UPCC 21520

The LIMIT *KRAS* Mutant NSCLC Trial: Lysosome Inhibition to Enhance MAPK Inhibition Targeting KRAS Mutant NSCLC: A Phase 2 Open Label Trial of Binimetinib and Hydroxychloroquine in Patients with Advanced *KRAS* Mutant Non-Small Cell Lung Cancer

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STUDY SUMMARY

Title	The LIMIT *KRAS* Mutant NSCLC Trial: Lysosome Inhibition to Enhance MAPK Inhibition Targeting *KRAS* Mutant NSCLC: A Phase 2 Open Label Trial of Binimetinib and Hydroxychloroquine in Patients with Advanced *KRAS* Mutant Non-Small Cell Lung Cancer
Protocol Number	UPCC 21520
Phase	Phase 2
Methodology	Single Arm
Study Duration	2 years
Study Center(s)	Single Center
Objectives	Primary Objectives • Determine the Objective Response Rate (ORR) of binimetinib (B) and hydroxychloroquine(HCQ), as measured by RECIST 1.1 • Determine the safety/tolerability of B+ HCQ, as measured by CTCAE 5.0 Secondary Objectives • Progression-Free Survival • Overall Survival • Measurement of changes in circulating tumor DNA (ctDNA) KRAS allelic frequency • Measurement of changes in biomarkers related to autophagy
Number of Subjects	Stage 1: 10 patients Stage 2: 19 patients Total: Up to 29 patients
Diagnosis and Main Inclusion/Exclusion Criteria	• Must be ≥ 18 years of age. • Metastatic or incurable NSCLC • Presence of a non-synonymous mutation in *KRAS* • Must have received at least one prior systemic therapy for metastatic NSCLC, or be intolerant/otherwise ineligible for such therapy • Must have ECOG performance status of 0 or 1 • Must have adequate renal, liver, cardiac and hematological function • Must be able to provide written informed consent. • Must have at least one measurable lesion according to Response Evaluation Criteria for Solid Tumors (RECIST 1.1) • Patients with asymptomatic or treated, stable brain metastases are allowed to enroll. Patients with carcinomatous meningitis are excluded.

Study Product, Dose, Route, Regimen	Hydroxychloroquine 400 mg by mouth every 12 hours (obtained commercially), binimetinib 45 mg 2 times daily
Duration of administration	Until progression, unacceptable toxicity, or withdrawal of consent.
Study design	Phase 2: Optimal Simon 2-stage design with the first stage recruiting 10 patients, and 19 additional patients if there are ≥ 2 responses seen.

Duration of trial	• Approximately 2 years
	• Follow-up:
	  ○ All subjects will be evaluated per study calendar for 6 months after discontinuation of study treatment
	  ○ 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 Objectives

- To assess the clinical efficacy of HCQ + B by ORR.
- Determine the safety/tolerability of HCQ + B as measured by CTCAE 5.0.

1.2 Secondary Objectives

- To measure the progression-free survival among patients treated with HCQ+B
- To measure the overall survival among patients treated with HCQ+B.

1.3 Correlative Objectives

- To evaluate the correlation between response to HCQ + B and pre-treatment tumor staining for PPT1, LC3, ALDH1A1, and HLTF by immunohistochemistry.
- To monitor changes on a repeat biopsy after 3 weeks of treatment with HCQ + B in PPT1, LC3, ALDH1A1, and HLTF by immunohistochemistry, as well as in gene signatures.
- To measure the change in median number of autophagic vesicles/cell (mAV/cell) in serially collected tumor tissue with HCQ + B.
- To determine if blood based candidate biomarkers of autophagy modulation (including IL-8, Interleukin-1 Beta, LIF, DKK3, and FAM3C, and other serum cytokines) reflect autophagy dynamics in tumors of patients treated with HCQ + B.
- To characterize HCQ pharmacokinetics when HCQ is combined with binimetinib
- To monitor changes in allelic fraction of KRAS in circulating tumor DNA (ctDNA) among patients treated with HCQ + B

2.0 BACKGROUND AND RATIONALE

2.1 Patient population

Non-small cell lung cancer (NSCLC) is the leading cause of cancer death for both men and women in the United States. The use of targeted therapy based on molecular profile has completely changed the treatment approach for patients with genetic alterations in the EGFR, ALK, and ROS1 genes. Such patients are now routinely treated with tyrosine kinase inhibitors(TKIs), and the overall survival seen in these patient populations is substantially longer than the general population of patients with NSCLC. Despite being the most commonly mutated gene in non-squamous NSCLC, KRAS has been historically very difficult to target. The relative resistance of *KRAS* mutant NSCLC to TKI based therapy is likely related to the very strong affinity of KRAS for its natural ligand. While there have been some emerging advances for *KRAS G12C* mutant NSCLC using allosteric inhibitors, there have been nearly no advances in the targeted therapy of patients with other *KRAS* mutations.

2.2 Rationale for combined Lysosomal and Autophagy inhibition

The lysosome has been found to be critical for the growth of pancreatic cancer (Perera *Nature* PMC5086585). Kimmelman's group has published multiple papers demonstrating that autophagy is critical for pancreatic cancer growth and targeting with chloroquine derivatives produces cytotoxicity in most pancreatic cancer cell lines and patient derived xenografts (Yang *Cancer Discovery* 2014; PMC4125497). The University of Pittsburgh group has done extensive preclinical and clinical work on combining HCQ with chemotherapy. A phase I/II trial of 35 patients examined the neoadjuvant treatment with HCQ and gemcitabine in patients with borderline resectable pancreatic adenocarcinomas (Boone Annals of Surgery 2015; PMC4663459). 1200 mg/day of HCQ was taken for 31 days up until the day of surgery combined with two doses of a fixed-dose 1500 mg/m^2 gemcitabine. DLT or grade 4/5 events did not occur during this trial. 19/35 patients had a decrease in surrogate biomarker response (CA 19-9) and 29/35 patients underwent surgical resection as scheduled. This trial demonstrated that HCQ with gemcitabine is safe and tolerable, and showed encouraging activity in the neoadjuvant setting. A randomized phase II trial with gemcitabine, abraxane, with or without HCQ in resectable pancreatic cancer patients has been launched (NCT01978184), and a randomized phase II study of gemcitabine, abraxane, with or without HCQ in the metastatic setting (NCT01506973) found that HCQ added to chemotherapy significantly improved response rate (Karasic JAMA Oncology 2019; PMC6547080). The safety and activity of combining dabrafenib (BRAF inhibitor) trametinib (MEK inhibitor) and HCQ has been preliminarily demonstrated in an ongoing Phase I/II trial in BRAF mutant melanoma patients (See below).

Recently published data supporting MEK inhibitor + HCQ combination for *Ras* mutant cancer.
Three separate teams of investigators at the University of Utah, University of North Carolina Chapel Hill/NYU, and NCI/UCSF have found stunning activity of combining HCQ with either MEK (University of Utah) or ERK inhibition (UNC) in preclinical models of *KRAS*-driven pancreas cancer. The Utah group included melanoma colon and lung cancer xenografts with *RAS* mutations. In all cases the combination of a MEK inhibitor with HCQ was synthetically lethal to *RAS* mutant cancers, with tumors regressing in each case (Kinsey Nature Medicine 2019 PMID:30833748; Bryant Nature Medicine 2019 PMID:30833752; Lee PNAS 2019 PMC6410784). This data provides the rationale for studying a MEK inhibitor in combination with HCQ in *KRAS* mutant non-small cell lung cancer.

2.3 Binimetinib

Binimetinib (also known as MEK162, ARRY-438162 or ONO-7703) is an orally bioavailable, selective and potent mitogen-activated protein (MAP) kinase kinase (MEK)1 and MEK2 inhibitor. Binimetinib, given in combination with encorafenib, has received marketing approval in several jurisdictions for the treatment of patients with *BRAF*-mutant melanoma as per local prescribing information.

2.3.1 Binimetinib: Clinical Safety

Binimetinib has been administered as a single agent to 943 patients with cancer across multiple studies. The most frequently reported adverse events with single agent binimetinib treatment have been blood creatine kinase elevation, diarrhea, acneiform dermatitis, peripheral edema, rash, nausea and fatigue. The majority of these adverse events have been grade 1-2, with the exception of blood creatine kinase levations, which was seen in 21% at a grade 3-4 level in patients with melanoma and 29% of patients with low grade serous carcinoma of the ovary, fallopian tube, or primary peritoneum.

