Abbreviated Title: Trametinib and HCQ in BTC
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Title: Phase II Study of combination of Trametinib (MEK inhibitor) and Hydroxychloroquine (HCQ) (autophagy inhibitor) in patients with KRAS mutation refractory bile tract carcinoma (BTC)

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Investigational Device:

Device Name:	TruSight™ Oncology 500
IDE Number:	Non-significant risk (NSR) device
Sponsor:	CCR/NCI
Manufacturer:	Illumina

Commercial Agents: Hydroxychloroquine (generic), Trametinib (Novartis)

PRÉCIS

Background:

- Among the new cases of bile tract carcinoma (BTC) that are diagnosed every year in the United States, there are approximately 6,500 cases of gallbladder carcinoma, 3,000 cases of extrahepatic cholangiocarcinoma, and 3,000 cases of intrahepatic cholangiocarcinoma.
- Current treatment options for patients with cholangiocarcinoma are limited and take no account of the known biological and genetic heterogeneity in these diseases. Median survival for advanced disease remains poor at approximately 1 year.
- Activating KRAS mutations are frequently detected in all subtypes of BTC and can be found in up to 40% of BTC, predominantly in perihilar and distal cholangiocarcinoma (CCA). However, pharmacological inhibition of mutated KRAS has demonstrated little clinical benefit in general.
- Trametinib is a reversible, highly selective allosteric inhibitor of mitogen-activated extracellular signal regulated kinases MEK1 and MEK2. Tumor cells with KRAS mutations commonly have hyperactivated extracellular signal-related kinase (ERK) pathways in which activated MEK is a critical component. However, tumors are able to overcome MEK signaling inhibition by trametinib through upregulation of autophagy pathway.
- Hydroxychloroquine (HCQ) inhibits lysosomal acidification and prevents the degradation of autophagosomes, to suppress autophagy.
- Trametinib has been approved by FDA for the treatment of melanoma as a single agent or for the treatment of other cancers if tumors carry BRAF mutation. Hydroxychloroquine are approved for the treatment of malaria, lupus erythematosus and acute or chronic rheumatoid arthritis.
- Preclinical studies have shown that combined treatment of trametinib plus HCQ elicited striking tumor regression in animal model.

Objective:

- To determine whether the 5-month progression free survival (PFS) of the trametinib plus hydroxychloroquine (HCQ) combination in subjects with refractory bile tract carcinoma (BTC) with KRAS mutation exceeds 25%.

Eligibility:

- Histopathological confirmation of BTC or carcinoma highly suggestive of a diagnosis of BTC
- Tumor must have KRAS mutation.
- Patients must have disease that is not amenable to potentially curative resection, transplantation or ablation.
- Age ≥18 years
- Patients must have measurable lesion by RECIST 1.1.

- At least two weeks washout period from previous therapy
- ECOG ≤ 2
- Adequate renal, hepatic and bone marrow function

Design:

- The study is open-labeled phase 2 study. It is designed to enroll total 30 patients with refractory BTC, to test the hypothesis that treatment with a combination of HCQ and trametinib prevents cancer progression/recurrence. We propose that this combination will have relative safety profile and antitumor efficacy in BTC patients with KRAS mutation.

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 INTRODUCTION

1.1 Study Objectives

1.1.1 Primary Objective:

- To determine whether the 5-month PFS of the trametinib plus hydroxychloroquine (HCQ) combination in subjects with refractory bile tract carcinoma (BTC) with KRAS mutation exceeds 25%.

1.1.2 Secondary Objectives:

- To determine the safety, tolerability and feasibility of the trametinib plus HCQ combination in subjects with refractory BTC with KRAS mutation.
- To evaluate the response rate (RR) (CR+PR) in patients with refractory BTC with KRAS mutation treated with the combination of trametinib plus HCQ
- To access overall survival (OS).

1.1.3 Exploratory Objectives:

- To evaluate the bioactivity of the combination of trametinib plus HCQ against the MEK1/2 and autophagy pathways.
- To measure changes in immune parameters in the peripheral blood and tumors of patients with refractory KRAS mutation BTC treated with the combination of trametinib plus HCQ.
- To explore mechanism of primary and secondary resistance to the combination of trametinib plus HCQ in patients with refractory KRAS mutation BTC.
- To evaluate the relationship between the RR, OS and immune parameters, in patients with refractory KRAS mutation BTC treated with the combination of trametinib plus HCQ.

1.2 Background and Rationale

1.2.1 Bile tract carcinoma and KRAS mutation

BTC is a hepatobiliary malignancy and incidence has been steadily increasing [1]. Among the new cases of BTC that are diagnosed every year in the United States, there are approximately 6,500 cases of gallbladder carcinoma, 3,000 cases of extrahepatic cholangiocarcinoma, and 3,000 cases of intrahepatic cholangiocarcinoma[2]. Surgical resection is the only curative approach for patients with local disease, however, the majority of patients present with advanced unresectable disease limiting the success of curative surgery [3-5]. The combination of gemcitabine and cisplatin chemotherapy has become the standard first-line treatment regimen for advanced BTC with a median survival of 9 months [6, 7]. For patients who progress on first-line therapy, there is no standard second-line option [8]. RAS genes include HRAS, KRAS, and NRAS. The three RAS genes encode four 188–189 amino acid proteins that share 82%–90% amino acid sequence identity and share near-identical structural and biochemical properties[9]. However, they are differentially expressed and mutated with different frequencies in cancer[10, 11]. KRAS is the predominant mutated RAS gene in cancers (84% of all RAS missense mutations), followed by NRAS (12%), with HRAS rarely mutated (4%) (COSMIC v80)[9]. Activating KRAS mutations are frequently detected in all subtypes of BTC and can be found in up to 40% of BTC [12, 13]. Notably, the incidence of KRAS mutations increases with disease stage[14]. KRAS has been associated with perineural invasion and poor prognosis [15].

Newly developed targeted therapies have shown promising clinical efficacy against chemotherapy refractory CCA. In a phase II study of patients with FGFR-altered advanced biliary tract cancer, a selective pan-FGFR kinase inhibitor showed impressive anti-tumor activity and a disease-control rate (DCR) of 82% [16]. Additionally, in a phase I study of patients with IDH1-positive CCA, which represents approximately 25% of all CCA cases, IDH1 inhibitor, ivosidenib exhibited a well-tolerated safety profile with an overall response rate (ORR) of 5% and an OS of 13.8 months [17]. Unfortunately, these promising targeted therapies only apply to a relatively small percentage of CCA patients with specific mutations.

Within the last decade, immunotherapy has become a major pillar of cancer therapy. Immune checkpoint inhibitors (ICIs) act by targeting dysregulated immune checkpoints such as programmed death protein 1 (PD-1) and programmed death ligand 1 (PD-L1), which are present in many types of cancers. Accumulating data suggest encouraging results with ICIs alone in hepatocellular carcinoma (HCC), with response rates as high as 15-20% [18, 19]. However, there is minimal efficacy of ICIs in the treatment of CCA [20, 21]. In our recent study of advanced BTCs that were treated with a combination of an ICI and microwave ablation, the ORR was 12.5% [22]. Novel strategies to combat CCA progression are thus urgently needed.

1.2.2 Challenge of targeting mutant KRAS

Despite the recognized frequency and significance of KRAS mutations, targeting this pathway remains challenging and a clinically effective anti-RAS therapy remains elusive. The main current strategies for developing therapeutics to block mutant KRAS function have focused on indirect approaches: to target proteins that support KRAS function and promote KRAS-driven cancer growth, including inhibition of activated downstream signaling molecules such as MEK, AKT or mTOR [23]. Early evidence of efficacy of MEK inhibitor (MEKi) was reported in a single arm study of selumetinib in advanced bile duct cancer[24]. Of the 28 patients enrolled, 3 patients had confirmed partial responses. In this study, no BRAF V600E mutations were found. Recently, the

ABC-04 study of selumetinib in combination with gemcitabine and cisplatin in advanced or metastatic bile duct cancer (9/13 CCA) demonstrated a RR of 37.5%, a median PFS of 6.4 months and manageable toxicities [25].

However, it is likely that KRAS drives tumorigenesis by the integrated result of multiple effector signaling pathways, with multiple KRAS effector pathways contributing to the tumor etiology, that likely contributes to treatment resistance to MEKi. Given the intense crosstalk between the signaling pathways downstream of KRAS and possible resistance mechanisms, combination of inhibitors targeting different redundant signaling pathways seems to be the most promising strategy. Co-targeting with a MEKi and the multi-kinase inhibitor ponatinib for example has shown promising effects in pancreatic cancer cells and in-vivo models[26]. Further trials targeting the KRAS signaling pathway with MEKi combined with other therapeutics are ongoing (NCT02042443; NCT01438554). The other interesting target strategy to be combined with MEKi is to interfere autophagy process.

In this protocol for confirmation of KRAS mutations we plan to use TruSight™ Oncology 500 panel assay performed by NCI Laboratory of Pathology or use results of tests approved by FDA to evaluate KRAS mutation status. There is a list of next generation sequencing-based *in-vitro* diagnostic tests developed and approved by FDA for tumor genetic profiling (see Study Instrument submitted with this amendment), including KRAS mutation, which is one of most commonly detected mutations in various tumor types. These approvals were based on the analytical validity of the tests for specific biomarkers and clinical evidence. Since they all detect mutation profiling with next generation sequencing method, the results derived from different companies or kits are expected similar though different kits cover various detected gene panels.

1.2.3 Autophagy

1.2.3.1 Autophagy with bile tract carcinoma

Progressive cancer growth requires reprogramming energy and nutrient metabolism to support their elevated proliferative state [27]. These increased needs are met by reprogramming various metabolic processes to either recycle intracellular fuel sources or to scavenge extracellular components.

Autophagy is one of critical process that provides this refuel. It is a cellular degradation or “self-eating” pathway highly conserved throughout all life kingdoms [28]. This multistep and fine-tuned process is regulated by autophagy-(ATG-) related proteins originally discovered in autophagy-defective yeast mutants [29]. It is a tightly orchestrated process that sequesters proteins, and damaged or aged organelles in double-membrane vesicles called autophagosomes, which ultimately fuse to lysosomes, leading to the degradation of the sequestered components [30]. Autophagy plays an important role in maintaining cellular homeostasis and is therefore constitutively active at a basal level in most cell types. However, during different stress conditions, such as those induced by nutrient starvation, organelle damage, accumulation of abnormal proteins, or during development and cell differentiation [31], autophagy is additionally enhanced to meet the cellular needs.

In the last two decades, accumulating evidence pointed to the importance of autophagy in various human diseases [32]. Studies have indicated that autophagy impairments are root causes of numerous diseases such as cancer, neurodegenerative disorders (Alzheimer disease, Parkinson disease), infectious and inflammatory diseases (Crohn's disease), diabetes, obesity, and

cardiovascular and muscular diseases [33]. Therefore, the number of studies focusing on the autophagy modulation as a perspective and promising therapeutic target is constantly increasing.

The complex and paradoxical role of autophagy in modulating cancer progression has been widely studied. The determination of tumor cell fate by autophagy depends on the cancer type, stage, and genetic context [34]. As a physiological quality control process, autophagy exerts a cytoprotective effect by removing misfolded proteins, damaged organelles and reactive oxygen species (ROS), hence limiting the genomic damage that leads to aberrant mutations and ultimately cancer. However, as cancer progresses, the stress-mitigating properties of autophagy are hijacked by tumor cells to meet the heightened metabolic requirements necessary for tumor survival and rapid proliferation [35, 36].

Cholangiocarcinoma (CCA) reportedly follows a stepwise carcinogenesis process via the precursor lesion biliary intraepithelial neoplasia (BilIN). The involvement of autophagy in multistep cholangiocarcinogenesis was evaluated by testing expression of beclin-1, and p62/sequestosome-1, as well as tumor suppressor gene product p53 [37]. The status of KRAS mutations at codons 12 and 13 was examined in selected cases of BilIN-1/2. The results showed the expression of LC3 (a component of the autophagosome), beclin-1, and p62 was significantly higher in BilIN-1/2, BilIN-3, intraductal papillary neoplasm, and invasive carcinoma than in large bile duct and peribiliary gland. The results suggest deregulation of autophagy is associated with BTC tumorigenesis. Genetically engineered mouse model of intrahepatic cholangiocarcinoma (iCCA) with somatic activation of KRAS G12D and deletion of TP53 has been established[38]. In nutrient-rich conditions, cell lines derived from KRAS-TP53 iCCAs have elevated LC3, a marker of autophagy, compared with normal and KRAS-TP53 mutant liver. Visualization of GFP-LC3 autophagic puncta and chloroquine (CQ) inhibition of autophagic flux confirmed this biochemical observation [39]. Furthermore, CQ treatment inhibits the growth of four separate cell lines from both well and poorly differentiated iCCA[39]. There is no clinical trial available to directly study the effect of autophagy on BTC. However, abovementioned preclinical data indicates that the inhibition of this metabolic pathway may provide a new therapeutic option in the treatment of BTC.

