Local Protocol #: **14-Multi-14-MCC**

TITLE: A Pilot Study to Determine the Biological Effects of Hydroxychloroquine on PAR-4 Levels in Patients with Resectable Solid Tumors

Coordinating Center: Markey Cancer Center

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SUMMARY

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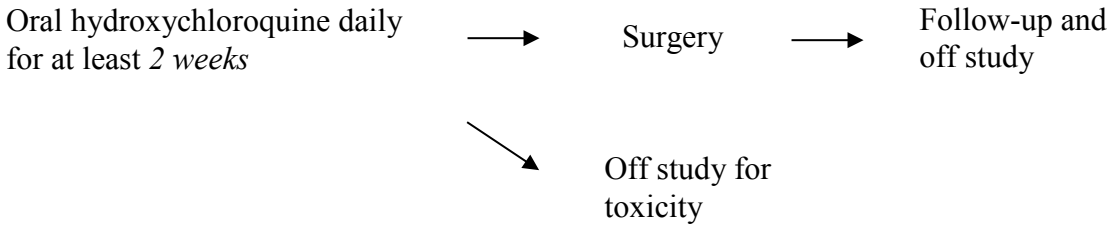

Dose Schedule	
Dose Level	**Dose**
	Hydroxychloroquine (mg/day)
Level -1	200 mg/day
STARTING DOSE: Level 1	400 mg/day
Level 2	800 mg/day
Level 3	1200 mg/day

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1. OBJECTIVES

1.1 Primary Objectives

1.1.1 To characterize the effects of hydroxychloroquine (HCQ) on plasma PAR-4 levels in adults with resectable solid tumors at the time of and after resection and to compare them to pre-HCQ treatment plasma levels

1.2 Secondary Objectives

1.2.1 To evaluate the toxicity profile and pharmacokinetics of hydroxychloroquine given in this setting

1.2.2 To assess the ability of hydroxychloroquine to alter PAR-4 expression in tumors.

2. BACKGROUND

2.1 Introduction: PAR-4

Prostate apoptosis response-4 (PAR-4, also called PAWR) is a tumor suppressor protein that induces apoptosis in diverse cancer cells but not in normal cells.[1] PAR-4 is ubiquitously expressed in normal cells and tissues, but is often inactivated, down-regulated or mutated in several types of cancers.[2] PAR-4 is located in various cell compartments, including the cytoplasm, endoplasmic reticulum (ER), and the nucleus, and both intracellular PAR-4 as well as secreted PAR-4 have been shown to play a role in apoptosis induction by caspase-dependent mechanisms.[3] Moreover, PAR-4 sensitizes cells to the action of diverse therapeutic agents.[4] Accordingly, loss of PAR-4 in tumors contributes to recurrent tumors and a decrease in overall patient survival.[5] PAR-4 protein is secreted in cell culture-conditioned medium (CM) or systemically in mice by normal cells, and extracellular Par-4 binds to its receptor GRP78 on the cancer cell surface and induces apoptosis.[2,6] By contrast, normal cells express low to undetectable levels of cell surface GRP78 and are resistant to apoptosis by extracellular PAR-4.[2,6] Our recent studies have indicated that GRP78 levels can be increased on the surface of diverse cancer cells to overcome PAR-4-resistance by inhibition of NF-κB activity, which is usually elevated in most cancer cells.[6] Recombinant PAR-4 (made in E. coli) induces apoptosis of tumor cells and inhibits tumor growth in mice.[7]

2.2 Hydroxychloroquine

Chloroquine [CQ; *(RS)*-*N'*-(7-chloroquinolin-4-yl)-*N*,*N*-diethyl-pentane-1,4-diamine) is an antimalarial drug introduced into clinical practice in 1947 for the prophylactic treatment of malaria.[8] CQ has been repurposed to treat inflammatory diseases such as rheumatoid arthritis and lupus. Hydroxychloroquine (HCQ) differs from chloroquine by the presence of a hydroxyl group at the N-ethyl substituent is beta-hydroxylated, but has similar pharmacokinetics to CQ, with quick gastrointestinal absorption and is eliminated by the kidney with minimal side effects on short term treatments. Side effects of chronic treatment are restricted to the eye. Overdose symptoms, which can occur within a half-hour of taking the medication, include convulsions, drowsiness, headache, heart problems or heart failure, difficulty breathing and vision problems. Both CQ and HCQ are potent inhibitors of autophagy, a cellular survival process during starvation to maintain cellular energy by degradation of unnecessary or dysfunctional

components through the actions of lysosomes. CQ and HCQ are lysosomotropic agents, which inhibit autophagy by raising the lysosomal pH and leads to inhibition of both fusion of autophagosome with lysosome and lysosomal protein degradation. Moreover, by inhibiting autophagy, CQ activates caspases and sensitizes the cells to apoptosis. These apoptosis sensitizing features of HCQ or CQ enhance the anti-tumor effects of a broad range of cancer therapeutics.[9,10] In fact, CQ prophylaxis against malaria in Tanzania was associated with a reduction in the number of cases of Burkitt's lymphoma.[11] Accordingly, HCQ and CQ are being studied in a number of clinical trials that use these compounds either pre-operatively or in conjunction with chemotherapeutic agents or radiation (http://www.cancer.gov/clinicaltrials/search/results?protocolsearchid=12327270)

2.3 Rationale for Studying PAR-4 Expression in subjects treated with Chloroquine
As the baseline levels of PAR-4 secreted by normal cells are generally inadequate to cause massive apoptosis in cancer cells, drugs that bolster the secretion of PAR-4 would constitute an important therapeutic advance. Our recent studies identified several small molecules that show robust secretion of Par-4 in cell culture and mouse models, and secreted PAR-4 induces apoptosis of cancer cells.[12] However, these small molecules are not FDA-approved for human use. We therefore sought to identify FDA-approved small molecule drugs that show robust secretion of PAR-4 in normal cells and therefore can be repurposed for treatment of cancer. These studies (unpublished) identified HCQ as a potent inducer of PAR-4 secretion from normal cells under conditions that show no normal cell death. As normal cells in any patient far outnumber cancer cells, FDA-approved PAR-4 secretagogues may play a valuable role in elevating systemic and local levels of PAR-4 protein and thereby induce paracrine apoptosis in primary and metastatic tumors. Systemic PAR-4 is also expected to induce apoptosis in circulating cancer cells that contribute to metastasis. Because PAR-4 produces apoptosis in diverse tumors, and HCQ is expected to produce elevated levels of PAR-4 secretion in a broad range of patients to prevent the growth of tumors. Thus, our findings will potentially have broad clinical significance.

2.4 Correlative Studies Background
Studies in cell culture and mice indicated that HCQ induced robust secretion of PAR-4 in normal fibroblast and epithelial cell cultures, and elevated PAR-4 protein levels in the plasma of immunocompetent mice. Moreover, examination of plasma samples from renal cell carcinoma patients who were given HCQ (400 mg daily for 2 weeks) prior to surgery indicated that HCQ increased the secretion of PAR-4 protein levels in 4 of 5 patients (Figure 1). However, HCQ pharmacokinetics is highly variable and we expect an exposure dependent (area under the cureve) effect on Par-4 secretion rather than a dose-dependent effect.

