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NCI Comprehensive Cancer Center
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STUDY NUMBER:	CASE 1216
ClinicalTrials.gov #:	NCT02861300
PROTOCOL DATE:	December 16, 2020
STUDY TITLE:	Phase I/II Study of CB-839 and Capecitabine in Patients with Advanced Solid Tumors and Fluoropyrimidine Resistant PIK3CA Mutant Colorectal Cancer

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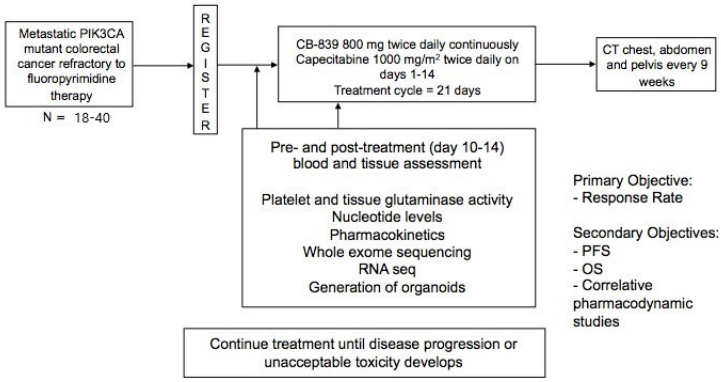
STUDY COORDINATOR:	[redacted]
SPONSOR:	Case Comprehensive Cancer Center
SUPPORT/FUNDING:	GI SPORE 1P50 CA150964
SUPPLIED AGENT:	CB-839
IND #:	130616
OTHER AGENT:	Capecitabine

SUMMARY OF CHANGES

Protocol Date 12/16/2020	Section	Change
	Title Page 2	*Erin Anderson, RN* is replacing Janette Gortz
	Protocol Summary, Sample Size	~~Phase I: 9-24 patients~~ ~~Phase II: 10-29 patients~~ ~~Males and females permitted~~ *Phase I: 9-24 patients* *Phase II: 18-40 patients* *Males and females permitted*
		STUDY SCHEMA—PHASE II  **(schema updated to reflect new "N" number)** **Phase II:** Patients will be treated with CB-839 *800 mg* by mouth twice daily for 21 days (continuous dosing) as well *as capecitabine 1000 mg/m²* by mouth twice daily for 14/21 days *as were determined to be safe doses during the phase I portion of the study.* ~~Doses to be administered will be determined in the Phase 1 portion of the study.~~ Pre- and post-treatment (post-treatment being day 10-15) blood samples will be obtained for assessment of glutaminase activity. Baseline tissue biopsies and a day 10-15 tissue biopsy will be obtained to assess tissue *glutaminase activity* and ~~UPP1 gene expression, glutathione levels,~~ nucleotide levels *as well as for whole exome sequencing, RNA seq and for the production of organoids* ~~and 5FU incorporation into RNA and DNA~~. Blood and tissue specimens will be collected the same day. Post-15 day treatment pharmacokinetics will be obtained and correlated with other laboratory assessments. Patients will

		undergo a disease assessment with CT imaging of the chest, abdomen and pelvis following 3 cycles (9 weeks) of treatment.
	3.2	3.2 Study design for phase II component The phase II portion of the study is a single arm design, assessing the disease control rate of combination therapy with CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who have developed fluoropyrimidine resistance. Patients will receive combination therapy with CB-839 and capecitabine for 21 day cycles and will remain on treatment until either disease progression, development of unacceptable toxicity or discontinuation per patient/physician preference. Patients will receive CB-839 800 mg orally twice daily for 21 days (continuous administration) and capecitabine 1000 mg/m2 orally twice daily for 14/21 days as was determined during the phase I portion of the study. Patients will undergo blood and tissue sampling at baseline and 10-15 days post-treatment for assessment of glutaminase activity. Tissue specimens will also be assessed for changes in nucleotide levels as well as for a whole exome analysis, an RNA seq analysis and for the generation of organoids. Patients will undergo a CT of the chest, abdomen and pelvis following 3 cycles of therapy (every 9 weeks).
	3.2	**3.2 Number of Patients** A total of 9-24 patients will be enrolled in the phase I portion of this trial and a total of 18-40 patients will be enrolled in the phase II portion of this trial.
	4.2.1	Patients must have histologically or cytologically confirmed, metastatic colorectal cancer. PIK3CA mutant status must be confirmed by tumor sequencing conducted in a CLIA certified lab. *Genetic sequencing performed on tissue specimens or circulating DNA from peripheral blood samples are allowed.* *Abnormalities in PIK3CA considered to be variants of unknown significance (VUS) will not be eligible*

