Document Coversheet

Study Title: Phase 2 Study of Hydroxychloroquine to Increase Tumor Suppressor PAR-4 Levels in Oligometastatic Prostate Cancer

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SUMMARY OF CHANGES

MCC Study ID No.: MCC-19-GU-72
Date: 06/16/2023
RE: Submission of Amendment 4 (pvd 16JUN2023)

The approved protocol (Amendment 3, pvd 05JAN2022) is being revised for PI reassignment. Dr. James (PI) is transitioning to a new institution and is being reassigned as external Co-Principal Investigator. Dr. Patrick Hensley is assuming the role of co-PI at Markey. We added Dr. Zhengyan Huang as co-statistician; we removed Dr. Peng Wang from the personnel list. **The revised protocol is Amendment 4, protocol version date 16JUN2023**.

Additional administrative edits comprising:

- All pages: new version date updated in footer.
- Cover Page: updates to Protocol Version and Version Date.
- Protocol History: updated the table.

MCC Protocol #: 19-MCC-GU-72
ClinicalTrials.gov Identifier: NCT04011410

Phase 2 Study of hydroxychloroquine to increase tumor suppressor PAR-4 levels in oligometastatic prostate cancer

Short Study Title: HCQ on PAR-4 in Oligometastatic Prostate Cancer

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Commercially Available Agent: Hydroxychloroquine
IND Status: Study Exempt from IND Requirements per 21 CFR 312.2(b).

Funding Source: Internal Markey CCSG Pilot Award, 11/1/2018

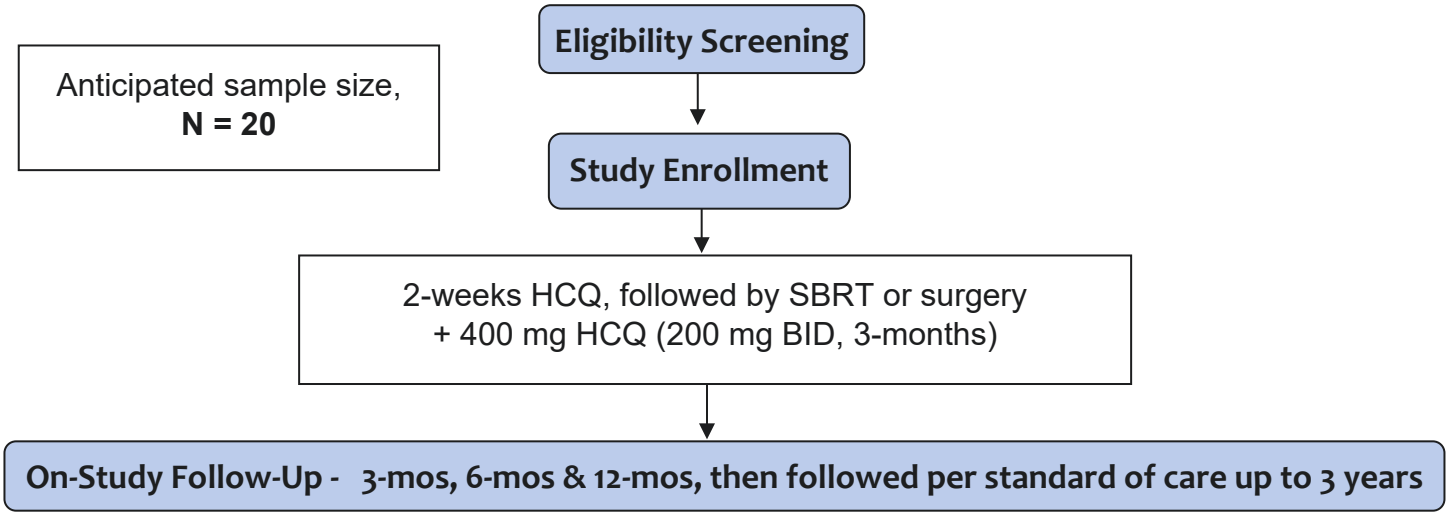
Protocol Version # / Version Date: Amendment 3, 05.January.2022
Amendment 4, 16.June.2023

Protocol History -- Summary of Changes from Original Version to Current Version:

Version No. & Date	Revision(s)
Original, 1/22/2019	First full draft, January 22, 2019 (aka "V1") ; 2/4/19: Reviewed by FRC, Approved, minor edits. 2/7/19: PI responses to FRC. FRC OK'd move to PRMC. 2/22/19: Reviewed by PRMC, Approved. PRMC OK'd proceed to IRB with incorporations of edits from FRC and PRMC.
Original, Revision 1, 3/8/2019	Revision to original. Dosing schedule and imaging clarified; size of CPT to collect PBMCs clarified; Study Calendar completely rearranged & revised to improve clarity re: research-related tests (versus standard of care).
3/21/2019	PRMC approved revision dated 3/8/2019.
3/12/2019	IRB Submission – review of Version Date 3.8.2019
4/29/2019 5/03/2019	IRB Meeting Notification of IRB withheld approval – requested edits to Informed Consent form
5/7/2019	Changes made to informed consent form, resubmitted to IRB
5/13/2019	Medicare coverage analysis returned.
5/20/2019	IRB initial approval received.
Original, Revision 2, 6/27/2019	Minor clarifications to Study Calendar from the Medicare Coverage Analysis findings. Budget updated to include MCC member discount to SRF fees, and research-related costs.
7/3/2019	PRMC approved Revision 2, dated 27 June 2019.
10/15/2019	PRMC approved Protocol Version Date 9/ 27 /19
12/3/2019	**OPEN TO ACCRUAL**
Amendment 1, 11/18/2020	Protocol revised to relax eligibility criteria (cataracts, and 5 lesions for oligomets).
Amendment 2, 6/7/2021	Protocol revised to clarify follow-up timepoints post-treatment.
Amendment 3, 01/05/2022	Protocol revised to include PSMA scan.
Amendment 4, 06/14/2023	PI changed to Dr. Hensley; Dr. James noted as external co-PI. Dr. Zhengyan Huang added as co-statistician; Dr. Peng Wang removed from the personnel list
Amendment 5, *Add date*	*Placeholder for future amendment.*
Amendment 6, *Add date*	*Placeholder for future amendment.*

Figure 1. SCHEMA

Inclusion Criteria:	**Exclusion Criteria**:
- Men ages 18 and older with recurrent prostate cancer following primary treatment of localized disease with either surgery or radiation - Recurrence defined as PSA ≥ 0.2 ng/dl following prostatectomy *OR* a PSA increase ≥2 above nadir following radiotherapy of primary tumor - ≤ 5 synchronous metastatic lesions on CT C/A/P and/or fluciclovine or prostate-specific membrane antigen scan - ECOG 0-2	- Brain or CNS mets - Prior XRT/surgery to a metastatic site; orchiectomy - Prior ADT for metastatic disease or as adjuvant therapy in past two years - Hx of fracture or spinal cord compression - Currently treated with abiraterone, enzalutamide, apalutamide, chemotherapy, immunotherapy or radioisotopes - Macular degeneration - Serum testosterone less than 50 ng/ml - Receipt of HCQ in the past 6 months



STUDY ENDPOINTS

Primary: 3-mos PAR-4 levels

Secondary:

Median Progression-Free Survival and 1-yr ADT-Free Survival up to 3 years
HCQ Toxicity and Tolerability
Quality of Life

Correlatives: PBMCs (days 0, 30, 60 of HCQ treatment), immune function and activation, systematic inflammation, and autophagy induction

1. STUDY OBJECTIVES

1.1 Primary Objective

Assess the rate of attainment of a 50% increase in tumor suppressor PAR-4 levels from baseline in patients treated with 90-days of hydroxychloroquine (HCQ) in combination with radiation or surgery for recurrent, oligometastatic prostate cancer.

> *We hypothesize the successful attainment of a 50% increase from baseline will occur among 50% or more of study participants within the three-months administration period of HCQ.*

1.2 Secondary Objectives

- Median Progression-Free Survival (time to clinical progression)
- 1- and 3-year Androgen Deprivation Therapy (ADT) Free Survival
- HCQ Treatment toxicity (AEs and SAEs during HCQ administration) and radiation toxicity to be assessed and recorded utilizing the CTCAE v5.0. Surgical Complications will be scored using the Clavien-Dindo Classification (Appendix F).
- Immunological effect of HCQ by analyzing peripheral blood mononuclear cells (PBMCs) at day 0 and Day 30 of treatment, and also, Day 60 (+ 6 weeks) of treatment.
- Quality of life as measured by the EORTC QLQ-C30 + QLQ-PR25

2. BACKGROUND

2.1 Prostate Cancer and its Treatment

Among men in the U.S., prostate cancer is the most common cancer and the second most common cause of cancer mortality.[1] In 2018, an estimated 164,960 men will be diagnosed with this disease, representing 19% of all new cancer cases and 9% of male cancer-specific mortality.[1] The prevalence of prostate cancer is projected to rapidly rise over the next 15 years, stemming from "the greying of the U.S. population." Given the link between increasing age and cancer, aging baby boomers will significantly increase cancer incidence and mortality. For the first time in two decades, there was an increase in rates of prostate cancer deaths from 2017 to 2018.[2]

Early stage prostate cancer has few symptoms while men with more advanced disease may experience significant dysfunction, including weak or interrupted urine flow, urinary urgency, frequency and pain and bloody urine.[2] Initial treatment options include surgery, external beam radiation or brachytherapy.[2-4]

2.2 Oligometastatic Prostate Cancer

Advanced prostate cancer commonly spreads to the bones, which can cause pain in the hips, spine, ribs or other areas.[2,4-6] For advanced disease (metastases or recurrence), hormonal therapy in conjunction with surgery or radiation is used.[7-9] Historically, treatment of these patients has comprised primarily androgen deprivation therapy (ADT), guided by the premise that aggressive treatment of the primary tumor and metastatic lesions conferred considerable morbidity and was unlikely to result in cure.[4,7-8] The survival benefit of ADT is juxtaposed against significant sequelae including cardiovascular morbidity, skeletal fractures, diabetes, sexual dysfunction, and a decrease in cognitive function.[10]

Advancements in molecular testing have highlighted distinctly different mutations in the lesions of patients with limited metastatic prostate disease as compared to those of patients with diffuse metastatic disease, possibly explaining the different biologic behavior of these tumors.[11-13] Among men with limited metastatic disease, there is a potential benefit to treatment of oligometastatic prostate lesions.[14] Previous studies have identified a potential delay in the initiation of ADT in men treated with stereotactic radiotherapy.[15,16] Additionally, a prospective, multicenter trial showed that metastasis-directed therapy delayed initiation of ADT in patients with oligometastatic disease.[17]

Given increased rates of metastatic disease and projected increases in incidence and mortality, innovative enhancement of current prostate cancer treatment regimens is vital. Novel therapeutic approaches for oligometastatic prostate cancer are needed, given ADT-induced sequelae such as cardiovascular morbidity, diabetes, cognitive deficits and sexual dysfunction.

