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Protocol #: CTMS 16-0076

Study Title:

Modulation of Sorafenib induced autophagy using
hydroxychloroquine in hepatocellular cancer

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Summary of Changes

Protocol CTMS# 16-0076: Modulation of Sorafenib induced autophagy using hydroxychloroquine in hepatocellular cancer (CTMS# 16-0076)

Protocol Version 9.0 dated January 19, 2021

Protocol Title Page
Updated version, version date and pharmacy contact.
Rationale – Updated due to protocol revisions and change in pharmacy staff.
Primary Objective
Changed to read: To determine the clinical efficacy with time to tumor progression (TTP) of the combination of HCQ and sorafenib in sorafenib-naïve advanced HCC (cohort 1); and the addition of HCQ to sorafenib in patients who progress on sorafenib (cohort 2) duplicate “or” in exclusion criteria 4.
Rationale – This is to add a clarification given the changing landscape of treatments for liver cancer (HCC), and that patients may receive immunotherapy now before sorafenib. Also, this is congruence with study design that says “Cohort 1: SOR-naïve patients receive SOR”.
Schema
Updated schema figure.
Rationale – Updated to be consistent with: “Cohort 1: SOR-naïve patients receive SOR”.
Cohort 1
Updated wording for Cohort 1.
Rationale – This is to add a clarification given the changing landscape of treatments for liver cancer (HCC), and that patients may receive immunotherapy now before sorafenib. Also, this is congruence with study design above that says “Cohort 1: SOR-naïve patients receive SOR”.



Modulation of Sorafenib induced autophagy using hydroxychloroquine in hepatocellular cancer (CTMS# 16-0076)

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ABBREVIATIONS

AE	Adverse Event
BISR	Biostatistics and Informatics Shared Resource
CAI	Corrective Action Item
CQ	Chloroquine
DSM	Data Safety Monitoring
DSMB	Data Safety Monitoring Board
DSMC	Data Safety Monitoring Committee
DSMP	Data Safety and Monitoring Plan
DSO	Data and Safety Officer
DQA	Director of Quality Assurance
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HCQ	Hydroxychloroquine
IDD	Institute for Drug Development
IDEAS	Informatics Data Exchange Acquisition System
IIS	Investigator Initiated Protocol
IND	Investigational New Drug
IRB	Institutional Review Board
NSABP	National Surgical Adjuvant Breast and Bowel Project
NIH	National Institutes of Health
NCI	National Cancer Institute
NCI-CTEP	National Cancer Institute – Cancer Therapy Evaluation Program
NCI-CTSU	National Cancer Institute – Cancer Trials Support Unit
O&L	Operations and Logistics Committee
ORA	Office of Research Administration
OSP	Office of Sponsored Programs (UTHSCSA)
QAD	Quality Assurance Division
PALS	Priority of Audit Level Score
PI	Principal Investigator
PRC	Protocol Review Committee
PRF	Protocol Review Form
PRMS	Protocol Review and Monitoring System
PSD	Pharmacokinetic Sampling Department
RTOG	Radiation Therapy Oncology Group
SAE	Serious Adverse Event
SWOG	South West Oncology Group
UPIRSO	Unanticipated Problem Involving Risks to Subjects or Others
UTHSCSA	University of Texas Health Science Center at San Antonio
VAH	Veterans Administration Hospital (Audie L. Murphy)

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1.0 Synopsis

Title of Study: Modulation of Sorafenib induced autophagy using hydroxychloroquine in hepatocellular cancer
Investigators: PI: Sukeshi Patel Arora, MD Statistician: Joel Michalek, PhD
Study Center(s): UT Health Science Center at San Antonio
Concept and Rationale: In the U.S., HCC currently has the second highest increase in incidence and the highest increase in death rates of any tumor over the last decade.¹ Unfortunately, most patients present with advanced disease not amenable to curative therapy. The only systemic therapy approved for with patients with advanced HCC is sorafenib, based on the SHARP study that reported a median time to radiologic progression (mTTP) of 5.5 months with sorafenib vs. 2.8 months for placebo, and median overall survival (mOS) of 7.9 months with placebo vs. 10.7 months with sorafenib.² Given the modest improvement, it is postulated that HCC tumors may have primary or acquired resistance to sorafenib therapy. Recent studies suggest that therapy-induced autophagy promotes cancer cell survival, and thus, may diminish the efficacy of some therapeutic agents.³⁻⁵ Sorafenib-induced autophagy has been reported to promote survival of hepatoma cells, and thereby, may be a cellular adaptive response related to primary resistance to this compound.⁶ Furthermore, sorafenib treatment in HCC cells induced the morphological and biochemical hallmarks of autophagy, such as the generation of autophagosomes, GFP-LC3 redistribution and LC3-II accumulation.⁷ Additionally, the combination of sorafenib and HCQ, an inhibitor of autophagy, led to desired effects via endoplasmic reticulum (ER) stress-related apoptosis and growth inhibition in a pre-clinical model of human HCC.⁷ Based on this preclinical data, we conducted a phase I clinical study evaluating the safety and pharmacokinetics of the combination sorafenib with HCQ, that further support autophagy inhibition to enhance cancer therapeutics. Base on phase I data, we have determined the recommended phase 2 dose of sorafenib 400 mg by mouth (PO) twice daily and HCQ 400mg by mouth daily and showed clinical activity in a range of tumor types. These findings support a phase II study combining the autophagy inhibitor HCQ with sorafenib either in combination or sequentially added to sorafenib on progression of HCC. <i>We <u>hypothesize</u> that sorafenib/HCQ will have improved efficacy when compared to sorafenib alone and in patients progressing of sorafenib the addition of HCQ would lead to disease stability in patients with advanced HCC.</i> This hypothesis is based on the observations that: 1. Sorafenib, an oral multi-kinase inhibitor of Raf/MEK/ERK, is the only systemic approved therapy for advanced HCC but has shown only modest improvements in survival.² 2. Resistance to sorafenib in pre-clinical models has been attributed to autophagy induction.⁶ 3. Autophagy inhibition with HCQ enhanced sorafenib-induced cell death and apoptosis in pre-clinical and early clinical studies.⁷ 4. Preliminary data from the phase I study of sorafenib plus HCQ in advanced solid tumors at our institution demonstrates clinical safety and efficacy. Our overall objective is to answer these questions: will autophagy inhibition with HCQ improve efficacy of sorafenib and can biomarkers be identified that will predict clinical efficacy of autophagy inhibition with HCQ plus sorafenib in advanced HCC?
Primary Objective(s): • To determine the clinical efficacy with time to tumor progression (TTP) of the combination of HCQ and sorafenib in sorafenib-naive advanced HCC (cohort 1); and the addition of HCQ to sorafenib in patients who progress on sorafenib (cohort 2).
Secondary Objective(s):

- To determine the overall survival in advanced HCC patients receiving HCQ and sorafenib in both cohort 1 and 2
- To evaluate the tumor response rate in advanced HCC patients receiving HCQ and sorafenib
- To further define the safety in advanced HCC patients receiving HCQ and sorafenib
- To identify biomarkers of autophagy modulation and immune markers that correlate with clinical efficacy in advanced HCC patients receiving HCQ and sorafenib.

Primary Endpoint(s):

- TTP: will be measured for each patient from the start of the first treatment the patient receives until the date the criteria for progression are met or the date the patient is taken off study for any reason, whichever is shorter.

Secondary Endpoint(s):

- OS: measured from the start of treatment on trial until the death of the patient.
- Response: measured as best response, complete response (CR) or partial response (PR), as defined by Response Evaluation Criteria in Solid Tumors (RECIST 1.1) Committee.
- Adverse events: grade 1-5 adverse events according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v4.0; measured at baseline, each cycle, and at off study date.
- Biomarkers of efficacy of autophagy modulation: evaluate biomarkers such as cathepsin D and LC2-II levels in PBMCs (RT-PCR), and autophagic vesicles in tumor (electron microscopy). Immune markers, such as IL-6, will be measured.

Study Design:

Phase 2 study with two cohorts (Figure 1):

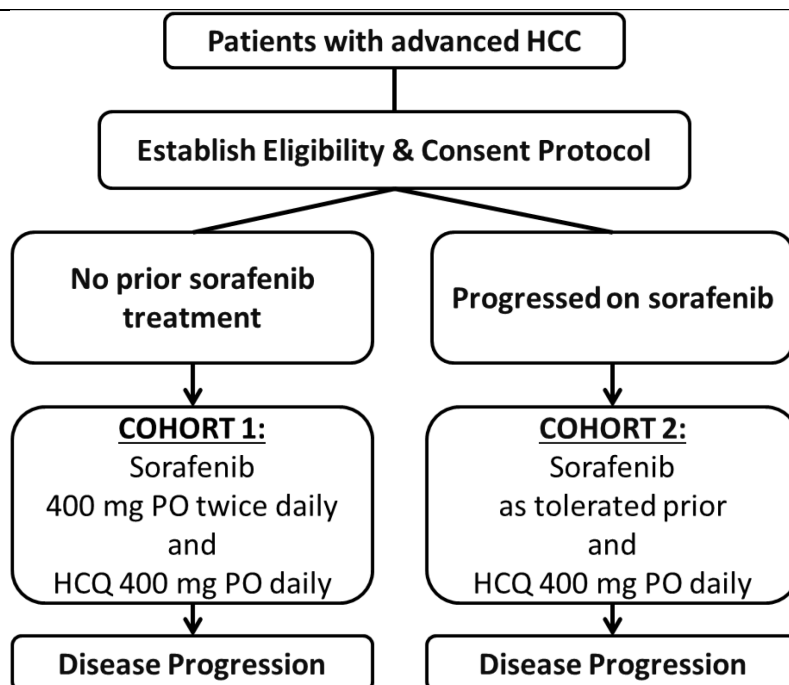
Cohort 1: SOR-naïve patients receive SOR 400 mg by PO twice daily on Cycle1/Day1 (C1D1). In clinical practice, dose reduction of SOR is often required⁸. Therefore, on C1D15, the clinician will dose-reduce sorafenib based on toxicity and HCQ 400 mg PO daily will be started. Alternatively, the treating physician can start the dose at 200 mg PO twice daily and titrate up to 400 mg PO twice daily based on safety and tolerability for patients with Child Pugh B cirrhosis or other factors that the physician feels the patient should start at a lower dose. C2D1 of each cohort, toxicity of HCQ will be assessed. Dose reductions due to adverse events (AEs) to each agent are allowed for SOR per standard of care and/or HCQ for grade 3+ AE (Table 2).

Cohort 2: As second-line treatment, we will add HCQ to SOR dose the patient was tolerating at the time of progression.

Blood samples will be collected at before treatment (baseline or C1D1), C1D15 and C2D1 to assess for biomarkers.

Dose reductions due to adverse events are allowed for both sorafenib per standard of care and/or HCQ for grade 3 or more adverse event was related to study medication. Dose reductions are also permitted based on investigator clinical decision.

Schema:



In cohort 1, we will administer sorafenib 400 mg by mouth (PO) twice daily and HCQ 400 mg PO daily in 4-week cycles. Alternatively, for patients with Child-Pugh B cirrhosis, the treating physician can start the dose at 200 mg PO twice daily and titrate up to 400 mg PO twice daily based on safety and tolerability, as per standard of care. In cohort 2, we will add HCQ to the dose of sorafenib the patient (pt) was using at the time of progression.

Number of Patients: Cohort 1 (sorafenib in combination with HCQ): Historically, the mTTP for patients treated with sorafenib is 5.5 mos. We predict addition of HCQ will improve mTTP by 50% to 8.2 months. We expect 5% lost to follow-up. Assuming a 24-month accrual period with 1 year for follow up, a one-parameter exponential model, and $\alpha=5\%$, 49 subjects are necessary to obtain 80% power. With 5% lost to follow-up the sample size required is 52 ($=49/0.95$).

Cohort 2 (Addition of HCQ on sorafenib progression): Historically, the mTTP for patients following progression on sorafenib 2.1 mos. We predict addition of HCQ will improve mTTP to 4.2 months. We expect 10% lost to follow-up. Assuming a 24-month accrual period with 1 year for follow up, a one-parameter exponential model, and $\alpha=5\%$, 19 subjects are necessary to obtain 80% power. With 10% lost to follow-up the sample size required is 21 ($=19/0.95$).

Main Criteria for Inclusion/Exclusion:

Inclusion Criteria:

1. Cytologically or histologically confirmed advanced or metastatic HCC. If no histological diagnosis, patient must have imaging studies compatible with HCC.
2. Age 18 years and above
3. ECOG performance status of 0 or 1
4. Not a candidate for curative treatments (i.e., resection, transplantation)
5. Child-Pugh class A or B7-8 liver function
6. Measurable disease as defined by RECIST 1.1
7. Patients who received prior local therapy (e.g., TACE) are eligible.
8. Documented virology status of hepatitis, as confirmed by at least 1 of the following: screening HBsAg, anti-HBc, or anti-HCV.
9. Life expectancy > 3 months

10. For women who are not postmenopausal (12 months of amenorrhea) or surgically sterile (absence of ovaries and/or uterus): agreement to use two adequate methods of contraception. For men: agreement to use a barrier method of contraception during the treatment period
11. Hematologic, Biochemical, and Organ Function within 21 days prior to Cycle 1 Day 1: Granulocyte count > 1500/mm³, Platelet count > 75,000/mm³, Hemoglobin > 8 g/dL; Total bilirubin < 2.0; AST (SGOT) and ALT (SGPT) < 5 x ULN; Serum creatinine < 1.5 x ULN

Cohort 1 (with sorafenib): No previous systemic therapy with sorafenib. Previous immunotherapy, TACE and local treatments are permitted.

