nerves that causes pain, weakness, and paralysis in the extremities (Guillain-Barré syndrome), inflammation of the optic nerve that may cause a complete or partial loss of vision

- Inflammation of the eyes, skin, in and the membranes of the ears, brain and spinal cord (Vogt-Koyanagi-Harada disease), red eye

- Fluid around the heart

- Asthma

- Inflammation of the oesophagus (passage between throat and stomach)

- Inflammation of the bile duct

- Skin rashes and blistering on the legs, arms, and abdomen (pemphigoid), skin disease with thickened patches of red skin, often with silvery scales (psoriasis), hives (itchy, bumpy rash) inflammation of the muscles causing weakness, swelling, and pain, disease in which the immune system attacks the glands that make moisture for the body, such as tears and saliva (Sjogren's syndrome), inflammation of muscles causing pain or stiffness, inflammation of the joints (painful joint disease), disease in which the immune system attacks its own tissues, causing widespread inflammation and tissue damage in the affected organs, such as joints, skin, brain, lungs, kidneys, and blood vessels (systemic lupus erythematosus)

- Inflammation of the kidney

- Absence of sperm in the semen.

- Changes in the results of tests carried out by your doctor may show:
  increase in level of c-reactive protein
  red blood cell sedimentation rate increased.

### 7.9 Monitoring and Dose Delay Criteria

Patients should be assessed for toxicity prior to each dose; dosing will occur only if the clinical assessment and laboratory test values are acceptable. If toxicity occurs as indicated below, patients in both study arms should have study drug(s) held. In patients who are in the combined treatment arm, both drugs should be held for any toxicity. Dose delays are designed to maximize treatment for those who derive clinical benefit from treatment while ensuring patient safety.

Nivolumab, nivolumab-relatlimab or nivolumab and ipilimumab administration may be delayed for the following reasons:

- Grade ≥2 non-skin, drug-related AEs with the following exceptions:
- Grade 2 drug-related fatigue or laboratory abnormalities do not require a treatment delay
- Any Grade 3 skin, drug-related AE
- Any Grade 3 drug-related laboratory abnormalities with the following exceptions for lymphopenia, leukopenia, AST, ALT or total bilirubin
- Grade 3 lymphopenia or leukopenia does not require dose delay
- If a patient has a baseline AST, ALT or total bilirubin that is within normal limits, delay dosing for drug-related Grade ≥2 toxicity
- If a patient has a baseline AST, ALT or total bilirubin that is within the Grade 1 toxicity range, delay dosing for drug-related Grade ≥3 toxicity
- Any AE, laboratory abnormalities, or intercurrent illness, which in the judgment of the Investigator warrants delaying the dose of study drug.
- All troponin elevations (including asymptomatic elevations) will require the participant to undergo a cardiac evaluation (via prompt cardiology consultation) and a confirmatory repeat evaluation within 24 hours. If troponin elevation is not confirmed within 24 hours in an asymptomatic participant, a dose delay for the next dose may not be required provided that the cardiac evaluation is completed and and based on the Investigator's judgment to proceed with treatment.
- Otherwise, if the troponin elevation is confirmed, dosing may only resume when the AE resolves to baseline, and permanent discontinuation of treatment should be considered.

### 7.10 Management guidelines for immune related adverse events

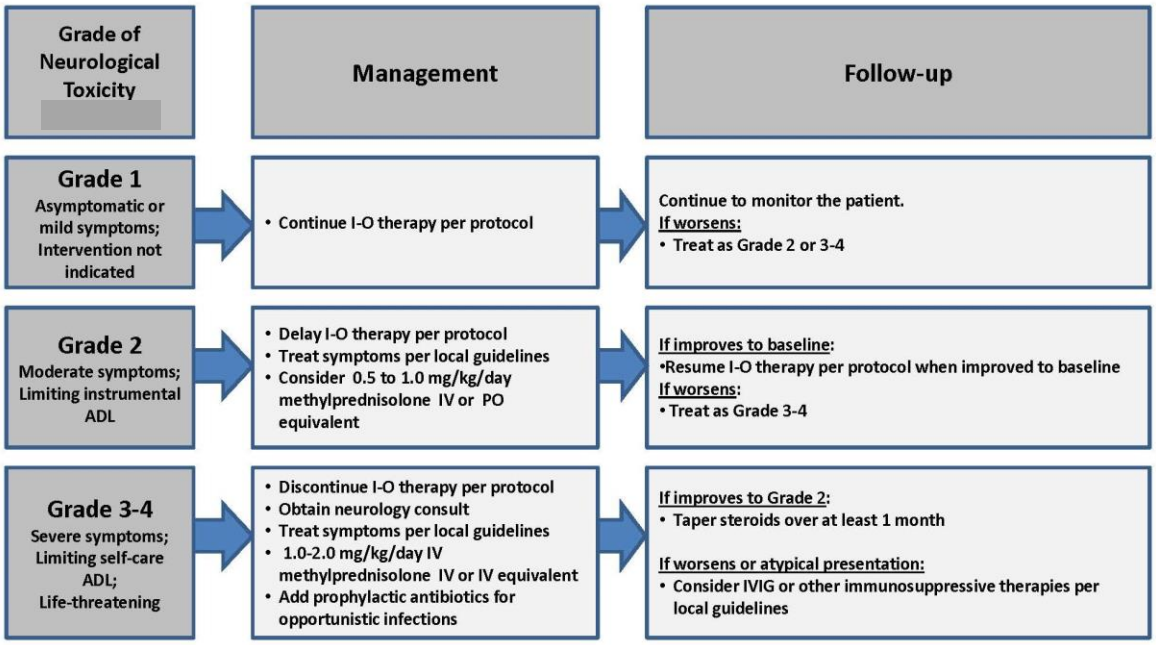
Immuno-oncology (I-O) agents are associated with AEs that can differ in severity and duration than AEs caused by other therapeutic classes. Nivolumab, ipilimumab, and relatlimab are considered immuno-oncology agents in this protocol. Management algorithms have been developed to assist investigators in assessing and managing the following groups of AEs: Gastrointestinal, Renal, Pulmonary, Hepatic, Endocrinopathies, Skin, and Neurological. Early recognition and intervention are recommended according to the management algorithms; and in addition include ophthalmologic evaluations for any visual symptoms in order to evaluate for immune checkpoint-inhibitor related uveitis.

For patients expected who require more than 4 weeks of corticosteroids or other immunosuppressants to manage an AE, consider the following recommendations.

- Antimicrobial/antifungal prophylaxis per institutional guidelines to prevent opportunistic infections such as *Pneumocystis jiroveci* and fungal infections.
- Early consultation with an infectious disease specialist should be considered.
- Depending on the presentation, consultation with a pulmonologist for bronchoscopy or a gastroenterologist for endoscopy may also be appropriate.
- In patients who develop recurrent AEs in the setting of ongoing or prior immunosuppressant use, an opportunistic infection should be considered in the differential diagnosis.