1.2.3.2 KRAS Regulates Autophagy

Recent studies have implicated mutant KRAS in the regulation of autophagy [40, 41]. It has been established that KRAS signaling leads to the scavenging of extracellular proteins and lipids and activates self-eating and recycling of proteins through autophagy [42]. The role of autophagy in cancer is extremely complex[43] and while it appears clear that cancer cells depend on autophagy for growth, the role of oncogenic KRAS in this dependence remains unclear. When tissue samples from 71 patients with pancreatic ductal adenocarcinoma (PDAC) were analyzed via immunohistochemical staining for LC3 protein, it was determined that high expression of LC3 was correlated with large tumor size, short-disease free period, and overall poor clinical outcome [44]. Genetic or pharmacological inhibition of autophagy results in increased reactive oxygen species, elevated DNA damage, and mitochondrial defects that lead to decreased proliferation of pancreatic cancer cell lines in vitro, as well as substantial tumor regression and sustained survival in in vivo models of pancreatic cancer [45, 46]. In support of a cooperative role between RAS expression and proliferation fueled by autophagy, immortal, non-tumorigenic baby mouse kidney epithelial cells ectopically expressing oncogenic HRAS or KRAS experienced defects in mitochondrial respiration upon autophagy inhibition [45]. A recent study has shown the inhibitor of KRAS→RAF→MEK→ERK signaling in cancer cell lines elicits autophagy, which

protects cancer cells from the potentially cytotoxic effects of pathway inhibition[47]. This indicates the antitumor efficacy of the combination of MEK blockade with autophagy inhibitor.

1.2.4 Rationale for combining inhibitors of MEK and Autophagy

Current clinical trials have shown that autophagy inhibition by HCQ as a monotherapy is not sufficient [48]. One possible explanation as to why the combined therapies work better than HCQ monotherapy is because the autophagy could be required to degrade harmful material generated as a result of chemotherapeutic drug insults to the cancer cells. The other reason could be because the inhibition of a single possible pathway is simply not sufficient. In the other words, combination of autophagy inhibition with other modality would be a logical strategy. For instance, sorafenib is a multi-kinase inhibitor, including MEK pathway. Earlier studies have demonstrated that sorafenib treatment led to accumulation of autophagosomes due to activation of autophagic flux. Pharmacological inhibition of autophagic flux by CQ increased apoptosis and decreased cell viability in hepatoma cells. Furthermore, sorafenib induced autophagy in Huh7 xenograft tumors in nude mice and co-administration with CQ significantly suppressed tumor growth compared with sorafenib alone[49]. Additionally, it was found autophagy inhibitor rapamycin induced the apoptosis of malignancy cell line from uterus, and this apoptosis was enhanced by MEKi U0126[50].

1.2.4.1 Preclinical experience of combining inhibitors of MEK and autophagy

Recent publication has shown combined inhibition of MEK1/2 and autophagy with CQ displays synergistic anti-proliferative effects against PDAC cell lines in vitro [47]. Most strikingly, whereas single agent therapy had modest effects, combined treatment of xenografted patient derived PDAC tumors with trametinib plus CQ/HCQ elicited striking tumor regression. The regression elicited by trametinib plus CQ/HCQ was superior to gemcitabine/ nab-paclitaxel standard of care chemotherapy in preclinical models. These finding suggest that combination of trametinib and CQ/HCQ may be efficacious in patients with KRAS mutant bearing cancer.

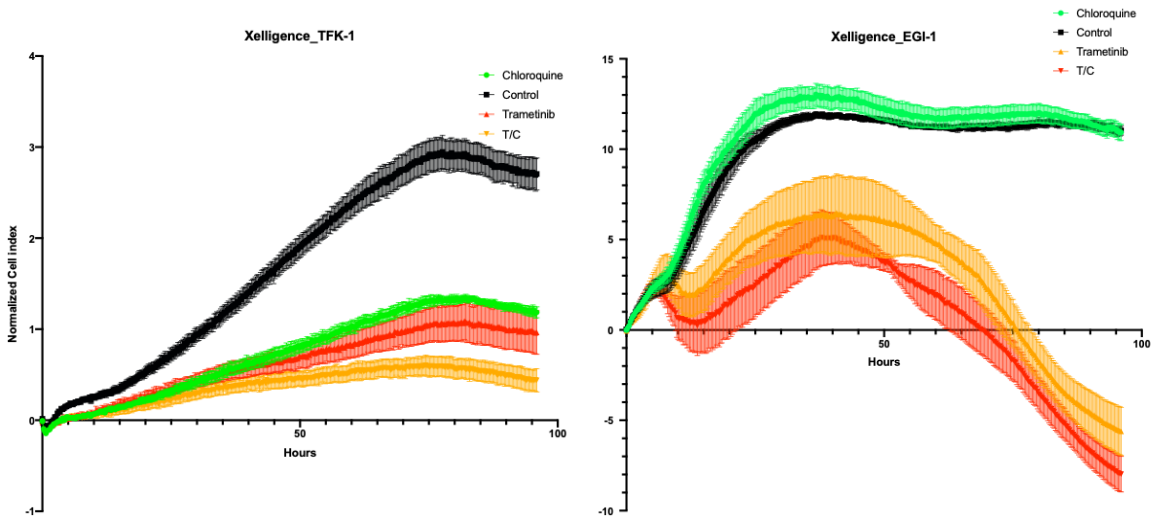


Figure 1 **Antitumor effect trametinib/HCQ in human CCA cell line TFK-1 (KRAS WT, left panel) and EGI (KRAS Mutant right panel) cells.** Cholangiocarcinoma cell growth curve of the TFK-1 cell line

(RAS Wild Type) and of the EGI-1 cell line (RAS mutated) treated with chloroquine (20uM), trametinib (450 nM) and chloroquine /trametinib (20uM/450 nM). The cells with KRAS WT show reduced proliferation with chloroquine, trametinib or the combination, whereas cells with KRAS mutant exhibit cell death starting by 50 hour of treatment with trametinib or the combination.

In our lab, combined treatment of trametinib plus CQ shows significantly antitumor effect in human CCA cell lines with KRAS mutant (**Figure 1**). These data indicate this combination has antitumor efficacy in CCA with KRAS mutant

1.2.4.2 Clinical experience of combining inhibitors of MEK and autophagy

More recently, a study showed the elicits autophagy in pancreatic ductal adenocarcinoma (PDAC) cell lines by activation of the LKB1→AMPK→ULK1 signaling axis, a key regulator of autophagy. Furthermore, combined inhibition of MEK1/2 plus autophagy displays synergistic anti-proliferative effects against PDAC cell lines in vitro and promotes regression of xenografted patient-derived PDA tumors in mice. The observed effect of combination trametinib plus chloroquine was not restricted to PDAC as other tumors, including patient-derived xenografts (PDX) of NRAS-mutated melanoma and BRAF-mutated colorectal cancer displayed similar responses[47]. Interestingly, treatment of a patient with PDAC with the combination of trametinib plus HCQ resulted in a partial, but nonetheless striking disease response[47]. So far, 10 patients (7 patients with PDAC and 3 patients with KRAS mutated malignancies) have been treated at Huntsman Cancer Institute with trametinib plus HCQ. All patients were heavily pretreated and out of standard options. All patients were treated with 1200 mg HCQ and 2 mg of trametinib. Common toxicities observed in these patients and attributed to the drug combination have been grade 1-2: rash, fatigue, nausea, and diarrhea. However, most patients have seen dramatic decline in tumor marker values and tumor burden with the eventual development resistance.

Our GI oncology team in NIH CC also treated a heavily treated PDAC patient with KRAS mutation indicating stable tumor control and decline of tumor specific marker (**Figure 2**).

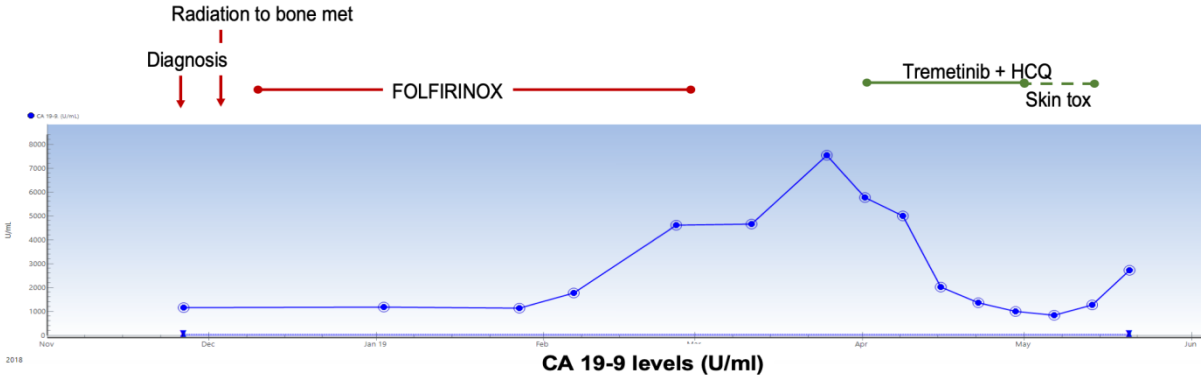


Figure 2. Antitumor effect of trametinib/HCQ in PDAC patient with KRAS mutation.

1.2.5 Trametinib

1.2.5.1 Mechanism of action

Trametinib is a reversible inhibitor of mitogen-activated extracellular signal-regulated kinase 1 (MEK1) and MEK2 activation and of MEK1 and MEK2 kinase activity. MEK proteins are

upstream regulators of the extracellular signal-related kinase (ERK) pathway, which promotes cellular proliferation. BRAF V600E mutations result in constitutive activation of the BRAF pathway which includes MEK1 and MEK2. Trametinib inhibits cell growth of various BRAF V600 mutation-positive tumors in vitro and in vivo.

1.2.5.2 Toxicity

Cardiomyopathy

METRIC clinical trial is an international multicenter randomized (2:1), open-label, active-controlled trial in 322 patients with BRAF V600 E or V600 K mutation-positive, unresectable or metastatic melanoma trial. Cardiomyopathy [defined as cardiac failure, left ventricular dysfunction, or decreased left ventricular ejection fraction (LVEF)] occurred in 7% (14/211) of patients treated with trametinib; no chemotherapy-treated patient in Trial 1 developed cardiomyopathy. The median time to onset of cardiomyopathy in patients treated with trametinib was 63 days (range 16 to 156 days); cardiomyopathy was identified within the first month of treatment with trametinib in five of these 14 patients. Four percent of patients in METRIC trial required discontinuation (4/211) and/or dose reduction (7/211) of trametinib. Cardiomyopathy resolved in 10 of these 14 (71%) patients.

Across clinical trials of trametinib at the recommended dose (N = 329), 11% of patients developed evidence of cardiomyopathy (decrease in LVEF below institutional lower limits of normal with an absolute decrease in LVEF ≥10% below baseline) and 5% demonstrated a decrease in LVEF below institutional lower limits of normal with an absolute decrease in LVEF of ≥20% below baseline. Assess LVEF by echocardiogram or multigated acquisition (MUGA) scan before initiation of trametinib, one month after initiation of trametinib, and then at 2- to 3-month intervals while on treatment. Withhold treatment if absolute LVEF value decreases by 10% from pre-treatment values and is less than the lower limit of normal. Permanently discontinue trametinib for symptomatic cardiomyopathy or persistent, asymptomatic LVEF dysfunction that does not resolve within 4 weeks.

Retinal pigment Epithelial Detachment (RPED)

RPED can occur during treatment with trametinib. In the METRIC trial, where ophthalmologic examinations including retinal evaluation were performed pretreatment and at regular intervals during treatment, one patient (0.5%) receiving trametinib developed RPED and no cases of RPED were identified in chemotherapy-treated patients. Across all clinical trials of trametinib, the incidence of RPED was 0.8% (14/1749). Retinal detachments were often bilateral and multifocal, occurring in the macular region of the retina. RPED led to reduction in visual acuity that resolved after a median of 11.5 days (range: 3 to 71 days) following the interruption of dosing with trametinib, although Ocular Coherence Tomography (OCT) abnormalities persisted beyond a month in at least several cases. Perform ophthalmological evaluation at any time a patient reports visual disturbances and compare to baseline, if available. Withhold trametinib if RPED is diagnosed. If resolution of the RPED is documented on repeat ophthalmological evaluation within 3 weeks, resume trametinib at a reduced dose.

Retinal Vein Occlusion (RVO)

Across all clinical trials of trametinib, the incidence of RVO was 0.2% (4/1749). An RVO may lead to macular edema, decreased visual function, neovascularization, and glaucoma. Urgently (within 24 hours) perform ophthalmological evaluation for patient-reported loss of vision or other

visual disturbances. Permanently discontinue trametinib in patients with documented retinal vein occlusion.