Cohort 2 (on progression of sorafenib): Patients who have received prior sorafenib therapy for at least 4 weeks and has confirmation of disease progression on CT/MRI. Prior surgery or local therapy within 4 weeks prior to Cycle 1 Day 1, with the exception of palliative radiation therapy to the bone

Exclusion Criteria:

1. Patients receiving prior therapy with HCQ.
2. Patients with uncontrolled brain metastases. Patients with brain metastases must be asymptomatic and off corticosteroids for at least one week.
3. Due to risk of disease exacerbation, patients with psoriasis are ineligible unless the disease is well controlled, and they are under the care of a specialist for the disorder who agrees to monitor the patient for exacerbations.
4. Patients with previously documented macular degeneration or untreated diabetic retinopathy (stable retinopathy is allowed).
5. Patients may not be receiving any other investigational agents.
6. History of allergic reactions attributed to compounds of similar chemical or biologic composition to HCQ.
7. Patients requiring the use of enzyme-inducing anti-epileptic medication (phenytoin, carbamazepine, phenobarbital, primidone or oxcarbazepine) are not eligible for entry into the study.
8. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements
9. Major surgery or significant traumatic injury occurring within 21 days prior to treatment.
10. QTc > 500 milliseconds (ms) at baseline (on a single EKG) or average of 3 determinations at 10 minute intervals
11. Gastrointestinal tract disease resulting in an inability to take oral medication or a requirement for IV alimentation, prior surgical procedures affecting absorption, or active peptic ulcer disease. Patients with NG-tube, J-tube, or G-tube will not be allowed to participate.
12. Pregnant women are excluded from this study because sorafenib has the potential for teratogenic or abortifacient effects. For this reason, women of childbearing potential and men must also agree to use adequate contraception (hormonal or barrier method of birth control) prior to study entry and for the duration of study participation.
13. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with sorafenib, breastfeeding should be discontinued.

14. Informed Consent - No study specific procedures will be performed without a written and signed informed consent document. Patients who do not demonstrate the ability to understand or the willingness to sign the written informed consent document will be excluded from study entry.

Inclusion of Women and Minorities:

Both men and women and members of all ethnic groups are eligible for this trial.

Intervention and Mode of Delivery: In cohort 1, we will administer sorafenib 400 mg by mouth (PO) twice daily and HCQ 400 mg PO daily in 4-week cycles. Alternatively, the treating physician can start the dose at 200 mg PO twice daily and titrate up to 400 mg PO twice daily based on safety and tolerability for patients with Child Pugh B cirrhosis or other factors that the physician feels the patient should start at a lower dose. In cohort 2, we will add HCQ to the dose of sorafenib the patient (pt) was using at the time of progression.

Duration of Intervention and Evaluation: Follow-up: A repeat CT/MRI scan will be performed after 2 cycles of treatment regimen to evaluate response based on RECIST criteria. Serum tumor marker AFP (every cycle) and CT/MRI scans will be repeated at least every 2 cycles, or 8 weeks, to ensure no progression of disease. Patients will continue sorafenib/HCQ until disease progression, unacceptable toxicity, withdrawal of consent by the patient, or decision of physician for patient's best interest. We will follow the last patient for 12 months, at which time, the study will be completed for data analysis.

Statistical Methods:

Analytic plan for primary objective:

- TTP will be measured for each patient from the start of the first treatment the receives until the date the criteria for progression are met or the date the patient is taken off study for any reason, whichever is shorter. mTTP will be illustrated with Kaplan-Meier plots, the estimate of median TTP with its 95% confidence interval. Treatment groups will be contrasted with regard to the response rate with a logistic regression model adjusted for age, sex, race, Child Pugh score, BCLC stage and AFP levels; the odds ratio, its 95% confidence interval, and the p-value for testing the null hypothesis that the log odds ratio is zero will be presented. If the logistic model fails due to small cell counts then treatments will be contrasted with regard to response using Fisher's Exact Test.

Analytic plan for secondary objectives:

- OS: OS will be measured for each patient from start of treatment to time of death. The analyses of median OS will be performed in the intent-to-treat population. OS will be illustrated with Kaplan-Meier plots, the estimate of median OS with its 95% confidence interval.
- Response rates: Response rates will be measured as the proportion of patients with the best response, complete response (CR) or partial response (PR), as defined by RECIST 1.1 Criteria and a 95% confidence interval.
- Adverse events: All adverse events will be listed by type and grade.
- Biomarkers of efficacy of autophagy modulation: The significance of variation in biomarkers, including immune markers, with treatment will be assessed with linear models with and without adjustment for age, sex, race, Child Pugh score, BCLC stage and AFP levels. The dependent variables will be log transformed (base 2) if necessary. The relation between treatment efficacy, as measured by TTP, and biomarkers will be assessed with proportional hazards models in terms of treatment, the biomarker, the biomarker by treatment interaction, age, sex, race, Child Pugh score, BCLC stage and AFP levels. Significant effects will be described and illustrated, as appropriate, with Kaplan Meier plots by strata determined by biomarker level and treatment group, for example. Also, electron microscopy will be used to visualize and quantify the autophagic vesicles (AV) in tumor samples (optional biopsy). The average number of autophagic vesicles per cell for patients will be correlated with survival.

Sample size justification: Cohort 1 (sorafenib in combination with HCQ): Historically, the mTTP for patients treated with sorafenib is 5.5 mos. We predict addition of HCQ will improve mTTP by 50% to 8.2 months. We expect 5% lost to follow-up. Assuming a 24-month accrual period with 1 year for follow up, a one-parameter exponential model, and $\alpha=5\%$, 49 subjects are necessary to obtain 80% power. With 5% lost to follow-up the sample size required is 52 ($=49/0.95$).

Cohort 2 (Addition of HCQ on sorafenib progression): Historically, the mTTP for patients following progression on sorafenib 2.1 mos. We predict addition of HCQ will improve mTTP to 4.2 months. We expect 10% lost to follow-up. Assuming a 24-month accrual period with 1 year for follow up, a one-parameter exponential model, and $\alpha=5\%$, 19 subjects are necessary to obtain 80% power. With 10% lost to follow-up the sample size required is 21 ($=19/0.95$).

Funding, Regulatory, and Feasibility Issues: Sorafenib is standard of care treatment for HCC, so will be covered by the patient's insurance. HCQ is already being purchased at low market cost ($<\$50/\text{month}$ per patient). Thus, the sorafenib/HCQ combination provides a cost-effective study drug combination. We have a large referral volume of advanced HCC patients to our institution for consideration of clinical studies, so we do not expect problems with enrollment since we currently enroll 3-4 advanced HCC patients/month onto clinical trials.

Patient Acceptability/Ethics and Consent Issues: The combination of sorafenib/HCQ will be given in the first-line or second-line setting to patients with advanced HCC, for which sorafenib is the only systemic treatment option. Since there is a clear need to identify better treatments to improve survival in advanced HCC, this study gives patients a treatment that is likely superior, based on preliminary data. Stopping rules will be in place after 50% enrollment. See below. We expect that sorafenib/HCQ will be well tolerated based on our Phase I preliminary data, with no grade 3, 4 or 5 adverse events observed. However, if there is unexpected toxicity, there will be early stopping for toxicity as follows:

Early stopping for toxicity: The stopping rule for toxicity is not based on conditional statistical power but rather on observed adverse events. A toxicity event for the purposes of safety stopping is defined as a grade 4 or 5 toxicity of any kind due to treatment with sorafenib and/or HCQ. If at any time during the study there are 2 or more grade 5 toxicities that are attributed to sorafenib and/or HCQ, then the study will be terminated. If $> 50\%$ of patients are having similar type of grade 3 and 4 toxicity, then the study will be terminated. If HCQ causes grade 4 peripheral retinal toxicity, there will be prompt drug discontinuation and ophthalmologic evaluation, with consideration for termination of study.

Data safety and monitoring Board (DSMB): All protocols conducted at UT Health Cancer Center are covered under the auspices of the Institutional Data Safety Monitoring Plan (DSMP). The Institutional DSMP global policies provide individual trials with institutional policies and procedures, as well as monitoring adverse events of study agents that are overseen by the Data Safety Monitoring Board (DSMB). Baseline events and adverse events will be captured in Redcap for each patient. The PI will work closely with the DSMB to ensure all safety requirements for this study are met. The DSMB will meet every 6 months, to discuss the safety update of this study.

2.0 Background

2.1 HCC: Hepatocellular carcinoma (HCC) is the sixth most common cancer globally and is the third leading cause of cancer deaths. There are over 700,000 new cases diagnosed each year worldwide with large geographic variation in both risk factors and incidence.^{10,11} HCV is the major cause of HCC in the United States and Europe. The incidence of HCC in the United States has tripled in the last 30 years and continues to rise largely because of chronic HCV, alcoholic liver disease, and nonalcoholic steatohepatitis.^{11,12}

2.2 Treatment of HCC: Up to 80% of patients first presenting with HCC have advanced unresectable or metastatic disease. For tumors that are confined to the liver, available treatment options include ablative therapies, such as radiofrequency ablation, or transarterial chemoembolization (TACE), all of which achieve modest response. Systemic therapy is the only option for patients with advanced metastatic disease.

2.2.1 Treatment advanced HCC: Sorafenib, a multi-kinase inhibitor of Raf/MEK/ERK signaling and the receptor tyrosine kinase, was shown to induce apoptosis and inhibit tumor proliferation as well as angiogenesis in a variety of tumors.¹³ The molecular involvement of the Raf-1 and tyrosine kinase signaling pathways has been well established in the pathogenesis of HCC and provides a rationale for the investigation of sorafenib in HCC treatment.¹⁴ In pre-clinical experiments, sorafenib reduced tumor angiogenesis and increased tumor apoptosis in a mouse xenograft model of human HCC.¹⁴ Sorafenib is the only drug which has been demonstrated as effective in a recent randomized, placebo-controlled study (SHARP), with mTTP improved from 2.8 months with placebo to 5.5 months with sorafenib, and mOS improved from 7.9 months with placebo to 10.7 months with sorafenib.² *However, development of resistance and limited efficacy of sorafenib in advanced HCC patients are now pivotal issues in the field and further research is warranted to reveal the precise mechanism of sorafenib resistance, which is largely unknown.*

2.3 Autophagy as a mechanism for cancer survival: Autophagy is a conserved protein degradation process that involves the vacuolar sequestration of long-lived cytoplasmic proteins and organelles into a structure called an autophagosome.¹⁵ Autophagosomes fuse with lysosomes, which results in the proteolytic degradation of their contents. In eukaryotic cells, autophagy plays an important role in the disposal of damaged organelles and proteins and also serves to generate alternative sources of energy for cell survival during cellular stress through the catabolization of protein substrates.¹⁶ The activation of stress response genes such as p53 by anticancer therapies can stimulate autophagy in addition to apoptosis.¹⁷ *Although prolonged autophagy can result in cancer cell death, recent investigations suggest that therapy-induced autophagy is a reversible response that promotes cancer cell survival in patients and thus, may diminish the efficacy of some therapeutic agents.*³⁻⁵ Therefore, autophagy may significantly contribute to resistance to a number of anticancer therapeutic modalities.

2.3.1 Autophagy as a mechanism of resistance to anti-cancer therapeutics. Accumulating evidence indicates that cancer therapies such as irradiation and administration of cytotoxic drugs and chemicals induce autophagy and autophagic cell death in a variety of tumor cells. The induction of autophagy has been observed in malignant cells after treatments with P13K/AKT inhibitors, tamoxifen, imatinib, arsenic trioxide, histone deacetylase (HDAC) inhibitors, and rapamycin.¹⁸⁻²² However, autophagy induction by all these anti-cancer agents is not beneficial to patients rather counterproductive as survival of cancer cells is promoted. It is thought that autophagy promotes the survival of a subpopulation of tumor cells that are localized in hypoxic tumor regions and refractory to cell death.

2.3.2 Autophagy as a mechanism of resistance to sorafenib and role of autophagy in HCC. Sorafenib-induced autophagy appears to promote survival of hepatoma cells, and thereby, may be a cellular adaptive response related to primary resistance to this compound.⁶ Studies have shown that sorafenib treatment in HCC cells induced the morphological and biochemical hallmarks of autophagy, for example, the generation of autophagosomes, GFP-LC3 redistribution and LC3-II accumulation.⁷ Sorafenib-induced autophagy was preceded by ER stress and with rapidly induced increase in cytosolic calcium level, which is a typical cellular response to ER stress that may be largely independent of Raf-

MEK1/2-ERK1/2 signaling cascade in HCC.⁷ Thus, blocking of autophagy mediated survival by sorafenib-targeted therapy should be a rational approach to overcome resistance to sorafenib.