## Neurological Adverse Event Management Algorithm

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




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

## Renal Adverse Event Management Algorithm

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

| Grade of Creatinine Elevation | Management | Follow-up |
|---|---|---|
| **Grade 1**<br>Creatinine > upper limit of normal (ULN) and > than baseline but ≤ 1.5x baseline | • Continue I-O therapy per protocol<br>• Monitor creatinine weekly | <u>If returns to baseline</u>:<br>•Resume routine creatinine monitoring per protocol<br><u>If worsens:</u><br>•Treat as Grade 2 or 3/4 |
| **Grade 2-3**<br>Creatinine > 1.5x baseline to ≤ 6x ULN | • Delay I-O therapy per protocol<br>• Monitor creatinine every 2-3 days<br>• 0.5 to 1.0 mg/kg/day methylprednisolone IV or oral equivalent<br>• Consider renal biopsy | <u>If returns to Grade 1:</u><br>•Taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy and routine creatinine monitoring per protocol<br><u>If elevations persist > 7 days or worsen:</u><br>•Treat as Grade 4 |
| **Grade 4**<br>Creatinine > 6x ULN | • Discontinue I-O therapy per protocol<br>• Monitor creatinine daily<br>• 1.0-2.0 mg/kg/day methylprednisolone IV or IV equivalent<br>• Consult nephrologist<br>• Consider renal biopsy | <u>If returns to Grade 1</u>: Taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections |

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

## Endocrinopathy Management Algorithm

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

| | | |
|---|---|---|
| **Asymptomatic thyroid stimulating hormone (TSH) elevation** | • Continue I-O therapy per protocol<br>• If TSH < 0.5 x lower limit of normal (LLN), or TSH > 2 x ULN, or consistently out of range in 2 subsequent measurements: include free thyroxine (fT4) at subsequent cycles as clinically indicated; consider endocrinology consult | |
| **Symptomatic endocrinopathy** | • Evaluate endocrine function<br>• Consider pituitary scan<br><br><u>Symptomatic with abnormal lab/pituitary scan:</u><br>• Delay I-O therapy per protocol<br>• 1-2 mg/kg/day methylprednisolone IV or by mouth (PO) equivalent<br>• Initiate appropriate hormone therapy<br><br><u>No abnormal lab/pituitary MRI scan but symptoms persist:</u><br>• Repeat labs in 1-3 weeks /MRI in 1 month | <u>If improves (with or without hormone replacement):</u><br>• Taper steroids over at least 1 month and consider prophylactic antibiotics for opportunistic infections<br>• Resume I-O therapy per protocol<br>• Patients with adrenal insufficiency may need to continue steroids with mineralocorticoid component |
| **Suspicion of adrenal crisis (e.g. severe dehydration, hypotension, shock out of proportion to current illness** | • Delay or discontinue I-O therapy per protocol<br>• Rule out sepsis<br>• Stress dose of IV steroids with mineralocorticoid activity<br>• IV fluids<br>• Consult endocrinologist<br>• If adrenal crisis ruled out, then treat as above for symptomatic endocrinopathy | |

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

## Pulmonary Adverse Event Management Algorithm

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

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

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

## Skin Adverse Event Management Algorithm

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

| Grade of Rash | Management | Follow-up |
|---|---|---|
| **Grade 1-2**<br>Covering ≤ 30% body surface area (BSA)* | • Symptomatic therapy (e.g. antihistamines, topical steroids)<br>• Continue I-O therapy per protocol | If persists > 1-2 weeks or recurs:<br>• Consider skin biopsy<br>• Delay I-O therapy per protocol<br>• Consider 0.5-1.0 mg/kg/day methylprednisolone IV or oral equivalent. Once improving , taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol<br>If worsens:<br>• Treat as Grade 3-4 |
| **Grade 3-4**<br>Covering >30% BSA; Life threatening consequences* | • Delay or discontinue I-O therapy per protocol<br>• Consider skin biopsy<br>• Dermatology consult<br>• 1.0-2.0 mg/kg/day IV methylprednisolone IV or IV equivalent | If improves to Grade 1:<br>• Taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections<br>• Resume I-O therapy per protocol |

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

## Hepatic Adverse Event Management Algorithm

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

| Grade of Liver Test Elevation | Management | Follow-up |
|---|---|---|
| **Grade 1**<br>AST or ALT > ULN to 3.0 x ULN and/or Total bilirubin (T. bili) > ULN - 1.5 x ULN | • Continue I-O therapy per protocol | • Continue liver function tests (LFT) monitoring per protocol<br>If worsens:<br>• Treat as Grade 2 or 3-4 |
| **Grade 2**<br>AST or ALT > 3.0 to ≤ 5 x ULN and/or T. bili > 1.5 to ≤ 3 x ULN | • Delay I-O therapy per protocol<br>• Increase frequency of monitoring to every 3 days | If returns to baseline:<br>•Resume routine monitoring, resume I-O therapy per protocol<br>If elevations persist > 5-7 days or worsen :<br>• 0.5-1 mg/kg/day methylprednisolone or oral equivalent and when LFT returns to grade 1 or baseline, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol |
| **Grade 3-4**<br>AST or ALT > 5 x ULN and /or T.bili >3 x ULN | • Discontinue I-O therapy*<br>• Increase frequency of monitoring to every 1-2 days<br>• 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent**<br>• Add prophylactic antibiotics for opportunistic infections<br>• Consult gastroenterologist | If returns to grade 2:<br>• Taper steroids over at least 1 month<br>If does not improve in >3-5 days, worsens or rebounds:<br>• Add mycophenolate mofetil 1 gram (g) twice daily (BID)<br>• If no response within an additional 3-5 days, consider other immunosuppressants per local guidelines |

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

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

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

## GI Adverse Event Management Algorithm

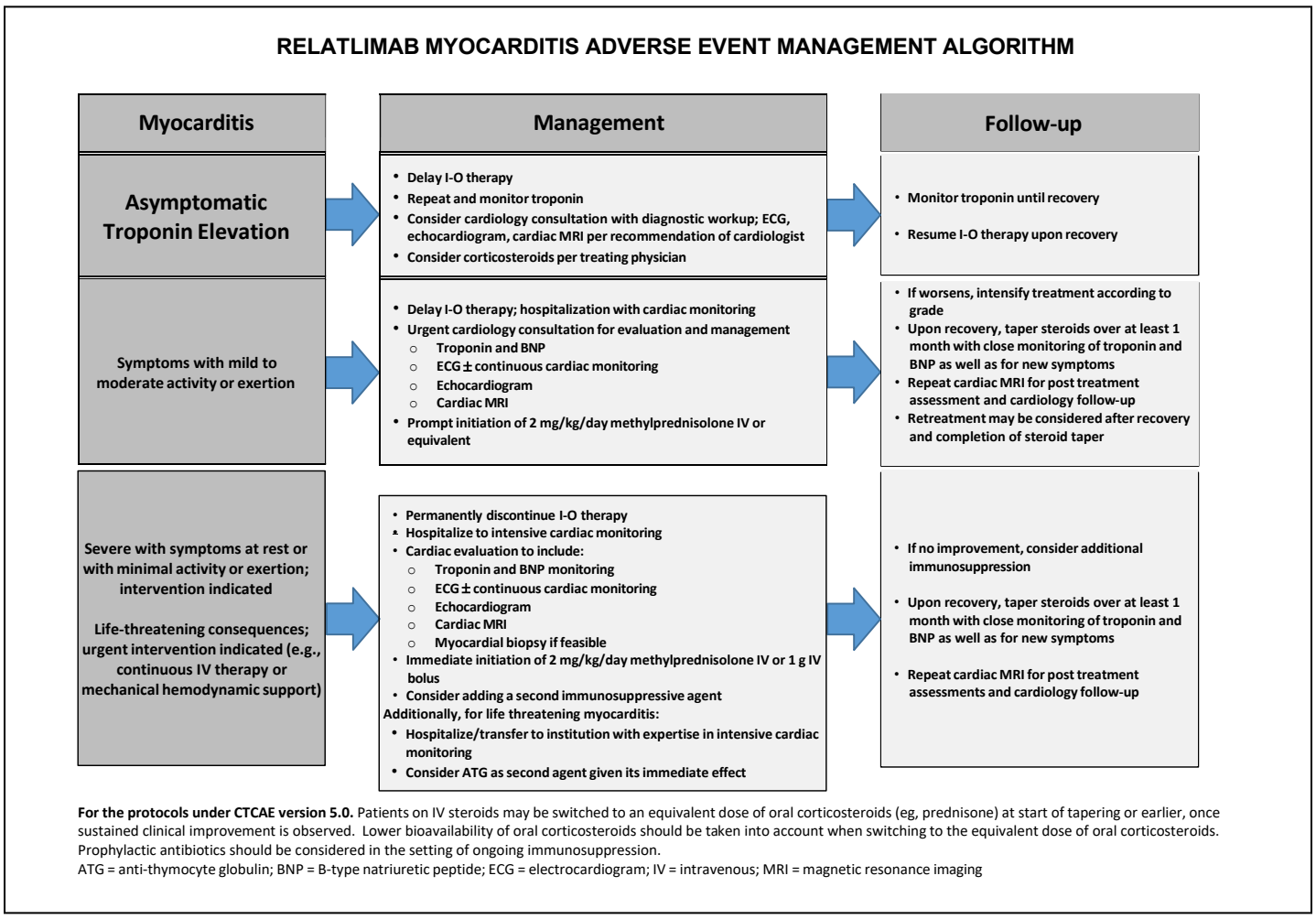
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.

