COVER PAGE FOR PROTOCOL AND STATISTICAL ANALYSIS PLAN

Official Study Title: Modulation of Autophagy: A Clinical Study of Vorinostat Plus Hydroxychloroquine Versus Regorafenib in Refractory Metastatic Colorectal Cancer (mCRC) Patients

NCT number: NCT02316340

IRB Approval Date: 06.14.2017

Unique Protocol ID: CTMS# 14-2015

Modulation of Autophagy: A Clinical Study of Vorinostat plus Hydroxychloroquine versus Regorafenib in Refractory Metastatic Colorectal Cancer (mCRC) Patients

Date: June 14, 2017

Coordinating Institution:
UT Health Cancer Center, 7979 Wurzbach Road, San Antonio, TX 78229

Study Chairperson: Dr. Sukeshi Patel Arora
Phone: 210-450-1015

Study Statistician: Joel Michalek, PhD

Protocol ID: CTMS# 14-2015

Protocol Amendment 5.0
Protocol Amendment 4.0
Protocol Amendment 3.0
Protocol Amendment 2.0
Protocol Version 1.0

INVESTIGATOR'S AGREEMENT

I have read and understand the contents of this clinical protocol for Protocol UT Health Cancer Center CTMS#14-2015 and will adhere to the study requirements as presented, including all statements regarding confidentially. In addition, I will conduct the study in accordance with current international conference on harmonization (ICH) guidance, Good Clinical Practice (GCP) guidance, the Declaration of Helsinki, US Food and Drug Administration (FDA) regulations and local IRB and legal requirements.

Name of Clinical Investigator: _____
Institution: _____

Investigator Signature: _____ Date:_____

ABBREVIATIONS

AE	Adverse Event
BISR	Biostatistics and Informatics Shared Resource
CAI	Corrective Action Item
DSM	Data Safety Monitoring
DSMB	Data Safety Monitoring Board
DSMC	Data Safety Monitoring Committee
DSMP	Data Safety and Monitoring Plan
DSO	Data and Safety Officer
DQA	Director of Quality Assurance
FDA	Food and Drug Administration
GCP	Good Clinical Practice
IDD	Institute for Drug Development (UT Health Cancer Center)
IDEAS	Informatics Data Exchange Acquisition System
IIS	Investigator Initiated Protocol
IND	Investigational New Drug
IRB	Institutional Review Board
NCI	National Cancer Institute
QAD	Quality Assurance Division
PALS	Priority of Audit Level Score
PI	Principal Investigator
PSD	Pharmacokinetic Sampling Department
SAE	Serious Adverse Event
UPIRSO	Unanticipated Problem Involving Risks to Subjects or Others

Table of Contents:

1.0 Synopsis

Title of Study: Modulation of autophagy: A clinical study of vorinostat plus hydroxychloroquine versus Regorafenib in refractory metastatic colorectal cancer (mCRC) patients.

Investigators: PI: Sukeshi Patel Arora, MD
Statistician: Joel Michalek, PhD

Study Center(s): UT Health Cancer Center

Concept and Rationale: Colorectal cancer (CRC) is the second most common cause of cancer death in the United States (1). Recently, Regorafenib (RGF), an oral multi-kinase inhibitor with predominant angiogenic or vascular endothelial growth factor (VEGF) inhibition, was approved for patients with mCRC who have progressed on standard therapies, including anti-VEGF agent bevacizumab (2). Compared to placebo, the phase III CORRECT study showed RGF improved median overall survival (mOS) from 5.0 to 6.4 months (mo) and median progression-free survival (mPFS) from 1.7 to 1.9 mo, regardless of K-Ras mutational status (3). Given the modest improvements in survival and clinical studies showing lack of efficacy with continued VEGF inhibition following progression of anti-VEGF therapies, such as bevacizumab, novel therapies continue to be desperately needed in the third-line setting (4). Investigators at our institution have shown that autophagy inhibition using hydroxychloroquine (HCQ) enhanced the apoptotic activity of the histone deacetylase (HDAC) inhibitor, vorinostat (VOR), via ubiquitinated protein accumulation in pre-clinical CRC models (5). Based on this preclinical data, we completed an NIH-funded phase I clinical study evaluating the safety, efficacy, and pharmacokinetics of the combination VOR and HCQ and found that patients with mCRC obtain prolonged clinical benefit with the combination therapy with VOR and HCQ (6). This study led to a Phase II single-arm study of VOR 400 mg PO (orally) daily plus HCQ 600 mg PO daily in patients with mCRC, which has completed accrual of a planned 18 patients but follow-up is presently on going. To date, 5 patients have received 6+ cycles (preliminary mPFS of >3 months), further supporting its activity in mCRC.

Further evaluation of biomarkers of autophagy, such as cathepsin D, and biomarkers of VOR, CDKN1A, that correlate with improved efficacy will help identify patients who benefit from autophagy inhibition (6). The expression of high-mobility group box 1 (HMGB1) increases with autophagy in CRC, and HMGB1 acts upon binding to RAGE ("receptor for advanced glucated end products") (7, 8).

In addition, HDAC has been well-recognized as potential targets for the treatment of numerous aging-related disorders (9) such as cancer (10). Therefore, we plan to evaluate the potential role of biomarkers of aging with the combination of HCQ plus VOR.

These findings support a phase II study VOR/HCQ versus standard therapy RGF in patients failing standard therapies.

*We **hypothesize** that VOR/HCQ will have improved efficacy when compared to RGF in treatment-refractory mCRC patients.* This hypothesis is based on the observations that:

1. In refractory mCRC patients, the role of continued VEGF inhibition in mCRC has not been established (4).
2. Only a modest improvement in survival was reported with the use of RGF, an oral multi-kinase inhibitor with predominantly angiogenic inhibition, for mCRC patients who have failed standard therapies (3).
3. Autophagy inhibition enhances vorinostat-induced apoptosis via ubiquitinated protein accumulation in CRC models pre-clinically (5).
4. Clinical activity based on treatment-refractory mCRC patients enrolled in phase I (6) and the ongoing single arm Phase II study of VOR with HCQ therapy suggests a median PFS of > 3 months.

Our overall objective is to answer these questions: will VOR/HCQ be more efficacious than RGF, will VOR/HCQ be safe, and can biomarkers be identified that will predict clinical efficacy of VOR/HCQ and RGF in refractory mCRC patients?

Primary Objective(s):

- To determine the clinical efficacy with progression-free survival (PFS-1) of the combination of VOR plus HCQ when compared to RGF in treatment-refractory mCRC.

Secondary Objective(s):

- To determine the overall survival in refractory mCRC patients receiving VOR plus HCQ when compared to RGF.
- To determine the progression-free survival (PFS-2) of the crossover treatment, which is optional.
- To evaluate the tumor response rate in refractory mCRC patients receiving VOR plus HCQ when compared to RGF.
- To further define the safety of the combination of VOR/HCQ when compared to RGF in treatment-refractory mCRC.
- To identify biomarkers of autophagy and anti-angiogenic inhibition that are associated with clinical efficacy of VOR/HCQ and RGF, respectively, in mCRC.

Primary Endpoint(s):

- PFS-1: measured from the start of the first treatment the patient is randomized to until the date the criteria for progression are met or the date the patient is taken off study for any reason, whichever is shorter.

Secondary Endpoint(s):

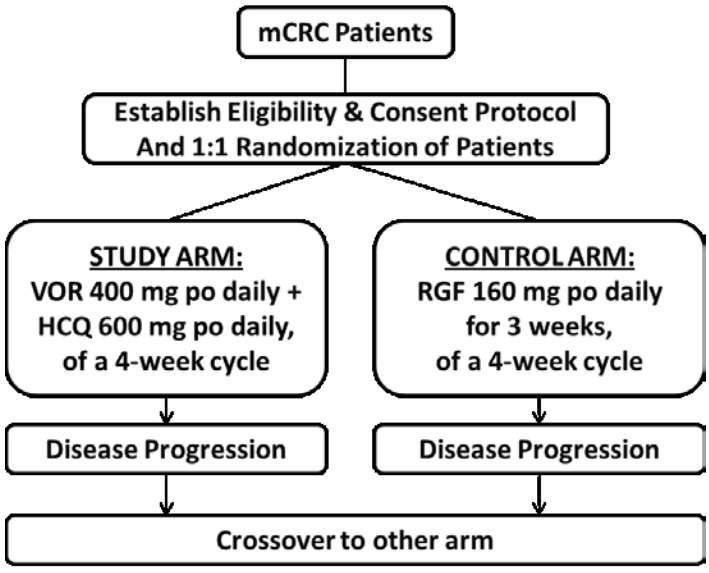
- OS: measured from the start of treatment on trial until the death of the patient.
- mPFS-2**:** measured from the start of the crossover treatment (second regimen, which is optional) until the date the criteria for progression are met or the date the patient is taken off study for any reason, whichever is shorter.

- Response: measured as best response, complete response (CR) or partial response (PR), as defined by Response Evaluation Criteria in Solid Tumors (RECIST 1.1) Committee.
- Adverse events: grade 1-5 adverse events according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v4.0**;** measured at baseline, each cycle, and at off study date.
- Biomarkers of efficacy of VOR/HCQ and RGF: evaluate biomarkers of autophagy and anti-angiogenic inhibition that are associated with clinical efficacy of VOR/HCQ and RGF, respectively, in mCRC.

Study Design:

This will be a randomized phase II clinical trial of patients with histologic documentation of metastatic colorectal cancer, who have received local and currently approved standard therapies, excluding RGF. We will give VOR 400 mg PO daily and HCQ 600 mg PO daily in 4-week cycles. Patients will require imaging up to 6 weeks prior to enrollment and will be assessed for measureable evidence of mCRC. This will be a randomized, controlled phase II clinical trial of patients with histological documentation of metastatic colorectal cancer, who have received locally and currently approved standard therapies, excluding RGF. Patients will be randomized 1:1 to RGF or VOR/HCQ (see schema below). Also, crossover is optional after first progression on the initial therapy, and based on physician discretion and in the best interest of the patient. If crossover is not done, then the patient will be off study and can go on to receive other treatments.

Schema:



****Crossover is optional and at the discretion of the physician and in the best interest for the patient**

Number of Patients: Historically, the mPFS for previously treated mCRC patients treated with RGF is 1.9 mos. We predict VOR/HCQ will improve mPFS to 3.8 months. We expect 5% lost to follow-up. Assuming a 36-month accrual period with 1 year for follow up, 36 subjects per group are necessary to obtain 80% power. With 5% lost to follow-up the sample size required per group is 38 (=36/0.95), therefore, the total required sample size is 76 subjects. As a result, the target accrual rate is 2-4 patients/month to achieve a target accrual at 36 months with an additional 12-month follow-up.

Main Criteria for Inclusion/Exclusion: Inclusion Criteria:

1. Histological documentation of metastatic colorectal cancer (mCRC).
2. ECOG performance status of 0-2.
3. Radiographical documentation of metastatic disease with imaging up to 6 weeks prior to enrollment.
4. Patients with mCRC must have been previously treated with irinotecan and/or oxaliplatin and/or VEGF/EGFR therapy or intolerant to these agents
5. Documentation of K-Ras mutational status
6. Adequate hematologic, renal and liver function (i.e. absolute neutrophil count > 1000/mm^3, platelets > 75,000/mm^3); creatinine < 2 times the upper limits of normal (ULN) total bilirubin < 1.5 mg/dl, ALT and AST< 3 times above the ULN, ALT and AST can be < 5 times ULN if patients have hepatic involvement.
7. Able to provide written informed consent.
8. Patients with the potential for pregnancy or impregnating their partner must agree to follow acceptable birth control methods to avoid conception. Women of childbearing potential must have a negative pregnancy test within 72 hours prior to receiving the investigational product.
9. Tumor blocks available from previous surgery/biopsy, or if not available, patients willing to have biopsy.

Exclusion Criteria:

1. Patients receiving prior therapy with RGF, VOR, and/or HCQ.
2. Patients with uncontrolled brain metastases. Patients with brain metastases must be asymptomatic and off corticosteroids for at least one week.
3. Due to risk of disease exacerbation, patients with porphyria are not eligible.
4. Due to risk of disease exacerbation, patients with psoriasis are ineligible unless the disease is well controlled, and they are under the care of a specialist for the disorder who agrees to monitor the patient for exacerbations.
5. Patients with previously documented macular degeneration or untreated diabetic retinopathy (stable retinopathy is allowed).
6. Patients who have had chemotherapy or radiotherapy within 2 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study. For investigational targeted therapies, patients will need to clear for 5 half-lives (not applicable to standard of care therapies).
7. Patients may not be receiving any other investigational agents.
8. Patients should not have taken valproic acid or another histone deacetylase inhibitor for at least 2 weeks prior to enrollment.
9. History of allergic reactions attributed to compounds of similar chemical or biologic composition to VOR or HCQ.
10. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac

arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements

11. Major surgery or significant traumatic injury occurring within 21 days prior to treatment.
12. QTc > 500 ms at baseline.
13. Gastrointestinal tract disease resulting in an inability to take oral medication or a requirement for IV alimentation, prior surgical procedures affecting absorption, or active peptic ulcer disease. Patients with NG-tube, J-tube, or G-tube will not be allowed to participate.
14. Pregnant women are excluded from this study because vorinostat has the potential for teratogenic or abortifacient effects. For this reason, women of childbearing potential and men must also agree to use adequate contraception (hormonal or barrier method of birth control) prior to study entry and for the duration of study participation.
15. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with vorinostat, breastfeeding should be discontinued.
16. Informed Consent - No study specific procedures will be performed without a written and signed informed consent document. Patients who do not demonstrate the ability to understand or the willingness to sign the written informed consent document will be excluded from study entry.

Inclusion of Women and Minorities:

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

Intervention and Mode of Delivery: If randomized to VOR/HCQ arm, we will administer VOR 400 mg PO daily and HCQ 600 mg PO daily in 4-week cycles. If randomized to RGF arm, we will administer RGF 160 mg PO daily for 3 weeks, of a 4week cycle. Crossover to the other arm will be allowed, and is OPTIONAL, if the patient has disease progression or unacceptable toxicity. The option to get the crossover treatment will be left to the physician discretion, and if in the best interest of the patient.

Duration of Intervention and Evaluation: Follow-up: A repeat CT scan will be performed after 2 cycles of treatment regimen to evaluate response based on RECIST 1.1 criteria. CT scans will be repeated at least every 2 cycles, or 8 weeks, to ensure no progression of disease. Patients will continue VOR/HCQ or RGF until disease progression, unacceptable toxicity, withdrawal of consent by the patient, or decision of physician for patient's best interest. Crossover to the other arm will be allowed, but OPTIONAL, and will be determined by the treating physician for the patient's best interest. Each patient will be followed for 1 year. We will follow the last patient for 12 months, at which time, the study will be completed for data analysis.

Interim analysis: The O'Brien-Fleming procedure will be used to conduct an interim analyses for efficacy when 50% of the patients complete the study. A statistical basis for stopping the study at the first look will be achieved if the test statistic indicates benefit in the VOR/HCQ Arm and exceeds 2.96259, corresponding to a significance level of 0.003.

If the test statistic does not exceed 2.96259 at the first look then the trial will proceed to the final analysis. A treatment effect will be declared significant and beneficial at the final analysis if the test statistic indicates benefit and exceeds 1.96857, corresponding to a significance level of 0.047. If a treatment effect is not declared at the interim analysis, a statistical basis for stopping the study for futility will be attained if the conditional power is less than or equal to 20%.

Statistical Methods:

Analytic plan for primary objective:

- mPFS-1: Historically, the mPFS for previously treated mCRC patients treated with RGF is 1.9 mos. We predict VOR/HCQ will improve mPFS to 3.8 months. We expect 5% lost to follow-up. Assuming a 36-month accrual period with 1 year for follow up, 36 subjects per group are necessary to obtain 80% power. With 5% lost to follow-up the sample size required per group is 38 (=36/0.95), therefore, the total required sample size is 76 subjects. As a result, the target accrual rate is 2-4 patients/month to achieve a target accrual at 36 months with an additional 12-month follow-up.

Interim analysis: The O'Brien-Fleming procedure will be used to conduct an interim analyses for efficacy when 50% of the patients complete the study. A statistical basis for stopping the study at the first look will be achieved if the test statistic indicates benefit in the VOR/HCQ Arm and exceeds 2.96259, corresponding to a significance level of 0.003. If the test statistic does not exceed 2.96259 at the first look then the trial will proceed to the final analysis. A treatment effect will be declared significant and beneficial at the final analysis if the test statistic indicates benefit and exceeds 1.96857, corresponding to a significance level of 0.047. If a treatment effect is not declared at the interim analysis, a statistical basis for stopping the study for futility will be attained if the conditional power is less than or equal to 20%.

Analytic plan for secondary objectives:

- OS: The analyses of median OS will be performed in the intent-to-treat population. OS will be illustrated with Kaplan-Meier plots, the estimate of median OS with its 95% confidence interval.
- mPFS-2: The analyses of median PFS-2 will be performed in the crossover population (crossover is optional). mPFS-2 will be illustrated with Kaplan-Meier plots, the estimate of median PFS-2 with its 95% confidence interval. Treatment groups will be contrasted with regard to the response rate with a logistic regression model adjusted for age, sex, race and the baseline tumor K-RAS status (mutated, wild type); the odds ratio, its 95% confidence interval, and the p-value for testing the null hypothesis that the log odds ratio is zero will be presented. If the logistic model fails due to small cell counts then treatments will be contrasted with regard to response using Fisher's Exact Test.
- Response rates: Response rates will be measured as the proportion of patients with the best response, complete response (CR) or partial response (PR), as defined by RECIST 1.1 Criteria and a 95% confidence interval.
- Adverse events: All adverse events will be listed by type and grade.

- Biomarkers of efficacy of VOR/HCQ and RGF: The significance of variation in biomarkers with treatment will be assessed with linear models with and without adjustment for age, sex, race and the baseline tumor K-RAS status (mutated, wild type). The dependent variables will be log transformed (base 2) if necessary. The relation between treatment efficacy, as measured by PFS-1, and biomarkers will be assessed with proportional hazards models in terms of treatment, the biomarker, the biomarker by treatment interaction, age, sex, race, and baseline tumor K-RAS status (mutated, wild type). Significant effects will be described and illustrated, as appropriate, with Kaplan Meier plots by strata determined by biomarker level and treatment group, for example.