2.4 Rationale for combining autophagy inhibitors with sorafenib. *Sorafenib induces autophagy and inhibition of this process with CQ could lead to enhanced anticancer activity as shown in HCC studies.*^{6,7}

Autophagy inhibition augments the efficacy of anticancer agents as observed in a variety of tumor histologies in multiple preclinical models.^{23,24} On the basis of reports that effective autophagy inhibition can be achieved *in vivo* with the antimalarial drug, chloroquine (CQ),²³ clinical trials for patients with refractory malignancies were undertaken.²⁵

Chloroquine (CQ) is a synthetic 4-aminoquinoline that has been used for more than 60 years in humans for the prophylaxis and treatment of malaria²⁶ and rheumatoid arthritis²⁷. CQ is an orally available and inexpensive drug that has CNS penetration and a broad therapeutic index. Its most predictable cumulative toxicity is retinopathy, which can be prevented by stopping administration of the drug.^{28,29} CQ derivatives, such as hydroxychloroquine (HCQ), are still widely used in the treatment of rheumatologic diseases and have a greater therapeutic index than CQ. Derivatives of CQ, such as HCQ, function as weak bases and are trapped in acidic cellular compartments, including lysosomes.³⁰ This deacidification of lysosomes by CQ and its derivatives impairs the activity of most lysosomal enzymes due to their strict pH requirements.³⁰ Consequently, CQ and its derivatives inhibit the last step in the autophagic degradation process. *When autophagy is inhibited by CQ, cells dependent on autophagy for survival increase the generation of autophagosomes and undergo either apoptotic or non-apoptotic cell death.*

2.4.1 Preclinical studies of autophagy inhibition with chloroquine in HCC models: Preclinical studies have shown that chemical and genetic autophagy inhibitors, 3-MA, chloroquine (CQ) and Atg5 siRNA knockdown, potentiated sorafenib-induced cell death.⁷ *In vivo*, sorafenib plus CQ treatment resulted in a marked increase in TUNEL-positive apoptotic cells when compared with the sorafenib-treated tumors.⁷ Of note, CQ co-treatment with sorafenib did not result in an additional liver toxicity in the treated animals.⁷

In addition to the modulation of well-known target Raf/MEK/MAPK pathway, sorafenib has also been shown to inhibit the mTORC1 pathway. Because mTOR inhibition by rapamycin activates autophagosome formation in hepatoma cells, it is reasonable to postulate that sorafenib-induced inhibition of the mTORC1 pathway might be involved in sorafenib-mediated induction of autophagy.⁶ Sorafenib-induced adaptive autophagy has also been shown to occur independently of p53.⁶ Of importance are the findings that sorafenib-induced autophagy in a xenograft model and that co-administration of CQ and sorafenib led to better suppression of xenograft tumor than sorafenib alone.⁶ However, studies are still ongoing to evaluate the underlying mechanisms by which sorafenib induces autophagy.

In summary, preclinical data has shown that autophagy inhibition with CQ enhances the anti-tumor effect of sorafenib in the liver cancer xenograft model.

2.5 Preliminary data:

2.5.1 Clinical safety of autophagy inhibition with sorafenib in advanced cancer patients. Based on the data above, our institution conducted A Phase 1 Dose-Escalation Trial of Oral Hydroxychloroquine Plus Oral Sorafenib to Treat Patients with Refractory or Relapsed Solid Tumors. The primary objective of this study was to determine the maximum tolerated dose (MTD) of HCQ plus sorafenib in patients with advanced solid tumors. Although the MTD was not reached, due to observed clinically efficacy in cohort 4 as well as tolerable toxicity, the recommended phase 2 dose is sorafenib 400 mg PO twice daily and HCQ 400 mg PO daily. Preliminary data presented here support autophagy inhibition to enhance the efficacy of sorafenib (Table 1).

Given limited treatment options for patients with advanced HCC, *these findings support a phase II study combining the autophagy inhibitor HCQ with sorafenib either in combination or sequentially added to sorafenib on progression of HCC.*

Cohort (SOR/ HCQ)	Patient Number	Age	Tumor type	Cycles (28 days)	Adverse Events†
1 (200 mg daily / 200 mg daily)	1	57	Ovarian	2	none
	2	56	Ovarian	2	G1 early satiety, G1 vomiting
	3	66	Ovarian	2	none
2 (200 mg BID / 200 mg daily)	5	64	Ovarian	2	G1 rash
	6	49	Cervical	2	none
	7	64	Pancreas	2	G1 anorexia
3 (400 mg BID / 200 mg daily)	8	52	Ovarian	2	G1 fatigue, G1 rash
	13	85	Ovarian	4	G1 fatigue, G1 nausea
	14	65	Breast	3	G1 fatigue, G1 dry hands
	16	59	Non-small cell lung	4	G1 elevated transaminases, G1 rash
4 (400 mg BID / 400 mg daily)	17	68	Ovarian	6+	G2 rash*
	18	77	Sarcoma	7+	G2 nausea/vomiting
	19	68	Ovarian	2	G1 fatigue, G1 nausea

Table 1: Adverse events for patients on Phase I study of SOR and HCQ in advanced solid tumors at our institutions. † National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v4.0. + Pt is still on study. *required dose reduction. 19 patients were screened for this study.

2.6 **Biomarkers of Autophagy:**

2.6.1 Biomarkers of Autophagy modulation solid tumors. *Our experience in colorectal cancer patients receiving autophagy modulators* has shown that specific biomarkers are induced when autophagy is observed. These include the induction of biomarkers of autophagy such as cathepsin D (CTSD) and LC3-II levels after drug treatment. CTSD and LC3-II, marker of autophagy inhibition, increase both in PBMC and pt's primary tumor using RT-PCR (Figure 1) and correlate with efficacy of autophagy inhibition.²⁵ Also, increased autophagosomes are seen with autophagy inhibition in the patient's tumor on electron microscopy. Further, elevations in cleaved caspase-3 were observed after treatment, which is a marker of apoptosis.

2.6.2 Biomarkers of Autophagy inhibition in preclinical models of HCC. Studies have shown that sorafenib treatment in HCC cells induced the morphological and biochemical hallmarks of autophagy, for example, the generation of autophagosomes, GFP-LC3 redistribution and LC3-II accumulation.⁷ Sorafenib-induced autophagy was preceded by ER stress and with rapidly induced increase in cytosolic calcium level, which is a typical cellular response to ER stress that may be largely independent of Raf-MEK1/2-ERK1/2 signaling cascade in HCC.⁷ *Thus, blocking of autophagy mediated survival by sorafenib-targeted therapy should be a rational approach to overcome resistance to sorafenib.*

2.6.2.1 Biomarkers of autophagy inhibition in HCC patients. In the phase I study of sorafenib and HCQ at our institution, biomarkers specific to autophagy modulation were not evaluated in the patient's tumor or blood. Therefore, we suggest the prospective incorporation of such predictive biomarkers in this study, and the assessment of such biomarkers relative to the efficacy of sorafenib and HCQ. We plan to evaluate the following biomarkers: GRP78 (marker of ER stress), cathepsin D and LC3-II (marker of autophagy inhibition), and cleaved caspase-3 (marker of apoptosis). Establishing a predictive biomarker associated with clinical sensitivity to autophagy inhibition is necessary for the optimal development of this class of drugs for cancer therapy.

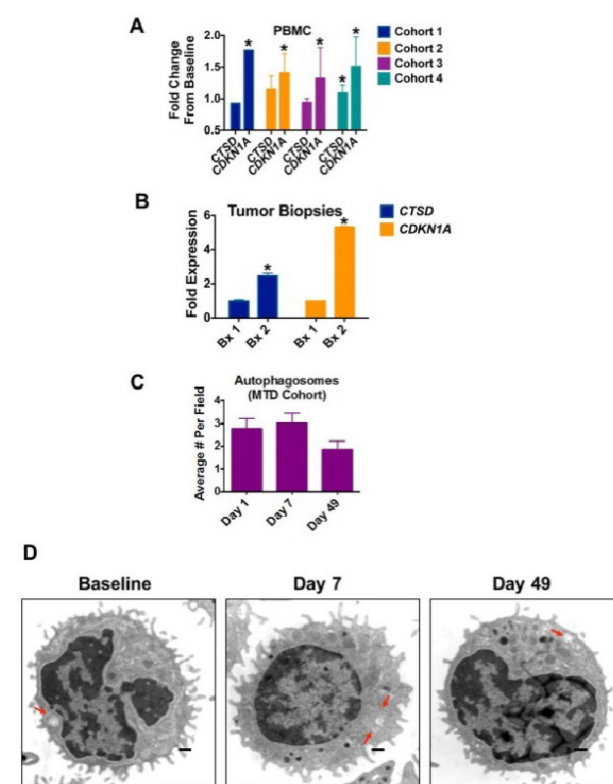


Figure 1: HCQ and vorinostat stimulate the expression of CTSD and CDKN1A and the accumulation of autophagic vacuoles. (A) Fold change from baseline in the levels of CTSD and CDKN1A in PBMC specimens in individual cohorts. PBMC specimens were collected at baseline and on C1D7. Gene expression was quantified by qRT-PCR and normalized to GAPDH. *Indicates a significant change from baseline, $P < 0.05$. (B) Tumor biopsies were obtained from 2 patients with colorectal cancer at baseline and on d 49 (Patient #1 = unmutated RAS, Patient #2 = mutant KRAS). The fold change from baseline in the levels of CTSD and CDKN1A expression in tumor specimens was quantified by qRT-PCR and normalized to GAPDH. *Indicates a significant change from baseline, $P < 0.05$. (C and D) Effects of treatment on autophagic vacuoles. PBMC specimens were collected from patients at baseline and post-treatment on d 7 and 49. Transmission electron microscopy was utilized to visualize and quantify autophagic vacuoles in PBMCs. The average number of autophagic vacuoles per cell for patients enrolled in the MTD cohort (cohort 3) is shown in (C). Representative images for one patient's specimens collected at baseline, d 7 and d 49 are shown in (D).

2.6.3 Autophagy and modulation of immune response in HCC patients.

Immunosurveillance, the infiltration of immune cells, and antitumor immunity are currently recognized as key factors shaping the tumor microenvironment.²⁸ Although the role of cancer cell-associated autophagy has not been directly analyzed within the context of cancer immunoediting or immunosurveillance, accumulating observations suggest that autophagy may play an important immunomodulatory role by regulating the cancer cell secretion of various factors, including inflammatory cytokines and growth factors that would promote tumor growth. Studies using conditional knockout of FIP200 (a component of the ULK complex of the autophagy pathway) in a mouse model of breast cancer driven by the PyMT oncogene, it has been indicated that suppressing autophagy halts tumorigenesis by not only affecting energy metabolism and proliferation but also by increasing host antitumor immunosurveillance.²⁹ FIP200 deletion in mice increases intra-tumoral infiltration of antitumorigenic interferon (IFN)- γ^+ CD8 $^+$ T cells,²⁹ triggered by increased production of key chemokines, such as CXCL10, from FIP200-null tumor cells. Antibody-based depletion of CD8 $^+$ T cells (i.e., cytotoxic T lymphocytes) in this set-up restored accelerated mammary tumor initiation, thus delineating a key role for cancer cell-associated autophagy in subverting antitumor immunity.²⁹ In line with this model, the inhibition of autophagy by silencing Atg5 or Beclin 1 in lung cancer cell lines revealed that autophagy is a protective mechanism against killing by CD8 $^+$ T cells under hypoxic conditions.³⁰ *All together, these observations suggest that cancer cell-associated autophagy could derail cancer immunosurveillance or antitumor immunity within a tumor, thereby supporting tumor progression.*

Inflammation plays a critical role in the development of cancer, with many cancers arising from sites of chronic inflammation.⁵¹ Chronic inflammatory states, such as hepatitis C virus (HCV)-related cirrhosis predispose patients to HCC. HCV core protein interacts with STAT3 protein, which is a transcription factor that mediates cytokine signaling that leads to tumor cell proliferation.^{35,36} Chronic HCV infection results in elevated IL-6, which activates the STAT3 signal pathway contributing to HCC pathogenesis.^{37,38} Further, males have a higher incidence of HCC than females.⁵⁶ This may be a result of females having more estrogen, which inhibits IL-6, and thus serving as an anti-inflammation effect resulting in the decreased incidence of HCC.⁵⁷⁻⁵⁹ Similarly, males have higher levels of IL-6 than females, which further supports a causal relationship between the inflammatory state and HCC.⁶⁰ Therefore, we hypothesize that manipulation of the immune system, such as decreasing pro-inflammatory cytokines such as IL-6, might be an effective approach to prevent the occurrence of HCC and/or to treat patients with HCC. Blocking cancer cell autophagy with CQ treatment or by knocking down Atg5 or Atg7 dampens ATP secretion from dying cancer cells in response to DNA-damaging agents, blunts T cell-mediated anti-cancer immunity and decreases pro-inflammatory cytokines⁴⁴. Additionally, when IL-6 is downregulated, HCC tumors are more sensitive to SOR.¹² Our preliminary data from the recently completed phase 1 study does support the reduction of IL-6

observed with SOR and HCQ combination (Figure 2). We also saw that SOR+HCQ reduced T cell exhaustion (reduced PD-1 and Lag3, data now shown), consistent with improved anti-tumor immunity⁶¹. Therefore, if autophagy inhibition downregulates pro-inflammatory cytokines, improves T cell-mediated anti-tumor immunity and enhances sensitivity to SOR, this further supports the rationale of combining HCQ with SOR to treat HCC.