2.4 Hydroxychloroquine (HCQ)

Chloroquine (CQ) is a synthetic 4-aminoquinoline that has been used for 80 years in humans for malaria prophylaxis and treatment, rheumatoid arthritis, and human immunodeficiency virus (HIV). It is an inexpensive orally available drug that has CNS penetration. It has a large therapeutic index, and its most predictable cumulative toxicity is retinopathy (Marmor et al., 2011). It is this toxicity and worldwide malarial resistance to CQ that lead to discontinuation of extensive research into CQ's non-malarial applications. Chloroquine derivatives such as HCQ are still used extensively in rheumatoid arthritis and lupus erythematosis and have a larger therapeutic index. The chemical structure of CQ derivatives allows them to serve as a weak base which is trapped in acidic cellular compartments (Poole and Ohkuma, 1981). Thus chloroquine derivatives deacidify lysosomes, inhibiting the last step in autophagy. With this last step blocked, a cell reliant on autophagy will increase the generation of autophagosomes and will eventually undergo either apoptotic or non-apoptotic cell death. Evidence in mouse models and human cancer cell lines suggest CQ may have significant anti-tumor activity by inhibiting autophagy induced by cancer therapy (Amaravadi *Cancer Discovery* 2019 PMID:31434711)

2.4.1 Clinical Experience of HCQ in cancer trials

We have previously published the first 6 clinical trials involving combinations of HCQ and cancer drugs (Vogl Autophagy 2014 PMC4203515: Rosenfeld Autophagy 2014 PMC4203513; Rangwala Autophagy 2014 PMC4203514; Rangwala Autophagy 2014 PMC4203516; Mahalingam Autophagy 2014 PMC4203517; Barnard Autophagy 2014 PMC4203518). This effort has demonstrated the safety of the approach in patients with advanced solid tumors. Our preliminary data in 2 phase I studies (presented at the Society of Melanoma Research 2016) demonstrates the safety and striking activity of combined BRAF and autophagy inhibition in *BRAF* mutant melanoma. We launched a Phase I trial of vemurafenib and HCQ in $BRAF^{V600E}$ melanoma patients (NCT01897116). 7 patients in the 1st dose level (vemurafenib 960 po bid + HCQ 400 po bid) had 2 dose limiting toxicities (DLT; grade 3 rash and grade 3 transaminitis evaluable for response had PR or CR (median max target shrinkage -62% [-38- -100%]). Prolonged PFS was seen in 1 CR (36+ mo) and 1 PR (20 mo). Combined BRAF/MEK inhibition was adopted widely so this trial was closed, the BAMM trial, a Novartis funded multi-institution phase I/II trial of dabrafenib (D), trametinib (T) and HCQ was opened (NCT02257424). Phase I was completed with no DLT (n=7). Recommended phase II dose was HCQ 400 bid with D+T. Phase II enrollment continues. D+T+HCQ was well tolerated, with no evidence of significant ocular toxicity on extensive serial ophthalmological exams (Nti Retina 2018; PMC6039280).

Striking responses were observed: 90% patients have responded and 48% of patients have had an investigator-assessed complete response. Median PFS is roughly 18 months. The only non-responder was found to have *BRAF*^{V600E} amplification, and had pyrexia that required frequent dose interruptions.

2.5 Rationale for correlative studies

To test the hypothesis that HCQ could enhance the therapeutic efficacy of B by inhibiting autophagy, this clinical trial will incorporate several correlative studies. First, we will include biopsies before and after starting treatment with HCQ + B. 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. Next, through serial blood draws, we can investigate non-invasive markers by which autophagic dynamics may be detected. We have selected certain candidate biomarkers such as IL-8, Interleukin-1 Beta, LIF, DKK3, and FAM3C, based on in vitro data that shows elevated serum levels in human patients during high autophagy states (Kraya *Autophagy* 2015 PMC4502670). Finally, we aim to investigate the immune consequences of autophagy inhibition by measuring serum cytokine levels using luminex technology.
For all patients enrolled, 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). 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: Phospho ERK, cleaved caspase 3, PPT1. Additional biomarkers and Immune cell staining will be conducted as the concept is developed further.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

Each subject must meet all of the following inclusion criteria to be enrolled in the study:

1) Metastatic or incurable NSCLC
2) Presence of a non-synonymous mutation in KRAS
3) Patient must have received at least one prior systemic therapy for metastatic NSCLC or be intolerant/ineligible/refuse available therapies with known benefit
4) Ability and willingness to sign a written informed consent document
5) Age ≥18 years old
6) At least one measureable lesion according to Response Evaluation Criteria in Solid Tumors (RECIST) 1.1
7) ECOG performance status 0-1

8) Adequate organ function, as defined below

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,250/mcL
Platelets	≥100,000/mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L
Renal	
Serum creatinine OR Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) OR ≥50 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN OR
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN OR ≤ 5 X ULN for subjects with liver metastases
[a]Creatinine clearance should be calculated per institutional standard.	

9) Women of childbearing potential must have a negative serum pregnancy test performed within 72hours of the first dose of study therapy. Subjects of reproductive potential must agree to use acceptable birth control methods (see Appendix B for childbearing potential).

10) Qtc < 500 mSec on EKG

11) Must be able to swallow tablets

12) Must be willing to comply with protocol procedures (including completion of diaries and outcome measures

3.2 Exclusion Criteria

A subject will not be eligible for inclusion in this study if the following criteria apply:

1) Currently participating in or has participated in a study of an investigational agent or anticipated use of an investigational device within 4 weeks of the first dose of study treatment.

2) Untreated symptomatic central nervous system (CNS) metastases and/or carcinomatous meningitis.

3) Prior monoclonal antibody within 4 weeks prior to enrollment, or individuals who have not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.

4) Known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin, non-invasive bladder tumors, or in situ cervical cancer

5) Active infection requiring systemic therapy with IV antibiotics

6) History or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.
7) Known psychiatric or substance abuse disorders as documented in the chart that, in the opinion of the investigator, would interfere with cooperation with the requirements of the trial.
8) Pregnant or breastfeeding women
9) Anticipated receipt of any live vaccine within 30 days prior to the first dose of trial treatment.
10) Have a known immediate or delayed hypersensitivity reaction or idiosyncrasy to drugs chemically related to study drug, or excipients or to dimethyl sulfoxide (DMSO).
11) Patients receiving cytochrome P450 enzyme-inducing anticonvulsant drugs (EIADs) (i.e. phenytoin, carbamazepine, Phenobarbital, primidone or oxcarbazepine) *within 4 weeks of the start of the study treatment*
12) Known Hepatitis B Virus (HBV), or Hepatitis C Virus (HCV) infection (subjects with laboratory evidence of cleared HBV and/or HCV will be permitted)
13) Patients with a previously documented retinal vein occlusion.
14) History or evidence of increased cardiovascular risk including any of the following:
    - Current clinically significant uncontrolled arrhythmias.

      Exception: Subjects with controlled atrial fibrillation for > 30 days prior to randomization are eligible.
    - History of acute coronary syndromes (including myocardial infarction and unstable angina), coronary angioplasty, or stenting within 6 months prior to randomization.
    - Ejection fraction of ≤50% as measured by echocardiography or MUGA
15) Any other conditions judged by the investigator that would limit the evaluation of the subject.

4.0 TREATMENT PLAN

4.1 Overview

Patients will be treated with B 45 mg two times daily and HCQ 400 mg twice daily beginning on day 1. The dose of HCQ is based on an ongoing Phase 1 trial, and may be modified in a future amendment prior to the first patient enrolled. Efforts will be made to ensure dose homogeneity throughout the trial. Treatment will be administered on an outpatient basis on a 28 day cycle. Reported adverse events and potential risks for HCQ and B are described in Section 6. Appropriate dose modifications for HCQ and B are described in Sections 5 and 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

4.2 Hydroxychloroquine 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. Tablets of HCQ are available in 200 mg strength. HCQ will be administered in divided doses (every 12 hours) with or without food. **To enhance compliance and assist in pharmacokinetic analysis, 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. Modification of the schedule in case of GI symptoms is allowed. Missed doses do not need to be replaced. On days of clinic visits, patients will be asked the date / time / dose of last HCQ taken. This information will be documented on the CRF. Patients will be asked to take HCQ continuously, as prescribed.

4.3 Binimetinib administration

The starting dose for B is 45 mg BID. B will be administered in divided dose (every 12 hours) on either an empty or full stomach. The two daily doses of B should be taken 12 hours apart, for example, 9 AM and 9 PM.