Sample size justification: Historically, the mPFS for previously treated mCRC patients treated with RGF is 1.9 mos. We predict VOR/HCQ will improve mPFS to 3.8 months. We expect 5% lost to follow-up. Assuming a 36-month accrual period with 1 year for follow up, 36 subjects per group are necessary to obtain 80% power. With 5% lost to follow-up the sample size required per group is 38 (=36/0.95), therefore, the total required sample size is 76 subjects. As a result, the target accrual rate is 2-4 patients/month to achieve a target accrual at 36 months with an additional 12-month follow-up.

Funding, Regulatory, and Feasibility Issues: The study will be funded by a CPRIT grant. We previously obtained VOR through Merck Pharmaceuticals for both our phase 1 and 2 studies. However, with VOR coming off patent in late 2014, the purchase of generic VOR at low cost is likely. HCQ is already being purchased at low market cost (<$50/month per patient. Thus, the VOR/HCQ combination provides a cost-effective study drug combination. We have a large referral volume of mCRC patients to our institution for consideration of clinical studies, therefore we do not expect problems with enrollment since we currently enroll 3-4 refractory mCRC patients/month onto phase I clinical trials.

Patient Acceptability/Ethics and Consent Issues: The combination of VOR/HCQ will be given in the third-line setting to patients with refractory mCRC failing first- and second-line therapies; therefore, this treatment competes with RGF or best supportive care. Since there is a clear need to identify better treatments to improve survival in mCRC, this study gives patients a treatment that is likely superior, based on preliminary data, when compared to current standard of care. Stopping rules are in place for lack of efficacy, as discussed above. We expect that VOR/HCQ will be well tolerated based on our Phase I and II preliminary data, with no grade 4 or 5 adverse events observed. However, if there is unexpected toxicity, there will be early stopping for toxicity as follows:

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

terminated. If HCQ causes grade 4 peripheral retinal toxicity, there will be prompt drug discontinuation and ophthalmologic evaluation, with consideration for termination of study.

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

2.0 Background

2.1 Metastatic colorectal cancer:

Colorectal cancer is the second most common cause of cancer death in the United States, with 20% of patients presenting with distant metastatic disease at the time of presentation (1). Patients with distant metastases have a 5-year survival rate of 13%, indicating the need for better treatments to improve survival in patients with mCRC.

2.2 VEGF inhibition in mCRC:

The VEGF pathway is essential to angiogenesis, a process that is exploited by cancer for cell proliferation and growth (13-15). Cancers of the gastrointestinal tract, including colorectal cancer, have been shown to express VEGF (16). Thus, this led to the initial phase II study of bevacizumab with a chemotherapy backbone showing encouraging improvements in survival and serving as groundwork for future studies (17). Agents targeting the angiogenic pathway have been the cornerstone of mCRC treatment for the last 10 years. Standard therapy includes systemic chemotherapy, in combination or in sequence, consisting of fluoropyrimidines, oxaliplatin, and irinotecan with monoclonal antibodies that target VEGF, bevacizumab or zivaflibercept (18). The benefit of adding bevacizumab was first demonstrated in the AVF2017 phase III study of 813 previously untreated patients randomized to irinotecan plus bolus fluorouracil and leucovorin (IFL) with placebo or bevacizumab. The study showed improvement with bevacizumab in median overall survival (mOS) (15.6 to 20.3 months, $P<0.001$) and in median progression-free survival (mPFS) (6.2 to 10.6 months, $P<0.001$) (19). Based on this study, the US FDA approved bevacizumab in 2004 as a first-line treatment for mCRC in combination with chemotherapy. In 2004, the N9741 study reported that IFL was an inferior backbone compared to fluorouracil, folinic acid, and oxaliplatin (FOLFOX) which led to most US physician adopting FOLFOX as their preferred first line regimen (20).

The well-designed first-line NO16966 trial randomized, in a 2 x 2 factorial design, 1401 previously untreated mCRC patients either to capecitabine and oxaliplatin (XELOX) or FOLFOX4, and then to bevacizumab or placebo. Adding bevacizumab to chemotherapy increased mPFS from 8.0 to 9.4 months ($P=0.0023$) and mOS from 19.9 to 21.3 months ($P=0.077$); despite a statistically significant improvement in PFS, a similar improvement in OS was not observed (21). Subsequent studies showed similar efficacy of the FOLFIRI and

FOLFOX regimens in chemotherapy-naïve mCRC patients (22, 23); consequently, bevacizumab is often combined with either FOLFOX or FOLFIRI chemotherapy. Results of these and other studies have been the basis for the prominent role of VEGF inhibition in bevacizumab-naïve mCRC patients (24, 25).

Preclinical and clinical observations suggested a rebound or flare-up of angiogenesis when VEGF-targeted therapy was withheld (26, 27). Such findings favored continuing anti-angiogenic therapy after initial clinical and/or radiological progression in the first- or second-line setting. After first progression on prior chemotherapy with three or more months of bevacizumab, the TML study was the first prospective study to show improvement in mPFS, 4.1 versus 5.7 months (P<0.0001), and mOS, 9.8 versus 11.2 months (P=0.0062), favoring bevacizumab continuation when combined with chemotherapy backbone (28), although statistically significant, gains in mOS were modest. Conversely, the phase III GONO trial randomized mCRC patients treated first-line with bevacizumab and fluoropyrimidines (FOLFIRI, FOLFOX or FOLFOXIRI) to receive mFOLFOX6 or FOLFIRI with or without bevacizumab. mPFS improved from 5.0 to 6.7 months with bevacizumab (P=0.0065), but mOS was 14.3 versus 15.9 months (P=0.11) (29). *The improvement of 1.6 months in mOS was not statistically significant. Despite this very modest difference in OS, many clinicians choose to continue patients on VEGF inhibitors.*

These landmark clinical trials have demonstrated that gains from VEGF inhibition seem to diminish when used beyond first progression, with some phase III studies showing better mPFS with VEGF inhibition, while no difference in mOS was observed (21, 29).

2.2.1 VEGF inhibition in refractory mCRC:

In refractory mCRC, the role of VEGF inhibition in mCRC patients has not been established (30). Recently, Regorafenib (RGF), an oral multi-kinase inhibitor, including with angiogenic inhibition, was approved for mCRC patients who have failed standard therapies (2). In the CORRECT trial, patients with mCRC were randomized in a 2:1 ratio to receive best supportive care with oral RGF 160 mg or placebo by mouth once daily, for 3 weeks of a 4-week cycle (3). The primary endpoint was overall survival. *Compared to placebo, RGF improved mPFS from 1.7 to 1.9 months (P<0.000001) and mOS from 5.0 to 6.4 months (P=0.005), regardless of K-RAS status (3).* The most common treatment-related toxicities were fatigue (47% vs 28%, RGF vs placebo), hand-foot syndrome (47% vs 8%), diarrhea (34% vs 8%), anorexia (30% vs 15%), voice changes (29% vs 6%), hypertension (28% vs 6%), oral mucositis (27% vs 4%), rash/desquamation (26% vs 4%), nausea (14% vs 11%), weight loss (14% vs 2%), and fever (10% vs 3%). In this study, 54% patients had grade 3 or more adverse events versus 14% with placebo, with the most common grade 3 fatigue (9% vs 5%) and grade 3 hand-foot syndrome (17% vs <1%). In the third-line setting, even when statistical significance is reached, gains in PFS and OS are modest, i.e., 0.2 months (6 days) improvement in mPFS and the 1.4 months (42 days) benefit in mOS seen with RGF over placebo (3). Given the modest improvements of RGF in survival, VEGF inhibition may not be beneficial to all patients with mCRC refractory to VEGF inhibitors; therefore, more therapies are needed in patients with mCRC progressing despite VEGF inhibition.

2.3 Autophagy as a mechanism for cancer survival:
Autophagy is a conserved protein degradation process that involves the vacuolar sequestration of long-lived cytoplasmic proteins and organelles into a structure called an autophagosome (31). Autophagosomes fuse with lysosomes, which results in the proteolytic degradation of their contents. In eukaryotic cells, autophagy plays an important role in the disposal of damaged organelles and proteins and serves to generate alternative sources of energy for cell survival during cellular stress through the catabolization of protein substrates (32). The activation of stress response genes such as p53 by anticancer therapies can stimulate autophagy in addition to apoptosis (33). *Although prolonged autophagy can result in cancer cell death, recent investigations suggest that therapy-induced autophagy is a reversible response that promotes cancer cell survival and thus, may diminish the efficacy of some therapeutic agents* (34-36). Therefore, autophagy may significantly contribute to resistance to a number of anticancer therapeutic modalities.

2.3.1 Autophagy as a mechanism of resistance to anti-cancer therapeutics.
The induction of autophagy has been observed in malignant cells after treatments, such as tamoxifen, imatinib, arsenic trioxide, histone deacetylase (HDAC) inhibitors, and rapamycin (3741). Histone deacetylase inhibitors, such as Vorinostat (VOR), have shown selective anti-cancer activity in preclinical and clinical models (10). The main effect of vorinostat, VOR, is inhibition of histone deacetylase (HDAC) activity, which causes hyperacetylation of all core histone proteins, H2A, H2B, H3 and H4. Hypoacetylation of histones is associated with condensed chromatin structure, which results in the repression of the transcription of many genes including those implicated in the regulation of cell survival, proliferation, differentiation and apoptosis. Through HDAC inhibition, vorinostat can restore the expression of silenced genes by remodeling the tightly coiled chromatin, leading to the subsequent induction of differentiation and apoptosis (42). VOR induces cell cycle arrest in either the G1 or G2M phases, downregulates cyclin Dl and D2, upregulates p53, p21 and p27 expression, and stimulates apoptosis.

The p53 tumor suppressor is a transcription factor whose activity is modulated by protein stability and post-translational modifications including acetylation. Acetylation promotes p53 stability and function. There is evidence that deacetylation of p53 is mediated by a histone deacetylase-1-containing complex, which modulates p53-mediated cell growth arrest and apoptosis (43, 44). In pre-clinical studies vorinostat also inhibited VEGF-induced expression of the VEGF receptors (VEGFR1, VEGFR2) and neuropilin, thus blocking angiogenic signaling, and reducing circulating levels of inflammatory cytokines (TNF-alpha, IL1 beta, IL6 and IFN gamma induced liposaccharide) (45, 46).

VOR was the first HDAC inhibitor to obtain FDA approval for cancer therapy. It is currently indicated for the treatment of cutaneous T-cell lymphoma (CTCL) and has demonstrated modest activity in patients with advanced solid tumors with ongoing clinical studies in advanced CRC (47). Studies have identified key mechanisms of resistance to HDAC inhibitors with the primary goals of identifying patients likely to benefit from treatment with this class of agents and developing new strategies to overcome resistance (48-50). *Pre-clinical studies have shown that HDAC inhibitor-induced autophagy blunts its anticancer activity (36).*

2.3.2 Preclinical studies of autophagy inhibition in CRC models:

Chloroquine (CQ) is a synthetic 4-arninoquinoline that has been used for more than 60 years in humans for the prophylaxis and treatment of malaria (51) and rheumatoid arthritis (52). CQ is an orally available and inexpensive drug that has CNS penetration and a broad therapeutic index. It is most predictable cumulative toxicity is retinopathy, which can be prevented by stopping administration of the drug (53, 54). CQ derivatives such as hydroxychloroquine (HCQ) are still widely used in the treatment of rheumatoid arthritis and lupus erythematosis and have a greater therapeutic index than CQ. By nature of their chemical structures, CQ derivatives function as weak bases and are trapped in acidic cellular compartments, including lysosomes (55). The deacidification of lysosomes by CQ and its derivatives impairs the activity of most lysosomal enzymes due to their strict pH requirements. Consequently, CQ and its derivatives inhibit the last step in the autophagic degradation process. When autophagy is inhibited by CQ, cells dependent on autophagy for survival increase the generation of autophagosomes and undergo either apoptotic or non-apoptotic cell death.

Our group's preclinical data have shown that autophagy inhibition can reduce tumor burden and induce apoptosis in the colon cancer xenograft model (5). CQ has been shown to be an inhibitor of autophagy by causing ubiquitinated protein accumulation enhances VOR-induced apoptosis via ubiquitinated protein accumulation, which may be a potential therapy in mCRC (5). Preclinical investigations conducted in mouse models and human cancer cell lines indicate that CQ may have significant anticancer activity due to its ability to inhibit autophagy induced by cancer therapeutics, such as VOR (5, 56). *Therapy-induced increase in the levels of lysosomal protease cathepsin D was identified as a key downstream pharmacodynamic mediator of the proapoptotic effects vorinostat when combined with the autophagy inhibitor, chloroquine (CQ) (5, 56).*

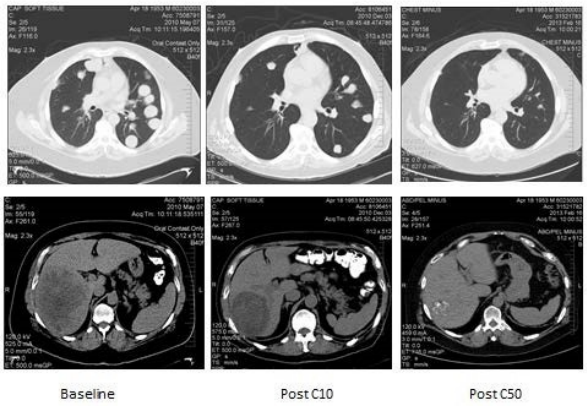


Figure 1: Representative tumor scan images demonstrating response to VOR/HCQ in phase I study.

Treatment with HCQ and VOR yielded a prolonged partial response in a patient with refractory RCC that has been durable for more than 50 cycles of therapy. MRI scans obtained at baseline and post cycles 10 and 50 (C10 and C50, respectively) are shown.

2.4 Rationale for combining VOR and HCQ.
The HDAC inhibitor vorinostat (VOR) is approved for the treatment of CTCL and is being investigated for the treatment of many other types of cancer. Vorinostat has pleiotropic effects and induces both apoptosis and autophagy. Considering that autophagy may promote cancer cell survival, we hypothesized that inhibiting autophagy would enhance the anticancer activity of VOR. Our group have reported that the autophagy inhibitor CQ dramatically augmented the antineoplastic effects of VOR in chronic myelogenous leukemia (CML) cell lines and primary CML cells expressing wild-type and imatinib-resistant mutant forms of BCR-Abl, including T315I(56). This combination regimen demonstrated selectivity for malignant cells in *in vitro* studies and its efficacy was not diminished by RNA-mediated impairment of p53 function, another factor that contributes to the resistance to imatinib and many other anticancer agents. Inhibiting autophagy with CQ promoted VOR–induced superoxide generation, triggered relocalization and marked increases in the expression of the lysosomal protease cathepsin D, and reduced the expression of the cathepsin D substrate thioredoxin. Knockdown of cathepsin D diminished the efficacy of this combination, demonstrating its role as a critical mediator of this therapeutic response (56).

We extended our preclinical evaluation of this combination to *in vitro* and *in vivo* models of colon cancer (5). Our results showed that HCQ significantly increased the pro-apoptotic effects of VOR. HCQ was well tolerated and significantly reduced the tumor burden of nude mice bearing xenografts of the HCT8 and HT29 human colon cancer cell lines as compared with mice treated with vorinostat alone. Our data suggest that the autophagy inhibitors CQ/HCQ significantly increase the anti-cancer activity of VOR and that this combination represents a promising new strategy to treat refractory cancer patients that fail conventional therapy.

2.4.1 Clinical safety of autophagy inhibition in mCRC patients.
Based on the data described above, we conducted an NCI-funded study Phase 1 Safety, Tolerability, and Phamacokinetics (PK) of HCQ in Combination with the VOR in Patients (Patients) with Advanced Solid Tumors. The primary objective of this study was to determine the maximum tolerated dose (MTD) of HCQ in combination with VOR in patients with advanced solid tumors. Patients with ECOG PS 0-2 and adequate organ function received by mouth (PO) VOR 300-400 mg daily and HCQ 400-1000 mg daily in 21-day cycles. Thirty-one patients were enrolled, with 27 evaluable for DLT due to three patients having rapid clinical deterioration related to underlying disease in cycle 1 (6). *The recommended dose is VOR 400 mg PO daily plus HCQ 600 mg PO daily. Grade 3 AE were as follows: fatigue (3), anemia (1), neutropenia (1), thrombocytopenia (1). Fatigue and gastrointestinal AE were dose-limiting toxicities.* Treatment-related toxicities were primarily grade 1-2: nausea (11 patients), diarrhea (8), fatigue (6), anorexia (4), weight loss (4), anemia (4), and elevated creatinine (4). Grade 3 toxicities were fatigue (3), anemia (1), thrombocytopenia (1) and neutropenia (1). No drugrelated deaths or grade 4 toxicities were noted. These findings indicate that administration of VOR plus HCQ at the established MTD is safe and generally well tolerated.

Tumor response was evaluated by RECIST 1.0 criteria (57). *One patient with renal cell carcinoma (RCC) who had failed seven lines of prior therapy had a confirmed durable partial/ near complete response (cohort 2, active on study with more than 50 cycles received or 3 years)*

(See Figure 1). Two patients with K-RAS mutated mCRC had prolonged stable disease (≥6 cycles). The addition of HCQ did not significantly impact the PK profile of VOR.

2.5. Preliminary data

2.5.1 Clinical efficacy of autophagy inhibition in mCRC.

This led to a Phase II single arm study of vorinostat (VOR) plus hydroxychloroquine (HCQ) in patients with advanced CRC (through UT Health Cancer Center funding). Based on phase I data, the recommended phase II dose is HCQ 600 mg PO daily with VOR 400 mg PO daily as the MTD. To date, 18 of planned 18 patients with mCRC have been enrolled to date. Of 14 patients evaluable, 4 patients have received 6+ cycles (preliminary median PFS > 3 months), indicating a potential new agent in refractory mCRC patients. Similar the phase I study, the safety profile demonstrated tolerance of VOR/HCQ, with dose reductions to 300 mg/400 mg mainly for G2/G3 nausea performed following 1 cycle, respectively, in about 35% of all patients (See Table 1).