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

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

### 7.11 Immune-Mediated Myocarditis

Immune checkpoint inhibitors can cause immune-mediated myocarditis, which is defined as requiring use of steroids and no clear alternate etiology. The diagnosis of immune-mediated myocarditis requires a high index of suspicion. Patients with cardiac or cardio-pulmonary symptoms should be assessed for potential myocarditis. If myocarditis is suspected based on troponin elevation or clinical symptoms, withhold further therapy,confirm th elevation within 24 hours, arrange for a prompt cardiology followup and consider promptly initiating high dose steroids (prednisone or methylprednisolone 1 to 2 mg/kg/day). If clinically confirmed, permanently discontinue nivolumab-relatlimab and HCQ for Grade 2-4 myocarditis.




**For the protocols under CTCAE version 5.0.** Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids. Prophylactic antibiotics should be considered in the setting of ongoing immunosuppression.
ATG = anti-thymocyte globulin; BNP = B-type natriuretic peptide; ECG = electrocardiogram; IV = intravenous; MRI = magnetic resonance imaging

### 7.12 Hydroxychloroquine Dose Reduction

Any AE of ≥ Grade 3 and attributed as possibly, probably or definitely related solely to HCQ will result in the dose being held until the AE has resolved to ≤ grade 1 or baseline while nivolumab, nivolumab and ipilimumab, or nivlumab-relatlimab dosing may continue uninterrupted. If the AE resolves, reinstitution of treatment can occur but at a reduced dose as described in Table 5. If the AE recurs at the reduced dose, treatment will be held until the AE has resolved to ≤ grade 1 and when resolved treatment can be reinstituted at the next lower dose level. No more than 2 dose reductions are allowed during the maintenance cycles.

**Table 5: Hydroxychloroquine Dose Reduction Schema**

| Dose Level | Dose mg/day | First dose reduction | Second Dose reduction |
|---|---|---|---|

| | | | |
|---|---|---|---|
| 1 | 400 mg twice daily | 400mg am and 200mg pm | 200 mg in am and pm |
| 2 | 600 mg twice daily | 600mg am and 400mg pm | 400 mg in am and pm* |
| 1.5 | 400 mg am/ 600 mg pm | 400mg twice daily | 200 mg in am and pm |

- A third dose reduction by 50% of the dose may be pursued in patients deemed to be deriving clinical benefit from the combination to HCQ 200 mg in am and pm at the discretion of the treating physician.

Toxicities that may be attributable to HCQ include: nausea, anorexia, vomiting, constipation, diarrhea, rash, and visual field deficit. If any of these AEs occur at grade ≤ 2, HCQ may be continued and the AE managed with supportive care. For any AE with a grade ≥ 3, HCQ dose will be held until the toxicity resolves to ≤ grade 2, after which HCQ may be restarted at a reduced dose as described in table 5. With particular regard to visual field deficits patients should be cautioned to report any visual symptoms, particularly difficulty seeing entire words or faces, intolerance to glare, decreased night vision, or loss of peripheral vision. **These symptoms of retinal toxicity or subclinical evidence of retinal toxicity on eye exam should prompt drug discontinuation and ophthalmologic evaluation.** Patients who discontinue HCQ permanently may stay on nivolumab and stay on study at the discretion of the PI.

### 7.13 Dose reductions for toxicities that overlap nivolumab, ipilimumab and HCQ

Colitis, rash and fatigue can be seen with nivolumab, ipilimumab, and nivolumab-relatlimab and HCQ making it difficult to assign association with three drugs combined. For rash and fatigue, HCQ can be attempted to be dose modified first before nivolumab and ipilimumab, or nivolumab-relatlimab. However, given the potential life threatening nature of colitis, all three drug should be stopped for Grade 3 diarrhea or grade 2 diarrhea with other associated symptoms (abdominal pain, fever) that is clearly not responsive to conservative diarrhea prevention measures.

## 8.0 CORRELATIVE STUDIES

### 8.1 Estimated number of subjects

Maximum 70 subjects (combined enrollment in Phase 1a Nivolumab + HCQ dose escalation, Phase 2 nivolumab + HCQ, Phase 2 nivolumab-relatlimab + HCQ, and Phase 1b nivolumab/ipilimumab + HCQ). Currently correlative studies will only be done in anti-PD-1 refractory patients due to funding constraints . However, tissue and blood will be collected on Phase 2b Cohort (PD-1 naïve) patients as well.

### 8.2 Serial Tumor Biopsies and analysis

**Collection of Serial Tumor Biopsies**

The study will include optional serial tumor biopsies in any patient deemed appropriate for serial biopsy by the physician. If the tumor is not accessible by ultrasound guided biopsy or in office procedures, the patient may be deemed not appropriate for biopsy. Easily accessible lesions that do not require radiological guidance will be conducted either by the medical oncologists, surgical oncologist, dermatologists or dermatological surgeons. The procedures involved include 4 mm punch biopsies, excisional biopsies, excisions of large tumors in the operating room, and core needle biopsies. Lesions that are not easily accessible can be biopsied with the assistance of ultrasound guidance. This will involve core needle biopsies conducted by a radiologist. In all cases patients will be consented separately for the surgical procedure and provide their written informed consent. Tumor biopsy material will be divided on ice by the operator and fixed in

1) formalin
2) snap frozen
3) At the HUP main PENN site, fresh tumor tissue will be processed for flow cytomtery to assess TIL immunophenotyping

Timepoints for serial tumor biopsies:

1) baseline
2) 12+/- 2 days after IO therapy + HCQ is started
3) at progression

We anticipate 20 patients out of 51 anti PD-1 refractory patients will be amenable to biopsy and with an average of 2.5 biopsies per patient, we anticipate a total of 50 biopsies. We will budget 50 radiology-assisted biopsies.

### 8.3 Immunophenotyping on serially collected blood and tumor tissue

Checkpoint blockade results in a change in the subsets of tumor infiltrating lymphocytes, and response to anti-PD1 therapy is correlated with an increase in CD8 T cell infiltrate and PDL-1 expression in the tumor. There is also increasing data that suggest that HCQ modulates immunosuppressive myeloid populations in the tumor microenvironment. We have in vitro data that M2 (immunosuppressive) to M1 (anti-tumor) macrophage phenotype switching occurs with HCQ treatment. We have in vivo data that HCQ + PD1 results in a significant reduction in polymorphonuclear myeloid derived suppressor cells (PMN-MDSC).

Dr. Alexander Huang at the University of Pennsylvania has pioneered immunophenotyping of peripheral blood (measuring re-invigoration of exhausted phenotype CD8 T cells) in melanoma patients treated with anti-PD1 antibody pembrolizumab (Huang et al. *Nature 2017*). It is currently unknown whether a similar immunological response can be identified in patients on IO and autophagy targeted therapies. In patients undergoing serial biopsy, we will collect paired blood (Peripheral Blood Mononuclear Cells (PBMC)) and tumor specimens at pre-treatment, and 12 days , 4 weeks, 8 weeks, 12 weeks and 12 months on treatment (for nivo + HCQ or nivolumab + relatlimab + HCQ), or 3 weeks 6 weeks, 9 weeks, 12 weeks, 12 months for ipi + nivo. We will also use the freshly collected tumor piece to isolate immune infiltrates for 4-8 color multicolor immunofluorescence to interrogate the components of the immune system (CD8, Tregs, myeloid) in the tumor microenvironment and changes after nivolumab and HCQ or nivolumab and ipilimumab and HCQ.