4.4 Concomitant Medication and Procedures

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. Zonisamide is not recommended as this drug accumulates in red blood cells where HCQ also accumulates. 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 (see Section 4.5). Binimetinib does not need to be taken fasting. All other concomitant medications are permitted, but P-gp and BCRP inhibitors are to be administered with caution.

4.5 Duration of Protocol Treatment and Follow-up

Treatment will continue until disease progression and may continue at the discretion of the treating physician in consultation with the Principal Investigator, if there is an isolated progression. Treatment may also be discontinued for adverse events that delay treatment greater than 28 days and as outlined in Toxicities and Dose Modifications. Please refer to Off Treatment/Off Study for additional reasons for study withdrawal. Subjects will be evaluated for at least 6 months 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 6 month follow-up evaluations prior to one year from the start of treatment.

5.0 TOXICITY CRITERIA, MONITORING, DOSE DELAYS AND MODIFICATIONS

5.1 Toxicity Criteria

This study will utilize the CTCAE version 5 for toxicity and Adverse Event Reporting. A copy of the CTCAE version 5.0 can be downloaded from the CTEP home page (http://ctep.cancer.gov). All appropriate treatment areas should have access to a copy of the CTCAE version 5.0.

5.2 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 it 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 is not resolved in ≤ 28 days, patient will be taken off treatment, unless there is an exception granted by the medical monitor.

5.3 Toxicities and Dose Modifications

Guidelines for specific toxicities are outlined below, but general guidelines for toxicities not listed are provided in Table 5. Of note, if a toxicity is attributable only to one of the investigational drugs, the other investigational agent can be continued if tolerated.

General Dose modification guidelines for binimetinib for toxicities attributable to only binimetinib. These are general guidelines but for specific toxicities dose modification instructions may be different Doses of binimetinib should be adjusted for AEs throughout the study (see Table 1 Recommended Dose Levels). In general, doses should not be reduced or interrupted for Grade 1 AEs unless the AE is a specific ocular AE referred to in the Recommended Dose Modifications Table (see Table 2) but treatment to control symptoms should be provided as appropriate, if applicable.

An individual patient may have their dose of binimetinib reduced to the dose levels in Table 1 below:

Table 1 Recommended Dose Levels

	Binimetinib (mg BID)
Starting Dose	45
Dose Level -1	30
Dose Level -2	15

The lowest recommended dose level of binimetinib is 15 mg BID. When the AE that resulted in a dose reduction improves to and remains stable at the patient's baseline level for a minimum of 14 days, the dose can be re-escalated to the next dose level at the discretion of the Investigator, provided there are no other concomitant toxicities that would prevent drug re-escalation. There is no limit to the number of times the patient can have their dose reduced or re-escalated, however:

- No dose re-escalation of binimetinib is allowed after a dose reduction due to LVEF dysfunction
- No dose re-escalation of binimetinib is allowed after a dose reduction due to retinal toxicity ≥ Grade 2.

Please refer to Dose Modification Table for recommended dose modifications for binimetinib, if applicable, based on the occurrence of treatment-related AEs.

Table 2 Recommended Dose Modifications

In all cases except retina disorders, HCQ may be continued at the discretion of the treating physician

Severity of Adverse Event	Dose Modifications
Cardiomyopathy	
Asymptomatic, absolute decrease in LVEF of > 10% from baseline that is also below the LLN	Withhold binimetinib for up to 4 weeks, evaluate LVEF every 2 weeks. Resume binimetinib at a reduced dose if the following are present: • LVEF is at or above the LLN and • Absolute decrease from baseline is 10% or less and • Patient is asymptomatic. If LVEF does not recover within 4 weeks permanently discontinue binimetinib.
Grade 3-4 (Symptomatic congestive heart failure or absolute decrease in LVEF of > 20% from baseline that is also below LLN)	Permanently discontinue binimetinib. Closely monitor LVEF until resolution or up to 16 weeks.
Venous Thromboembolism	
Uncomplicated DVT or PE	Withhold binimetinib. • If improves to Grade 0-1, resume at a reduced dose. • If no improvement, permanently discontinue binimetinib.
Life threatening PE	Permanently discontinue binimetinib.
Serous Retinopathy	
Symptomatic serous retinopathy/ Retinal pigment epithelial detachments	Withhold binimetinib for up to 10 days. • If improves and becomes asymptomatic, resume at the same dose. • If not improved, resume at a lower dose level or permanently discontinue binimetinib.
Retinal Vein Occlusion (RVO)	
Any Grade	Permanently discontinue binimetinib.
Uveitis	
Grade 1-3	If Grade 1 or 2 does not respond to specific ocular therapy, or for Grade 3 uveitis, withhold binimetinib for up to 6 weeks. • If improved, resume at same or reduced dose. • If not improved, permanently discontinue binimetinib.
Grade 4	Permanently discontinue.

Severity of Adverse Event	Dose Modifications
Other Eye Disorders (i.e., Non-retinal Events, non-Uveitis Events	
Grade 1-2	Maintain dose level of binimetinib and increase frequency of ophthalmic monitoring to at least every 14 days until stabilization or resolution.
Grade 3	Interrupt dosing of binimetinib and refer patient to ophthalmologist within 7 days. • If resolved to Grade ≤ 1 in ≤ 21 days, resume treatment at 1 reduced dose level of binimetinib. • If not resolved to Grade ≤ 1 in ≤ 21 days, permanently discontinue binimetinib and close follow-up with ophthalmic monitoring until stabilization or resolution.
Grade 4	Permanently discontinue binimetinib and immediate follow-up with ophthalmic monitoring until stabilization or resolution.
Interstitial Lung Disease	
Grade 2	Withhold binimetinib for up to 4 weeks. • If improved to Grade 0-1, resume at a reduced dose. • If not resolved within 4 weeks, permanently discontinue.
Grade 3 or Grade 4	Permanently discontinue.
Hepatotoxicity	
Grade 2 AST or ALT increased	Maintain binimetinib dose. If no improvement within 2 weeks, withhold binimetinib until improved to Grade 0-1 or to pretreatment/baseline levels and then resume at the same dose.
Grade 3 or 4 AST or ALT increased	See Other Adverse Reactions
Rhabdomyolysis or Creatine Phosphokinase (CPK) elevations	
Grade 4 asymptomatic CPK elevation or Any Grade CPK elevation with symptoms or with renal impairment	Withhold binimetinib dose for up to 4 weeks. If improved to Grade 0-1 resume at a reduced dose. If not resolved within 4 weeks, permanently discontinue binimetinib.
Dermatologic	
Grade 2	If no improvement within 2 weeks, withhold until Grade 0-1. Resume at same dose if first occurrence or reduce dose if recurrent.
Grade 3	Withhold until Grade 0-1. Resume at same dose if first occurrence or reduce dose if recurrent. If recurrent and already on dose level -2, resume at that dose.
Grade 4	Permanently discontinue
Nausea/Vomiting	
Grade 1-2	Maintain dose level of binimetinib. Promptly institute antiemetic measure.

Severity of Adverse Event	Dose Modifications
Grade 3	Interrupt dosing of binimetinib until resolved to Grade ≤ 1. Then resume treatment at 1 reduced dose level of binimetinib. Resume treatment with binimetinib at the current dose if, in the judgment of the Investigator, the toxicity is considered to be unrelated to binimetinib, or at 1 reduced dose level. Note: Interrupt dosing of binimetinib for ≥ Grade 3 vomiting or Grade 3 nausea only if the vomiting or nausea cannot be controlled with optimal antiemetics (as per local practice).
Grade 4	Permanently discontinue binimetinib.
Other Adverse Reactions (including haemorrhage)	
Recurrent Grade 2 or First occurrence of any Grade 3	Withhold for up to 4 weeks. • If improves to Grade 0-1 or to pretreatment/baseline levels, resume at reduced dose. • If no improvement, permanently discontinue.
First occurrence of any Grade 4	Permanently discontinue or withhold for up to 4 weeks. • If improves to Grade 0-1 or to pretreatment/baseline levels, then resume at a reduced dose. • If no improvement, permanently discontinue.
Recurrent Grade 3	Consider permanently discontinuing.
Recurrent Grade 4	Permanently discontinue.

Investigators should always err on the side of caution in these settings if treatment-related toxicity is a possibility.

5.3.1 Ocular Toxicity

Due to the known ocular toxicities associated with MEK inhibitors and the potential for toxicity with HCQ, ophthalmic exams will be mandatory for all patients until protocol treatment is discontinued, as indicated in study calendar. In addition, an ophthalmologist should be consulted if changes in vision develop. However, if the visual changes are clearly unrelated to study treatment (e.g., allergic conjunctivitis), then monitor closely as it may be reasonable to defer ophthalmic examination.