Patient	Age	KRAS status	Cycles	Adverse Events†	Dose Reduction*
1	64	Wild	10	G3 n/v, G1 fatigue	Yes
2	65	Wild	2	G2 n/v, G2 fatigue	No
3	45	Wild	8	G1 fatigue, G1 n/v	No
4	72	Mutated	2	G2 n/v, G2 fatigue	No
5	45	Mutated	2	G2 n/v, G2 fatigue	Yes
6	74	Mutated	2	G1 fatigue	No
7	70	Mutated	2	G3 n/v	Yes
8	64	Wild	2	G4 thrombocytopenia G1 fatigue	Yes
9	58	Wild	10+	G1 fatigue, G1 n/v	No
10	53	Mutated	3	G2 n/v, G1 fatigue	Yes
11	60	Mutated	6+	none	No

Table 1: Adverse events for CRC patients receiving 2 or more cycles on single-arm VOR/HCQ on Phase II study at our institutions. † National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v4.0. *Dose reduction to VOR 300 mg and HCQ 400 mg PO daily. n/v = nausea/vomiting. + Patient is still on study.

Additionally, peripheral blood specimens were collected from patients on Days 1, 7, and 49 of treatment. Tumor biopsies were also obtained from 2 patients with CRC at baseline and posttreatment on Day 49. Quantitative RT-PCR analyses revealed that significant increases in the levels of the cyclin-dependent kinase inhibitor *CDKN1A* from baseline, an established biomarker of VOR, could be detected in PBMCs from patients in all four treatment cohorts (See Figure 2A). Considering this dose level is above the MTD, these findings indicate that PBMC specimens may not be appropriate for monitoring of HCQ-driven correlative PD endpoints.

In contrast, significant increases from baseline in both *CDKN1A* and *CTSD* were readily observed in tumor biopsy specimens obtained from 2 patients treated at the MTD of VOR plus HCQ (See Figure 2B). Although the number of tumors that we were able to obtain for these analyses is small, our preliminary results suggest that tumor specimens may be more valuable than PBMCs with respect to quantifying treatment-related markers associated with autophagy inhibition. This possibility is further supported by our analyses of autophagic vesicles (AV) in PBMCs. Notably, the number of AVs per cell in PBMC specimens collected from patients treated in the MTD cohort was not significantly affected by treatment with VOR and HCQ at any time points. A very modest, but statistically insignificant rise in the number of AVs/cell was noted on Day 7. However, by Day 49, this effect was gone and the mean AVs/cell was actually slightly below baseline levels (See Figure 2C-D).

Treatment-related increases in the expression of p21 and cathepsin D were more pronounced in tumor biopsies than peripheral blood mononuclear cells (PBMCs). However, given small number of patients in this phase study, these biomarkers of treatment need to be further correlated with efficacy and investigated in a larger clinical trial. These findings support a large phase II study evaluating VOR/HCQ in patients with refractory mCRC.

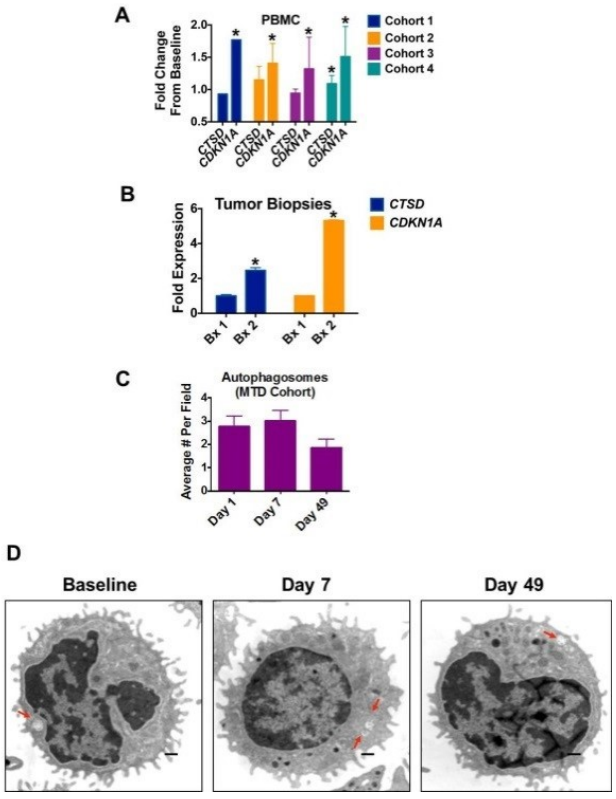


Figure 2: HCQ and VOR stimulate the expression of *CTSD* and *CDKN1A* and the accumulation of autophagic vesicles. (A) Fold change from baseline in the levels of *CTSD* and *CDKN1A* in PBMC specimens in individual cohorts. PBMC specimens were collected at baseline and on C1D7. Gene expression was quantified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

2.6. Correlative Studies

2.6.1 Correlative Studies of autophagy inhibition in relation to efficacy.

Furthermore, preliminary data at our institution has shown that the induction of biomarkers of autophagy cathepsin D (CTSD) and CDKN1A correlate both in PBMCs and patient's primary tumor using RT-PCR and electron microscopy (EM) (Fig 2 A-D) with efficacy of VOR/HCQ(6). We also observed increases in SQSTM1/p62 levels in PBMCs in patients treated with VOR/HCQ. (6) In addition, expression of high-mobility group box 1 (HMGB1) increases with autophagy in CRC, and HMGB1 acts upon binding to RAGE ("receptor for advanced glucated

end products") (7, 8). Also, LDH has been associated with necrotic cell death due to autophagy, which should decrease with autophagy inhibition (58). *Thus, we hypothesize that with VOR/HCQ, and thus autophagy inhibition, CTSD, CDKN1A, and SQSTM1/p62 will increase, and that HMGB and RAGE levels will decrease*. The change in expression levels of these various cellular proteins appears to be correlated with clinical efficacy.

Therefore, we suggest prospectively incorporating the identification of such predictive biomarkers in this study and the assessment of such biomarkers relative to the efficacy of agents evaluated. Establishing a predictive biomarker associated with clinical sensitivity to autophagy inhibitors is necessary for the optimal development of this class of drugs for cancer therapy. In addition, delineation of patients who would benefit from autophagy inhibition should reduce unnecessary costs and toxicities.

In addition, HDAC has been well-recognized as potential targets for the treatment of numerous aging-related disorders (9) such as cancer (10). Therefore, we plan to evaluate the potential role of biomarkers of aging with the combination of HCQ plus VOR.

2.6.2 Correlative Studies of anti-angiogenic inhibition in relation to efficacy.
Patients receiving RGF had modest improvements in survival, but the study has not yet identified which biomarkers predict improved efficacy to anti-angiogenic therapies in patients with refractory mCRC. We know that there are biomarkers that correlate with response to anti-VEGF therapy. Recently, the AVAGAST trial demonstrated that plasma VEGF-A and tumor neuropilin-1 are strong biomarkers for predicting clinical outcome in patients with advanced gastric cancer treated with Bev (59). For mCRC patients receiving Bev, low levels of baseline angiopoetin-2, a key regulator of vascular remodeling in conjunction with VEGF, has been associated with better response rates, PFS and OS (60, 61). Additionally, in pre-clinical studies VOR also inhibited VEGF-induced expression of the VEGF receptors (VEGFR1, VEGFR2) and neuropilin, thus blocking angiogenic signaling, and reducing circulating levels of inflammatory cytokines (TNF-alpha, IL1 beta, IL6 and IFN gamma induced liposaccharide) (45, 46).

Therefore, we will prospectively incorporate the identification of such predictive biomarkers in this study and the assessment of such biomarkers relative to the efficacy of agents evaluated. Establishing a predictive biomarker associated with clinical sensitivity to autophagy inhibitors is necessary for the optimal development of this class of drugs for cancer therapy. Also, delineation of patients who would benefit from VEGF inhibition and alternative non-VEGF targeting agents should reduce unnecessary costs and toxicities.

2.6.3 Correlative studies of HDAC inhibitors in relation to aging.
Alterations in protein misfolding, aggregation, and degradation are widely implicated in an increasing number of aging-related diseases, providing for novel therapeutic opportunities targeting protein homeostasis (proteostasis). The proteostasis network is essential at the cellular and organismal level for development and lifespan (62). Components of this system include the ubiquitin-proteasome and the autophagic-lysosomal systems that allow for degradation and clearance, along with HDAC that regulate epigenetic chromatin remodeling (63). Inhibitors of histone deacetylase (HDAC) exhibit neuroprotective and neuroregenerative properties in animal

models of various brain diseases (64). In preclinical models, HDAC inhibitors promote functional recovery of aging axons (65). Also, administration of the short fatty acid 4phenylbutyrate, an HDAC inhibitor, to a mouse model of Alzheimer's disease restored learning behavior as a result of elevated levels of H4 acetylation and increased synthesis of proteins involved in synaptic function (9). Further, elevated acetylation of histones (66) were obtained in mouse models of Alzheimer's disease treated with the pan-HDAC inhibitor vorinostat (VOR) (67). Therefore, the effects of HDAC inhibitors on aging need to be further investigated in humans.

2.6.3.1 Biomarkers of aging.

Chronologic aging has been associated with an increase in senescent cell populations throughout the body (68). Most senescent cells appear to express $p16^{Ink4a}$, a cyclin-dependent kinase inhibitor and tumor suppressor, and are known to increase with aging in pre-clinical and clinical models, including cancer patients (69-71). The causal relationship between cellular senescence and aging is not understood, but it is thought that pro-inflammatory factors produced by senescent cells (IL-1, IL-6, IL-8, TNFalpha, MCP1, MMP3), known as the senescenceassociated secretory phenotype (SASP) mediate the aging phenotype (72). **Therefore, the above findings support the evaluation of the effects of proteostasis modulation with HDAC inhibitors on aging in humans.** *We hypothesize that HDAC inhibitors will decrease biomarkers of aging, and therefore, slow aging when compared to normal controls.*

3.0 Statement of Study Objectives

Primary Objective:

- To determine the clinical efficacy with progression-free survival (PFS-1) of the combination of VOR plus HCQ when compared to RGF in treatment-refractory mCRC.

Secondary Objectives:

- To determine the overall survival in refractory mCRC patients receiving VOR plus HCQ when compared to RGF.
- To determine the progression-free survival (PFS-2) of the crossover treatment (crossover is optional).
- To evaluate the tumor response rate in refractory mCRC patients receiving VOR plus HCQ when compared to RGF.
- To further define the safety of the combination of VOR/HCQ when compared to RGF in treatment-refractory mCRC.
- To identify biomarkers of autophagy and anti-angiogenic inhibition that are associated with clinical efficacy of VOR/HCQ and RGF, respectively, in mCRC.

4.0 Inclusion and Exclusion Criteria Inclusion

Criteria:

1. Patients 18 years of age or older with histological documentation of metastatic colorectal cancer (mCRC).
2. ECOG performance status of 0-2.

3. Radiographical documentation of metastatic disease with imaging up to 6 weeks prior to enrollment.
4. Patients with mCRC must have been previously treated with irinotecan and/or oxaliplatin and/or VEGF/EGFR therapy or intolerant to these agents
5. Documentation of K-Ras mutational status
6. Adequate hematologic, renal and liver function (i.e. absolute neutrophil count > 1000/mm^3, platelets > 75,000/mm^3); creatinine < 2 times the upper limits of normal (ULN) total bilirubin < 1.5 mg/dl, ALT and AST< 3 times above the ULN, ALT and AST can be < 5 times ULN if patients have hepatic involvement.
7. Able to provide written informed consent.
8. Patients with the potential for pregnancy or impregnating their partner must agree to follow acceptable birth control methods to avoid conception. Women of childbearing potential must have a negative pregnancy test within 72 hours prior to receiving the investigational product.
9. Tumor blocks available from previous surgery/biopsy, or if not available, patients willing to have biopsy.

Exclusion Criteria:

1. Patients receiving prior therapy with RGF, VOR, and/or HCQ.
2. Patients with uncontrolled brain metastases. Patients with brain metastases must be asymptomatic and off corticosteroids for at least one week.
3. Due to risk of disease exacerbation, patients with porphyria are not eligible.
4. Due to risk of disease exacerbation, patients with psoriasis are ineligible unless the disease is well controlled, and they are under the care of a specialist for the disorder who agrees to monitor the patient for exacerbations.
5. Patients with previously documented macular degeneration or untreated diabetic retinopathy (stable retinopathy is allowed).
6. Patients who have had chemotherapy or radiotherapy within 2 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study. For investigational targeted therapies, patients will need to clear for 5 half-lives (not applicable to standard of care therapies).
7. Patients may not be receiving any other investigational agents.
8. Patients should not have taken valproic acid or another histone deacetylase inhibitor for at least 2 weeks prior to enrollment.
9. History of allergic reactions attributed to compounds of similar chemical or biologic composition to VOR or HCQ.
10. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements
11. Major surgery or significant traumatic injury occurring within 21 days prior to treatment.
12. QTc > 500 ms at baseline
13. Gastrointestinal tract disease resulting in an inability to take oral medication or a requirement for IV alimentation, prior surgical procedures affecting absorption, or active peptic ulcer disease. Patients with NJ, J or G tube will not be allowed to participate.

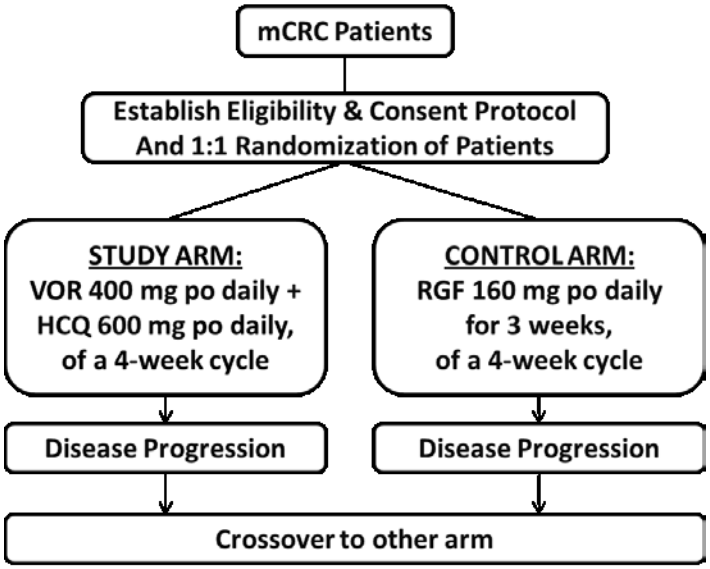
14. Pregnant women are excluded from this study because vorinostat has the potential for teratogenic or abortifacient effects. For this reason, women of childbearing potential and men must also agree to use adequate contraception (hormonal or barrier method of birth control) prior to study entry and for the duration of study participation.
15. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with vorinostat, breastfeeding should be discontinued.
16. Informed Consent - No study specific procedures will be performed without a written and signed informed consent document. Patients who do not demonstrate the ability to understand or the willingness to sign the written informed consent document will be excluded from study entry.

Inclusion of Women and Minorities:
Both men and women and members of all ethnic groups are eligible for this trial.

5.0 Study Design:
This will be a phase II randomized clinical trial of patients with histologic documentation of metastatic colorectal cancer, who have received local and currently approved standard therapies, excluding RGF. Patients will be randomized 1:1 to RGF or VOR/HCQ (see schema below) and stratified by KRAS status (wild-type or mutated). We will give VOR 400 mg PO daily and HCQ 600 mg PO daily in 4-week cycles. Patients will require imaging up to 6 weeks prior to enrollment and will be assessed for measureable evidence of mCRC. Crossover to the other arm will be allowed, but OPTIONAL, and will be determined by the treating physician for the patient's best interest.

5.1 Sample size:
Historically, the mPFS for previously treated mCRC patients treated with RGF is 1.9 mos. We predict VOR/HCQ will improve mPFS to 3.8 months. We expect 5% lost to follow-up. Assuming a 36-month accrual period with 1 year for follow up, 36 subjects per group are necessary to obtain 80% power. With 5% lost to follow-up the sample size required per group is 38 (=36/0.95), therefore, the total required sample size is 76 subjects. As a result, the target accrual rate is 2-4 patients/month to achieve a target accrual at 36 months with an additional 12month follow-up.

5.2 Study Schema:



*** Crossover to the other arm will be allowed, but OPTIONAL, and will be determined by the treating physician for the patient's best interest.

Randomization:
Randomization list will be sent to pharmacy. Once the patient is registered onto the study, the pharmacist will assign the patient to one of the treatment arms. The patient should start the treatment within 14 days of randomization.

Upon randomization, regorafenib prescription will be sent to specialty pharmacy. If the patient receives the medication in <14 days, then the patient can start at home. The start date will be C1D1. If the patient receives ≥14 days, then the patient will need a C1D1 clinical visit and labs: CBC, CMP, CEA and biomarkers.

5.3 Treatment Requirements
All eligible patients who consent to this study must have a baseline evaluation (CT or MRI) within 6 weeks of the start of treatment.

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

5.3.1 Duration of Intervention and Evaluation:
Follow-up: A repeat CT scan will be performed after 2 cycles of treatment regimen to evaluate response based on RECIST 1.1 criteria (See Appendix 13.1 for definitions of response) (73). CT scans will be repeated at least every 2 cycles, or 8 weeks, to ensure no progression of disease. Patients will continue on VOR/HCQ or RGF until disease progression, unacceptable toxicity, withdrawal of consent by the patient, or decision of physician for patient's best interest. Crossover to the other arm will be allowed, but OPTIONAL, and will be determined by the treating physician for the patient's best interest. If patient is not given crossover treatment, then the patient will be off study and will be allowed to receive subsequent treatments. Each patient

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

Interim analysis: The O'Brien-Fleming procedure will be used to conduct an interim analyses for efficacy when 50% of the patients complete the study. A statistical basis for stopping the study at the first look will be achieved if the test statistic indicates benefit in the VOR/HCQ Arm and exceeds 2.96259, corresponding to a significance level of 0.003. If the test statistic does not exceed 2.96259 at the first look then the trial will proceed to the final analysis. A treatment effect will be declared significant and beneficial at the final analysis if the test statistic indicates benefit and exceeds 1.96857, corresponding to a significance level of 0.047.

5.3.2 VOR and HCQ Administration

The starting dose will be VOR 400 mg PO daily and HCQ 600 mg PO daily, as determined by the phase I study in advanced cancer and as given in the expansion cohort of mCRC patients.

Dose can be decreased due to toxicity if deemed by the clinical investigator to be related to VOR. The dose reductions will be 300 mg (level -I), 200 mg (level -2).