Interstitial Lung Disease

In clinical trials of trametinib at the recommended dose (N = 329), interstitial lung disease (ILD) or pneumonitis occurred in 1.8% of patients. In Trial 1, 2.4% (5/211) of patients treated with trametinib developed ILD or pneumonitis; all five patients required hospitalization. The median time to first presentation of ILD or pneumonitis was 160 days (range: 60 to 172 days). Withhold trametinib in patients presenting with new or progressive pulmonary symptoms and findings including cough, dyspnea, hypoxia, pleural effusion, or infiltrates, pending clinical investigations. Permanently discontinue trametinib for patients diagnosed with treatment related ILD or pneumonitis.

Serious Skin Toxicity

In this trial, the overall incidence of skin toxicity including rash, dermatitis, acneiform rash, palmar-plantar erythrodysesthesia syndrome, and erythema was 87% in patients treated with trametinib and 13% in chemotherapy-treated patients. Severe skin toxicity occurred in 12% of patients treated with trametinib. Skin toxicity requiring hospitalization occurred in 6% of patients treated with trametinib, most commonly for secondary infections of the skin requiring intravenous antibiotics or severe skin toxicity without secondary infection. In comparison, no patients treated with chemotherapy required hospitalization for severe skin toxicity or infections of the skin. The median time to onset of skin toxicity in patients treated with trametinib was 15 days (range: 1 to 221 days) and median time to resolution of skin toxicity was 48 days (range: 1 to 282 days). Reductions in the dose of trametinib were required in 12% and permanent discontinuation of trametinib was required in 1% of patients with skin toxicity. Monitor patients receiving trametinib for skin toxicities and for secondary infections.

Embryofetal Toxicity

Based on its mechanism of action, trametinib can cause fetal harm when administered to a pregnant woman. trametinib was embryotoxic and abortifacient in rabbits at doses greater than or equal to those resulting in exposures approximately 0.3 times the human exposure at the recommended clinical dose. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to a fetus.

Advise female patients of reproductive potential to use highly effective contraception during treatment with trametinib and for 4 months after treatment. Advise patients to contact their healthcare provider if they become pregnant, or if pregnancy is suspected, while taking trametinib.

1.2.5.3 Clinical trial experience

Trametinib as a single agent was evaluated in 329 patients including 107 (33%) exposed for ≥ 6 months and 30 (9%) exposed for ≥ one year. Trametinib as a single agent was studied in open-label, single-arm trials (N = 118) and in an open- label, randomized, active-controlled trial (N = 211; the METRIC study, a randomized, open-label trial of patients with BRAF V600E or V600K mutation-positive unresectable or metastatic melanoma). The median age was 54 years, 60% were male, > 99% were White, and all patients had unresectable or metastatic melanoma. All patients received 2 mg once-daily doses of trametinib or chemotherapy (N = 99) (either dacarbazine 1,000 mg/m^2 every 3 weeks or paclitaxel 175 mg/m^2 every 3 weeks). Patients with abnormal LVEF, history of acute coronary syndrome within 6 months, or current evidence of Class II or greater

congestive heart failure (New York Heart Association) were excluded. The median duration of treatment with trametinib was 4.3 months.

In this study, 9% of patients receiving trametinib experienced adverse reactions resulting in permanent discontinuation of trial medication. The most frequent adverse reactions resulting in permanent discontinuation of trametinib were decreased left ventricular ejection fraction (LVEF), pneumonitis, renal failure, diarrhea, and rash. Adverse reactions led to dose reductions in 27% of patients treated with trametinib. Rash and decreased LVEF were the most frequent reasons cited for dose reductions of trametinib. **Table 1 and Table 2** present adverse reactions and laboratory abnormalities, respectively, of trametinib as a single agent in the METRIC study.

Table 1. Select Adverse Reactions Occurring in ≥ 10% of Patients Receiving Trametinib and at a Higher Incidence (≥ 5%) than in the Chemotherapy Arm or ≥ 2% (Grades 3 or 4) Adverse Reactions in METRIC

Adverse Reactions	Trametinib N = 211		Chemotherapy N = 99	
	All Grades [a, b]	Grades 3 [b] and 4 [c, b]	All Grades[a]	Grades 3 and 4 [c, b]
Skin and subcutaneous tissue				
Rash	57	8	10	0
Acneiform dermatitis	19	<1	1	0
Dry skin	11	0	0	0
Pruritus	10	2	1	0
Paronychia	10	0	1	0
Gastrointestinal				
Diarrhea	43	0	16	2
Stomatitis [d]	15	2	2	0
Abdominal pain [e]	13	1	5	1
Vascular				
Lymphedema [f]	32	1	4	0
Hypertension	15	12	7	3
Hemorrhage [g]	13	<1	0	0

[a]Events included are higher in the trametinib arm compared with chemotherapy by ≥5% in 141 overall incidence or by ≥2% Grade 3-4 adverse reactions higher in trametinib arm compared 142 with chemotherapy.
[b]National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.
[c]Grade 4 adverse reactions were limited to rash (n = 1) in trametinib arm and diarrhea (n = 1) in the chemotherapy arm.
[d]Includes the following terms: stomatitis, aphthous stomatitis, mouth ulceration, and mucosal inflammation.
[e]Includes the following terms: abdominal pain, abdominal pain lower, abdominal pain upper, and abdominal tenderness.
[f]Includes the following terms: lymphedema, edema, and peripheral edema.
[g]Includes the following terms: epistaxis, gingival bleeding, hematochezia, rectal hemorrhage, melena, vaginal hemorrhage, hemorrhoidal hemorrhage, hematuria, and conjunctival hemorrhage.

Other clinically important adverse reactions observed in ≤ 10% of patients (N = 329) receiving trametinib were: bradycardia, dry mouth, folliculitis, rash pustular, cellulitis, rhabdomyolysis, dizziness, dysgeusia, blurred vision, dry eye

Table 2. Laboratory Abnormalities Occurring at a Higher Incidence in Patients Treated with Trametinib in the METRIC Study [Between-arm Difference of ≥ 5% (All Grades) or ≥ 2% (Grades 3 or 4)[a]]

Laboratory Abnormality	Trametinib N = 211		Chemotherapy N = 99	
	All Grades	Grades 3 and 4	All Grades	Grades 3 and 4
Increased aspartate aminotransferase (AST)	60	2	16	1
Hypoalbuminemia	42	2	23	1
Increased alanine aminotransferase (ALT)	39	3	20	3
Anemia	38	2	26	3
Increased alkaline phosphatase	24	2	18	3

[a]Only grade 3 adverse reactions were reported in either treatment arm

Further information on these risks (e.g. presenting symptoms) can be found in the current version of the trametinib package insert.

1.2.6 Hydroxychloroquine

1.2.6.1 Mechanism of Action

HCQ can inhibit lysosomal acidification and prevent the degradation of autophagosomes, thereby suppressing autophagy. The mechanism by which chloroquine derivatives interfere with autophagy is still not very well understood.

1.2.6.2 Toxicity

Ocular:

Irreversible retinal damage has been observed in some patients who had received HCQ sulfate. Significant risk factors for retinal damage include daily doses of HCQ sulfate greater than 6.5 mg/kg (5 mg/kg base) of actual body weight, durations of use greater than five years, subnormal glomerular filtration, use of some concomitant drug products such as tamoxifen citrate and concurrent macular disease. A baseline ocular examination is recommended within the first year of starting HCQ. The baseline exam should include best corrected distance visual acuity (BCVA), an automated threshold visual field (VF) of the central 10 degrees (with retesting if an abnormality is noted), and spectral domain ocular coherence tomography (SD-OCT). For individuals with significant risk factors (daily dose of HCQ sulfate greater than 5.0 mg/kg base of actual body weight, subnormal glomerular filtration use of tamoxifen citrate or concurrent macular disease) monitoring should include annual examinations which include BCVA, VF and SD-OCT.

For individuals without significant risk factors, annual exams can usually be deferred until five years of treatment. In individuals of Asian descent, retinal toxicity may first be noticed outside the macula. In patients of Asian descent, it is recommended that visual field testing be performed in the central 24 degrees instead of the central 10 degrees. It is recommended that HCQ be discontinued if ocular toxicity is suspected and the patient should be closely observed given that retinal changes (and visual disturbances) may progress even after cessation of therapy.

Cardiac Effects, including Cardiomyopathy and QT prolongation:

Post marketing cases of life-threatening and fatal cardiomyopathy have been reported with use of HCQ as well as with use of chloroquine. Patients may present with atrioventricular block, pulmonary hypertension, sick sinus syndrome or with cardiac complications. ECG findings may include atrioventricular, right or left bundle branch block. Signs or symptoms of cardiac compromise have appeared during acute and chronic treatment. Clinical monitoring for signs and symptoms of cardiomyopathy is advised, including use of appropriate diagnostic tools such as ECG to monitor patients for cardiomyopathy during HCQ therapy. Chronic toxicity should be considered when conduction disorders (bundle branch block/atrio-ventricular heart block) or biventricular hypertrophy are diagnosed. If cardiotoxicity is suspected, prompt discontinuation of HCQ may prevent life-threatening complications. HCQ prolongs the QT interval. Ventricular arrhythmias and torsade's de pointes have been reported in patients taking HCQ. Therefore, HCQ should not be administered with other drugs that have the potential to prolong the QT interval

Worsening of psoriasis and porphyria:

Use of HCQ in patients with psoriasis may precipitate a severe attack of psoriasis. When used in patients with porphyria the condition may be exacerbated. The preparation should not be used in these conditions unless in the judgment of the physician the benefit to the patient outweighs the possible hazard.

Proximal Myopathy and Neuropathy:

Skeletal muscle myopathy or neuropathy leading to progressive weakness and atrophy of proximal muscle groups, depressed tendon reflexes, and abnormal nerve conduction, have been reported. Muscle and nerve biopsies have been associated with curvilinear bodies and muscle fiber atrophy with vacuolar changes. Assess muscle strength and deep tendon reflexes periodically in patients on long-term therapy with HCQ.

Neuropsychiatric events, including suicidality:

Suicidal behavior has been rarely reported in patients treated with HCQ.

Hypoglycemia:

HCQ has been shown to cause severe hypoglycemia including loss of consciousness that could be life threatening in patients treated with or without antidiabetic medications. Patients treated with HCQ should be warned about the risk of hypoglycemia and the associated clinical signs and symptoms. Patients presenting with clinical symptoms suggestive of hypoglycemia during treatment with HCQ should have their blood glucose checked and treatment reviewed as necessary.

1.2.6.3 Toxicities of the combination of trametinib and HCQ

There is no data available in terms of this combination though there should have this information accessible in the near future since several trials involved with this combination is ongoing. However, there is safety data from the BAMM trial (NCT02257424), that is designed for patients with advanced BRAF mutant melanoma treated with dabrafenib, trametinib, and hydroxychloroquine. The doses of trametinib and hydroxychloroquine used in the BAMM trial is the exact same as we plan in our trial except that we are not planning BRAF inhibitor dabrafenib. Therefore, this precious data will provide important reference for our trial. From personal communication with Dr. Ravi Amaravadi (Primary Investigator of BAMM trial, University of Pennsylvania), we summarize the safety profile from BAMM trial as showed in the following **Table 3**.

Table 3. Safety profile in the BAMM Study

Adverse Event	Dabrafenib + trametinib + HCQ (BAMM)	
	Grade 1-2	Grade 3
Chills	14 (56%)	1 (4%)
Diarrhea	13 (52%)	0
Fever	13 (52%)	1 (4%)
Nausea	12 (48%)	1 (4%)
Fatigue	11 (44%)	0
Headache	9 (36%)	0
Myalgia	9 (36%)	0
Rash	9 (36%)	3 (12%)
Anorexia	8 (32%)	0
Dry mouth	7 (28%)	0
Pruritus	7 (28%)	0
Arthralgia	6 (24%)	0
Abdominal pain	5 (20%)	0
Vomiting	5 (20%)	0
Constipation	4 (16%)	0
Dysgeusia	4 (16%)	0
Creatinine increased	3 (12%)	0
Dehydration	3 (12%)	2 (8%)
QTc prolonged	3 (12%)	1 (4%)
Increased alanine aminotransferase	1 (4%)	2 (8%)
Ejection fraction decreased	0	1 (4%)

With the triplet combination, the most common adverse effects are chills, diarrhea, fever, nausea, rash, and fatigue with grade 1-2. No grade 4 AEs are reported. This information indicates the less adverse effects we foresee with trametinib and hydroxychloroquine in our trial.