In this study, we would like to explore the effects of SOR+HCQ with studies such as T cell exhaustion (CD3/CD4/CD8/PD-1/Lag3/Tim3), activation (CD44/CD62L) and cytokines (IFN- γ , TNF- α , IL-10, IL-6) will be assessed.

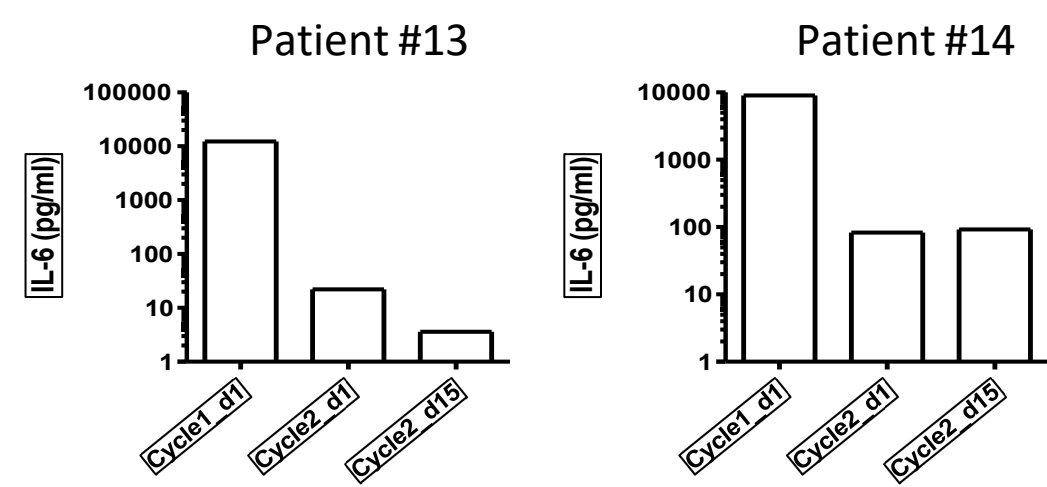


Figure 2: IL-6 levels in patients with advanced cancer receiving SOR during Cycle 1 with HCQ added at Cycle 2. Cycle1_d1 represents baseline IL-6 levels prior to treatment. SOR was started on C1D1, and Cycle2_d1 represents decreased IL-6 levels with SOR. HCQ was added to SOR at C2D1, and Cycle2_d15 represents IL-6 levels after the addition of HCQ

3.0 Statement of Study Objectives

Primary Objective:

- To determine the clinical efficacy with to determine the clinical efficacy with time to tumor progression (TTP) of the combination of HCQ and sorafenib in sorafenib-naïve advanced HCC (cohort 1); and the addition of HCQ to sorafenib in patients who progress on sorafenib (cohort 2).

Secondary Objectives:

- To determine the overall survival in advanced HCC patients receiving HCQ and sorafenib in both cohort 1 and 2
- To evaluate the tumor response rate in advanced HCC patients receiving HCQ and sorafenib
- To further define the safety in advanced HCC patients receiving HCQ and sorafenib
- To identify biomarkers of autophagy modulation and immune markers that correlate with clinical efficacy in advanced HCC patients receiving HCQ and sorafenib

4.0 Inclusion and Exclusion Criteria

Inclusion Criteria:

1. Cytologically or histologically confirmed advanced or metastatic HCC. If no histological diagnosis, patient must have imaging studies compatible with HCC.
2. Age 18 years and above

3. ECOG performance status of 0 or 1
4. Not a candidate for curative treatments (i.e., resection, transplantation)
5. Child-Pugh class A or B7-8 liver function
6. Measurable disease as defined by RECIST 1.1
7. Patients who received prior local therapy (e.g., TACE) are eligible.
8. Documented virology status of hepatitis, as confirmed by at least one of the following: screening HBsAg, anti-HBc, /or anti-HCV
9. Life expectancy > 3 months
10. For women who are not postmenopausal (12 months of amenorrhea) or surgically sterile (absence of ovaries and/or uterus): agreement to use two adequate methods of contraception. For men: agreement to use a barrier method of contraception during the treatment period
11. Hematologic, Biochemical, and Organ Function within 21 days prior to Cycle 1 Day 1: Granulocyte count > 1500/mm³, Platelet count > 75,000/ mm³, Hemoglobin > 8 g/dL; Total bilirubin < 2.0; AST (SGOT) and ALT (SGPT) < 5 x ULN; Serum creatinine < 1.5 x ULN
12. Able to provide written informed consent.
13. Patients with the potential for pregnancy or impregnating their partner must agree to follow acceptable birth control methods to avoid conception. Women of childbearing potential must have a negative pregnancy test within 72 hours prior to receiving the investigational product.

Cohort 1 (with sorafenib): No previous systemic therapy with sorafenib or. Previous immunotherapy, TACE and local treatments are permitted.

Cohort 2 (on progression of sorafenib): Patients who have received prior sorafenib therapy for at least 4 weeks and has confirmation of disease progression on CT/MRI. Prior surgery or local therapy within 4 weeks prior to Cycle 1 Day 1, with the exception of palliative radiation therapy to the bone

Exclusion Criteria:

1. Patients receiving prior therapy with HCQ.
2. Patients with uncontrolled brain metastases. Patients with brain metastases must be asymptomatic and off corticosteroids for at least one week.
3. Due to risk of disease exacerbation, patients with psoriasis are ineligible unless the disease is well controlled, and they are under the care of a specialist for the disorder who agrees to monitor the patient for exacerbations.
4. Patients with previously documented macular degeneration or untreated diabetic retinopathy (stable retinopathy is allowed).
5. Patients may not be receiving any other investigational agents.
6. History of allergic reactions attributed to compounds of similar chemical or biologic composition to HCQ.
7. Patients requiring the use of enzyme-inducing anti-epileptic medication (phenytoin, carbamazepine, phenobarbital, primidone or oxcarbazepine) are not eligible for entry into the study.
8. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements
9. Major surgery or significant traumatic injury occurring within 21 days prior to treatment.
10. QTc > 500 ms at baseline (on a single EKG) or average of 3 determinations at 10-minute intervals
11. Gastrointestinal tract disease resulting in an inability to take oral medication or a requirement for IV alimentation, prior surgical procedures affecting absorption, or active peptic ulcer disease. Patients with NJ, J or G tube will not be allowed to participate.
12. Pregnant women are excluded from this study because sorafenib has the potential for teratogenic or abortifacient effects. For this reason, women of childbearing potential and men must also agree

to use adequate contraception (hormonal or barrier method of birth control) prior to study entry and for the duration of study participation.

13. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with sorafenib, breastfeeding should be discontinued.
14. Informed Consent - No study specific procedures will be performed without a written and signed informed consent document. Patients who do not demonstrate the ability to understand or the willingness to sign the written informed consent document will be excluded from study entry.

Inclusion of Women and Minorities:

Both men and women and members of all ethnic groups are eligible for this trial.

5.0 Study Design:

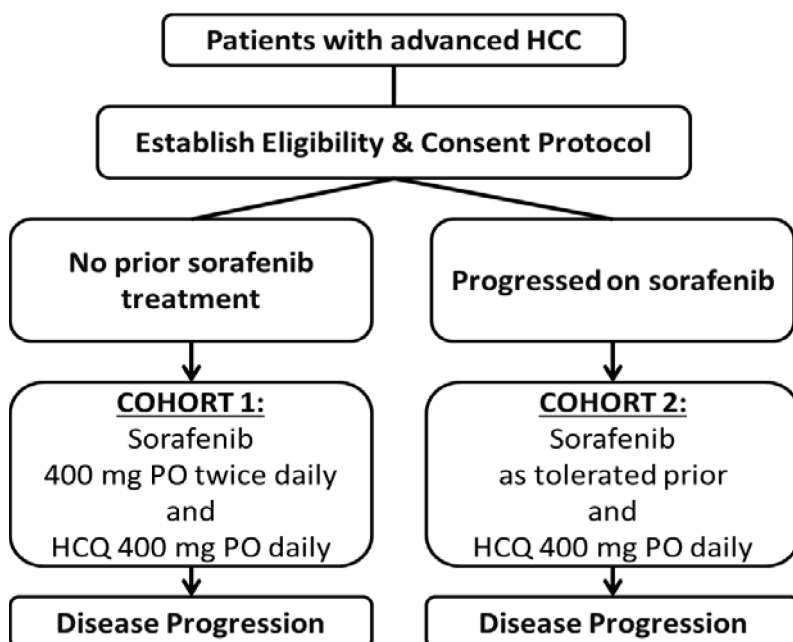
Phase 2 study with two cohorts (Figure 1) - Cohort 1: SOR-naïve patients receive SOR 400 mg by PO twice daily on Cycle1/Day1 (C1D1). In clinical practice, dose reduction of SOR is often required. {Al-Rajabi, 2015 #58} Therefore, on C1D15, the clinician will dose-reduce sorafenib based on toxicity and HCQ 400 mg PO daily will be started. Alternatively, the treating physician can start the dose at 200 mg PO twice daily and titrate up to 400 mg PO twice daily based on safety and tolerability for patients with Child Pugh B cirrhosis or other factors that the physician feels the patient should start at a lower dose. Cohort 2: As second-line treatment, we will add HCQ to SOR dose the patient was tolerating at the time of progression. C2D1 of each cohort, toxicity of HCQ will be assessed. Dose reductions due to adverse events (AEs) to each agent are allowed for SOR per standard of care and/or HCQ for grade 3+ AE (Table 2). Blood samples will be collected at C1D1, C1D15 and C2D1 to assess for pharmacokinetics, if necessary.

5.1 Sample size:

Cohort 1 (sorafenib in combination with HCQ): Historically, the mTTP for patients treated with sorafenib is 5.5 mos. We predict addition of HCQ will improve mTTP by 50% to 8.2 months. We expect 5% lost to follow-up. Assuming a 24-month accrual period with 1 year for follow up, a one-parameter exponential model, and $\alpha=5\%$, 49 subjects are necessary to obtain 80% power. With 5% lost to follow-up the sample size required is 52 ($=49/0.95$).

Cohort 2 (Addition of HCQ on sorafenib progression): Historically, the mTTP for patients following progression on sorafenib 2.1 mos. We predict addition of HCQ will improve mTTP to 4.2 months. We expect 10% lost to follow-up. Assuming a 24-month accrual period with 1 year for follow up, a one-parameter exponential model, and $\alpha=5\%$, 19 subjects are necessary to obtain 80% power. With 10% lost to follow-up the sample size required is 21 ($=19/0.95$).

5.2 Study Schema:



Cohort 1: SOR-naïve patients receive SOR 400 mg by PO twice daily on Cycle1/Day1 (C1D1). In clinical practice, dose reduction of SOR is often required {Al-Rajabi, 2015 #58}. Therefore, on C1D15, the clinician will dose-reduce sorafenib based on toxicity and HCQ 400 mg PO daily will be started. Alternatively, the treating physician can start the dose at 200 mg PO twice daily and titrate up to 400 mg PO twice daily based on safety and tolerability for patients with Child Pugh B cirrhosis or other factors that the physician feels the patient should start at a lower dose.

Cohort 2: As second-line treatment, we will add HCQ to SOR dose the patient was tolerating at the time of progression. SOR-naïve patients receive SOR 400 mg by PO twice daily on Cycle1/Day1 (C1D1). In clinical practice, dose reduction of SOR is often required.⁹¹² Therefore, on C1D15, the clinician will dose-reduce sorafenib based on toxicity and HCQ 400 mg PO daily will be started. C2D1 of each cohort, toxicity of HCQ will be assessed. Dose reductions due to adverse events (AEs) to each agent are allowed for SOR per standard of care and/or HCQ for grade 3+ AE (Table 2). Blood samples will be collected at C1D1, C1D15 and C2D1 to assess for pharmacokinetics, if necessary.

5.3 Treatment Requirements

All eligible patients who consent to this study must have a baseline evaluation (CT or MRI) within 28 days of the start of treatment.

Patient must meet all eligibility requirements to be entered onto the protocol.

5.3.1 Duration of Intervention and Evaluation:

Follow-up: A repeat CT/MRI scan will be performed after 2 cycles of treatment regimen to evaluate response based on RECIST 1.1 criteria (See Appendix 13.1 for definitions of response).⁴⁷ Serum tumor marker AFP (every cycle) and CT/MRI scans will be repeated at least every 2 cycles, or 8 weeks, to ensure no progression of disease. Patients will continue on Sorafenib/HCQ until disease progression, unacceptable toxicity, withdrawal of consent by the patient, or decision of physician for patient's best

interest. Each patient will be followed for 1 year. We will follow the last patient for 12 months, at which time, the study will be completed for data analysis.

5.3.2 Sorafenib and HCQ Administration

The starting dose will be Sorafenib 400 mg PO twice daily and HCQ 400 mg PO daily, as determined by the phase I study in advanced cancer patients.

Dose can be decreased due to toxicity if deemed by the clinical investigator to be related to sorafenib. The dose reductions will be 200 mg twice daily (level -1), 200 mg (level -2). For dose level -1, this can be taken 400mg daily or 200mg twice daily based on patient tolerance. As in standard of care clinical practice with sorafenib, patients usually cannot tolerate prolonged courses of sorafenib without adjustments in their schedule. Therefore, if the patient continues to have clinical benefit from the study drug, then the dosing schedule may change as per routine clinical care (ie, 2 weeks on, 1 week off).