	4.2.3	Patients must have received and progressed on fluoropyrimidine or fluoropyrimidine based therapy. *Patients must have a history of disease progression during treatment with fluoropyrimidine, or within 3 months of a dose of a fluoropyrimidine-containing regimen. Patients who have only progressed during treatment holiday of 3 months or greater will not be eligible.* Receipt of either oxaliplatin or irinotecan in combination with a fluoropyrimidine is required in the front line setting unless either of these agents are otherwise contraindicated in the opinion of the treating physician, in which case a fluoropyrimidine only may be used. Prior regorafenib or TAS-102 therapy is not required.
	4.2.6	▪ Serum creatinine ~~within the normal institutional limits~~ *below the institutional upper limit of normal.*
	10.1.3	The pre-treatment biopsy must be done after the 4-week washout from previous systemic therapy. Four core biopsies will be obtained for each patient during each biopsy. Two cryovials should contain approximately 1.5 ml sterile PBS for placement of a fresh specimen and then will be placed on wet ice (see section 10.4 for further details).
	11.4	[c] Tumor biopsy may be done up to 14 days prior to initiation of treatment. Please allow 28 days washout from prior systemic therapy prior to pre-treatment biopsy.
	14.1.1	Phase II: *Progression free survival (FPS) on combination CB-839 and capecitabine determined by clinical assessment and* ~~Disease control rate (inclusive of CR, PR or SD) as assessed by~~ RECIST criteria ~~of combination CB-839 and capecitabine chemotherapy~~ in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy. *Progression free survival is defined as the time from randomization to documented progression or death without progression.*
	14.1.2	Phase II: *Response rate as assessed by RECIST criteria of combination CB-839 and capecitabine chemotherapy in* ~~Progression free survival of~~ patients

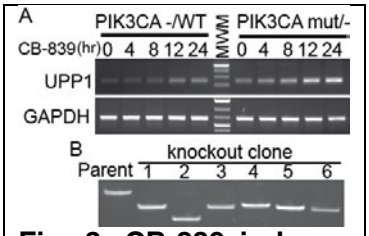
		with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy. ~~Progression free survival is defined as the time from randomization to documented progression or death without progression.~~
	14.2	In the phase II component of this study, the primary endpoint is *progression free survival at 6 months* ~~disease control rate (which is to include patients with a complete response, partial response or stable disease)~~, with a null hypothesis of *18*~~5~~% vs. a target of *36*~~20~~%. Employing a 2-stage design, and assuming 80% power and a 0.05 significance level, *40*~~29~~ total patients will be required with *18*~~10~~ in the first stage and the probability of early stopping if the null hypothesis is correct (*<3/18 patients with stable disease at 6 months* ~~0/10 responses~~) will be *0.589*~~0.5987~~. If *3 or more patients in 18* has stable disease or better at 6 months~~at least 1 patient in 10 has disease control~~, an additional *22*~~19~~ patients will be treated (total of *40*~~29~~ patients). If antitumor activity is identified in the PIK3CA mutant population, we will consider amending the phase 2 trial to assess an additional cohort of patients with wild-type PIK3CA. The statistical considerations will follow the same rules as in the original mutant cohort. Response rate will be determined using RECIST criteria.
	14.4	All correlative studies will be performed in biospecimens from the intent to treat population of 10-29 patients from the phase II portion of the study. Pre- and post-treatment tumor samples from the same patients will be used for assessments, except glutaminase activity, which will be performed on post-treatment samples only (tumor samples and platelet samples). All correlative continuous measurements will be summarized using mean +/- SEM, range and median; all categorical measurements will be summarized using frequencies and proportions. Change in these measurements in tumors before and after therapy will be assessed using paired Wilcoxon tests. Unpaired Wilcoxon tests will be used for assessment of glutaminase actively in post-treatment samples only. ~~Assuming a maximum of 29 patients (0.05 significance level and 80% power), for the paired Wilcoxon tests we can detect normalized differences of 5.4 to 21.6% for standard deviations of the difference between pairs ranging from 0.10 to 0.40. Correlations of plasma trough levels and platelet glutaminase activity will be calculated using Spearmans correlation coefficients. Assuming a maximum of 29 patients, 0.05 significance level,~~