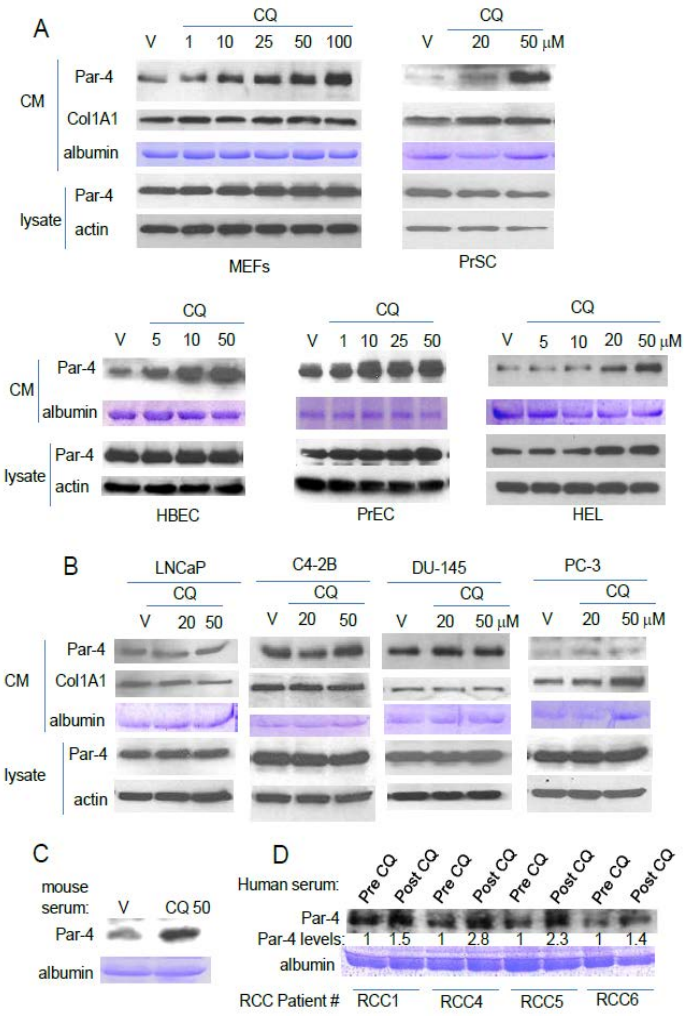


Figure 1. Chloroquine induces Par-4 secretion. A and B. Various cell lines such as mouse embryonic fibroblasts (MEFs, early passage 4 or 5), human primary prostate fibroblast cells (PrSC), human primary prostate epithelial cells (PrEC), human lung embryonic fibroblasts (HEL), human bronchial epithelial cells (HBEC) (A) or prostate cancer cell lines (B), were treated with the indicated amounts of chloroquine diphosphate (CQ) or vehicle (V) control for 24 h. The conditioned medium (CM), as well as the whole-cell lysates were subjected to Western blot analysis with the indicated antibodies. Collagen (Col1A1) was used as a loading control for protein secretion, as it is generally unchanged in response to the treatments. The samples were also subjected to SDS-PAGE and Coomassie blue staining to determine albumin levels in serum from the CM as another loading control. C. C57/BL6 mice were injected intraperitoneally with a single dose (50 mg/kg body weight) of CQ and serum samples were collected after 24 h and processed by Western blot analysis.

D. Plasma samples were collected from renal cell carcinoma patients who were treated with HCQ pre-operatively for 2-weeks at 400 mg/day were collected pre- and post-HCQ treatment at the University of Pittsburgh as part of a clinical trial on HCQ in 2010-2011. The archival (frozen) samples were processed by Western blot analysis at the University of Kentucky in 2013-2014.

2.5 Hypothesis

That hydroxychloroquine will induce at least 2-fold increase in systemic (plasma) PAR-4 levels compared to pre-treatment plasma levels in patients with resectable solid tumors.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have a histologically confirmed or highly suspected (as determined by treating physician) solid tumor that is planned for surgical resection.

3.1.2 Age ≥18 years.

3.1.3 ECOG performance status ≤2 (Karnofsky ≥60%, see Appendix A).

3.1.4 Patients must be able to ingest oral medications (crushing and administering via PEG tube is acceptable).

3.1.5 Patients must have normal organ and marrow function as defined below:

– absolute neutrophil count	≥1,500/mcL
– platelets	≥100,000/mcL
– total bilirubin	Less than 1.5 x ULN
– AST(SGOT)/ALT(SGPT)	≤2.5 × institutional upper limit of normal
– creatinine	less than institutional ULN OR
– Creatinine clearance	≥60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal.

3.1.6 Patients must be able to undergo surgical resection of their tumor as determined by the treating surgeon.

3.1.7 The effects of hydroxychloroquine on the developing human fetus are unknown. For this reason and because anti-malarial agents as well as other therapeutic agents used in this trial are known to be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and 4 months after completion of hydroxychloroquine administration.

3.1.8 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.1 Patients with metastatic cancer and/or cancer that is not amenable to surgery (i.e. must be curative intent; locally advanced is acceptable).

3.2.2 Patients with significant malabsorption as determined by the treating physician.

3.2.3 Patients who are receiving any other investigational agents.

3.2.4 Patients with known brain metastases are excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events. Patients with primary brain tumors amenable to surgery are allowed on this protocol.

3.2.5 History of allergic reactions attributed to compounds of similar chemical or biologic composition to hydroxychloroquine.

3.2.6 Caution should be taken with the use of hydroxychloroquine and any drugs known to interact with it (Appendix C). Because the lists of these agents are constantly changing, it is important to regularly consult a frequently-updated list such as http://medicine.iupui.edu/clinpharm/ddis/table.aspx; medical reference texts such as the Physicians' Desk Reference may also provide this information. As part of the enrollment/informed consent procedures, the patient will be counseled on the risk of interactions with other agents, and what to do if new medications need to be prescribed or if the patient is considering a new over-the-counter medicine or herbal product. Appendix C contains a list of known drug interactions with hydroxychloroquine.

3.2.7 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.

3.2.8 Pregnant women are excluded from this study. While hydroxychloroquine has not been formally assigned to a pregnancy category by the FDA, animal studies have revealed that the drug passed rapidly across the placenta, accumulated selectively in the melanin structures of the fetal eyes, and was retained in the ocular tissues for five months after the drug had been eliminated from the rest of the body. There are no controlled data in human pregnancy. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with hydroxychloroquine, breastfeeding should be discontinued if the mother is treated with hydroxychloroquine. These potential risks may also apply to other agents used in this study.

3.2.9 HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with hydroxychloroquine. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in patients receiving combination antiretroviral therapy when indicated.

3.2.10 Patients that are on enzyme-inducing anti-epileptic medications

3.3 Inclusion of Women and Minorities

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

Accrual Targets					
Ethnic Category	**Sex/Gender**				
	Females		**Males**		**Total**
Hispanic or Latino	1	+	1	=	2
Not Hispanic or Latino	8		8	=	16
Ethnic Category: Total of all subjects	9	+	9	=	18
Racial Category					
American Indian or Alaskan Native	0	+	0	=	0
Asian	1	+	1	=	2
Black or African American	1	+	1	=	2
Native Hawaiian or other Pacific Islander		+		=	
White	7	+	7	=	14
Racial Category: Total of all subjects	9	+	9	=	18

4. REGISTRATION PROCEDURES

4.1 Protocol Review and Monitoring Committee and Institutional Review Board Review

Before implementing this study, the protocol, the proposed informed consent form and other information to subjects, must be reviewed by the Markey Cancer Center's Protocol Review and Monitoring Committee and the University of Kentucky Institutional Review Board (IRB). A signed and dated statement that the protocol and informed consent have been approved by the IRB must be maintained in the Markey Cancer Center Clinical Research and Data Management Shared Resource Facility (MCC CRDM SRF) regulatory binder. Any amendments to the protocol, other than administrative ones, must be reviewed and approved by the PRMC, study sponsor and the UK IRB.