2.3 Prostate Apoptosis Response-4 (PAR-4): tumor suppressor protein

Prostate apoptosis response-4 (PAR-4) is a tumor suppressor protein that facilitates apoptosis in numerous types of cancer cells.[18] PAR-4 is ubiquitously expressed in normal cells and tissues, but it is often inactivated, down-regulated or mutated in several types of cancer cells.[18,19] PAR-4 sensitizes cells to the action of diverse therapeutic agents.[18] The loss of PAR-4 in tumors contributes to recurrence and decreased overall patient survival. The baseline levels of PAR-4 secreted by normal cells are generally inadequate to cause massive apoptosis in cancer cells. Given the apoptosis-PAR-4 association, drugs that successfully bolster PAR-4 secretion constitute an important therapeutic advance.

2.4 Hydroxychloroquine (HCQ)

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 and 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.

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, leading 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.[20,21] Institutional experiences with chloroquine administered concurrently with conventional therapy in adjuvant setting provided longer survival.[22] These studies provide a basis for the hypothesis that hydroxychloroquine can elevate PAR-4 levels and thus provide tumor suppression systemically. Accordingly, HCQ and CQ are being studied either pre-operatively or in conjunction with chemotherapeutic agents or radiation.[23] Chloroquine has been found to induce PAR-4 expression and subsequently promote apoptosis of cancer cells and inhibit metastatic progression.[23,24]

2.5 Study Rationale

Treatment of oligometastatic prostate cancer may be enhanced by the addition of hydroxychloroquine to either surgical resection or radiation treatment of metastatic lesions. Potential benefits of hydroxychloroquine include delayed disease progression and delayed initiation of ADT, lessening morbidity and increasing quality of life in men with oligometastatic prostate cancer. 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.[19,24] 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 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, thereby inducing paracrine apoptosis in primary and metastatic prostate tumors. Systemic PAR-4 is also expected to induce apoptosis in circulating cancer cells that contribute to metastasis. PAR-4 produces apoptosis and HCQ is expected to produce elevated levels of PAR-4 secretion that will prevent the growth of tumors. HCQ has also been reported to both inhibit and enhance immune responses via changes in Th1, Th2, Th17 and Treg subsets and reduce inflammatory markers. It also acidifies lysosomes that potentially inhibits antigen presentation by antigen-presenting cells and can be measured with LysoSensor Yellow/Blue DND-160 by flow cytometry. HCQ blocks autophagy by preventing the fusion of the autophagosome with the lysosome, and auto- phagosome cargo protein p62/Sequestosome-1 (also known as the ubiquitin-binding protein), and microtubule-associated protein LC-3II levels (analyzed by Western blot analysis or IHC) are elevated in the normal cells, as well as tumor cells in the patient.

2.6 Study Hypothesis: In men who develop recurrent oligometastatic disease following primary treatment of prostate cancer, treatment with surgery or radiation in combination with administration of hydroxychloroquine (400 mg per day for a 90-day period) will induce an elevation of PAR-4 expression among a majority of participants within three months of HCQ administration.

> *We hypothesize successful attainment of a 50% increase in PAR-4 from baseline levels among 50% or more of participants within the 90-day administration period of HCQ.*

3. PATIENT SELECTION – ELIGIBILITY

3.1 Inclusion Criteria

3.1.1 Males age 18 and over

3.1.2 Histologically confirmed prostate cancer that has recurred following primary definitive treatment of localized prostate cancer (either surgery, radiotherapy or both)
PSA biochemical recurrence defined as either:
PSA ≥ 0.2 ng/dl following radical prostatectomy
PSA increase ≥ 2 ng/mL above PSA nadir value following radiotherapy of primary tumor

3.1.3 Five or fewer synchronous metastatic lesions (on imaging) with no evidence of residual local disease

3.1.4 ECOG performance status 0 – 2 (*see* **Appendix A**)

3.1.5 Approval by screening eye exam (disqualifying baseline conditions listed in 3.2.2, below)

3.1.6 Ability to provide informed consent

3.2 Exclusion Criteria

3.2.1 Hydroxychloroquine-specific rule-outs:
3.2.1a. Receipt of HCQ within the past 6 months
3.2.1b. History of allergic reactions attributed to compounds of similar chemical or biologic composition to hydroxychloroquine
3.2.1c. Use of contraindicated medications, including but not limited to combination antiretroviral therapy and enzyme-inducing anti-epileptics (*see* **Appendix B**)

3.2.2 Macular degeneration or concurrent macular disease. Eligibility in the presence of other eye / visual impairments (cataracts, severe retinopathy, severe visual impairment at baseline, presence of only one functional eye) will be at the discretion of the treating physician based on eye exam by ophthalmologist.

3.2.3 Prior treatment with ADT including:
ADT for treatment of metastatic disease
ADT as adjuvant therapy prior to surgery/radiation within 1 year of enrollment

3.2.4 Previous history of radiation or surgery to a metastatic site

3.2.5 Previous treatment with any of the following:
-Abiraterone -Enzalutamide -Apalutamide -antiretroviral therapy
-chemotherapy -immunotherapy -radioisotopes

3.2.6 Serum testosterone less than 50 ng/ml

3.2.7 History of orchiectomy

3.2.8 History of pathologic fracture or spinal cord compression

3.2.9 Brain or CNS metastases

3.2.10 History of G-6-PD (glucose-6-phosphate dehydrogenase) deficiency

3.2.11 Uncontrolled intercurrent illness including but not limited to the following:
-ongoing or active infection -symptomatic congestive heart failure
-unstable angina pectoris -cardiac arrhythmia

3.2.12 Psychiatric illness and/or social situations that would limit compliance with study requirements.

3.2.13 Patients taking other investigational agents

3.3 Inclusion of Women, Minorities and Children

Prostate cancer does not occur in women nor in male children; as such, these groups are excluded from this study. No eligible male participants will be excluded from this study on the basis of race or ethnicity.

4. STUDY REGISTRATION PROCEDURES

4.1 Protocol Review and Monitoring Committee and Institutional Review Board Review

Before implementing this study, the protocol must be reviewed by the Markey Cancer Center's Protocol Review and Monitoring Committee. The protocol, the proposed informed consent form and other information to subjects must be reviewed by the University of Kentucky Institutional Review Board (IRB). A signed and dated UK IRB initial review approval memo must be maintained in the Markey Cancer Center Clinical Research Office (MCC CRO) 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

Patients who have received previous treatment for prostate cancer with the intent to cure who have biochemical recurrence will undergo metastatic evaluation with imaging at baseline including fluciclovine or prostate-specific membrane antigen (PSMA) PET scan (preferred), bone scan, PET/CT and/or CT of the chest, abdomen, and pelvis as needed to assess for metastatic disease.

Biochemical recurrence for patients who have previously undergone radical prostatectomy is defined as a PSA ≥0.2 ng/dl. Patients who have biochemical recurrence following radical prostatectomy should have received postoperative radiotherapy to the prostate bed if no evidence of metastatic disease.

For patients who have previously undergone primary treatment of their prostate cancer with radiotherapy, biochemical recurrence is defined as an increase of 2 or more ng/dl above the nadir. Patients who have undergone primary radiotherapy will have pelvic MRI to rule out local disease as part of the requisite baseline imaging. Suspicious lesions within prostate bed will be assessed by biopsy.

Patients with 1-3 metastatic lesions on metastatic evaluation will be further screened per inclusion and exclusion criteria as noted above, Section 3. Initial localization of metastases will be recorded (node vs bone vs visceral).
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 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 may withdraw from the study at any time and that withdrawal of consent will not affect his 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. No patient can enter the study before his 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 study 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 CRO. The study PI or designee is also responsible for notifying the Data and Safety Monitoring Committee of the MCC 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, ophthalmologic exam and radiographic studies should be completed within eight weeks prior to initiation of HCQ treatment.

Complete history, physical examination, and evaluation of performance status will also be performed during this 8-week timeframe.

Required lab work will include:

complete blood count (CBC); serum chemistry tests to include alkaline phosphatase, glucose, creatinine, electrolytes, AST (SGOT), total testosterone, and total bilirubin.

5.2 Metastatic site-directed treatment and hydroxychloroquine administration

Modality of site-directed treatment of oligometastatic lesions will be decided by multidisciplinary team consisting of urologic surgery, radiation oncology, and medical oncology in consultation with the patient.

5.3 HCQ Administration

Hydroxychloroquine will be administered daily on an outpatient basis beginning 2 weeks before metastasectomy or initiation of radiation treatment to metastatic lesions. HCQ administration will be held the day of surgery for those patients undergoing surgical resection of their metastatic site(s) and will resume on post-operative day 1, and the missed dose will not be made up. Hydroxychloroquine will be administered orally at a total daily dose of 400 mg (200 mg BID) for 90-days. Tablets of HCQ are available in 200 mg strength.