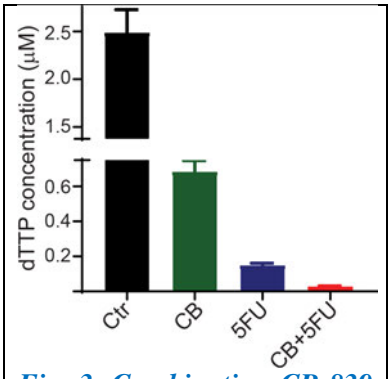
		~~we have >95% power to detect a Spearmans correlation coefficient of 0.60 or higher.~~ All correlative studies will be performed in biospecimens from the intent to treat population of 18-40 patients from the phase II portion of the study. Pre- and post-treatment tumor samples from the same patients will be used for assessments, except glutaminase activity, which will be performed on post-treatment samples only (tumor samples and platelet samples). All correlative continuous measurements will be summarized using mean +/- SEM, range and median; all categorical measurements will be summarized using frequencies and proportions. Change in these measurements in tumors before and after therapy will be assessed using paired Wilcoxon tests. Unpaired Wilcoxon tests will be used for assessment of glutaminase actively in post-treatment samples only. *Assuming a maximum of 40 patients (2-sided test with 0.05 significance level and 80% power), for the paired T-test we can detect normalized differences of 4.5 to 18.2% for standard deviations of the difference between pairs ranging from 0.10 to 0.40. Correlations of plasma trough levels and platelet glutaminase activity will be calculated using Spearman correlation coefficients. Assuming a maximum of 40 patients, 2-sided test with 0.05 significance level, we have >98% power to detect a Spearman correlation coefficient of 0.60 or higher.* Whole exome sequencing and RNA sequencing will be performed on pre and post treatment tissue samples and compared for differences for all patients (n=~~29~~*40* patients maximum). All of these analyses are exploratory, hence no power calculations are shown.
	14.5	In the phase II portion of the study, disease control rate is the primary endpoint and the study will be conducted using a Simon two-stage design. As outlined in section 14.2, a minimum of *18*~~10~~ and a maximum of *40*~~29~~ patients will be required.

	14.6	Accrual for this study will occur at the University Hospitals Seidman Cancer Center and the Cleveland Clinic Taussig Cancer Institute, both of the Case Comprehensive Cancer Center. For the phase I portion of the trial, we anticipate that it will take approximately 6-7 months to complete this portion of the trial if all doses levels need to be evaluated. For the phase II portion of the study, between the two institutions, 719 colorectal cancer patients are seen annually. We estimate that approximately 30% of our patients will have measureable, metastatic disease and that 15% of patients will have a PIK3CA mutation, resulting in 32 eligible patients per year. Based on our proposed sample size of *18-40* ~~10 29~~ patients, we anticipate that it will take approximately *18 months* ~~one year~~ to accrue to the phase II portion of the trial.