Budget. We will focus immune analysis on patients who also undergo serial tumor biopsy and we anticipate there will be 20 patients who undergo serial tumor biopsy (pre and post). For these 20 patients, we will collect a peripheral blood at 4 time points, pre-treatment and weeks 2, 4, 8 post treatment for a total of 80 peripheral blood samples. In addition, CD45 cells will be isolated from 50 tumors. Funds are requested to purchase flurochrome tagged antibodies for flow cytometry analysis. Twenty-three separate antibodies to different targets will be purchased. There will be total of 130 samples (see above) each of which will be analyzed with 23 antibodies for a total of 2070 stains.

### 8.4 Tracking tumor specific T cells using combinatorial tetramer

We will test whether blockade of PD-1 and autophagy pathways alter the number, phenotype, and function of tumor-specific and viral-specific T cells in the tumor. We have recently established the combinatorial tetramer panel where we can track 10 antigen-specific T cell populations using 4 flow cytometry channels (Fig. 4). We will use this platform to screen for tumor and viral-specific T cells and track the kinetics of the immune response to PD1+autophagy blockade, and interrogate transcriptional profile, inhibitory expression, and function of tumor-specific T cells. On-target effects (tumor-specific changes), and off-target effects (viral-specific changes) can then be associated with clinical outcomes such as clinical response and irAEs.

### 8.5 Immunohistochemistry for markers of tumor immunity in serial biopsies

In parallel immune markers well be measured by IHC in serial tumor biopsies including MART1 (melanoma), CD8+ (cytotoxic T cells), F4/80 (TAM), Ly6G/C (MDSC). Using multiplexed IHC we will be able to determine if the addition of HCQ reduces PMN-MDSC, or converts M2 to M1 TAMs, and results in increased infiltration of T cells into the tumor.

### 8.6 Nanostring on serially collected tumor tissue

While informative, there are limited antibodies to a limited amount of targets for a comprehensive evaluation of the tumor microenvironment by immunofluorescence. It has been demonstrated that gene expression analysis is highly effective in characterizing the immune contexture of tumors using validated immune signatures. We will nanostring platform, which measures expression patterns for focused number of genes in paraffin-embedded tumors, before and after treatment, to compare changes in immune and tumor signatures after treatment with nivolumab, or nivolumab and ipilimumab, plus HCQ. 33

### 8.7 FFPE archival tumor tissue for immunohistochemistry

For all anti PD-1 refractory patients (51), we plan to obtain 20 unstained slides from archival FFPE blocks of ideally the latest metastatic lesion that was biopsied will be obtained. IHC assays for correlative autophagy biomarkers: We have identified a 2-gene signature involving Aldehyde dehydrogenase and helicase like transcription factor (HLTF) that predicts antitumor sensitivity of cancer cell lines to HCQ. We have developed IHC protocols for these genes. We hypothesize the patterns of expression of these genes previously identified in HCQ-S and HCQ-R cancer cell lines (Piao *Autophagy* 2017) will correlate with PFS. In addition, the following proteins will be measured by IHC: PDL1, HLTF, ALDH1A1, PPT1. Additional biomarkers and Immune cell staining will be conducted as the concept is developed further.

## 9.0 STUDY CALENDAR (TABLE 6)

(SEE APPENDIX B FOR RECOMMENDED VISIT BY VISIT ASSESSMENTS)

| | | During Protocol Therapy | | | | Post-Treatment |
|---|---|---|---|---|---|---|
| | **Baseline / Pre-RX[a]** | **Day 12 +/- 2 days** | **Each cycle[1]** | **End of protocol treatment (any reason)** | **Disease Progression** | **Follow-up***** |
| History and physical examination | X | | X | | | Safety follow-up 100 days after last dose of study drug |
| Vital Signs (particularly BP) | X | | X | | | |
| EKG[2] | X | | X | | | |
| Brain CT or MRI | X | | Every 3-6 cycles if brain metastases are present on baseline scan | | | As clinically indicated |
| Chest CT[3] | X | | Every 3 cycles and as clinically indicated | | | As clinically indicated |
| Abdomen/pelvis CT or MRI[3] | X | | Every 3 cycles and as clinically indicated<br>Confirmatory scans at least 4 weeks following initial documentation of objective response | | | As clinically indicated |
| CBC with differential | X | | X | X | | |
| Troponin Sodium, potassium, BUN, serum creatinine, glucose, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, LDH, albumin; TSH | X | | X | X | | |

| | | During Protocol Therapy | | | | Post-Treatment |
|---|---|---|---|---|---|---|
| | **Baseline / Pre-RX[a]** | **Day 12 +/- 2 days** | **Each cycle[1]** | **End of protocol treatment (any reason)** | **Disease Progression** | **Follow-up*****|
| PT/PTT | X | | | | | |
| Serum BHCG[4] | X | | | | | |
| Urine pregnancy test | X (within 24 hours of starting) | | Before each cycle (within 3 days of dosing) | | | |
| | | | | | | |
| **TREATMENT** | | | | | | |
| | | | | | | |
| **Phase 1a, 2a, 2b, 2c (HCQ+nivolumab)** | | | | | | |
| Nivolumab or nivolumab-relatlimab | | | X every 4 weeks | | | |
| Hydroxychloroquine | | | Twice every day | | | |
| | | | | | | |
| **Phase 1b (HCQ+nivolumab+ipilimumab)** | | | | | | |
| Nivolumab | | | X every 3 weeks x4 cycles<br><br>followed by Maintenance every 4 weeks (starting 6 weeks after last dose of ipi/nivo) | | | |
| Ipilimumab | | | X every 3 weeks x4 cycles | | | |

| | | During Protocol Therapy | | | | Post-Treatment |
|---|---|---|---|---|---|---|
| | **Baseline / Pre-RX[a]** | **Day 12 +/- 2 days** | **Each cycle[1]** | **End of protocol treatment (any reason)** | **Disease Progression** | **Follow-up*****|
| Hydroxychloroquine | | | Twice every day | | | |
| **CORRELATIVE STUDIES[8]** | | | | | | |
| Research tumor biopsy in biopsiable patients[5] | X | X | | | X | |
| Plasma collection for autophagy biomarker (1 purple top) | X for all patients | X day 12 only for biopsy pts | X[6] for all patients | X | | |
| PBMC collection (for biopsy patients only)[7] | X | X | X (4, 8, 12 weeks only for nivo + HCQ or nivo-rela + HCQ, or 3, 6, 9, 12 weeks for ipi + nivo). All patients with serial PBMC collection will have one additional draw at 12 months | | | |
| Archival Tumor tissue | X[9] | | | | | |

Standard of care *To account for major holidays and unforeseen events such as inclement weather, scheduled visits and testing can occur +/- 1 week, as long as there is documentation as to why they did not occur according to intended schedule

*** Follow-up: all subjects will be evaluated for at least 100 days after discontinuation of study treatment and will be followed for a minimum of one year from the start of treatment, unless disease progression is reported prior to one year. One-year survival information will be extracted from the medical record or by phone call for subjects who have discontinued protocol treatment and completed the 100 day follow-up evaluations prior to one year from the start of treatment.

a- eligibility labs need to be completed within 14 days before start of treatment. Eligibility/baseline scans need to be within 28 days of starting treatment.

**NOTE:** All visceral areas originally involved by tumor should be re-imaged every 3 cycles. In the case of bone scan, if negative at baseline, it may be deferred until the development of bone pain or a newly increased alkaline phosphatase.