Instructions on taking HCQ (patient version) are located in **Appendix C**.

To minimize nausea, HCQ is recommended to be administered in divided doses (one 200-mg tablet twice per day). The divided doses should be taken with meals, once in the morning and once at night. Should a subject have emesis and regurgitate the HCQ 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.

Study participants will be required to keep a medication diary and to present the completed diary at the end of the treatment (**Appendix D**). Additionally, at the PI's discretion, study staff will call the participants periodically on an as-needed basis to monitor ongoing completion of the pill diaries while patient is on-study, e.g., if a patient misses a clinic visit.

Appropriate dose modifications are described in Section 6.

Adverse events and potential risks are described in Section 7.

5.4 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 PI 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 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 that may cause adverse ocular or skin reactions.

5.5 Duration of Therapy

In the absence of treatment delays due to adverse event(s), hydroxychloroquine treatment will continue for 3 months or until one of the following criteria applies:

- Disease Progression, assessed by standard of care and defined as either:
  - PSA progression (an increase in PSA > 25% above baseline at study entry), or
  - Symptomatic progression (increasing pain from metastatic lesion), or
  - Progression of baseline-detected lesions (soft-tissue lesions as assessed by RECIST and bone lesions as assessed by the MDA criteria (respectively, Appendix H.1 and Appendix H.2).
    Additionally, CT C/A/P and/or fluciclovine or PSMA PET scans may be performed at the time of either Symptomatic or PSA Progression at discretion of the treating physician.
  - Appearance of new metastatic lesions
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition that render the patient unacceptable for further treatment in the judgment of the investigator

5.6 Duration of Follow-up

Patients will be followed for up to three years post-therapy or until death, whichever occurs first. Patients removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

6. DOSING DELAYS AND DOSE MODIFICATIONS

Dose schedule and dose reduction schedule for hydroxychloroquine (HCQ):

Dose Level	***HCQ* Dose**
1	*200 mg, twice a day (total daily dose, 400 mg)*
-1	*200 mg, daily*

If a participant is unable to tolerate 200 mg per day of HCQ, he will be removed from the study.

6.1 HCQ Dose Modifications for Non-Hematologic Toxicities that are deemed *Possibly, Probably or Definitely* related to Hydroxychloroquine

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.	
Recommended management: Re-educate patient to take with meals and anti-emetics. Adjunct anti-nausea therapy is permitted and should be recorded when used.	

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.	
Recommended management: Loperamide antidiarrheal therapy Dosage schedule:4 mg at first onset, followed by 2 mg with each loose bowel movement until diarrhea-free for 12 hours (maximum dosage:16 mg/24 hours) 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 or greater	Permanently discontinue.
The methods recommended for early diagnosis of "chloroquine retinopathy" consist of (1) fundoscopic examination of macula for fine pigmentary disturbances or loss of foveal reflex & (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.	

6.2 HCQ 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.
*Patients requiring a delay of >2 weeks 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.	

NOTE: All other grade 3 or 4 toxicities should be discussed with the study PIs.

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 if the event requires expedited reporting (via Medwatch Forms) **in addition** to routine reporting.

7.1 Expected Toxicities for Hydroxychloroquine

7.1.1 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.

Hydroxychloroquine Retinitis is both dose- and duration-dependent with increased risk occurring at doses exceeding 5mg/kg of actual body weight and chronic use exceeding 5 years. This trial uses an FDA-approved dose of hydroxychloroquine and is 90 days in duration. Thus, risks of hydroxychloroquine retinitis are small (less than 1%) and will be further minimized by baseline screening for retinal abnormalities, (see eligibility criteria 3.1.5 and 3.2.2), monthly assessment of adverse effects, repeat visual examinations for ocular toxicity and permanent discontinuation of hydroxychloroquine for any grade 2 or greater ocular toxicity (see 6.1, Visual Changes. 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 **HCQ Overdosage or Sensitivity**, it is suggested that ammonium chloride (8 g daily in divided doses) 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 20-90%. However, caution must be exercised in patients with impaired renal function and/or metabolic acidosis. See Section 8.7 for details.

7.1.2 Adverse Reactions

The Council for International Organizations of Medical Sciences (CIOMS) frequency rating is used as follows, when applicable:

CIOMS Frequency Rating *(expected % of population experiencing adverse reactions)*			
Inadequate data	***Not Known***	0.1% - 0.9%	***Uncommon***
10% or greater =	***Very Common***	0.01 – 0.09%	***Rare***
1% – 9%	***Common***	Less than 0.01%	***Very Rare***

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, i.e., 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 treatment.

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 annularecentrifugum), toxic epidermal necrolysis, erythema multiforme, Stevens-Johnson syndrome, Drug Rash with Eosinophilia and Systemic Symptoms (DRESS syndrome), photosensitivity, exfoliative dermatitis, acute generalized exanthematouspustulosis (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.

Metabolism and nutrition disorders

Common: Anorexia (usually resolves immediately upon reducing dose or stopping treatment).

Not known: hypoglycemia

HYDROXYCHLOROQUINE may exacerbate porphyria.

7.1.2 Adverse Reactions from HCQ – CONTINUED

Nervous system disorders

Common: Headache
Uncommon: Dizziness
Not known: Convulsions

Psychiatric disorders

Common: Affect lability
Uncommon: Nervousness
Not known: Psychosis, suicidal behavior

Blood and lymphatic system disorders

Not known: Bone marrow depression, anemia, aplastic anemia, agranulocytosis, leucopenia, and 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.

Immune system disorders

Not known: Urticaria, angioedema, bronchospasm

Ear and labyrinth disorders

Uncommon: Vertigo, tinnitus
Not known: Hearing loss including cases of irreversible hearing loss.

Hepatobiliary disorders

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

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.

For a comprehensive list of adverse reactions, please refer to the HCQ package insert.

7.2 Adverse Event Characteristics

7.2.1 CTCAE v.5.0: For AE reporting, descriptions and grading scales of the NCI Common Terminology Criteria for Adverse Events CTCAE version 5.0 will be utilized. All appropriate treatment areas have access to a copy of the CTCAE version 5.0.
Additionally, a copy of the CTCAE version 5.0 can be downloaded from the CTEP web site *http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.*

7.2.2 *For expedited reporting purposes only*:
AEs for HCQ noted in Section 7.1 should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.

Other AEs that do not require expedited reporting are outlined in Expedited Adverse Event Reporting (Section 7.3).

7.2.3 Attribution of the Adverse Event (AE):

Definite – The AE *is clearly related* to the HCQ.
Probable – The AE *is likely related* to the HCQ.
Possible – The AE *may be related* to the HCQ.
Unlikely – The AE *is doubtfully related* to the HCQ.
Unrelated – The AE *is clearly NOT related* to the HCQ.

7.3 Expedited Adverse Event Reporting

7.3.1 For MCC Investigator-Initiated Trials (IITs), study investigators and staff must report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of HCQ, during the 90-day HCQ administration (study treatment), or within 30 days of the last dose of HCQ on the SAE form.

This applies to the following categories:

- Grade 2 ocular or visual changes – any changes, regardless of attribution to the HCQ
- Grade 3 (severe) *Medical* Events – Only events that are Unexpected and Possibly, Probably or Definitely Related / Associated with the HCQ.
- 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 are a grade 5.

7.3.2 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 by MCC investigator of HCQ, a commercially available agent (non-IND and non-IDE)	**Grade 2 eye/visual changes, any attribution**. 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 events (fatal)	FDA: Suspected AE that is serious and unanticipated (not listed in IDB or in the consent)	OnCore and DSMC reporting only	Voluntary Medwatch 3500 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

7.3.3 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 AE Expected	Gr. 3 AE Unexpected	Gr. 4 AE Expected	Gr. 4 AE Unexpected	Gr. 5 AE Expected or Unexpected
Unrelated Unlikely	Only for eye/visual changes	Not required	5 calendar days#	5 calendar days	24 hours*
Possible Probable Definite	Only for eye/visual changes	5 calendar days	5 calendar days#	5 calendar days	24 hours*
# If listed in protocol as Expected and Not Requiring expedited reporting, the adverse event does not need to be reported.					
* For participants enrolled and actively participating in the study ***or*** for SAEs occurring within 30 days of the last intervention, the SAE should be reported within 24 business hours of learning of the event.					

7.4 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.

7.5 Routine Adverse Event Reporting

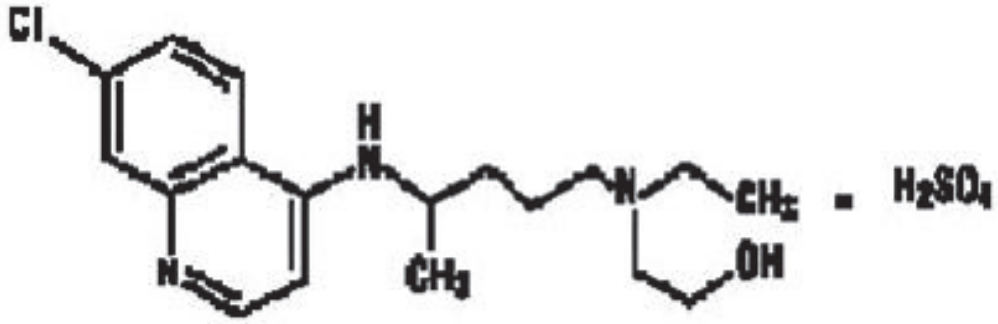
All AEs must be reported in routine study data submissions to the PI on the OnCore case report forms. AEs reported through expedited processes (e.g., reported to the IRB) must also be reported in routine study data submissions.