Protocol Date	**Section**	**Change**
01/30/18	Title page	David Bajor, MD is replacing Jennifer Eads, MD as the PI
	Title page	All sub-investigators have been removed as they are listed on the delegation log instead
	Title page	The Study Coordinators for Phase 1 and Phase 2 have been added
	Protocol Summary	Phase II Primary Objective: To determine the ~~response~~ *disease control* rate of combination CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancers who are refractory to fluoropyrimidine based therapy.

Protocol Date	Section	Change
	Protocol Summary	Phase II Correlative Objectives: ~~To determine the change in UPP1 gene expression in tumor specimens following treatment with CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy.~~ ~~To determine the change in glutathione levels in tumor specimens following treatment with CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy.~~ *To determine genomic changes in both DNA and RNA in tumor specimens following treatment with CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy.* ~~To determine the level of 5-fluorouracil incorporation into RNA and DNA in tumor specimens following treatment with CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy.~~ *To generate organoids from patient tissue biopsies obtained prior to and following treatment in patients treated with CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who are refractory of fluoropyrimidine based therapy.*
	Protocol Summary	Phase II: Patients will be treated with CB-839 *800 mg* by mouth twice daily for 21 days (continuous dosing) as well *as capecitabine 1000 mg/m^2* by mouth twice daily for 14/21 days *as were determined to be safe doses during the phase I portion of the study.* ~~Doses to be administered will be determined in the Phase 1 portion of the study.~~ Pre- and post-treatment (post-treatment being day 10-15) blood samples will be obtained for assessment of glutaminase activity. Baseline tissue biopsies and a day 10-15 tissue biopsy will be obtained to assess tissue *glutaminase activity* and ~~UPP1 gene expression, glutathione levels,~~ nucleotide levels *as well as for whole exome sequencing, RNA seq and for the production of organoids* ~~and 5FU incorporation into RNA and DNA~~. Blood and tissue specimens will be collected the same day. Post-15 day treatment pharmacokinetics will be obtained and correlated with other laboratory assessments. Patients will undergo a disease assessment with CT imaging of the chest, abdomen and pelvis following 3 cycles (9 weeks) of treatment.
	Abbreviations	*PBS: phosphate buffered saline* TR*SR*~~PC~~: Translational Research ~~and Pharmacology Core~~ *Shared Resource* UHCMC: University Hospitals ~~Case~~ *Cleveland* Medical Center

Protocol Date	Section	Change
	Table of Contents	Section 10.0 10.2 *Pharmacokinetic analysis of CB839* ~~UPP1 Gene Expression~~ 10.3 ~~Pharmacokinetic analysis of CB839~~ *Additional pharmacodynamic analyses* *10.4 Generation of organoids*
	1.2 CB-839	~~*CB-839 treatment induces UPP1, an enzyme that plays an important role in converting 5-fluorouracil to an active compound*~~ ~~The mechanism of cytotoxicity of 5-fluorouracil has been ascribed to the mis-incorporation of fluoronucleotides into RNA and DNA and to the inhibition of the nucleotide synthetic enzyme thymidylate synthase (13). To interrogate the molecular mechanisms by which CB-839 enhances the tumor inhibitory effect of 5-fluorouracil, we noted that our gene expression analysis shows that glutamine deprivation up-regulates uridine phosphorylase 1 (UPP1) in PIK3CA mutant cells (Fig. 3). UPP1 facilitates conversion of 5-FU to FdUTP and FUTP (14), which can be incorporated into DNA and RNA. It has been shown that UPP1 -/- embryonic stem cells are much more resistant to 5-FU (15), whereas overexpression of UPP1 enhances cytotoxicity of 5-FU (16). We examined UPP1 gene expression in the isogenic HCT116 PIK3CA mut/- and WT/- cell lines. CB-839 induced more UPP1 in PIK3CA mut/- cells than in the WT/- cells. Similar results were observed in the DLD1 PIK3CA mut/- and WT/- cell lines. We therefore hypothesize that in addition to blocking glutamine metabolism, CB-839 treatment also induces UPP1 gene expression and thus enhances 5-FU toxicity.~~  **~~Fig. 3. CB-839 induces more UPP1 in PIK3CA-mutant cells.~~** ~~(**A**) HCT116 PIK3CA -/WT and mut/- cell lines were treated with 3 μM CB-839 for the indicated times. RNAs were extracted for RT-PCR analyses. MWM: molecular weight marker. (**B**) Knockout of UPP1 in HCT116 cells. Genomic fragments encompassing the CRISP cutting site (68 bp) of parent and knockout clones.~~