4.2 Enrollment Guidelines

Eligible patients will be identified by the principal investigator and co-investigators of this study. Potentially eligible patients will be screened in the University of Kentucky Markey Cancer Center clinics by the investigators, study personnel, and the Principal Investigator (PI). Upon obtaining proper consent, patients will be enrolled into the study.

4.3 Informed Consent

The goal of the informed consent *process* is to provide people with sufficient information so they can make informed choices about whether to begin or continue participation in clinical research. The process involves a dynamic and continuing exchange of information between the research team and the participant throughout the research experience. It includes discussion of the study's purpose, research procedures, risks and potential benefits, and the voluntary nature of participation.

The informed consent *document* provides a summary of the clinical study and the individual's rights as a research participant. The document acts as a starting point for the necessary exchange of information between the investigator and potential research participant. Also, research participants and their families may use the consent document as an information resource and reference throughout participation in the trial. The informed consent *document* is often considered the foundation of the informed consent process; it does not, however, represent the entirety of the process. Nor is the informed consent document a risk-management tool for the investigator and/or institution.

The investigator must explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

This informed consent should be given by means of a standard written statement, written in non-technical language. The subject should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If the subject cannot read or sign the documents, oral presentation may be made or signature given by the subject's legally appointed representative, if witnessed by a person not involved in the study, mentioning that the patient could not read or sign the documents. No patient can enter the study before his/her informed consent has been obtained. The informed consent form is considered to be part of the protocol, and must be submitted by the investigator with the protocol at the time of IRB review.

4.4 Compliance with Laws and Regulations

The study will be conducted in accordance with U.S. Food and Drug Administration (FDA) and International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice (GCP), the Declaration of Helsinki, any applicable local health authority, and Institutional Review Board (IRB) requirements. The PI or designee will be responsible for obtaining continuing and not less than annual IRB re-approval throughout the duration of the study. Copies of the Investigator's annual report to the IRB and copies of the IRB continuance of approval must maintained by the MCC CRDM SRF. The PI or designee is also responsible for notifying the Data and Safety Monitoring Committee of the MCCC and the UK IRB of any significant adverse events that are serious and/or unexpected, as per SOP's of those entities. The MCC DSMC will review all

adverse events of this IIT as per its SOP.

5. TREATMENT PLAN

5.1 Enrollment and Screening Process

Prior to any study-required tests, subjects must first provide written informed consent to participate in this study. All lab tests and radiographic studies should be completed within 4 weeks prior to initiation of treatment. Complete history, physical examination, and evaluation of Performance Status. Required lab work will include: complete blood count (CBC) with differential; serum chemistry tests to include alkaline phosphatase, glucose, creatinine, electrolytes, AST (SGOT), and total bilirubin.

5.2 Agent Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Dose Schedule	
Dose Level	**Dose**
	Hydroxychloroquine (mg/day)
Level -1	200 mg/day
STARTING DOSE: Level 1	400 mg/day
Level 2	800 mg/day
Level 3	1200 mg/day

Subjects will receive HCQ every day for 14 days, starting at least 14d before planned surgery and optimally ending day prior to surgery. Dosing can be extended 7 days if necessary due to unexpected delays. HCQ can be taken at any time of the day with meals. Tablets of HCQ are available in 200 mg strengths. HCQ will be administered in divided doses (twice a day) for doses above 200 mg/day to minimize nausea. The divided doses should be taken in the AM and at night with meals. Subjects will also be required to keep a medication diary and to present this at the end of the treatment. Should a subject have emesis and regurgitate the medication within 30 minutes of taking it, the dose may be repeated once, but if vomiting occurs longer than 30 minutes after ingestion, the dose should not be repeated. Subjects taking antacids, proton-pump inhibitors or H2-blockers should not take hydroxychloroquine within 4 hours of these medicines.

5.3 See Statistical Considerations for description of Dose-Escalation Process

An adaptive, nonparametric, isotonic regression model will be employed in order to determine the biological effect of HCQ as well as to assess safety. Patients will be enrolled starting at dose level 1. Dose escalation and de-escalation is described in detail in the Statistical Considerations section below. Briefly,

1. Treat a cohort of 3 patients at dose level 1
2. Let j^t denote the highest level tried thus far. At the current dose level j, based on the observed data, determine the dose j* that has the highest isotonic estimate of biological effect probability among the tried doses.
3. If $j^* > j$, escalate to dose j+1
4. If $j^* < j$, de-escalate to dose j-1
5. If $j^* = j < j^t$, stay at dose level j; otherwise if $j^* = j = j^t$, escalate to dose j+1

Once the maximum sample size is reached (n = 18), select the lowest dose that has the highest estimate of biological effect based on PAR-4 levels.

5.4 Definition of Dose-Limiting Toxicity

Subjects will be evaluated for toxicity according to the Common Terminology Criteria for Adverse Events (CTCAE) of the National Cancer Institute (NCI) version 4.0 available at: www.ctep.gov. A DLT is defined as any of the below treatment emergent toxicities with attribution (possibly, probably or definitely related) to the study medication that occurs during or within 30 days of the last dose of hydroxychloroquine.

Non-Hematologic:

- ≥ Grade 3 or 4 neuropathy
- ≥ Any Grade 3 or 4 adverse event (excluding grade 3 nausea, vomiting, diarrhea lasting < 7 days or grade 3 fatigue). Note: Abnormal non-hematologic laboratory values > grade 3 will be considered a DLT if they are determined to be clinically significant and possibly, probably or definitely related to study drug. If baseline value is elevated (or decreased) prior to enrollment, an increase (or decrease) will not be considered a DLT unless it worsens by 2 grades and is determined to be clinically significant by the treating investigator.
- ≥ Grade 3 nausea, vomiting, or diarrhea lasting > 7 days despite maximal antiemetic/antidiarrheal therapy
- Grade 5 toxicity

Hematologic:

- Grade 4 neutropenia (ANC < 0.5 x 109/L) lasting for ≥ 7 days
- Febrile neutropenia (ANC < 1.0 x 109/L with a fever ≥ 38.3ºC)
- Grade 4 thrombocytopenia (platelets < 25.0 x 109/L) lasting ≥ 7 days despite dose delay
- Grade 3-4 thrombocytopenia associated with bleeding
- Grade 5 toxicity

Management and dose modifications associated with the above adverse events are outlined in Section 6.

5.5 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of hydroxychloroquine with other concomitantly administered drugs through the cytochrome P450 system, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential to affect selected CYP450 isoenzymes. Appendix B presents guidelines for identifying medications/substances that could potentially interact with the hydroxychloroquine. While not strictly prohibited, these medications should be avoided if at all possible. Hydroxychloroquine should also be used with caution in patients taking medicines which may cause adverse ocular or skin reactions.

5.6 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment will continue for 14 days or until one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

5.7 Duration of Follow Up

Patients will be followed for 30 days after surgery or 30 days after completion of the last dose of hydroxychloroquine if no surgery is completed. Patients removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event. Follow-up visit should be combined with subject standard of care post-operative visit. If that visit occurs prior to the 30 days post op, subject should be contacted 30 days or after completion of the last dose for adverse event assessment.

