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# Phase I/II Trial of Regorafenib, Hydroxychloroquine, and Entinostat in Metastatic Colorectal Cancer

**Principal Investigator:** Peter J O'Dwyer, MD
Abramson Cancer Center
3400 Civic Center Blvd
Philadelphia, PA 19104
peter.o'dwyer@uphs.upenn.edu

**Co-Investigators:** Thomas B. Karasic, MD
Mark O'Hara, MD
Ursina Teitelbaum, MD
Ravi Amaravadi, MD
Nevena Damjanov, MD
Kim Reiss-Binder, MD
Charles J. Schneider, MD

**Statistician:** Wei-Ting Hwang, PhD
University of Pennsylvania School of Medicine
Center for Clinical Epidemiology & Biostatistics
628 Blockley Hall
423 Guardian Drive
Philadelphia, PA 19104

**Medical Monitor:** Vivek Narayan, MD

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| Title | **A Phase I/II Trial of Regorafenib, Hydroxychloroquine, and Entinostat in Metastatic Colorectal Cancer** |
| Short Title | Regorafenib/HCQ/Entinostat in colorectal cancer |
| IND # | 135547 |
| IRB Protocol Number | 827226 |
| UPCC Number | UPCC 31216 |
| Phase | Phase I/II |
| Methodology | Single Arm |
| Number of Patients | 32-44 |
| Study Duration | 18 months |
| Study Center | University of Pennsylvania Abramson Cancer Center |
| Primary Objectives | Phase I:<br>To determine doses of hydroxychloroquine and entinostat with acceptable toxicity in combination with regorafenib<br><br>Phase II:<br>To determine the response rate of the three-drug combination in metastatic colorectal cancer that has progressed on standard agents |
| Secondary Objectives | 1. To measure overall survival, progression-free survival, and duration of response<br>2. To describe the toxicity associated with the regimen<br>3. To describe the mutational profiles of responders and non-responders using next generation sequencing<br>4. To evaluate the pharmacodynamics of entinostat by measuring protein lysine acetylation in peripheral blood mononuclear cells (PBMCs), and lymphocyte subsets<br>5. To assess and quantitate autophagy pharmacodynamics using Western blotting of PBMC extracts and serum markers<br>6. To describe gene expression alterations as result of therapy using paired biopsy specimens at baseline and 3 weeks<br>7. To analyze FOXO1 expression and subcellular localization as a marker of treatment effect using paired biopsy specimens at baseline and 3 weeks |

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| Main Inclusion/Exclusion Criteria | 1. Must have measurable disease by RECIST 1.1 criteria<br>2. ECOG PS 0-1, age at least 18 years<br>3. Sufficient renal, hepatic, and bone marrow function by standard laboratory testing<br>4. Must be able to understand and sign written informed consent |
| Study Products | 1. Entinostat: an oral inhibitor of class I histone deacetylases<br>2. Hydroxychloroquine: an oral inhibitor of autophagy |
| Duration of Therapy | Until disease progression or unacceptable toxicity |
| Statistical Analysis | Phase I: Standard 3+3 design, determination of MTD<br>Phase II: Single-arm, overall response rate as primary endpoint |

## 1.0 OBJECTIVES:

### *1.1 Phase I Primary Endpoint*

1.1.1 To determine doses of hydroxychloroquine and entinostat with acceptable toxicity in combination with regorafenib

### *1.2 Phase II Primary Endpoint*

1.2.1 To determine the response rate of a three drug combination of regorafenib, hydroxychloroquine, and entinostat in patients with colorectal cancer that has progressed on standard agents

### *1.3 Secondary Endpoints*

1.3.1 To describe the toxicity profile of the three drug combination

1.3.2 To measure overall survival, progression-free survival, and duration of response

1.3.3 To describe mutational profiles of responders and non-responders using next-generation sequencing

1.3.4 To evaluate the pharmacodynamics of entinostat by measuring protein lysine acetylation in peripheral blood mononuclear cells (PBMCs), and lymphocyte subsets

1.3.5 To assess and quantitate autophagy pharmacodynamics using Western blotting of PBMC extracts and serum markers

1.3.6 To describe gene expression alterations as result of therapy using paired biopsy specimens at baseline and 3 weeks (Phase II portion only, when funding available)

1.3.7 To analyze FOXO1 expression and subcellular localization as a marker of treatment effect using paired biopsy specimens at baseline and 3 weeks (Phase II portion only, when funding available)

## 2.0 BACKGROUND:

### *2.1 Antiangiogenic activity in colorectal cancer*

Antiangiogenic therapy has been a mainstay in colon cancer since the approval of bevacizumab over a decade ago.[1] Angiogenesis is thought of as a hallmark of cancer, and microvascular density and other markers of increased angiogenesis have been shown to predict worse outcomes in colorectal cancer.[2,3] The use of bevacizumab, the prototypic antiangiogenic agent, has been shown to decrease tumor perfusion, microvascular density, and interstitial fluid pressure in rectal tumors,[4] treatment of patients with advanced disease is associated with improved survival, both in initial therapy, and in second-line, in combination with different chemotherapy.[1,5]

The role of VEGF pathway inhibition through antibodies and antibody fragments continues to expand. Bevacizumab has now demonstrated benefit in both the first- and second-line setting in combination with chemotherapy in colorectal cancer.[5,6] Aflibercept, a recombinant protein that combines VEGF1R and VEGF2R segments with the Fc portion of human IgG, shows survival benefit in the second-line setting.[7] Antibody targeting of VEGFR2 through ramucirumab, a fully human IgG1 antibody specific to VEGFR2, also improves survival in the second-line setting.[8]

More elusive in colorectal cancer has been the development of oral small molecule inhibitors of angiogenesis. Numerous tyrosine kinase inhibitors (TKIs) have been or are

currently being developed to target angiogenesis through the VEGF pathway. Various combinations using these drugs have ultimately failed during clinical trials in colorectal cancer.[9-13] Some considerations to be borne in mind for these drugs include multiple off-target effects, limited pharmacodynamic grounding, frequent combination with chemotherapy, which may not be the optimal setting, and questions about optimal scheduling for drugs with short half-lives. We recently published a randomized phase II trial showing lack of effect of the addition of sorafenib to chemotherapy. Nonetheless, as will be described in detail below, regorafenib was FDA-approved for use in colorectal cancer, on the basis of a single-agent trial. It has demonstrated a modest survival benefit in the third-line setting as a single agent with very few objective responses, suggesting a cytostatic effect[14]. Since regorafenib and sorafenib are almost identical in their preclinical and clinical characteristics, we expect the combinations proposed here to be safe, and any improvement in activity to be readily evident.

***2.2 Resistance to Antiangiogenic therapy***

The mechanisms through which angiogenesis inhibitors result in greater tumor cell kill have been studied extensively but remain incompletely understood. The prevailing hypothesis is that antiangiogenic therapy works to "normalize" the disrupted vasculature of tumors, resulting in increased drug delivery.[15,16] In this scenario, the disordered angiogenesis that characterizes colon cancers is normalized through inhibition of endothelial proliferation, to normalize interstitial pressure and improve chemotherapy delivery. We have proposed an alternative hypothesis, that antiangiogenic therapy chokes off the vascular supply of nutrients and oxygen and, by inducing hypoxia, adds to the cellular stress of cytotoxic chemotherapy.[17,18] Preclinical models respond to bevacizumab in vivo proportionally to their susceptibility to hypoxic cell death.[17,19] Additional mechanistic insights have been provided by the work of Kerbel and colleagues who have elucidated the contribution of circulating marrow-derived stem cells, which may have a profound effect on the tumor microenvironment.[20,21] As yet, these proposed mechanisms have not yielded useful markers to define the population of patients that may benefit from antiangiogenic therapy.

### *2.3 Autophagy as a mechanism of anti-angiogenic therapy resistance*

Hypoxia and low vascularity environments are frequent obstacles to proliferation encountered by tumor cells, and they have developed pathways of resistance. One such pathway is autophagy, in which tumor cells autodigest organelles and cytoplasm to generate energy in low nutrient and/or oxygen-poor environments.[22,23] Autophagy is a multi-step process that involves the vesicular sequestration of cytoplasmic proteins and damaged organelles into a structure called the autophagic vesicle (AV). AVs fuse with lysosomes and AV contents are degraded by acid-dependent enzymes. This process is activated by numerous cell stresses including growth factor or nutrient limitation, hypoxia, chemotherapy, and radiation.[24] The anti-malarial drugs CQ and HCQ induce the death of cells that rely on autophagy for survival by impairing lysosomal function, blocking the last step of autophagy. This strategy has demonstrated early promise in multiple tumor types, especially in combination with antiangiogenic therapy.[25]

We have performed multiple Phase I trials that all show combinations of cytotoxic drugs with therapeutic doses of HCQ to be tolerable. We performed a Phase I/II trial of the combination of HCQ and FOLFOX/bevacizumab in patients with metastatic colorectal cancer. Eligible patients had Stage IV colorectal cancer, with no prior therapy for metastatic disease, good PS (0,1), and adequate hematologic and biochemical indices. We entered 16 patients in the Phase I portion, 6M/10F, median age 57.5, and PS 0 (9) or 1 (7). One patient had received prior adjuvant treatment 6 months or more prior to being enrolled. Three patients were treated with 300 mg BID PO continuously in the first cohort, 8 patients were treated with 400 mg PO BID, and 5 patients were treated with 600mg PO BID. No dose-limiting toxicities were seen. The most common adverse events were fatigue, nausea, diarrhea, and neuropathy. In the first cohort, one patient had MRSA port infection and one patient had a pulmonary embolism, two patients had grade 3 neutropenia and one had grade 3 fatigue in the second cohort, and in the third cohort there was one cardiac arrest and pulmonary embolism and four patients with grade 3 neutropenia. The adverse events were not thought to be related to the addition of hydrochloroquine to FOLFOX and bevacizumab. In all cohorts, 44% of patients had partial response, 37% had stable disease, and 19% had progressive disease. We

concluded that full doses of FOLFOX/bevacizumab are well-tolerated in combination with HCQ 600mg PO BID continuously.