1. A cycle constitutes 28 days or 4 weeks of nivolumab + HCQ or nivolumab-relatlimab+HCQ therapy and 21 days for the first 4 combined treatments with nivolumab + ipilimumab and HCQ, and 28 days thereafter for maintenance nivolumab and HCQ. Monthly tests and procedures should be done on Day 1 of each cycle +/- 1 week. Screening labs can be used for cycle 1 day 1 if obtained within 14 days.
2. If HCQ is discontinued, EKGs are only required if clinically indicated.
3. Standard of care imaging will be utilized for tumor measurements while on study. The preferred type of imaging is high resolution CT scans of the chest abdomen and pelvis with contrast. In cases of elevated creatinine or anaphylactic reaction to contrast dye, CT scans without contrast or MRI with gadolinium may be substituted for the abdomen and non-contrast CT for the chest. PET/CT scans may not be substituted unless the CT portion of the PET/CT is a high-resolution scan with contrast. Scans will be obtained every 12 weeks +/- 1 week. Ipi/nivo patients would be getting first ontreatment scans after 4 cycles and then after every 3 cycles of maintenance nivolumab.
4. For women of childbearing potential. Serum HCG within 28 days of starting treatment and urine pregnancy test within 24 hours of starting should be obtained.
5. Tumor biopsies will be obtained on patients that have easily accessible lesions, amenable to at least one biopsy, and whose biopsiable tumor is not the only RECIST measurable disease. Window for tumor biopsy will be +/- 2 days.
6. Plasma will be collected every cycle for the first 3 months in all patients.
7. Please refer to Section 8.3 for details of sample collection.
8. Patients are asked to continue drug treatment as prescribed and not hold any drug doses.
9. Archival tumor tissue can be obtained at any point during study enrollment and treatment timeline.

## 10.0 MEASUREMENT OF EFFECT

Patients will be evaluable for response if they have completed 75% of their expected dose of HCQ for 4 weeks of combination therapy with immune checkpoint inhibitor. These restrictions do not apply to patients that had a DLT (see Section 5.2 for rules regarding evaluability for patients experiencing DLT). For the purposes of this study, patients should be re-evaluated for response every 3 cycles (12 weeks). In addition to a baseline scan, confirmatory scans should also be obtained (not less than 4) weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. **Investigator assessment of RECIST response at each site will be used to make treatment decisions in real time.**

### 10.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with combined treatment with nivolumab and HCQ.

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, if they have completed 75% of their expected dose of HCQ for 4 weeks of combination therapy with nivolumab, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

### 10.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm by chest x-ray, as ≥10 mm with CT scan, or ≥10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

**Note**: Tumor lesions that are situated in a previously irradiated area are not considered measurable.

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

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

**Note**: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected based on their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible

repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion, which can be measured reproducibly, should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

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

### 10.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ³10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

### 10.4 Response Criteria

10.4.1 Evaluation of Target Lesions

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

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

Confirmed Partial Response (cPR): 2 scans in a row separate by 4 weeks that show a partial response.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

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

10.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis)

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

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

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

10.4.3 Evaluation of Best Overall Response

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

**Table 7. Determination of Best Overall Response**

| **Target Lesions** | **Non-Target Lesions** | **New Lesions** | **Overall Response** | **Best Overall Response when Confirmation is Required*** |
|---|---|---|---|---|
| CR | CR | No | CR | ≥4 wks. Confirmation** |
| CR | Non-CR / Non-PD | No | PR | ≥4 wks. Confirmation** |
| CR | Not evaluated | No | PR | |
| PR | Non-CR / Non-PD / not evaluated | No | PR | |
| SD | Non-CR / Non-PD/not evaluated | No | SD | Documented at least once ≥4 wks. from baseline** |
| PD | Any | Yes or No | PD | no prior SD, PR or CR |
| Any | PD*** | Yes or No | PD | |
| Any | Any | Yes | PD | |

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

** Only for non-randomized trials with response as primary endpoint.

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

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as

"*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

### 10.5 Duration of Response

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

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

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

### 10.6 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of first progression, death due to any cause or last patient contact alive and progression-free. In patients treated beyond progression, time to first progression (isolated) and second progression (multifocal) will be recorded.

### 10.7 Off Treatment/Off Study

Each subject has the right to withdraw from the study at any time without prejudice. The investigator may discontinue any subject's participation for any reason, including adverse event or failure to comply with the protocol. Should a subject withdraw from the study, the reason(s) must be stated on the case report form, and a final evaluation of the subject should be performed. Reasons for withdrawal include the following:

Progression of Disease: Remove patient from protocol therapy at the time progressive disease is documented.

Extraordinary Medical Circumstance: If at any time the treating physician feels constraints of this protocol are detrimental to the patient's health remove the patient from protocol therapy.

Patient's refusal to continue treatment: In this event, document the reason(s) for withdrawal.

Failure to comply with protocol (as judged by the investigator such as compliance below 80%, failure to maintain appointments, etc.).

Delay in treatment > 28 days due to toxicity

## 11.0 ADVERSE EVENTS AND REPORTING

The timely reporting of adverse events (including toxic deaths) is required by the Food and Drug Administration. The reporting of toxicities is part of the data reporting for this study. The investigator is responsible for ensuring that all adverse events (AEs) and significant adverse events (SAEs) that are observed or reported during the study are collected and reported to the FDA, appropriate IRB(s), in accordance with CFR 312.32 (IND Safety Reports).

### 11.1 Adverse Events

An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study that does not necessarily have a causal relationship with this treatment. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms

- leads to additional treatment or to further diagnostic tests is considered by the investigator to be of clinical significance

A ***non-serious adverse event*** is an AE not classified as serious.

**Note:** Although not always adverse events by regulatory definition, the following events associated with a BMS product must be reported.

- Exposure (to fetus) during pregnancy, exposure (to infant) during lactation, and paternal exposure
- Overdose
- Lack of efficacy
- Abuse
- Misuse
- Off-label use
- Occupational exposure
- Medication error and potential medication error
- Suspected transmission of an infectious agent, e.g., any organism, virus or infectious particle pathogenic or non-pathogenic, via the medicinal product.

**Adverse Event Reporting Period.**

The study period during which adverse events must be reported is defined as the period from the initiation of the first study treatment to 100 days following the last administration of study treatment.

**Post-study Adverse Event.**

All unresolved adverse events should be followed by the investigator until the events are resolved, the patient is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each patient to report any subsequent event(s) that the patient, or the patient's personal physician, believes might reasonably be related to participation in this study.

**Abnormal Laboratory Values.**

A clinical laboratory abnormality should be documented as an adverse event if the abnormality is of a degree, typically at least grade 2 and not present as grade 1 or better at baseline, that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation.

### 11.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. Adverse events will be measured and graded in accordance with the document entitled "Common Terminology Criteria for Adverse Events (CTCAE)", NCI version 5.0 issued by the US Department of Health and Human Services. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be at least possibly related to the study treatment or study participation should be recorded and reported immediately.

11.2.1 Serious Adverse Events

Adverse events are classified as serious or non-serious.

A serious adverse event is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- Suspected transmission of an infectious agent (e.g., pathogenic or nonpathogenic) via the study drug is an SAE.
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

**ADVERSE EVENT COLLECTION AND REPORTING INFORMATION:**

- All Serious Adverse Events (SAEs) that occur following the subject's written consent to participate in the study through 100 days of discontinuation of dosing must be reported to BMS Worldwide Safety, whether related or not related to study drug. If applicable, SAEs must be collected that relate to any later protocol-specified procedure (e.g., a follow-up skin biopsy).
- Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, are collected, including those thought to be associated with protocol-specified procedures. The investigator should report any SAE occurring after these aforementioned time periods, which is believed to be related to study drug or protocol-specified procedure.
- An SAE report should be completed for any event where doubt exists regarding its seriousness;
- If the investigator believes that an SAE is not related to study drug, but is potentially related to the conditions of the study (such as withdrawal of previous therapy or a complication of a study procedure), the relationship should be specified in the narrative section of the SAE Report Form.

An appropriate SAE form (e.g. ex-US = CIOMS form or USA = MedWatch form) should be used to report SAEs to BMS. If you prefer to use your own Institutional form, it must be reviewed by the BMS Protocol Manager prior to study initiation to ensure that at a minimum all of the data elements on the CIOMS form are present. Note: Please include the BMS Protocol number on the SAE form or on the cover sheet with the SAE form transmission.