Monitor patients for visual signs and symptoms (such as, change in vision, photophobia and eye pain) during therapy. Special attention should be given to retinal findings (e.g., retinal pigment epithelial detachment (RPED) or retinovascular abnormalities (i.e., branch or central retinal vein occlusions (RVO).

The ocular component of this clinical trial will be important in better understanding ophthalmic risk inherent to the drug combination. All key subjective complaints, exam findings and testing

data will be entered into a study specific case report form for analysis and summarization at the conclusion of the trial.

Table 3 Ocular Examination timeline

	Examination components[1]				
Timepoint[2]	Comprehensive eye exam[3]	OCT	10-2 Humphrey visual fields	Fundus photography[4]	Fundus autofluorescence[4]
Baseline[a]	X	X	X	X	X
Cycle 2[b]	X	X			
Cycle 7[b]	X	X	X		
Cycle 13[c]	X	X	X	X	X
Cycle 19[c]	X	X	X		
Cycle 25[d]	X	X	X	X	X
End of study[e]	X	X	X	X	X

Footnotes (1-4):

1- to be performed OU in both eyes.

2- if ophthalmic AE's are reported, an unscheduled exam should be performed including comprehensive exam and if clinically indicated, HVF 10-2, macular OCT, and fundus photos.

3-To assure the ocular safety of the subjects, at each ocular exam visit the Ophthalmology sub-Investigator will perform a comprehensive eye examination of both eyes. The specified assessments within the ophthalmic examination are reflective of consensus guidelines [American Academy of Ophthalmology Preferred Practice Patterns Committee, 2005] and will be performed according to the Investigator's usual practice in addition to the assessment of visual acuity in the study and will include:

-Ocular alignment and motility, pupillary function, and visual fields by confrontation

-External examination of the eyelids and eyelashes

-Slit-lamp biomicroscopic examination of the anterior segment structures: eyelid margins, conjunctiva, sclera, cornea, anterior chamber, iris, lens, and anterior vitreous

-Intraocular pressure (IOP) measurement

-Following dilation of the pupil, continued slit-lamp biomicroscopic examination of anterior segment structures with clinical grading of lens opacity

-Fundus slit-lamp biomicroscopic examination, including use of accessory diagnostic lenses, to view the vitreous, retina, macula, vasculature, and optic nerve

4-if changes on exam or ancillary testing are noted, fundus photography and autofluorescence should be utilized if clinically indicated.

Footnotes (a-e)

a- Within 56 days of starting therapy

b- +/- 1 week to allow for scheduling difficulties

c- +/- 2 weeks to allow for scheduling difficulties

d- After 2 years patients will continue to be followed every six cycles (months). At the half-year follow-ups (e.g. 2.5 y, 3.5 y, etc), exams will comprise the same components as the 1.5 y exam. At the annual follow-ups (e.g. 3y, 4y, etc), the exam will comprise the same components as the 2 year exam.

e- If end of study occurs within 3 cycles (months) of the most recent scheduled exam, no additional testing is required unless ophthalmic concerns played a role in discontinuation in which case a full end of study exam is conducted. If end of study falls outside of this timeframe, the end of study eye exam should be captured when feasible.

Table 4 Management and Dose Modification Guidelines for Visual Changes and/or Ophthalmic Examination Findings

CTCAE Grade[a]	Adverse Event Management	Action and Dose Modification
Grade 1[b]	• Consult ophthalmologist within 7 days of onset	• If dilated fundus examination cannot be performed within 7 days of onset, interrupt binimetinib and hydroxychloroquine until RVO, and visual field deficit can be excluded by retina specialist/ophthalmologist. • If and RVO excluded, continue (or restart) binimetinib and HCQ at same dose level • <u>If RVO diagnosed:</u> **Permanently discontinue binimetinib and report as SAE.**
Grade 2 and Grade 3	• Consult ophthalmologist immediately • Interrupt binimetinib and HCQ.	• If RVO excluded, restart binimetinib and HCQ at same dose level. • <u>If RVO diagnosed:</u> **Permanently discontinue binimetinib and report as SAE**
Grade 4	• Consult ophthalmologist immediately • Interrupt binimetinib and HCQ.	• If visual field deficit and RVO excluded, may consider restarting binimetinib at same or reduced dose after discussion with study medical monitor • **If RVO or visual field deficit diagnosed, permanently discontinue binimetinib and report as SAE.**

Abbreviations: RPED = retinal pigment epithelial detachment; CTCAE = Common Terminology Criteria for Adverse Events; RVO= retinal vein occlusion; SAE = serious adverse event

a. Refers to CTCAE Version 5.0 'Eye disorders – Other, specify'

b. If visual changes are clearly unrelated to study treatment (e.g., allergic conjunctivitis), monitor closely but ophthalmic examination is not required.

Table 5 Recommended dose modifications for binimetinib for retinal pigment epithelial detachments (RPED)

CTCAE Grade	Action and Dose Modification
Grade 1 RPED (Asymptomatic; clinical or diagnostic observations only)	• Continue treatment with retinal evaluation each cycle (monthly) until resolution. If RPED worsens follow instructions below
Grade 2-3 RPED (Symptomatic with mild to moderate decrease in visual acuity; limiting instrumental ADL)	• Interrupt binimetinib and hydroxychloroquine • Retinal evaluation each cycle (monthly) • If improved to ≤ Grade 1, restart binimetinib at lower dose or discontinue in patients taking lowest dose

Table 6 Management of Ocular Toxicities

Ocular Toxicity	Uveitis, conjunctivitis	Retinal Vein occlusion, retinal pigment epithelial detachment (RPED), papilledema.	Visual field deficits
Action	Continue all therapies as long as effective local therapies can control inflammation	See table 3, 4, 5	Discontinue HCQ

5.3.2 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 binimetinib 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.

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 scheduled eye exams should prompt drug discontinuation and ophthalmologic evaluation.**

6.0 PHARMACEUTICAL INFORMATION

Array BioPharma Inc. is providing Binimetinib (Mektovi®) in order to conduct this study. Hydroxychloroquine is commercially available.
Drug accountability logs and pharmacy records must be maintained at each pharmacy location and must be available for review upon request. Drug accountability logs and pharmacy records will be reviewed during monitoring visits, and by the ACC Department of Compliance and Monitoring (DOCM) at the time of each audit.

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

6.1 Hydroxychloroquine

Generic name: Hydroxychloroquine sulfate

Commercial name: Plaquenil

Chemical name: 7-Chloro-4-[4-[ethyl-(2-hydroxyethyl)amino]-1-methylbutylamino] quinolone

Source: Commercially available

6.2 Binimetinib

Generic name: Binimetinib

Commercial name: Mektovi

Chemical name: 5-[(4-bromo-2-fluorophenyl)amino]-4-fluoro-N-(2 hydroxyethoxy)-1-methyl-1H-benzimidazole-6-carboxamide

Source: Study provided (see the Manual of Procedures for ordering instructions)

Storage and Handling: Binimetinib film-coated tablets are packaged in plastic bottles or in blister packs acceptable for pharmaceutical use.

- Binimetinib film-coated tablets packaged in plastic bottles should not be stored above 25 degrees Centigrade and should be protected from the light
- Binimetinib film-coated tablets packaged in opaque polyvinyl chloride (PVC)/polyvinylidene chloride (PVDC) blister packs do not require any specific storage conditions

Binimetinib oral suspension prepared from 15 mg binimetinib tablets should not be stored above 25 degrees Centigrade and should not be refrigerated. The constituted suspension should be used within 30 days after preparation.

7.0 CORRELATIVE STUDIES

7.1 Overview of Correlative Studies.

A number of blood and tissue collections are involved in this study to address important research questions. For patients with safely biopsiable disease, a biopsy may be performed prior to study enrollment and again after 3 weeks of therapy. This biopsy is encouraged, but optional. We aim to have 5 patients undergo biopsy. We will also collect plasma at each visit for autophagy biomarkers, whole blood for HCQ PK, and archival tumor tissue will be collected for all patients.