Dose can be decreased due to toxicity if deemed by the clinical investigator to be related to HCQ. The dose reductions will be 200 mg (level I)

If toxicity is thought to be due to Sorafenib and HCQ, then dose reductions can be done for each drug as above.

Table 2: Dose reductions for sorafenib plus HCQ in the Phase II study of SOR/HCQ in advanced HCC patients

Dose Level	HCQ	SORAFENIB
0 (<i>Standard Daily Dose</i>)	400 mg daily	400 mg twice daily
-1	200 mg daily	200 mg twice daily*
-2	n/a	200 mg daily

* Patient can take either 400mg once daily or 200mg twice daily depending on tolerance

5.3.3 Concomitant Medications and Supportive Care Guidelines

Diarrhea Management: Treat diarrhea promptly with appropriate supportive care, including loperamide. Instruct patients to begin taking loperamide at the first signs of: 1) poorly formed or loose stool, 2) occurrence of more bowel movements than usual in one day, or 3) unusually high volume of stool. Loperamide should be taken in the following manner: 4 mg at first onset of diarrhea, then 2 mg after each unformed stool. Daily dose should not exceed 16 mg/day. Loperamide should not be taken prophylactically. Advise patients to drink plenty of clear fluids to help prevent dehydration caused by diarrhea. Avoid loperamide if there is the presence of blood or mucus in the stool or if diarrhea is accompanied by fever. If grade 3 or 4 diarrhea develops, discontinue further treatment with sorafenib.

Anti-emetic Prophylaxis: During dosing, promethazine (25 mg PO) or metoclopramide (10 mg PO) every 6 hours may be initiated, as needed. For patients who suffer diarrhea, promethazine rather than metoclopramide must be used. For patients who experience nausea and vomiting despite this antiemetic treatment, dexamethasone (4 mg PO) every 12 hours and granisetron (1 mg PO) QD, or equivalent 5HT3 antagonist, may be administered. All patients should have antiemetic medications available once discharged from the clinic. Oral anti-emetic medications should be prescribed and administered as needed, and adjusted during the cycle at the discretion of the treating investigator. The prophylactic use of a 5-HT3-antagonist can be administered before sorafenib administration if deemed indicated by the investigator. Due to its interaction with P450 enzymes, aprepitant use should be avoided. If needed, then its use must be recorded. Control of nausea and vomiting may require institution of multiple anti-emetic medications including phenothiazines or 5-HT3 antagonists.

Hydration Management: Adequate hydration is important in the event of dysgeusia. Advise patients that popsicles and Gatorade may be useful in this event.

Corticosteroids: If the patient receives steroids an effort will be made to keep the patient on this steroid dose until the next scan is obtained. Corticosteroid dose can be tapered as clinically indicated if the patient appears to be responding to therapy as judged by serial scans.

Anti-convulsants: Because HCQ has known effects on P450 enzymes, patients requiring anti-convulsants may be treated with any of the non-enzyme inducing anti-convulsants, which include: felbamate, valproate acid, gabapentin, lamotrigine, tiagabine, topiramate, zonisamide, or levetiracetam. Patients requiring the use of enzyme-inducing anti-epileptic medication (phenytoin, carbamazepine, phenobarbital, primidone or oxcarbazepine) are not eligible for entry into the study. If these agents are required during the study, patients will discontinue study treatment.

Growth factors: Patients are allowed to continue hematological growth factors such as Aranesp if initiated before the protocol. G-CSF or GM-CSF will not be allowed as prevention in cycle 1, but could be used as needed for treatment of grade 4 or complicated neutropenia on cycle 1 or beyond. If prophylaxis is considered necessary by the clinical investigator beyond cycle 1, this is allowed.

Given the possibility of metabolic interaction through the P450 2D6 enzyme the concomitant administration of HCQ with digoxin, B blockers, cyclosporine and cimetidine should be closely monitored.

5.4 Dose Modification for Toxicity

Toxicity Criteria - This study will utilize the CTCAE version 4.0 for toxicity and Adverse Event Reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP home page (<http://ctep.cancer.gov>). All appropriate treatment areas will have access to a copy of the CTCAE version 4.0.

Dose reductions due to adverse events are allowed for both sorafenib per standard of care and/or HCQ for grade 3 or more adverse event was related to study medication. Dose reductions are also permitted based on investigator clinical decision.

5.4.1 Major Events

Major Events are non-treatment-related grade 3 and 4 hematologic and non-hematologic toxicities. Treatment should be delayed for major events if the study drug may further complicate the non-treatment related event. If a major event requires a delay of treatment, treatment must be delayed until toxicity is resolved ($\leq$ Grade 2 or $\leq$ Baseline). If toxicity is not resolved in ≤ 2 weeks, patient will be taken off treatment except if the patient experiences clinical benefit.

5.4.2 Dose Reduction for sorafenib and/or HCQ (see Table 2)

Dose can be decreased due to toxicity if deemed by the clinical investigator to be related to sorafenib. The dose reductions will be 200 mg twice daily (level -1), 200 mg daily (level -2).

Dose can be decreased due to toxicity if deemed by the clinical investigator to be related to HCQ. The dose reductions will be 200 mg daily (level -1).

If toxicity is thought to be due to Sorafenib and HCQ, then dose reductions can be done for each drug as above.

5.4.2.1 HCQ Dose Modification

Any AE of $\geq$ Grade 3 and attributed as possibly, probably or definitely related to HCQ will result in the dose being held until the AE has resolved to $\leq$ grade 1 or baseline. If the AE resolves, reinstitution of treatment can occur but at a reduced dose as described in Table 2.

If the AE recurs at the reduced dose, treatment will be held until the AE has resolved to $\leq$ grade 1 and when resolved treatment can be reinstituted at the next lower dose level. No more than 2 dose reductions are allowed during the maintenance cycles.

With particular regard to visual field deficits patients should be cautioned to report any visual symptoms, particularly difficulty seeing entire words or faces, intolerance to glare, decreased night vision, or loss of peripheral vision. **These symptoms of peripheral retinal toxicity should prompt drug discontinuation and ophthalmologic evaluation.**

5.4.2.2 Sorafenib Dose Modification

Known sorafenib hematological or non-hematological toxicities will not be attributed to HCQ and will only be attributed to sorafenib and may result in sorafenib dose modifications. Toxicities having an attribution to sorafenib of possible, probable or definite will result in the following sorafenib dose modifications:

Sorafenib administration will be delayed, reduced or discontinued based on weekly assessment of according to hematological and non-hematological toxicity criteria, as specified below.

If the administration of sorafenib has to be interrupted, the treatment with HCQ will proceed normally and no catch-up days of sorafenib. The total number of days of sorafenib administration and total dose of sorafenib will be recorded in the CRF.

5.5 Use of Hematologic Growth Factors

The use of hematologic growth factors (filgrastim, pegfilgrastim, epoetin alfa) is permitted to provide optimal care for patients with severe myelosuppression at the Investigator's discretion

5.6 Pharmaceutical Information

5.6.1 Hydroxychloroquine

Generic name: Hydroxychloroquine sulfate

Commercial name: Plaquenil

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

How Supplied: As a generically available agent, multiple companies supply Hydroxychloroquine as 200 mg tablets, equal to 155 mg of the base compound. The brand name Plaquenil (Sanofi Synthelabo) is a white to off white film coated tablet. The inactive ingredients in each tablet include dibasic calcium phosphate, hydroxypropyl methylcellulose, magnesium stearate, polyethylene glycol 400, polysorbate 80, starch, and titanium dioxide. Hydroxychloroquine 200 mg tablets are supplied in bottles containing 100, 500 and 1000 tablets.

HCQ will dispensed from the Cancer Center pharmacy or may be dispensed from a commercial pharmacy directly to the study participant.

Storage:

Store Hydroxychloroquine capsules at room temperature, 15 to 30 °C (59 to 86 °F). Do not store above 30 °C. Avoid excess if moisture. Dispense in a light resistant container

Stability: Stability of each bottle is marked on the container

Route of Administration: Orally

Method of Administration: Hydroxychloroquine should be administered with food or milk. Magnesium containing antacids should be avoided 1 hour prior and 2 hour post Hydroxychloroquine dosing.

Potential Drug Interactions: Hydroxychloroquine is metabolized extensively in the liver and appears to be mediated via CYP450 2D6. In vivo studies of Hydroxychloroquine effects on agents metabolized by CYP450 2D6 are mixed. In healthy volunteers metoprolol levels following administration of Hydroxychloroquine were increased, however, dextromethorphan levels were not significantly altered. Other interactions of note include digoxin and cimetidine.

Special Handling: None.

Patient Care Implications: Patients should be instructed to notify a physician if vision changes occur; ringing in ears or hearing loss; fever; sore throat; unusual bleeding or bruising; unusual pigmentation of the skin; bleaching or loss of hair; or mood changes.

Known Potential Toxicities of Hydroxychloroquine

Central nervous system: Irritability, nervousness, emotional changes, nightmares, psychosis, headache, dizziness, vertigo, seizure, ataxia, lassitude.

Dermatologic: Bleaching of hair, alopecia, pigmentation changes (skin and mucosal; black-blue color), rash (urticarial, morbilliform, lichenoid, maculopapular, purpuric, erythema annulare centrifugum, Stevens-Johnson syndrome, acute generalized exanthematous pustulosis, and exfoliative dermatitis).

Gastrointestinal: Anorexia, nausea, vomiting, diarrhea, abdominal cramping.

Hematologic: Aplastic anemia, agranulocytosis, leukopenia, thrombocytopenia, hemolysis (in patients with glucose-6-phosphate deficiency).

Hepatic: Abnormal liver function, hepatic failure (isolated cases).

Neuromuscular & skeletal: Myopathy leading to progressive weakness and atrophy of proximal muscle groups (may be associated with mild sensory changes, loss of deep tendon reflexes, and abnormal nerve conduction).

Ocular: Disturbance in accommodation, keratopathy, corneal changes/deposits (visual disturbances, blurred vision, photophobia - reversible on discontinuation), macular edema, atrophy, abnormal pigmentation, retinopathy (early changes reversible – may progress despite discontinuation if advanced), optic disc pallor/atrophy, attenuation of retinal arterioles, pigmentary retinopathy, scotoma, decreased visual acuity, nystagmus.

5.6.2 Sorafenib

Generic name: Sorafenib tosylate

Chemical Name: 4-(4-{3-[4-Chloro-3-(trifluoromethyl)phenyl]ureido}phenoxy)²*N* methylpyridine-2-carboxamide 4-methylbenzenesulfonate

Commercial Names: Nexavar®

Classification: Antineoplastic

CAS Registry Number: 475207-59-1

Description: a kinase inhibitor that is the tosylate salt of sorafenib.

Mode of Action:

Sorafenib is a kinase inhibitor that decreases tumor cell proliferation *in vitro*. Sorafenib was shown to inhibit multiple intracellular (CRAF, BRAF and mutant BRAF) and cell surface kinases (KIT, FLT-3, RET, VEGFR-1, VEGFR-2, VEGFR-3, and PDGFR-β). Several of these kinases are thought to be involved in tumor cell signaling, angiogenesis, and apoptosis. Sorafenib inhibited tumor growth and angiogenesis of human hepatocellular carcinoma and renal cell carcinoma, and several other human tumor xenografts in immunocompromised mice.

How Supplied: Sorafenib is supplied by Bayer HealthCare Pharmaceuticals, Inc., and Onyx Pharmaceuticals, Inc. Nexavar tablets are supplied as round, biconvex, red film-coated tablets, debossed with the “Bayer cross” on one side and “200” on the other side, each containing sorafenib tosylate equivalent to 200 mg of sorafenib.

Bottles of 120 tablets.

Storage: Store at 25°C (77°F); excursions permitted to 15–30°C (59–86°F) (see USP controlled room temperature). Store in a dry place.

Route of Administration: Orally

Method of Administration: The recommended daily dose of NEXAVAR is 400 mg (2 x 200 mg tablets) taken twice daily without food (at least 1 hour before or 2 hours after a meal). Treatment should continue until the patient is no longer clinically benefiting from therapy or until unacceptable toxicity occurs.

Potential Drug Interactions: Concomitant strong CYP3A4 inducers: The use of concomitant strong CYP3A4 inducers may decrease sorafenib plasma concentrations and should be avoided (for example, St. John's Wort, dexamethasone, phenytoin, carbamazepine, rifampin, rifabutin, phenobarbital). Although a dose increase has not been studied, if a strong CYP3A4 inducer must be co-administered, a NEXAVAR dose increase may be considered. If the dose of NEXAVAR is increased, the patient should be monitored carefully for toxicity

- **CYP2B6 and CYP2C8 Substrates** - Sorafenib inhibits CYP2B6 and CYP2C8 *in vitro* with Ki values of 6 and 1–2 micromolar, respectively. Systemic exposure to substrates of CYP2B6 and CYP2C8 is expected to increase when co-administered with NEXAVAR. Caution is recommended when administering substrates of CYP2B6 and CYP2C8 with NEXAVAR.