5.8 Criteria for Removal from Study

Patients will be removed from study when any of the criteria listed in Section 5.6 applies. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

6. DOSING DELAYS/DOSE MODIFICATIONS

The following dose levels will be considered:

Dose Schedule	
Dose Level	**Dose**
	Hydroxychloroquine (mg/day)
Level -1	200 mg/day
STARTING DOSE: Level 1	400 mg/day
Level 2	800 mg/day
Level 3	1200 mg/day

At the discretion of the PI and investigative team, additional dose levels may be considered, if the primary endpoint cannot adequately be determined by the dose levels listed above.

6.1 Study Specified Dose Modifications for Non-Hematologic Toxicities

Nausea and/or Vomiting	**Management/Next Dose for Hydroxychloroquine**
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3 or 4	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**
*Patients requiring a delay of >2 weeks should go off protocol therapy. **Patients requiring > two dose reductions should go off protocol therapy.	
Recommended management: antiemetics.	

Diarrhea	**Management/Next Dose for Hydroxychloroquine**
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3 or 4	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**
*Patients requiring a delay of >2 weeks should go off protocol therapy. **Patients requiring > two dose reductions should go off protocol therapy.	
Recommended management: Loperamide antidiarrheal therapy Dosage schedule: 4 mg at first onset, followed by 2 mg with each loose motion until diarrhea-free for 12 hours (maximum dosage: 16 mg/24 hours)	

Diarrhea	**Management/Next Dose for Hydroxychloroquine**
Adjunct anti-diarrheal therapy is permitted and should be recorded when used.	

Visual Changes	**Management/Next Dose for Hydroxychloroquine**
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3 or 4	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**
*Patients requiring a delay of >2 weeks should go off protocol therapy. **Patients requiring > two dose reductions should go off protocol therapy.	
The methods recommended for early diagnosis of "chloroquine retinopathy" consist of (1) fundoscopic examination of the macula for fine pigmentary disturbances or loss of the foveal reflex and (2) examination of the central visual field with a small red test object for pericentral or paracentral scotoma or determination of retinal thresholds to red.	

Pustular or Bullous Dermatitis	**Management/Next Dose for Hydroxychloroquine**
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3 or 4	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**
*Patients requiring a delay of >2 weeks should go off protocol therapy. **Patients requiring > two dose reductions should go off protocol therapy.	

6.2 Study Specified Dose Modifications for Hematologic Toxicities

Neutropenia	**Management/Next Dose for Hydroxychloroquine**
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3 or 4	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**
Grade 4	Off protocol therapy
*Patients requiring a delay of >2 weeks should go off protocol therapy. **Patients requiring > two dose reductions should go off protocol therapy.	

Thrombocytopenia	**Management/Next Dose for Hydroxychloroquine**
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3 or 4	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**
*Patients requiring a delay of >2 weeks should go off protocol therapy. **Patients requiring > two dose reductions should go off protocol therapy.	

All other grade 3 or 4 toxicities should be discussed with the PI.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting (via Medwatch Forms) **in addition** to routine reporting.

7.1 Expected Toxicities for Hydroxychloroquine

7.2 Precautions

Dermatologic reactions to hydroxychloroquine sulfate tablets may occur and, therefore, proper care should be exercised when they were administered to any patient receiving a drug with a significant tendency to produce dermatitis.

The methods recommended for early diagnosis of "chloroquine retinopathy" consist of (1) fundoscopic examination of the macula for fine pigmentary disturbances or loss of the foveal reflex and (2) examination of the central visual field with a small red test object for pericentral or paracentral scotoma or determination of retinal thresholds to red. Any unexplained visual symptoms, such as light flashes or streaks should also be regarded with suspicion as possible manifestations of retinopathy.

If serious toxic symptoms occur from overdosage or sensitivity, it has been suggested that ammonium chloride (8 g daily in divided doses for adults) be administered orally three or four days a week for several months after therapy has been stopped, as acidification of the urine increases renal excretion of the 4-aminoquinoline compounds by 20 to 90 percent. However, caution must be exercised in patients with impaired renal function and/or metabolic acidosis.

7.3 Adverse Reactions

The following Council for International Organizations of Medical Sciences (CIOMS) frequency rating is used, when applicable: Very common ≥ 10 %; Common ≥ 1 and <10 %; Uncommon ≥ 0.1 and < 1 %; Rare ≥ 0.01 and <0.1 %; Very rare < 0.01 %; Not known (frequency cannot be estimated from available data).

Blood and lymphatic system disorders
Not known: Bone marrow depression, anemia, aplastic anemia, agranulocytosis, leucopenia, thrombocytopenia.

Cardiac disorders
Not known: Cardiomyopathy, which may result in cardiac failure and in some cases a fatal outcome.
Chronic toxicity should be considered when conduction disorders (bundle branch block/ atrioventricular heart block) as well as biventricular hypertrophy are found. Drug discontinuation may lead to recovery.

Ear and labyrinth disorders
Uncommon: Vertigo, tinnitus
Not known: Hearing loss including cases of irreversible hearing loss.

Eye disorders
Common: Blurring of vision due to a disturbance of accommodation which is dose dependent and reversible.
Uncommon: Maculopathies which may be irreversible.
Retinopathy with changes in pigmentation and visual field defects. In its early form it appears reversible upon discontinuation of the drug. If allowed to develop however, there may be a risk of progression even after treatment withdrawal.
Patients with retinal changes may be asymptomatic initially, or may have scotomatous vision with paracentral, pericentral ring types, temporal scotomas, abnormal color visions, reduction in visual acuity, night blindness, difficulty reading and skipping words.
Corneal changes including edema and opacities. They are either symptomless or may cause disturbances such as halos around lights especially at night, blurring of vision or photophobia. They may be transient or are reversible upon discontinuation of therapy.
Not known: Macular degeneration which may be irreversible.

Gastrointestinal disorders
Very common: Abdominal pain, nausea
Common: Diarrhea, vomiting
These symptoms usually resolve immediately upon reducing the dose or upon stopping the treatment.

Hepatobiliary disorders
Uncommon: Abnormal liver function tests
Not known: Fulminant hepatic failure

Immune system disorders
Not known: Urticaria, angioedema, bronchospasm.

Metabolism and nutrition disorders
Common: Anorexia (usually resolves immediately upon reducing the dose or upon stopping the treatment).
Not known: hypoglycemia
HYDROXYCHLOROQUINE may exacerbate porphyria.

Musculoskeletal and connective tissue disorders
Uncommon: Sensory motor disorders
Not known: Skeletal muscle palsies or skeletal muscle myopathy or neuromyopathy leading to progressive weakness and atrophy of proximal muscle groups. Depression of tendon reflexes, abnormal results of nerve conduction tests. Myopathy may be reversible after drug discontinuation, but recovery may take many months.

Nervous system disorders
Common: Headache
Uncommon: Dizziness

Not known: Convulsions

Psychiatric disorders
Common: Affect lability
Uncommon: Nervousness
Not known: Psychosis, suicidal behaviour

Skin and subcutaneous tissue disorders
Common: Skin rash, pruritus
Uncommon: Pigmentary changes in skin and mucous membranes, bleaching of hair, alopecia. These usually resolve readily upon cessation of therapy.
Not known: Bullous eruptions (including urticarial, morbilliform, lichenoid, maculopapular, purpuric, erythema annulare centrifugum), toxic epidermal necrolysis, erythema multiforme, Stevens-Johnson syndrome, Drug Rash with Eosinophilia and Systemic Symptoms (DRESS syndrome), photosensitivity, exfoliative dermatitis, acute generalized exanthematous pustulosis (AGEP).
AGEP has to be distinguished from psoriasis, although HYDROXYCHLOROQUINE may precipitate attacks of psoriasis. It may be associated with fever and hyperleukocytosis. Outcome is usually favorable after discontinuation of drug.