8. PHARMACUETICAL INFORMATION

A list of the adverse events and potential risks associated with HCQ administered in this study is found in Section 7.1.

8.1 Hydroxychloroquine

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, pre-gelatinized 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 63304-296-0	Bottles of 10
NDC 63304-296-01	Bottles of 100
NDC 63304-296-05	Bottles of 500
NDC 63304-296-10	Bottles of 1000

8.2 HCQ Storage Requirements:

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

8.3 HCQ Stability:

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

8.4 Route of Administration for HCQ:

Hydroxychloroquine is taken orally twice a day, each dose to be taken with a meal or a glass of milk. If patients miss the dose, take if within 2 hours. Outside of this 2-hour window, just skip the dose and take the next dose at the regular scheduled time. See **Appendix C**.

8.5 HCQ Contraindications

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

8.6 HCQ 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.

8.7 HCQ Overdose: Symptoms of Overdose and Treatment for HCQ Overdose

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

8.7.1 Symptoms of HCQ 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 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.

8.7.2 Treatment of HCQ 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 over dosage or sensitivity, it is suggested that ammonium chloride (8 g daily in divided doses for adults) three or four days a week be 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. IMMUNOLOGICAL EFFECT OF HCQ

To assess the immunological effect of HCQ therapy in patients, we will analyze peripheral blood mononuclear cells (PBMCs) at days 0 and 30 of treatment, and also, Day 60 (+ 6-week study visit window) of treatment. PBMC and plasma separation and storage will be handled by the Biospecimen Procurement and Translational Pathology Shared Resource Facility, per their standard processes until samples are ready for analysis.

Changes in markers of immune function/activation will be determined by measuring the percentage and absolute number of activated, naïve, and memory T cell subsets within CD4 helper, CD8 cytotoxic, and Treg subsets using cell marker antibodies to: CD4, CD8, CD45RA, CD45RO, CD25, and Foxp3. Th1, Th2 and Th17 subsets will be identified in the CD4 T cell subset by intracellular staining for IFN- ɣ, IL-10 and IL-17 in ex vivo activated PBMCs. Markers for systemic inflammation (C-reactive protein, IFN-gamma and TNFa) will be quantified in plasma samples. As a lysomotrophic compound, HCQ reduces pH in lysosomes which can promote antigen retention and can promote autophagy. The pH levels in intracellular vesicles will be evaluated by flow cytometry in CD4 and CD8 T cell subsets and in monocytes using LysoSensor Yellow/Blue DND-160. Induction of autophagy will be monitored in CD4 and CD8 T cell subsets and in monocytes by evaluating expression levels of the autophagy marker, LC3B.

To assess PAR-4 levels we will analyze plasma (or serum) at Day 0, 2 weeks prior to surgery or initiation of radiation treatment (corresponds to roughly Day 14 of HCQ therapy); 30-, 60- and 90-days post-HCQ initiation; and at the 6-month and 12-month follow-up timepoints (that correspond to a standard of care clinic visit 6- and 12-months after cessation of HCQ therapy).

See Study Lab Manual for additional details – a draft of the lab manual is **Appendix E**

10. Study Calendar / Schedule of Activities

Baseline evaluations are to be conducted within 8 weeks prior to start of HCQ. Scans and ophthalmologic exam must be completed less than 8 weeks prior to the start of HCQ regimen. In the event the patient's condition is deteriorating, laboratory evaluations should be repeated more frequently, as clinically indicated and at the discretion of the treating physician. At PSA progression or symptomatic progression: PSA, history and physical/toxicity assessment, CT C/A/P and/or fluciclovine or PSMA PET scans to be performed at discretion of treating physician, per standard of care.

Study Calendar

Procedures	Pre-study Screening	Enrollment/Baseline Visit 1, Day 0	Day 14 post-HCQ initiation	Day 30 post-HCQ initiation	Day 60 post-HCQ initiation	90 days post-HCQ initiation	6-months post-HCQ cessation	1 Year Post- HCQ cessation	2 Years Post-HCQ cessation	3 Years Post-HCQ cessation	Off-Study
HCQ Drug Dispensing		R		**R**	**R**	**R**					
Adverse Event Evaluation		R	**R**	**R**	**R**	**R**					
HCQ compliance (pill diary)				**R**	**R**	**R**					
PAR-4 blood sample [1]		**R**	**R**	**R**	**R**	**R**	**R**	**R**			
PBMCs blood collection [2]		**R**		**R**	**R**						
Quality of Life [3]		**R**				**R**	**R**	**R**			
Informed Consent	**R**										
Demographics	**R**										
Ophthalmologic Exam [4]	**R**	← ---------- **S** ** ----------→									
PSA (serum, one tube) [5]	S	**R**		**R**		S	S	S			
Imaging [6]											
Fluciclovine or PSMA PET or PET/CT scan [6a]	S**										
Bone scan [6a]	S**										
CT chest, abdomen, pelvis [6a]	S**										
MRI of pelvis [6b]	S *										
Assessment of Disease Progression [6c]		S * , **									
CBC w/ differential	S	*	*	S	S	S	**	**			
CMP **	S	*	*	S	S	S	**	**			
Concomitant Medications [7]	S	S	S	S	S	S					
Medical History	S										
Height [8]		S									
Physical Exam with vitals (including weight) and ECOG Performance status	S	**	S**	S	S	S	**	**			
Survival Status									**R**	**R**	**R**
Record date of ADT initiation [9]											

Study Calendar

Procedures	Pre-study Screening	Enrollment/Baseline Visit 1, Day 0	Day 14 post-HCQ initiation	Day 30 post-HCQ initiation	Day 60 post-HCQ initiation	90 days post-HCQ initiation	6-months post-HCQ cessation	1 Year Post- HCQ cessation	2 Years Post-HCQ cessation	3 Years Post-HCQ cessation	Off-Study

NOTES: * per standard of care ** at the discretion of the treating physician.

Legend: S = standard of care; R = research; PSA, prostate-specific antigen; PET, positron emission tomography scan; HCQ, hydroxychloroquine; PAR-4, prostate apoptosis response-4; PSMA, prostate-specific membrane antigen PET scan

1: Blood (1 tube) will be drawn at each of the 7 noted timepoints for PSA and PAR-4 levels; plasma is preferred (serum is acceptable).
Timepoints may be +/- 72-hour window at Pre-study and Enrollment.
For Day 14, Timepoints may be +/- 120-hour window.
Timepoints may be +/- 28-day window at the 3-, 6- and 12-month f/u, coinciding with a clinic visit.

2: for collection of PBMCs (peripheral blood mononuclear cells): one tube per collection timepoint, slated for Day 0, Day 30 and Day 60. Day 0 and Day 30 timepoints have a +/- 14-day window. Day 60 has a +6 week window. This is explicated in greater detail in study lab manual.

3: QOL is assessed via EORTC QLQ-C30 + QLQ-PR25 at 4 timepoints
- at a clinic visit prior to initiation of HCQ
- at a clinic visit; either the end of HCQ administration OR when HCQ is discontinued (if stop it early)
- at 6-mos and 12-mos post-treatment. These final two QOL timepoints can be +/- 8 weeks, and conducted during a regularly scheduled, standard of care clinic visit.

4: Requisite pre-study eye exam; Eye exam may be repeated during HCQ administration if vision complaints.

5: One tube of blood will be drawn at each of the 6 noted timepoints for serum PSA; this is standard of care for baseline and every 3 months (4 of the 6 draws), with two additional draws scheduled. Timepoints may be +/- 72-hour window at screening and enrollment.

6: *Imaging at baseline, pre-study*: Patients who have confirmed recurrent disease of the prostate are deemed ineligible for this trial. As such, all eligible patients will undergo standard of care imaging to identify metastases (initial evaluation of status/extent of recurrent disease), including:
6a: All eligible patients will undergo standard of care imaging to assess extent of metastatic disease at baseline; fluciclovine or PSMA PET scans are preferred; other scans (PET/CT scan, bone scan and/or CT of the chest, abdomen and pelvis) may be performed as needed at baseline to assess extent of metastatic disease, per standard of care.
6b: *Additionally*, eligible patients who have undergone radiation prior to study enrollment will have an MRI of pelvis (in addition to previously named imaging studies) to assess for recurrent local disease. Suspicious lesions of the prostate seen on MRI will be biopsied.

6c: *Imaging on Study as work-up for disease progression*: Once a patient is enrolled on-study, imaging to assess disease progression will proceed per standard of care, based on clinical indicators such as rising PSA and/or other clinical symptoms. Per RECIST 1.1, tumor re-evaluation scheduled every 6-8 weeks at the discretion of the treating physician based on clinical indicators. Notably, a rising PSA and/or other clinical symptoms could trigger a scan/imaging in an earlier timeframe.

7: Concomitant Medications will be assessed per standard of care at the discretion of the treating physician; during HCQ administration, Con Meds will be assessed with a particular focus on medications contraindicated for use with HCQ.

8: Height will be evaluated once while on-study. Height can either be assessed via the patient's medical record, and/or assessed directly (in conjunction with vital signs during a clinic visit)

9: ADT initiation: record date and reason for ADT initiation. Reasons for ADT initiation include: PSA/biochemical progression, Symptomatic Progression, Local Progression of baseline-detected lesions (either soft-tissue or bone lesions), or Appearance of new metastatic lesions.

11. MEASUREMENT OF HCQ EFFECT

11.1 PRIMARY OBJECTIVE

To assess the effect of HCQ on PAR-4, first compliance with HCQ planned dose regimen should be ascertained.

HCQ regimen compliance: pill diaries (Appendix D) and/or patient-reported HCQ compliance will be evaluated to assess participant adherence to HCQ daily regimen at follow-up clinic visits with study staff. Additionally, study staff may institute a telephone check with study participants on a PRN basis, should compliance to the HCQ regimen need to be monitored outside regular clinic visit schedule.