1.2.7 Drug dose rationale

Given there is a previously described efficacious dosing for trametinib at 2 mg once daily for metastatic melanoma with described de-escalation for trametinib associated side-effects, we will keep this dosing static and only de-escalate if trametinib specific, dose limiting side effects are encountered. As we do not know the dosing of HCQ needed to inhibit trametinib-induced autophagy, we will use flat dose as 600 mg twice daily used in previous mentioned PDAC trial and de-escalate if HCQ specific, dose limiting side effects are encountered. Both drugs are orally bioavailable and will be dosed orally. Drug-drug interactions between trametinib and HCQ are not expected due to separate metabolism and transporter pathways.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 Eligibility Criteria

2.1.1 Inclusion Criteria

- Histopathological confirmation of:
  - biliary tract carcinoma (BTC)

  OR

- carcinoma in the setting of clinical and radiological characteristics which, together with the pathology, are highly suggestive of a diagnosis of BTC

Note: The term BTC includes intra- or extra- hepatic cholangiocarcinoma (CCA), gallbladder cancer or ampullary cancer.

- The tumor must have KRAS mutation(s) of clinical significance, confirmed by NCI Laboratory of Pathology or by FDA approved test.
- Patients must have received or been intolerant of at least one line of chemotherapy.
- Patients must have at least 1 measurable lesion by RECIST version 1.1 (see section **6.3**)
- Patients must have disease that is not amenable to potentially curative resection, ablation or transplantation.
- Age ≥ 18 years.
- Performance status (ECOG) 0-2 (see **Appendix A**)
- If liver cirrhosis is present, patient must have a Child-Pugh score <7 (Class A) (Section **Appendix B**)
- Patients must have adequate organ and marrow function as defined below:

ANC	≥ 1,500/mcL
platelets	≥ 100,000/mcL
hemoglobin	≥ 9 g/dL
total bilirubin	If cirrhosis present: Part of Child Pugh requirement If no cirrhosis: bilirubin should be ≤ 1.5 x ULN
ALT or AST	≤ 5 x ULN.
Creatinine OR Measured or calculated creatinine clearance (CrCl) (eGFR may also be used in place of CrCl) [A]	< 1.5x institution upper limit of normal OR ≥ 30 mL/min/1.73 m^2 for participant with creatinine levels ≥ 1.5 X institutional ULN
ALT (SGPT)=alanine aminotransferase (serum glutamic pyruvic transaminase); AST (SGOT)=aspartate aminotransferase (serum glutamic oxaloacetic transaminase); GFR=glomerular filtration rate; ULN=upper limit of normal. [A] Creatinine clearance (CrCl) or eGFR should be calculated per institutional standard.	

- Patients must have at least 1 focus of disease that is amenable to mandatory tumor biopsies and be willing to undergo this. Ideally, the biopsied lesion should not be one of the target measurable lesions, although this can be up to the discretion of the investigators.
- The study drugs are harmful for developing human fetus. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier

method of birth control; abstinence) at the study entry, for the duration of study treatment and up to 4 months after the last dose of the study drug(s). Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

- Patients must be able to understand and be willing to sign a written informed consent.

2.1.2 Exclusion Criteria

- Patients who have had standard-of-care anti-cancer therapy within 2 weeks of treatment initiation or therapy with investigational agents (e.g. chemotherapy, immunotherapy, endocrine therapy, targeted therapy, biologic therapy, tumor embolization, monoclonal antibodies or other investigation agents), large field radiotherapy, or major surgery within 4 weeks of treatment initiation.
- Any unresolved toxicity NCI CTCAE v.5 Grade ≥2 from previous anticancer therapy with the exception of alopecia, vitiligo, and the laboratory values defined in the inclusion criteria. Patients with Grade ≥2 neuropathy will be evaluated on a case-by-case basis.
- Has biliary duct obstruction, unless a treatable, clinically relevant obstruction has been relieved by internal endoscopic drainage/stenting, palliative by-pass surgery or percutaneous drainage prior to treatment initiation.
- Patients with known brain metastases are excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.
- Patients with signs of liver failure, e.g. clinically significant ascites, encephalopathy, or variceal bleeding within six months before treatment initiation.
- History or current evidence of retinal vein occlusion (RVO) or current risk factors for RVO (e.g. uncontrolled glaucoma or ocular hypertension, history of hyper viscosity or hypercoagulability syndromes)
- Current evidence of uncontrolled, significant intercurrent illness including, but not limited to, the following conditions:
  - Cardiovascular disorders: Congestive heart failure New York Heart Association class 3 or 4, unstable angina pectoris, serious cardiac arrhythmias, stroke (including transient ischemic attack [TIA]), myocardial infarction (MI), or other ischemic event, or thromboembolic event (e.g., deep venous thrombosis, pulmonary embolism) within 3 months before treatment initiation
  - History of glucose-6-phosphate dehydrogenase (G6PD) deficiency
  - History of seizures
  - Patients who are planning on embarking on a new strenuous exercise regimen after first dose of study treatment. Muscular activities, such as strenuous exercise, that can result in significant increases in plasma creatine kinase (CK) levels should be avoided while on study treatment

- Patients who have neuromuscular disorders that are associated with elevated CK (e.g., inflammatory myopathies, muscular dystrophy, amyotrophic lateral sclerosis, spinal muscular atrophy)
- Impairment of gastrointestinal function or gastrointestinal disease (e.g., ulcerative disease, uncontrolled nausea, vomiting, diarrhea, malabsorption syndrome, or small bowel resection that under the judgment of the principal investigator (PI) may impair absorption of study drugs)
- Any other condition that would, in the Investigator's judgment, contraindicate the patient's participation in the clinical study due to safety concerns or compliance with clinical study procedures, e.g., infection/inflammation, intestinal obstruction, unable to swallow medication (patients may not receive drug through a feeding tube), social/ psychological issues, etc.

- Screening corrected QT interval by Fridericia's (QTcF) > 500 msec
- Known infection with human immunodeficiency virus (HIV), unless patient is on effective anti-retroviral therapy with undetectable viral load within 6 months of treatment initiation
- Known chronic hepatitis B virus, unless hepatitis B virus (HBV) viral load is undetectable.
- Known history of hepatitis C virus (HCV) infection, unless completed treatment and cured with undetectable HCV viral load.
- Known prior severe hypersensitivity to study drugs or any component in its formulations (CTCAE v5.0 grade >= 3).
- Pregnant women are excluded from this study because study therapy can cause fetal harm. Because there is potential risk for adverse events in nursing infants secondary to treatment of the mother with study therapy, breastfeeding should be discontinued if the mother is treated with study drugs.

2.1.3 Recruitment Strategies

This protocol may be abstracted into a plain language announcement posted on NIH websites, including www.clinicaltrials.gov and the CCR website, and on NIH social media platforms. Outside providers and colleagues may directly refer patients for screening into this study.

2.2 Screening Evaluation

2.2.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes.

2.2.2 Screening activities performed after a consent for screening has been signed

The following activities will be performed only after the subject has signed the study consent OR the consent for study 01-C-0129 (provided the procedure is permitted on that study) on which screening activities may also be performed. Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a patient has signed the consent.

The following must be completed within 28 days prior to initiation of study treatment unless otherwise noted below:

- Complete Medical History and Physical Evaluation (including height, weight, vital signs, and ECOG performance status).
- EKG
- Echocardiogram
- Cardiology Consultation (if clinically indicated)
- Ophthalmological evaluation
- CT scan of chest, abdomen and pelvis (or MRI of chest, abdomen and pelvis if clinically indicated)
- MRI of brain (if clinically indicated)
- Laboratory Evaluation
    - Hematological Profile: CBC with differential and platelet count.
    - Biochemical Profile: electrolytes, BUN, creatinine, AST, ALT, total and direct bilirubin, calcium, phosphorus, albumin, magnesium.
    - 24-hour urine collection (if clinically indicated)
    - Serum or urine pregnancy test for female participants of childbearing potential (within 2 weeks of enrollment).
    - HIV, Hepatitis B and C serology and/or viral load (if clinically indicated)
    - TB testing (if clinically indicated)
    - Troponin I (if clinically indicated)
- Documentation of histologic confirmation of BTC or carcinoma highly suggestive of a diagnosis of BTC (at any time point prior to enrollment). If there is no available documentation, biopsy will be performed to confirm the diagnosis.
- KRAS mutation status will be confirmed by NCI Laboratory of Pathology with TruSight™ Oncology 500 panel (at any time point prior to enrollment). If there is no available tumor sample, biopsy will be performed to confirm KRAS mutational status. Note: if participant has documentation of KRAS mutation status performed with FDA approved test (see Study Instrument) from certified laboratory, confirmation with TruSight™ Oncology 500 panel can be omitted.

2.3 Participant Registration and Status Update Procedures

Registration and status updates (e.g. when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found here.

2.3.1 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a not definite KRAS status may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening

2.3.2 Treatment Assignment Procedures (for registration purposes only):

Cohorts

Number	Name	Description
1	Cohort 1	Subjects with BTC or carcinoma in the setting of clinical and radiological characteristics which, together with the pathology, are highly suggestive of a diagnosis of BTC.

Arms

Number	Name	Description
1	Arm 1	Trametinib + hydroxychloroquine (HCQ)

Patients in Cohort 1 will be directly assigned to treatment in Arm 1.

2.4 Baseline Evaluation

Tests done at screening do not need to be repeated on baseline if performed in designated time frame prior to start of study treatment.

Within 28 days prior to study treatment initiation:

- CT scan of chest, abdomen and pelvis (or MRI of chest, abdomen and pelvis if clinically indicated)
- Baseline research biopsy (does not need to be repeated if biopsy was done during screening)
- Concomitant medications
- Baseline signs and symptoms
- Urinalysis

3 STUDY IMPLEMENTATION

3.1 Study Design

The proposed study is an open label, single-arm phase II study of trametinib in combination with hydroxychloroquine (HCQ) in BTC patients (**Schema 1**).

Initially, 6 patients will be enrolled into safety run-in portion of the trial (section **3.1.2**). If safe, we will continue enrollment as planned, if not, we will submit next amendment with new study design.

Initially, 10 patients will be enrolled and evaluated for progression. Enrollment will be temporarily halted after the 10th patient has been accrued, unless we know that 3 patients have passed the 5-month point without progression. If 3 or more of the first 10 patients enrolled have not progressed at the 5-month evaluation, then accrual will continue until a total of 17 patients have been entered. If, among the first 10 patients accrued, 0 to 2 are able to be progression-free at the 5-month evaluation, then no further patients will be enrolled after such a determination has been made. If 6 or more of the total cohort of 17 patients have been found to be progression-free at 5 months, then this will indicate an adequate progression free probability to justify further consideration of trametinib in combination with hydroxychloroquine in this population of patients. On the other hand, if 3 to 5 of 17 are progression-free at 5 months, this will be considered insufficient.

Treatment with trametinib and HCQ will be delivered in cycles consisting of 4 weeks (+/- 3 days) and start on cycle 1 day 1.

Both drugs will be given orally on daily basis: trametinib (2 mg once a day) and HCQ (600 mg twice a day – 1,200 mg total dose).

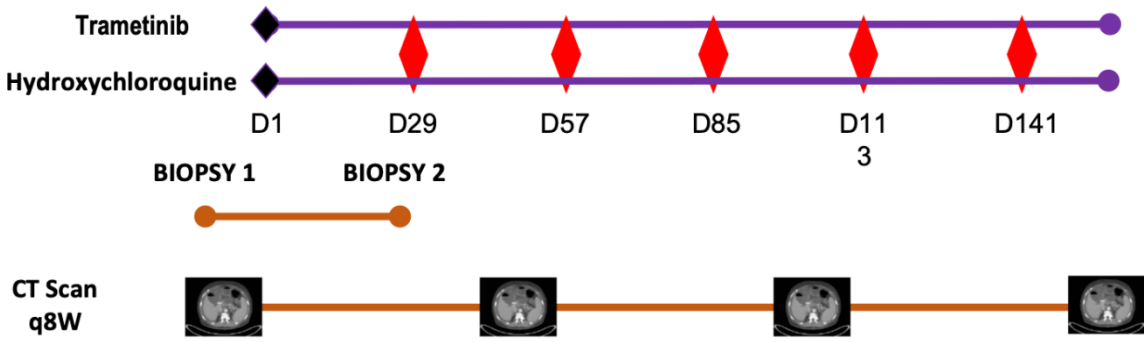
Treatment with study drugs will continue until off treatment criteria are met (see section **3.6.1**).

If one of the study drugs is permanently discontinued because of toxicity, per PI discretion the patient may continue the other study drug. It is unclear in terms of the attitude and duration of antitumor efficacy with this drug combination in BTC. If patient's disease is controlled with the combination but the treatment is held because of AEs from one of the agents, we think it would be beneficial for participants to continue the other agent and continue monitoring the disease progress accordingly.

Patients will be monitored every 8 (+/- 1) weeks with imaging studies.

Patients will be evaluated for toxicity and malignancy per Study Calendar **3.4**.

Patients will undergo mandatory research biopsies at the baseline and any day during week 3 of cycle 2. If patient disease progresses before C3D1, post-treatment biopsy might be performed per PI discretion at the time of progression.