Summary of timing for correlative study collections:

I. HCQ PK: Pre-treatment (baseline and/or C1D1 prior to therapy), during combination therapy: at every cycle (monthly) visit

II. Biopsy (Optional) at

1) Baseline
2) C1D21 ± 7 days

III. Plasma for autophagy dynamics (All enrolled subjects):

1) Pre-treatment (baseline and/or C1D1 prior to therapy)
2) D1 of each cycle for C1-4
3) Sample obtained at progression

IV. Archival tissue (All enrolled subjects)

Obtain 20 unstained slides from archival FFPE blocks

Table 7 Blood collection schedule

Sample Type	Pre-treatment	C1D1*	C2D1	C3D1	C4D1	CXD1
Plasma	X	X	X	X	X	
HCQ PK	X	X	X	X	X	X

**At least one collection pre-treatment is required prior to C1D1 treatment, both time points are encouraged*

7.2 HCQ Pharmacokinetics

7.2.1 Clinical Pharmacology of HCQ and rationale for population PK Studies

Preclinical studies have demonstrated that high nanomolar to low micromolar concentrations of CQ or HCQ are needed to effectively inhibit autophagy resulting in the accumulation of AV in cancer cells. In order to characterize autophagy inhibition in patients receiving HCQ in combination B, PK measurements will be correlated with PD measurements to determine the exposure of HCQ that is clinically achievable in this population and the effect on autophagy at these concentrations. Comparison of PK measurements of HCQ when given in this combination regimen to previously reported PK measurements of HCQ alone, will also identify potential drug-drug interactions that require further study. All PK analyses will be done by Lisa Davis, PhD at the University of Arizona through a subcontract and MTA.

The PK properties of HCQ as a single agent have been extensively described in patients with rheumatoid arthritis. In one study patients received HCQ at doses of 400 mg, 800 mg or 1200

mg po daily (Carmichael et al., 2003). HCQ is metabolized by the p450 enzyme system into a number of active metabolites. The investigators measured the blood levels of HCQ, and its active metabolites desethylhydroxychloroquine (DHCQ), desethylchloroquine (DCQ) and bisdesethylchloroquine (BDCQ). Despite differences in interindividual PK measurements, HCQ concentration increased linearly in the blood of patients with dose. In this study peak concentrations of HCQ approach 2-3 μM in higher dose cohorts supporting the need for dose escalation of HCQ beyond the 400 mg po daily dose that is commonly prescribed for rheumatoid arthritis patients. A clear dose-response relationship was observed between peak HCQ, DHCQ, and BCQ concentrations and improvement in arthritis. In addition a clear association between peak HCQ concentrations and GI toxicity, mainly nausea and vomiting, was observed. The mean elimination half-life of each of these metabolites is between 30-50 days. Due to budgetary constraints binimetinib PK studies are not planned. If there is a significant difference between HCQ exposure in this study compared to previous studies and there is sufficient activity to warrant a larger phase II study a more complete drug-drug interaction study can be conducted as part of that study.

A population PK approach has been used in the past for HCQ, and has several advantages compared to a more traditional intensive sampling PK study design. First, it allows the analysis of relatively sparse data which can be more easily obtained over time in an outpatient setting. Furthermore, a population PK analysis provides estimates of population central PK parameter values, as well as estimates of inter- and intra-patient variability in HCQ blood concentrations. To obtain these estimates, samples must be obtained from each patient on more than one occasion. In patients that remain on study for this time, blood concentration determinations during the late phases of the study will be important to derive PK parameter estimates that are most representative of those at steady-state conditions. Samples obtained during initial and late phases of the study will be used to build the population PK model.

7.2.2 Pharmacokinetic Sample Collections

A sparse sampling approach will be applied to all patients that consists of blood collection pre-treatment, and monthly after start of B+HCQ. A 5 mL venous blood sample for determination of HCQ in anticoagulated whole blood will be collected in a heparinized green-top tube and placed on ice until it can be aliquotted into three polypropylene tube and stored at -80^{o}C. Centrifugation is not required. All specimens will be labeled with the patient's assigned study number, day on study, date and time. A separate log of patient samples will also record this information, and in addition, the time of last HCQ and last binimetinib dose, and time of last meal, and a description of concomitant medications at the time of collection. The PK blood draw should be scheduled for the convenience of the subject. <u>For these sparse sampling collections, patients will be asked to continue their drug dosing as scheduled and not hold any doses even on days of blood draws.</u> For HCQ, one sample will be collected during each visit for the first year that will also ensure random collections at times both pre- and post-HCQ dose. The time of last administration of HCQ and the time of the blood draw should be recorded.

7.2.3 Bioanalytic Methods and PK analysis.

The bioanalytic method and pharmacokinetic analysis will be performed at the University of Arizona under the aegis of Lisa Davis, PhD.

<u>Hydroxychloroquine.</u> Whole blood concentrations of HCQ and its metabolites (DHCQ, DCQ, BDCQ) will be measured using a modification of published high-performance liquid chromatography methods using fluorescence and tandem mass spectrometry detection (HPLC-MS/MS). CQ will be used as the internal standard. Briefly, the blood will be alkalinized and then subjected to ether extraction to extract HCQ and its metabolites. The ether extracts will be

evaporated to dryness and reconstituted in mobile phase. A methanol-based mobile phase will be used to separate the analytes. Elution of the analytes will be analyzed simultaneously using fluorescence and mass detection with a Varian LC 1200 triple quadrupole mass spectrometer. Whole blood concentrations of HCQ will be calculated for each sample based on a standard curve of peak area ratios of the drug and internal standard (CQ).

A population PK model will be developed to define the full HCQ PK profile over the entire patient population, which includes patients receiving HCQ in conjunction with other agents under investigation. In the population approach, data from individual patients are treated as random samples from a larger population. Estimates of the population central values for HCQ, as well as inter- and intra-patient variability are determined using nonlinear mixed-effects modeling software (NONMEM). The population PK parameters that will be estimated using this approach are apparent clearance (Cl/F) and volume of distribution of the central compartment (Vd/F). Since the absolute bioavailability of HCQ (F) cannot be calculated for oral dosing alone, bioavailability will be represented by a fixed constant for each of the parameter estimates. Comparison of these PK parameters of HCQ when given in combination with D+T to previously reported PK parameters of HCQ alone may identify a potential drug interaction that requires further study.

The results from the population analysis will be used as prior information to obtain Bayesian estimates of each individual patient's PK parameters (Cl and Vd) from his/her blood HCQ concentrations. The Bayesian approach incorporates data from a population pharmacokinetic model and each individual patient to estimate the patient's pharmacokinetic parameters. The a priori pharmacokinetic parameters of the population model are used as a starting estimate for the individual's parameters, which are then adjusted based on the patient's measured drug levels and with consideration of the variability of the parameters and drug concentration measurements. With this approach, the population model itself is incorporated into the estimation procedure.

This algorithm will determine the individual estimates of blood clearance (Cl/F) and volume of distribution (Vd/F) from which the following PK parameters of HCQ exposure in individual patients will be calculated: 1) area under the concentration-versus time curve (AUC) 2) peak (Cmax) blood concentrations. 3) trough (Cmin) blood concentrations. AUC will be computed as follows: AUC (mcg * h/mL) = Dose (mg)/Cl (L/h). The Cmin and Cmax levels will be derived from model-predicted concentrations just prior to and following a dose of HCQ, respectively. These data will be summarized by dose for the phase I portion of the trial and used for correlative toxicity and efficacy analyses.

7.3 Pharmacodynamic Studies in tumor tissues

7.3.1 Serial Tumor Biopsies and analysis.

Optional serial tumor biopsies are included in the budget for this study. We anticipate that 5 patients will complete at least two serial biopsies (10 total biopsies). Biopsy of easily accesible 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: excisional biopsies, excisions of large tumors in the operating room, and core needle biopsies. For core needle biopsies we request 4 cores if possible. The radiologist m ay choose the size of the needle. Lesions that are not easily accesible can be biopsied with the assistance of imaging guidance. This will involve core needle biopsies conducted by a radiologist. Processing of cores will be as follows:

Core 1) formalin- provided by the institution's pathology department. Sample is processed through surgical pathology. Once all samples are obtained, or at an intermediate timepoint in the study, a request for 20 unstained slides or the tissue block from this specimen will be made.

Core 2) snap frozen.- handled by institutions path department. Samples should be stored at -80ºC or in liquid nitrogen until batch shipment on dry ice.

Core 3) EM fixative- provided by Dr. Amaravadi's lab (please email ravi.amaravadi@Uphs.upenn.edu to request EM fixative).

A sesame seed size sample of tissue can be fixed in EM fix and stored at 4ºC (regular refrigerator) until batch shipment.

Core 4) additional research sample

Timepoints for serial tumor biopsies will be

1) baseline
2) C1D21 ± 7 days

These timepoints may be changed/modified at the discretion of the prinicipal investigator/study chair since the precise timing of successfully measuring the biological effect of drug intervention is not known.