Participants who completed 80% of the planned HCQ doses will be considered evaluable for the Primary Endpoint, PAR-4 increase.

PAR-4 level will be assessed for each participant at the following timepoints:

Enrollment/Baseline visit
Day 14 post- HCQ initiation
Day 30 of HCQ
Day 60 of HCQ
Day 90 of HCQ
6-month follow-up (during a standard of care visit)
12-month follow-up (during a standard of care visit)

It is expected that HCQ will induce an elevation of PAR-4 expression among 50% of the participants within the 90-day administration of HCQ. The elevation is expected to be a 50% increase from baseline PAR-4 levels among half of the study participants.

11.2 SECONDARY OBJECTIVES:

11.2.1 HCQ safety analysis and Treatment Toxicity: frequency and incidence tables of toxicity and AEs will be generated. Toxicity will be assessed using Common Terminology Criteria for Adverse Events V5.0 (for HCQ and for radiation therapy), as well as the Clavien-Dindo classification of surgical complications in the case of patients who underwent surgery (Appendix F). All patients will be evaluable for toxicity (HCQ) from the time of their first treatment with Hydroxychloroquine.

11.2.2 HCQ immunologic effect on PAR-4 Levels: as measured by serum (or plasma) blood collected at multiple timepoints. PAR-4 secretion is expected to come primarily from normal cells of the patient. The contribution of the tumor cells to serum levels of PAR-4 is expected to be minimal, but this has not been directly tested. Association between PAR-4 levels and clinical stage will be assessed.

11.2.3 Quality of Life: This is assessed by two validated patient-reported outcomes measures, the EORTC QLQ-C30 and QLQ-PR25 questionnaires. All items assessing symptoms and functional domains employ a 4-point Likert scale, with responses ranging from “Not At All”, “A Little”, “Quite a Bit” and “Very Much.” Study participants will be instructed to choose one response per item. The EORTC **QLQ-C30** is a 30-item questionnaire assessing multiple domains of global health-related quality of life in cancer populations. It includes one global QOL rating, 5 functioning scales (physical, cognitive, emotional, role and social) and 9 symptom-specific subscales (fatigue, N/V, pain,

dyspnea, insomnia, constipation, diarrhea, appetite loss). The EORTC **QLQ-PR25** is a prostate cancer module meant for use among patients with prostate cancer in varying in disease stage and treatment modality (i.e., surgery, chemotherapy, radiotherapy, etc.), which complements the QLQ-C30. The QLQ-PR25 module comprises 25 items assessing disease symptoms, treatment side effects, sexual functioning and sexual activity. The functional scales are sexual activity and sexual functioning; the 4 symptom scales are urinary symptoms, bowel symptoms, hormonal treatment-related symptoms, and incontinence aid. All participants will complete items 1-19 as well as items 20-21 (which query current sexual activity). Completion of Items 22-25 are conditional on being sexually active (and comprise the Sexual Functioning subscale), and thus will only be completed by a subgroup of study patients. For interpretation of scores on the two EORTC QOL questionnaires, all of the scales and single-item measures range in score from 0 – 100. A high scale score represents a higher response level – so that a higher score represents a higher ("better") level of functioning OR a higher ("worse) level of symptoms. See **Appendix G** for additional details and the patient self-report questionnaires.

11.3 Four Types of Disease Progression

1. PSA or Biochemical Progression: defined as a PSA increase of ≥ 25% and ≥ 2 ng/mL if PSA was ≥ 2 ng/mL from baseline (OR a PSA increase of ≥ 25% if PSA was < 2 ng/mL at study enrollment).

2. Symptomatic Progression: defined as increasing pain from baseline-detected metastatic lesion. Time to first symptomatic event will be calculated from study enrollment until the event of symptoms due to metastatic disease.

3. Local Progression of baseline-detected lesions depend on site:

- 3a. *soft-tissue metastatic lesion*: local progression is defined as an increase of ≥ 20% in the largest tumor dimension with a minimum absolute increase of 5 mm. All lesions are considered target lesions, irrespective of size, if they are suspicious on choline PET-CT. See Appendix H.1 for RECIST v1.1.

- 3b. *bone metastases*: local progression is assessed using the MD Anderson Cancer Center-criteria[26-27], and defined as a ≥ 25% increase in size of a measurable lesion or a ≥ 25% increase in the size of ill-defined lesions on CT considered to be progression. MDA bone-specific response criteria in Appendix H.2.

4. Distant Progression: appearance of new metastatic lesions

11.4 Definition of Disease Assessments to evaluate Progression-Free Survival and ADT-Free Survival

11.4.1 ADT-Free Survival

Indications to start Androgen Deprivation Therapy include appearance of new metastases (i.e., progression to more than the three baseline-detected metastases, “distant progression”), or local progression of baseline-detected metastases.
ADT initiation can be considered at Symptomatic Progression or at PSA Progression. Additionally, CT C/A/P and/or fluciclovine or PSMA PET scans may be performed at the time of either Symptomatic or PSA Progression at discretion of the treating physician.

Date of ADT initiation will be recorded for each participant, accompanied by the reason for initiation.

ADT-free Survival is defined as the time between study enrollment until the start of ADT or death as a result of any cause. Patients who do not start ADT at the time of last follow-up will be censored at that time point.

- Initiation of ADT will be recorded if it occurs after initiation of HCQ. This will also be recorded for enrolled patients who have ADT initiation occurring after completion of surgery/radiation but prior to HCQ initiation (as this is exclusion criterion, the patient will be removed from the study).
- After HCQ administration is completed, survival status will be assessed at ongoing clinic visits for one year post-enrollment, and afterwards, annually for up to 2 additional years (i.e., at Year 2 post-enrollment and at Year 3 post-enrollment).

11.4.2 Progression-Free Survival (time to clinical progression)

Four types of progression are defined in 11.3. Progression is calculated from time of study enrollment until progression (per the 4 specified definitions) or death. Events for PFS are:

1. PSA or biochemical progression: defined as a PSA increase of ≥ 25% and ≥ 2 ng/mL (if PSA was ≥ 2 ng/mL from baseline)
2. Symptomatic progression: increasing pain from metastatic lesion
3. Local progression of baseline-detected lesions: each metastasis is a target lesion independently assessed as:
   - soft-tissue lesions assessed per RECIST v1.1 (Appendix H.1)
   - bone lesions assessed per MDA bone-specific response criteria (Appendix H.2)
4. Distant progression: appearance of new metastatic lesions.

If an event is identified, then the time to the event will be recorded as the first instance where any of the above four situations occur.

12. DATA REPORTING AND REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.

12.1 Method of Data Reporting

This study will require data submission and reporting via the OnCore Database, which is the official database of the MCC CRO. Instructions for submitting data are listed in the Data Management Plan created by the Markey Data Management staff in conjunction with the MCC CRO and the QA Office, per their SOPs (see S13.4, below).

12.2 Responsibility for Data Submission and Oversight

Study staff is responsible for submitting study data and/or data forms to OnCore as per the MCC CRO SOP's. This trial will be monitored by the MCC DSMC on a schedule determined by the PRMC at the initial PRMC review. The CRO SRF staff is responsible for compiling and submitting data for all participants and for providing the data to the PI for review.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design, Sample Size Considerations and Accrual Rate

13.1.1 Study Design

A single-arm, Phase II trial will be employed to obtain initial assessment of the effect of HCQ in modulation of PAR4 levels among patients with oligometastatic prostate cancer treated with surgery or radiation. The primary endpoint is based on obtaining a 50% increase in PAR-4 levels from baseline within the 3-month period of HCQ administration.

13.1.2 Sample size justification

Based on an ongoing Phase I trial of HCQ, we will assume that the proportion of patients who will exhibit a 50% induction in PAR-4 from baseline levels is equal to 0.50 compared to a null hypothesis of 0.20. A sample of 18 patients will provide 84% power to detect this hypothesized difference in proportion based on a two-sided test with 5% significance level. Assuming no more than a 10% drop out rate, we will enroll a total of 20 patients into the study. No patients dropped out of our Phase 1 study, which ascertained 200 mg daily as the optimal biological dose.[25]

13.1.3 Accrual Rate

The accrual rate is estimated to be one patient per month. Based on this enrollment rate, we will complete the accrual of 20 patients in about 18 months.

13.2 Interim Monitoring for Efficacy

We propose to assess the proportion of patients who will exhibit induction levels in PAR-4 after half of the patients have been enrolled into the trial. Bayesian interim monitoring methods using posterior probabilities, credible intervals and predictive probabilities will be calculated based on proportion of patients exhibiting a 50% induction in PAR-4 levels. Briefly, a beta distribution will be assumed for the proportion of PAR-4 response and uninformative and neutral prior distributions will be utilized.

After half of the participants are enrolled on-study (n = 10-11), the proportion of patients with PAR-4 induction will again be estimated and posterior distributions and predictive posterior distributions will be calculated. Accrual will continue during interim analysis. Based on posterior probabilities and credible intervals from the interim analysis, we will then determine whether to continue or stop accrual efforts to attain the planned sample size of 20.

13.3 Analysis of Primary and Secondary Endpoints

To be considered “evaluable” a patient must have completed 80% of the 90-day planned dose of HCQ.

13.3.1 PAR-4 Levels

Patient compliance with planned HCQ regimen will be assessed by the treating physician and the patient-reported pill diary will be reviewed. For each evaluable patient, descriptive statistics will be calculated to summarize PAR-4 levels at each time point of follow-up. Percent change from baseline PAR-4 compared to each follow-up time point will likewise be calculated. The proportion of patients who exhibit a 50% induction in PAR-4 compared to baseline within the 3-month therapy period will be calculated and a one-sample test for proportion will be performed.