We continued to the Phase II portion of the trial, and again the combination showed no unexpected toxicity. Among the first 25 patients, the response rate was 57%, with 2 CR, and many patients were taken to metastatic resection, making interpretation of PFS very difficult. Based on the results, a randomized Phase II trial of 160 patients has been proposed for ECOG-ACRIN, and will be proposed to the GI Steering Committee in a few months.

***2.4 Epigenetic regulation and autophagy***

Adaptive responses to tumor stress such as autophagy require complex coordination, influenced by numerous tumor suppressor genes (e.g. TP53, PTEN) and oncogenes (e.g. EGFR) as well as epigenetic modification of gene expression.[25] While the mutational profiles of tumors have been increasingly well described through efforts such as The Cancer Genome Atlas, the role of epigenetic modification in many tumors remains incompletely understood. Histone deacetylases (HDACs) are a family of proteins that influence gene expression through multiple mechanisms and have been demonstrated to be pathogenic in a variety of tumors, including colorectal cancer.[27] The HDACs have been classified based on homology to yeast proteins into classes I, II, and IV, and at least 11 separate human HDAC proteins have been identified. They initially were shown to alter gene expression through cleavage of acetyl-lysine bonds on histones, with resultant alterations in chromatin structure and DNA promoter access.[28] Additionally, they have been shown to interact with a variety of non-histone proteins, including p53, E2F, NF-κB, α-tubulin, and heat shock protein 90 (HSP90), which may further alter cell-signaling pathways.[29]

Despite incomplete understanding of the different mechanisms through which HDACs exert their effects *in vivo*, small molecule inhibitors of HDACs have shown efficacy in a variety of tumor types. Three HDAC inhibitors, vorinostat, belinostat and romidepsin, are approved in cutaneous T cell lymphoma, and a fourth HDAC inhibitor, panobinostat, is approved in multiple myeloma. Preclinical mechanistic studies have demonstrated multiple antitumor effects, including increased apoptotic death, G1/S cell cycle arrest, downregulation of angiogenesis, and modification of tumor immunogenicity.[29] The

relative importance of each of these effects varies considerably based on tumor type and specificity of the HDAC inhibitor for the various HDAC isotypes.[30] Additionally, the alterations in cell signaling are highly dependent upon the cellular environment, and stressors such as nutrient deprivation, hypoxia, or chemotherapy. This variability hampers the applicability of preclinical models and makes combination therapy effects less predictable. Nevertheless, the benefit of HDAC inhibitor monotherapy in solid tumors is modest, and promising combinations are emerging.[31] Entinostat, also known as MS-275, is an HDAC inhibitor that selectively inhibits class I HDACs, resulting in a more favorable side effect profile.[32] It is currently undergoing investigation as monotherapy and in combination in a variety of tumor types.

Studies in our lab at Penn have shown that HDAC inhibitors are strong inducers of autophagy in colon cancer cells lines, especially under hypoxia, and that IC50 values are variable among the lines. Coadministration of the autophagy inhibitor CQ markedly sensitizes the cell lines to HDAC inhibition, with CI values that demonstrate marked synergy. This positive interaction is even more enhanced under hypoxic conditions. The combination of hydroxychloroquine and vorinostat was recently shown to be safe in phase I clinical trial.[33]

**Table 1: Interaction of HDAC inhibition with Autophagy Inhibition**

| Cell Line | IC50 SAHA (μM) Oxic | IC50 SAHA (μM) Hypoxic | IC50 CQ (μM) Oxic | IC50 CQ (μM) Hypoxic | CI S + H* Oxic | CI S + H* Hypoxic |
|---|---|---|---|---|---|---|
| 1.HT29 | 0.35 | 1.57 | 3.9 | 2.1 | 0.54 | 0.45 |
| 2.HCT116 | 0.12 | 0.32 | 10.8 | 7.2 | 0.45 | 0.33 |
| 3.HCT15 | 3.42 | 7.14 | 10.4 | 6.3 | 0.25 | 0.18 |
| 4.RKO | 1.89 | 4.52 | 12 | 6.7 | 0.3 | 0.15 |
| 5.DLD1 | 0.82 | 0.98 | 11.5 | 9.2 | 0.65 | 0.09 |
| 6.SW620 | 0.54 | 1.62 | 10.1 | 7.2 | 0.22 | 0.22 |
| 7.Colo205 | 0.26 | 0.31 | 9.0 | 5.4 | 0.8 | 0.37 |

*Combinatorial Index. A CI value <0.9 is defined as synergistic, 0.9-1.1 additive, and >1.1 antagonistic

An extensive literature documents a positive interaction between HDAC inhibitors and DNA damaging drugs, but for various reasons this has never been translated effectively to the clinic. These interactions will be explored more fully in a front-line trial with FOLFOX.

### *2.5 Regorafenib Clinical Experience*

Regorafenib is a multikinase inhibitor with effects on angiogenesis via the VEGF pathway as well as various cell growth pathways. It is closely related to sorafenib, differing in structure by a single fluorine molecule. The principle tyrosine kinase targets of regorafenib include VEGFR1-3, PDGR-β, KIT, RAF-1, B-RAF, B-RAF$^{V600E}$, RET, and to a lesser extent FGFR2 and TIE2.[34]

Phase I trials established a dosing regimen of 160 mg daily for 21 days of a 28 day cycle and demonstrated hand-foot syndrome, fatigue, diarrhea, and hypertension as the predominant side effects.[35] Regorafenib demonstrated consistent bioavailability between subjects. $T_{1/2}$ ranged from 20-40 hours, with relative stability of plasma concentrations between doses. It is metabolized into two pharmacologically active compounds, M2 and M5, which reach similar concentrations as the parent compound at steady state.

Regorafenib is an FDA-approved therapy for metastatic colorectal cancer that has progressed on prior therapy with 5-fluorouracil, oxaliplatin, irinotecan, anti-angiogenic therapy, and, if KRAS wild-type, anti-EGFR therapy. This approval was based on a proven survival benefit from the phase III CORRECT trial, which compared regorafenib to placebo in a 2:1 ratio in colorectal cancer that had progressed on all standard therapies.[36] Participants randomized to regorafenib in that trial received 160 mg daily for three weeks out of a four-week cycle. Overall survival improved in the regorafenib arm to 6.4 months, compared to 5.0 months in the placebo arm (hazard ratio 0.77, 95% CI 0.64-0.94, p=0.0052. Grade 3-4 toxicities in this trial with regorafenib included hand-foot syndrome (17%), fatigue (10%), diarrhea (7%), hypertension (7%), and rash (6%). Very few objective responses were seen with regorafenib (1% versus 0.4% with placebo), implying a cytostatic effect on the tumor.

A second phase III trial conducted in Asia, the CONCUR study, identical in design to the CORRECT trial, confirmed the results.[37] In this study, overall survival improved with regorafenib (8.8 months) compared to placebo (6.3 months) (HR 0.55, CI 0.40-0.77, p=0.00016). The objective response rate was 4% in the regorafenib arm. Grade 3-4 adverse events were similar to those seen in the CORRECT trial, including hand-foot syndrome (16%), hypertension (11%), liver abnormalities (7% hyperbilirubinemia, 7% elevated ALT, 6% elevated AST), hypophosphatemia (7%), lipase elevation (6%), and rash (6%). Additionally, two grade 5 adverse events (cardiac arrest and death NOS) were possibly attributable to the drug.

### *2.6 Hydroxychloroquine Clinical Experience*

Chloroquine (CQ) is a synthetic 4-aminoquinoline that has been used for 60 years in humans for malaria prophylaxis and treatment,[38] rheumatoid arthritis,[39] and human immunodeficiency virus (HIV)[40]. It is an inexpensive orally available drug that has CNS penetration. It has a large therapeutic index, and its most predictable cumulative toxicity is retinopathy, which can be prevented by discontinuation of the drug.[41] It is this toxicity and worldwide malarial resistance to CQ that lead to discontinuation of extensive research into CQ's non-malarial applications. Chloroquine derivatives such as HCQ are still used extensively in rheumatoid arthritis and lupus erythematosis and have a larger therapeutic index. The chemical structure of CQ derivatives allows them to serve as a weak base which is trapped in acidic cellular compartments.[42] Thus chloroquine deacidifies lysosomes, inhibiting the last step in autophagy. With this last step blocked, a cell reliant on autophagy will increase the generation of autophagosomes and will eventually undergo either apoptotic or non-apoptotic cell death. Evidence in mouse models and human cancer cell lines suggest CQ may have significant anti- tumor activity by inhibiting autophagy induced by cancer therapy.[43]

Adding chloroquine to improve the efficacy of anticancer therapy has already been tested in a randomized clinical trial. A small single-institution placebo-controlled phase III trial testing the addition of CQ at an oral daily dose of 150 mg to RT and carmustine in patients with newly diagnosed glioblastoma multiforme (GBM) yielded surprising

results.[44] Median overall survival was significantly longer in the CQ-treated patients (24 months) than in controls (11 months). At the end of the observation period, six patients (40%) treated with CQ were alive at 59, 45, 30, 20 (1 each) and 27 (2 patients) months after surgery. In contrast, patients in the control group survived 32, 25, and 22 months. Although not statistically significantly different, the rate of death over time was almost half as large in the CQ group compared to the placebo group (hazard ratio, 0.52 [95% CI, 0.21 to 1.26]; p= 0.139).