Schema 1. Treatment and monitoring schedule

3.1.1 Dose Limiting Toxicity (DLT) (Safety Run-In only)

The DLT period is one cycle, 28 days, starting on Day 1 of cycle 1 after study drugs administration.

The following events will be considered DLTs (CTCAE v5.0) if attributable (definite, probable, possible) to the combination of study drugs:

Toxicity Category	**DLT Criteria (grade per CTCAE 5.0)**
Hematology	Grade ≥ 4 neutropenia lasting ≥ 7 consecutive days and/or not resolving within 48 hours to a grade 2 or less toxicity after correctible treatment
	Grade ≥ 4 thrombocytopenia lasting ≥ 7 consecutive days and/or not resolving within 48 hours to a grade 2 or less toxicity after correctible treatment
	Grade ≥ 3 neutropenia with fever (temperature ≥ 38.5 °C), not correctible within 48 hours to a grade 2 or less toxicity
Skin and subcutaneous tissue disorders	Grade ≥ 3 rash, hand-foot skin reaction or photosensitivity lasting > 7 consecutive days and/or not resolving within 48 hours to a grade 2 or less toxicity after correctible treatment
Eye disorders	Grade ≥ 2 retinal events lasting > 14 consecutive days confirmed by ophthalmologic examination
	Grade ≥ 3 retinal events confirmed by ophthalmologic examination, not correctible within 48 hours to a grade 2 or less toxicity
Gastrointestinal	Grade ≥ 3 nausea or vomiting lasting ≥ 7 days and/or not resolving within 48 hours to a grade 2 or less toxicity after correctible treatment
	Grade ≥ 3 diarrhea lasting ≥ 7 days and/or not resolving within 48 hours to a grade 2 or less toxicity after correctible treatment

Hepatic	Grade ≥ 3 total bilirubin not related to progression of disease, not correctible within 48 hours to a grade 2 or less toxicity
	Grade ≥ 3 AST or ALT (isolated increases in AST without concomitant increases in ALT will not be considered DLT because of the non-specific nature of AST), not correctible within 48 hours to a grade 2 or less toxicity. Patients with liver metastases will require AST or ALT levels 10 x ULN (isolated increases in AST without concomitant increases in ALT will not be considered DLT because of the nonspecific nature of AST).
	Grade ≥ 4 alkaline phosphatase lasting ≥ 7 consecutive days and/or not resolving within 48 hours to a grade 2 or less toxicity after correctible treatment
ECG QT Interval	QTcF interval ≥ 501 ms on at least two separate ECGs, not correctible within 48 hours to a grade 2 or less toxicity
Renal	Serum creatinine > 2 × ULN, not correctible within 48 hours to a grade 2 or less toxicity
Exceptions to DLT criteria	Grade 3 alopecia
	< 5 days of Grade 3 fatigue
	< 5 days of Grade 3 edema
	Grade ≤ 4 lymphopenia
	Grade ≤ 4 laboratory abnormalities that are responsive to oral supplementation or deemed by the investigator to be clinically insignificant.

3.1.2 Safety Run-In:

Six patients will be enrolled in a staggered fashion with at least 7 days minimum between each patient enrolled. If < 2 patients experience DLTs, as defined in section **3.1.1**, we will proceed to the following portion of the trial. If ≥ 2 patients experience DLT, we will stop enrollment and will submit next amendment with new study design.

Subjects who do not start or do not complete the DLT observation period for reasons other than a DLT will be replaced and not included in the evaluation.

In case of DLT, DLT will be documented and patient, per PI discretion, may continue study treatment if toxicity could be managed by interruption of study treatment or dose reductions (See Section **3.3**).

Patient will be eligible for the DLT evaluation if at least ≥80 percent of trametinib and HCQ scheduled doses were taken within the DLT period.

3.2 Drug Administration

3.2.1 Trametinib

Trametinib will be given orally at designated dose once a day on every day of every 28-day cycle.

Trametinib should be taken orally at least 1 hour before and 2 hours after a meal with approximately 200 mL of water. If a subject vomits after taking study treatment, the subject should be instructed not to retake the dose but should be instructed to take the next scheduled dose. If a subject misses a dose of trametinib, the subject may take the dose immediately if the next dose is scheduled for at least 12 hours later. If the next scheduled dose is due in less than 12 hours, the subject should skip the dose and resume dosing at the next scheduled dose.

Missing of 20% of the doses for any given cycle is allowed.

Patients will complete and return Patient's Diary (**Appendix C).**

3.2.2 Hydroxychloroquine (HCQ)

Hydroxychloroquine will be given orally at designated dose twice a day on every day of every 28-day cycle.

Hydroxychloroquine should be taken at approximately the same times each day. Doses should be taken within 2 hours of the scheduled time.

In case of a missed dose (more than 2 hours late) or vomiting after taking hydroxychloroquine, patients will be instructed not to make up the missed dose.

Hydroxychloroquine will be taken orally with a meal or a glass of milk.

Missing of 20% of the doses for any given cycle is allowed.

Patients will complete and return Patient's Diary (**Appendix C).**

3.3 Dose Modifications/Delay

In case of unbearable toxicity definitely attributed to one of the study drugs, patient will be taken off this drug and per PI discretion may continue treatment with the other drug only.

When, at the beginning of a treatment cycle, treatment delay related to one of the drugs is indicated, both study drugs should be held and resumed concurrently.

If, in the opinion of the investigator, a toxicity is considered to be due solely to one drug, the dose of the other drug may not require modification per PI discretion.

Dose interruptions for study treatment-related AEs are allowed as per the dose modification recommendations. Doses of any investigational product that were not administered due to toxicity will not be replaced within the same cycle. In addition to dose interruption, the need for a dose reduction at the time of treatment resumption should also be considered based on the dose modifications recommendations. If a toxicity-related dose delay of HCQ and trametinib for >28 days is required, treatment will be discontinued permanently, and the patient should be removed from study treatment.

3.3.1 Dose reduction

Following dosing interruption due to treatment related toxicity, the offending agent may need to be resumed at a reduced dose as per the dose modification recommendations as following. Dose

reduction should proceed by decreasing the administered dose by 0.5 mg of original dose of trametinib or 400 mg of original dose of HCQ.

3.3.2 Hydroxychloroquine

Table 4 Guidelines for hydroxychloroquine-related toxicities

Adverse Event	1st Occurrence	2nd Occurrence
Hematology Grade 4 neutropenia lasting ≥ 7 consecutive days Grade 4 thrombocytopenia ≥ 7 consecutive days Grade ≥ 3 neutropenia with fever (temperature ≥ 38.5 °C)	Hold HCQ and trametinib. Continue weekly hematologic monitoring. Once AE resolves to ≤Grade 1, restart study drugs, with HCQ at next lower dose level.	Discontinue HCQ and trametinib
Skin and subcutaneous tissue disorders Grade ≥ 3 rash, hand-foot skin reaction or photosensitivity lasting > 7 consecutive days despite skin toxicity treatment	See **Table 5**	Discontinue HCQ and trametinib
Eye disorders Grade 2 retinal events lasting > 14 consecutive days confirmed by ophthalmologic examination Grade ≥ 3 retinal events confirmed by ophthalmologic examination	Hold HCQ and trametinib. Monitor until AE resolves to Grade ≤ 1. Patients should be referred for ophthalmologic exam. Restart study drugs, with HCQ at next lower dose level.	Discontinue HCQ and trametinib
Gastrointestinal Grade ≥ 3 nausea or vomiting lasting ≥ 7 days despite optimal anti-emetic therapy	Hold HCQ and trametinib. Monitor until AE resolves to Grade ≤ 1. Restart study drugs, with HCQ at next lower dose level.	Discontinue HCQ and trametinib
Grade ≥ 3 diarrhea lasting ≥ 7 days despite optimal anti-diarrhea treatment	See **Table 6**	Discontinue HCQ and trametinib
Hepato-biliary Grade ≥ 3 total bilirubin felt to be not related to progression of disease Grade ≥ 3 ALT (isolated increases in AST without concomitant increases in ALT will not be considered dose-limiting because of the non-specific nature of AST) Grade 4 alkaline phosphatase lasting ≥ 7 consecutive days felt to be not related to progression of disease	Hold HCQ and trametinib. Monitor with weekly CMP until AE resolves to Grade ≤ 1. Restart study drugs, with HCQ at next lower dose level.	Discontinue HCQ and trametinib
ECG QT Interval	Hold HCQ and trametinib. Monitor	Discontinue HCQ

Adverse Event	1st Occurrence	2nd Occurrence
QTcF interval ≥ 501 ms on at least two separate ECGs	with ECG until AE resolves to Grade ≤ 1. Restart study drugs, with HCQ at next lower dose level.	and trametinib
Renal Serum creatinine ≥ Grade 2	Check cystatin-C levels. If GFR calculated by cystatin C is ≥ 30 mL/min/1.73 m2, continue HCQ and trametinib. If GFR calculated by cystatin C is ≤ 30 mL/min/1.73 m2, hold HCQ and trametinib. Monitor with weekly CMP and cystatin C until AE resolves to ≤Grade 1. Restart study drugs, with HCQ at next lower dose level.	Check cystatin-C levels. If GFR calculated by cystatin C is ≥30 mL/min/1.73 m2, continue HCQ and trametinib. If GFR calculated by cystatin C is ≤30 mL/min/1.73 m2, discontinue HCQ and trametinib.* *2nd occurrence of elevated serum creatinine will only be determined if 1st occurrence included elevated cystatin C and dose reduction.
Hypoglycemia	Hold HCQ if Grade 2. Monitor AE until resolution to Grade ≤ 1. Restart study drugs, with HCQ at next lower dose level. Hold HCQ if Grade 3. Monitor AE until resolution to Grade ≤ 1. Restart study drugs HCQ at next lower dose level. Discontinue HCQ permanently if Grade 4	Discontinue HCQ and trametinib
Non-hematologic events Grade ≥3, except for the exclusions noted below: • Grade 3 alopecia • < 5 days of Grade 3 fatigue • < 5 days of Grade 3 edema • Grade 3 laboratory abnormalities that are responsive to oral supplementation	Hold HCQ and trametinib. Monitor AE until resolution to Grade ≤ 1. Restart study drugs, with HCQ at next lower dose level.	Discontinue HCQ and trametinib

Adverse Event	1st Occurrence	2nd Occurrence
or deemed by the investigator to be clinically insignificant		

3.3.3 Trametinib

Dose reductions for adverse reactions associated with trametinib are presented in **Table 5**

Table 5:Recommended Dose Reductions for Trametinib for Adverse Reactions

Action	Recommended Dose
First Dose Reduction	1.5 mg orally once daily
Second Dose Reduction	1 mg orally once daily
Subsequent Modification	Permanently discontinue if unable to tolerate trametinib 1 mg orally once daily

Dosage modifications for adverse reactions associated with trametinib are presented in **Table 6**.

Table 6 Recommended Dosage Modifications for Trametinib for Adverse Reactions

Severity of Adverse Reaction[a]	Dosage Modification for Trametinib [b]
Venous Thromboembolism	
Uncomplicated deep venous thrombosis (DVT) or pulmonary embolism (PE)	Withhold trametinib for up to 3 weeks. • If improved to Grade 0-1, resume trametinib at lower dose. • If not improved, permanently discontinue trametinib.
Life threatening PE	Permanently discontinue trametinib.
Cardiomyopathy	
Asymptomatic, absolute decrease in left ventricular ejection fraction (LVEF) of 10% or greater from baseline and is below institutional lower limit of normal (LLN) from pretreatment value	Withhold trametinib for up to 4 weeks. • If improved to normal LVEF value, resume trametinib at lower dose. • If not improved to normal LVEF value, permanently discontinue

Severity of Adverse Reaction[a]	Dosage Modification for Trametinib [b]
	trametinib.
Symptomatic cardiomyopathy Absolute decrease in LVEF of greater than 20% from baseline that is below LLN	Permanently discontinue trametinib.
Ocular Toxicities	
Retinal pigment epithelial detachments (RPED)	Withhold trametinib for up to 3 weeks. • If improved, resume trametinib at same or lower dose. • If not improved, permanently discontinue trametinib or resume trametinib at lower dose.
Retinal vein occlusion (RVO)	Permanently discontinue trametinib.
Pulmonary	
Interstitial lung disease (ILD)/pneumonitis	Permanently discontinue trametinib.
Febrile Reactions	
Fever higher than 104°F Fever complicated by rigors, hypotension, dehydration, or renal failure	Withhold trametinib until fever resolves, then resume trametinib at same or lower dose.
Skin Toxicities	
Intolerable Grade 2 Grade 3 or 4	Withhold trametinib for up to 3 weeks. • If improved, resume trametinib at lower dose. • If not improved, permanently discontinue.
Severe cutaneous adverse reactions (SCARs)	Permanently discontinue trametinib.
Other Adverse Reactions, including Hemorrhage	

Severity of Adverse Reaction[a]	**Dosage Modification for Trametinib [b]**
Intolerable Grade 2 Any Grade 3	Withhold trametinib • If improved to Grade 0-1, resume at lower dose. • If not improved, permanently discontinue.
First occurrence of any Grade 4	• Withhold trametinib until improves to Grade 0-1, then resume at lower dose. Or • Permanently discontinue trametinib.
Recurrent Grade 4	Permanently discontinue trametinib.