Secondary analyses of the primary endpoint will also be performed. Continuous changes in PAR-4 levels will also be assessed using paired t-test or nonparametric analog. Linear mixed models will also be employed to analyzed repeatedly measured levels of PAR-4 and association with clinical parameters as well as PSA levels. Other secondary analyses will include summary of the PAR-4 induction profile over the 3-month treatment period and after end of therapy period.

13.3.2 Analysis of Secondary Endpoints

Clinical Endpoints.

Progression-free survival (PFS) will be estimated using the Kaplan-Meier method and estimates of median PFS and PFS rates at specific time points will be calculated.

Likewise, median, 1-year and 3-year ADT-free survival will be estimated using the Kaplan-Meier method.

Exploratory analysis for association of PFS and ADT-free survival with PAR-4 and PSA levels will be assessed using the Cox regression model.

Correlative and QOL endpoints. PSA levels will be assessed according to the schedule described by the study calendar above and PSA doubling time will be calculated. Association of PSA levels with PAR-4 levels will be determined using Spearman or Pearson’s correlation coefficient. Quality of life (QOL) measured by the EORTC QLQ-C30 supplemented with QLQ-PR25 will be evaluated and descriptive statistics of QOL scores will be calculated. Association of QOL with PAR-4, PSA and clinical endpoints will be determined in an exploratory manner using correlations and survival analysis models.

Safety analysis. All patients who received study drug will be included in the safety analysis. Frequency and incidence tables of toxicity and AEs will be generated in the overall patient group. We will generate a safety report for review by the Markey Cancer Center’s Data Safety and Monitoring Committee (DSMC) based on an intent-to-treat population.

13.4 Data Management

Data management will be performed by cross-team members at MCC, including members from the Cancer Research Informatics (CRI) SRF and BB SRF, who will work\ closely with the MCC CRO. A protocol-specific Data Management Plan (DMP) will be authored by a senior data manager in collaboration with the biostatistician and CRO, each team will be expected to review and sign off on the DMP prior to finalization. In order to maintain best clinical practices in data management, the DMP may include, but not be limited to CRF/eCRF design, database build and design, database training, edit check/validation specifications, study database testing/ release, data and paper workflow, report, metrics, query/discrepancy management, management of external (including lab) data, medical coding, SAE handling/reconciliation, data transfers and database lock. The protocol-specific DMP will additionally define the schedule at which data will be accessed by study statisticians to perform statistical programming for conduct of data quality, data control, data management, generation of interim reports and statistical analysis. Furthermore, the CRI will utilize the Labkey system to develop a web-based data entry system for the PAR-4 and other correlative biomarkers that will be evaluated using western blot and ELISA assays from Dr. Rangnekar' s laboratory. The study statistician will access to both Oncore clinical databases and Labkey system. Cross-team members will collaborate to establish procedures and timelines for quality control, audits, query resolution, interim safety and final data analysis.

The study statistician and staff from the Biostatistics and Bioinformatics Shared Resource (BB SRF) of the Markey Cancer Center (MCC) will work closely with the study team, the Clinical Research Office (CRO) at MCC and the Cancer Research Informatics (CRI) Shared Resource at MCC to develop the Data Management Plan (DMP) for this trial. The DMP will provide details for development of eCRFs in the OnCore system, trial-specific processes for data entry, generation of reports, data management and statistical analysis. Specifically, the statistician will attend several meetings including the eCRF development meeting, Data Management and Development meeting and the Protocol Initiation meeting. Appropriate and accurate collection of primary and secondary study endpoints and inclusion of valid values and range checks for data fields will be designed for the eCRFs. The OnCore clinical trial management system, managed by Markey's CRO and CRI, will be the primary database repository of clinical data from all patients enrolled into this trial. Furthermore, the CRI at MCC has established workflows for receipt, storage and secure access of high-throughput bioinformatics data (next generation sequencing) using the university's high performance computing facility. The study statistician and Dr. Chi Wang from the BB SRF have access to both OnCore clinical databases and bioinformatics data. Data will be accessed by the study statistician on a regularly-scheduled basis as defined in the DMP to perform statistical programming for conduct of data quality control, data management, generation of interim reports and statistical analysis. In collaboration with the study team, procedures will be developed for timelines for data quality control, resolution of data queries, interim safety reporting and final data analysis.

A Data Management Specialist (DMS) from CRI SRF will be assigned to build the study-specific calendar and appropriate eCRF for data collection. User Acceptance Testing (UAT) will be performed and reviewed by study team members, Data Management Specialist Sr, (DMSr), CRA, and export files by statistician, to assure all data collection tools are appropriate for the study data capture and all data export formatting is appropriate for analysis; eCRF's will be updated and retested as needed. Additional data management study start-up activities, (i.e. writing of Data Management Plan, data validation programs, reporting and review tools) will be conducted concurrent to database build and testing.

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16. APPENDICES

Appendix A: Performance Status Scales
Appendix B: Medications known to interact with HCQ
Appendix C: Instructions for taking HCQ
Appendix D: Pill Dairy (sample / patient version)
Appendix E: Lab Manual for FCIM – assays
Appendix F: Clavien-Dindo Classification
Appendix G: EORTC Quality of Life Measures Summary of the two complimentary QOL measures EORTC QLQ - C30 EORTC QLQ-PR25 Scoring Instructions for the QLQ-PR25
Appendix H: Response Criteria - Disease Assessment H.1 RECIST v1.1 for soft-tissue lesions H.2 MDAnderson bone-specific response criteria

Appendix A. PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	*Description*	*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. POSSIBLE DRUG INTERACTIONS WITH HYDROXYCHLOROQUINE

Proper Name	Effect/clinical comment
Any medication known to cause Adverse ocular reactions AND/OR Adverse skin reactions	
Agalsidase	theoretical risk of inhibition of intra-cellular α-galactosidase activity when HCQ is co-administered with agalsidase.
Amiodarone	There may be an increased risk of inducing ventricular arrhythmias if HCQ is used concomitantly with other arrhythmogenic drugs.
Antacids	As with chloroquine, antacids may reduce absorption of HCQ so it is advised that a four-hour interval be observed between HCQ and antacid dosing.
Anti-diabetics	May enhance the effects of a hypoglycemic treatment, a decrease in doses of antidiabetic drugs may be required.
Anti-epileptics	The activity of antiepileptic drugs might be impaired if co-administered with HCQ.
Anti-malarials known to lower the convulsion threshold	HCQ can lower the convulsive threshold. Co-administration of HCQ with other anti-malarials known to lower the convulsion threshold (e.g., mefloquine) may increase the risk of convulsions.
Mefloquine	HCQ can lower the convulsive threshold. Co-administration of HCQ 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 HCQ is used concomitantly with other arrhythmogenic drugs.
Cyclosporine	An increased plasma ciclosporin level was reported when ciclosporin and HCQ were co-administered.
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.
Moxifloxacin	There may be an increased risk of inducing ventricular arrhythmias if HCQ is used concomitantly with other arrhythmogenic drugs.
Due to its similarity in structure and pharmacokinetics with chloroquine, a similar effect may be expected for HCQ with the following medications and their derivatives, although specific reports have not appeared regarding their use with HCQ	
Praziquantel	Chloroquine has been reported to reduce the bioavailability of praziquantel. A similar effect may be expected for HCQ.
Pyridostigmine	Potential antagonism of effect of pyridostigmine.
Human diploid cell rabies vaccine	Reduction of the antibody response to primary immunization with intradermal human diploid cell rabies vaccine.
Neostigmine	Antagonism of effect of neostigmine.
Aminoglycoside antibiotics	Potentiation of its direct blocking action at the neuromuscular junction by aminoglycoside antibiotics.
Cimetidine	Inhibition of its metabolism by cimetidine, which may increase plasma concentration of the antimalarial.

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 HCQ 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 Dr. Andrew James at (859) 257-4488 or Dr. Peng Wang at (859) 323-6522 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 that you think may be due to this medicine, speak with Dr. Andrew James or Dr. Peng Wang (after business hours or on weekends call 859-323-5321), or your pharmacist.

Appendix D. PILL DIARY (HCQ oral administration log) – sample draft

Day of course	Date	AM	PM	Side effects
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Day of course	Date	AM	PM	Side effects
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Day of course	Date	AM	PM	Side effects
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APPENDIX E. Lab Manual and summary of work from Flow Cytometry & Immune Monitoring SRF

Personnel:

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Immune monitoring procedures and assays to be performed:

Overview: Blood from HCQ-treated patients will be drawn into BD Vacutainer® CPT™ Mononuclear Cell Preparation Tube (8ml) containing Sodium Heparin at days 0, 30, and 60 of treatment. At each time point, de-identified blood samples in CPT tubes will be sent to FCIM for immediate separation of plasma and peripheral blood mononuclear cells (PBMCs) and cryopreservation. All cryopreserved blood samples will be assayed in bulk following collection of all patient samples. At that time, PBMCs will be assessed for immune phenotypes and function as detailed in the following section. Plasma will be assessed for cytokine levels as detailed below.

PBMC assays

1. Immunophenotyping of T cell subsets
2. Intracellular cytokine (IL-10, IL-17, and IFN-ɣ) in T cell subsets
3. Lysosomal acidification in T cell subsets
4. Expression of autophagy in T cell subsets

Plasma assays

1. Concentration of IFNɣ in plasma
2. Concentration of TNFα in plasma
3. Concentration of C-reactive protein in plasma

Immunophenotyping of T cell subsets:

1. Freshly thawed aliquots of each PBMC sample (2 x 10^6 cells/vial) will be resuspended in FACS staining Buffer (100µl) containing Human IgG Fc-receptor blocking antibody for 20 min.
2. After blocking, antibody cocktail (T cell cocktail) (50µl) will be added to each tube and incubated @ 4^{o}C for 60 min in the dark.