Hydroxychloroquine (HCQ) is commonly prescribed for rheumatoid arthritis and lupus at doses of 400 mg po daily. A pharmacokinetic/pharmacodynamic study of escalating doses of HCQ at 400 mg/800 mg/1200 mg po daily in patients with rheumatoid arthritis followed by maintenance doses of 400 mg po daily found that doses of up to 1200 mg po daily were well tolerated.[45] Dose limiting toxicities of nausea, vomiting and abdominal pain were observed at 800 and 1200 mg po daily. This toxicity correlated with blood HCQ levels, but not to blood levels of the other active metabolites, desethylhydroxychloroquine (DHCQ), desethylchloroquine (DCQ), or bisdesethylchloroquine (BDCQ). Improvement of symptoms in rheumatoid arthritis correlated with blood DHCQ levels, suggesting a dose-response relationship. Chloroquine derivatives are metabolized through the p450 enzyme system and CQ may inhibit the metabolism of CYP2D6- metabolized drugs.[45,46]

A predictable cumulative toxicity associated with CQ is retinopathy, and this is another reason why dose escalation with CQ would be limited. While a link between HCQ and retinopathy has also been made, it occurs infrequently and only after a prolonged exposure. A study using multifocal electroretinography to detect early pre-clinical retinal changes in long-term HCQ users, found that 10 out of 11 patients that developed early pre-clinical changes had been taking HCQ at doses of 400 mg po daily for greater than 5 years.[47] No overt retinopathy was noted in the 19 patients followed. This suggests that at a cumulative dose of 730 g, the risk of retinal changes increases, but techniques such as multifocal electroretinography can detect early changes and prevent overt visual loss.

We have previously conducted several trials of HCQ in combination with anticancer agents ranging from HCQ/alkylating therapy for glioblastoma, through HCQ/temsirolimus in Phase I, as well as FOLFOX/bevacizumab/HCQ in colorectal cancer. A CNS consortium trial (led from the University of Pennsylvania) to test the addition of HCQ at escalating doses to RT/temozolomide found excessive myelosuppression compared to temozolomide alone and reached a daily dose of only 600mg HCQ, which incompletely inhibited autophagy and was ineffective in the phase II portion.[48] With temsirolimus, tolerable doses of HCQ have been higher, and a dose level of 1200 mg is currently being explored. Dermatologic effects and fatigue associated with mTOR inhibitors do not appear to be worsened by HCQ at doses up to 1000 mg daily. In the frontline setting for metastatic colorectal cancer, 600mg twice daily dosing was used along with FOLFOX as the phase II dose without any additional toxicity.

The pharmacokinetics of hydroxychloroquine (HCQ) studied in patients with rheumatoid arthritis, malaria, and healthy volunteers demonstrate marked intra- and interpatient variability, with a two-fold range in total clearance (4-10 L/h) (26-8). The variability in the rate of absorption from oral dosing has been reported to be as high as 87%, contributing to differences in peak blood concentrations (Cmax) and time to peak concentration (Tmax) among patients receiving identical doses (26-8). Due to its long terminal elimination half-life of approximately 40 days, at least 120 days of continuous dosing are required before blood HCQ levels reach 90% of steady-state concentrations. Predicted blood concentrations are 898 ng/mL and 1796 ng/mL for patients receiving 200 mg and 400 mg daily of HCQ sulfate, respectively.[49] A one- compartment population pharmacokinetic model with a lag time was developed to estimate individual HCQ pharmacokinetic parameters in 36 patients who participated in a dose-escalation trial of HCQ in conjunction with radiation therapy and temozolomide for glioblastoma multiforme[48]. Population values for apparent volume of distribution (Vd) and total clearance were 604 L and 10.7 L/hr, with CV% of 23% and 5%, respectively. Mean individual estimated HCQ pharmacokinetic values for Vd were 573 L (range, 205-1291 L) and clearance 10.5 L/hr (range, 6.8-13.7 L/hr). Mean estimated Cmax values were linear and proportional to total daily oral HCQ sulfate doses at 200, 400, 600, and 800 mg

daily and consistent with predicted and observed blood concentrations from the published literature.[50-52] The population pharmacokinetic estimates are comparable to data from a population pharmacokinetic study in patients with rheumatoid arthritis where the Vd was 605 L and total clearance was 9.9 L/h.[53] Although pharmacokinetic analyses of HCQ sulfate at doses up to 1200 mg daily combined with other agents in phase I and II trials at the University of Pennsylvania have not been completed, dose-proportional HCQ concentrations and concentration-response relationships for daily doses between 400 and 1200 mg daily have been reported.[50]

***2.7 Entinostat Clinical Experience***

Entinostat, also known as MS-275, is a synthetic benzamide derivative that has been demonstrated to preferentially inhibit class I HDACs, especially HDAC type 1. Preclinical animal models of entinostat demonstrated a $T_{max}$ of 30-40 minutes and $T_{1/2}$ of one hour. Based on this data, the initial phase I trial of entinostat started dosage at 2 $mg/m^2$ daily for the first 28 days of a 42 day cycle.[54] Both patients on this daily protocol experienced dose-limiting toxicity during the first cycle, and pharmacokinetic data suggested a half-life of 30-50 times longer than that observed in the animal models. As a result, dosing was changed to biweekly, and all subsequent phase I trials used either weekly or biweekly dosing strategies. Plasma clearance of entinostat in humans follows bi-exponential kinetics suggestive of enterohepatic circulation with a $T_{1/2}$ of approximately 50 hours. Pharmacodynamic studies have used measurement of histone H3 acetylation in peripheral-blood mononuclear cells (PMBCs) as a surrogate for HDAC inhibition, although this does not nearly reflect the full spectrum of HDAC inhibitor activity.[55] Western blot and flow cytometric assays of this using entinostat demonstrated significant interpatient variability in both magnitude and timing of H3 hyperacetylation but no clear dose-dependence.[56]

The most common side effects observed in the initial phase I study were fatigue (100%), nausea (83%), anorexia (56%), myelosuppression (neutropenia 74%, thrombocytopenia 61%, anemia 26%), headache (52%), hypoalbuminemia (48%), and electrolyte abnormalities (hypocalcemia 43%, hyponatremia 35%, hypophosphatemia 26%). The

most frequent grade 3 toxicities in the 23 patients were nausea (4), hypophosphatemia (4), fatigue (3), diarrhea (3), and neutropenia (3). The only grade 4 event was leukopenia and neutropenia in a single patient. The MTD in this study was 10 mg/m$^2$ every 2 weeks.

Additional phase I studies have explored alternative dosing schedules without clear definition of an optimal regimen. A dose of 2 mg/m$^2$ twice weekly was excessively toxic and discontinued in favor of weekly or biweekly dosing in a phase I trial in patients with solid tumors and lymphoma.[56] All subsequent studies have of entinostat have used weekly or biweekly dosing. In a phase I trial in patients with refractory leukemia, 8 mg/m$^2$ weekly for the first 4 weeks of a 6-week cycle was determined to be the MTD[57] In a phase I trial in patients with refractory solid tumors, the MTD was 6 mg/m$^2$ weekly for the first 4 weeks of a 6-week cycle.[58] Later phase II trials used fixed dose instead of weight-based dosing of entinostat, with a range of doses and weekly or biweekly scheduling.[59-61] Most recently, a phase I trial of entinostat in combination with sorafenib in solid tumors demonstrated tolerability of standard full doses of sorafenib (400mg twice daily) with entinostat 10 mg every 2 weeks.[62] The toxicity profile in this trial was similar to that of the initial phase I study, with few events of grade 3 or higher. Therefore the combination with regorafenib, an almost identical VEGF-R2 inhibitor, is expected to be tolerable.

### *2.8 Rationale for Clinical Trial*

In summary, previous studies have indicated that autophagy may be a mechanism of resistance to both antiangiogenic and cytotoxic drugs. Multiple phase I trials have shown that HCQ is well-tolerated with multiple forms of chemotherapy, and specifically with the combination of FOLFOX/bevacizumab in colorectal cancer, as well as with sorafenib. Based on very striking (Table 1) preclinical data that support the further addition of HDAC inhibition, this trial is proposed as a test of principle that augmented autophagy inhibition can render a purely antiangiogenic therapy more effective. Since regorafenib alone is associated with a 1% response rate, we would view a 15% response rate with the combination as promising and worth developing further.

## 3.0 ELIGIBILITY

### *3.1 Inclusion Criteria*

3.1.1 Histologic or cytologic confirmation of metastatic colorectal cancer

3.1.2 Measurable disease based on modified RECIST 1.1 criteria

3.1.3 Patients should have received adequate therapy with prior 5-fluorouracil, oxaliplatin, and irinotecan, unless contra-indicated, not tolerated or declined.