Protocol Date	Section	Change
		Combination of CB-839 and 5-fluorouracil depletes cellular dTTP levels *Given that glutamine is a precursor for nucleotide synthesis and that 5-FU is a thymidylate synthase inhibitor, we set to determine how the drug combination impacts cellular dTTP levels. As shown in Fig. 3, either drug alone significantly reduced amounts of dTTP, whereas the combination of CB-839 and 5-FU nearly completely depleted cellular dTTP. These data suggest that CB-839 enhances the cytotoxicity of 5-FU by further depleting cellular dTTP.*  ***Fig. 3. Combination CB-839 and 5-FU depletes cellular dTTP levels.*** *HCT116 cells were treated with the indicated drugs. Cellular dTTP amounts were measured by LC-MS/MS.*

Protocol Date	Section	Change
	1.4	**Rationale for clinical and correlative studies** It has been demonstrated that CB-839, via targeting glutaminase, inhibits xenograft growth in 2 of 2 PIK3CA mutant colon cancer test xenograft models but does not inhibit growth in PIK3CA WT models, supporting that there is an addiction of PIK3CA mutant colorectal cancers to glutamine metabolism. Furthermore, CB-839 plus 5-FU shrinks 2 of 2 PIK3CA mutant xenografts that both grow through maximally tolerated doses of 5-FU alone. Based on these findings, we propose to test responses of the CB-839 ~~plus 5-FU~~ *and fluoropyrimidine* combination in ~~5-FU~~ *fluoropyrimidine* resistant human patients. Based on our preclinical mechanistic studies described above, we propose a phase I clinical trial to determine ~~the *in vivo* pharmacodynamic effects of CB-839 in patients with metastatic colorectal cancer who harbor a PIK3CA mutation, and to determine the~~ maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D) *and dose limiting toxicities* of CB-839 plus capecitabine in patients with *advanced solid tumors.* ~~PIK3CA mutant metastatic colorectal cancer. We will test the hypothesis that CB-839 results in reduced glutaminase activity in tumors and platelets, as well as induction of UPP1 expression in tumors, with trough plasma levels associated with these pharmacodynamic effects.~~ The data derived from this trial will be used for a subsequent phase II clinical trial where we will estimate the antitumor activity and pharmacodynamic effects of CB-839 and capecitabine in patients with PIK3CA mutant colorectal cancers that are resistant to fluoropyrimidine therapy. We hypothesize that CB-839 in combination with capecitabine will overcome fluoropyrimidine resistance and that the addition of capecitabine to CB-839 will result in the inhibition of glutaminase in tumors and platelets *and* the reduction *in* ~~of glutathione and~~ nucleotide levels in tumors~~, the increase in 5-FU incorporation into RNA and DNA in tumors and the induction of UPP1 gene expression in tumors.~~ *Genomic abnormalities on the DNA and RNA level will also be explored via a whole exome sequencing analysis and an RNA seq analysis.* *As of January 2018, the phase I portion of the study has been fully accrued. A total of 16 patients were treated on the study and we were able to assess all of the four planned dose levels. No dose limiting toxicities were observed and we have therefore determined that the recommended phase II dose be CB-839 800 mg by mouth twice daily continuously and capecitabine 1000 mg/m^2 orally twice daily on days 1-14 of a 21 day treatment cycle*