Dose can be decreased due to toxicity if deemed by the clinical investigator to be related to HCQ. The dose reductions will be 400 mg (level -I), 200 mg (level -2).

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

Table 2: Dose reductions for VOR plus HCQ in the Phase II study of VOR/HCQ versus RGF in previously treated mCRC patients

If toxicity due to both VOR and HCQ:		
Dose Level	**VOR**	**HCQ**
0 *(Standard Daily Dose)*	400 mg	600 mg
-1	300 mg	400 mg
-2	200 mg	200 mg
If toxicity due to VOR:		
Dose Level	**VOR**	**HCQ**
0 *(Standard Daily Dose)*	400 mg	600 mg
-1	300 mg	600 mg
-2	200 mg	400 mg
If toxicity due to HCQ:		
Dose Level	**VOR**	**HCQ**
0 *(Standard Daily Dose)*	400 mg	600 mg

-1	400 mg	400 mg
-2	300 mg	200 mg

5.3.3 RGF Administration

The starting dose will be RGF 160 mg PO daily, for 3 weeks, of a 4-week cycle, as determined by the phase III study in mCRC patients (3). All patients will be given a handout on how to take Regorafenib (Appendix 12.3).

Dose can be decreased due to toxicity if deemed by the clinical investigator to be related to RGF. The dose reductions will be 120 mg (level -I), 80 mg (level -2).

Table 3: Dose reductions for RGF in the Phase II study of VOR/HCQ versus RGF in previously treated mCRC patients

If toxicity due to RGF:	
Dose Level	**RGF**
0 *(Standard Daily Dose)*	160 mg
-1	120 mg
-2	80 mg

5.3.4 Concomitant Medications and Supportive Care Guidelines

Diarrhea Management: Treat diarrhea promptly with appropriate supportive care, including loperamide. Instruct patients to begin taking loperamide at the first signs of: 1) poorly formed or loose stool, 2) occurrence of more bowel movements than usual in one day, or **3)** unusually high volume of stool. Loperamide should be taken in the following manner: 4 mg at first onset of diarrhea, then ***2*** mg after each unformed stool. Daily dose should not exceed 16 mg/day.

Loperamide should not be taken prophylactically. Advise patients to drink plenty of clear fluids to help prevent dehydration caused by diarrhea. Avoid loperamide if there is the presence of blood or mucus in the stool or if diarrhea is accompanied by fever. If grade 3 or 4 diarrhea develops, discontinue further treatment with vorinostat.

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

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

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

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

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

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

5.4 Dose Modification for Toxicity

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

5.4.1 Major Events

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

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

Dose can be decreased due to toxicity if deemed by the clinical investigator to be related to VOR. The dose reductions will be 300 mg (level -I), 200 mg (level -2).

Dose can be decreased due to toxicity if deemed by the clinical investigator to be related to HCQ. The dose reductions will be 400 mg (level -I), 200 mg (level -2).

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

5.4.2.1 HCQ Dose Modification

[redacted]

reinstitution of treatment can occur but at a reduced dose as described in Table 2.

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

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

5.4.2.2 VOR Dose Modification

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

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

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

5.4.3 Dose Modification for RGF (Table 3)
The starting dose will be RGF 160 mg PO daily, for 3 weeks, of a 4-week cycle, as determined by the phase III study in mCRC patients (3).

Dose can be decreased due to toxicity if deemed by the clinical investigator to be related to RGF. The dose reductions will be 120 mg (level -I), 80 mg (level -2).

Due to known hepatic toxicity, we will monitor liver function tests (ALT, AST and bilirubin) before initiation of RGF and monitor at least every two weeks during the first 2 months of treatment. Thereafter, monitor every cycle or more frequently as clinically indicated. Monitor liver function tests weekly in patients experiencing elevated liver function tests until improvement to less than 3 times the ULN or baseline. Temporarily hold and then reduce or permanently discontinue RGF depending on the severity and persistence of hepatotoxicity as manifested by elevated liver function tests or hepatocellular necrosis. See below for specific recommendations:

<u>Interrupt Regorafenib for the following</u>:
- NCI CTCAE Grade 2 hand-foot skin reaction (HFSR) [palmar-plantar erythrodysesthesia (PPE)] that is recurrent or does not improve within 7 days despite dose reduction; interrupt therapy for a minimum of 7 days for Grade 3 HFSR
- Symptomatic Grade 2 hypertension
- Any NCI CTCAE Grade 3 or 4 adverse reaction

<u>Reduce the dose of Regorafenib to 120 mg</u>:
- For the first occurrence of Grade 2 HFSR of any duration
- After recovery of any Grade 3 or 4 adverse reaction
- For Grade 3 aspartate aminotransferase (AST)/alanine aminotransferase (ALT) elevation; only resume if the potential benefit outweighs the risk of hepatotoxicity

<u>Reduce the dose of Regorafenib to 80 mg</u>:
- For re-occurrence of Grade 2 HFSR at the 120 mg dose
- After recovery of any Grade 3 or 4 adverse reaction at the 120 mg dose (except hepatotoxicity)

<u>Discontinue Regorafenib permanently for the following</u>:
- Failure to tolerate 80 mg dose
- Any occurrence of AST or ALT more than 20 times the upper limit of normal (ULN)
- Any occurrence of AST or ALT more than 3 times ULN with concurrent bilirubin more than 2 times ULN
- Re-occurrence of AST or ALT more than 5 times ULN despite dose reduction to 120 mg

- For any Grade 4 adverse reaction; only resume if the potential benefit outweighs the risks

5.5 Use of Hematologic Growth Factors
The use of hematologic growth factors (filgrastim, pegfilgrastim, epoetin alfa) is permitted to provide optimal care for patients with severe myelosuppression at the investigator's discretion.

5.6 Pharmaceutical Information
5.6.1 Hydroxychloroquine
Generic name: Hydroxychloroquine sulfate

Commercial name: Plaquenil

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

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

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

Stability: Stability of each bottle is marked on the container

Route of Administration: Orally

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

Potential Drug Interactions: Hydroxychloroquine is metabolized extensively in the liver and appears to be mediated via CYP450 2D6. In vivo studies of Hydroxychloroquine effects on agents metabolized by CPY450 2D6 are mixed. In health volunteers metoprolol levels following administration of
Hydroxychloroquine were increased, however, dextromethorpan levels were not significantly altered.

Other interactions of note include digoxin and cimetidine.

Special Handling: None.

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

Known Potential Toxicities of Hydroxychloroquine

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

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

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

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

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

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

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

5.6.2 Vorinostat
Chemical Name: N-hydroxy-N'-phenyl-octane-l,8-diotic acid diamide; **N-hydroxyl-N'phenyl(9CI)** octanediamide; suberoylanilide hydroxamic acid

Other Names: Zolinza®, Vorinostat, L-00 1079038, WIN 64652, MSK390, AP390

Classification: Antineoplastic

CAS Registry Number: 149647-78-9

Molecular Formula: $C_{14}H_{20}N_2O_3$ M.W.: 264.32

Approximate Solubility: Water ≤ 5 mg/mL

Description: Histone deacetylase (HDAC) inhibitor

Mode of Action:
Histone deacetylases (HDACs) are a family of enzymes that regulate chromatin remodeling and gene transcription via the dynamic process of acetylation and deacetylation of core histones.

Vorinostat, a potent inhibitor of HDAC activity, binds directly to the catalytic pocket of HDAC enzymes. It causes Gl or G2 phase cell-cycle arrest, apoptosis, or differentiation in cultured transformed cells.

How Supplied: Vorinostat is supplied by Merck & Co., Inc. and distributed by the CTEP, DCTD, NCI. Vorinostat is supplied as a white, opaque gelatin, size 3 capsule, containing 100 mg of vorinostat. The inactive ingredients contained in each capsule are microcrystalline cellulose, sodium croscarmellose, and magnesium stearate.

Vorinostat 100 mg capsules are supplied in bottles containing 120 capsules.
Storage: Store vorinostat capsules at room temperature, 15 to 30 ºC (59 to 86 ºF). Do not store above 30 ºC. Avoid exposure to excessive moisture.

Stability: Shelf life stability studies of the intact bottles are on-going.

Route of Administration: Orally

Method of Administration: Unless otherwise stated in the protocol, vorinostat capsules must be administered whole with **8** oz. of water. Administer doses of vorinostat with food, if possible.

Potential Drug 1nteractions: The major pathways of vorinostat metabolism involve glucuronidation and p-oxidation. As vorinostat is not eliminated via CYP450 pathways, no drugdrug interactions are expected with known CYP450 inhibitors or inducers. Although vorinostat was not a potent reversible.

CYP450 inhibitor, studies performed to monitor gene expression changes indicated some potential for CYP2C9 and CYP3A4 activity suppression. However, these changes were observed at concentrations higher than the pharmacologically relevant concentration of 2 mcM (C_{max}).

Prothrombin time and INR prolongations have been reported in patients taking vorinostat concomitantly with coumarin derivative anticoagulants. Monitor these patients more frequently for alterations in their coagulation parameters.

Special Handling: Vorinostat is an anticancer drug. Clean powder spills from broken or damaged vorinostat capsules carefully minimizing inhalation. Wash spill area at least 3 times with ethyl alcohol, followed by water.

Patient Care Implications: Because vorinostat's dose limiting toxicities are anorexia, dehydration, diarrhea, and fatigue, patients should maintain adequate fluid and food intake. Encourage patients to seek a nutritional consult.

Women of child bearing potential / men capable of fathering a child:
All women of childbearing potential MUST have a negative pregnancy test within 72 hours prior to receiving the investigational product. If the pregnancy test is positive, the patient must not receive investigational product and must not be enrolled in the study.

Vorinostat can cause fetal harm when administered to a pregnant woman. There are no adequate and well-controlled studies of vorinostat in pregnant women. Results of animal studies indicate that vorinostat crosses the placenta and is found in fetal plasma. Doses up to 50 and 150 mg/kg/day were tested in rats and rabbits, respectively (-0.5 times the human exposure based on AUC 0-24). Treatment-related developmental effects including decreased mean live fetal weights, incomplete ossifications of the skull, thoracic vertebra, sternum, metacarpals and skeletal variations (cervical ribs, supernumerary ribs, vertebral count and sacral arch variations) in rats and rabbits at the highest doses of vorinostat tested. The no observed effect level (NOEL) for these effects was 15 and 50 mg/kg/day (<0.1 times the human exposure based on AUC) in rats and rabbits. If the drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus.

During the course of the trial, all patients of childbearing potential should be instructed to contact the treating physician immediately if they suspect they might have conceived a child. In addition, a missed or late menstrual period should be reported to the treating physician. If a female patient or the treating physician suspects that the female patient may be pregnant prior to administration of study drugs, the study drugs must be withheld until the results of a pregnancy test are available. If pregnancy is confirmed the patient must not receive study medications and must be withdrawn from the study. Throughout the entire pregnancy, additional contact should be made with the patient, and in some cases with the healthcare provider, to identify spontaneous abortions and elective terminations, as well as any medical reasons for elective termination. In addition, the study investigator should include perinatal and neonatal outcome. Infants should be followed for a minimum of 4 weeks.

If a male patient is suspected of having fathered a child while on study drugs, the pregnant female partner must be notified and counseled regarding the risk to the fetus. In addition, the treating physician must follow the course of the pregnancy, including prenatal and neonatal outcome. Infants should be followed for a minimum of eight weeks.

Upon live-birth delivery, the minimum information that should be collected includes date of birth, length of pregnancy, sex of infant, major and minor anomalies identified at birth. Outcomes can be obtained via mailed questionnaires, maternal interviews, medical record

abstraction, or a combination of these methods. All serious adverse event reports relating to the pregnancy, including spontaneous abortion, elective abortion and congenital anomalies, should be forwarded to the FDA & Merck & Co. Inc. (See Safety reporting section).

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

Known Potential Toxicities of Vorinostat Cardiovascular:

Peripheral edema (13%)

Central nervous system: Fatigue (52%), chills (16%), dizziness (15%), headache (12%), fever (11%)

Dermatologic: Alopecia (19%), pruritus (12%)

Endocrine & metabolic: Hyperglycemia (8% to 69%; grade 3: 5%), dehydration (1% to 16%)

Gastrointestinal: Diarrhea (52%), nausea (41%), taste alteration (28%), anorexia (24%), weight loss (21%), xerostomia (16%), constipation (15%), vomiting (15%), appetite decreased (14%)

Hematologic: Thrombocytopenia (26%; grades 3/4: 6%), anemia (14%; grades 3/4: 2%)

Neuromuscular & skeletal: Muscle spasm (20%)

Renal: Proteinuria (51%), creatinine increased (16% to 47%)

Respiratory: Cough (11%), upper respiratory infection (11%) 1% to 10%:

Cardiovascular: QT_c prolongation (3% to 4%)

Dermatologic: Squamous cell carcinoma (4%)

Respiratory: Pulmonary embolism (5%)

≤1% (Limited to important or life-threatening): Abdominal pain, angioneurotic edema, blurred vision, chest pain, cholecystitis, deafness, diverticulitis, dysphagia, DVT, enterococcal infection, exfoliative dermatitis, gastrointestinal bleeding, gastrointestinal hemorrhage, Guillain-Barré syndrome, hemoptysis, hypertension, hypokalemia, hyponatremia, infection, lethargy, leukopenia, MI, neutropenia, pneumonia, renal failure, sepsis, spinal cord injury, streptococcal bacteremia, stroke (ischemic), syncope, T-cell lymphoma, tumor hemorrhage, ureteric obstruction, ureteropelvic junction obstruction, urinary retention, vasculitis, weakness

5.6.3 Regorafenib

Chemical Name: 4-[4-({[4-chloro-3-(trifluoromethyl) phenyl] carbamoyl} amino)-3fluorophenoxy]-N-methylpyridine-2-carboxamide monohydrate

Other Names: Stivarga®

Classification: Antineoplastic

Molecular Formula: $C_{21}H_{15}ClF_4N_4O_3 \cdot H_2O$ M.W 500.83

Approximate Solubility: insoluble in water, slightly soluble in acetonitrile, methanol, ethanol, and ethyl acetate and sparingly soluble in acetone.

Description: kinase inhibitor

Mode of Action:
Regorafenib is a small molecule inhibitor of multiple membrane-bound and intracellular kinases involved in normal cellular functions and in pathologic processes such as oncogenesis, tumor angiogenesis, and maintenance of the tumor microenvironment. In in vitro biochemical or cellular assays, regorafenib or its major human active metabolites M-2 and M-5 inhibited the activity of RET, VEGFR1, VEGFR2, VEGFR3, KIT, PDGFR-alpha, PDGFR-beta, FGFR1, FGFR2, TIE2, DDR2, TrkA, Eph2A, RAF-1, BRAF, BRAFV600E, SAPK2, PTK5, and Abl at concentrations of Regorafenib that have been achieved clinically. In in vivo models, Regorafenib demonstrated anti-angiogenic activity in a rat tumor model, and inhibition of tumor growth as well as anti-metastatic activity in several mouse xenograft models including some for human colorectal carcinoma

In colorectal cancer, Regorafenib is indicated for the treatment of patients with metastatic colorectal cancer (CRC) who have been previously treated with fluoropyrimidine-, oxaliplatin- and irinotecan-based chemotherapy, an anti-VEGF therapy, and, if KRAS wild type, an antiEGFR therapy.

How Supplied: Regorafenib is supplied by Bayer HealthCare Pharmaceuticals Inc.

Regorafenib is a 40 mg, light pink, oval shaped, film-coated tablet, debossed with ‘BAYER’ on one side and ‘40’ on the other side. Each tablet contains 40 mg of Regorafenib in the anhydrous state, which corresponds to 41.49 mg of Regorafenib monohydrate, and the following inactive ingredients: cellulose microcrystalline, croscarmellose sodium, magnesium stearate, povidone, and colloidal silicon dioxide. The film-coating contains the following inactive ingredients: ferric oxide red, ferric oxide yellow, lecithin (soy), polyethylene glycol 3350, polyvinyl alcohol, talc, and titanium dioxide.

Regorafenib tablets are supplied in packages containing three bottles, with each bottle containing 28 tablets, for a total of 84 tablets per package (NDC 50419 -171-03).

Storage: Store Regorafenib at 25°C (77°F); excursions are permitted from 15 to 30°C (59 to 86°F) [See USP Controlled Room Temperature].

Store tablets in the original bottle and do not remove the desiccant. Keep the bottle tightly closed after first opening.

Discard any unused tablets 28 days after opening the bottle. Dispose of unused tablets in accordance with local requirements.

Stability: Discard any unused tablets 28 days after opening the bottle. Dispose of unused tablets in accordance with local requirements.

Route of Administration: Orally

Method of Administration: Take Regorafenib at the same time each day. Swallow tablet whole with a low-fat breakfast that contains less than 30% fat. Examples of a low-fat breakfast include 2 slices of white toast with 1 tablespoon of low-fat margarine and 1 tablespoon of jelly, and 8 ounces of skim milk (319 calories and 8.2 g fat); or 1 cup of cereal, 8 ounces of skim milk, 1 slice of toast with jam, apple juice, and 1 cup of coffee or tea (520 calories and 2 g fat). Do not take two doses of Regorafenib on the same day to make up for a missed dose from the previous day.

Potential Drug 1nteractions:

- Strong CYP3A4 inducers: Avoid strong CYP3A4 inducers.
- Strong CYP3A4 inhibitors: Avoid strong CYP3A4 inhibitors.

Effect of Strong CYP3A4 Inducers on Regorafenib:
Co-administration of a strong CYP3A4 inducer (rifampin) with a single 160 mg dose of Regorafenib decreased the mean exposure of Regorafenib, increased the mean exposure of the active metabolite M-5, and resulted in no change in the mean exposure of the active metabolite M-2. Avoid concomitant use of Regorafenib with strong CYP3A4 inducers (e.g. rifampin, phenytoin, carbamazepine, phenobarbital, and St. John's Wort).

Effect of Strong CYP3A4 Inhibitors on Regorafenib:
Co-administration of a strong CYP3A4 inhibitor (ketoconazole) with a single 160 mg dose of Regorafenib increased the mean exposure of Regorafenib and decreased the mean exposure of the active metabolites M-2 and M-5. Avoid concomitant use of Regorafenib with strong inhibitors of CYP3A4 activity (e.g. clarithromycin, grapefruit juice, itraconazole, ketoconazole, nefazadone, posaconazole, telithromycin, and voriconazole).