3.1.4 No prior therapy with regorafenib or other anti-angiogenic tyrosine kinase inhibitor

3.1.5 No prior or current therapy with an HDAC inhibitor

3.1.6 Age 18 years or older

3.1.7 ECOG performance status of 0 or 1

3.1.8 If a female of childbearing potential, has a negative serum blood pregnancy test during screening and a negative urine pregnancy test within 3 days prior to receiving the first dose of study drug. If the screening serum test is done within 3 days prior to receiving the first dose of study drug, a urine test is not required. If a patient is of childbearing potential the patient must agree to use effective contraception (see Appendix C for acceptable methods) during the study and for 120 days after the last dose of study drug. Non-childbearing potential is defined as (by other than medical reasons):

- ≥45 years of age and has not had menses for >2 years
- Amenorrheic for <2 years without a hysterectomy and oophorectomy and a follicle-stimulating hormone value in the postmenopausal range upon pre-study (screening) evaluation
- Post hysterectomy, oophorectomy or tubal ligation. Documented hysterectomy or oophorectomy must be confirmed with medical records of the actual procedure or confirmed by an ultrasound. Tubal ligation must be confirmed with medical records of the actual procedure otherwise the patient must be willing to use 2 adequate barrier methods throughout the study, starting with the screening visit through 120 days after the last dose of study drug

3.1.9 If male, agrees to use an adequate method of contraception (see Appendix C) starting with the first dose of study drug through 120 days after the last dose of study drug

3.1.10 Life expectancy of greater than 3 months

3.1.11 Patients must have the ability to understand and the willingness to sign a written informed consent document.

3.1.12 Adequate bone-marrow, liver, and renal function as assessed by the following laboratory requirements within 4 weeks of starting treatment

- Absolute neutrophil count ≥1,500 per uL
- Hemoglobin ≥ 9 g/dL
- Platelets ≥100,000 per uL
- Creatinine ≤ 1.5 x ULN **OR** Creatinine clearance (CrCl) ≥ 60 by Cockcroft-Gault Equation if Creatinine >1.5
- AST and ALT ≤ 2.5 x ULN (≤ 5 x ULN if documented liver metastases)
- Total bilirubin ≤1.5 ULN **OR** direct bilirubin ≤ ULN if total bilirubin > 1.5 x ULN
- INR ≤ 2.0

3.1.13 Experienced resolution of toxic effect(s) of the most recent prior anti-cancer therapy to Grade <1 (except alopecia or neuropathy). If patient underwent major surgery or radiation therapy of >30 Gy, they must have recovered from the toxicity and/or complications from the intervention.

### *3.2 Exclusion Criteria*

3.2.1 History or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the study, interfere with the patient's participation for the full duration of the study, or is not in the best interest of the patient to participate, in the opinion of the treating Investigator, including, but not limited to:

a) Myocardial infarction or arterial thromboembolic events within 6 months prior to screening or severe or unstable angina, New York Heart Association (NYHA) Class III or IV disease, or a QTc interval > 470 msec.

b) Uncontrolled hypertension or diabetes mellitus.

c) Another known malignancy that is progressing or requires active treatment.

d) Any prior history of other cancer within the prior 5 years with the exception of adequately treated basal cell carcinoma or cervical intraepithelial neoplasia [CIN]/cervical carcinoma in situ or melanoma in situ).
e) Active infection requiring systemic therapy
f) Known active central nervous system (CNS) metastases and/or carcinomatous meningitis.

3.2.2 Any contraindication to oral agents or significant nausea and vomiting, malabsorption, or significant small bowel resection that, in the opinion of the investigator, would preclude adequate absorption.

3.2.3 Allergy to benzamide, inactive components of entinostat, or any of the other administered therapies

3.2.4 Known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the study.

3.2.5 Currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of study drug.

3.2.6 If female, is pregnant or breastfeeding.

3.2.7 Known G6PD deficiency, severe psoriasis, porphyria, macular degeneration, or severe diabetic retinopathy due to greater potential HCQ toxicity

3.2.8 Patients with pre-existing hypertension should be on a stable antihypertensive regimen and have a blood pressure ≤ 150/100 mmHg at the time of enrollment.

3.2.9 Evidence or history of bleeding diathesis. Any hemorrhage or bleeding event of CTCAE grade 3 or higher within 4 weeks of start of study medication

3.2.10 Non-healing wound, ulcer, or bone fracture

3.2.11 Patients using warfarin are excluded. Patients using other oral or parenteral anticoagulation are not excluded provided they are on a stable dose of anticoagulant but must undergo more frequent platelet count monitoring (see section 4.8).

## 4.0 TREATMENT PLAN

**Table 2: Dosing Schedule**

| Drug | Dose | Route | Schedule |
|---|---|---|---|
| Regorafenib | 160 mg daily | Oral | Daily for 21 days of each 28-day cycle |
| Entinostat | 2-5 mg weekly | Oral | Days 1, 8, 15, and 22 of each 28-day cycle |
| Hydroxychloroquine | 400-1200 mg daily | Oral | Days 1-28 of each 28-day cycle |

### *4.1 Regorafenib Dosing*

All patients will receive regorafenib at the FDA-approved dose of 160 mg by mouth daily for days 1-21 of a 28-day cycle. As noted below, dose decreases of regorafenib are often required in the initial cycle, followed by tolerance of full doses in subsequent cycles: such manipulations of the regorafenib dose are standard, and will not be taken into account in determining the MTD. Should excess toxicity attributable to regorafenib be repeatedly observed, the principal investigator may institute a standard initial dose escalation period. This will consist of regorafenib 80mg daily for the first 7 days of therapy, followed by increase to 120 mg for days 8-14 if 80mg tolerated, and 160 mg for days 15 and beyond if 120 mg tolerated.

### *4.2 Phase I Dose Escalation of Hydroxychloroquine and Entinostat*

We expect that the full doses of each drug will be well-tolerated based on previous studies. Patients will begin treatment with weekly oral entinostat and daily oral HCQ given in divided doses, per Table 3 below. The starting phase I total daily dose for HCQ is 600 mg, based on our previous Phase I trials. Weekly dosing of entinostat has been established as the best balance between toxicity due to accumulation and drug pharmacodynamics. Entinostat is currently undergoing study at a dose of 5mg weekly in a phase III trial in breast cancer, and 5mg weekly will be the target dose for this study. Given the potential for overlapping toxicities in this combination, an initial dose of 3 mg will be used, with subsequent escalation.

Three patients will be treated at the starting doses, with escalation to an entinostat dose of 5mg at dose level 2 followed by dose escalation of HCQ to 1200 mg/daily at dose level 3, with expansion to a total of 6 patients once safety has been established. Dose levels 2A and 2B will not be used during the dose escalation phase, they will be used only if de-escalation from dose level 3 is required. Toxicity attributable to hydroxychloroquine is not expected given its excellent tolerability in multiple phase II studies with combination chemotherapy using 1200 mg per day. In the event that dose level 3 exceeds the MTD (as described below), provision is made for stepwise dose de-escalation by 200 mg to dose levels 2B (1000 mg total daily dose HCQ) then 2A (800 mg total daily dose HCQ).

Before accrual to the next dose level may begin, all patients in the first cohort must complete the first 4 weeks of treatment, permitting toxicities to be assessed. The target DLT rate is ≤ 33%. The MTD will be defined as a) the dose producing DLT in 1 out of 6 patients, or b) the dose level below the dose which produced DLT in ≥ 2 out of 3 patients, or in ≥ 2 out of 6 patients. The rules for dose escalation and cohort size are outlined in Table 3. No intra-patient dose escalation is planned.

**Table 3: Hydroxychloroquine and Entinostat Phase I: Dose Escalation Schema**

| Dose Level | Dose Hydroxychloroquine (mg/day) | Schedule of HCQ Administration | Dose Entinostat |
|---|---|---|---|
| -1 | 400 mg | 200 mg q12h | 2 mg weekly |
| 1 | 600 mg | 200 mg qAM/ 400 mg qPM | 3 mg weekly |
| 2 | 600 mg | 200 mg qAM/ 400 mg qPM | 5 mg weekly |
| 2A | 800 mg | 400mg qAM/400mg qPM | 5 mg weekly |
| 2B | 1000 mg | 400mg qAM/600mg qPM | 5 mg weekly |
| 3 | 1200 mg | 600 mg q12h | 5 mg weekly |

Tablets of HCQ are available in 200 mg strengths. HCQ will be administered in two divided doses daily. When taking HCQ twice daily, the two daily doses should be taken as close as possible to 12 hours apart. Patients will be instructed to swallow the whole tablet in rapid succession without chewing. Patients receiving antacids, sucralfate, cholestyramine, and/or bicarbonate should have the HCQ and entinostat drug doses administered at least 1 hour before or 2 hours after these medications.

### *4.3 Phase II Dose Expansion*

To determine the recommended phase II dose (RP2D), at least 6 patients must be treated at the MTD for 2 cycles. In order to fulfill this criterion, patients who go off study before the completion of 2 cycles for reasons other than toxicity (e.g. disease progression) will be replaced in this cohort. The RP2D will be defined as a dose in which fewer than 33% of patients have a DLT and at which chronic administration is tolerated. To evaluate for chronic toxicity, tolerability is defined as 4 out of 6 patients treated for 2 cycles at the RP2D receiving during cycle 2:

1) At least 80% (in total mg per cycle) of the regorafenib dose at which the patient begins cycle 2
2) At least 75% of entinostat doses, with no more than one dose reduction
3) At least 80% (in total mg per cycle) of the HCQ doses

Should chronic tolerability fail to be demonstrated at the initial RP2D, the previous dose level (or a lower dose level at the discretion of the principal investigator) will be expanded until at least 6 patients have been treated at this new RP2D for 2 cycles. Evaluation for chronic tolerability will be performed as above after 2 cycles until the RP2D is determined.

After determination of the RP2D, an additional 20 patients will be enrolled at this dose level in the Phase II portion.

### *4.4 Experimental Drug Compliance*

Treatment compliance to entinostat and HCQ will be assessed at the end of each cycle. Patients will complete a diary to document their weekly intakes. They will be instructed to return all unused drugs (partially used and empty containers) and their diary at each visit. Site staff will perform accountability of the returned drug and will assess patient compliance. Site staff must ensure that the patient clearly understands the directions for self-medication and follows the schedule.