- CYP3A4 Inducers** - Continuous concomitant administration of NEXAVAR and rifampicin resulted in an average 37% reduction of sorafenib AUC. Other inducers of CYP3A4 activity (for example, *Hypericum perforatum* also known as St. John's wort, phenytoin, carbamazepine, phenobarbital, and dexamethasone) may also increase metabolism of sorafenib and thus decrease sorafenib concentrations
- CYP3A4 Inhibitors and CYP Isoform Substrates** - *In vitro* data indicate that sorafenib is metabolized by CYP3A4 and UGT1A9 pathways. Ketoconazole (400 mg), a potent inhibitor of CYP3A4, administered once daily for 7 days did not alter the mean AUC of a single oral 50 mg dose of sorafenib in healthy volunteers. Therefore, sorafenib metabolism is unlikely to be altered by CYP3A4 inhibitors. Studies with human liver microsomes demonstrated that sorafenib is a competitive inhibitor of CYP2C19, CYP2D6, and CYP3A4 as indicated by K_i values of 17 micromolar, 22 micromolar, and 29 micromolar, respectively. Administration of NEXAVAR 400 mg twice daily for 28 days did not alter the exposure of concomitantly administered midazolam (CYP3A4 substrate), dextromethorphan (CYP2D6 substrate), and omeprazole (CYP2C19 substrate). This indicates that sorafenib is unlikely to alter the metabolism of substrates of these enzymes *in vivo*. Studies with human liver microsomes demonstrated that sorafenib is a competitive inhibitor of CYP2C9 with a K_i value of 7–8 micromolar. The possible effect of sorafenib on the metabolism of the CYP2C9 substrate warfarin was assessed indirectly by measuring PT-INR. The mean changes from baseline in PT-INR were not higher in NEXAVAR patients compared to placebo patients, suggesting that sorafenib did not inhibit warfarin metabolism *in vivo*
- P-glycoprotein Substrates** - Sorafenib is an inhibitor of P-glycoprotein *in vitro*, therefore may increase the concentrations of concomitant drugs that are P-glycoprotein substrates.
- Neomycin** - The average plasma exposure (AUC) of sorafenib was decreased by 54% in healthy volunteers who first received neomycin 1 g three times daily for 5 days orally. Therefore, the coadministration of sorafenib with oral neomycin should be carefully considered. Effects of other antibiotics on sorafenib pharmacokinetics have not been studied

Overdosage: There is no specific treatment for NEXAVAR overdose. The highest dose of NEXAVAR studied clinically is 800 mg twice daily. The adverse reactions observed at this dose were primarily diarrhea and dermatologic. No information is available on symptoms of acute overdose in animals because of the saturation of absorption in oral acute toxicity studies conducted in animals. In cases of suspected overdose, NEXAVAR should be withheld and supportive care instituted.

Women of child bearing potential / men capable of fathering a child:

All women of childbearing potential **MUST** have a negative pregnancy test within 7 days prior to receiving the investigational product. If the pregnancy test is positive, the patient must not receive investigational product and must not be enrolled in the study.

Physicians should inform female patients that NEXAVAR may cause birth defects or fetal loss and that they should not become pregnant during treatment with NEXAVAR and for at least 2 weeks after stopping treatment. Both male and female patients should be counseled to use effective birth control during treatment with NEXAVAR and for at least 2 weeks after stopping treatment. Female patients should also be advised against breast-feeding while receiving NEXAVAR

Known Potential Toxicities:

>10%:

Cardiovascular: Hypertension (9% to 41%; grade 3: 3% to 4%; grade 4: <1%; grades 3/4: 10%, onset: ~3 weeks)
 Central nervous system: Fatigue (37% to 46%), headache ($\leq 10\%$ to 17%), mouth pain (14%), voice disorder (13%), peripheral sensory neuropathy ($\leq 13\%$), pain (11%)
 Dermatologic: Palmar-plantar erythrodysesthesia (21% to 69%; grade 3: 6% to 8%; grades 3/4: 19%), alopecia (14% to 67%), skin rash (including desquamation; 19% to 40%; grade 3: $\leq 1\%$; grades 3/4: 5%), pruritus (14% to 20%), xeroderma (10% to 13%), erythema ($\geq 10\%$)
 Endocrine & metabolic: Hypoalbuminemia ($\leq 59\%$), weight loss (10% to 49%), hypophosphatemia (35% to 45%; grade 3: 11% to 13%; grade 4: <1%), increased thyroid stimulating hormone level (>0.5 mU/L: 41%; due to impairment of exogenous thyroid suppression), hypocalcemia (12% to 36%), increased amylase (30% to 34% [usually transient])
 Gastrointestinal: Diarrhea (43% to 68%; grade 3: 2% to 10%; grade 4: <1%), increased serum lipase (40% to 41% [usually transient]), abdominal pain (11% to 31%), decreased appetite (30%), anorexia (16% to 29%), stomatitis (24%), nausea (21% to 24%), constipation (14% to 16%), vomiting (11% to 16%)
 Hematologic & oncologic: Lymphocytopenia (23% to 47%; grades 3/4: $\leq 13\%$), thrombocytopenia (12% to 46%; grades 3/4: 1% to 4%), increased INR ($\leq 42\%$), neutropenia ($\leq 18\%$; grades 3/4: $\leq 5\%$), hemorrhage (15% to 17%; grade 3: 2%), leukopenia
 Hepatic: Increased serum ALT (59%; grades 3/4: 4%), increased serum AST (54%; grades 3/4: 2%), hepatic insufficiency ($\leq 11\%$; grade 3: 2%; grade 4: 1%)
 Infection: Infection
 Neuromuscular & skeletal: Limb pain (15%), weakness (12%), myalgia
 Respiratory: Dyspnea ($\leq 14\%$), cough ($\leq 13\%$)
 Miscellaneous: Fever (11%)

1% to 10%:

Cardiovascular: Ischemic heart disease (including myocardial infarction; $\leq 3\%$), cardiac failure (2%, congestive), flushing
 Central nervous system: Depression, glossalgia
 Dermatologic: Hyperkeratosis (7%), acne vulgaris, exfoliative dermatitis, folliculitis
 Endocrine & metabolic: Hypokalemia (5% to 10%), hyponatremia, hypothyroidism
 Gastrointestinal: Dysgeusia (6%), dyspepsia, dysphagia, gastroesophageal reflux disease, mucositis, xerostomia
 Genitourinary: Erectile dysfunction, proteinuria
 Hematologic & oncologic: Squamous cell carcinoma of skin (3%; grades 3/4: 3%), anemia
 Hepatic: Increased serum transaminases (transient)
 Neuromuscular & skeletal: Muscle spasm (10%), arthralgia ($\leq 10\%$), myalgia
 Renal: Renal failure
 Respiratory: Epistaxis (7%), flu-like symptoms, hoarseness, rhinorrhea

<1% (Limited to important or life-threatening): Acute renal failure, amyotrophy, anaphylaxis, angioedema, aortic dissection, cardiac arrhythmia, cardiac failure, cerebral hemorrhage, cholangitis, cholecystitis, dehydration, eczema, erythema multiforme, gastritis, gastrointestinal hemorrhage, gastrointestinal perforation, gynecomastia, hepatic failure, hepatitis, hypersensitivity reaction (skin reaction, urticaria), hypertensive crisis, hyperthyroidism, increased serum alkaline phosphatase, increased serum bilirubin, interstitial pulmonary disease (acute respiratory distress, interstitial pneumonia, lung inflammation, pneumonitis, pulmonitis, radiation pneumonitis), malignant neoplasm of skin (keratoacanthomas), nephrotic syndrome, ostealgia, osteonecrosis (jaw), pancreatitis, pleural effusion, prolonged QT interval on ECG, respiratory tract hemorrhage, reversible posterior leukoencephalopathy syndrome (RPLS), rhabdomyolysis, Stevens-Johnson

syndrome, thromboembolism, toxic epidermal necrolysis (TEN), transient ischemic attacks, tumor lysis syndrome, tumor pain

Pregnancy

Pregnancy Category D

Based on its mechanism of action and findings in animals, NEXAVAR may cause fetal harm when administered to a pregnant woman. Sorafenib caused embryo-fetal toxicities in animals at maternal exposures that were significantly lower than the human exposures at the recommended dose of 400 mg twice daily. There are no adequate and well-controlled studies in pregnant women using NEXAVAR. Women of childbearing potential should be advised to avoid becoming pregnant while on NEXAVAR. 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 the fetus.

When administered to rats and rabbits during the period of organogenesis, sorafenib was teratogenic and induced embryo-fetal toxicity (including increased post-implantation loss, resorptions, skeletal retardations, and retarded fetal weight). The effects occurred at doses considerably below the recommended human dose of 400 mg twice daily (approximately 500 mg/m²/day on a body surface area basis). Adverse intrauterine development effects were seen at doses ≥ 0.2 mg/kg/day (1.2 mg/m²/day) in rats and 0.3 mg/kg/day (3.6 mg/m²/day) in rabbits. These doses result in exposures (AUC) approximately 0.008 times the AUC seen in patients at the recommended human dose. A NOAEL (no observed adverse effect level) was not defined for either species, since lower doses were not tested.

Nursing Mothers

It is not known whether sorafenib is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from NEXAVAR, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

Following administration of radiolabeled sorafenib to lactating Wistar rats, approximately 27% of the radioactivity was secreted into the milk. The milk to plasma AUC ratio was approximately 5:1

5.7 Primary and Secondary Endpoints:

Primary endpoint:

- TTP: will be measured for each patient from the start of the first treatment the patient receives until the date the criteria for progression are met or the date the patient is taken off study for any reason, whichever is shorter.

Secondary endpoints:

- OS: measured from the start of treatment on trial until the death of the patient.
- Response: measured as best response, complete response (CR) or partial response (PR), as defined by Response Evaluation Criteria in Solid Tumors (RECIST 1.1) Committee.
- Adverse events: grade 1-5 adverse events according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v4.0; measured at baseline, each cycle, and at off study date.
- Biomarkers of efficacy and immune markers of autophagy modulation: evaluate biomarkers such as cathepsin D and LC3-II levels in PBMCs (RT-PCR) and autophagic vesicles in tumor (Electron microscopy). Immune markers, such as IL-6, will be measured.

5.8 Biomarker Analysis.

Five blood samples will be collected during the timepoints listed above:

- Baseline or Cycle 1 Day 1
- Cycle 1 Day 15
- Cycle 2 Day 1

Blood samples will be collected as follows:

- One – 10-ml EDTA tubes for plasma
- Five – 8 -ml CPT tubes for PBMC samples for immune marker analysis

The blood samples will be processed, and stored at Pharmacokinetic Sampling Department (PSD), Institute of Drug Development, at the Cancer Therapy and Research Center.

Preparation of Plasma samples:

- 1) Collect 10 mL of whole venous blood into 1- 10 mL EDTA tube
- 2) Centrifuge tubes at 2000 g for 15 minutes, at 4 C, to separate the cells from the Plasma.
- 3) Prepare the labels for the cryovials. The label for each tube should be written in black water fast ink.
- 4) Carefully transfer the top layer of the plasma from the EDTA tube being careful not to disturb the Buffy Coat (white layer just below the plasma). Transfer up to 2 mL aliquots of plasma from the blood collection tubes into each of the 3 – 3.6 ml skirted bottom externally threaded cryovials.
- 5) Transfer the white thick layer above the red blood cell pellet from the blood collection tube into 1 – 2 ml cryovials.
- 6) Affix each label lengthwise to its corresponding tube and secure each label with a strip of clear plastic tape wrapped completely around the tube to ensure the label remains affixed to the tube during the freezing storage.
- 7) Immediately place the labeled tubes of plasma and buffy coat in an upright position in a -20°C freezer.
- 8) **Within 12 hours, transfer samples to -80°C freezer for long-term storage.** Samples will be stored in PSD until all of the sample is used to evaluate the endpoints of the study.

Preparation of PMBC samples for immune marker analysis:

- 1) Draw blood a total of 40 mls prior to C1D1 (day -21 to day 0), C1D15 and C2D1 in 5 - 8 ML CPT tubes.
- 2) The lab drawn will be labeled at the PSD department at UT Health Cancer Center, accessioned, placed on ice in <30 minutes, collected and stored at at -20°C. Within 12 hours, transfer samples to -70°C freezer for long-term storage. Samples will be stored in PSD until all of the sample is used to evaluate the endpoints of the study.

6.0 Schedule of events / Study Calendar:

Table 3: Study calendar for Phase II study of sorafenib plus hydroxychloroquine in advanced HCC.