Special Handling: Safely throw away (discard) any unused Regorafenib tablets after 28 days of opening the bottle

Patient Care Implications:
Regorafenib may cause severe or life-threatening liver damage. Inform patients that they will need to undergo monitoring for liver damage and to immediately report any signs or symptoms of severe liver damage to their health care provider.

- Regorafenib can cause severe bleeding. Advise patients to contact their health care provider for any episode of bleeding.
- Regorafenib can cause hand-foot skin reactions or rash elsewhere. Advise patients to contact their health care provider if they experience skin changes associated with redness, pain, blisters, bleeding, or swelling.

- Regorafenib can cause or exacerbate existing hypertension. Advise patients they will need to undergo blood pressure monitoring and to contact their health care provider if blood pressure is elevated or if symptoms from hypertension occur including severe headache, lightheadedness, or neurologic symptoms.
- Regorafenib increased the risk for myocardial ischemia and infarction. Advise patients to seek immediate emergency help if they experience chest pain, shortness of breath, or feel dizzy or like passing out.
- Contact a healthcare provider immediately if they experience severe pains in their abdomen, persistent swelling of the abdomen, high fever, chills, nausea, vomiting, severe diarrhea (frequent or loose bowel movements), or dehydration.
- Regorafenib may complicate wound healing. Advise patients to inform their health care provider if they plan to undergo a surgical procedure or had recent surgery.
- Inform patients that Regorafenib can cause fetal harm. Advise women of reproductive potential and men of the need for effective contraception during Regorafenib treatment and for up to 2 months after completion of treatment. Instruct women of reproductive potential to immediately contact her health care provider if pregnancy is suspected or confirmed during or within 2 months of completing treatment with Regorafenib.
- Advise nursing mothers that it is not known whether Regorafenib is present in breast milk and discuss whether to discontinue nursing or to discontinue Regorafenib.
- Inform patients to take any missed dose on the same day, as soon as they remember, and that they must not take two doses on the same day to make up for a dose missed on the previous day.
- Inform patients to store medicine in the original container. Do not place medication in daily or weekly pill boxes. Any remaining tablets should be discarded 28 days after opening the bottle. Tightly close bottle after each opening and keep the desiccant in the bottle

Known Potential Toxicities of Regorafenib:

Hepatotoxicity: Severe drug induced liver injury with fatal outcome occurred in 0.3% of 1200 Stivarga-treated patients across all clinical trials. Liver biopsy results, when available, showed hepatocyte necrosis with lymphocyte infiltration. In Study 1, fatal hepatic failure occurred in 1.6% of patients in the Regorafenib arm and in 0.4% of patients in the placebo arm; all the patients with hepatic failure had metastatic disease in the liver. In Study 2, fatal hepatic failure occurred in 0.8% of patients in the Regorafenib arm. Obtain liver function tests (ALT, AST and bilirubin) before initiation of Stivarga and monitor at least every two weeks during the first 2 months of treatment. Thereafter, monitor monthly or more frequently as clinically indicated. Monitor liver function tests weekly in patients experiencing elevated liver function tests until improvement to less than 3 times the ULN or baseline. Temporarily hold and then reduce or permanently discontinue Stivarga depending on the severity and persistence of hepatotoxicity as manifested by elevated liver function tests or hepatocellular necrosis.

>10%:

Cardiovascular: Hypertension (30% to 59%; grade ≥3: 8% to 28%)

Central nervous system: Fatigue (52% to 64%), dysphonia (30% to 39%), pain (29%), fever (21% to 28%), headache (10% to 16%)

Dermatologic: Palmar-plantar erythrodysesthesia (45% to 67%; grade ≥3: 17% to 22%), rash (26% to 30%; grade ≥3: 6% to 7%), alopecia (8% to 24%)

Endocrine & metabolic: Hypocalcemia (17% to 59%), hypophosphatemia (55% to 57%), hyponatremia (30%), hypokalemia (21% to 26%), hypothyroidism (4% to 18%)

Gastrointestinal: Appetite decreased (31% to 47%), lipase increased (14% to 46%), diarrhea (43% to 47%), mucositis (33% to 40%), weight loss (14% to 32%), amylase increased (26%), nausea (20%), vomiting (17%)

Hematologic: Anemia (79%; grade 3: 5%; grade 4: 1%), lymphopenia (30% to 54%; grade 3: 8% to 9%), thrombocytopenia (13% to 41%; grade 3: 1% to 2%; grade 4: <1%), INR increased (24%), hemorrhage (11% to 21%; grade ≥3: 2% to 4%), neutropenia (3% to 16%; grade 3: 1% to 2%)

Hepatic: AST increased (58% to 65%; grade 3: 3% to 5%; grade 4: 1%), ALT increased (39% to 45%; grade 3: 4% to 5%; grade 4: 1%), hyperbilirubinemia (33% to 45%)

Neuromuscular & skeletal: Stiffness (14%)

Renal: Proteinuria (33% to 60%; grade 3: 3%)

Miscellaneous: Infection (31% to 32%; grade ≥3: 5% to 9%) 1% to 10%:

Cardiovascular: Myocardial ischemia and infarction (1%)

Gastrointestinal: Taste disturbance (8%), xerostomia (5%), gastroesophageal reflux (1%)

Neuromuscular & skeletal: Tremor (2%)

Respiratory: Dyspnea (2%)

<1% **(Limited to important or life-threatening):** Bradycardia, erythema multiforme, gastrointestinal fistula, hypertensive crisis, liver injury (severe), liver failure, reversible posterior encephalopathy syndrome (RPLS), skin cancer (keratoacanthoma, squamous cell carcinoma), Stevens-Johnson syndrome, toxic epidermal necrolysis

Pregnancy Category D:

Risk Summary Based on its mechanism of action, Regorafenib can cause fetal harm when administered to a pregnant woman. There are no adequate and well-controlled studies with Regorafenib in pregnant women. Regorafenib was embryo lethal and teratogenic in rats and rabbits at exposures lower than human exposures at the recommended dose, with increased incidences of cardiovascular, genitourinary, and skeletal malformations. If this drug is used during pregnancy or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to a fetus

5.7 Primary and Secondary Endpoints: **Primary endpoint**:

- PFS-1: measured from the start of the first treatment the patient is randomized to until the date the criteria for progression are met or the date the patient is taken off study for any reason, whichever is shorter.

Secondary endpoints:

- OS: measured from the start of treatment on trial until the death of the patient.

- mPFS-2**:** measured from the start of the crossover treatment (OPTIONAL, second regimen) until the date the criteria for progression are met or the date the patient is taken off study for any reason, whichever is shorter.
- Response: measured as best response, complete response (CR) or partial response (PR), as defined by Response Evaluation Criteria in Solid Tumors (RECIST 1.1) Committee.
- Adverse events: grade 1-5 adverse events according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v4.0**;** measured at baseline, each cycle, and at off study date.
- Biomarkers of efficacy of VOR/HCQ and RGF: We will obtain blood samples for plasma and PBMCs before start of treatment, prior to cycle 2, and off study treatment.

5.8 Biomarker Analysis.
Five blood samples will be collected during the timepoints listed above:
- Baseline (within 14 days of starting Cycle 1)
- Pre-Cycle 2
- Off Study Treatment

Blood samples will be collected in three tubes:
- Two - 10-ml EDTA tubes for plasma
- Three - 8 mL Vacutainer CPT tube for PBMCs

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

Preparation of Plasma samples:
1) Collect 20 mL of whole venous blood into 2- 10 mL EDTA tubes
2) Centrifuge tubes at 2000 g for 15 minutes, at 4 C, to separate the cells from the Plasma.
3) Prepare the labels for the cryovials. The label for each tube should be written in black water fast ink.
4) Carefully transfer the top layer of the plasma from the EDTA tubes being careful not to disturb the Buffy Coat (white layer just below the plasma). Transfer up to 2 mL alloquots of plasma from the blood collection tubes into each of the 6 – 3.6 ml skirted bottom externally threaded cryovials.
5) Transfer the white thick layer above the red blood cell pellet from the blood collection tube into 2 – 2 ml cryovials.
6) Affix each label lengthwise to its corresponding tube and secure each label with a strip of clear plastic tape wrapped completely around the tube to ensure the label remains affixed to the tube during the freezing storage.
7) Immediately place the labeled tubes of plasma and buffy coat in an upright position in a 20°C freezer.
8) **Within 12 hours, transfer samples to -70°C freezer for long-term storage.**

Preparation of PBMC samples:

- Draw 8 mL of whole venous blood directly into each 8 mL Vacutainer CPT tubes and gently invert each tube 8 times.
- After inverting the tubes, centrifuge both tubes at room temperature at 1500-1 800 g (2800 rpm) for 20 minutes. NOTE: Centrifuge must be capable of generating at least 1500 RCF at the tube bottom.
- Draw off the clear plasma layer and discard, leaving behind cloudy PBMC layer lying just above the gel barrier.
- Transfer the PBMC layer into one 14 mL polypropylene centrifuge tubes.
- Add 10 mL of PBS-DEPC (at room temperature) to each tube and invert 3 to 4 times.
- Centrifuge at room temperature at 300 g (-1200 rpm) for 5 minutes.
- Pour off and discard supernatant, leaving PBMC pellet behind.
- Cap tube and store at -20°C.
- **Within 12 hours, transfer samples to -70°C freezer for long-term storage.**

Tumor biopsy
At baseline, archival tissue from diagnosis will be documented, but if not available, we need documentation of mCRC. The archival tissue may be used for PD analysis. In such instances, TEN-FIFTEEN 15 micron (also called thick sections) paraffin-embedded tissue sections (unstained) and at least 1 H&E stained slide from the tumor biopsy.

Optional tumor biopsy may be done for accessible tumors at Cycle 2. If a patient cross-overs to the other therapy arm following progression, another optional biopsy may be performed at second cycle of crossover treatment. This allows for analysis of tumor cytotoxic effects of therapy.

Procedure for collection of Cycle 2 tumor biopsy:

2 cores will be obtained and sent to PK lab for analysis.

All planned PD analysis pertaining to the tumor biopsies obtained from patients with colorectal cancer, at baseline and again at 2 cycle (treatment arm or crossover arm) will be under supervision of the PI of the study.

6.0 Schedule of events:
Table 4: Study Calendar for Phase II study of VOR plus HCQ versus RGF in previously treated mCRC patients.

	Baseline	**Every cycle (+/- 3 days)**	**C1D15 and C2D15 only (+/- 3 days)**	**Every other cycle (+/- 3 days)**	**Pre-cycle 2 (+/- 3 days)**	**Off treatment (+/- 3 days)**
Informed Consent°	**X**α					

History & Physical✓	**X€**	**X**	**Ω**			**X**
Pathology review and KRAS status documentation ✓	**X**					
Vital Signs✓	**X**	**X**				**X**
ECOG✓	**X**	**X**				**X**
CBC, CMP✓	**X**	**X^**	**X$^{\Omega}$**			**X**
CEA,✓ LDH✓	**X**	**X^**				**X**
PT/PTT✓	**X**					
Serum Pregnancy Test°	**X**					
EKG✓	**X**	**X#**			**X#**	
Tumor Evaluation (CTs)±✓	**X**			**X**		**X**
Ophthalmologic Exam*°	**X***					
Tissue biopsy °	**X∞**				**X∞**	
PBMCs & Plasma/Serum Biomarkers°¶	**X**				**X**	**X**
Adverse Events°%	**X**	**X**				**X**
Concurrent medication review°	**X£**	**X**				**X**

✓ Standard of care

° Research purpose

α Consent should be done <30 days from C1D1, otherwise re-consent prior to C1D1

€ Baseline History and Physical should ask for porphyria since that is an exclusion criteria and make sure patient has not taken Regorafenib, hydroxychloroquine or vorinostat in the past. ^ For Cycle 1, **for patients who are assigned to VOR/HCQ arm,** if baseline labs were done <14 days from C1D1, then can omit CBC, CMP, CEA and LDH. Upon randomization, regorafenib prescription will be sent to specialty pharmacy. If the patient receives the medication in <14 days, then the patient can start at home. This start date will be C1D1. If the patient receives $\geq$14 days, then the patient will need a C1D1 clinical visit and labs: CBC, CMP, CEA, LDH.

Repeat EKG after cycle 2 only if clinically relevant

$^{\Omega}$ For Cycle 1 Day 15 and Cycle 2 Day 15, we will check CBC and CMP to evaluate for toxicity. Specifically, for RGF, we will monitor ALT, AST and bilirubin. For VOR, we will monitor blood counts. History and physical exam only as needed or if clinically indicated.

± Baseline CT should be Day -36 to Day -1. Every other cycle CT scans can be done +/- 4 days. Off treatment CT scan is done if not done at disease progression or in the last 28 days. (See Appendix 13.1 for definitions of response).

* ONLY required for patients receiving HCQ on study: If the patient is randomized to VOR/HCQ arm, and the patient is 70 year or older or has baseline ophthalmologic pathology (i.e., diabetic retinopathy), then a comprehensive eye exam will be done at baseline. If the

patient continues to receive HCQ on study at 6 months, a repeat comprehensive eye exam will be done at 6 months and then every 6 months while receiving HCQ on study. If the patient has visual disturbances while receiving HCQ, then an ophthalmologic evaluation will be done at that time. If the ophthalmologist recommends discontinuation of HCQ due to eye toxicity, then the patient will be taken off study for toxicity.

Patients receiving RGF are not required to have baseline or follow- up eye exams, unless there is crossover to the VOR/HCQ arm. If patient moves onto VOR/HCQ crossover arm, eye exam is as stated above (patient is 70 year or older or has baseline ophthalmologic).

∞ At baseline, archival tissue from diagnosis, but if not available, we need documentation of mCRC. Pre-cycle 2 tumor biopsy is optional. Follow instructions in **section 5.8**. ¶ Baseline should be done within 14 days of starting Cycle 1. Pre-cycle 2 and Off Treatment blood draws can occur +/- 3 days. Blood samples will be collected in five tubes: Two - 10-ml EDTA tubes for plasma, and Three - 8 mL Vacutainer CPT tubes for PBMCs. Follow instructions in **section 5.8.**

% NCI-CTCAE v4.0 will be used to document adverse events

£ Patients should not have taken valproic acid or another histone deacetylase inhibitor for at least 2 weeks prior to enrollment.

BASELINE:

- HISTORY AND PHYSICAL
- PATHOLOGY REVIEW AND KRAS STATUS DOCUMENTATION
- VITAL SIGNS
- ECOG
- LABS: CBC, CMP, CEA, PT/PTT
- SERUM PREGNANCY TEST,
- PBMCS AND PLASMA BIOMARKERS
- EKG
- TUMOR EVALUATION (CT)
- OPHTALMOLOGY EXAM - Baseline ophthalmologic evaluation to evaluate in patients randomized to VOR/HCQ ARM who are 70 years and older or have underlying eye disease.
- ADVERSE EVENTS EVALUATION
- CONCURRENT MEDICATION REVIEW

DAY 1 OF EVERY CYCLE

- HISTORY AND PHYSICAL EXAM
- VITAL SIGNS
- ECOG
- LABS : CBC, CMP
- EKG
- ADVERSE EVENTS EVALUATION
- CONCURRENT MEDICATION REVIEW

DAY 15 OF CYCLE 1

- LABS : CBC, CMP
- HISTORY AND PHYSICAL EXAM ONLY IF CLINICALLY INDICATED (AS NEEDED)

DAY 1 OF CYCLE 2

- HISTORY AND PHYSICAL EXAM
- VITAL SIGNS
- ECOG
- LABS : CBC, CMP
- PBMCS AND PLASMA BIOMARKERS
- EKG (Repeat EKG after cycle 2 only if clinically relevant)
- ADVERSE EVENTS EVALUATION
- CONCURRENT MEDICATION REVIEW

DAY 15 OF CYCLE 2

- LABS : CBC, CMP

- HISTORY AND PHYSICAL EXAM ONLY IF CLINICALLY INDICATED (AS NEEDED)

EVERY OTHER CYCLE

- TUMOR EVALUATION (CT)

DISEASE PROGRESSION:

- HISTORY AND PHYSICAL
- VITAL SIGNS
- ECOG
- LABS: CBC, CMP, CEA
- TUMOR EVALUATION (CT)
- ADVERSE EVENTS EVALUATION
- CONCURRENT MEDICATION REVIEW

OFF STUDY EVALUATION:

- HISTORY AND PHYSICAL
- VITAL SIGNS
- ECOG
- LABS: CBC, CMP, CEA, LDH
- PBMCS AND PLASMA BIOMARKERS
- TUMOR EVALUATION (CT) [if not done at disease progression or in last 28 days]
- ADVERSE EVENTS EVALUATION
- CONCURRENT MEDICATION REVIEW

6.1 AE /SAE Relationship

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

- ***Unrelated***
  The adverse event is clearly not related to the investigational agent(s).

- ***Unlikely***
  The adverse event is doubtfully related to the investigational agent(s).

- ***Possible***
  The adverse event may be related to the investigational agent(s).

- ***Probable***
  The adverse event is most likely related to the investigational agent(s).

- ***Definite***
  The adverse event is clearly related to the investigational agent.

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

Definitions
Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria (73). Note: Lesions are either measurable or non-measurable using the criteria provided below. The term "evaluable" in reference to measurability will not be used because it does not provide additional meaning or accuracy.

See Appendix 13.1 for RECIST criteria

7.0 Data Collection
Data will be entered into Case Report Forms (CRF), which are currently in paper form.

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

- Lab values: CBC, CMP, CEA, PTT/INR (when collected)
- PFS-1: measured from the start of the first treatment the patient is randomized to until the date the criteria for progression are met or the date the patient is taken off study for any reason, whichever is shorter).
- OS: measured from the start of treatment on trial until the death of the patient (date of death)
- PFS-2**:** measured from the start of the crossover treatment (Optional, second regimen) until the date the criteria for progression are met or the date the patient is taken off study for any reason, whichever is shorter.
- Response: measured as best response at any given time: complete response (CR) or partial response (PR), as defined by Response Evaluation Criteria in Solid Tumors (RECIST 1.1) Committee (73). (See Appendix 13.1 for definitions of response).
- Adverse events: the number and grade 1-5 of all adverse events will be documented according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v4.0**;** measured at baseline, each cycle, and at off study date.