7.3.2 Measuring autophagy induction and autophagy inhibition in tumor tissue.

Currently the gold standard techniques for detection of autophagy in tissues are 1) electron microscopy (EM) and 2) immunoblotting against the autophagy protein LC3. The accumulation of characteristic double-membrane vesicles in cells can be quantified by EM. When autophagy is induced by cellular stresses, small AV can be detected with well-digested contents. When autophagy is inhibited with CQ derivatives, larger and more numerous AV with undigested contents accumulate. When AV vesicles accumulate, LC3 is cleaved to form LC3-I and LC3-II[39]. LC3-II is inserted into the membranes of AV making it a specific marker for AV formation. The ratio between LC3I and LC3II as measured by immunoblotting reflects the number of AV accumulated. The inhibition of autophagy with CQ derivatives leads to a rapid accumulation of AVs in tumor tissue. This can be measured by scoring the number of AV/cell by EM in tumor tissue[24].

7.3.3 Therapy-induced gene expression changes in the tissue microenvironment.

In order to best understand the mechanism of action, resistance and interaction with the tumor microenvironment for dabrafenib, trametinib and HCQ, mRNA expression analysis or proteomic analysis is best suited to provide a snapshot of each of these effects. We will use the Quantigene® platform (http://www.panomics.com/index.php?id=product_6) because it is compatible with both fresh and FFPE tumor tissue. The array will be customized and enriched for genes associated with the inflammatory response, kinase signaling pathways, and stress response pathways such as the endoplasmic reticulum (ER) stress response and autophagy. We anticipate there will be a total of 10 tissue samples from 5 patients from the phase II trial including pre -treatment, on treatment that are large enough to warrant this sort of analysis.

7.4 Blood based correlative studies

7.4.1 Plasma biomarkers for autophagy dynamics

Rationale: 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. (Kraya Autophagy 2015). We therefore propose to analyze plasma 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.

7.4.2 Methods for Collecting Plasma

Supplies (to be purchased and maintained by each institution's clinical research lab)

- Purple top EDTA Blood Collection Tubes (for example, BD vacutainers catalog # 366450)
- Centrifuge with swinging bucket rotor
- 15 ml polypropylene conical tubes (for example, Corning 430052, Fisher cat #05-538-53D)
- Sterile cryovials with writing surface (for example, Simport T311-2 or Fisher #05-669-57)
- 2ml, 5ml and 10 ml pipettes (for example, Fisher cat #13-678-11C, 13-678-11D, 13-678-11E)
- Disposable transfer pipettes (for example, Fisher cat #13-711-20)
- Automatic pipet aid
- Small ice bucket

Plasma Collection Procedure

1) At each time point, encourage filling of entire volume of 1 purple top EDTA tube:
2) After collection, gently mix the blood by inverting the tube 8 to 10 times. Store vacutainer tubes upright at 4ºC until centrifugation. Blood samples should be centrifuged within three hours of blood collection.
3) Centrifuge blood samples in a horizontal rotor (swing-out head) for 10 minutes at 1200 g (2500 rpm) at room temperature.
4) After centrifugation, plasma layer will be at the top of the tube. Mononuclear cells and platelets will be in a whitish layer, called the "buffy coat", just under the plasma and above the red blood cells
5) Collect plasma layer into a second tube. Spin secondary tube at 1200 xg (2500 rpm) for 5 mins. To remove remaining red blood cells.
6) Aliquot plasma (~4 ml) into cryotubes (500 µl per tube).
7) Transfer to -80 °C for archival storage. NOTE: Time from blood draw until freezing should be less than 3 hrs. (if greater than 3 hrs., note time and flag samples, but do not discard). Do NOT store blood at 4 °C.

Plasma Data points

1. Date and time of blood collection
2. Number and volume of aliquots prepared
3. Date and time into -80ºC

4. Date and time of shipping

5. Any freeze-thaw that occurs with a sample for any reason

6. Any variations or deviations from the SOP, problems, or issues

7.4.3 FFPE archival tumor tissue for genetic analysis.

For all patients enrolled, we plan to obtain 20 unstained slides from archival FFPE blocks of ideally the latest metastatic lesion that was biopsied. 10 slides will be submitted for Myseq® multiplex DNA sequencing using the Illumina Truseq Cancer Panel (**http://www.illumina.com/products/truseq_amplicon_cancer_panel.ilmn**), which consists of 46 oncogenes and tumor suppressor genes through the Center for Personalized Diagnostics at the University of Pennsylvania. This extensive characterization of common somatic mutations will potentially generate hypothesis that a genotype-therapeutic interaction exists for long term survivors treated with this regimen. In addition, tumor samples obtained at progression will also be subjected to Myseq sequencing to generate hypotheses of mechanisms of resistance to this combination. Archival tissue remaining from genetic analysis will be available for future studies focused on the ER stress response and autophagy using IHC techniques. Similar next generation sequencing platforms at other institutions or from outside vendors may be used. Slides should be requested within the first 6 months of enrollment and shipped to the address provided in 8.3.1 in batches on a periodic basis.

8.0 STUDY CALENDAR

Trial Period:	**Treatment Cycles (28 days, study assessments planned for D1 of each cycle)**									**End of Treatment**	**Post-Treatment**		
Treatment Cycle/Title:	**Screening Visit**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Beyond 8 Cycles[12] 9+**	**EOT**	**Safety Follow-up**	**Follow Up Visits**	**Survival Follow-Up**
Scheduling Window (Days):	**-28 to -1**		**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**At time of Discontinuation**	**30 days post EOT[7]**	**As per standard of care post discon of treatment[5]**	**Every 12 weeks[5]**
Informed Consent	X													
Inclusion/Exclusion Criteria Assessment	X													
Demographics and Medical History	X													
Prior and Concomitant Medication Review	X	X	X	X	X	X	X	X	X	X	X	X		
Drug distribution		X	X	X	X	X	X	X	X	X				
Post-study anticancer therapy status											X	X	X	X
Survival Status														X
Adverse Event Assessment	X	X	X	X	X	X	X	X	X	X	X	X		
Full Physical Examination	X	X	X	X	X	X	X	X	X	X	X	X		
Eye Exam[14]	X		X					X		X[4]				
EKG	X	X	X	X	X	X	X	X	X	X				
Vital Signs and Weight	X	X	X	X	X	X	X	X	X	X	X	X		
ECOG Performance Status	X	X	X	X	X	X	X	X	X	X	X	X		
Pregnancy Test – Urine or Serum β-HCG[3]	X	X												
PT/INR and aPTT	X[11]													
CBC with Differential[10]	X[11]		X	X	X	X	X	X	X	X	X	X	X	

Trial Period:	**Treatment Cycles (28 days, study assessments planned for D1 of each cycle)**										**End of Treatment**	**Post-Treatment**		
Treatment Cycle/Title:	**Screening Visit**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Beyond 8 Cycles[12] 9+**	**EOT**	**Safety Follow-up**	**Follow Up Visits**	**Survival Follow-Up**
Scheduling Window (Days):	**-28 to -1**		**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**± 3**	**At time of Discontinuation**	**30 days post EOT[7]**	**As per standard of care post discon of treatment[5]**	**Every 12 weeks[5]**
Comprehensive Serum Chemistry Panel[10]	X[11]		X	X	X	X	X	X	X	X	X	X	X	
Transthoracic Echo	X			X[4]			X[4]			X[4]				
Tumor Imaging[6]	X[8]		X[9]		X[9]		X[9]		X[9]	X[9]	X		X[13]	
Tumor biopsies[1]	X	X												
Research Blood Collection[2]	X	X	X	X	X	X	X	X	X	X	X			