### *4.5 DLT Definition and Escalation Decision Process*

Dose Limiting Toxicities will be defined by toxicity occurring during the first 4 weeks of this study. Any non-hematologic AE of Grade 3 or higher that is judged to be probably treatment-related will be considered a DLT with the exceptions of fatigue; rash attributable to regorafenib; or nausea, vomiting, or diarrhea that has improved to Grade < 1 within 72 hours. Any non-hematologic AE of Grade 4 or higher of any duration that is judged to be probably treatment-related will be considered a DLT except for elevations of lipase and/or amylase in the absence of clinical pancreatitis.

The following hematologic DLT will be considered if any occurs in the first cycle:
1) grade 4 neutropenia lasting more than 7 days
2) febrile neutropenia (T > 100.4 F in presence of ANC <500)
3) platelet count less than 25,000/$mm^3$
4) platelet count less than 50,000/$mm^3$ with clinically significant hemorrhage

If a DLT is observed in exactly 1 patient in the first cohort of 3, the cohort will be expanded to 6. If a DLT occurs in 2 or more patients per cohort, then the cohort one dose below will be the declared the MTD provided that at least 6 patients have been treated at that level with fewer than one third having DLTs. The rules for dose escalation and cohort size are outlined in Table 3. No intra-patient dose escalation is planned.

***Evaluable Patients:*** Patients will be evaluable for toxicity if they have taken one dose of HCQ and entinostat. Patients who experience a dose-limiting toxicity will be evaluable for the cohort after at least one dose of HCQ and one dose of entinostat. To be considered for evaluability in a Phase I cohort in the absence of dose-limiting toxicity, patients should have completed $\geq$ 85% of HCQ doses, and at least 3 of 4 entinostat doses. Phase II patients will be evaluable for response if they have completed 85% of their expected dose of HCQ and at least 3 doses of entinostat in the initial 4 weeks, in the absence of progression. Patients who do not meet these criteria will be replaced. In the unexpected event of excess toxicity at dose level 1, there is provision (Table 3) for a dose reduction of both HCQ and entinostat.

**Table 4: Phase I: Criteria for Dose Escalation and Cohort Size**

| Number of patients with DLT | Rule |
|---|---|
| 0/3 DLT | Escalate |
| 1/3 DLT | Increase cohort to 6 patients |
| ≥ 2/3 DLT | De-escalate dose |
| **If the cohort size is increased to 6 patients, the following rules apply** | |
| **Number of patients with DLT** | **Rule** |
| 1/6 DLT | Escalate |
| 2/6 DLT | MTD is next lower dose |
| ≥ 3/6 DLT | De-escalate dose |

The doses of entinostat and HCQ will remain the same for each patient throughout treatment unless there is a need for a dose decrease for toxicity. These DLT decisions are assuming the toxicity is characteristic of entinostat or HCQ.

DLT determination is complex in this three-drug combination, and should the DLT toxicities be more likely associated with regorafenib, a lower dose of regorafenib may be required.

### *4.6 Duration of Treatment*

Patients will continue therapy on 28-day cycles until disease progression or the constraints of this therapy are deemed to be detrimental to the patient's health. In this event, the protocol should be discontinued. Furthermore, the protocol will be discontinued should the patient withdraw consent. All patients in whom protocol therapy has been discontinued will be followed for progression of disease and survival.

### *4.7 Measurement of Response*

Patients will be assessed on regularly prescribed intervals in order to determine if there is evidence of disease progression. A CT or MRI of the abdomen/pelvis and chest (if thoracic disease is present) will be obtained at 8 and 16 weeks, then every 8-12 weeks per standard practice.

### *4.8 Laboratory Testing*

Patients will have laboratory testing at the pre-treatment screening visit, cycle 1 day 1, and cycle 1 day 15 as described in the schedule of events (Appendix A), including complete blood count with differential and comprehensive metabolic panel with magnesium and phosphorous. Complete blood counts will be continued every two weeks until week 8, and then every four weeks if the hematologic toxicity is tolerable. Comprehensive metabolic panel, magnesium, and phosphorous will be checked every 4 weeks after the initial testing unless there is concerning toxicity. CEA will be checked at baseline, and, if it is elevated above the laboratory upper limit of normal, will be checked on day 1 of each 28-day cycle. Prothrombin time (PT) and activated prothromboplastin time (aPTT) will be checked at baseline. Patients on non-prophylactic anticoagulation will have a complete blood count weekly for cycles 1 and 2, and every 2 weeks with subsequent cycles.

For correlative pharmacodynamic testing, blood samples for peripheral blood mononuclear cells (PBMCs) will be obtained on cycle 1 day 1 (prior to therapy administration), cycle 1 day 2, and cycle 1 day 15. As in our previous study, autophagy inhibition will be assessed using western analysis of autophagy markers in PBMCs. HDAC inhibition will be measured through multiparametric flow cytometric analysis of histone and non-histone targets of de-acetylation enzymes in PBMCs[63] in collaboration with Dr Jane Trepel, NIH. In addition, the effects of the treatment on lymphocyte subsets will be analyzed by flow cytometry.[64] The procedures for this are further described in the laboratory manual.

### *4.9 Tumor Biopsy*

All patients receiving treatment on this trial should have available archival tumor tissue for analysis. This tissue should be that which was procured at the time of diagnosis and prior to treatment either through colonoscopic biopsy, surgical excision, or core biopsy of a metastatic site. Formalin-fixed, paraffin-embedded tissue will suffice. About 20 unstained slides, 5 microns thick will be requested. During the phase II portion of the study, additional tumor biopsy samples will be obtained in consenting patients with accessible disease between day 15 and day 22 of cycle 1, as permitted by scheduling of the procedure. In these paired biopsy samples, analysis of antigens including FOXO1

expression, localization, phosphorylation, and acetylation will be performed before and after treatment. Gene expression will also be analyzed in these patients by RNA-Seq and cytokine analysis will be performed in collaboration with Drs Downes and Evans at Salk.

### *4.10 Gene Expression Profiling*

For the tumor genomic characterization, we plan to submit all patients' baseline tumor biopsies for NGS analysis in a CLIA-approved test developed in the Department of Pathology at Penn (CPD Solid Tumor Panel, version 2), or equivalent, or more in-depth analysis as funding may permit.

## 5.0 DOSE MODIFICATIONS

Toxicity will be graded using the NCI Common Toxicity Criteria, CTCAE v. 4.03, which is available on the NCI website www.ctep.cancer.gov. As specified in the following tables, dose modification should be based upon the worst grade of toxicity experienced. Dose reductions should be continued for subsequent cycles. Exceptions may be made after discussion with the Principal Investigator.

### *5.1. Regorafenib*

Interrupt regorafenib for the following:

• NCI CTCAE Version 4.03 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 v4.03 Grade 3 or 4 adverse reaction attributable to regorafenib

Reduce the dose of regorafenib to 120 mg:

• For the first occurrence of Grade 2 HFSR of any duration

• After recovery of any Grade 3 or 4 adverse reaction attributed to regorafenib

• For Grade 3 aspartate aminotransferase (AST)/ alanine aminotransferase (ALT) elevation (see Note below)

Reduce the dose of regorafenib to 80 mg:

• 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)

Discontinue regorafenib permanently for the following:

• Failure to tolerate 80 mg dose

• Any occurrence of AST or ALT more than 20 times the upper limit of normal (ULN) Grade 4

• Any occurrence of AST or ALT more than 3 times ULN with concurrent bilirubin more than 2 times ULN

• For any of the following complications: gastrointestinal perforation/fistula, hemorrhage (severe or life-threatening), reversible posterior leukoencephalopathy syndrome (RPLS), wound dehiscence

**Note:** With regorafenib, as many as a third of patients may require dose decreases. These can often be temporary and patients can often be re-dosed at starting doses after resolution of toxicity.

### *5.2 Entinostat*

All dose modifications should be based on the AE requiring the greatest modification and should be properly documented in source documents. Investigators may take a more conservative approach than the guidelines outlined below on the basis of clinical judgment that is in the best interest of the subject.

Management of toxicities that are at least possibly related to entinostat, with toxicities graded by the Investigator according to the NCI, CTCAE, version 4.03 should be managed as follows:

**Table 5: Dose Reductions for Entinostat-Related Non-Hematologic Toxicity[1]**

| Non-hematologic Toxicity | |
|---|---|
| **Toxicity** | **Dose Modifications** |
| Grade 4 | Administer symptomatic remedies/ start prophylaxis.<br>Hold doseP until recovery to Grade 1 or baseline under the following directions:<br>1. If recovered within 4 weeks of onset (ie: ≤3 missed doses) , resume study drug as follows:<br>• If receiving 5 mg, restart study drug at 3 mg<br>• If receiving 3 mg, restart study drug at 2 mg<br>• If receiving 2 mg, discontinue study treatment<br>2. If not recovered within 4 weeks, permanently discontinue study drug. |
| Grade 3 | Administer symptomatic remedies/ start prophylaxis. Hold dose until recovery to Grade 1 or baseline under the following directions:<br>1. If recovered within 1 week, resume study drug at prior dose. If not recovered within 1 week, continue to hold dose.<br>2. If recovered within 2-4 weeks, resume study drug as follows:<br>• If receiving 5 mg, restart study drug at 3 mg<br>• If receiving 3 mg, restart study drug at 2 mg<br>• If receiving 2 mg, permanently discontinue study drug<br>3. If not recovered within 4 weeks, permanently discontinue study drug. |
| Recurrence of the **same** ≥ Grade 3 toxicity despite dose reduction | If the **same** ≥ Grade 3 event **recurs:**<br>1. Administer symptomatic remedies/ start prophylaxis. Hold[1] dose until recovery to Grade 1 or baseline.<br>2. If recovered within 2 weeks, resume study drug as follows:<br>• If receiving 5 mg, restart study drug at 3 mg<br>• If receiving 3 mg, restart study drug at 2 mg<br>• If receiving 2 mg, permanently discontinue study drug<br>3. If the **same** ≥ Grade 3 event **recurs** (i.e., third occurrence) despite entinostat dose reduction to 2 mg, as described above, discontinue study drug. |
| ≤Grade 2 | Administer symptomatic remedies / start prophylaxis.<br>Dosing of study drug may be interrupted at the Investigator's discretion.<br>• If dose is held for 4 consecutive weeks, permanently discontinue study drug.[1]<br>• If toxicity resolves, resume entinostat at the original dose. |