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

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

- institutional policies and procedures for institutional data safety and monitoring,
- an institutional guide to follow,
- monitoring of protocol accrual by the UT Health Cancer Center Protocol Review Committee,
- review of study forms and orders by the Forms Committee,
- tools for monitoring safety events,
- independent monitoring and source data verification by the UT Health Cancer Center QA Monitor/Auditor
- monitoring of UPIRSO's by the Director of Quality Assurance and DSMB,
- determining level of risk (Priority of Audit Level Score – PALS) ,
- oversight by the Data Safety Monitoring Board (DSMB), and
- verification of protocol adherence via annual audit for all Investigator Initiated Studies by the UT Health Cancer Center Quality Assurance Division.

Monitoring Progress and Safety

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

Baseline events and adverse events will be captured using the UT Health Cancer Center Master Adverse Events Document for each patient using CTCAE V4.0 for the grading and attribution of adverse events. Usage of the UT Health Cancer Center Master Adverse Events Document centrally documents:

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

Safety Definitions:

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

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

Serious Adverse Event Definition: is any adverse event that:

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

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

A. unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied (note: the unfounded classification of a serious adverse event as "anticipated" constitutes serious non-compliance);
B. definitely related or probably related to participation in the research; and
C. suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized

<u>Reporting Requirements</u>

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

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

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

The endpoint for dose reduction is based on observed adverse events. To identify the clinical and hematologic adverse events, this study will use NCI-CTCAE v4.0. At each visit, including [redacted] definitely related to VOR/HCQ will result in the dose being held until the AE has resolved to ≤ grade 1 or baseline. If the AE resolves, reinstitution of treatment can occur, but at a reduced dose. Dose reductions will be made as per *Table below*.

For patients randomized to the VOR/HCQ arm, a baseline ophthalmologic exam will be done in all patients over the age of 70 years or if there is baseline eye pathology as chronic use of HCQ has been associated rarely with visual disturbances. If the patient remains on study receiving HCQ at 6 months, then repeat examination will be done every 6 months thereafter, if the patient continues to receive HCQ at that time, or sooner if the patient has any new ophthalmologic symptoms, with particular regard to visual field deficits. Patients should be cautioned to report any visual symptoms, particularly difficulty seeing entire words or faces, intolerance to glare, decreased night vision, or loss of peripheral vision. These symptoms of peripheral retinal toxicity should prompt drug discontinuation and ophthalmologic evaluation. If the patient has visual disturbances while receiving HCQ, then an ophthalmologic evaluation will be done at that time. If the ophthalmologist recommends discontinuation of HCQ due to eye toxicity, then the patient will be taken off study for toxicity.

Dose reductions for VOR plus HCQ Phase II study of VOR/HCQ in previously treated mCRC patients

If toxicity due to both VOR and HCQ:		
Dose Level	**VOR**	**HCQ**
0 *(Standard Daily Dose)*	400 mg	600 mg
-1	300 mg	400 mg
-2	200 mg	200 mg
If toxicity due to VOR:		
Dose Level	**VOR**	**HCQ**
0 *(Standard Daily Dose)*	400 mg	600 mg
-1	300 mg	600 mg
-2	200 mg	400 mg
If toxicity due to HCQ:		
Dose Level	**VOR**	**HCQ**
0 *(Standard Daily Dose)*	400 mg	600 mg
-1	400 mg	400 mg
-2	300 mg	200 mg

If toxicity due to RGF:	
Dose Level	**RGF**
0 *(Standard Daily Dose)*	160 mg
-1	120 mg
-2	80 mg

Early Stopping Rules:

Early stopping for futility: The O'Brien-Fleming procedure will be used to conduct an interim analyses for efficacy when 50% of the patients complete the study. A statistical basis for stopping the study at the first look will be achieved if the test statistic indicates benefit in the VOR/HCQ Arm and exceeds 2.96259, corresponding to a significance level of 0.003. If the test statistic does not exceed 2.96259 at the first look then the trial will proceed to the final analysis. A treatment effect will be declared significant and beneficial at the final analysis if the test statistic indicates benefit and exceeds 1.96857, corresponding to a significance level of 0.047. If a treatment effect is not declared at the interim analysis, a statistical basis for stopping the study for futility will be attained if the conditional power is less than or equal to 20%.

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

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

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

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

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

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

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

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

* For Hospitalization Only — Any medical event equivalent to CTC Grade 3, 4, 5 which precipitated hospitalization (or prolongation of existing hospitalization) must be reported regardless of requirements for Phase of study, expected or unexpected and attribution.

Assuring Compliance with Protocol and Data Accuracy

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

UT Health Cancer Center DSMB Membership

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

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

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

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

UT Health Cancer Center DSMB Charter and Responsibilities

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

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

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

9.0 Minority Recruitment Plan

Section 1: Minority Accrual Template

Purpose:

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

Preliminary information that Principal Investigators should consider include: ○ Overall Representation of Minorities in the target catchment area

○ The ethnic/racial distribution of the specific type of cancer that is being targeted

The following resources can be utilized to determine target sample sizes for ethnic groups: ○ Texas Health Data http://soupfin.tdh.state.tx.us/txhd.htm ○ Texas Cancer Registry www.dshs.tx.us/tcr/ ○ SEER Cancer Statistics Review www.seer.cancer.gov ○ ACS Cancer Statistics www.cancer.org

***Estimated Target Accrual (Total)**

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

Breakdown of subjects - Indicate the **total** targeted/planned accrual by **Race & Gender**		
Race	**% Male**	**% Female**
Black or African American	3	3
American Indian or Alaskan Native		
Asian	2	2
Native Hawaiian or Other Pacific Islander		

White	45	45
More Than One Race (Multiracial)		
Total	50	50

Breakdown of subjects - Indicate the **total** targeted/planned accrual by **Ethnicity & Gender**.		
Ethnicity	**% Male**	**% Female**
Hispanic or Latino	25	25
Not Hispanic or Latino	25	25
Total	50	50

Section 2: Clinical Investigator Tools for Recruitment of Minorities

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

Yes ☐ No ☐	11. Patient Friendly Fast Facts in English and Spanish ** *(IRB approval required)*
Yes ☐ No ☐	12. Outreach to advocacy or community organizations (including presentations or awareness campaigns). Please specify:
Yes ☐ No ☐	13. Other. Please Specify:

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

10.0 Statistical Section

Analytic plan for primary objective:

mPFS-1: Historically, the mPFS for previously treated mCRC patients treated with RGF is 1.9 mos. We predict VOR/HCQ will improve mPFS to 3.8 months. We expect 5% lost to followup. Assuming a 36-month accrual period with 1 year for follow up, 36 subjects per group are necessary to obtain 80% power. With 5% lost to follow-up the sample size required per group is 38 (=36/0.95), therefore, the total required sample size is 76 subjects. As a result, the target accrual rate is 3-4 patients/month to achieve a target accrual at 36 months with an additional 12month follow-up.

Treatments will be contrasted with regard to PFS-1 with a proportional hazards model with adjustment for age, sex, race, and the baseline tumor K-RAS status (mutated, wild type); the adjusted hazard ratio, its 95% confidence interval, and the p-value for testing the null hypothesis that the log hazard ratio is zero will be presented. Survival curves will be illustrated with Kaplan-Meier plots. Interactions between treatment and baseline covariates will be explored.

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

Interim analysis: The O'Brien-Fleming procedure will be used to conduct an interim analyses for efficacy when 50% of the patients complete the study. A statistical basis for stopping the study at the first look will be achieved if the test statistic indicates benefit in the VOR/HCQ Arm and exceeds 2.96259, corresponding to a significance level of 0.003. If the test statistic does not exceed 2.96259 at the first look then the trial will proceed to the final analysis. A treatment effect will be declared significant and beneficial at the final analysis if the test statistic indicates benefit and exceeds 1.96857, corresponding to a significance level of 0.047. If a treatment effect is not declared at the interim analysis, a statistical basis for stopping the study for futility will be attained if the conditional power is less than or equal to 20%.

Analytic plan for secondary objectives:

- OS: The analyses of median OS will be performed in the intent-to-treat population. OS will be illustrated with Kaplan-Meier plots, the estimate of median OS with its 95% confidence interval. Treatment groups will be contrasted with regard to the response rate with a logistic regression model adjusted for age, sex, race and the baseline tumor K-RAS status (mutated, wild type); the odds ratio, its 95% confidence interval, and the p-value for testing the null hypothesis that the log odds ratio is zero will be presented. If the logistic model fails due to small cell counts then treatments will be contrasted with regard to response using Fisher's Exact Test.
- mPFS-2: The analyses of median PFS-2 will be performed in the crossover population. Crossover to the other arm will be allowed, but OPTIONAL, and will be determined by the treating physician for the patient's best interest. mPFS-2 will be illustrated with KaplanMeier plots, the estimate of median PFS-2 with its 95% confidence interval. Treatment groups will be contrasted with regard to the response rate with a logistic regression model adjusted for age, sex, race and the baseline tumor K-RAS status (mutated, wild type); the odds ratio, its 95% confidence interval, and the p-value for testing the null hypothesis that the log odds ratio is zero will be presented. If the logistic model fails due to small cell counts then treatments will be contrasted with regard to response using Fisher's Exact Test.
- Response rates: Response rates will be measured as the proportion of patients with the best response, complete response (CR) or partial response (PR), as defined by RECIST 1.1 Criteria and a 95% confidence interval. (See Appendix 13.2 for RECIST 1.1 criteria)
- Adverse events: All adverse events will be listed by type and grade. All adverse events will be listed by treatment group, level of seriousness (serious, not serious). Treatments will be contrasted with regard to each adverse event with Fisher's Exact Test.
- Biomarkers of efficacy of VOR/HCQ and RGF: Biomarkers will be graphically displayed to demonstrate patterns across time and group and to identify the need for transformations and potential outliers. The significance of variation in biomarkers with treatment will be assessed with linear models with and without adjustment for age, sex, race and the baseline tumor KRAS status (mutated, wild type). The dependent variables will be log transformed (base 2) if necessary. The relation between treatment efficacy, as measured by PFS-1, and biomarkers will be assessed with proportional hazards models in terms of treatment, the biomarker, the biomarker by treatment interaction, age, sex, race, and baseline tumor K-RAS status (mutated, wild type). Significant effects will be described and illustrated, as appropriate, with Kaplan Meier plots by strata determined by biomarker level and treatment group, for example.

Sample size justification: Historically, the mPFS for previously treated mCRC patients treated with RGF is 1.9 mos. We predict VOR/HCQ will improve mPFS to 3.8 months. We expect 5% lost to follow-up. Assuming a 36-month accrual period with 1 year for follow up, 36 subjects per group are necessary to obtain 80% power. With 5% lost to follow-up the sample size required per group is 38 (=36/0.95), therefore, the total required sample size is 76 subjects. As a result, the target accrual rate is 3-4 patients/month to achieve a target accrual at 36 months with an additional 12-month follow-up.

11.0 References/Bibliography

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer Statistics, 2014. CA: a cancer journal for clinicians. 2014. Epub 2014/01/09. doi: 10.3322/caac.21208. PubMed PMID: 24399786.
2. Package Insert: STIVARGA (regorafenib) tablets, oral [Internet]. Wayne, New Jersey: Bayer HealthCare Pharmaceuticals 2012 [cited 2013 17 July]. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/203085lbl.pdf.
3. Grothey A, Van Cutsem E, Sobrero A, Siena S, Falcone A, Ychou M, Humblet Y, Bouche O, Mineur L, Barone C, Adenis A, Tabernero J, Yoshino T, Lenz HJ, Goldberg RM, Sargent DJ, Cihon F, Cupit L, Wagner A, Laurent D. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. Lancet. 2013;381(9863):303-12. Epub 2012/11/28. doi: 10.1016/S0140-6736(12)61900-X. PubMed PMID: 23177514.
4. Patel SR, Karnad AB, Ketchum NS, Pollock BH, Sarantopoulos J, Weitman S, Mahalingam D. Should we move beyond VEGF inhibition in metastatic colorectal cancer? Lessons from early phase clinical trials. Journal of gastrointestinal oncology. 2014;5(2):99-103. doi: 10.3978/j.issn.2078-6891.2014.002. PubMed PMID: 24772337; PubMed Central PMCID: PMC3999623.
5. Carew JS, Medina EC, Esquivel JA, 2nd, Mahalingam D, Swords R, Kelly K, Zhang H, Huang P, Mita AC, Mita MM, Giles FJ, Nawrocki ST. Autophagy inhibition enhances vorinostat-induced apoptosis via ubiquitinated protein accumulation. Journal of cellular and molecular medicine. 2010;14(10):2448-59. Epub 2009/07/09. doi: 10.1111/j.1582-4934.2009.00832.x. PubMed PMID: 19583815; PubMed Central PMCID: PMC2891399.
6. Mahalingam D, Mita M, Sarantopoulos J, Wood L, Amaravadi R, Davis LE, Mita A, Curiel TJ, Espitia CM, Nawrocki ST, Giles FJ, Carew JS. Combined autophagy and HDAC inhibition: A phase I safety, tolerability, pharmacokinetic, and pharmacodynamic analysis of hydroxychloroquine in combination with the HDAC inhibitor vorinostat in patients with advanced solid tumors. Autophagy. 2014;10(8). PubMed PMID: 24991835.
7. Livesey KM, Kang R, Zeh HJ, 3rd, Lotze MT, Tang D. Direct molecular interactions between HMGB1 and TP53 in colorectal cancer. Autophagy. 2012;8(5):846-8. doi: 10.4161/auto.19891. PubMed PMID: 22647615; PubMed Central PMCID: PMC3378423.
8. Livesey KM, Tang D, Zeh HJ, Lotze MT. Autophagy inhibition in combination cancer treatment. Current opinion in investigational drugs. 2009;10(12):1269-79. PubMed PMID: 19943199.
9. Ricobaraza A, Cuadrado-Tejedor M, Perez-Mediavilla A, Frechilla D, Del Rio J, Garcia-Osta A. Phenylbutyrate ameliorates cognitive deficit and reduces tau pathology in an Alzheimer's disease mouse model. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2009;34(7):1721-32. doi: 10.1038/npp.2008.229. PubMed PMID: 19145227.
10. Kelly WK, Marks PA. Drug insight: Histone deacetylase inhibitors--development of the new targeted anticancer agent suberoylanilide hydroxamic acid. Nature clinical practice Oncology. 2005;2(3):150-7. doi: 10.1038/ncponc0106. PubMed PMID: 16264908.
11. Liu Y, Sanoff HK, Cho H, Burd CE, Torrice C, Ibrahim JG, Thomas NE, Sharpless NE. Expression of p16(INK4a) in peripheral blood T-cells is a biomarker of human aging. Aging

cell. 2009;8(4):439-48. doi: 10.1111/j.1474-9726.2009.00489.x. PubMed PMID: 19485966; PubMed Central PMCID: PMC2752333.
12. Burd CE, Sorrentino JA, Clark KS, Darr DB, Krishnamurthy J, Deal AM, Bardeesy N, Castrillon DH, Beach DH, Sharpless NE. Monitoring tumorigenesis and senescence in vivo with a p16(INK4a)-luciferase model. Cell. 2013;152(1-2):340-51. doi: 10.1016/j.cell.2012.12.010. PubMed PMID: 23332765; PubMed Central PMCID: PMC3718011.
13. Samant RS, Shevde LA. Recent advances in anti-angiogenic therapy of cancer. Oncotarget. 2011;2(3):122-34. Epub 2011/03/15. PubMed PMID: 21399234; PubMed Central PMCID: PMC3260813.
14. Ferrara N. VEGF and the quest for tumour angiogenesis factors. Nature reviews Cancer. 2002;2(10):795-803. Epub 2002/10/03. doi: 10.1038/nrc909. PubMed PMID: 12360282.
15. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nature medicine. 2003;9(6):669-76. Epub 2003/06/05. doi: 10.1038/nm0603-669. PubMed PMID: 12778165.
16. Brown LF, Berse B, Jackman RW, Tognazzi K, Manseau EJ, Senger DR, Dvorak HF. Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in adenocarcinomas of the gastrointestinal tract. Cancer research. 1993;53(19):4727-35. Epub 1993/10/01. PubMed PMID: 8402650.
17. Kabbinavar F, Hurwitz HI, Fehrenbacher L, Meropol NJ, Novotny WF, Lieberman G, Griffing S, Bergsland E. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2003;21(1):60-5. Epub 2002/12/31. PubMed PMID: 12506171.
18. Benson AB, Bekaii-Saab T, Chan E, Chen YJ, Choti MA, Cooper HS, Engstrom PF, Enzinger PC, Fakih MG, Fenton MJ, Fuchs CS, Grem JL, Hunt S, Kamel A, Leong LA, Lin E, May KS, Mulcahy MF, Murphy K, Rohren E, Ryan DP, Saltz L, Sharma S, Shibata D, Skibber JM, Small W, Jr., Sofocleous CT, Venook AP, Willett CG, Gregory KM, FreedmanCass DA. Metastatic colon cancer, version 3.2013: featured updates to the NCCN Guidelines. Journal of the National Comprehensive Cancer Network : JNCCN. 2013;11(2):141-52; quiz 52. Epub 2013/02/16. PubMed PMID: 23411381.
19. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. The New England journal of medicine. 2004;350(23):2335-42. Epub 2004/06/04. doi: 10.1056/NEJMoa032691. PubMed PMID: 15175435.
20. Goldberg RM, Sargent DJ, Morton RF, Fuchs CS, Ramanathan RK, Williamson SK, Findlay BP, Pitot HC, Alberts SR. A randomized controlled trial of fluorouracil plus leucovorin, irinotecan, and oxaliplatin combinations in patients with previously untreated metastatic colorectal cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2004;22(1):23-30. Epub 2003/12/11. doi: 10.1200/JCO.2004.09.046. PubMed PMID: 14665611.
21. Saltz LB, Clarke S, Diaz-Rubio E, Scheithauer W, Figer A, Wong R, Koski S, Lichinitser M, Yang TS, Rivera F, Couture F, Sirzen F, Cassidy J. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a