[a] National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 5.0.

[b] See **Table 5** for recommended dose reductions of trametinib.

3.4 Study Calendar

	Screening	Baseline[1]	Day 1 of every cycle[1]	Weekly[2]	28 Days Safety FU[13, 15]	Long Term FU[14,15]
Trametinib[3]			X	X		
Hydroxychloroquine[4]			X	X		
Medical History	X					
Height	X					
Histologic confirmation of disease	X					
KRAS mutational status	X					
Ophthalmology consult[5]	X		X			
Cardiology evaluations[6]	X		X			
24-hour urine (if indicated)	X					
Infectious diseases testing [7]	X					
Physical exam, weight and ECOG	X		X		X	X
Vital Signs	X		X		X	
CBC w/differential, Platelets	X		X	X	X	X
Biochemical profile[8]	X		X	X	X	X
CK			X			
Urinalysis		X				
Pregnancy test[9]	X		X			
Thyroid tests TSH, T3, T4			X		X	
Uric acid, amylase and lipase			X	X	X	
PT, INR, PTT, fibrinogen			X	X	X	
Tumor marker CEA, AFP, CA19-9			X		X	
Baseline signs and symptoms		X				
Concomitant medications	X	X	X			
Adverse event evaluation			X		X	

	Screening	Baseline[1]	Day 1 of every cycle[1]	Weekly[2]	28 Days Safety FU[13, 15]	Long Term FU[14,15]
Radiologic Evaluation[10]	X	X	X			X
Tumor biopsy[11]		X		X		
Research blood for whole exome/panel		X				
Research blood for Immune monitoring[12]			X			
Research blood for serum p62 [12]			X			
Phone call or e-mail for survival every 6 month						X

[1] Baseline and C1D1 evaluations do not need to be repeated if performed on screening or baseline in designated time frame. Cycle=28+/-3 days. All evaluations will be done within 3 days before Day 1 of every cycle. Screening evaluations will be repeated if treatment does not start within 28 days after screening.

[2] Weekly until first restaging, after that every two weeks during treatment. Outside labs are acceptable.

[3] 2 mg of trametinib daily every day of every cycle.

[4] 600 mg of HCQ PO twice daily every day of every cycle.

[5] A standard ophthalmologic exam must be completed at screening, C2D1, and every 3 cycles thereafter (i.e. C5D1, C8D1, etc.) and as clinically indicated to assess for retinopathies. The screening ophthalmologic exam should include: best corrected distance visual acuity (BCVA), an automated threshold visual field (VF) of the central 10 degrees (with retesting if an abnormality is noted), and spectral domain ocular coherence tomography (SD-OCT). Outside evaluations are acceptable.

[6] EKG must be performed at screening and on Day 1 of every cycle. Echocardiogram must be completed at screening, C2D1, and every 3 cycles thereafter (i.e. C5D1, C8D1, etc.) and as clinically indicated. Troponin I test and cardiology consult only if clinically indicated.

[7] HIV, Hepatitis B and C serology. If screening serology results indicate potential HBV or HCV infection, viral load will be tested before starting investigational agents. TB testing will be performed only if clinically indicated.

[8] Biochemical Profile: electrolytes, BUN, creatinine, AST, ALT, total and direct bilirubin, calcium, phosphorus, albumin, magnesium.

[9] Pregnancy test for female participants of childbearing potential (serum or urine; if urine is positive it must be confirmed with serum test

[10] CT scan of chest, abdomen and pelvis (or MRI of chest, abdomen and pelvis if clinically indicated) every 8 (+/-1) weeks after start of study therapy. If patient taken off treatment for reason other than progression, CT scans will continue until progression or 5 months have passed since the start of study therapy whatever comes earlier. MRI of brain can be done at screening if clinically indicated.

[11] Mandatory tumor biopsies will be performed at baseline and any day during week 3 of cycle 2. If patient disease progresses before this, post-treatment biopsy might be performed per PI discretion at the time of progression.

[12] On Day 1 of cycles 1-4, then every 12 weeks until PD

[13] +/- 1 week

[14] After Safety Follow Up visit, subjects will be followed for adverse events by phone call or e-mail at 60 (+/- 14 days) and 90 (+/- 14 days) days. After that every 6 (± 1) months by phone call or e-mail for survival. If patient is taken off treatment for reason other than disease progression before 5 months have passed since the start of study therapy, we will continue to invite patient every 8 (+/-1) weeks for imaging studies until disease progression or 5 months have passed since the start of study therapy whatever comes earlier. Outside scans are acceptable.

[15] If patients are not willing to come to the NIH Clinical Center, they may be followed by phone call or e-mail.

3.5 Costs and Compensation

3.5.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

3.5.2 Compensation

No compensation is offered on this study.

3.5.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.6 Criteria for Removal from Protocol Therapy

Prior to removal from the study, effort must be made to have all subjects complete a safety visits approximately 30 days following the last dose of study therapy.

3.6.1 Criteria for Removal from Protocol Therapy

- Participant requests to be withdrawn from active therapy
- Unacceptable toxicity (both drugs) as described in Section **3.3**
- PI discretion
- Positive pregnancy test or intent to become pregnant
- Drugs become unavailable
- Initiation of therapy that prevents further administration of study treatment
- Confirmed disease progression based on radiographic progression per RECIST 1.1.
- Permanent loss of capacity to consent, per requirements of section **12.3**

3.6.2 Off Study Criteria

- Death
- Patient request to be withdrawn from study
- PI discretion
- Lost to follow up
- PI decision to end the study
- Screen failure.

3.6.3 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for 3 scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visits and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, an IRB approved certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up

4 CONCOMITANT MEDICATIONS/MEASURES

All routine and appropriate supportive care (including blood products) will be provided during this study, as clinically indicated, and in accordance with the standard of care practices. Clinical judgment should be utilized in the treatment of any adverse event experienced by the patient.

4.1 Prohibited Therapy

The following medications are not permitted to be taken concurrently with study therapy.

- Any other anti-cancer systemic therapy.
- Other investigational agents.
- Any live vaccines (Inactivated vaccines are allowed).
- The following QT prolonging agents: mesoridazine, thioridazine, pimozide, terfenadine, cisapride, sparfloxacin, saquinavir, ziprasidone, dronedarone, piperaquine, amisulpride (Other agents with the possibility of QT prolongation should be used with caution).
- Non-steroid anti-inflammatory medications (i.e. naproxen, ibuprofen, piroxicam, diclofenac, meloxicam).
- Anti-platelet agents (i.e. clopidogrel, prasugrel, dipyridamole).
- Serotonin reuptake inhibitors (SSRIs) should be used with caution.
- Tamoxifen.

5 CORRELATIVE STUDIES FOR RESEARCH

5.1 Biospecimen Collection

A description of correlative studies including a brief statement of rationale and processing information is made below.

Test/assay	Sample volume (approx.)	Type of tube/ tissue sample [a]	Collection point	Location of specimen analysis
Blood Samples				
Immune-monitoring by FACS	Blood, 100 ml	EDTA (Purple top tubes)	See Study Calendar **3.4**	BPC (Figg Lab)
Serum p62 by ELISA	4 ml	SST tube	See Study Calendar **3.4**	BPC (Figg Lab)
Whole exome/panel	Blood, 2.5 mL	1 PAX gene DNA tube	See Study Calendar **3.4**	BPC (Figg Lab)
Tumor Samples				
IHC for immune cell infiltration	Tumor sample		At time of biopsies	Laboratory of Pathology
RNA seq	Tumor sample		At time of biopsies	Laboratory of Pathology
Whole exome/panel	Tumor sample		At time of biopsies	Laboratory of Pathology
Proteomics	Tumor sample		At time of biopsies	Greten Laboratory
Metabolomics	Tumor sample		At time of biopsies	Greten Laboratory
RNA Nanostring analysis	Tumor sample		At time of biopsies	Greten Laboratory
IHC	Tumor sample		At time of biopsies	Greten Laboratory

a. Please note that tubes and media may be substituted based on availability with the permission of the PI or laboratory investigator.

5.1.1 Correlative studies of blood samples

Blood samples will be collected at time points indicated in the Study Calendar. Blood samples will be initially sent to the Blood Processing Core (BPC) for barcoding, storage and analysis.

5.1.1.1 Immune monitoring

We will analyze PBMC for quantitative and functional changes of effector cells by FACS. The effect on (i) CD4 T cell number and activity, (ii) CD8 T cell number and activity, (iii) NK cell number and activity, (iv) Treg number, (v) MDSC: frequency + functional assay, and (vi) the detection of tumor-associated antigens using tetramer assay. These data will provide further

information in terms of potential immune modulation effect with the combination therapy of trametinib/HCQ. These experiments will be done in BPC.

5.1.1.2 Serum p62 level

We predict that p62 levels may be elevated in serum with treatment as this has been demonstrated to be a biomarker for impaired autophagy [51]. We will test this hypothesis by obtaining blood samples from patients during routine blood collection and utilizing a commercially available ELISA assay to determine the concentration of p62 in the bloodstream. This study may also lead to a predictive biomarker for response to trametinib plus HCQ. These experiments will be done in BPC.

5.1.2 Tumor studies (non-genetic analysis)

Mandatory tumor biopsies will be performed at baseline and anytime during week 3 of Cycle 2. If patient disease progresses before week 3 of Cycle 2, post-treatment biopsy might be performed per PI discretion at the time of progression. It is preferred that at **least two core biopsies ≥18 gauge in diameter and ≥1 cm** in length will be obtained.

5.1.2.1 IHC

IHC will be performed on tumor tissue for assessment of immune cell infiltration (e.g. CD3+ CD4/8 cells, PDL1 etc.). Immunohistochemistry will be performed in the Laboratory of Pathology under the direction of Dr. David Kleiner.

5.1.2.2 Omics study

Tumor samples will be collected for proteomics and metabolomics studies to investigate the differential expression of protein and metabolite that might be associated with the treatment outcome or efficacy. These experiments will be done in Dr. Greten lab.

5.2 Samples for Genetic/Genomic Analysis

5.2.1 KRAS evaluation

KRAS mutation detection will be formed with TruSight™ Oncology 500 panel in Lab of Pathology.

5.2.2 RNA Nanostring analysis

Tumor samples will be used for gene expression profiling with Nanostring analysis (nCounterPan Cancer Immunology Profile) in BPC.

5.2.3 Whole exome/Panel (tumor/normal) and RNAseq (tumor)

Whole exome will be done on tumor and blood sample. RNAseq will be done on tumor for expressed somatic mutations and neoantigen discovery. Tumor material for these analyses will be sent to the Laboratory of Pathology, the laboratory of Drs. Javed Khan and Paul Meltzer (NCI Genetics Branch). Specifically, the expression of different signaling pathway, including interferon gamma signaling, immune cell functional molecular, e.g. perforin and granzyme, will be investigated

5.2.4 Management of Results

Subjects will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the

American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. (A list of current guidelines is maintained on the CCR intranet: https://ccrod.cancer.gov/confluence/display/CCRCRO/Incidental+Findings+Lists). Subjects will be contacted at this time with a request to provide a blood sample to be sent to a CLIA certified laboratory. If the research findings are verified in the CLIA certified lab, the subject will be offered the opportunity to come to NIH (at our expense) to have genetic education and counseling to explain this result. If the subject does not want to come to NIH, a referral to a local genetic healthcare provider will be provided (at their expense).

This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

5.3 Sample Storage, Tracking and Disposition

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside the National Institutes for Health (NIH) without appropriate approvals and/or agreements, if required.

All samples will be barcoded, with data entered and stored in the secure databases. These databases create a unique barcode ID for every sample and sample box, which cannot be traced back to patients without database access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80^{0}C according to stability requirements.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in database. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

5.3.1 Samples Managed by Dr. Figg's Blood Processing Core (BPC)

BPC contact information

Please e-mail at NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

5.3.2 Protocol Completion/Sample Destruction

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described in sections above. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed. The PI will record any loss or unanticipated destruction of samples as a deviation. Reports will be made per the requirements of section **7.2**.

If the patient withdraws consent the participant's data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

6 DATA COLLECTION AND EVALUATION

6.1 Data Collection

The PI will be responsible for overseeing entry of data into a 21 CFR Part 11-compliant data capture system provided by the NCI CCR and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the first study intervention, day 1 of cycle 1 through 90 days after the study agent (s) was/were last administered. Beyond 90 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded.

An abnormal laboratory value will be recorded in the database as an AE only if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization
- Is judged by the Investigator to be of significant clinical impact

If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

Information on all concomitant medications, administered blood products, as well as interventions occurring during the study must be recorded on the patient's eCRF.