1: *If greater than 50% of doses are missed during any 6 week period, patients may be discontinued from study drug treatment, in the absence of extenuating circumstances.*

**Table 6: Dose Reductions for Entinostat-Related Hematologic Toxicity[1]**

| Hematologic Toxicity | |
|---|---|
| **Toxicity** | **Dose Modifications** |
| ≥Grade 3 neutropenia, ≥Grade 3 uncomplicated thrombocytopenia, or Grade 2 complicated thrombocytopenia | Administer symptomatic remedies/ start prophylaxis.<br>Hold dose[1]P until recovery to Grade 1 or study baseline under the following direction:<br>1. If not recovered by next scheduled dose, skip the dose. If recovered by next scheduled dose, resume study drug at prior dose.<br>2. If receiving 2 mg dose, and not recovered by either of the next 2 scheduled doses, permanently discontinue study treatment. Otherwise, skip each dose. If recovered for either of these doses, resume study drug as follows:<br>• If receiving 5 mg, restart study drug at 3 mg.<br>• If receiving 3 mg, restart study drug at 2 mg.<br>3. If not recovered within 4 weeks, permanently discontinue study drug. |
| Recurrence of the **same** hematologic toxicity | If the same hematologic toxicity recurs:<br>1. Administer symptomatic remedies/ start prophylaxis. Hold[1] dose until recovery to Grade 1 or baseline.<br>2. If recovered within 2 weeks, resume study drug as follows:<br>• If receiving 5 mg, restart study drug at 3 mg<br>• If receiving 3 mg, restart study drug at 2 mg<br>• If receiving 2 mg, permanently discontinue study drug<br>3. If the same ≥ Grade 3 event recurs (i.e., third occurrence) despite entinostat dose reduction to 2 mg, as described above, permanently discontinue study drug. |

1: *If greater than 50% of doses are missed during any 6 week period, patients may be discontinued from study drug treatment, in the absence of extenuating circumstances.*

### *5.3 Hydroxychloroquine*

5.3.1 Dose Modification for Hydroxychloroquine

Any AE of ≥ Grade 3 and attributed as possibly, probably or definitely related to HCQ will result in the dose being held until the AE has resolved to ≤ grade 1 or baseline. If the AE resolves, reinstitution of treatment can occur at either the previous or reduced dose as described in Table 2 at the discretion of the investigator.

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.

Toxicities that may be attributable to HCQ include nausea, vomiting, diarrhea, rash, and visual field deficit. If any of these AEs occur at grade ≤ 2, HCQ may be continued and the AE managed with supportive care. For any AE with a grade ≥ 3, the dose of HCQ should be held until the toxicity resolves to grade 1 (or is found to be unrelated to HCQ).

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.**

**Table 7: Dose Reductions for Regorafenib**

| Drug | Starting Dose | Dose Level -1 | Dose Level -2 |
|---|---|---|---|
| Regorafenib | 160 mg daily | 120 mg daily | 80 mg daily |

## 6.0 DRUG INFORMATION

### *6.1 Regorafenib*

6.1.1 Other names: BAY 73-4506

6.1.2 Commericial name: Stivarga

6.1.3 Availability: Regorafenib is commercially available as 40 mg tablets and is available through the hospital pharmacy

6.1.4 Route of administration: Oral

6.1.5 Storage and Stability: Regorafenib tablets are supplied in packages containing three bottles, with each bottle containing 28 tablets, for a total of 84 tablets per package. Store regorafenib at 25°C (77°F); excursions are permitted from 15 to 30°C (59 to 86°F). 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

6.1.6 Nursing/Patient Instructions: Take regorafenib at the same time each day with a low-fat breakfast.

6.1.7 **Table 8: Potential Toxicities of Regorafenib (from Stivarga Package Insert)**

| Adverse Reactions | STIVARGA (N=500) | | Placebo (N=253) | |
|---|---|---|---|---|
| | Grade | | Grade | |
| | All % | ≥ 3 % | All % | ≥ 3 % |
| **General disorders and administration site conditions** | | | | |
| Asthenia/fatigue | 64 | 15 | 46 | 9 |
| Pain | 29 | 3 | 21 | 2 |
| Fever | 28 | 2 | 15 | 0 |
| **Metabolism and nutrition disorders** | | | | |
| Decreased appetite and food intake | 47 | 5 | 28 | 4 |
| **Skin and subcutaneous tissue disorders** | | | | |
| HFSR/PPES | 45 | 17 | 7 | 0 |
| Rash | 26 | 6 | 4 | <1 |
| **Gastrointestinal disorders** | | | | |
| Diarrhea | 43 | 8 | 17 | 2 |
| Mucositis | 33 | 4 | 5 | 0 |
| **Investigations** | | | | |
| Weight loss | 32 | <1 | 10 | 0 |
| **Infections and infestations** | | | | |
| Infection | 31 | 9 | 17 | 6 |
| **Vascular disorders** | | | | |
| Hypertension | 30 | 8 | 8 | <1 |
| Hemorrhage* | 21 | 2 | 8 | <1 |
| **Respiratory, thoracic and mediastinal disorders** | 30 | 0 | 6 | 0 |
| Dysphonia | | | | |
| **Nervous system disorders** | 10 | <1 | 7 | 0 |
| Headache | | | | |

* Fatal outcomes observed.

Other clinically important adverse reactions observed more commonly in less than 10% of Stivarga-treated patients and at a higher incidence than in placebo-treated patients included the following: alopecia (7.6% vs. 1.6%), taste disorder (7.6% vs. 2.4%), musculoskeletal stiffness (6.0% vs. 2.0%), dry mouth (4.8% vs. 2.0%), hypothyroidism (4.2% vs. 0.4%), tremor (2.0% vs. 0.0), gastroesophageal reflux (1.4% vs. 0.0), and gastrointestinal fistula (0.8% vs. 0.4%).

Keratoacanthoma/squamous cell carcinoma of the skin occurred in 0.09% of 1100 Stivarga-treated patients across open-label or placebo-controlled clinical trials.

**For complete information about regorafenib, please see the FDA package insert

### *6.2 Entinostat*

6.2.1 Other names: MS-275, SNDX0275

6.2.2 Availability: Entinostat is not commercially available and will be supplied by Syndax at no cost to participants

6.2.3 Route of administration: Oral

6.2.4 Nursing/Patient Instructions: Entinostat is to be taken on an empty stomach, at least 2 hours after a meal and at least 1 hour before the next meal. If entinostat is vomited, dosing should not be re-administered but instead the dose should be skipped.

*For weekly (or less frequent) dosing:*

If an entinostat dose is missed, it may be taken up to 48 hours after the scheduled dosing time. If it is not taken within the 48 hour window, the dose should not be taken and should be counted as a missed dose. The patient should take the next scheduled dose per protocol.

6.2.5 Storage and Stability: Entinostat is an oral drug supplied by Syndax as pink to light red (1 mg) or yellow (5 mg) as polymorph B coated tablets. Each tablet contains mannitol, sodium starch glycolate, hydroxypropyl cellulose, potassium bicarbonate, and magnesium stearate as inert fillers. The film coating consists of hypromellose, talc, titanium dioxide, and ferric oxide pigments (red and yellow) as colorants. Entinostat is to be stored at controlled room temperature (15ºC to 25ºC) in a secure, locked storage area to which access is limited. Entinostat is to be protected from light and not to be exposed to extremes of temperature (greater than 30ºC or less than 5ºC). The pharmacist should dispense the investigational material to the patient at appropriate intervals throughout the study in childproof containers.

### 6.2.6 **Table 9: Potential Toxicities of Entinostat**

| **Adverse Events with Possible Relationship to MS-275 (SNDX-275, entinostat) (CTCAE 4.0 Term) [n= 215]** | | |
|---|---|---|
| **Likely (>20%)** | **Less Likely (<=20%)** | **Rare but Serious (<3%)** |
| | | |
| BLOOD AND LYMPHATIC SYSTEM DISORDERS | | |
| Anemia | | |
| | Febrile neutropenia | |
| GASTROINTESTINAL DISORDERS | | |
| | Abdominal pain | |
| | Constipation | |
| Diarrhea | | |
| | Dyspepsia | |
| | Flatulence | |
| Nausea | | |
| Vomiting | | |
| GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS | | |
| | Edema limbs | |
| Fatigue | | |
| | Fever | |
| | Non-cardiac chest pain | |
| INFECTIONS AND INFESTATIONS | | |
| | Infection[2] | |
| INVESTIGATIONS | | |
| | Alanine aminotransferase increased | |
| | Alkaline phosphatase increased | |
| | Aspartate aminotransferase increased | |
| | Blood bilirubin increased | |
| | Creatinine increased | |
| | Lymphocyte count decreased | |
| Neutrophil count decreased | | |
| Platelet count decreased | | |
| | Weight loss | |
| | White blood cell decreased | |

| Adverse Events with Possible Relationship to MS-275 (SNDX-275, entinostat) (CTCAE 4.0 Term) [n= 215] | | |
|---|---|---|
| **Likely (>20%)** | **Less Likely (<=20%)** | **Rare but Serious (<3%)** |
| | | |
| METABOLISM AND NUTRITION DISORDERS | | |
| Anorexia | | |
| | Dehydration | |
| | Hyperglycemia | |
| Hypoalbuminemia | | |
| | Hypocalcemia | |
| | Hypokalemia | |
| | Hypomagnesemia | |
| Hyponatremia | | |
| Hypophosphatemia | | |
| MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS | | |
| | Arthralgia | |
| | Back pain | |
| | Generalized muscle weakness | |
| | Myalgia | |
| | Pain in extremity | |
| NERVOUS SYSTEM DISORDERS | | |
| | Dysgeusia | |
| Headache | | |
| RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS | | |
| | Cough | |
| Dyspnea | | |
| | Epistaxis | |
| SKIN AND SUBCUTANEOUS TISSUE DISORDERS | | |
| | | Erythema multiforme |
| SURGICAL AND MEDICAL PROCEDURES | | |
| | Surgical and medical procedures - Other (packed RBC transfusion) | |