Protocol Date	Section	Change
	2.0	**OBJECTIVES** The overall objectives for this study are to 1) determine the recommended phase II dose (RP2D) of CB-839 and capecitabine in patients with advanced solid tumors who have no further treatment options or patients for whom single agent capecitabine is an acceptable treatment regimen and 2) to determine the antitumor activity of CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy. The phase I portion of this study will also determine the dose-limiting toxicities of this treatment regimen as well as the pharmacokinetics. In the phase II portion of this study, the pharmacodynamic effects of CB-839 and capecitabine will be evaluated using multiple biomarker studies including glutaminase activity level, *and* ~~UPP1 gene expression, glutathione levels,~~ nucleotide levels *as well as a whole exome sequencing analysis and an RNA seq analysis.* ~~and incorporation of 5-FU into RNA and DNA.~~ We hypothesize that we will observe a reduction in glutaminase activity in both tumors and platelets *and* ~~, induction of UPP1 gene expression in tumors,~~ a reduction in ~~glutathione levels and~~ nucleotide levels following treatment. *The whole exome and RNA seq analyses will be exploratory.* ~~and an increase in the incorporation of 5-FU into RNA and DNA.~~ *The generation of organoids from tumor biopsy specimens both prior to and following treatment will also be exploratory and will be used for further potential studies as additional preclinical information becomes available.*
	2.1	**Primary Objective** 2.1.2 - Phase II: To determine the ~~response~~ *disease control* rate of combination CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancers who are refractory to fluoropyrimidine based therapy.

Protocol Date	Section	Change
	2.3	**Correlative Objective(s)** 2.3.1 Phase II ~~To determine the change in UPP1 gene expression in tumor specimens following treatment with CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy.~~ ~~To determine the change in glutathione levels in tumor specimens following treatment with CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy.~~ *To determine genomic changes in both DNA and RNA in tumor specimens following treatment with CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy.* ~~To determine the level of 5-fluorouracil incorporation into RNA and DNA in tumor specimens following treatment with CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy.~~ *To generate organoids from patient tissue biopsies obtained prior to and following treatment in patients treated with CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who are refractory of fluoropyrimidine based therapy.*

Protocol Date	Section	Change
	3.2	**Study design for phase II component** The phase II portion of the study is a single arm design, assessing the ~~antitumor activity~~ *disease control rate* of combination therapy with CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who have developed fluoropyrimidine resistance. Patients will receive combination therapy with CB-839 and capecitabine for 21 day cycles and will remain on treatment until either disease progression, development of unacceptable toxicity or discontinuation per patient/physician preference. Patients will receive CB-839 *800 mg* orally twice daily for 21 days (continuous administration) and capecitabine *1000 mg/m^2* orally twice daily for 14/21 days *as was determined during the phase I portion of the study*. ~~Doses to be administered will be determined during the phase I portion of the study.~~ Patients will undergo blood and tissue sampling at baseline and 10-15 days post-treatment for assessment of glutaminase activity. Tissue specimens will also be assessed for *changes in* ~~UPP1 gene expression, glutathione levels,~~ nucleotide *levels as well as for a whole exome analysis, an RNA seq analysis and for the generation of organoids.* ~~and 5-FU incorporation into DNA and RNA~~. Patients will undergo a CT of the chest, abdomen and pelvis following 3 cycles of therapy (every 9 weeks).
	5.0	**REGISTRATION** All patients will be registered through University Hospitals ~~Case~~ *Cleveland* Medical Center/Seidman Cancer Center and will be provided a study number by contacting the study coordinator.
	6.1.2	6.1.2 Phase II: Patients will receive combination therapy with CB-839 and capecitabine for 21 day cycles and will remain on treatment until either disease progression, development of unacceptable toxicity or discontinuation per patient/physician preference. Patients will receive CB-839 *800 mg* orally twice daily for 21 days (continuous administration) and capecitabine *1000 mg/m^2* orally twice daily for the first 14/21 days *as was determined during the phase I portion of the study*. ~~Doses to be administered will be determined during the phase I portion of the study.~~ Additionally, given that there are ongoing studies, the safety profile will continue to be monitored in these studies and the RP2D could be adjusted based on emerging safety and tolerability data.
	6.2	**General Agent Administration Guidelines** CB-839 will be supplied as 200 mg *capsules* ~~tablets~~.