randomized phase III study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2008;26(12):2013-9. Epub 2008/04/19. doi: 10.1200/JCO.2007.14.9930. PubMed PMID: 18421054.
22. Tournigand C, Andre T, Achille E, Lledo G, Flesh M, Mery-Mignard D, Quinaux E, Couteau C, Buyse M, Ganem G, Landi B, Colin P, Louvet C, de Gramont A. FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2004;22(2):229-37. doi: 10.1200/JCO.2004.05.113. PubMed PMID: 14657227.
23. Colucci G, Gebbia V, Paoletti G, Giuliani F, Caruso M, Gebbia N, Carteni G, Agostara B, Pezzella G, Manzione L, Borsellino N, Misino A, Romito S, Durini E, Cordio S, Di Seri M, Lopez M, Maiello E, Montemurro S, Cramarossa A, Lorusso V, Di Bisceglie M, Chiarenza M, Valerio MR, Guida T, Leonardi V, Pisconti S, Rosati G, Carrozza F, Nettis G, Valdesi M, Filippelli G, Fortunato S, Mancarella S, Brunetti C. Phase III randomized trial of FOLFIRI versus FOLFOX4 in the treatment of advanced colorectal cancer: a multicenter study of the Gruppo Oncologico Dell'Italia Meridionale. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(22):4866-75. Epub 2005/06/09. doi: 10.1200/JCO.2005.07.113. PubMed PMID: 15939922.
24. Giantonio BJ, Catalano PJ, Meropol NJ, O'Dwyer PJ, Mitchell EP, Alberts SR, Schwartz MA, Benson AB, 3rd. Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2007;25(12):1539-44. Epub 2007/04/20. doi: 10.1200/JCO.2006.09.6305. PubMed PMID: 17442997.
25. Ruff P, Lakomy R, Prausová J, Van Hazel G, Moiseyenko V, Bhargava P, Tabernero J, editors. Analysis of overall survival and safety during the course of the phase III VELOUR trial comparing FOLFIRI and aflibercept or placebo in patients with mCRC that progressed on prior oxaliplatin treatment [abstract]. Journal of clinical oncology : official journal of the American Society of Clinical Oncology; 2013.
26. Mancuso MR, Davis R, Norberg SM, O'Brien S, Sennino B, Nakahara T, Yao VJ, Inai T, Brooks P, Freimark B, Shalinsky DR, Hu-Lowe DD, McDonald DM. Rapid vascular regrowth in tumors after reversal of VEGF inhibition. The Journal of clinical investigation. 2006;116(10):2610-21. Epub 2006/10/04. doi: 10.1172/JCI24612. PubMed PMID: 17016557; PubMed Central PMCID: PMC1578604.
27. Batchelor TT, Sorensen AG, di Tomaso E, Zhang WT, Duda DG, Cohen KS, Kozak KR, Cahill DP, Chen PJ, Zhu M, Ancukiewicz M, Mrugala MM, Plotkin S, Drappatz J, Louis DN, Ivy P, Scadden DT, Benner T, Loeffler JS, Wen PY, Jain RK. AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. Cancer cell. 2007;11(1):83-95. Epub 2007/01/16. doi: 10.1016/j.ccr.2006.11.021. PubMed PMID: 17222792; PubMed Central PMCID: PMC2748664.
28. Bennouna J, Sastre J, Arnold D, Osterlund P, Greil R, Van Cutsem E, von Moos R, Vieitez JM, Bouche O, Borg C, Steffens CC, Alonso-Orduna V, Schlichting C, Reyes-Rivera I, Bendahmane B, Andre T, Kubicka S. Continuation of bevacizumab after first progression in metastatic colorectal cancer (ML18147): a randomised phase 3 trial. The lancet oncology.

2013;14(1):29-37. Epub 2012/11/22. doi: 10.1016/S1470-2045(12)70477-1. PubMed PMID: 23168366.
29. Masi G, Loupakis F, Salvatore L, Cremolini C, Fornaro L, Schirripa M, Granetto C, editors. Second-line chemotherapy (CT) with or without bevacizumab (BV) in metastatic colorectal cancer (mCRC) patients (pts) who progressed to a first-line treatment containing BV: Updated results of the phase III "BEBYP" trial by the Gruppo Oncologico Nord Ovest (GONO) [abstract] J Clin Oncol **31**: Suppl abstr 3615; 2013.
30. Patel S KN, Pollock B, Sarantopoulos J, Weitman S, Mahalingam D., editor. Beyond VEGF inhibition: Comparative efficacy analysis of novel VEGF and non-VEGF therapeutic agents in phase I trials for patients with metastatic colorectal cancer (mCRC). . ASCO; 2013.
31. Louis M, Rosato RR, Brault L, Osbild S, Battaglia E, Yang XH, Grant S, Bagrel D. The histone deacetylase inhibitor sodium butyrate induces breast cancer cell apoptosis through diverse cytotoxic actions including glutathione depletion and oxidative stress. International journal of oncology. 2004;25(6):1701-11. Epub 2004/11/18. PubMed PMID: 15547708.
32. Li H, Wu X. Histone deacetylase inhibitor, Trichostatin A, activates p21WAF1/CIP1 expression through downregulation of c-myc and release of the repression of c-myc from the promoter in human cervical cancer cells. Biochemical and biophysical research communications. 2004;324(2):860-7. Epub 2004/10/12. doi: 10.1016/j.bbrc.2004.09.130. PubMed PMID: 15474507.
33. Kuefer R, Hofer MD, Altug V, Zorn C, Genze F, Kunzi-Rapp K, Hautmann RE, Gschwend JE. Sodium butyrate and tributyrin induce in vivo growth inhibition and apoptosis in human prostate cancer. British journal of cancer. 2004;90(2):535-41. Epub 2004/01/22. doi: 10.1038/sj.bjc.6601510. PubMed PMID: 14735205; PubMed Central PMCID: PMC2409572.
34. Strait KA, Dabbas B, Hammond EH, Warnick CT, Iistrup SJ, Ford CD. Cell cycle blockade and differentiation of ovarian cancer cells by the histone deacetylase inhibitor trichostatin A are associated with changes in p21, Rb, and Id proteins. Molecular cancer therapeutics. 2002;1(13):1181-90. Epub 2002/12/14. PubMed PMID: 12479699.
35. Duan H, Heckman CA, Boxer LM. Histone deacetylase inhibitors down-regulate bcl-2 expression and induce apoptosis in t(14;18) lymphomas. Molecular and cellular biology. 2005;25(5):1608-19. Epub 2005/02/17. doi: 10.1128/MCB.25.5.1608-1619.2005. PubMed PMID: 15713621; PubMed Central PMCID: PMC549348.
36. Carew JS, Nawrocki ST, Cleveland JL. Modulating autophagy for therapeutic benefit. Autophagy. 2007;3(5):464-7. Epub 2007/05/15. PubMed PMID: 17495516.
37. Bursch W, Ellinger A, Kienzl H, Torok L, Pandey S, Sikorska M, Walker R, Hermann RS. Active cell death induced by the anti-estrogens tamoxifen and ICI 164 384 in human mammary carcinoma cells (MCF-7) in culture: the role of autophagy. Carcinogenesis. 1996;17(8):1595-607. Epub 1996/08/01. PubMed PMID: 8761415.
38. Ertmer A, Huber V, Gilch S, Yoshimori T, Erfle V, Duyster J, Elsasser HP, Schatzl HM. The anticancer drug imatinib induces cellular autophagy. Leukemia. 2007;21(5):936-42. Epub 2007/03/03. doi: 10.1038/sj.leu.2404606. PubMed PMID: 17330103.
39. Kanzawa T, Kondo Y, Ito H, Kondo S, Germano I. Induction of autophagic cell death in malignant glioma cells by arsenic trioxide. Cancer research. 2003;63(9):2103-8. Epub 2003/05/03. PubMed PMID: 12727826.

40. Shao Y, Gao Z, Marks PA, Jiang X. Apoptotic and autophagic cell death induced by histone deacetylase inhibitors. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(52):18030-5. Epub 2004/12/15. doi: 10.1073/pnas.0408345102. PubMed PMID: 15596714; PubMed Central PMCID: PMC539807.
41. Takeuchi H, Kondo Y, Fujiwara K, Kanzawa T, Aoki H, Mills GB, Kondo S. Synergistic augmentation of rapamycin-induced autophagy in malignant glioma cells by phosphatidylinositol 3-kinase/protein kinase B inhibitors. Cancer research. 2005;65(8):333646. Epub 2005/04/19. doi: 10.1158/0008-5472.CAN-04-3640. PubMed PMID: 15833867.
42. Marks PA, Richon VM, Breslow R, Rifkind RA. Histone deacetylase inhibitors as new cancer drugs. Current opinion in oncology. 2001;13(6):477-83. Epub 2001/10/24. PubMed PMID: 11673688.
43. Luo J, Su F, Chen D, Shiloh A, Gu W. Deacetylation of p53 modulates its effect on cell growth and apoptosis. Nature. 2000;408(6810):377-81. Epub 2000/12/01. doi: 10.1038/35042612. PubMed PMID: 11099047.
44. Ito A, Kawaguchi Y, Lai CH, Kovacs JJ, Higashimoto Y, Appella E, Yao TP. MDM2HDAC1-mediated deacetylation of p53 is required for its degradation. The EMBO journal. 2002;21(22):6236-45. Epub 2002/11/12. PubMed PMID: 12426395; PubMed Central PMCID: PMC137207.
45. Nimmanapalli R, Fuino L, Stobaugh C, Richon V, Bhalla K. Cotreatment with the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) enhances imatinib-induced apoptosis of Bcr-Abl-positive human acute leukemia cells. Blood. 2003;101(8):3236-9. Epub 2002/11/26. doi: 10.1182/blood-2002-08-2675. PubMed PMID: 12446442.
46. Rahmani M, Reese E, Dai Y, Bauer C, Payne SG, Dent P, Spiegel S, Grant S. Coadministration of histone deacetylase inhibitors and perifosine synergistically induces apoptosis in human leukemia cells through Akt and ERK1/2 inactivation and the generation of ceramide and reactive oxygen species. Cancer research. 2005;65(6):2422-32. Epub 2005/03/23. doi: 10.1158/0008-5472.CAN-04-2440. PubMed PMID: 15781658.
47. Spiegel S, Milstien S, Grant S. Endogenous modulators and pharmacological inhibitors of histone deacetylases in cancer therapy. Oncogene. 2012;31(5):537-51. Epub 2011/07/05. doi: 10.1038/onc.2011.267. PubMed PMID: 21725353; PubMed Central PMCID: PMC3356932.
48. Lane AA, Chabner BA. Histone deacetylase inhibitors in cancer therapy. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2009;27(32):545968. Epub 2009/10/15. doi: 10.1200/JCO.2009.22.1291. PubMed PMID: 19826124.
49. Robey RW, Chakraborty AR, Basseville A, Luchenko V, Bahr J, Zhan Z, Bates SE. Histone deacetylase inhibitors: emerging mechanisms of resistance. Molecular pharmaceutics. 2011;8(6):2021-31. Epub 2011/09/09. doi: 10.1021/mp200329f. PubMed PMID: 21899343; PubMed Central PMCID: PMC3230675.
50. Lee JH, Choy ML, Marks PA. Mechanisms of resistance to histone deacetylase inhibitors. Advances in cancer research. 2012;116:39-86. Epub 2012/10/24. doi: 10.1016/B978-0-12394387-3.00002-1. PubMed PMID: 23088868.
51. Peart MJ, Tainton KM, Ruefli AA, Dear AE, Sedelies KA, O'Reilly LA, Waterhouse NJ, Trapani JA, Johnstone RW. Novel mechanisms of apoptosis induced by histone deacetylase

inhibitors. Cancer research. 2003;63(15):4460-71. Epub 2003/08/09. PubMed PMID: 12907619.
52. Ruefli AA, Ausserlechner MJ, Bernhard D, Sutton VR, Tainton KM, Kofler R, Smyth MJ, Johnstone RW. The histone deacetylase inhibitor and chemotherapeutic agent suberoylanilide hydroxamic acid (SAHA) induces a cell-death pathway characterized by cleavage of Bid and production of reactive oxygen species. Proceedings of the National Academy of Sciences of the United States of America. 2001;98(19):10833-8. Epub 2001/09/06. doi: 10.1073/pnas.191208598. PubMed PMID: 11535817; PubMed Central PMCID: PMC58560.
53. Rynes RI. Ophthalmologic considerations in using antimalarials in the United States. Lupus. 1996;5 Suppl 1:S73-4. Epub 1996/06/01. PubMed PMID: 8803917.
54. Wolfe F, Marmor MF. Rates and predictors of hydroxychloroquine retinal toxicity in patients with rheumatoid arthritis and systemic lupus erythematosus. Arthritis care & research. 2010;62(6):775-84. Epub 2010/06/11. doi: 10.1002/acr.20133. PubMed PMID: 20535788.
55. Mayo MW, Denlinger CE, Broad RM, Yeung F, Reilly ET, Shi Y, Jones DR. Ineffectiveness of histone deacetylase inhibitors to induce apoptosis involves the transcriptional activation of NF-kappa B through the Akt pathway. The Journal of biological chemistry. 2003;278(21):18980-9. Epub 2003/03/22. doi: 10.1074/jbc.M211695200. PubMed PMID: 12649266.
56. Carew JS, Nawrocki ST, Kahue CN, Zhang H, Yang C, Chung L, Houghton JA, Huang P, Giles FJ, Cleveland JL. Targeting autophagy augments the anticancer activity of the histone deacetylase inhibitor SAHA to overcome Bcr-Abl-mediated drug resistance. Blood. 2007;110(1):313-22. PubMed PMID: 17363733.
57. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. Journal of the National Cancer Institute. 2000;92(3):205-16. Epub 2000/02/03. PubMed PMID: 10655437.
58. Amaravadi RK, Thompson CB. The roles of therapy-induced autophagy and necrosis in cancer treatment. Clinical cancer research : an official journal of the American Association for Cancer Research. 2007;13(24):7271-9. doi: 10.1158/1078-0432.CCR-07-1595. PubMed PMID: 18094407.
59. Van Cutsem E, de Haas S, Kang YK, Ohtsu A, Tebbutt NC, Ming Xu J, Peng Yong W, Langer B, Delmar P, Scherer SJ, Shah MA. Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: a biomarker evaluation from the AVAGAST randomized phase III trial. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2012;30(17):2119-27. Epub 2012/05/09. doi: 10.1200/JCO.2011.39.9824. PubMed PMID: 22565005.
60. Goede V, Coutelle O, Neuneier J, Reinacher-Schick A, Schnell R, Koslowsky TC, Weihrauch MR, Cremer B, Kashkar H, Odenthal M, Augustin HG, Schmiegel W, Hallek M, Hacker UT. Identification of serum angiopoietin-2 as a biomarker for clinical outcome of colorectal cancer patients treated with bevacizumab-containing therapy. British journal of cancer. 2010;103(9):1407-14. Epub 2010/10/07. doi: 10.1038/sj.bjc.6605925. PubMed PMID: 20924372; PubMed Central PMCID: PMC2990609.

61. Liu Y, Starr MD, Bulusu A, Pang H, Wong NS, Honeycutt W, Amara A, Hurwitz HI, Nixon AB. Correlation of angiogenic biomarker signatures with clinical outcomes in metastatic colorectal cancer patients receiving capecitabine, oxaliplatin, and bevacizumab. Cancer medicine. 2013;2(2):234-42. Epub 2013/05/02. doi: 10.1002/cam4.71. PubMed PMID: 23634291; PubMed Central PMCID: PMC3639662.
62. Calamini B, Morimoto RI. Protein homeostasis as a therapeutic target for diseases of protein conformation. Current topics in medicinal chemistry. 2012;12(22):2623-40. PubMed PMID: 23339312; PubMed Central PMCID: PMC3955168.
63. Powers ET, Morimoto RI, Dillin A, Kelly JW, Balch WE. Biological and chemical approaches to diseases of proteostasis deficiency. Annual review of biochemistry. 2009;78:959-91. doi: 10.1146/annurev.biochem.052308.114844. PubMed PMID: 19298183.
64. Fischer A, Sananbenesi F, Mungenast A, Tsai LH. Targeting the correct HDAC(s) to treat cognitive disorders. Trends in pharmacological sciences. 2010;31(12):605-17. doi: 10.1016/j.tips.2010.09.003. PubMed PMID: 20980063.
65. Baltan S. Histone deacetylase inhibitors preserve function in aging axons. Journal of neurochemistry. 2012;123 Suppl 2:108-15. doi: 10.1111/j.1471-4159.2012.07949.x. PubMed PMID: 23050648; PubMed Central PMCID: PMC3481173.
66. Fischer A, Sananbenesi F, Wang X, Dobbin M, Tsai LH. Recovery of learning and memory is associated with chromatin remodelling. Nature. 2007;447(7141):178-82. doi: 10.1038/nature05772. PubMed PMID: 17468743.
67. Kilgore M, Miller CA, Fass DM, Hennig KM, Haggarty SJ, Sweatt JD, Rumbaugh G. Inhibitors of class 1 histone deacetylases reverse contextual memory deficits in a mouse model of Alzheimer's disease. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2010;35(4):870-80. doi: 10.1038/npp.2009.197. PubMed PMID: 20010553; PubMed Central PMCID: PMC3055373.
68. Baker DJ, Wijshake T, Tchkonia T, LeBrasseur NK, Childs BG, van de Sluis B, Kirkland JL, van Deursen JM. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. Nature. 2011;479(7372):232-6. doi: 10.1038/nature10600. PubMed PMID: 22048312; PubMed Central PMCID: PMC3468323.
69. Kim WY, Sharpless NE. The regulation of INK4/ARF in cancer and aging. Cell. 2006;127(2):265-75. doi: 10.1016/j.cell.2006.10.003. PubMed PMID: 17055429.
70. Krishnamurthy J, Torrice C, Ramsey MR, Kovalev GI, Al-Regaiey K, Su L, Sharpless NE. Ink4a/Arf expression is a biomarker of aging. The Journal of clinical investigation. 2004;114(9):1299-307. doi: 10.1172/JCI22475. PubMed PMID: 15520862; PubMed Central PMCID: PMC524230.
71. Sanoff HK, Deal AM, Krishnamurthy J, Torrice C, Dillon P, Sorrentino J, Ibrahim JG, Jolly TA, Williams G, Carey LA, Drobish A, Gordon BB, Alston S, Hurria A, Kleinhans K, Rudolph KL, Sharpless NE, Muss HB. Effect of cytotoxic chemotherapy on markers of molecular age in patients with breast cancer. Journal of the National Cancer Institute. 2014;106(4):dju057. doi: 10.1093/jnci/dju057. PubMed PMID: 24681605; PubMed Central PMCID: PMC3982894.
72. Tchkonia T, Zhu Y, van Deursen J, Campisi J, Kirkland JL. Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. The Journal of clinical

investigation. 2013;123(3):966-72. doi: 10.1172/JCI64098. PubMed PMID: 23454759; PubMed Central PMCID: PMC3582125.
73. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45(2):228-47. Epub 2008/12/23. doi: 10.1016/j.ejca.2008.10.026. PubMed PMID: 19097774.