For a comprehensive list of adverse events please refer to the package insert.

7.4 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent(s) that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.
  - Other AEs for the protocol that do not require expedited reporting are outlined in the next section (Expedited Adverse Event Reporting) under the sub-heading of Protocol-Specific Expedited Adverse Event Reporting Exclusions.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.5 Expedited Adverse Event Reporting

7.5.1 For MCC Investigator-Initiated Trials (IITs), investigators **must** report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form. This applies to the following categories:

- **Grade 3 (severe) *Medical* Events** – Only events that are Unexpected and Possibly, Probably or Definitely Related / Associated with the Intervention.
- **ALL Grade 4 (life threatening or disabling) *Medical* Events** – Unless expected AND specifically listed in protocol as not requiring reporting.
- **ALL Grade 5 (fatal) Events** regardless of study phase or attribution

Note: If subject is in Long Term Follow Up, death is reported at continuing review.
Note: Abnormal laboratory values are not considered medical events, unless determined to be causative of SAE by the investigator or grade 5.

7.5.2 The following table outlines the required forms and reporting structure for clinical trials.

Study type	Expedited reporting to MCC	Expedited reporting to External Agency	Non-expedited AE	Form	IRB
IIT where MCC investigator holds the IDE or IND	• Grade 3 – Unexpected AE PLUS Possibly, Probably or Definitely Related • ALL Grade 4 Unless expected AND listed in protocol as not requiring reporting. • ALL Grade 5 (fatal) Events	FDA: Suspected AE that is serious and Unanticipated (not listed in IDB or consent)	OnCore and DSMC reporting only	Mandatory Medwatch 3500a for Serious and unanticipated OnCore for all AEs, including SAEs	Yes if it meets the IRB reporting requirements: Unanticipated Problem and/or Serious AE (use IRB AE reporting form for all correspondence with IRB)
IIT by MCC investigator of commercially available agent (non-IND and non-IDE)	• Grade 3 – Unexpected AE PLUS Possibly, Probably or Definitely Related • ALL Grade 4 Unless expected AND listed in protocol as not requiring reporting.	FDA: Suspected AE that is serious and Unanticipated (not listed in IDB or consent)	OnCore and DSMC reporting only	Voluntary Medwatch 3500 for Serious and unanticipated OnCore for all AEs, including SAEs	

	• ALL Grade 5 (fatal) Events				

7.5.3 MCC Expedited Reporting Guidelines for MCC IITs

Investigators within MCC will report SAEs directly to the MCC DSMC per the MCC DSMC SOP and the University of Kentucky IRB reporting policy.

Attribution	MCC Reportable AEs				
	Gr. 2 & 3 AE Expected	**Gr. 2 & 3 AE Unexpected**	**Gr. 4 AE Expected**	**Gr. 4 AE Unexpected**	**Gr. 5 AE Expected or Unexpected**
Unrelated Unlikely	Not required	Not required	5 calendar days#	5 calendar days	24 hours*
Possible Probable Definite	Not required	5 calendar days	5 calendar days#	5 calendar days	24 hours*
# If listed in protocol as expected and not requiring expedited reporting, event does not need to be reported.					
* For participants enrolled and actively participating in the study ***or*** for AEs occurring within 30 days of the last intervention, the AE should be reported within 24 business hours of learning of the event.					

Other investigative sites will report SAEs to their respective IRB according to the local IRB's policies and procedures in reporting adverse events. A copy of the submitted institutional SAE form should be forwarded to the Overall PI within the timeframes detailed in the table above.

The Overall PI will submit SAE reports from outside institutions to the MCC DSMC and UK IRB according to IRB policies and procedures in reporting adverse events: http://www.research.uky.edu/ori/SOPs_Policies/C2-0350-Unanticipated_Problems_Adverse_Events_SOP.pdf.

7.6 Expedited Reporting to External Agencies

The Overall PI will comply with the policies of all external funding agencies and the UK IRB regarding expedited reporting, as per the UK IRB's SOP: http://www.research.uky.edu/ori/SOPs_Policies/C4-0150-Mandated_Reporting_to_External_Agencies_SOP.pdf.

7.6.1 Expedited Reporting to the Food and Drug Administration (FDA)

Include this section and text for investigator-held IDE and IND studies, including gene transfer. If this is not an investigator-held IDE or IND study, this section and text should be deleted.

The Overall PI, as study sponsor, will be responsible for all communications with the FDA. The Overall PI will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

7.6.2 **Expedited Reporting to Hospital Risk Management**

Participating investigators will report to the UK Office of Risk Management any participant safety reports or sentinel events that require reporting according to institutional policy.

7.7 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions to the Overall PI on the OnCore case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must <u>also</u> be reported in routine study data submissions.**

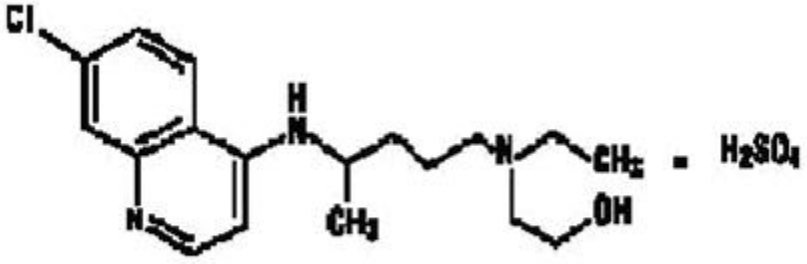
8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in Section 7.1.

8.1 Commercial Agent: Hydroxychloroquine.

8.2 Description

Hydroxychloroquine sulfate, USP is a colorless crystalline solid, soluble in water to at least 20 percent; chemically the drug is 2-[[4-[(7-Chloro-4-quinolyl) amino]pentyl] ethylamino] ethanol sulfate (1:1). Hydroxychloroquine sulfate, USP has the following structural formula:



Molecular Formula: C18H26ClN3O.H2SO4
Molecular Weight of 433.95

Each tablet, for oral administration, contains 200 mg hydroxychloroquine sulfate, USP (equivalent to 155 mg base). In addition, each tablet contains the following Inactive Ingredients: colloidal silicon dioxide, dibasic calcium phosphate, hypromellose, macrogol/PEG 3350, magnesium stearate, polysorbate 80, pregelatinized starch, talc, and titanium dioxide.

Hydroxychloroquine sulfate tablets, USP are white, to off-white, capsule-shaped tablets, debossed with "HCQS" on one side and plain on the reverse side and are available in bottles of 10, 100, 500 and 1000.

NDC 63304-296-03	Bottles of 10
NDC 63304-296-01	Bottles of 100
NDC 63304-296-05	Bottles of 500
NDC 63304-296-10	Bottles of 1000

8.3 Storage requirements
Dispense in a tight, light-resistant container as defined in the USP/NF.
Store at 20° - 25°C (68° - 77°F) excursions permitted to 15° - 30°C (59° - 86°F) [See USP Controlled Room Temperature].

Store out of the reach of children.

8.4 Stability:
When stored as listed above and light-resistant containers, hydroxychloroquine is stable for 3 years from manufacture date.

8.5 Route of administration:
Hydroxychloroquine is taken orally daily, each dose to be taken with a meal or a glass of milk.

8.6 CONTRAINDICATIONS
Use of this drug is contraindicated (1) in the presence of retinal or visual field changes attributable to any 4-aminoquinoline compound, (2) in patients with known hypersensitivity to 4-aminoquinoline compounds, and (3) for long-term therapy in children.