  - The CIOMS form is available at: http://www.cioms.ch/index.php/cioms-form-i
  - The MedWatch form is available at: MedWatch 3500 Form
- For studies with long-term follow-up periods in which safety data are being reported, include the timing of SAE collection.
- The Sponsor will reconcile the clinical database AE cases (**case level only**) transmitted to BMS Global Pharmacovigilance (Worldwide.Safety@bms.com).

- The Investigator will request from BMS GPV&E, aepbusinessprocess@bms.com the SAE reconciliation report and include the BMS protocol number every 3 months and prior to data base lock or final data summary
- GPV&E will send the investigator the report to verify and confirm all SAEs have been transmitted to BMS GPV&E.
- 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 (Worldwide.Safety@bms.com).
- In addition to the Sponsor Investigator's responsibility to report events to their local HA, suspected serious adverse reactions (whether expected or unexpected) shall be reported by BMS to the relevant competent health authorities in all concerned countries according to local regulations (either as expedited and/or in aggregate reports).
- In accordance with local regulations, BMS will notify sponsor investigators of all reported SAEs that are suspected (related to the investigational product) and unexpected (i.e., not previously described in the IB). An event meeting these criteria is termed a Suspected, Unexpected Serious Adverse Reaction (SUSAR). Sponsor investigator notification of these events will be in the form of either a SUSAR Report or a Semi-Annual SUSAR Report.
    - Other important findings which may be reported by BMS as an Expedited Safety Report (ESR) include: increased frequency of a clinically significant expected SAE, an SAE considered associated with study procedures that could modify the conduct of the study, lack of efficacy that poses significant hazard to study subjects, clinically significant safety finding from a nonclinical (e.g., animal) study, important safety recommendations from a study data monitoring committee, or sponsor or BMS decision to end or temporarily halt a clinical study for safety reasons.
    - Upon receiving an ESR from BMS, the investigator must review and retain the ESR with the IB. Where required by local regulations or when there is a central IRB for the study, the sponsor will submit the ESR to the appropriate IRB. The investigator and IRB will determine if the informed consent requires revision. The investigator should also comply with the IRB procedures for reporting any other safety information.

SAEs, whether related or not related to study drug, and pregnancies must be reported to BMS within 24 hours \ 1 Business Day of becoming aware of the event. SAEs must be recorded on either CIOMS, MedWatch, or approved site SAE form.

Pregnancies must be reported and submitted to BMS. BMS will perform due diligence follow-up using the BMS Pregnancy Form which the investigator must complete.

**SAE Email Address:** Worldwide.Safety@BMS.com

**SAE Facsimile Number:** +1 609-818-3804

If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported.)

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours to BMS using the same procedure used for transmitting the initial SAE report.

All SAEs should be followed to resolution or stabilization.

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

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

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

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

**All adverse events that do not meet any of the criteria for serious should be regarded as non-serious adverse events.**

**NONSERIOUS ADVERSE EVENT**

- Non-serious Adverse Events (AE) are to be provided to BMS in aggregate via interim or final study reports as specified in the agreement or, if a regulatory requirement [e.g., IND US trial] as part of an annual reporting requirement.
- Non-serious AE information should also be collected from the time of the subject's written consent to participate in the study.

**Non-serious Adverse Event Collection and Reporting**

The collection of non-serious AE information should begin following the subject's written consent to participate in the study. All non-serious adverse events (not only those deemed to be treatment-related) should be collected continuously during the treatment period and for a minimum of **100** days following the last dose of study treatment.

Non-serious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up is also required for non-serious AEs that cause interruption or discontinuation of study drug and for those present at the end of study treatment as appropriate.

**Laboratory Test Abnormalities**

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

The following laboratory abnormalities should be documented and reported appropriately:

- any laboratory test result that is clinically significant or meets the definition of an SAE
- any laboratory abnormality that required the participant to have study drug discontinued or interrupted
- any laboratory abnormality that required the subject to receive specific corrective therapy.

It is expected that wherever possible, the clinical rather than laboratory term would be used by the reporting investigator (e.g., anemia versus low hemoglobin value).

Hospitalization, Prolonged Hospitalization or Surgery.

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should not be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

### 11.3 Assessment of Adverse Events

All AEs and SAEs whether volunteered by the subject, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be reported appropriately. Each reported AE or SAE will be described by its duration (i.e., start and end dates), regulatory seriousness criteria if applicable, suspected relationship to the study drug (see following guidance), and actions taken. To ensure consistency of AE and SAE causality assessments, only the PI or sub-investigators may grade AEs, and investigators should apply the following general guideline:

#### 11.3.1 Relationship to study drug: Yes

There is a plausible temporal relationship between the onset of the AE and administration of the study drug, and the AE cannot be readily explained by the subject's clinical state, intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response to the study drug; and/or the AE abates or resolves upon discontinuation of the study drug or dose reduction and, if applicable, reappears upon re-challenge.

#### 11.3.2 Relationship to study drug: No

Evidence exists that the AE has an etiology other than the study drug (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to study drug administration (e.g., cancer diagnosed 2 days after first dose of study drug).

Expected adverse events are those adverse events that are listed or characterized in the Package Insert or current Investigator Brochure.

Unexpected adverse events are those not listed in the Package Insert (P.I.) or current Investigator Brochure (I.B.) or not identified. This includes adverse events for which the specificity or severity is not consistent with the description in the P.I. or I.B. For example, under this definition, hepatic necrosis would be unexpected if the P.I. or I.B. only referred to elevated hepatic enzymes or hepatitis.

#### 11.3.3 Diagnosis vs. Signs and Symptoms

If known at the time of reporting, a diagnosis should be reported rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis

or syndrome at the time of reporting, it is ok to report the information that is currently available. If a diagnosis is subsequently established, it should be reported as follow-up information.

11.3.4 Deaths

All deaths that occur during the protocol-specified AE reporting period (see Section 12.1), regardless of attribution, will be reported to the appropriate parties. When recording a death, the event or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report "Unexplained Death".

11.3.5 Preexisting Medical Conditions

A preexisting medical condition is one that is present at the start of the study. Such conditions should be reported as medical and surgical history. A preexisting medical condition should be re-assessed throughout the trial and reported as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

11.3.6 Pregnancy

If a female subject becomes pregnant while receiving investigational therapy or within 120 days after the last dose of study drug, the investigator must immediately notify Worldwide.Safety@BAMS.com of this event via the Pregnancy Surveillance From (provided from BMS upon request) in accordance with SAE reporting guidelines. Follow-up to obtain the outcome of the pregnancy should also occur. Abortion, whether accidental, therapeutic, or spontaneous, should always be classified as serious, and expeditiously reported as an SAE. Similarly, details of the birth, and the presence or absence of any congenital anomaly/birth defect or maternal and/or newborn complications in a child born to a female subject exposed to the study drug should be reported as an SAE. Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the Pregnancy Surveillance Form [provided upon request from BMS]. In order for Sponsor or designee to collect any pregnancy surveillance information from the female partner must sign an informed consent form for disclosure of this information. Any pregnancy that occurs in a female partner of a male study participant should be reported to BMS. Information on this pregnancy will be collected on the Pregnancy Surveillance Form. 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.

## 11.4 UPENN Abramson Cancer Center (ACC)'s Data and Safety Monitoring Committee (DSMC) Notification by all Sites

The Data Safety and Monitoring Committee (DSMC) of the University of Pennsylvania's Abramson Cancer Center will monitor the data quality and adherence to safety rules. Additionally, the DSMC will review all safety/toxicity data for the trial and recommend trial suspension or termination as needed. Specific details of monitoring and audit frequency will be included in the monitoring plan, but will be at least every 6 months.