12.0 Appendices

12.1 RECIST 1.1 criteria for Measurement of Effect

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

Definitions

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria (73). Note: Lesions are either measurable or non-measurable using the criteria provided below. The term "evaluable" in reference to measurability will not be used because it does not provide additional meaning or accuracy.

Measurable disease

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

Non-measurable disease

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

Target lesions

All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be

calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

Non-target lesions
All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Non-target lesions include measurable lesions that exceed the maximum numbers per organ or total of all involved organs as well as nonmeasurable lesions.

Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

Guidelines for Evaluation of Measurable Disease
All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

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

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment.

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

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

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

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

purposes in reference centers. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained.

Cytology, Histology: These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain). ***The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.***

Response Criteria
Evaluation of target lesions

- Complete Response (CR): Disappearance of all target lesions
- Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD
- Progressive Disease (PD): At least a 20% increase in the sum of the longest diameter (LD) of target lesions, taking as reference the smallest sum LD recorded since treatment started or the appearance of one or more new lesions.
- Stable Disease (SD): Neither sufficient shrinkage to qualify for a PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started.

Evaluation of non-target lesions

- Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level
- Incomplete Response/ Stable Disease (SD): Persistence of one or more non-target lesions(s) and/or maintenance of tumor marker level above the normal limits.
- Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions.

12.2 ECOG Performance Status

Grade	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours

3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

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

12.3 Handout for patients randomized to the regorafenib arm

Taking the Regorafenib Tablet

Please take Regorafenib at the same time each day.

Swallow tablet whole with a low-fat breakfast that contains less than 30% fat.

Examples of a low-fat breakfast include:

Menu 1
2 slices of white toast
1 tablespoon of low-fat margarine
1 tablespoon of jelly
8 ounces of skim milk

The food items in Menu 1 contain a total of 319 calories and 8.2 g fat.

Menu 2
1 cup of cereal
8 ounces of skim milk
1 slice of toast with jam
apple juice
1 cup of coffee or tea

The food items in Menu 2 contain a total of 520 calories and 2 g fat.

Missed doses:
Do not take two doses of Regorafenib on the same day to make up for a missed dose from the previous day.

13.0 Protocol Amendment Summary

Protocol Amendment 2.0 Dated 04/06/15 (Additions to a section are in **bold**, deleted have a line through)

Section 5.2 Randomization - The patient should start the treatment within **14** ~~10~~ days of randomization.

Upon randomization, regorafenib prescription will be sent to specialty pharmacy. If the patient receives the medication in <14 days, then the patient can start at home. The start date will be C1D1. If the patient receives ≥14 days, then the patient will need a C1D1 clinical visit and labs: CBC, CMP, CEA and biomarkers.

Section 6.0 Schedule of Events, footnote ^ - For Cycle 1, **for patients who are assigned to VOR/HCQ arm,** if baseline labs were done <14 days from C1D1, then can omit CBC, CMP, CEA and **LDH. Upon randomization, regorafenib prescription will be sent to specialty pharmacy. If the patient receives the medication in <14 days, then the patient can start at home. This start date will be C1D1. If the patient receives ≥14 days, then the patient will need a C1D1 clinical visit and labs: CBC, CMP, CEA, LDH.**

Reason for Change: Regorafenib, a standard of care drug, comes from a specialty pharmacy and can take some time for patient to receive medication.

Protocol Amendment 3.0 Dated 03/01/16 (Additions to a section are in **bold**, deleted have a line through)

Formatting, spelling and administrative changes were made throughout protocol.

Added Investigator Agreement signature page.

Deleted abbreviations not needed for this protocol.

Updated Table of Contents.

Exclusion Criteria:
5. Patients with previously documented macular degeneration or **untreated** diabetic retinopathy **(stable retinopathy is allowed)**.

Reason for Change: Clarifies patients with diabetic retinopathy are excluded.

Exclusion Criteria:
12. QTc > 500 ms at baseline ~~(average of 3 determinations at 10 minute intervals)~~.

Reason for Change: Only a single EKG is done.

5.3.1 Duration of Intervention and Evaluation:

Follow-up: A repeat CT scan will be performed after 2 cycles of treatment regimen to evaluate response based on RECIST 1.1 criteria (See Appendix 13.1 for definitions of response) (73). ~~Serum tumor marker CEA and~~ CT scans will be repeated at least every 2 cycles, or 8 weeks, to ensure no progression of disease. Patients will continue on VOR/HCQ or RGF until disease progression, unacceptable toxicity, withdrawal of consent by the patient, or decision of physician for patient's best interest. Crossover to the other arm will be allowed, but OPTIONAL, and will be determined by the treating physician for the patient's best interest. If patient is not given crossover treatment, then the patient will be off study and will be allowed to receive subsequent treatments. Each patient will be followed for 1 year. We will follow the last patient for 12 months, at which time, the study will be completed for data analysis.

Rational for Change: Serum tumor marker CEA will not be done.

5.8 Biomarker Analysis.

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

- Baseline (within 14 days of starting Cycle 1)
- Pre-Cycle 2
- Off Study Treatment

Blood samples will be collected in five tubes:

- Two - 10-ml EDTA tubes for plasma
- ~~One~~ **Three** - 8 mL Vacutainer CPT tubes for PBMCs

Rational for Change: Clarification on number of tubes for biomarker analysis.

Tumor biopsy

At baseline, archival tissue from diagnosis will be documented, but if not available, we need documentation of mCRC. The archival tissue may be used for PD analysis. In such instances, TENFIFTEEN 15 micron (also called thick sections) paraffin-embedded tissue sections (unstained) and at least 1 H&E stained slide from the tumor biopsy.

Optional tumor biopsy may be done for accessible tumors at Cycle 2. If a patient cross-overs to the other therapy arm following progression, another optional biopsy may be performed at second cycle of crossover treatment. This allows for analysis of tumor cytotoxic effects of therapy.

Procedure for collection of Cycle 2 tumor biopsy:

2 cores will be obtained and sent to PK lab for analysis.

All planned PD analysis pertaining to the tumor biopsies obtained from patients with colorectal cancer, at baseline and again at 2 cycle (treatment arm or crossover arm) will be under supervision of the PI of the study.

Rational for Change: This section clarifies the timing, processing and handling of tumor biopsies and the timing of the tumor biopsy for the crossover arm. This biopsy is optional.

6.0 Schedule of events:

Table 4: Study Calendar for Phase II study of VOR plus HCQ versus RGF in previously treated mCRC patients.

	Baseline	Every cycle (+/- 3 days)	C1D15 and C2D15 only (+/- 3 days)	Every other cycle (+/- 3 days)	Pre-cycle 2 (+/- 3 days)	Off treatment (+/- 3 days)
Informed Consent°	Xα					
History & Physical✓	X€	X	Ω			X
Pathology review and KRAS status documentation ✓	X					
Vital Signs✓	X	X				X
ECOG✓	X	X				X
CBC, CMP✓	X	X^	X$^{\Omega}$			X
CEA,✓ LDH✓	X	X^				X
PT/PTT✓	X					
Serum Pregnancy Test°	X					
EKG✓	X	X#			X#	
Tumor Evaluation (CTs)±✓	X			X		X
Ophthalmologic Exam*°	X*					
Tissue biopsy °	X∞				X∞	
PBMCs & Plasma/Serum Biomarkers°¶	X				X	X
Adverse Events°%	X	X				X
Concurrent medication review°	X£	X				X

✓ Standard of care ° Research purpose **α Consent should be done <30 days from C1D1, otherwise re-consent prior to C1D1**

€ Baseline History and Physical should ask for porphyria since that is an exclusion criteria and make sure patient has not taken Regorafenib, hydroxychloroquine or vorinostat in the past.

^ For Cycle 1, **for patients who are assigned to VOR/HCQ arm,** if baseline labs were done <14 days from C1D1, then can omit CBC, CMP, CEA and LDH. Upon randomization, regorafenib prescription will be sent to specialty pharmacy. If the patient receives the medication in <14 days, then the patient can start at home. This start date will be C1D1. If the patient receives ≥14 days, then the patient will need a C1D1 clinical visit and labs: CBC, CMP, CEA, LDH.

Repeat EKG after cycle 2 only if clinically relevant

Ω For Cycle 1 Day 15 and Cycle 2 Day 15, we will check CBC and CMP to evaluate for toxicity. Specifically, for RGF, we will monitor ALT, AST and bilirubin. For VOR, we will monitor blood counts. History and physical exam only as needed or if clinically indicated.

± Baseline CT should be Day -36 to Day -1. Every other cycle CT scans can be done +/- 4 days. Off treatment CT scan is done if not done at disease progression or in the last 28 days. (See Appendix 13.1 for definitions of response).

* ONLY required for patients receiving HCQ on study: ~~Ophthalmology evaluations at baseline if the patient is 70 years or older; it is repeated if any visual disturbances occurred while a patient was on study.~~ **If the patient is randomized to VOR/HCQ arm, and the patient is 70 year or older or has baseline ophthalmologic pathology (i.e., diabetic retinopathy), then a comprehensive eye exam will be done at baseline. If the patient continues to receive HCQ on study at 6 months, a repeat comprehensive eye exam will be done at 6 months and then every 6 months while receiving HCQ on study. If the patient has visual disturbances while receiving HCQ, then an ophthalmologic evaluation will be done at that time. If the ophthalmologist recommends discontinuation of HCQ due to eye toxicity, then the patient will be taken off sudy for toxicity.**

Patients receiving RGF are not required to have baseline or follow- up eye exams, unless there is crossover to the VOR/HCQ arm. If patient moves onto VOR/HCQ crossover arm, eye exam is as stated above (patient is 70 year or older or has baseline ophthalmologic).

∞ At baseline, archival tissue from diagnosis, but if not available, we need documentation of mCRC. Pre-cycle 2 tumor biopsy is optional. **Follow instructions in section 5.8.**

If the patient crosses over to the other treatment arm, then tumor biopsy is optional at cycle 2 of the crossover treatment.

¶ Baseline should be done within 14 days of starting Cycle 1. Pre-cycle 2 and Off Treatment blood draws can occur +/- 3 days. Blood samples will be collected in ~~three~~ **five** tubes: Two - 10-ml EDTA tubes for plasma, and ~~One~~ **Three** - 8 mL Vacutainer CPT tube for PBMCs. Follow instructions in **section 5.8.**

% NCI-CTCAE v4.0 will be used to document adverse events

£ Patients should not have taken valproic acid or another histone deacetylase inhibitor for at least 2 weeks prior to enrollment.

BASELINE:

- HISTORY AND PHYSICAL
- PATHOLOGY REVIEW AND KRAS STATUS DOCUMENTATION

- VITAL SIGNS
- ECOG
- LABS: CBC, CMP, CEA, PT/PTT
- SERUM PREGNANCY TEST,
- PBMCS AND PLASMA BIOMARKERS
- EKG
- TUMOR EVALUATION (CT)
- OPHTALMOLOGY EXAM – ~~Patients 70 years and older will need a baseline~~ Baseline ophthalmologic evaluation to evaluate ~~for macular degeneration or retinopathy~~ in patients randomized to VOR/HCQ ARM who are 70 years and older or have underlying eye disease.
- ADVERSE EVENTS EVALUATION
- CONCURRENT MEDICATION REVIEW

DAY 1 OF every CYCLE

- HISTORY AND PHYSICAL EXAM
- VITAL SIGNS
- ECOG
- LABS : CBC, CMP
- EKG
- ADVERSE EVENTS EVALUATION
- CONCURRENT MEDICATION REVIEW

DAY 15 OF CYCLE 1

- LABS : CBC, CMP
- HISTORY AND PHYSICAL EXAM ONLY IF CLINICALLY INDICATED (AS NEEDED)

DAY 1 OF CYCLE 2

- HISTORY AND PHYSICAL EXAM
- VITAL SIGNS
- ECOG
- LABS : CBC, CMP
- PBMCS AND PLASMA BIOMARKERS
- EKG (Repeat EKG after cycle 2 only if clinically relevant)
- ADVERSE EVENTS EVALUATION
- CONCURRENT MEDICATION REVIEW

DAY 15 OF CYCLE 2

- LABS : CBC, CMP
- HISTORY AND PHYSICAL EXAM ONLY IF CLINICALLY INDICATED (AS NEEDED)

EVERY OTHER CYCLE

- TUMOR EVALUATION (CT)

DISEASE PROGRESSION:

- HISTORY AND PHYSICAL
- VITAL SIGNS
- ECOG
- LABS: CBC, CMP, CEA, ~~PT/PTT~~ **LDH**
- TUMOR EVALUATION (CT)
- ADVERSE EVENTS EVALUATION
- CONCURRENT MEDICATION REVIEW

OFF STUDY EVALUATION:

- HISTORY AND PHYSICAL
- VITAL SIGNS
- ECOG
- LABS: CBC, CMP, CEA, ~~PT/PTT~~ **LDH**
- PBMCS AND PLASMA BIOMARKERS
- TUMOR EVALUATION (CT) [if not done at disease progression or in last 28 days]
- ADVERSE EVENTS EVALUATION
- CONCURRENT MEDICATION REVIEW

Rational for Change: Clarify the window of when patient should be consented again if there is a delay to start C1D1. Further clarifies management of patients receiving VOR/HCQ in regards to monitoring for eye toxicity that may be a side effect of HCQ. Clarifies management of patients with known eye disease, like diabetic retinopathy, as well as follow-up exam. Clarifies the timing of the tumor biopsy for the crossover arm. This biopsy is optional. Clarification on number of tubes for biomarker analysis. Clarification on eye exam requirements. Clarification to coincide with study calendar.

Monitoring Progress and Safety

Due to the risks associated with participation in this protocol, the UT Health Cancer Center DSMB *(DSMB#2)* in conjunction with the Principal Investigator will perform assessment of adverse events, adverse event trends and treatment effects on this study. The UT Health Cancer Center DSMB *(DSMB#2)* acts as an independent Data Safety Monitoring Board (DSMB) for IIS conducted at UT Health Cancer Center. The UT Health Cancer Center DSMB *(DSMB#2)* will monitor data throughout the duration of a study to determine if continuation of the study is appropriate scientifically and ethically. An additional layer of review is provided by the UT Health Cancer Center Data Safety Monitoring Committee (DSMC) who will review DSMB reports ~~every 3 months for the first year, and thereafter~~ every 6 months.

Rational for Change: Clarification on timing of the DSMB review.

The PI will provide the DSMB #2 with above findings ~~every 3 months for the first year, and thereafter~~ every 6 months, for discussion and review during their meetings.

Rational for Change: Clarification on timing of the DSMB review.

For patients randomized to the VOR/HCQ arm, a baseline ophthalmologic exam will be done in all patients over the age of 70 years **or if there is baseline eye pathology** ~~who receiving HCQ~~ as chronic use of HCQ has been associated rarely with visual disturbances. **If the patient remains on study receiving HCQ at 6 months, then** repeat examination will be **done every 6 months thereafter, if the patient continues to receive HCQ at that time, or sooner if the patient has** ~~due with~~ any new ophthalmologic symptoms, with particular regard to visual field deficits. Patients should be cautioned to report any visual symptoms, particularly difficulty seeing entire words or faces, intolerance to glare, decreased night vision, or loss of peripheral vision. These symptoms of peripheral retinal toxicity should prompt drug discontinuation and ophthalmologic evaluation. **If the patient has visual disturbances while receiving HCQ, then an ophthalmologic evaluation will be done at that time. If the ophthalmologist recommends discontinuation of HCQ due to eye toxicity, then the patient will be taken off study for toxicity.**

Rational for Change: Clarifies safety measures to monitor for rare toxicity by HCQ.

The PI ~~will review the Master Adverse Event documents to determine the significance of the reported events and~~ will provide **adverse events** findings using the Investigator Initiated Study DSMC Report Form ~~every 3 months for the first year, and thereafter~~ every 6 months, to the DSMB*#2*. The DSMB#2 will review the information provided by the PI and report to the UT Health Cancer Center DSMC ~~every 3 months for the first year, and thereafter every 6 months~~ **as reports are scheduled**, unless an emergent issue has been identified. The Investigator Initiated Study DSMC Report Form includes information on adverse events, current dose levels, number of patients enrolled, significant toxicities per the protocol, patient status (morbidity and mortality) dose adjustments with observed response, and any interim findings. Any trend consisting of three or more of the same event will be reported to the UT Health Cancer Center ~~DSMC~~ **DSMB** for independent review outside of the reporting cycle, which begins three months following protocol start up. The DSMB#2 will also provide its findings to the UT Health Cancer Center's Regulatory Affairs Division so that it may be provided to the UT Health San Antonio IRB with the protocol's annual progress report. Conflict of interest is avoided by the independent reviews of the UT Health Cancer CenterDSMB#2, UT Health Cancer Center ~~DSMC~~ **DSMB**, and by ongoing independent review of UPIRSO trends by the Director of Quality Assurance.

Rational for Change: PI will review the Master Adverse Events.

Protocol Amendment 4.0 Dated 7/13/16 (Additions to a section are in **bold**, deleted have a line through)

Exclusion Criteria:

6. Patients who have had chemotherapy or radiotherapy within 2 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study. For **investigational** targeted therapies, patients will need to clear for 5 half-lives **(not applicable to standard of care therapies)**.

Reason for Change: This amendment is a clarification regarding the exclusion criteria for INVESTIGATIONAL targeted therapies.

Protocol Amendment 5.0 Dated 06/14/2017

Changed PI from Devalingam Mahalingam, MD, PhD to Sukeshi Patel Arora, MD.

Rationale – Updated due to change in PI and changes in staffing

Throughout Protocol
Editorial changes made throughout to update site name CTRC to UT Health Cancer Center and UTHSCSA to UT Health San Antonio

Rationale – Update to coincide with UT Health San Antonio rebranding process