6.2.7 Prohibited Concomitant Medications

The following medications are excluded while the patient is receiving entinostat:

- Any other HDAC inhibitor, including valproic acid
- DNA methyltransferase inhibitors
- Any additional anticancer agents, such as chemotherapy, immunotherapy, targeted therapy, biological response modifiers, or endocrine therapy, will not be allowed, even if utilized as treatment of non-cancer indications.
- Any investigational agents
- Radiation therapy

  *Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be considered on an exceptional case-by-case basis after consultation with Sponsor. The patient must have clear measurable disease outside the radiated field. Administration of palliative radiation therapy will be considered clinical progression.*
- Traditional herbal medicines; these therapies are not fully studied and their use may result in unanticipated drug-drug interactions that may cause or confound the assessment of toxicity

Medications to be Avoided During the Study:

- Sensitive substrates of CYP1A2, CYP2C8, CYP3A with a narrow therapeutic window (see Appendix)
- Drugs that are known to inhibit or induce P-gp (see Appendix B)

### *6.3 Hydroxychloroquine*

6.3.1 Generic name: Hydroxychloroquine sulfate

6.3.2 Commercial name: Plaquenil

6.3.2 Availability: Hydroxychloroquine is commercially available and patients will be given prescriptions to be filled at their local pharmacy

6.3.4 Route of administration: Oral

6.3.5 Nursing/Patient Instructions: Hydroxychloroquine should be taken by swallowing the whole tablet in rapid succession without chewing. Patients receiving antacids, sucralfate, cholestyramine, and/or bicarbonate should have the HCQ drug dose administered at least 1 hour before or 2 hours after these medications.

6.3.6 **Table 10: 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 |

**For complete information about hydroxychloroquine, please see the FDA package insert

## 7.0 STATISTICS

### *7.1 Primary Endpoints*

#### 7.1.1 Phase I

The Phase I methodology will be a standard Phase I escalating trial with three patients per level, expanding to six patients per level in the event of dose-limiting toxicity at any level, and provision for expansion to 20 additional patients at the recommended Phase II dose to better characterize the optimal dose and the variation in toxicity at that level. A minimum of 4 weeks of combination therapy with hydroxychloroquine, entinostat, and regorafenib are required in the third patient before escalation to the next dose is initiated. At the MTD, at least 6 patients must be treated for 2 cycles to evaluate chronic toxicity and determine the RP2D (see section 4.3 for details).

We will calculate the percentage of subjects with toxicities at each dose level. We expect that doses close to full single agent doses will be tolerable. Three patients will be initially treated at each dose level. The plan for escalation is described below:

Three patients will initially be treated at each dose level.

If 0/3 patients experience DLT, then the dose will be escalated.

If 2-3/3 patients experience DLT, then the MTD has been exceeded. Three additional patients are treated at the previous dose (if only 3 had been treated) as a total of 6 patients must be treated at the MTD.

If 1/3 patients experience DLT, then 3 additional patients are treated. If none of the additional patients develop DLT, then the dose will be escalated, otherwise escalation ceases and the previous dose will be expanded to a total of 6 patients.

To assure safety in this phase I study, the operating characteristics of the escalation rule to be employed have been determined. The operating characteristics denote the probability of escalation to the next dose level, for a given true DLT rate. For a true DLT that is high, we desire the probability of escalation to be low. As noted in the table, the probability of escalating beyond a certain dose level, if that dose level truly has a 10% DLT rate, is 0.91. On the other hand, if the true DLT rate is 40%, there is only a 31% chance of dose escalation.

**Table 11: Operating Characteristics of the Escalation Rule**

| True DLT Rate | Probability of Escalation |
|---|---|
| .1 | .91 |
| .2 | .71 |
| .3 | .49 |
| .4 | .31 |
| .5 | .17 |
| .6 | .08 |
| .7 | .03 |
| .8 | .01 |

7.1.2 Phase II

Single agent regorafenib showed ORR of 1% in phase III trials in advanced colorectal cancers. We anticipate being able to improve this response rate substantially, perhaps to 15% or more. We will enroll a total 26 patients at the RP2D, including those treated during the phase I portion of the study. Sample size calculations are based on an assumption of at least 20 evaluable patients. With 20 patients, an exact binomial test with a 0.05 one-sided significance level will have 84% power to detect an improvement from the historical rate (null hypothesis proportion) of 1% to an improved rate (alternative proportion) of 15%.

### *7.2 Secondary endpoints*

Progression-free survival, overall survival, and duration of response will be calculated with 95% confidence intervals. Toxicity rates will also be calculated by category with 95% confidence intervals. With 20 subjects, we have a 88% chance of observing, at least once, any toxicity occurring at a rate of 10% or higher.

We will use response as the primary efficacy marker to investigate the relationship between changes in autophagy and protein lysine acetylation markers and the efficacy of treatment. We will summarize time-to-event outcomes (time to progression, survival time and duration of response) with Kaplan-Meier survival curves. We will also analyze toxicity including changes in counts, and degree of non-hematologic toxicity, according to the pharmacodynamic markers. Analyses of mutational profiles and correlatives from tumor biopsies (if obtained) will be exploratory.

### *7.3 Sample Size/Accrual Rate*

Phase I: The phase I study will involve six possible dose levels, although accrual at more than 3 or 4 levels is very unlikely. The total sample size will range from a minimum of 12 to a maximum of 24 patients. We expect accrual to be completed within 6-9 months.

Phase II: During the Phase II portion we will enroll up to 20 additional patients and expect an accrual time of approximately 1 year.

### *7.4 Statistical Software*

All analyses, except as otherwise noted, will be performed using either SAS or STATA.

# 8.0 SAFETY AND ADVERSE EVENTS

## *8.1 Definitions*

### 8.1.1 Adverse Event

An adverse event (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. The CTC version 4.0 will be used to grade toxicity. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- Results in study withdrawal
- Is associated with a serious adverse event
- Is associated with clinical signs or symptoms
- Leads to additional treatment or to further diagnostic tests
- Is considered by the investigator to be of clinical significance

### 8.1.2. Serious Adverse Event

Adverse events are classified as serious or non-serious. A serious adverse event is any AE that is:

- Fatal
- Life-threatening
- Requires or prolongs hospital stay
- Results in persistent or significant disability or incapacity
- A congenital anomaly or birth defect
- An important medical event
- Pregnancy

### 8.1.3 Important Medical Events

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the patient, and may require intervention to prevent one of the other serious outcomes noted above. All adverse events that do not meet any of the criteria for serious should be regarded as non-serious adverse events.

8.***2. Post-study***

All unresolved adverse events should be followed by the investigator until the events are resolved, the patient is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each patient to report any subsequent event(s) that the patient, or the patient's personal physician, believes might reasonably be related to participation in this study.

### *8.3 Abnormal Laboratory Values*

A clinical laboratory abnormality should be documented as an adverse event if the abnormality is of a degree, typically at least grade 2 and not present as grade 1 or higher at baseline, that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation.

### *8.4 Hospitalization, Prolonged Hospitalization or Surgery*

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should not be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

### *8.5 Recording of Adverse Events*

At each contact with the patient, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis. All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

### *8.6 Reporting of Serious Adverse Events*

8.6.1 Study Sponsor Notification by Investigator

A serious adverse event must be reported to the study sponsor within 24 hours of the event. Report serious adverse events by phone and facsimile to:

Peter O'Dwyer, MD
Phone: 215-360-0716
Fax: 215-349-8551
or via the HUP page operator at 215-662-4000

At the time of the initial report, the following information should be provided:

- Study identifier
- Study Center
- Patient number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

8.6.2 IRB Notification by Investigator-sponsor

Reports of all serious adverse events (including follow-up information) must be submitted to the IRB within 10 working days, according to IRB guidelines.

Reporting Process to IRB

Principal Investigators are required to submit reports of unanticipated problems posing risks to subjects or others that are probably or definitely study related via the HS-ERA system within 10 working days of the event.

For reportable deaths, the initial submission to the IRB may be made by contacting the appropriate IRB coordinator as soon as the death is known, with a report via HS-ERA within 10 days if death is from underlying disease and within 24 hours if study related activity is considered contributory to the death.

8.6.3 FDA Notification by Investigator-sponsor, Voluntary

The study sponsor shall notify the FDA by telephone or by facsimile transmission of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but no later than 7 calendar days from the sponsor's original receipt of the information. If a previous adverse event that was not initially deemed reportable is later found to fit the criteria for reporting, the study sponsor will submit the adverse event in a written report to the FDA as soon as possible, but no later than 15 calendar days from the time the determination is made.