1. On-study tumor tissue collection is optional and should be performed prior to treatment and then at C1D21±7 days . Preference will be made to biopsy the same site for both biopsies, if safe and feasible.
2. Up to 60mL of blood will be collected for research studies. Please refer to the Study Laboratory Manual for additional details.
3. Female subjects of reproductive potential only. A negative pregnancy test is required within 72 hours of Cycle 1/Day 1.
4. Required q12 weeks (i.e. approximately every 3 cycles). ECHO to be performed between D21-D28 for applicable cycle
5. Subjects who discontinue study treatment in the absence of disease progression will have post-treatment follow-up as per standard of care for disease status until one of the following events: (1) disease progression; (2) initiation of non-study cancer treatment; (3) withdrawal of study consent; (4) loss to follow-up; or (5) death. The subject will then enter into survival follow-up. Subjects who discontinue study treatment due to disease progression or initiation of a new anti-cancer therapy will enter survival follow-up directly. Survival follow-up will continue up to 1 year from initation of study treatment (Cycle 1/Day 1).
6. RECIST 1.1 will be used in this study for assessment of tumor response. While either CT or MRI may be utilized, as per RECIST 1.1, CT is the preferred imaging technique in this study. Tumor imaging timing should follow calendar days and should not be adjusted for delays in cycle starts or extension of cycle duration. All visceral areas originally involved by tumor should be re-imaged at each scan interval. 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.
7. The 30 day Safety Follow-up Visit must occur prior to the first dose of any new anti-cancer therapy.
8. Baseline tumor imaging to be performed within 30 days prior to Cycle 1/Day 1

9. Tumor imaging to be performed after every 2 cycles(approximately every 2 months after C1D1 on D21-D28 of applicable cycle).
10. CBC with differential including hemoglobin, white blood count, absolute neutrophil count, and platelet count. Serum chemistries including sodium (Na), potassium (K), chloride (Cl), bicarbonate (HCO3), blood urea nitrogen (BUN), creatinine (Cr), albumin, total bilirubin, AST, ALT, and alkaline phosphatase. After Cycle 1, pre-dose laboratory procedures can be conducted up to 72 hours prior to dosing. Results must be reviewed by the investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.
11. Screening laboratory tests are to be performed within 10 days prior to Cycle 1/Day 1.
12. Treatment will continue indefinitely in the absence of (1), documented disease progression, (2) unacceptable adverse event(s), (3) intercurrent illness that prevents further administration of treatment, (4) investigator's decision to withdraw the subject, (5) subject withdrawal of consent, (6) pregnancy of the subject, (7) noncompliance with trial treatment or procedure requirements, (8) institution of alternative systemic treatment, or (9) other administrative reasons.
13. Tumor imaging in post-treatment follow-up will be performed per routine care and at the discretion of the treating investigator.
14. See table 3 for required time-points. Suspicion for eye toxicity will prompt an unscheduled research eye exam. To assure the ocular safety of the subjects, at each ocular exam visit the Ophthalmology sub-Investigator will perform a comprehensive eye examination of both eyes. The specified assessments within the ophthalmic examination are reflective of consensus guidelines [American Academy of Ophthalmology Preferred Practice Patterns Committee, 2005] and will be performed according to the Investigator's usual practice in addition to the assessment of visual acuity in the study and will include:

    -Ocular alignment and motility, pupillary function, and visual fields by confrontation

    -External examination of the eyelids and eyelashes

    -Slit-lamp biomicroscopic examination of the anterior segment structures: eyelid margins, conjunctiva, sclera, cornea, anterior chamber, iris, lens, and anterior vitreous

    -Intraocular pressure (IOP) measurement

    -Following dilation of the pupil, continued slit-lamp biomicroscopic examination of anterior segment structures with clinical grading of lens opacity

    -Fundus slit-lamp biomicroscopic examination, including use of accessory diagnostic lenses, to view the vitreous, retina, macula, vasculature, and optic nerve

    -if changes on exam or ancillary testing are noted, fundus photography and autofluorescence should be utilized if clinically indicated.

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. Archival tumor tissue can be obtained at any point during study enrollment and treatment timeline

9.0 MEASUREMENT OF EFFECT

For the purposes of this study, patients should be re-evaluated for response every 2 cycles. 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 will be used to make treatment decisions in real time.** Scans will also be submitted for independent radiology review by the RECIST core in batches quarterly to provide an independent measure of response for research purposes. The investigator assessments and the independent radiology assessments will be reported separately. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

9.1 Definitions

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

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These 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.)

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

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

9.3 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 ≥10 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).

9.4 Response Criteria

9.4.1 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

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

9.4.2 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).

9.4.3 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 8 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.

9.4.4 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.

9.4.5 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.

9.4.6 Overall Survival

OS is defined as the duration of time from start of treatment to time of death due to any cause or last patient contact alive.

9.4.7 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 from treatment 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

Patients may also be removed from the trial after completing all relevant trial related procedures.

10.0 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), and Array in accordance with CFR 312.32 (IND Safety Reports).

10.1 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. 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

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 30 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.

10.2 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. 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.

10.2.1 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
- 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.

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

Events of progression of the subject's underlying cancer as well as events clearly related to progression of the subject's cancer (signs and symptoms of progression) should not be reported as a serious adverse event.

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.

10.3 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.

The severity rating of an AE refers to its intensity. The severity of each AE will be determined by the Investigator using the NCI CTCAE (cite version here). For any term that is not specifically listed in the CTCAE scale, severity should be assigned a Grade of 1 through 5 using the following CTCAE guidelines:

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated

Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental activities of daily living

Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living

Grade 4: Life-threatening consequences; urgent intervention indicated

Grade 5: Fatal

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:

10.3.1 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.

10.3.2 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).

Suspected unexpected serious adverse reactions (SUSARs) will be collected and reported by the Sponsor and/or designee to the competent authorities and relevant ethics committees in accordance with Directive 2001/20/EC or as per national regulatory requirements in participating countries.

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.

10.3.3 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.

10.3.4 Deaths

All deaths that occur during the protocol-specified AE reporting period (see Section 10.2), regardless of attribution, will be reported to the appropriate parties. Of note, if a death occurs due to progression without an accompanied adverse event, reporting to the drug manufacturer is not required. 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”.

10.3.5 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”).

10.3.6 Pregnancy

Pregnancy itself is not regarded as an AE unless there is a suspicion that the study medication may have interfered with the effectiveness of the contraceptive medication. If a female subject becomes pregnant while receiving investigational therapy or within 120 days after the last dose of study drug, a report should be completed and submitted to Array within 24 hours of learning of its occurrence.

Subjects who become pregnant during the study will not receive any additional study medication and will be withdrawn from the study. The Investigator must strongly encourage these subjects to return to the study site for the final protocol-specified visit and assessments. 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.

10.4 SAE Reporting to Array

All serious adverse events, regardless of suspected causality, occurring after the patient has provided the main informed consent and until at least 30 days after the patient has stopped study treatment must be reported to Array within 24 hours of learning of its occurrence. Send the completed, signed SAE report to the oncology Array Drug Safety and Epidemiology (DS&E) department (Fax 866-997-8322).

Any serious adverse event that occurs after the 30 days study period should only be reported to Array if the investigator suspects a causal relationship to the study treatment. Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up to the

original episode within 24 hours of the investigator receiving the follow-up information. An SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported one should be reported separately as a new event.

Other reportable events:

Deviations:

An one-time accidental or unintentional deviation from the approved protocol, identified retrospectively, that in the opinion of the investigator or as defined by the protocol, placed one or more participants at increased risk, compromises the rights or welfare of subjects, and/or disrupts the study design, is considered a reportable event and must be reported to the Study Principal Investigator, Study Medical Monitor, IRB, and ACC DSMC within 5 business days of knowledge. Principal Investigator and Medical Monitor approval/acknowledgement must be received first and included in with the IRB/DSMC submission.
Deviations to protect subjects from immediate harm/danger should be reported immediately following the event to the entities outlined above.

Exceptions:

An exception is defined as a one-time, intentional action or process that departs from the IRB and CTSRMC approved study protocol, intended for one occurrence. AND this action disrupts the study progress, such that the study design or outcome (endpoints) may be compromised, or the action compromises the safety and welfare of study subjects (i.e. requests to enroll and/or treat subjects outside of the current protocol criteria). Exceptions that meet this criteria will not be allowed unless reviewed and approved first by the Study Medical Monitor and the Study Principal Investigator/Sponsor-Investigator, and then subsequently by the ACC DSMC, and UPenn IRB prior to this subject being enrolled/treated. PI/Sponsor-Investigator and Medical Monitor approval must be received first and included in with the IRB/DSMC submission. All entities should be given sufficient time to evaluate this request.

Examples of Exceptions/Deviations that require submission include:

- Stopping rules that were not completed per protocol
- Dosing errors

Exceptions to eligibility, treatment/dosing, contraindicated treatment/therapies/interventions or safety tests will not be approved.

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

10.5 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.

Office of Regulatory Affairs, Institutional Review Board
3624 Market Street, Suite 301S, Philadelphia, PA 19104-6006
Phone: 215-573-2540
Fax: 215-573-9438

10.5.1 Reporting Process to IRB at Penn.

Unanticipated problems posing risks to subjects or others as noted above will be reported to the Penn IRB using the form: "Unanticipated Problems Posing Risks to Subjects or Others Including Reportable Adverse Events" or as 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).