Medical Monitor

This study will be monitored in accordance with the DSMC monitoring plan. Dr. Noelle Frey, an independent clinician in the Department of Medicine, Division of Hematology Oncology, will serve as medical monitor and will consult on decisions made as a part of this trial as noted above. The medical monitor will perform a regular review and assessment of the number and type of serious adverse events (at minimum yearly, but may occur more frequently as needed).

It is the responsibility of the Principal Investigator to oversee the safety of the study. This safety monitoring will include careful assessment and appropriate reporting of adverse events, as noted above, as well as the construction and implementation of a site data and safety monitoring plan.

Notifying the Data Safety Monitoring Committee (DSMC) for the following Adverse Events

1. All grade 3 or higher events regardless of attribution or expectedness within 10 business days of knowledge.
2. All unexpected deaths within two business days of knowledge.
3. All other deaths within 30 days of knowledge. Deaths of subjects greater than 90 days from the last study treatment/intervention are not reportable.

AEs will be submitted to the DSMC through the Velos Clinical Trial Management System.

**Other reportable events:**

**Deviations:**

A deviation is a one time, unintentional action or process that departs from the IRB and DSMC approved study protocol, involving one incident and identified retrospectively, after the event occurred. If the impact on the protocol disrupts the study design, may affect the outcome (endpoints) or compromises the safety and welfare of the subjects, the deviation must be reported to the DSMC within 5 business days and the IRB within 10 business days.

**Prospective Deviations:**

A one time, intentional action (planned prospectively) or process that departs from the IRB and CTSRMC approved study protocol, intended for one occurrence. Approval must be obtained from the Sponsor and Medical Monitor prior to submitting a prospective deviation request to the DSMC. IRB and DSMC approval or acknowledgment is required prior to implementation.

Events not deemed reportable as outlined above will require a PI assessment regarding study and/or safety impact. This assessment should be documented appropriately.

### 11.5 UPENN IRB Notification by Investigator-Sponsor

The University of Pennsylvania IRB (Penn IRB) requires expedited reporting of those events related to study participation that are unforeseen and indicate that participants or others are at increased risk of harm. The Penn IRB will not acknowledge safety reports or bulk adverse event submissions that do not meet the criteria outlined below. The Penn IRB requires researchers to submit reports of the following problems within 10 working days from the time the investigator becomes aware of the event:

- Any adverse event (regardless of whether the event is serious or non-serious, on-site or off-site) that occurs any time during or after the research study, which in the opinion of the principal investigator is:

**Unexpected** (An event is “unexpected” when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.)

**AND**

**Related to the research procedures** (An event is “related to the research procedures” if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.)

Deaths occurring for patients on-study and within 30 days of study drug administration that are considered unforeseen and indicates participants or others are at increased risk of harm (i.e. unexpected and probably/definitely related), must be reported to the IRB within 24 hours of notification.

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

For clinical drug trials, the following events are also reportable to the Penn IRB:

- Any adverse experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any adverse event that would cause the sponsor to modify the investigators brochure, protocol or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:

  -- An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.

  -- Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.

  -- A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

The IRB will accept other reports when the investigator is unsure whether the event should be reported, and the IRB will review such reports to determine whether the event meets the threshold for an unanticipated event presenting risk to the participant.

Institutional Review Board
3600 Civic Center Blvd., 9th Floor, Philadelphia, PA 19104
Phone: 215-573-2540
Fax: 215-573-9438

11.5.1 Reporting Process to IRB at Penn

Principal Investigators are encouraged to submit reports of unanticipated problems posing risks to subjects or others using the form: "**Unanticipated Problems Posing Risks to Subjects or Others Including Reportable Adverse Events**" via HS-ERA or a written report of the event (including a description of the event with

information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation) within 7 working days and the PI or her designee will enter the event into HS-ERA and VELOS for Penn and participating sites. Participating sites should follow local, institutional guidelines on Event Reporting. For reportable deaths, the initial submission to the IRB may be made by contacting the IRB Director or designee at (T: 215.573.1206). All deaths will be reported via HS-ERA, as well.

11.5.2 FDA Notification by Investigator-Sponsor

This study is IND exempt and reporting to the FDA is voluntary using a MedWatch 3500 or via the FDA's website for voluntary reporting.

### 11.6 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his site. This safety monitoring will include careful assessment and appropriate reporting of adverse events, as noted above, as well as the construction and implementation of a site data and safety monitoring plan. Medical monitoring by an independent clinician Dr. Noelle Frey, Department of Medicine, Division of Hematology-Oncology will include a regular assessment of the number and type of serious adverse events on a periodic basis. Dr. Frey will be reached at Noelle.frey@uphs.upenn.edu or via telephone at (215) 662-6901.

### 11.7 Study Monitoring Plan

This study will be monitored by the Principal Investigator and sub-investigators, as appropriate. Such monitoring will include at least weekly meetings of the study team to review accrual, toxicity, SAEs. Dose escalations and study finding. In addition, the PI will ensure that data are completed in a timely manner and he or his designee will review the data for accuracy, completeness and integrity. Further, the PI will view real-time toxicity and all laboratory results on an ongoing basis by accessing the Monitoring Report database and the Protocol Labs database.

### 11.8 Auditing and Inspecting

The investigator will permit the Cancer Center's Administrative Director of Compliance and Auditing, or her designee to review records, data and facilities at mutually agreeable times. This study will be audited by the Department of Compliance and Monitoring (DCOM) on behalf of the Abramson Cancer Center Data Safety and Monitoring Committee (DSMC) every six months from the first subject enrolled and every 6 months thereafter for the life of the study. The DCOM may modify the frequency of auditing based on acceptable outcomes. Scheduling will occur in accordance with the process approved by the National Cancer Institute (NCI) and detailed in the NCI approved Institutional Data Safety and Monitoring Plan.

## 12.0 STATISTICAL CONSIDERATIONS

### 12.1 Phase 1 design

This is a phase I/exploratory phase II study. For the phase I portion, the primary objective is determining the MTD of HCQ when given in combination with daily nivolumab or nivolumab and ipilimumab in patients with advanced melanoma. Secondary endpoints include response rates, toxicity rates, and pharmacodynamic and pharmacokinetic correlative endpoints. Three patients per cohort will be treated for dose escalation in each group, and the dose escalation will take a stepwise fashion. If 1 out of 3 patients were defined as having a DLT during the phase 1 at a dose level, the cohort will be expanded to 6 patients to obtain the MTD for HCQ within this regimen for this patient population. The target DLT rate is 33%. Dose reduction will be pursued if observed DLT rate is in excess of 33%. The MTD will be defined as a) the dose producing DLT in < 2 out of 6 patients, or b) the dose level below the dose which produced DLT in ≥ 2 out of 3 patients, or in ≥ 3 out of 6 patients (Section 6 Tables 3 and 4).

The table below provides the operating characteristics of the dose escalation plan, as a function of the true DLT rate. For example, if the true DLT rate is 10%, the probability of dose escalation is 91%. If the true DLT rate is 50%, the probability of dose escalation is only 17%.

**Table 8: Operating Characteristics of the Dose Escalation Plan**

| True DLT Rate | Escalation Probability |
|---|---|
| .1 | .91 |
| .2 | .71 |
| .3 | .49 |
| .4 | .31 |
| .5 | .17 |
| .6 | .08 |
| .7 | .03 |
| .8 | .01 |
| .9 | .001 |

### 12.2 Sample size calculation for phase 2

Once the MTD has been determined we study the safety and preliminary efficacy in an open label 2 cohort 2 stage phase 2 trial involving 43 patients with anti-PD1 antibody naive disease (Arm A) and 27 patients with anti-PD1 Antibody refractory disease

Cohort 2a (PD1 Ab-refractory): Assuming the null hypothesis of a 5% response rate, a sample size of 27 patients will provide an 80% power to identify a response rate of 20% with a 0.05 level of significance. This will be a 2 stage design. The first stage will be 13 patients. If 0 patients achieve a PR the study will be terminated. If 1/13 or more patients achieve a response the second stage will be activated. If < 3 patients out of 27 patients achieve a response no further development of this combination is warranted unless a third compound is added

Cohort 2b (PD1Ab-naïve): Assuming the null hypothesis of a 45% response rate, a sample size of 43 patients will provide an 80% power to identify a response rate of 65% with a 0.05 level of significance. This will be a 2 stage design. The first stage will be 15 patients: if < 7 patients achieve a PR the study will be terminated; if ≥ 7 patients achieve a response the second stage will be activated. If < 24 patients out of 43 patients achieve a response no further development of this combination is warranted unless a third compound is added. If 15 patients are not accrued to the study in 36 months then this arm will be closed due to lack of feasibility.