8.7 Precautions: General
Antimalarial compounds should be used with caution in patients with hepatic disease or alcoholism or in conjunction with known hepatotoxic drugs. Periodic blood cell counts should be made if patients are given prolonged therapy. If any severe blood disorder appears which is not attributable to the disease under treatment, discontinuation of the drug should be considered. The drug should be administered with caution in patients having G-6-PD (glucose-6-phosphate dehydrogenase) deficiency.

8.8 Overdose
Overdose with the 4-aminoquinolines is dangerous particularly in infants, as little as 1-2 grams having proved fatal.

8.9 Symptoms of Overdose:
The 4-aminoquinoline compounds are very rapidly and completely absorbed following ingestion and in accidental overdosage toxic symptoms may occur within 30 minutes. These consist of headache, drowsiness, visual disturbances, cardiovascular collapse, hypokalemia and convulsions, rhythm and conduction disorders, including QT prolongation, torsade de pointes, ventricular tachycardia and ventricular fibrillation, followed by sudden potentially fatal respiratory and cardiac arrest. Immediate medical attention is required, as these effects may appear shortly after overdose. The ECG may reveal atrial standstill, nodal rhythm, prolonged intraventricular conduction time, and progressive bradycardia leading to ventricular fibrillation and/or arrest.

8.10 Treatment of Overdose:

Treatment is symptomatic and must be prompt with immediate evacuation of the stomach by emesis (at home, before transportation to the hospital), or gastric lavage until the stomach is completely emptied. If finely powdered activated charcoal is introduced by the stomach tube, after lavage and within 30 minutes after ingestion of the tablets, it may inhibit further intestinal absorption of the drug. To be effective, the dose of activated charcoal should be at least five times the estimated dose of ingested hydroxychloroquine. Convulsions, if present, should be controlled before attempting gastric lavage. If due to cerebral stimulation, cautious administration of an ultrashort-acting barbiturate may be tried but, if due to anoxia, convulsions should be corrected by oxygen administration, artificial respiration or, in shock with hypotension, by vasopressor therapy. Because of the importance of supporting respiration, tracheal intubation or tracheostomy, followed by gastric lavage, has also been advised. Exchange transfusions have been used to reduce the level of 4-aminoquiolines in the blood. Consideration should be given to administering diazepam parenterally since studies have reported it beneficial in reversing chloroquine cardiotoxicity.

A patient who survives the acute phase and is asymptomatic should be closely observed for at least 6 hours. Fluids may be forced, and sufficient ammonium chloride may be administered for a few days to acidify the urine to help promote urinary excretion.

If serious toxic symptoms occur from overdosage or sensitivity, it has been suggested that ammonium chloride (8 g daily in divided doses for adults) three or four days a week be 11 administered for several months after therapy has been stopped, as acidification of the urine increases renal excretion of the 4-aminoquinoline compounds by 20 to 90 percent. However, caution must be exercised in patients with impaired renal function and/or metabolic acidosis.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Laboratory Correlative Studies

The plasma concentrations of PAR-4 will be measured to assess induction of PAR-4 in each patient.
The plasma concentration of hydroxychloroquine and its metabolites will also be measured to establish exposure – response (PAR-4 release) relationships. The extra aliquots of plasma after above studies will be stored in −80°C for future biomarker studies if needed.

<u>Blood collection for PAR-4</u>

<u>Collection of Specimens</u>

Blood plasma samples will be collected pre-dosing, and weekly during the preoperative period (+/- 1 day), as well as on or after surgery (+/- 1 day). A total of 8 time points will be required for each patient. 4 ml of venous blood will be withdrawn for pharmacokinetics; 4 ml of venous blood will be withdrawn for Par-4 and biomarker studies. Samples will be withdrawn into

sodium heparinized collection tube.

Handling of Specimens

After collection, blood and anti-coagulant will be mixed by inverting the tube 8–10 times. Blood samples will be placed on ice immediately and centrifuged within 30 min at 7200 g at 4°C for 2 min. Plasma will be transferred into amber plastic tubes and stored on dry ice prior to transferring at −80°C until analysis. Plasma concentrations of PAR-4 will be quantified using ELISA (according to manufacturer's instruction with a kit from CUSABIO). Moreover, Western blot analysis will be performed as described previously[2,12] to further confirm the quantitative increase in the ~40 kDa PAR-4 band. Aliquots of plasma samples will be subjected to SDS-PAGE to resolve the plasma proteins. The proteins will be transferred onto nylon membranes, and then subjected to Western blot analysis. The blots will be probed with PAR-4 polyclonal antibody (from SantaCruz Biotechnology, Inc.) Parallel SDS-PAGE and Coomassie blue staining of the albumin in the serum samples will be used as loading control [2,6].

Blood Collection and Handling of Specimens for Pharmacokinetics

Blood samples for pharmacokinetic analysis will be collected prior to the start of HCQ regimen and during day 1 of weeks 1 and 2 and on/after day or surgery (+/- 1 day), as specified in the Study Calendar. Blood samples (4 mL) will be collected in heparinized tubes. After collection, blood and anti-coagulant will be mixed by inverting the tube 8–10 times and samples will be placed on ice immediately. Further processing will be carried out in the bioanalytical lab by staff in the Center of Pharmaceutical Research and Innovation (CPRI) under the direction of Dr. Leggas. Concentrations of HCQ and metabolites will be quantified using a validated bioanalytical assay implemented for LC/MS/MS.

Shipping of Specimens for Pharmacokinetics

Samples will be collected by study personnel and will be forwarded to the CPRI for storage, processing and analysis.

Tumor Collection for Pharmacodynamics

If available, Diagnostic biopsies and paired resected tumor specimen will be store in formalin-fixed, paraffin-embedded blocks for potential autophagy and apoptosis analysis in the future. Each subject will sign the consent form for tissue collection before enrolled in the study.

Shipping of Specimens for Pharmacodynamics

Samples will be collected by study personnel in Dr. Rangnekar's lab for storage, processing and analysis. For PK/Par-4 sample pick up contact Sogol Kangarlou at 859-361-9015.

Site(s) Performing Correlative Study

University of Kentucky.

STUDY CALENDAR

Baseline evaluations are to be conducted within 2-4 weeks prior to start of protocol therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated more frequently, as clinically indicated. Study drug administration should begin optimally 2 weeks prior to date of surgery and continue until day prior to surgery. Dosing can be extended one week if necessary for any unanticipated delays.

Studies to be obtained	Pre-Study	Week 1 (D7)	Week 2 (D14)	Day of Surgery	Post-operative visit	Off study
History	X	X	X		X	X
Physical Exam with vital signs	X	X	X		X	
Height, Weight	X					
Performance Status	X					
CBC, differential, platelets	X	X	X		X	
CMP	X	X	X		X	
Pregnancy test (only females of childbearing potential)	X					
Tumor collection for Correlative Studies (if available)	X			X		
Correlative Blood work*	X	X	X		X	

Correlative Par-4, and Hydroxychloroquine levels will be as follows:

Date	**Lab Draw**	**Tubes**
Pre-study	once	Par-4 (1 tube) Hydroxychloroquine (1 tube)
Week 1 (day 7)	3 samples (2 hours apart +/- 15 minutes)	Par-4 (1 tube) HCQ (3 tubes total- two hours apart)
Week 2 (day 14)	3 samples (2 hours apart +/- 15 minutes)	Par-4 (1 tube) HCQ (3 tubes total- two hours apart)
Post-operative clinic visit	once	Par-4 (1 tube), HCQ (1 tube)

10. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

10.1 Data Reporting

10.1.1 Method

This study will require data submission and reporting via the OnCore Database, which is the official database of the Markey Cancer Center Clinical Research and Data Management Shared Resource Facility (CRDM SRF). Instructions for submitting data is listed in Study-Specific Data Management Plans created by CRDM SRF staff.