	Baseline	C1D1 (+/-4 days) ^Δ	C1D15(+/- 1 1 day)	C2D1 (+/-4 days)	Every cycle (+/- 4 days)	Every other cycle (pre-C3, C5, C7...) (+/- 4 days)	Off treatment (+/- 7 days)
Consent ^Λ	XΩ						
History & Physical [✓]	X€	X°	X	X	X		X
Vital Signs [✓]	X	X°	X	X	X		X
ECOG [✓]	X	X°	X	X	X		X
EKG+ ^Λ	X						
CBC, CMP, PTT/INR, AFP [✓]	X	X°	X	X	X		X
Serum Pregnancy Test for female of child-bearing potential [✓]	X						
HBsAg, anti-HBc, or anti- HCV# [✓]	X#						
Start Sorafenib		X					
Dispense HCQ [◇]		X	X	X	X		
Dispense Drug Diary [◇]	X	X	X	X	X		
Drug Reconciliation and collection of drug diary from previous cycle			X	X	X		
Tumor Evaluation (CT or MRI) [✓]	X±					X	X
Ophthalmologic Exam* [✓]	X*						
Plasma samples for biomarkers ^Λ	Xα	Xα	Xα	Xα			
Immune markers ^Λ	X¶	X¶	X¶	X¶			
AEs & Medication Review ^Λ	X	X°	X	X	X		X
Tumor biopsy (optional) [∞] ✓	X∞						

- ✓ Standard of care
- ^ Research purpose
- Ω If C1D1 is >30 days from baseline consent, then repeat consent.
- * If the patient has baseline ophthalmologic pathology (i.e., diabetic retinopathy), then a comprehensive eye exam will be done at baseline as standard of care. In these patients, if they continue to receive HCQ on study at 6 months, a repeat comprehensive eye exam will be done at 6 months and then every 6 months while receiving HCQ on study. If the patient has visual disturbances while receiving HCQ, then an ophthalmologic evaluation will be done at that time. If the ophthalmologist recommends discontinuation of HCQ due to eye toxicity, then the patient will be taken off study for toxicity.
- € Baseline History and Physical should ask to make sure patient has not taken hydroxychloroquine in the past.
- + If EKG is clinically significant per treating physician at baseline, then repeat every cycle until off treatment.
- ° For Cycle 1 Day 1, if baseline labs were done <21 days from C1D1, then can omit CBC, CMP, AFP, PTT/INR for C1D1. If baseline history and physical, ECOG, vital signs, AEs, and medication review is < 21 days from C1D1, then can omit for C1D1
- Δ If patient has completed all eligibility requirements within the 21 days from C1D1, then C1D1 can start at home for Cohort 1 only. Patient will notify study coordinator of start date. For Cohort 2, C1D1 must occur in clinic since HCQ and drug diaries are dispensed on this day.
- # At baseline, if status not documented previously.
- ± Baseline CT/MRI should be Day -28 to Day -1. Every other cycle CT/MRI scans can be done +/- 4 days. Off treatment CT/MRI scan is done if not done at disease progression or in the last 28 days. Type of scan is dependent upon patient's insurance and treating physician. (See Appendix 13.1 for definitions of response).
- ∞ At baseline, tumor biopsy is optional and will be sent to PK lab for analysis. If a baseline biopsy has been done as standard of care, it will be sent to the PK lab for analysis for the secondary endpoint. If a biopsy is done as standard of care, while on study, then the tissue will be sent to the PK lab for analysis.
- α If blood draw done at baseline, C1D1 draw is not needed. Draw whole venous blood directly into One - 10-ml EDTA tubes for plasma (See Section 5.8 for processing)
- ¶ If blood draw done at baseline, C1D1 draw is not needed. Draw whole venous blood directly into FIVE – 8-ml CPT tubes for PBMC analysis (See Section 5.8 for processing)
- ◇ HCQ Dispense and HCQ drug diary will only occur on C1D15 if patient is in Cohort 1. HCQ dispense and HCQ drug diary will occur on C1D1 for Cohort 2. Sorafenib drug diary can be given at baseline for both Cohorts and dispensed every cycle thereafter.

6.1 AE /SAE Relationship

The Investigator will be asked to document his/her opinion of the relationship of the event to study medication as follows:

- **Unrelated**
The adverse event is clearly not related to the investigational agent(s).
- **Unlikely**
The adverse event is doubtfully related to the investigational agent(s).
- **Possible**
The adverse event may be related to the investigational agent(s).
- **Probable**
The adverse event is most likely related to the investigational agent(s).
- **Definite**
The adverse event is clearly related to the investigational agent.

6.2 Measurement of Effect

For the purposes of this study, patients should be reevaluated for response every **8** weeks.

Definitions

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria ⁴⁷. Note: Lesions are either measurable or non-measurable using the criteria provided below. The term "evaluable" in reference to measurability will not be used because it does not provide additional meaning or accuracy.

See Appendix 12.1 for RECIST criteria

7.0 Data Collection

Data will be entered and managed with a web-based system named Redcap. Redcap will be used as a research data system for clinical and laboratory data, designed, and maintained by the Department of Epidemiology and Biostatistics at UTHSCSA. Redcap monitors subject and site data quality metrics, protocol compliance with inclusion and exclusion criteria, site and protocol enrollment rates, identifies late, missing, erroneous, and suspect data entries, generates queries to the submitting site and resolves issues (like data quality, and patient safety) through means ranging from e-mail notifications to secure encrypted and auditable web-based communications between users at both the individual (user to user) and role (data monitor, or data entry technician) level.

For each patient on study, the following will be documented:

- Lab values: CBC, CMP, PTT/INR, AFP (when collected)
- TTP: will be measured for each patient from the start of the first treatment the patient receives until the date the criteria for progression are met or the date the patient is taken off study for any reason, whichever is shorter
- OS: measured from the start of treatment on trial until the death of the patient (date of death)
- Response: measured as best response at any given time: complete response (CR) or partial response (PR), as defined by Response Evaluation Criteria in Solid Tumors (RECIST 1.1) Committee ⁴⁷. (See Appendix 13.1 for definitions of response).
- Adverse events: the number and grade 1-5 of all adverse events will be documented according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v4.0; measured at baseline, each cycle, and at off study date.
- Biomarkers of efficacy and immune markers of autophagy modulation: changes in concentration of biomarkers in PBMCs, immune markers, AND visualize and quantify number of autophagic vesicles on electron microscopy in patient's tumor.

8.0 Data and Safety Monitoring Plan

Data and Safety Monitoring Oversight

A Data and Safety Monitoring Plan is required for all individual protocols conducted at UT Health Cancer Center. All protocols conducted at UT Health Cancer Center are covered under the auspices of the UT Health Cancer Center Institutional Data Safety Monitoring Plan (DSMP).

The UT Health Cancer Center Institutional DSMP global policies provide individual trials with:

- institutional policies and procedures for institutional data safety and monitoring,
- an institutional guide to follow,
- monitoring of protocol accrual by the UT Health Cancer Center Protocol Review Committee,
- review of study forms and orders by the Forms Committee,
- tools for monitoring safety events,

- independent monitoring and source data verification by the UT Health Cancer Center QA Monitor/Auditor
- monitoring of UPIRSO's by the Director of Quality Assurance and DSMC,
- determining level of risk (Priority of Audit Level Score – PALS) ,
- oversight by the Data Safety Monitoring Committee (DSMC), and
- verification of protocol adherence via annual audit for all Investigator Initiated Studies by the UT Health Cancer Center Quality Assurance Division.

Monitoring Progress and Safety

Due to the risks associated with participation in this protocol, the UT Health Cancer Center DSMB (*DSMB#2*) in conjunction with the Principal Investigator will perform assessment of adverse events, adverse event trends and treatment effects on this study. The UT HEALTH CANCER CENTER DSMB (*DSMB#2*) acts as an independent Data Safety Monitoring Board (DSMB) for IIS conducted at UT HEALTH CANCER CENTER. The UT HEALTH CANCER CENTER DSMB (*DSMB#2*) will monitor data throughout the duration of a study to determine if continuation of the study is appropriate scientifically and ethically. An additional layer of review is provided by the UT HEALTH CANCER CENTER Data Safety Monitoring Committee (DSMC) who will review DSMB reports every 6 months.

Baseline events and adverse events will be captured in Redcap for each patient using CTCAE V4.0 for the grading and attribution of adverse events. Redcap will be used to centrally document:

- the event and grades the seriousness of the event,
- if the event was a change from baseline,
- the determination of the relationship between the event and study intervention,
- if the event was part of the normal disease process, and
- what actions were taken as a result of the event.

Safety Definitions:

For this study, the following safety definitions will be applicable:

Adverse Event Definition: An adverse event (AE) is defined as any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research. For this study, all adverse events will be documented starting with the first dose of the study drug and ending 30 days after the last dose of study drug is received.

Serious Adverse Event Definition: is any adverse event that:

1. results in death;
2. is life-threatening (places the subject at immediate risk of death from the event as it occurred);
3. results in inpatient hospitalization or prolongation of existing hospitalization;
4. results in a persistent or significant disability/incapacity;
5. results in a congenital anomaly/birth defect; or
6. based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition

Unanticipated Problems Involving Risks to Subjects or Others Definition: Unanticipated problem involving risk to subjects or others includes any incident, experience or outcome that meets all of the following criteria:

- A. unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol

and informed consent document; and (b) the characteristics of the subject population being studied (note: the unfounded classification of a serious adverse event as “anticipated” constitutes serious non-compliance);

- B. definitely related or probably related to participation in the research; and
- C. suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized

Reporting Requirements

For this study, the adverse events collected on patients for this protocol will be reviewed by the Principal Investigator to determine if a serious safety problem has emerged that result in a change or early termination of a protocol such as:

- dose modification,
- suspending enrollment due to safety or efficacy, or
- termination of the study due to a significant change in risks or benefits.

The PI will provide the DSMB #2 with above findings every 6 months, for discussion and review during their meetings.

The endpoint for dose reduction is based on observed adverse events. To identify the clinical and hematologic adverse events, this study will use NCI-CTCAE v4.0. At each visit, including baseline, each adverse event (AE), its grade and attribution made by the treating physician and will be documented in IDEAS. Any AE of $\geq$ Grade 3 and attributed as possibly, probably or definitely related to SOR/HCQ will result in the dose being held until the AE has resolved to $\leq$ grade 1 or baseline. If the AE resolves, reinstitution of treatment can occur, but at a reduced dose. Dose reductions will be made as per *Table below*.

A baseline ophthalmologic exam will be done in patients with baseline eye pathology, as chronic use of HCQ has been associated rarely with visual disturbances. If the patient has baseline eye pathology and remains on study receiving HCQ at 6 months, then repeat examination will be done every 6 months thereafter, if the patient continues to receive HCQ at that time, or sooner if the patient has any new ophthalmologic symptoms, with particular regard to visual field deficits. Patients should be cautioned to report any visual symptoms, particularly difficulty seeing entire words or faces, intolerance to glare, decreased night vision, or loss of peripheral vision. These symptoms of peripheral retinal toxicity should prompt drug discontinuation and ophthalmologic evaluation. If the patient has visual disturbances while receiving HCQ, then an ophthalmologic evaluation will be done at that time. If the ophthalmologist recommends discontinuation of HCQ due to eye toxicity, then the patient will be taken off study for toxicity.

Table 4. Dose reductions for sorafenib plus HCQ Phase II study of sorafenib and HCQ in advanced HCC patients

If toxicity due to both sorafenib and HCQ:		
Dose Level	sorafenib	HCQ
0 (<i>Standard Daily Dose</i>)	400 mg twice daily	400 mg daily
-1	200 mg twice day	200 mg daily
-2	200 mg daily	n/a

If toxicity due to sorafenib:		
Dose Level	sorafenib	HCQ
0 (<i>Standard Daily Dose</i>)	400 mg twice daily	400 mg daily
-1	200 mg twice daily*	400 mg daily
-2	200 mg daily	n/a
If toxicity due to HCQ:		
Dose Level	sorafenib	HCQ
0 (<i>Standard Daily Dose</i>)	400 mg twice daily	400 mg daily
-1	400 mg twice daily	200 mg daily
-2	200 mg twice daily	n/a

* Patient can take either 400mg once daily or 200mg twice daily depending on tolerance

Early stopping for toxicity:

The stopping rule for toxicity is not based on conditional statistical power but rather on observed adverse events. A toxicity event for the purposes of safety stopping is defined as a grade 4 or 5 toxicity of any kind due to treatment with sorafenib and/or HCQ. If at any time during the study there are 2 or more grade 5 toxicities that are attributed to sorafenib and/or HCQ, then the study will be terminated. If > 50% of patients are having similar type of grade 3 and 4 toxicity, then the study will be terminated. If HCQ causes grade 4 peripheral retinal toxicity, there will be prompt drug discontinuation and ophthalmologic evaluation, with consideration for termination of study.

As per the UT Health Cancer Center DSMP, any protocol modifications, problematic safety reports, unanticipated problems, and suspension or early termination of a trial must be reported to the DSMB #2 and all members of the research team. Furthermore, the PI of this study will promptly notify all study affiliates, the UTHSCSA IRB, the UT Health Cancer Center DSMC, and the FDA via a FDA Form 3500Aa written IND safety report of any adverse events that are either serious and/or unexpected. Suspension and early termination of a trial must also be reported immediately to the Director of Quality Assurance who will promptly notify the sponsor and the UTHSCSA IRB.

The PI will provide adverse events findings using the Investigator Initiated Study DSMC Report Form every 6 months, to the DSMB#2. The DSMB#2 will review the information provided by the PI and report to the UT Health Cancer Center DSMC as reports are scheduled, unless an emergent issue has been identified. The Investigator Initiated Study DSMC Report Form includes information on adverse events, current dose levels, number of patients enrolled, significant toxicities per the protocol, patient status (morbidity and mortality) dose adjustments with observed response, and any interim findings. Any trend consisting of three or more of the same event will be reported to the UT Health Cancer Center DSMB for independent review outside of the reporting cycle, which begins three months following protocol start up. The DSMB#2 will also provide its findings to the UT Health Cancer Center's Regulatory Affairs Division so that it may be provided to the UTHSCSA IRB with the protocol's annual progress report. Conflict of interest is avoided by the independent reviews of the UT Health Cancer Center DSMB#2, UT Health Cancer Center DSMB, and by ongoing independent review of UPIRSO trends by the Director of Quality Assurance.