Table 1: T cell antibody cocktail (fluorochromes are tentative)

#	Antibody	Fluorochrome	Dilution (final)
1	Anti-human CD3	FITC	Optimal dose determined by titration.
2	Anti-human CD4	BV420	Optimal dose determined by titration.
3	Anti-human CD8	PercP	Optimal dose determined by titration.
4	Anti-human CD45RA	APC	Optimal dose determined by titration
5	Anti-human CD45RO	PE	Optimal dose determined by titration.
6	Anti-human CD25	Texas-red	Optimal dose determined by titration.
NOTE: Single antibody controls will be prepared with each of the above antibodies using OneComp eBeads (eBioscience). Appropriate FMO controls will be run to identify some cell subsets.			

3. Cells will be washed twice by adding 4 ml of PBS and centrifuging cells @1400 RPM for 5 min.
4. Cells then will be fixed using 100ul of paraformaldehyde (4% in PBS) and incubated for 15 min @4^{o}C.
5. Cells will be washed twice with PBS and resuspended in FACS buffer for flow cytometric analysis.

Intracellular cytokine staining for detection of IL-10, IL-17, and IFN-ɣ:

1. Freshly thawed aliquots of each PBMC sample (2 x 10^6 cells/vial) will be resuspended in 1 ml of cell culture media (1X10^6 cells/ml).
2. PBMCs will be cultured in IL-2 containing media overnight to allow for recovery of cells from thawing.
3. PBMCs will be cultured in the presence of PMA/ionomycin (60ng/3µM) for 6hrs in a cell culture media supplemented with BD GolgiStop.
4. After incubation, cells will be washed twice with FACS cell staining buffer and resuspended in FACS staining buffer (100µl) containing Human IgG Fc-receptor blocking antibody for 20 min.
5. After blocking, antibody cocktail (Intracellular cytokine cocktail) (50µl) will be added to each tube and incubated @ 4^{o}C for 60 min in the dark.

Table 2: Intracellular cytokine cocktail (flurochromes are tentative)

#	Antibody	Fuorochrome	Dilution (final)
1	Anti-human CD3	FITC	Optimal dose determined by titration.
2	Anti-human CD4	BV420	Optimal dose determined by titration.
3	Anti-human CD8	PerCP	Optimal dose determined by titration.
NOTE: Single antibody controls will be prepared with each of the above antibodies using OneComp eBeads (eBioscience). Appropriate FMO controls will be run to identify cell subsets and cytokine expression.			

6. Cells will be washed twice by adding 4 ml of PBS and centrifuging cells @1400 RPM for 5 min.
7. Cells will be fixed using 100ul of fix/perm buffer for 30 min in the dark to permeabilize cells
8. Cells will be resuspended in 50 µl perm buffer containing APC-Cy7 anti-human IL-17, PE-Cy7 anti-human IFN-ɣ, and APC anti-human IL-10 and incubated for 45 min in dark @4^{o}C.
9. Cells will be washed twice with perm wash buffer and resuspend in FACS buffer for flow analysis.

Detection of lysosomal acidification:

1. Freshly thawed aliquots of each PBMC sample (2 x 10^6 cells/vial) will be resuspended in 1 ml of cell culture media (1X10^6 cells/ml).
2. Lyso sensor yellow/blue DND-10 will be added to each sample as defined by manufacturer.
3. Cells will be washed in FACS cell staining buffer containing sodium azide and resuspended in FACS staining buffer (100µl) containing Human IgG Fc-receptor blocking antibody for 20 min.
4. After blocking, antibody cocktail (Lysosomal cocktail) (50µl) to each tube and incubate cells @ 4^{o}C for 30 min in the dark.

Table 1: Lysosomal cocktail (flurochromes are tentative)

#	Antibody	Fluorochrome	Dilution (final)
1	Anti-human CD3	FITC	Optimal dose determined by titration.
2	Anti-human CD4	PE	Optimal dose determined by titration.
3	Anti-human CD8	PerCP	Optimal dose determined by titration.
4	Anti-human CD14	APC	Optimal dose determined by titration.
NOTE: Single antibody controls will be prepared with each of the above antibodies using OneComp eBeads (eBioscience). Appropriate FMO controls will be run to identify cell subsets and lysosomal acidification.			

5. Cells will be washed twice by adding 4 ml of PBS and centrifuging cells @1400 RPM for 5 min.
6. Cells then will be fixed using 100ul of paraformaldehyde (4% in PBS) and incubated for 15 min @4^{o}C.
7. Cells will be washed twice with PBS and resuspended in FACS buffer for flow cytometric analysis.

Detection of cell autophagy:

1. Freshly thawed aliquots of each PBMC sample (2 x 10^6 cells/vial) will be resuspended in FACS staining Buffer (100µl) containing Human IgG Fc-receptor blocking antibody for 20 min.
2. After blocking, antibody cocktail (Autophagy cocktail) (50µl) will be added to each tube and incubated @ 4^{o}C for 60 min in the dark.

Table 1: Autophagy cocktail (flurochromes are tentative)

#	Antibody	Fluorochrome	Dilution (final)
1	Anti-human CD3	FITC	Optimal dose determined by titration.
2	Anti-human CD4	PE	Optimal dose determined by titration.
3	Anti-human CD8	PerCP	Optimal dose determined by titration.
4	Anti-human CD14	APC	Optimal dose determined by titration.
5	Anti-human LC3B (autophagy marker)	PE-Cy7	Optimal dose determined by titration.
6	Anti-SQSTM1 / p62 antibody (autophagy marker)	APC-Cy7	Optimal dose determined by titration.
NOTE: Single antibody controls will be prepared with each of the above antibodies using OneComp eBeads (eBioscience). Appropriate FMO controls will be run to identify cell subsets and autophagy.			

3. Cells will be washed twice by adding 4 ml of PBS and centrifuging cells @1400 RPM for 5 min.
4. Cells will be fixed using 100ul of fix/perm buffer for 30 min in the dark to permeabilize cells
5. Cells will be resuspended in 50 µl perm buffer containing Autophagy cocktail #2 and incubated for 45 min in dark @4^{O}C.
6. Cells will be washed twice with perm wash buffer and resuspend in FACS buffer for flow analysis.

Table 2: Autophagy cocktail #2 (flurochromes are tentative)

#	Antibody	Fluorochrome	Dilution (final)
1	Anti-human LC3B (autophagy marker)	PE-Cy7	Optimal dose determined by titration.
2	Anti-SQSTM1 / p62 antibody (autophagy marker)	APC-Cy7	Optimal dose determined by titration.
NOTE: Single antibody controls will be prepared with each of the above antibodies using OneComp eBeads (eBioscience). Appropriate FMO controls will be run to identify cell subsets and autophagy.			

<u>Cytokine detection by ELISA</u>

1. Frozen aliquots of plasma samples will be allowed to thaw at room temperature and then vortexed to mix.
   ELISA assays for IFNɣ, TNFα, and CRP will be performed as described in the manufacturer protocols and measured in a multiwall plate reader.

APPENDIX F. Clavien-Dindo Classification of Surgical Complications [28]

Grades	Definition
Grade I:	Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic and radiological interventions.
	Acceptable therapeutic regimens are: drugs as antiemetics, antipyretics, analgetics, diuretics and electrolytes and physiotherapy. This grade also includes wound infections opened at the bedside.
Grade II:	Requiring pharmacological treatment with drugs other than such allowed for Grade I complications.
	Blood transfusions and total parenteral nutrition are also included.
Grade III:	Requiring surgical, endoscopic or radiological intervention
Grade III-a:	intervention not under general anesthesia
Grade III-b:	intervention under general anesthesia
Grade IV:	Life-threatening complication (including CNS complications)‡ requiring IC/ICU-management
Grade IV-a:	single organ dysfunction (including dialysis)
Grade IV-b:	multi organ dysfunction
Grade V:	Death of a patient
Suffix 'd':	If the patient suffers from a complication at the time of discharge (see examples in Appendix B, http://Links.Lww-.com/SLA/A3), the suffix "d" (for 'disability') is added to the respective grade of complication. This label indicates the need for a follow-up to fully evaluate the complication.

‡ brain hemorrhage, ischemic stroke, subarrachnoidal bleeding, but excluding transient ischemic attacks (TIA);
IC: Intermediate care;
ICU: Intensive care unit
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APPENDIX G. Quality of Life: EORTC's Validated, Patient-Report Questionnaires

Quality of Life: This is assessed by two validated patient-reported outcomes measures, the EORTC QLQ-C30 and QLQ-PR25 questionnaires. All items assessing symptoms and functional domains employ a 4-point Likert scale, with responses ranging from "Not At All", "A Little", "Quite a Bit" and "Very Much." Study participants will be instructed to choose one response per item. The EORTC **QLQ-C30** is a 30-item questionnaire assessing multiple domains of global health-related quality of life in cancer populations. It includes one global QOL rating, 5 functioning scales (physical, cognitive, emotional, role and social) and 9 symptom-specific subscales (fatigue, N/V, pain, dyspnea, insomnia, constipation, diarrhea, appetite loss). The EORTC **QLQ-PR25** is a prostate cancer module meant for use among patients in varying in disease stage and treatment modality (i.e., surgery, chemotherapy, radiotherapy, etc.), which complements the QLQ-C30. The QLQ-PR25 module comprises 25 items assessing disease symptoms, treatment side effects, sexual functioning and sexual activity. The functional scales are sexual activity and sexual functioning; the 4 symptom scales are urinary symptoms, bowel symptoms, hormonal treatment-related symptoms, and incontinence aid. All participants will complete items 31-49 as well as items 50-51 (which query current sexual activity).

For interpretation of scores on the two EORTC QOL questionnaires, all of the scales and single-item measures range in score from 0 – 100. A high scale score represents a higher response level – so that a higher score represents a higher ("better") level of functioning OR a higher ("worse) level of symptoms.