10.1.2 Responsibility for Data Submission

Study staff is responsible for submitting study data and/or data forms to OnCore as per the Markey Cancer Center CRDM SRF SOP's. This trial will be monitored by the MCC Data and Safety Monitoring Committee (DSMC) on a schedule determined by the Protocol Review and Monitoring Committee at the initial PRMC review. The CRDM SRF staff is responsible for compiling and submitting data for all participants and for providing the data to the Principal Investigator for review.

11. STATISTICAL CONSIDERATIONS

11.1 Study Design/Endpoints

Based on previous work in renal cell carcinoma (Figure 1, Burikhanov, Rangnekar et al., unpublished data), there is on average a 2-fold increase in PAR-4 levels from pre to post plasma samples as measured by ELISA and Western Blot. We are interested in determining the dose wherein 70% of patients exhibit a 2-fold increase in PAR-4 levels and with an acceptable toxicity rate of no more than 30%. For calculation of biological effect, we will utilize changes in PAR-4 levels from pre-study and the last time point prior to resection.

Given the established safety and minimal toxicity of HCQ in several studies[13,14] our choice of study design in this pilot trial will be based on determining the dose with the target biological effect as well as acceptable toxicity. Specifically, a nonparametric approach based on a double-sided isotonic regression will be utilized which can also accommodate non-monotonically increasing biological effect rates (Zang, Y., Lee, J. and Yuan, Y. (2014) Adaptive Designs for Identifiying Optimal Biological Dose for Molecularly Targeted Agents, *Clinical Trials*, To appear). Simulations were generated for varying scenarios of biological effect rates and dose toxicity rates across dose levels to determine the operating characteristics of this design (see Table 1). Briefly, the first four scenarios assuming low toxicity indicate close to a 50% selection probability for the correct dose with the target biological rate while the next four scenarios

assuming moderate toxicity also indicate at least a 50% selection probability for the correct dose with the target biological rate. Finally, the last two scenarios indicate good selection probability for the dose with the highest biological rate that is closest to the target rate of 70%. Furthermore, all scenarios in Table 1 indicate that there is a clear winner (i.e. a much higher selection probability for the correct dose compared to the probabilities for the other dose levels). Finally, the simulations also confirmed that more patients will be allocated to the correct dose.

The study will accrue a total of 18 patients with 3 cohorts per dose level. Estimates of biological effects as described above will be calculated after every cohort of 3 patients and recommendations for the next cohort will be based on these estimates. Specifically, the study will proceed as follows:

1. Treat a cohort of 3 patients at dose level 1
2. Let j^t denote the highest level tried thus far. At the current dose level j, based on the observed data, determine the dose j* that has the highest isotonic estimate of biological effect probability among the tried doses.
3. If $j^* > j$, we escalate to dose j+1
4. If $j^* < j$, we de-escalate to dose j-1
5. If $j^* = j < j^t$, we stay at dose level j; otherwise if $j^* = j = j^t$, we escalate to dose j+1

6. Once the maximum sample size is reached, we select the lowest dose that has the highest estimate of biological effect based on PAR-4 levels. Decisions on the dose that will be utilized for subsequent trials will be based on biological, clinical and statistical considerations.

Table 1. Selection Probabilities for Varying Scenarios of Biological Effect and Toxicity Rates

Scenario 1	Dose -1	Dose 1	Dose 2	Dose 3
Biological rates	0.40	0.70	0.60	0.50
Toxicity rates	0.02	0.05	0.10	0.20
Selection Probabilities	8.9	53.3	28.5	9.3
Scenario 2	Dose -1	Dose 1	Dose 2	Dose 3
Biological rates	0.40	0.60	0.75	0.55
Toxicity rates	0.02	0.05	0.10	0.20
Selection Probabilities	15.5	24.0	49.1	11.4
Scenario 3	Dose -1	Dose 1	Dose 2	Dose 3
Biological rates	0.70	0.50	0.30	0.20
Toxicity rates	0.02	0.05	0.10	0.20
Selection Probabilities	69.8	24.4	5.2	0.6
Scenario 4	Dose -1	Dose 1	Dose 2	Dose 3

Biological rates	0.20	0.50	0.75	0.70
Toxicity rates	0.02	0.05	0.10	0.20
Selection Probabilities	9.8	13.7	46.9	29.6

Scenario 5	Dose -1	Dose 1	Dose 2	Dose 3
Biological rates	0.20	0.50	0.70	0.70
Toxicity rates	0.05	0.10	0.20	0.40
Selection Probabilities	9.9	22.2	50.7	17.2

Scenario 6	Dose -1	Dose 1	Dose 2	Dose 3
Biological rates	0.40	0.70	0.60	0.50
Toxicity rates	0.05	0.10	0.20	0.40
Selection Probabilities	9.9	55.1	30.8	4.2

Scenario 7	Dose -1	Dose 1	Dose 2	Dose 3
Biological rates	0.40	0.60	0.75	0.55
Toxicity rates	0.05	0.10	0.20	0.40
Selection Probabilities	15.7	27.2	50.9	6.2

Scenario 8	Dose -1	Dose 1	Dose 2	Dose 3
Biological rates	0.70	0.50	0.30	0.20
Toxicity rates	0.05	0.10	0.20	0.40
Selection Probabilities	71.2	23.6	4.5	0.7

Scenario 9	Dose -1	Dose 1	Dose 2	Dose 3
Biological rates	0.20	0.60	0.50	0.40
Toxicity rates	0.02	0.05	0.10	0.20
Selection Probabilities	4.6	53.6	31.4	10.4

Scenario 10	Dose -1	Dose 1	Dose 2	Dose 3
Biological rates	0.20	0.30	0.60	0.50
Toxicity rates	0.05	0.10	0.20	0.40
Selection Probabilities	20.0	16.2	52.6	11.2

11.2 Sample Size/Accrual Rate

A total of 18 patients will be enrolled in cohorts of 3 and dose escalation or de-escalation will be implemented as described in section 12.1 above. We expect to enroll 1 patient per month for expected study duration of 18 months.

11.3 Assessment of Safety

The isotonic regression design as described in 12.1 incorporates toxicity assessment. The dose with the highest selection probability accounts for both the dose with the highest biological effect and toxicity that is closest to the target rate of 30%.

11.4 Stratification Factors

Not applicable for this trial

11.5 Analysis of Safety

All patients who received study drug will be included in the safety analysis of this study. Adverse event data and corresponding toxicity grades during the days of treatment will be summarized in each tumor cohort and in the overall patient population. Incidence tables will be generated to summarize incidence of patients reporting at least one episode of each specific adverse event, incidence of adverse events causing withdrawal and incidence of serious adverse events. The total number of episodes for each event reported (Frequency Table), the severity and attribution to study therapy of each episode reported (Severity Table and Attribution Table) will also be displayed.

Listings of adverse events by patients will include the time to onset, the duration of each event, the severity of each event, and the relationship of the event to study therapy, whether it was a serious event, and whether it caused withdrawal. Safety data will be summarized for the overall patient group and by tumor cohort. Toxicities will be graded according to Common Toxicity Criteria (CTCAE) v4.0.