Cohort 2c (PD-1 Ab-refractory): Assuming the null hypothesis of a 12% response rate, a sample size of 35 patients will provide an 80% power to identify a response rate of 30% with a 0.05 level of significance. This is an optimal 2-stage design that provides a 61% chance of stopping early for futility. The first stage will be 11 patients. If <2 patients achieve a PR the study will be terminated. If 2 or more patients achieve a response the second stage of 24 patients will be activated. If <8 patients out of 35 patients achieve a response no further development of this combination is warranted.

### 12.3 Analysis of Secondary Endpoints

For secondary and correlative endpoints, analyses will be primarily descriptive in nature in keeping with the study design. All adverse events and clinical responses will be tabulated and summarized. Exact 95% confidence intervals will be produced for adverse event and response rates at the MTD. For all patients treated, we will use waterfall plots to present tumor response measured using RECIST 1.1 criteria. We will present Kaplan-Meier curves for time-to-event endpoints including PFS for patients at the MTD. We assume, in the following plan, that any variables requiring transformation because of skewing or other violations of distributional assumptions will be appropriately transformed prior to analysis. Summary statistics (mean, median, standard deviation, range) will be used to describe distributions of various correlative markers at baseline and post-treatment timepoints. Fold change (post-treatment/baseline) of these outcomes will also be summarized, and the association with clinical response metrics will be evaluated. The association between

continuous outcome measures will be assessed using Pearson or Spearman correlation coefficients. Associations between categorical measures (e.g., gender and response) will be assessed using exact tests of association implemented in StatXact. The receiver operating characteristic curve (ROC) and the AUC will be computed to evaluate biomarkers that can potentially predict treatment response (e.g. exosomal PDL1). All analyses, except as otherwise noted, will be performed using either StatExact, SAS or STATA.

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**APPENDIX A**

| **ECOG PERFORMANCE STATUS*** | |
|---|---|
| **Grade** | **ECOG** |
| 0 | Fully active, able to carry on all pre-disease performance without restriction |
| 1 | Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work |
| 2 | Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours |
| 3 | Capable of only limited self-care, confined to bed or chair more than 50% of waking hours |
| 4 | Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair |
| 5 | Dead |

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The ECOG Performance Status is in the public domain therefore available for public use. To duplicate the scale, please cite the reference above and credit the Eastern Cooperative Oncology Group, Peter O'Dwyer, MD PhD, Group Chair.

**APPENDIX B**

| **Recommended Assessments by Visit Phase I, II ( See Calendar Section 9 for more details)** | |
|---|---|
| Visit | Assessments |
| Consent to Enroll on study | History, Physical |
| | EKG |
| | Troponin, CBC, Sodium, potassium, BUN, serum creatinine, glucose, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, LDH, albumin, PT, PTT, serum HCG; TSH |
| | Order CT/MRI chest abdomen and pelvis |
| | Tumor biopsy |
| Cycle 1 Day 1 | History, Physical |
| | EKG |
| | Troponin, CBC, Sodium, potassium, BUN, serum creatinine, glucose, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, LDH, albumin, urine HCG (within 24 hours of treatment); TSH |
| | Research blood draw per section 9 |
| Cycle 1 Day 12 | Tumor biopsy |
| | Research Blood (See Calendar) |
| Cycle 2 Day 1 | History, Physical |
| | EKG |
| | Troponin, CBC, Sodium, potassium, BUN, serum creatinine, glucose, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, LDH, albumin,); urine HCG (within 3 days of dosing); TSH |
| | Order CT/MRI chest abdomen and pelvis prior to next cycle |
| | Research Blood (See Calendar) |
| Cycle 3 Day 1 | History, Physical |
| | EKG |
| | Troponin, CBC, Sodium, potassium, BUN, serum creatinine, glucose, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, LDH, albumin,); urine HCG (within 3 days of dosing); TSH |
| | Research Blood (see Calendar) |
| Cycle 4 and beyond | Repeat Cycle 1-3 as above |
| End of Study/Disease Progression | History, Physical |
| | Troponin, CBC, Sodium, potassium, BUN, serum creatinine, glucose, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, LDH, albumin,; TSH |
| | Research Blood (see Calendar) |
| | Tumor Biopsy |

| **Recommended Assessments by Visit Phase I (4-drug) (See Calendar Section 9 for details)** | |
|---|---|
| Visit | Assessments |
| | |
| Consent to Enroll on study | History, Physical |
| | EKG |
| | Troponin, CBC, Sodium, potassium, BUN, serum creatinine, glucose, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, LDH, albumin, PT, PTT, serum HCG; TSH |
| | Order CT/MRI chest abdomen and pelvis |
| | Tumor biopsy |
| | |
| Cycle 1 Day 1 | History, Physical |
| | EKG |
| | Troponin, CBC, Sodium, potassium, BUN, serum creatinine, glucose, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, LDH, albumin, urine HCG (within 24 hours of treatment); TSH |
| | Research blood draw per section 9 |
| | |
| Cycle 1 Day 12 | Tumor biopsy |
| | Research Blood (See Calendar) |
| | |
| Cycle 2 Day 1 | History, Physical |
| | EKG |
| | Troponin, CBC, Sodium, potassium, BUN, serum creatinine, glucose, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, LDH, albumin; urine HCG (within 3 days of dosing); TSH |
| | Research Blood (See Calendar) |
| | |
| Cycle 3 Day 1 | History, Physical |
| | EKG |
| | Troponin, CBC, Sodium, potassium, BUN, serum creatinine, glucose, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, LDH, albumin,; urine HCG (within 3 days of dosing); TSH |
| | Research Blood (see Calendar) |
| | Order CT/MRI chest abdomen and pelvis prior to next cycle |
| | |
| Cycle 4 | History, Physical |
| | EKG |
| | Troponin, CBC, Sodium, potassium, BUN, serum creatinine, glucose, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, LDH, albumin,); urine HCG (within 3 days of dosing); TSH |
| | Research Blood (See Calendar) |
| | |
| Maintenance Cycle 1 | History, Physical |
| | EKG |
| | Troponin, CBC, Sodium, potassium, BUN, serum creatinine, glucose, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, LDH, albumin; urine HCG (within 3 days of dosing); TSH |
| | Research Blood (see Calendar) |
| | |

| **Recommended Assessments by Visit Phase I (4-drug) (See Calendar Section 9 for details)** | |
|---|---|
| Visit | Assessments |
| | |
| Maintenance Cycle 2 | History, Physical |
| | EKG |
| | Troponin, CBC, Sodium, potassium, BUN, serum creatinine, glucose, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, LDH, albumin, urine HCG (within 3 days of dosing); TSH |
| | Research Blood (see Calendar) |
| | Order CT/MRI chest abdomen and pelvis prior to next cycle |
| | |
| Maintenance Cycle 3 | History, Physical |
| | EKG |
| | Troponin, CBC, Sodium, potassium, BUN, serum creatinine, glucose, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, LDH, albumin,; urine HCG (within 3 days of dosing); TSH |
| | Research Blood (see Calendar) |
| | |
| Maintenance Cycle 4 and beyond | Repeat Maintenance Cycle 1-3 |
| | |
| End of Study/Disease Progression | History, Physical |
| | Troponin, CBC, Sodium, potassium, BUN, serum creatinine, glucose, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, LDH, albumin, TSH |
| | Research Blood (see Calendar) |
| | Tumor Biopsy |