EORTC	Item / Scale	# of Items	Responses	Item No.s	Score Interpretation
QLQ-C30	Global QOL	2	1 - 7	29, 30	Higher score = better global QOL
QLQ-C30	Physical Functioning	5	1 - 4	1-5	Higher score = better functioning
	Cognitive Functioning	2		20, 25	
	Emotional Functioning	4		21-24	
	Role Functioning	2		6, 7	
	Social Functioning	2		26, 27	
QLQ-C30 Symptoms	Fatigue	3	1 - 4	10, 12, 18	Higher score = more/worse symptoms
	Nausea / Vomiting	2		14, 15	
	Pain	2		9, 19	
	Dyspnea	1		8	
	Insomnia	1		11	
	Appetite Loss	1		13	
	Constipation	1		16	
	Diarrhea	1		17	
	Financial Difficulties	1		28	
QLQ-PR25	Sexual Activity	2	1 - 4	50, 51	Higher score = better functioning
	Sexual Functioning *	4		52-55	
QLQ-PR25 Symptoms	Urinary Symptoms	8	1 – 4	31-37, 39	Higher score = more/worse symptoms
	Bowel Symptoms	4		40-43	
	Hormonal Treatment-related Symptoms	6		44-49	
	Incontinence Aid	1			

* Completion of Items 52-55 are conditional on being sexually active (and comprise the Sexual Functioning subscale), and thus, these 4 items will only be completed by a subgroup of study patients. Response to item #52 keeps original scoring, while responses to items 53-55 are reverse-scored. Specifically, for items 53-55, a response of "4" is recoded as "1", a response of "3" is recoded as "2", a response of "2" is recoded as "3", and a response of "1" is recoded as "4"). This reverse-scoring preserves the Functional scale property, where a higher response indicates higher functioning.

EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials:
Your birthdate (Day, Month, Year):
Today's date (Day, Month, Year):

	Not at All	A Little	Quite a Bit	Very Much
1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2. Do you have any trouble taking a long walk?	1	2	3	4
3. Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4. Do you need to stay in bed or a chair during the day?	1	2	3	4
5. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:

	Not at All	A Little	Quite a Bit	Very Much
6. Were you limited in doing either your work or other daily activities?	1	2	3	4
7. Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8. Were you short of breath?	1	2	3	4
9. Have you had pain?	1	2	3	4
10. Did you need to rest?	1	2	3	4
11. Have you had trouble sleeping?	1	2	3	4
12. Have you felt weak?	1	2	3	4
13. Have you lacked appetite?	1	2	3	4
14. Have you felt nauseated?	1	2	3	4
15. Have you vomited?	1	2	3	4
16. Have you been constipated?	1	2	3	4

Please go on to the next page

During the past week:	Not at All	A Little	Quite a Bit	Very Much
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1 2 3 4 5 6 7

Very poor Excellent

30. How would you rate your overall quality of life during the past week?

1 2 3 4 5 6 7

Very poor Excellent

Please go on to the next page

EORTC QLQ - PR25

Patients sometimes report that they have the following symptoms or problems. Please indicate the extent to which you have experienced these symptoms or problems during the past week. Please answer by circling the number that best applies to you.

During the past week	Not at all	A little	Quite a bit	Very much
31. Have you had to urinate frequently **during the day**?	1	2	3	4
32. Have you had to urinate frequently **at night**?	1	2	3	4
33. When you felt the urge to pass urine, did you have to hurry to get to the toilet?	1	2	3	4
34. Was it difficult for you to get enough sleep, because you needed to get up frequently at night to urinate?	1	2	3	4
35. Have you had difficulty going out of the house because you needed to be close to a toilet?	1	2	3	4
36. Have you had any unintentional release (leakage) of urine?	1	2	3	4
37. Did you have pain when you urinated?	1	2	3	4
38. Answer this question only if you wear an incontinence aid. Has wearing an incontinence aid been a problem for you?	1	2	3	4
39. Have your daily activities been limited by your urinary problems?	1	2	3	4
40. Have your daily activities been limited by your bowel problems?	1	2	3	4
41. Have you had any unintentional release (leakage) of stools?	1	2	3	4
42. Have you had blood in your stools?	1	2	3	4
43. Did you have a bloated feeling in your abdomen?	1	2	3	4
44. Did you have hot flushes?	1	2	3	4
45. Have you had sore or enlarged nipples or breasts?	1	2	3	4
46. Have you had swelling in your legs or ankles?	1	2	3	4

Please go to the next page

During the last 4 weeks…	Not at all	A little	Quite a bit	Very much
47. Has weight **loss** been a problem for you?	1	2	3	4
48. Has weight **gain** been a problem for you?	1	2	3	4
49. Have you felt less masculine as a result of your illness or treatment?	1	2	3	4
50. To what extent were you interested in sex?	1	2	3	4
51. To what extent were you sexually active (with or without intercourse)?	1	2	3	4

PLEASE ANSWER THE NEXT FOUR QUESTIONS ONLY IF YOU HAVE BEEN SEXUALLY ACTIVE OVER THE LAST 4 WEEKS

52. To what extent was sex enjoyable for you?	1	2	3	4
53. Did you have difficulty getting or maintaining an erection?	1	2	3	4
54. Did you have ejaculation problems (eg dry ejaculation)?	1	2	3	4
55. Have you felt uncomfortable about being sexually intimate?	1	2	3	4

EORTC
European Organisation for Research and Treatment of Cancer

EORTC Quality of Life

Prostate cancer module: QLQ-PR25

Scope

The prostate cancer module is meant for use among patients with prostate cancer varying in disease stage and treatment modality (i.e. surgery, chemotherapy, radiotherapy, etc.). It should always be complemented by the QLQ-C30.

Scoring

	Scale name	Number of items	Item range	QLQ-C30+PR25 Item numbers	QLQ-PR25 item numbers
Functional scales					
Sexual activity	PRSAC	2	3	50, 51	20, 21
Sexual functioning	PRSFU	4	3	52-55 (reverse code responses to 53-55)	22 – 25 (reverse code responses to 23-25)
Symptom scales					
Urinary symptoms	PRURI	8	3	31-37, 39	1 – 7, 9
Bowel symptoms	PRBOW	4	3	40-43	10 – 13
Hormonal treatment-related symptoms	PRHTR	6	3	44-49	14 – 19
Incontinence aid	PRAID	1	3	38	8

Remarks

- Items 20 and 21 can be completed by all patients
- Items 22-25 are conditional on being sexually active, and thus will only be completed by a subgroup of patients. This will require reversing the response categories of questions 23-25 but not of 22.
- If reporting scale level data, it is highly recommended that some basic psychometric analyses be carried out. Minimally, one would want to look at the internal consistency of the scales (using a reliability program of a statistical software package that calculates a Cronbach's alpha coeffieient). This coefficient should preferably be above 0.70 for purposes of group comparisons. You do not need to recode the items to perform the reliability analysis.

Reference

van Andel G, Bottomley A, Fosså SD, et al. An International Field Study of the EORTC QLQ-PR25: a Questionnaire for Assessing the Health-Related Quality of Life of Patients with Prostate Cancer. *Eur J Cancer* 44:2418-2424, 2008.

APPENDIX H 1-2 – Response Criteria for Disease Progression

- **H.1 RECIST v1.1 for soft-tissue metastatic lesions**
- **H.2 MDA Criteria for bone-specific response criteria**

H.1 Response Evaluation Criteria in Solid Tumors (RECIST 1.1)* to be used for soft-tissue metastatic lesions

Response category	Criteria
Complete response	Disappearance of all target lesions
	Reduction in short axis of target lymph nodes to < 10 mm
Partial response	Decrease in target lesion diameter sum ≥ 30%†
Progressive disease	Increase in target lesion diameter sum ≥ 20%‡
	≥ 5 mm increase in target lesion diameter sum
	New, malignant FDG uptake in the absence of other indications of progressive disease or an anatomically stable lesion, and confirmed on contemporaneous or follow-up CT
	Unequivocal progression of nontarget lesions
Stable disease	Does not meet other criteria‡

*Measurements are based on the sum of the unidimensional measurement of the greatest diameter of a maximum 5 lesions.
†Reference standard: baseline sum. ‡Reference standard: smallest recorded sum. Table modified from Eisenhauer et al.

H.2 MD Anderson Cancer Center-Criteria for bone response criteria for bone metastases *

Response category	Criteria
Complete response	Complete sclerotic fill-in of lytic lesions on XR or CT
	Normalization of bone density on XR or CT
	Normalization of signal intensity on MRI
	Normalization of tracer uptake on SS
Partial response	Development of a sclerotic rim or partial sclerotic fill-in of lytic lesions on XR or CT.
	Osteoblastic flare - Interval visualization of lesions with sclerotic rims or new sclerotic lesions in the setting of other signs of PR and absence of progressive bony disease
	≥ 50% decrease in measurable lesions on XR, CT, or MRI
	≥ 50% subjective decrease in the size of ill-defined lesions on XR, CT, or MRI
	≥ 50% subjective decrease in tracer uptake on SS
Progressive disease	≥ 25% increase in size of measurable lesions on XR, CT, or MRI
	≥ 25% subjective increase in the size of ill-defined lesions on XR, CT, or MRI
	≥ 25% subjective increase in tracer uptake on SS
	New bone metastases
Stable disease	No change
	< 25% increase or < 50% decrease in size of measurable lesions
	< 25% subjective increase or < 50% subjective decrease in size of ill-defined lesions
	No new bone metastases

*Measurements are based on the sum of a perpendicular, bidimensional measurement of the greatest diameters of each individual lesion.
Abbreviations: XR, radiography; CT, computed tomography; SS, skeletal scintigraphy; MRI, magnetic resonance imaging.
Table modified from Hamaoka et al. (27).