11.6 Pharmacokinetics Modeling and Pharmacodynamics Analysis

For assessment of biological effect, we will utilize PAR-4 levels from pre-treatment and PAR-4 from the last time point prior to resection. Changes in quantitative levels of PAR-4 measured via ELISA and Western Blot will be calculated and the proportion of patients exhibiting at least a 2-fold induction will be estimated overall and in each dose level along with exact 95% binomial confidence intervals. Isotonic regression will be fitted for the total sample to obtain estimates of biological effect at each dose level. Other exploratory analyses will include: paired tests to evaluate changes in PAR-4 quantitative levels from baseline and at each time point of measurement; correlations between several biological markers indicated in Section 9.1 for Correlative Studies will be estimated using Pearson or Spearman's correlation coefficient; repeated measures modeling of several biomarker measurements over time; and descriptive statistics of biomarker levels for each disease group (if there are enough in each disease site). Similar analyses plans will be employed for biomarker assessments in tissue specimens.

Population pharmacokinetic studies have previously been conducted [15]. This will allow the use of population parameters to be used as priors and thus enable forecasting the HCQ exposure for each patient, using a Bayesian approach, despite the limited sampling strategy [16, 17]. Data analysis will be carried out with ADAPT V [18].

11.7 Data Management

The study statistician and staff from the Biostatistics Shared Resource Facility (BSRF) of the Markey Cancer Center will work closely with the study PI and the Clinical Protocol and and Data Management (CPDM SRF) at Markey in the development of eCRFs for the study.

Specifically, the statisticians will attend several meetings including the Protocol Initiation Meeting (PIM) to address all statistical considerations for this protocol including incorporation of dose-escalation and de-escalation plans for the adaptive design in this Phase I trial, appropriate and accurate collection of primary and secondary study endpoints and inclusion of valid values and range checks for data fields. The OnCore clinical trial management system, managed by Markey's CPDM, will be the primary database repository of clinical data from all patients enrolled into this trial. Data will be accessed by the study statistical on a regularly-scheduled basis to perform statistical programming for conduct of data quality control, data management, generation of interim reports and statistical analysis. The BSRF has developed an automated mechanism for generating a trigger for assessment of biological response as well as dose limiting toxicities based on the dose escalation rules in section 12.1above. In collaboration with the study team, procedures will be developed for timelines for data quality control, resolution of data queries, interim reporting and final data analysis.

APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B INFORMATION ON POSSIBLE DRUG INTERACTIONS

A table with potential drug interaction with HYDROXYCHLOROQUINE is included below. HYDROXYCHLOROQUINE should also be used with caution in patients taking medicines which may cause adverse ocular or skin reactions.

Drug Interaction:

Proper Name	Effect/clinical comment
Agalsidase	There is a theoretical risk of inhibition of intra-cellular α-galactosidase activity when HYDROXYCHLOROQUINE is co-administered with agalsidase.
Aminoglycoside antibiotics	HYDROXYCHLOROQUINE may also be subject to several of the known interactions of chloroquine even though specific reports have not appeared including potentiation of its direct blocking action at the neuromuscular junction by aminoglycoside antibiotics.
Amiodarone	There may be an increased risk of inducing ventricular arrhythmias if HYDROXYCHLOROQUINE is used concomitantly with other arrhythmogenic drugs.
Antacids	As with chloroquine, antacids may reduce absorption of HYDROXYCHLOROQUINE so it is advised that a 4 hour interval be observed between HYDROXYCHLOROQUINE and antacid dosing.
Anti-diabetic drugs	May enhance the effects of a hypoglycemic treatment, a decrease in doses of antidiabetic drugs may be required.
Antiepileptic drugs	The activity of antiepileptic drugs might be impaired if co-administered with HYDROXYCHLOROQUINE.
Antimalarials known to lower the convulsion threshold	HYDROXYCHLOROQUINE can lower the convulsive threshold. Co-administration of HYDROXYCHLOROQUINE with other antimalarials known to lower the convulsion threshold (e.g. mefloquine) may increase the risk of convulsions.
Arrhythmogenic drugs	There may be an increased risk of inducing ventricular arrhythmias if HYDROXYCHLOROQUINE is used concomitantly with other arrhythmogenic drugs.
Cyclosporine	An increased plasma ciclosporin level was reported when ciclosporin and HYDROXYCHLOROQUINE were co-administered.
Cimetidine	HYDROXYCHLOROQUINE may also be subject to several of the known interactions of chloroquine even though specific reports have not appeared including inhibition of its metabolism by cimetidine which may increase plasma concentration of the antimalarial.

Digoxin	May result in increased serum digoxin levels; serum digoxin levels should be closely monitored in patients receiving concomitant treatment.
Insulin	May enhance the effects of a hypoglycemic treatment, a decrease in doses of insulin may be required.
Mefloquine	HYDROXYCHLOROQUINE can lower the convulsive threshold. Co-administration of HYDROXYCHLOROQUINE with other antimalarials known to lower the convulsion threshold (e.g. mefloquine) may increase the risk of convulsions.
Moxifloxacin	There may be an increased risk of inducing ventricular arrhythmias if HYDROXYCHLOROQUINE is used concomitantly with other arrhythmogenic drugs.
Neostigmine	HYDROXYCHLOROQUINE may also be subject to several of the known interactions of chloroquine even though specific reports have not appeared including antagonism of effect of neostigmine.
Praziquantel	Chloroquine has been reported to reduce the bioavailability of praziquantel. Due to the similarities in structure and pharmacokinetic parameters between hydroxychloroquine and chloroquine, a similar effect may be expected for HYDROXYCHLOROQUINE.
Pyridostigmine	HYDROXYCHLOROQUINE may also be subject to several of the known interactions of chloroquine even though specific reports have not appeared including antagonism of effect of pyridostigmine.
Vaccine: Human diploid cell rabies vaccine	HYDROXYCHLOROQUINE may also be subject to several of the known interactions of chloroquine even though specific reports have not appeared including reduction of the antibody response to primary immunization with intradermal human diploid cell rabies vaccine.

APPENDIX C ORAL HYDROXYCHLOROQUINE ADMINISTRATION

Hydroxychloroquine should be taken with a meal or a glass of milk. Try to take your hydroxychloroquine at about the same time every day. Please record any side effects from the medication in the medication diary given to you by your study team.

- Use of nausea medicines (Phenergan, Compazine, or Zofran) may be helpful, and these medicines are best given about 30-60 minutes before the hydroxychloroquine. Please discuss this with your doctor.

- If you are unable to take the medicine, call your doctor for further instructions

- If you vomit within 30 minutes of taking the medicine, you may take a second dose of the medication. Do not take more than two doses of medication in the same day.

- If you vomit longer than 30 minutes after taking the medicine, do not take any more hydroxychloroquine that day.

- If you have any questions, call your doctor for further instructions.

Common hydroxychloroquine side-effects	**What can I do if I experience this?**
Feeling sick, stomach pain, loss of appetite, diarrhea	Remember to take the tablets after food or with a drink of milk. Stick to simple foods - avoid spicy or rich meals
Headache	Ask your doctor or pharmacist to recommend a suitable painkiller
Eye problems (for example, blurred vision or sensitivity to light)	Let your doctor know about this as soon as possible
Skin rash or itching	If this becomes troublesome, speak with your doctor

If you have any other symptoms which you think may be due to this medicine, speak with your doctor or pharmacist.

APPENDIX D ORAL HYDROXYCHLOROQUINE ADMINISTRATION DIARY

Patient Number_____

Day of course	Date	Time	Side effects
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			

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