8.6.4 Sponsor Notification

The investigator must inform Syndax in writing using a SAE form or MEDWATCH 3500A form of any SAE within 24 hours of being aware of the event. The date of awareness should be noted on the report. The written report must be completed and supplied to Syndax within 24 hours/1 business day at the latest on the following working day. The initial report must be as complete as possible, including details of the current illness and (serious) adverse event, and an assessment of the causal relationship between the event and the investigational product(s). Information not available at the time of the initial report (e.g., an end date for the adverse event or laboratory values received after the report) must be documented on a follow-up report. A final report to document

resolution of the SAE is required. If this is a multicenter trial, participating study sites must report SAEs to Syndax as described and within 24 hours of awareness. Participating sites should also report SAEs to the primary study site.

**SAES should be sent via email or fax to:**

aereporting@syndax.com
1-781-419-1420

## 9.0. DATA HANDLING AND RECORD KEEPING

### *9.1 Confidentiality*

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period

### *9.2 Source Documents*

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

### *9.3 Case Report Forms*

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. Data will be collected using CRFs designed, stored and secured in Velos.

### *9.4 Study Monitoring, Auditing and Inspecting*

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, government regulatory bodies, and the ACC CRU monitors of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.). Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices. Vivek Narayan, MD will act as medical monitor for this study.

## 10.0 MEASUREMENT OF EFFECT

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [Eur J Ca 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

### *10.1 Definitions*

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment on study.

Evaluable for objective response: Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response: Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re- evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

### *10.2 Disease Parameters*

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

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be >15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease: All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥ 10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable. Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non- measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions: All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions: All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

### *10.3 Methods for Evaluation of Measurable Disease*

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

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

#### 10.3.1 Clinical lesions

Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and <10 mm diameter as assessed using calipers (e.g.,

skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

#### 10.3.2 Conventional CT and MRI

This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

#### 10.3.3 PET-CT

At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

#### 10.3.4 Ultrasound

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

#### 10.3.5 Tumor markers

Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

#### 10.3.6 Cytology, Histology

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.

#### 10.3.7 FDG-PET

While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

- Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
- No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the

initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

***10.4 Response Criteria***

10.4.1. Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

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

10.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis)

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

10.4.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

**Table 12: Evaluation of Patients with Measurable Disease**

| Target Lesions | Non-Target Lesions | New Lesions | Overall Response | Best Overall Response when Confirmation is Required* |
|---|---|---|---|---|
| CR | CR | No | CR | >4 wks. Confirmation** |
| CR | Non- CR/Non-PD | No | PR | >4 wks. Confirmation** |
| CR | Not evaluated | No | PR | |
| PR | Non- CR/Non-PD/not evaluated | No | PR | |
| SD | Non- CR/Non-PD/not evaluated | No | SD | documented at least once >4 wks. from baseline** |
| PD | Any | Yes or No | PD | no prior SD, PR or CR |
| Any | PD*** | Yes or No | PD | |
| Any | Any | Yes | PD | |

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion. ** Only for non-randomized trials with response as primary endpoint.*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "symptomatic deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment.

**Table 13: Evaluation of Patients with Non-Measurable Disease**

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

10.4.3 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

## 11.0 ETHICAL CONSIDERATIONS

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of

the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator- designated research professional obtaining the consent.

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## Appendix A: Schedule of Events

Pre-study evaluations including scans are to be conducted within 28 days prior to start of protocol therapy. Laboratory assessments are to be done within 5 days of scheduled date. If correlative biopsies are not obtained due to lack of funding, these omissions will not be considered protocol deviations.

| | Pre-Study | Cycle 1 (28 days) | | | Cycle 2 (28 days) | | Cycle 3 and later cycles (28 days) |
|---|---|---|---|---|---|---|---|
| | | Day 1 | Day 2 | Day 15 | Day 1 | Day 15 | Day 1 |
| Office Visit[1] | X | X | | X | X | | X |
| **Treatment** | | | | | | | |
| HCQ[A] | | X---------- | | | | | ----------X |
| Regorafenib[B] | | X---------- | | | | | ----------X |
| Entinostat[C] | | X---------- | | | | | ----------X |
| **Tests and Observations** | | | | | | | |
| Informed consent | X | | | | | | |
| Inclusion/exclusion criteria | X | | | | | | |
| Demographics | X | | | | | | |
| Medical history | X | | | | | | |
| Concurrent meds | X | X | | X | X | | X |
| Physical exam[2] | X | X | | X | X | | X |
| Vital signs[3] | X | X | | X | X | | X |
| Height | X | | | | | | |
| Weight | X | X | | X | X | | X |
| Performance status | X | X | | X | X | | X |
| Adverse event evaluation | | X | | X | X | | X |
| **Laboratory Studies** | | | | | | | |
| Complete blood count with differential[4] | X | X | | X | X | X | X |
| PT/aPTT[5] | X | | | | | | |
| Chemistry profile[6] | X | X | | X | X | | X |
| CEA[7] | X | X | | | X | | X |
| Urine or Serum B-HCG (women of childbearing potential)[8] | X | X[8] | | | | | |
| **Radiologic Evaluations** | | | | | | | |
| CT/MRI[9] | X | | | | | | X |
| **Research Correlates** | | | | | | | |
| PBMCs for PD Analysis[10] | | X | X | X | | | |
| Tumor Biopsies[11] | | | | X | | | |

A: HCQ will be administered according to Section 4.2 (days 1-28). See Section 5.0 for dose modifications.

B: Regorafenib will be administered at 160 mg (Days 1-21), unless adjusted as per Section 4.1. See Section 5.0 for dose modifications.

C: Entinostat will be administered according to Section 4.2 (Days 1, 8, 15, 22). See Section 5.0 for dose modifications.

1: The pre-study visit must be within 28 days of starting therapy (Cycle 1 Day 1). Office visits should occur within 1 week of the originally scheduled date. Longer delays should be discussed with the principal investigator.

2: Complete physical exam will be completed at baseline; focused physical examinations will be conducted thereafter.

3: Blood pressure, heart rate, oxygen saturation, temperature

4: After Cycle 2, done Day 1 of each cycle, provided that hematologic toxicity is tolerable. Patients on non-prophylactic anticoagulation must have CBC weekly for cycles 1 and 2 and every 2 weeks with subsequent cycles.

5: Screening or Cycle 1 Day 1 only.

6: Na, K, Ca, Mg, phosphorus, BUN, creatinine, glucose, alkaline phosphatase, AST, ALT, total bilirubin, albumin, total protein. After Cycle 1, done Day 1 of each cycle unless concerning toxicity

7: CEA will be checked at baseline. If it is elevated, it will be checked on day 1 of each cycle, otherwise it will not be routinely followed.

8: Serum B-HCG will be checked at screening and either urine or serum B-HCG must be checked within 3 days of initiation of study drug

9: Radiologic evaluations (CT preferred, MRI acceptable; PET/CT only if required per treating physician) of the abdomen/pelvis and chest (if thoracic disease is present) and tumor measurements will be performed at baseline (within 4 weeks of study therapy), at Cycle 3 Day 1 and Cycle 5 Day 1 (or within 2 weeks), then every 8-12 weeks per standard practice. Scans may be evaluated for texture analysis as a baseline for exploratory radiomic endpoints.

10: Sample handling as in Laboratory Manual.

11: Tumor biopsies to be taken in phase II portion (when funding obtained) in subjects who meet eligibility at Cycle 1 between day 15 and day 22 if possible. Archived tumor material prior to treatment will also be analyzed, as discussed in section 4.9.

## Appendix B: Concomitant Medications to Avoid

Examples of sensitive *in vivo* CYP substrates and CYP substrates with narrow therapeutic range are summarized below.

**Examples of substrates that may be affected by entinostat**

| CYP Enzymes | Substrates with narrow therapeutic range[1] |
|---|---|
| CYP1A2 | Theophylline, tizanidine |
| CYP2C8 | Paclitaxel |
| CYP3A[2] | Alfentanil, astemizole3, cisapride3, cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus, tacrolimus, terfenadine3 |

*1 CYP substrates with narrow therapeutic range refers to drugs whose exposure-response relationship indicates that small increases in their exposure levels by the concomitant use of CYP inhibitors may lead to serious safety concerns (e.g., Torsades de Pointes).*

*2 Because a number of CYP3A substrates (e.g., darunavir, maraviroc) are also substrates of P-gp, the observed increase in exposure could be due to inhibition of both CYP3A and P-gp.*

*3 Withdrawn from the United States market because of safety reasons.*

**P-gp Inhibitors and Inducers**

| Inhibitors | Inducers |
|---|---|
| Amiodarone, azithromycin, captopril, carvedilol, clarithromycin, conivaptan, diltiazem, dronedarone, felodipine, lopinavir,quercetin, ranolazine,ticagrelor, ritonavir, cyclosporine, verapamil erythromycin, ketoconazole, itraconazole, quinidine | Avasimibe, carbamazepine, phenytoin, rifampin, St John's Wort, tipranavir/ritonavir |

## Appendix C: Medically Acceptable Methods of Birth Control

Females:
Women of child-bearing potential (as per section 3.1.8) must agree to use 2 of the following forms of contraception OR completely refrain from intercourse during the study and for at least 120 days following the last dose of study drug.

Males:
Men with partners of child-bearing potential (as per section 3.1.8) must agree along with their partner to use 2 of the following forms of contraception OR completely refrain from intercourse during the study and for at least 120 days following the last dose of study drug.

Acceptable methods include:

- Condoms
- Diaphragm
- Cervical cap
- Intra-uterine device
- Surgical sterilization (tubal ligation or vasectomy)
- Oral contraceptives

Abstinence at certain times of the cycle, such as during ovulation or after ovulation, or withdrawal are not acceptable methods. The list of methods is not exhaustive and additional contraception methods not included above may also be acceptable. The study doctor must approve the contraceptive methods in subjects with child-bearing potential.