All SAE and UPRISO's will be reported following UT Health Cancer Center, UTHSCSA institutional and FDA guidelines.

UTHSCSA SAE/UPRISO REPORTING REQUIREMENTS		
Type Event	Report to	Timeframe
All AE, SAE and UPIRSO	Regulatory Affairs & DQA	Same as other notification timeframes except for SAE/AE which should be reported on Monday for the prior week
SAE	Sponsor	within 24 hours
AE/SAE	UTHSCSA IRB	Annually
UPIRSO - all	Sponsor	within 24 hours of the PI determining a UPIRSO exists
UPIRSO - life threatening	UTHSCSA IRB	within 48 hours of the PI determining a UPIRSO exists
UPIRSO- non-life threatening	UTHSCSA IRB	within 7 days of the PI determining a UPIRSO exists

AE's and SAE events that occur during clinical trials with or without an Investigational New Drug (IND) application are mandatory reports submitted to FDA via Medwatch FDA F3500A *within 15 days for events that have at least a possible relationship with the drug.*

Expedited Reporting for Phase II Studies (including hospitalization)*-

UNEXPECTED EVENT		EXPECTED EVENT	
GRADES 2 - 3 Attribution of Possible, Probable or Definite	GRADES 4 - 5 Regardless of Attribution	GRADES 1 - 3	GRADES 4 - 5 Regardless of Attribution
Expedited report within 10 working days Grade 1 - Adverse Event Expedited Reporting NOT required.)	Report by phone to IDB within 24 hrs. Expedited report to follow within 10 working days. This includes all deaths within 30 days of the last dose of treatment with an investigational agent regardless of attribution.	Adverse Event Expedited Reporting NOT required.	Expedited report, including Grade 5 Aplasia in leukemia patients, within 10 working days. This includes all deaths within 30 days of the last dose of treatment with an investigational agent regardless of attribution. Any late death attributed to the agent (possible, probable, or definite) should be reported within 10 working days.

	Any late death attributed to the agent (possible, probable, or definite) should be reported within 10 working days.		Grade 4 Myelosuppression or other Grade 4 events that do not require expedited reporting will be specified in the protocol.
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* For Hospitalization Only — Any medical event equivalent to CTC Grade 3, 4, 5 which precipitated hospitalization (or prolongation of existing hospitalization) must be reported regardless of requirements for Phase of study, expected or unexpected and attribution.

Assuring Compliance with Protocol and Data Accuracy

As with all studies conducted at UT Health Cancer Center, the PI has ultimate responsibility for ensuring protocol compliance, data accuracy/integrity and responding to recommendations that emanate from monitoring activities. *Source verification of data will be performed once a year as we have sufficient experience and safety evidence with this drug.* Protocol compliance, data accuracy and reporting of events is further ensured by an annual audit conducted by the Data Safety Officer, whose audit report is shared with the PI, the research team, and will be reviewed by the UT Health Cancer Center DSMC.

UT Health Cancer Center DSMB Membership

The UT Health Cancer Center has two DSMB's with a primary set of members specific to the histology of the study consisting of UTHSCSA faculty and staff. This Protocol will utilize DSMB#2 for Solid Tumor Studies.

As per NCI guidelines and to eliminate conflict of interest (financial, intellectual, professional, or regulatory in nature), the UT Health Cancer Center DSMB specific to this study will not treat patients on this protocol. Usage of the DSMB specific to the histology has been created to ensure that experts in that histology are represented on the DSMB assembled for this protocol, but may be expanded, at the PI's discretion, to include other members, which may include:

- experts in the fields of medicine and science that are applicable to the study (if not currently represented on the DSMB),
- statistical experts,
- lay representatives,
- multidisciplinary representation, from relevant specialties including experts such as bioethicists, biostatisticians and basic scientists, and
- others who can offer an unbiased assessment of the study progress.

Additional or alternate membership of in the DSMB is selected by the DSMC chair, in conjunction with the PI of this protocol.

UT Health Cancer Center DSMB Charter and Responsibilities

The UT Health Cancer Center DSMB will provide information on the membership composition, including qualifications and experience to both the UTHSCSA IRB and UT Health Cancer Center PRC for review. The UT Health Cancer Center DSMB for this study will act as an independent advisory board to the PI and will report its findings and recommendations to the PI, the UTHSCSA IRB and the UT Health Cancer Center DSMC. UT Health Cancer Center DSMB reports will utilize the Investigator Initiated Study DSMC Report Form and meetings will occur as scheduled, to review any updates from the prior meeting.

Once the protocol is activated, if not already established elsewhere in the protocol the UT Health Cancer Center DSMB will establish and provide:

- procedures for maintaining confidentiality;
- statistical procedures including monitoring guidelines, which will be used to monitor the identified primary, secondary, and safety outcome variables;
- consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the study;
- plans for changing frequency of interim analysis as well as procedures for recommending protocol changes;
- recommendation of termination due to unfavorable benefit-to-risk or inability to answer study questions;
- recommendation of continuation of ongoing studies;
- recommend modification of sample sizes based on ongoing assessment of event rates; and
- review of final results and publications.

9.0 Minority Recruitment Plan

Section 1: Minority Accrual Template

Purpose:

To assist the Principal Investigators (PIs) conducting Investigator Initiated Studies (IIS) in determining whether or not they are meeting the NIH and NCI guidelines and recommendations for the inclusion of minorities in Clinical Trials.

Preliminary information that Principal Investigators should consider include:

- Overall Representation of Minorities in the target catchment area
 - The ethnic/racial distribution of the specific type of cancer that is being targeted
- The following resources can be utilized to determine target sample sizes for ethnic groups:
- Texas Health Data <http://soupfin.tdh.state.tx.us/txhd.htm>
 - Texas Cancer Registry www.dshs.tx.us/tcr/
 - SEER Cancer Statistics Review www.seer.cancer.gov
 - ACS Cancer Statistics www.cancer.org

***Estimated Target Accrual (Total)**

This section asks for a **projection** of the total ethnic/racial and gender mix of the subjects

Breakdown of subjects - Indicate the <u>total</u> targeted/planned accrual by <u>Race</u> & Gender		
Race	% Male	% Female
Black or African American	3	3
American Indian or Alaskan Native		
Asian	2	2
Native Hawaiian or Other Pacific Islander		
White	45	45
More Than One Race (Multiracial)		
Total	50	50

Section 2: Clinical Investigator Tools for Recruitment of Minorities

Tool included in study	LIST OF TOOLS AND ACTIONS FOR INCREASING MINORITY ACCRUAL TO CLINICAL TRIALS <i>For assistance with submitting IRB documents, developing materials in English and Spanish, and scheduling public service announcements, please contact MAtools@uthscsa.edu</i>
Yes ☐ No ☐	1. Include Clinical Trial information on UT Health Cancer Center website in both English and Spanish (<i>Please notify MAtools@uthscsa.edu</i>).
Yes ☐ No ☐	2. Use of Bilingual Research Team Member or Translation services
Yes ☐ No ☐	3. Identification of bilingual Patient Navigator representative of the Target Population Please Specify:
Yes ☐ No ☐	4. Informed Consent available in Spanish
Yes ☐ No ☐	6. Information Brochures in English and Spanish** (<i>IRB approval required</i>)
Yes ☐ No ☐	7. Flyers in English and Spanish (two sided, printed in English on one side and Spanish on the other).** (<i>IRB approval required</i>)
Yes ☐ No ☐	8. Public Service Announcements (PSAs) or Advertisements- Spanish Radio** (<i>IRB approval required</i>)
Yes ☐ No ☐	9. PSA's or Advertisements -Spanish newspapers (e.g., La Prensa)** (<i>IRB approval required</i>)
Yes ☐ No ☐	10. PSA's or Advertisements -Spanish Television (e.g., Univision)** (<i>IRB approval required</i>)
Yes ☐ No ☐	11. Patient Friendly Fast Facts in English and Spanish ** (<i>IRB approval required</i>)
Yes ☐ No ☐	12. Outreach to advocacy or community organizations (including presentations or awareness campaigns). Please specify:
Yes ☐ No ☐	13. Other. Please Specify:

** - It is recommended to submit any patient materials to the IRB as an amendment after the initial IRB approval has been granted

10.0 Statistical Section

Analytic plan for primary objective:

Analytic plan for primary objective: mTTP for cohort 1 and cohort 2: TTP will be measured for each patient from the start of the first treatment the patient receives until the date the criteria for progression are met or the date the patient is taken off study for any reason, whichever is shorter. mTTP will be illustrated with Kaplan-Meier plots, the estimate of median TTP with its 95% confidence interval. Treatment groups will be contrasted with regard to the response rate with a logistic regression model adjusted for age, sex, race, Child Pugh score, BCLC stage and AFP levels; the odds ratio, its 95% confidence interval, and the p-value for testing the null hypothesis that the log odds ratio is zero will be presented. If the logistic model fails due to small cell counts then treatments will be contrasted with regard to response using Fisher's Exact Test.

If a patient does not complete Cycle 1 and is off study, then this patient will not be evaluable for efficacy but will be evaluable for toxicity.

Analytic plan for secondary objectives:

- OS for cohort 1 and cohort 2: OS will be measured for each patient from start of treatment to time of death. The analyses of median OS will be performed in the intent-to-treat population. OS will be illustrated with Kaplan-Meier plots, the estimate of median OS with its 95% confidence interval.
- Response rates: Response rates will be measured as the proportion of patients with the best response, complete response (CR) or partial response (PR), as defined by RECIST 1.1 Criteria and a 95% confidence interval.
- Adverse events: All adverse events will be listed by type and grade.
- Biomarkers of efficacy and immune markers of autophagy modulation: The significance of variation in biomarkers with treatment will be assessed with linear models with and without adjustment for age, sex, race, Child Pugh score, BCLC stage and AFP levels. The dependent variables will be log transformed (base 2) if necessary. The relation between treatment efficacy, as measured by TTP, and biomarkers will be assessed with proportional hazards models in terms of treatment, the biomarker, the biomarker by treatment interaction, age, sex, race, Child Pugh score, BCLC stage and AFP levels. Significant effects will be described and illustrated, as appropriate, with Kaplan Meier plots by strata determined by biomarker level and treatment group, for example. Also, electron microscopy will be used to visualize and quantify the autophagic vesicles (AV) in tumor samples (optional biopsy). The average number of autophagic vesicles per cell for patients will be correlated with survival.

Sample size justification: Cohort 1 (sorafenib in combination with HCQ): Historically, the mTTP for patients treated with sorafenib is 5.5 mos. We predict addition of HCQ will improve mTTP by 50% to 8.2 months. We expect 5% lost to follow-up. Assuming a 24-month accrual period with 1 year for follow up, a one-parameter exponential model, and $\alpha=5\%$, 49 subjects are necessary to obtain 80% power. With 5% lost to follow-up the sample size required is 52 ($=49/0.95$).

Cohort 2 (Addition of HCQ on sorafenib progression): Historically, the mTTP for patients following progression on sorafenib 2.1 mos. We predict addition of HCQ will improve mTTP to 4.2 months. We expect 10% lost to follow-up. Assuming a 24-month accrual period with 1 year for follow up, , a one-parameter exponential model, and $\alpha=5\%$, 19 subjects are necessary to obtain 80% power. With 10% lost to follow-up the sample size required is 21 ($=19/0.95$).

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12.0 Appendices

12.1 RECIST criteria for Measurement of Effect

12.2 ECOG Performance Status

12.1 RECIST criteria for Measurement of Effect
For the purposes of this study, patients should be re-evaluated for response every **8** weeks.

Definitions

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria⁴⁷. Note: Lesions are either measurable or non-measurable using the criteria provided below. The term "evaluable" in reference to measurability will not be used because it does not provide additional meaning or accuracy.

Measurable disease

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as > 20 mm with conventional techniques (CT, MRI, x-ray) or as > 10 mm with spiral CT scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Non-measurable disease

All other lesions (or sites of disease), including small lesions (longest diameter < 20 mm with conventional techniques or < 10 mm using spiral CT scan), are considered nonmeasurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

Target lesions

All measurable lesions up to a maximum of five lesions per organ and 10 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) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

Non-target lesions

All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Non-target lesions include measurable lesions that exceed the maximum numbers per organ or total of all involved organs as well as nonmeasurable lesions. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

Guidelines 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.

Note: Tumor lesions that are situated in a previously irradiated area are not to be considered evaluable for response, unless documented progression prior to study enrollment.

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 when both methods have been used to assess the antitumor effect of a treatment.

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

Conventional CT and MRI: These techniques should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

Ultrasound (US): When the primary endpoint of the study is PFS evaluation, US should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions, and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

Endoscopy, Laparoscopy: The utilization of these techniques for objective tumor evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor response should be restricted to validation purposes in reference centers. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained.

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.***

Response Criteria

Evaluation of target lesions

- Complete Response (CR): Disappearance of all target lesions
- Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD

- Progressive Disease (PD): At least a 20% increase in the sum of the longest diameter (LD) of target lesions, taking as reference the smallest sum LD recorded since treatment started or the appearance of one or more new lesions.
- Stable Disease (SD): Neither sufficient shrinkage to qualify for a PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started.

Evaluation of non-target lesions

- Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level
- Incomplete Response/ Stable Disease (SD): Persistence of one or more non-target lesions(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.

12.2 ECOG Performance Status

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

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