End of study procedures: Data will be stored according to HHS, FDA, and NIH Intramural Records Retention Schedule regulations as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in section **7.2.1**.

6.2 Data Sharing Plan

6.2.1 Human Data Sharing Plan

The PI will share coded linked human data generated in this research for future research

- in a NIH-funded or approved public repository clinicaltrials.gov and dbGaP
- in BTRIS
- in publication and/or public presentations

at the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Plan

Unlinked genomic data will be deposited in public genomic databases such as dbGaP in compliance with the NIH Genomic Data Sharing Policy

6.3 Response Criteria

For the purposes of this study, patients should be re-evaluated every 8 (+/-1) weeks. In addition, confirmatory scans should also be obtained 4-8 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). 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.

6.3.1 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:

- By chest x-ray: >20 mm;
- By CT scan:
    - Scan slice thickness 5 mm or under as >10 mm
    - Scan slice thickness >5 mm: double the slice thickness
- With calipers on clinical exam: >10 mm.

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

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/pneumonitis, 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.

6.3.2 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.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

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).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable

globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used, and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound during the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Cytology, Histology: These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

6.3.3 Response Criteria

6.3.3.1 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 of 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 progression).

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

6.3.3.2 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).

6.3.3.3 Evaluation of Best Overall Response (BOR)

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.

For Patients with Measurable Disease (i.e., Target Disease)

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.
** 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.

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

6.3.3.4 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.

6.3.3.5 Modified Immune-mediated response criteria (imRC)

Modified immune-mediated response criteria (imRC) will also be employed in this study. This new classification is based on the recent learning from clinical studies with cancer immunotherapies that even if some new lesions appear at the beginning of a treatment or if the total tumor burden does not increase substantially, tumor regressions or stabilizations might still occur later. The irRC were created using bi-dimensional measurements (as previously widely used in the World Health Organization criteria). For this trial, the concepts of the irRC are combined with RECIST 1.1 to come up with the modified imRC. Please refer to **Appendix D** for further details.

Progression-Free Survival: PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

Overall Survival: Overall survival is defined as the duration of time from the start of treatment to time of death of any cause.

6.4 Toxicity Criteria

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50). All appropriate treatment areas should have access to a copy of the CTEP Active Version of CTCAE).

7 NIH REPORTING REQUIREMENTS / DATA SAFETY MONITORING PLAN

7.1 Definitions

Please refer to definitions provided in Policy 801: Reporting Research Events found here.

7.2 OHSRP Office of Compliance and Training/IRB Reporting

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802: Non-Compliance Human Subjects Research found here. Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 NIH Intramural IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found here.

7.2.3 NCI Clinical Director Reporting

Problems expeditiously reported to the OHSRP in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email to the Clinical Director unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to Dr. Dahut at NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.3 NIH Required Data and Safety Monitoring Plan

The clinical research team will meet on a weekly basis when patients are being actively treated on the trial to discuss each patient.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8 SPONSOR PROTOCOL/SAFETY REPORTING

8.1 Definitions

8.1.1 Unanticipated Adverse Device Effect

Unanticipated adverse device effect is any serious adverse effect on health or safety, any life-threatening problem or death caused by, or associated with a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the application; or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

8.2 Assessment of Adverse Device Effects

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Investigator or Record Form as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

UADEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Investigator or Record Form as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Investigator or Record Form as the site principal investigator or sub-investigator.

8.3 Reporting of Unanticipated Adverse Device Effects

Any UADE must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form.

All UADE reporting must include the elements described in Section **8.2**.

UADE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 Safety Reporting Criteria to the Pharmaceutical Collaborators

Reporting will be per the collaborative agreement.

8.5 Sponsor Protocol Deviation Reporting

A Protocol Deviation is defined as any non-compliance with the clinical trial Protocol, Manual of Operational Procedures (MOP) and other Sponsor approved study related documents, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the study site staff inclusive of site personnel performing procedures or providing services in support of the clinical trial.
It is the responsibility of the study Staff to document any protocol deviation identified by the Staff or the site Monitor in the CCR Protocol Deviation Tracking System (PDTS) online application. The entries into the PDTS online application should be timely, complete, and maintained per CCR PDTS user requirements.

In addition, any deviation to the protocol should be documented in the participant's source records and reported to the reviewing IRB per their guidelines. OSRO required protocol deviation reporting is consistent with E6(R2) GCP: Integrated Addendum to ICH E6(R1): 4.5 Compliance with Protocol; 5.18.3 (a), and 5.20 Noncompliance; and ICH E3 16.2.2 Protocol deviations.

9 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights of the participants are protected, that the study is implemented per the approved protocol, Good Clinical Practice and standard operating procedures, and that the quality and integrity of study data and data collection methods are maintained.

Monitoring for this study will be performed by NCI CCR Office of Sponsor and Regulatory Oversight (OSRO) Sponsor and Regulatory Oversight Support (SROS) Services contractor. Clinical site monitoring activities will be based on OSRO standards, FDA Guidance E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) March 2018, and applicable regulatory requirements.

Details of clinical site monitoring will be documented in a Clinical Monitoring Plan (CMP) developed by OSRO. CMPs will be protocol-specific, risk-based and tailored to address human subject protections and integrity of the study data. OSRO will determine the intensity and frequency of monitoring based on several factors, including study type, phase, risk, complexity, expected enrollment rate, and any unique attributes of the study and the site. The Sponsor will conduct a periodic review of the CMP to confirm the plan's continued appropriateness. A change to the protocol, significant or pervasive non-compliance with GCP, or the protocol may trigger CMP updates.

OSRO SROS Monitoring visits and related activities will be conducted throughout the life cycle of each protocol. The first activity is before the study starts to conduct a Site Assessment Visit (SAV) (as warranted), followed by a Site Initiation Visit (SIV), Interim Monitoring Visit(s) (IMVs), and a study Close-Out Visit (COV).

Some monitoring activities may be performed remotely, while others will take place at the study site(s). Monitoring visit reports will describe visit activities, observations, findings of protocol non-adherence and associated action items or follow-up required for resolution of findings. Monitoring reports will be distributed to the study PI, NCI CCR QA, coordinating center (if applicable) and the OSRO regulatory file.

The site Monitor will inform the study team of any deviations observed during monitoring visits. If unresolved, the Monitor will request that the site Staff enter the deviations in the CCR Protocol Deviation Tracking System (PDTS) for deviation reporting to the Sponsor and as applicable per institutional and IRB guidance.

10 STATISTICAL CONSIDERATIONS

10.1 Statistical hypothesis

10.1.1 Primary Objective

- To determine whether the 5-month PFS of the trametinib plus hydroxychloroquine (HCQ) combination in subjects with refractory bile tract carcinoma (BTC) with KRAS mutation exceeds 25%.

10.1.2 Secondary Objectives

- To determine the safety, tolerability and feasibility of the combination with trametinib plus HCQ in refractory BTC with KRAS mutation.
- To evaluate the response rate and overall survival the combination with trametinib plus HCQ in patients with refractory BTC with KRAS mutation.

10.2 Sample Size Determination

The primary objective of this study is to determine in a preliminary, limited size trial whether trametinib in combination with HCQ is associated with a 5-month progression free probability, which exceeds 25% when used to treat patients with cholangiocarcinoma.

Based on our analysis of data from previous trials of patients with similar eligibility requirements the median progression free survival in BTC is 2.5 months[8]. This translates to 25% without progressive disease at 5 months following an exponential failure model. Based on these results, it would be useful to demonstrate whether trametinib in combination with hydroxychloroquine is able to be associated with a median PFS of 5 months, which would correspond to 50% of patients having stable disease at 5 months. The trial will be conducted using a two-stage optimal design [52]. In order to attempt to determine if the agent offers any improvement, using alpha=0.10 and beta =0.10 as acceptable error probabilities, the trial will target 50% as the desirable proportion of patients who are still without progression at the five-month evaluation (p1=0.50), and will be considered inadequate if only a fraction consistent with 20% are without progression by the same evaluation time (p0=0.20).

Initially, 10 patients will be enrolled and evaluated for progression. Enrollment will be temporarily halted after the 10th patient has been accrued, unless we know that 3 patients have passed the 5-month point without progression. If 3 or more of the first 10 patients enrolled have not progressed at the 5-month evaluation, then accrual will continue until a total of 17 patients have been entered. If, among the first 10 patients accrued, 0 to 2 are able to be progression-free at the 5-month evaluation, then no further patients will be enrolled after such a determination has been made. If 6 or more of the total cohort of 17 patients have been found to be progression-free at 5 months, then this will indicate an adequate progression free probability to justify further consideration of trametinib in combination with hydroxychloroquine in this population of patients. On the other hand, if 3 to 5 of 17 are progression-free at 5 months, this will be considered insufficient. Under the null hypothesis (20% progression free at 5 months), the probability of being able to stop accrual after 10 patients have been evaluated at 5 months is 68%.

In addition to evaluation of the proportion of patients that are progression-free at 5 months, the progression-free survival for patients will also be analyzed via a Kaplan-Meier curve. This curve will be compared informally to other published results in similar patients. As well, the overall response rate will be reported, and the overall survival will be reported using a Kaplan-Meier curve. It is anticipated that up to 10 patients per year will be able to enroll onto this protocol. Thus, it is expected that accrual of up to 17 total patients can be completed in approximately 2 years. In order to allow for a small number of unevaluable patients and screen failures, the accrual ceiling will be set at 30 patients.

To evaluate the response rate, proportion of patients obtaining CR and PR per RECIST 1.1 criteria of all evaluable patients will be analyzed.

To address safety, tolerability and feasibility of trametinib in combination with hydroxychloroquine, adverse events will be tabulated by grade according to CTCAE and analyzed and reported descriptively. The fraction of patients who experience a response will be reported along with confidence intervals.

10.3 Population for Analysis

Any patient who receives at least one dose of both agents will be included in the efficacy evaluations.

Any patients who receive at least one dose of either agent will be included in the safety evaluations.

10.4 Statistical analysis

10.4.1 General approach

The fraction of patients who do not have progressive disease by 5 months will be reported.

10.4.2 Analysis of the Primary Endpoints

The fraction of patients who are able to not have progressive disease at 5 months will be reported along with a 95% confidence interval. A Kaplan-Meier curve of PFS will also be created and reported along with the 5-month PFS probability and its 95% confidence interval.

10.4.3 Analysis of the Secondary Endpoint

The safety of the treatment will be monitored, and any toxicities identified will be reported by type and grade. The feasibility and tolerability will be assessed and reported descriptively.

In each cohort, the fraction of patients who experience a response (PR + CR) will be reported along with 80% and 95% two-sided confidence intervals.

Overall survival will be calculated from the on-study date using the Kaplan-Meier method.

10.4.4 Safety analysis

The safety of the treatment will be monitored, and any toxicities identified will be reported by type and grade.

10.4.5 Baseline Descriptive Statistics

Baseline demographic characteristics will be reported.

10.4.6 Planned Interim Analyses

There are no planned interim analyses for efficacy.

10.4.7 Sub-group analyses

None are planned.

10.4.8 Tabulations of individual participant data

None.

10.4.9 Exploratory Analyses

The following objectives will result in descriptive or comparative analyses when adequate data exist to perform them:

- To evaluate the bioactivity of the combination of trametinib plus HCQ against the MEK1/2 and autophagy pathways by immunohistochemistry.
- To measure changes in immune parameters in the peripheral blood and tumors of patients with refractory KRAS mutation BTC treated with the combination of trametinib plus HCQ.
- To explore mechanism of primary and secondary resistance to the combination of trametinib plus HCQ in patients with refractory KRAS mutation BTC.
- To evaluate the relationship between the RR, OS and immune parameters, in patients with refractory KRAS mutation BTC treated with the combination of trametinib plus HCQ.
- Any exploratory evaluations which generate quantitative measures will be done using descriptive statistics including confidence intervals when appropriate. Any statistical tests performed for evaluation of exploratory objectives will be done without formal adjustment for multiple comparisons, but in the context of the number of tests performed.

11 COLLABORATIVE AGREEMENTS

11.1 Cooperative Research and Development Agreement (CRADA)

CRADA for this protocol is currently being negotiated between Thoracic & GI Malignancy Branch, NCI, NIH and Novartis, the manufacturer of trametinib.

12 HUMAN SUBJECTS PROTECTIONS

12.1 Rationale for Subject Selection

No individual who meets the criteria for eligibility will be excluded from participation based on their race, ethnicity, gender, or socioeconomic status. Particular attention will be made to acquire a broad and diversified population.

12.2 Participation of Children

Children (younger than 18 years) will not be included in this protocol due to the limited data on this combination in children and the different biology of childhood malignancy.