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

10.5.2 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.

10.6 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at the 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. Reiss-Binder, Department of Medicine, Division of Hematology-Oncology will review comprehensive safety data at least bi-annually. This will include a real-time review of unexpected and related SAEs and any deaths that are unexpected or occur from the start until 90 days from the last study treatment. The Medical Monitor will also be consulted in the case of exception requests or the evaluation of deviations that may compromise subject safety or disrupt the design of the study. This information will be communicated to the Medical Monitor via email and filed in the Regulatory Binder/Subject Chart appropriately. As applicable, correspondence will be included in IRB/ACC DSMC submission of these events. Dr. Reiss Binder will be reached at Kim.Reissbinder@pennmedicine.upenn.edu . Ophthalmic safety concerns that develop during the study will be forwarded to Array for review, as well as the independent medical monitor.

10.7 Study Monitoring Plan

This study will be monitored in accordance with the Cancer Center's Clinical Trials Scientific Review and Monitoring Committee (CTSRMC) Plan, approved by NCI during the Core Grant's most recent review. This plan requires that the investigator submit a study-specific plan outlining how data will be reviewed In addition, the CTSRMC plan calls for an internal audit by the Cancer Center's Data Safety Committee twice yearly. The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.8 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection

instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

If an audit of this or any other study is requested by any regulatory authority, the Investigator will inform Array of the request. The study site will permit access to all necessary records.

11.0 STATISTICAL CONSIDERATIONS

11.1 Design

This is a single arm phase II study of Binimetinib and Hydroxychloroquine for patients with advanced KRAS mutant NSCLC. This study will be conducted in two stages using Simon's Optimal Design (Controlled Clinical Trials 10:1-10, 1989). A number of innovative correlative studies are planned.

11.2 Objectives

The primary objectives are to determine:

- objective response rate (ORR)
- safety & tolerability

The secondary objectives are to determine:

- progression-free survival (PFS) and overall survival (OS)
- changes in ctDNA KRAS allelic frequency (blood)

The correlative objectives are to determine:

- association between objective response and pre-treatment tumor expression of PPT1, LC3, ALDH1A1 and HLTF biomarkers
- differences in pre-treatment and Week 6 tumor expression of PPT1, LC3, ALDH1A1 and HLTF biomarkers
- changes autophagic vesicles/cell in serial tumor tissue samples
- evaluate pharmacokinetics and pharmacodynamics of HCQ, to determine whether changes in blood-based autophagy biomarkers reflect tissue-based changes in autophagy (See Section 18)

11.3 Endpoints

- Objective Response will be scored using RECIST. The objective response rate is defined as the percentage of patients who achieve a complete or partial response. Following an intent-to-treat analysis plan, all patients who receive at least one dose of the combination regimen will be included in the analysis of response.
- Duration of response: from time of first catageroization as a PR or CR to the time of progressive disease.
- Safety: NCI Common toxicity criteria (CTCAE v5.0) will be employed to grade toxicities.

- Progression-free survival will be defined as days from start of treatment to first documented disease progression, death due to any cause or last patient contact. Patients who are alive and have not progressed at the time of statistical analysis will be censored on the most recent date that progression-free status is documented (i.e., imaging date). For patients treated beyond progression (in the setting of an isolated lesion) with consent of study chair, PFS will be time to first progression but time to second progression will be recorded separately.
- Overall survival will be defined as days from start of treatment to death due to any cause or last patient contact. Patients who are alive at the time of statistical analysis will be censored for survival.
- Biomarkers: PPT1, LC3, ALDH1A1 and HLTF will be measured in pre-treatment tissue and at Week 6.
- Median number of autophagic vesicles/cell in tumor tissue
- HCQ PK-PD (See Section 11.5)

11.4 Simon 2-stage Design

After treatment with standard of care chemotherapy or chemo-immunotherapy, the standard of care for the treatment of progressive NSCLC is docetaxel. The ORR to docetaxel is 14% (Garon et al Lancet 2014). This experimental regimen will be considered not worthy of further development if the ORR is 10% (clearly inferior to docetaxel) and considered worthy of further development if the ORR is 30%.

- Stage 1: Ten patients will be entered into the first stage of the study. If 1 or fewer of these 10 patients respond, then the trial will be terminated. If at least 2 of these 10 patients respond, then proceed to Stage 2.
- Stage 2: Nineteen additional will be entered (total of 29) into the second stage of the study. If 5 or fewer of the 29 patients respond, then it will be concluded that the regimen does not merit further investigation. If 6 or more of the 29 patients respond, then it will be concluded that the regimen does merit further investigation.

The trial is designed with the following characteristics:

- False positive error: If the true response rate is 10%, then the probability of recommending the regimen for further investigation, is no more than 0.05.
- Power: If the true response rate is 30%, then the probability of recommending the regimen for further investigation, is at least 0.80.
- If the true response rate is 10%, then the probability of early termination (PET) is 0.74 after 10 patients have been treated.

11.5 Plans for Data Analysis

Primary

- The objective response rate and 95% exact confidence interval will be calculated.
- All observed toxicities will be graded and tabled (worse grade experienced in all cycles). The overall rate of $\geq$ grade 3 toxicity and 95% exact confidence interval will be calculated.

Secondary

- Progression-free survival and overall survival will be estimated by the method of Kaplan and Meier. Median values and 95% confidence intervals will be calculated.

Exploratory

- Associations between objective response and expression of biomarkers pre-treatment will be tested using nonparametric Wilcoxon rank sum test. Comparisons of pre- and post-treatment biomarker expression will be conducted using Wilcoxon signed ranks test for paired data.
- Describe autophagic vesicles/cell in tumor tissue at various time points using descriptive statistics and scatter plots.
- To evaluate HCQ pharmacokinetics and pharmacodynamics to determine whether changes in blood-based autophagy biomarkers (ctDNA KRAS allelic frequency) reflect tissue-based changes in autophagy (autophagic vesicles/cell), the nonlinear mixed–effects modeling approach (NONMEM) will be used to perform a full population PK analysis. PK-PD analyses to be conducted by University of Sciences of Philadelphia. Analysis methods – See Section 18. The key variables for the population include the Cmax and half life. Population PK will be assessed and qualitatively compared to population PK findings from other HCQ studies. No hypothesis testing will be performed for any of the vairables.

11.6 Sample Size

The total sample size ranges from 10 to 29 patients.

11.7 Study Duration

With an estimated accrual of 20 patients per year, accrual will continue for approximately 18 months. ORR will be estimated when all patients have been followed for at least 6 months. In addition, all patients will be followed for at least 12 months, to provide estimates of PFS and OS.

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APPENDIX A: ECOG PERFORMANCE STATUS

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

* As published in Am. J. Clin. Oncol.:
Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

The 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, Robert Comis M.D., Group Chair.

APPENDIX B: DEFINITION OF NON-CHILDBEARING POTENTIAL AND MEDICALLY ACCEPTABLE METHODS OF BIRTH CONTROL

Non-childbearing potential is defined as any of the following (by other than medical reasons):

1. ≥45 years of age and has not had menses for >2 years

2. Amenorrhoeic for <2 years without a hysterectomy and oophorectomy and a follicle-stimulating hormone value in the postmenopausal range upon pre-study (screening) evaluation

3. Post hysterectomy, oophorectomy or tubal ligation

Females:
Women of childbearing potential must have a negative serum pregnancy test within 7 days of the first dose of study therapy and agree to use a highly effective contraception method during the treatment period and for at least 3 months following the last dose of study therapy

Males:
Men with partners of child-bearing potential must agree along with their partner to use a highly effective contraception method during the treatment period and for at least 3 months following the last dose of study therapy

Acceptable methods include:
- Condoms
- Diaphragm
- Cervical cap
- Intra-uterine device
- Surgical sterilization (tubal ligation or vasectomy)
- Oral contraceptives

If condoms are used as a barrier method, a spermicidal agent should be added as a double barrier protection.

Cases of pregnancy that occur during maternal exposures to study therapy should be reported. If a patient is determined to be pregnant following study therapy initiation, she must discontinue treatment immediately. Data on fetal outcome and breast-feeding are to be collected for regulatory reporting and drug safety evaluation.

Abstinence at certain times of the cycle, such as during ovulation or after ovulation, or withdrawal are not acceptable methods. The list of methods above is not exhaustive and additional contraception methods may also be acceptable if approved by the study doctor.