12.3 Participation of Subjects Unable to Give Consent

Adults unable to consent are excluded from enrolling in the protocol. However, it is possible that subjects enrolled in the protocol may permanently lose the capacity to consent for themselves during the course of this study. If a subject loses the capacity to consent during the course of the study for reasons unrelated to protocol participation and is receiving direct benefit from the study therapy in the opinion of the PI, the subject may be retained on the study therapy with the permission of the alternate decision maker. If the permanent loss of capacity is due to toxicity from the study therapy or disease progression, the participant will be removed from study therapy as required per protocol, but may be retained on study to be followed for safety reasons and/or overall survival as described in the Study Calendar **3.4**. The follow-up procedures involve non-invasive, low risk studies, with the exception of scanning. Scanning during follow-up will be performed only in participants who are taken of treatment for reasons other than disease progression and only within 5 months of study initiation. These scans are clinically indicated. The benefit of a safety follow up would outweigh the risks of such procedures. All subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another

person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Please see section **12.5** for the consent procedure.

12.4 Risk/Benefit Assessment for All Participants

12.4.1 Known Potential Risks

The primary risk to patients participating in this research study is from the toxicity of study drugs.

All care will be taken to minimize study treatment side effects, but they can be unpredictable in nature and severity.

12.4.1.1 Risk of Biopsy

All care will be taken to minimize risks that may be incurred by tumor sampling. However, there are procedure-related risks (such as bleeding, infection, visceral injury, hematoma and death) that will be explained fully during informed consent. However, it may be noted that we have not experienced a single bleeding event in approximately 250 patients who have undergone liver biopsy procedures recently for other standard of care or research testing. The biopsies will be performed in interventional radiology.

12.4.1.2 Risks of Exposure to Ionizing Radiation

The study will involve radiation from the following sources:

- Up to 7 CT scans per year for disease assessment
- 2 CT scans for the collection of 2 mandatory biopsies

Subjects in this study may be exposed to approximately 9.3 rem per year. This amount is more than would be expected from everyday background radiation. Being exposed to excess radiation can increase the risk of cancer. The risk of getting cancer from the radiation exposure in this study is 0.9 out of 100 (0.9%) and of getting a fatal cancer is 0.5 out of 100 (0.5%).

12.4.1.3 Risks of CT Scans

In addition to the radiation risks discussed above, CT scans may include the risks of an allergic reaction to the contrast. Participants might experience hives, itching, headache, difficulty breathing, increased heartrate and swelling.

12.4.1.4 Risks of sedation

Biopsies will be done under sedation. Potential side effects of sedation include headache, nausea and drowsiness. These side effects usually go away quickly.

12.4.1.5 Risks of ophthalmologic exam

Risks of ophthalmologic exam include discomfort, blurry vision and light sensitivity.

12.4.1.6 Risks of Blood Collection

Risks of blood draws include pain and bruising in the area where the needle is placed, lightheadedness, and rarely, fainting. When large amounts of blood are collected, low red blood cell count (anemia) can develop. Up to the 170 ml of blood may be collected at any visit, but no more than 550 ml per 8 weeks period.

12.4.1.7 Risks of EKG

Risks include some minor skin irritation from the electrodes.

12.4.1.8 Risks of Echocardiogram

Risks include mild discomfort during the test.

12.4.1.9 Other Risks

Risks include the possible occurrence of any of a range of side effects which are listed in the Consent Document or this protocol document. Frequent monitoring for adverse effects will help to minimize the risks associated with administration of the study agents.

12.4.1.10 Risk of losing data

This includes the risk that data obtained during this study, including data related to genotype, DNA sequencing or risk for disease tendency or trait can be released to members of the public, insurers, employers, or law enforcement agencies. Although there are no plans to release results to the patients, family members or health care providers, this risk will be included in the informed consent document.

12.4.1.11 Risk of receiving unwanted information

Anxiety and stress may arise as a result of the anticipation that unwanted information regarding disease related DNA sequencing or disease tendencies, or misattributed paternity. Patients will be clearly informed that the data related to DNA sequencing and genetic analysis is coded, investigational and will not be shared with patients, family members or health care providers.

12.4.2 Known Potential Benefits

The study drug may help to control the disease. The results may help the investigators learn more about the disease and develop new treatments for patients with this disease

12.4.3 Assessment of Potential Risks and Benefits

BTC treatment needs improved therapy options. Preclinical studies suggest that study therapy may have tremendous anti-tumor efficacy.

A number of clinically appropriate strategies to minimize risks to patients have been built into the protocol through the means of inclusion/exclusion criteria, monitoring strategies, and management guidelines. Overall, the potential benefit of the study treatment in subjects with BTC outweigh the risks associated with study drugs.

The potential benefit to a patient that participates in this study is better control of their tumor growth and disease recurrence which may or may not have a favorable impact on symptoms and/or survival.

Potential adverse reactions attributable to the administration of the study drug utilized in this trial are discussed in Sections **1.2.5.2 and 1.2.6.2**. All care will be taken to minimize side effects, but they can be unpredictable in nature and severity.

12.5 Consent Process and Documentation

The informed consent document will be provided as a physical or electronic document to the participant or consent designee(s) as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the

associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below, with a manual (non-electronic) signature on the electronic document. When required, witness signature will be obtained similarly as described for the investigator and participant.

Manual (non-electronic) signature on electronic document:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,
- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location but is not required.

Both the investigator and the subject will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found here.

12.5.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in section **12.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **12.5**

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and as applicable, Food and Drug Administration (FDA).

13.2 Quality Assurance and Quality Control

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

13.3 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NCI has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

13.4 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence.

No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites will be secured and password protected. At the end of the study, all study databases will be archived at the NIH Clinical Center.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

14 PHARMACEUTICAL INFORMATION

14.1 Trametinib

14.1.1 Source

Commercial supplies of trametinib will be supplied by Novartis

14.1.2 Acquisition and Accountability

Trametinib will be provided by Novartis and delivered directly to the NIH Pharmacy. Individual bottles with tablets will be prepared for each study participant according to assigned dose by NIH Pharmacy personnel. Patients will pick up bottles at NIH Pharmacy and will return bottles and not-used tablets after completion of every cycle together with Medication Diary to Study Coordinator. After review of leftover tablets and Medication Diary, unused tablets will be returned to pharmacy and disposed by pharmacy personnel.

14.1.3 Formulation, Appearance, Packaging, and Labeling

- Please refer to the package insert for additional information.

14.1.4 Product Storage and Stability

Please refer to the package insert for additional information.

14.1.5 Preparation

N/A.

14.2 HYDROXYCHLOROQUINE (generic)

14.2.1 Source

Hydroxychloroquine will be provided by the NIH Clinical Center Pharmacy according to standard pharmacy procedures

14.2.2 Acquisition and Accountability

Hydroxychloroquine will be delivered directly to the NIH Pharmacy. Individual bottles with tablets will be prepared for each study participant according to assigned dose by NIH Pharmacy personnel. Patients will pick up bottles at NIH Pharmacy and will return bottles and not-used tablets after completion of every cycle together with Medication Diary to Study Coordinator. After review of leftover tablets and Medication Diary, unused tablets will be returned to pharmacy and disposed by pharmacy personnel.

14.2.3 Formulation, Appearance, Packaging, and Labeling

Please refer to the package insert for additional information.

14.2.4 Product Storage and Stability

Please refer to the package insert for additional information.

14.2.5 Preparation

N/A.

14.3 TruSight™ Oncology 500 panel

TruSight™ Oncology 500 panel assay is not FDA approved; it is being used as a treatment determining in-vitro diagnostic device in this study. According to 21 CFR 812.3(m), a significant risk device presents a potential for serious risk to the health, safety and welfare of a subject and meets the significant risk criteria listed in the table below along with the sponsor's conclusions with regard to the applicability of these criteria to the current study. The device has been assessed by the sponsor as non-significant risk (NSR) per the below.

Significant Risk Criteria	Applicable to current study	Justification
Is an implant	No	TruSight™ Oncology 500 panel is not introduced into the subject
Is used in supporting or sustaining human life	No	The device is diagnostic
Is of substantial importance in diagnosing mitigating or	No	While the device is diagnostic, we do not believe in presents a potential for serious risk to the health and welfare of the subject. The

Significant Risk Criteria	Applicable to current study	Justification
treating disease or preventing impairment of human health		assessment of KRAS mutation status is only used to help to increase the possibility that all persons enrolling on the study might derive benefit from therapy. Persons that are deemed ineligible to enroll on the basis of this test are eligible for studies within TGMB that are not reliant on this test.
Otherwise poses a risk	No	Testing will be performed on fresh sample that is collected at screening for confirmation of diagnosis. No additional collection of sample will occur for purposes of KRAS testing.

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16 APPENDICES

16.1 Appendix A - Performance Status Criteria

ECOG Performance Status Scale

Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

16.2 Appendix B Child-Pugh Classification System

Parameter	Points assigned		
	1	2	3
Ascites	Absent	Slight	Moderate
Bilirubin	<2 mg/dL (<34.2 micromol/liter)	2-3 mg/dL (34.2 to 51.3 micromol/liter)	>3 mg/dL (>51.3 micromol/liter)
Albumin	>3.5 g/dL (35 g/liter)	2.8-3.5 g/dL (28 to 35 g/liter)	<2.8 g/dL (<28 g/liter)
Prothrombin time			
Seconds over control	<4	4-6	>6
INR	<1.7	1.7-2.3	>2.3
Encephalopathy	None	Grade 1-2	Grade 3-4

Modified Child-Pugh classification of the severity of liver disease according to the degree of ascites, the plasma concentrations of bilirubin and albumin, the prothrombin time, and the degree of encephalopathy. A total score of 5-6 is considered grade A (well-compensated disease); 7-9 is grade B (significant functional compromise); and 10-15 is grade C (decompensated disease). These grades correlate with one- and two-year patient survival: grade A - 100 and 85 percent; grade B - 80 and 60 percent; and grade C - 45 and 35 percent.

16.3 Appendix C - Patient's Medication Diary

Cycle_____ Patient's ID _____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each cycle of treatment
2. You will take trametinib once a day and HCQ twice a day every day during 28-day cycle
3. HCQ should be taken every 12 hours at approximately the same times each day. Doses should be taken within 2 hours of the scheduled time.
4. In case of a missed dose (more than 2 hours late) or vomiting after taking HCQ, do not make up the missed dose of HCQ.
5. Take trametinib on empty stomach: at least 1 hour before or 2 hours after a meal with a glass of water. Take HCQ with a meal or a glass of milk.
6. Record the date, the number of tablets that you took, and when you took them.
7. If you have any comments or notice any side effects, please record them in the comment's column.
8. Please bring this form and your bottles (even it is empty) when you come for your clinic visits.

Day	**Date**	**# of Oral HCQ Tablets taken (every 12 hours)**		**# of Oral trametinib Tablets taken**	**Comments (side effects or missed doses)**
		AM	**PM**		
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					

Day	Date	# of Oral HCQ Tablets taken (every 12 hours)		# of Oral trametinib Tablets taken	Comments (side effects or missed doses)
		AM	PM		
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					

Patient's signature: _____

16.4 Appendix D: Modified immune-related response criteria (irRC)

This new classification is based on the recent learning from clinical studies with cancer immunotherapies that even if some new lesions appear at the beginning of a treatment or if the total tumor burden does not increase substantially, tumor regressions or stabilizations might still occur later. The irRC were created using bi-dimensional measurements (as previously widely used in the World Health Organization criteria). For this trial, the concepts of the irRC are combined with RECIST 1.1 to come up with the modified irRC.
For modified irRC, only target and measurable lesions are taken into account. In contrast to the RECIST 1.1 criteria, the modified irRC criteria (a) require confirmation of both progression and response by imaging at 6 weeks after initial imaging and (b) do not necessarily score the appearance of new lesions as progressive disease if the sum of lesion diameters of target lesions (minimum of 10 mm per lesion, maximum of 5 target lesions, maximum of 2 per organ) and measurable new lesions does not increase by ≥ 20%.
The same method of assessment and the same technique should be used to characterize each identified and reported target lesion(s) at baseline, during the trial, and at the end of trial visit.
All measurements should be recorded in metric notation. The modified irRC based on RECIST 1.1 are displayed below.

Modified immune-related response criteria are defined as follows:

New measurable lesions: Incorporated into tumor burden.

New non-measurable lesions: Do not define progression but precludes (irCR).

Overall irCR:	Complete disappearance of all lesions (whether measurable or not) and no new lesions. All measurable lymph nodes also must have a reduction in short axis to 10 mm.
Overall irPR:	Sum of the longest diameters of target and new measurable lesions decreases ≥ 30%.
Overall irSD:	Sum of the longest diameters of target and new measurable lesions neither irCR, irPR, (compared to baseline) or irPD (compared to nadir).
Overall irPD:	Sum of the longest diameters of target and new measurable lesions increases ≥ 20% (compared to nadir), confirmed by a repeat, consecutive observations at least 4 weeks (normally it should be done at 6 weeks) from the date first documented.