Protocol Date	Section	Change
	6.2.1	6.2.1 CB-839 Administration CB-839 will be given orally twice daily in combination with capecitabine chemotherapy during a 21 day treatment cycle. Administration of CB-839 will be continuous throughout the 21 day treatment cycle. During the phase I component of the study, the dose of CB-839 administered will be dictated by the dose escalation table. During the phase II component of the study, the dose of CB-839 administered will be *800 mg by mouth twice daily as was determined during the phase I portion of the study* ~~determined during the phase 1 component of the study~~.
	6.2.2	Capecitabine Administration Patients will receive capecitabine chemotherapy orally by mouth twice daily for the first 14 days of each 21 day treatment cycle. During the phase I component of the study, the dose of capecitabine administered will be dictated by the dose escalation table. During the phase II component of the study, the dose of capecitabine administered will be *1000 mg/m^2 twice daily on days 1-14 of a 21 day treatment cycle as was* ~~that~~ determined during the phase I component of the study.
	8.5.1	**SAE Reporting Requirements** All SAEs should be reported to the study principal investigator: ~~Jennifer Eads~~ *David Bajor*, MD Phone: [redacted]

Protocol Date	Section	Change
	9.1	**CB-839** Name of Agent: CB-839 Other Names: NA **Product description:** CB-839 is provided as 200 mg opaque Swedish orange gelatin capsules *or as tablets that are manufactured, packaged, and labeled according to current Good Manufacturing Practices (cGMP).* **Storage requirements:** CB-839 capsules should be stored as will be indicated on the study drug label - *this should be in a clinical site pharmacy at controlled room temperature, defined as between 20° and 25°C +/- 5°C (i.e. between 15° and 30°C (59° to 86°F).* ~~at room temperature between 15° 30°C (59° 86°F).~~ Stability studies being carried out on the phase I clinical supplies support storage for CB-839 capsules for at least 24 months at room temperature. *CB-839 HCl tablets must be stored in a secure area with controlled access and separately from commercially available and other investigational drugs, preferably in a separate location. Any breach of investigational product storage conditions including temperature excursions outside the range 15° and 30°C must be reported to the Sponsor upon detection and the IP in question must be quarantined until the Sponsor authorizes usage or otherwise*. Patients will be instructed to store medication according to storage conditions noted on the label, out of reach of children or other cohabitants.
		Packaging and labeling: *Tablets and* capsules are packaged in white plastic bottles with a label on the outside identifying the investigational product, capsule strength, storage conditions, investigational drug supplier, lot number and date of manufacture. Each bottle is sealed with a tamper-evident seal and a child-proof cap. Each bottle contains fifty (50) *tablets or* capsules.

Protocol Date	Section	Change
	10.1.3	Collection of Specimens During the phase II component of the study, patients will undergo either CT or US guided biopsy of a metastatic focus both prior to the initiation of treatment and 10-15 days following initiation of treatment. The pre-treatment biopsy may be performed up to 14 days prior to the initiation of treatment. Tumor Biopsies: Patients will undergo biopsy in the University Hospitals ~~Case~~ *Cleveland* Medical Center (UHCMC) Department of Radiology ~~by study interventional radiologist, Dr. Dean Nakamoto~~. If being treated at the Taussig Cancer Institute (TCI) of the Cleveland Clinic, patients will undergo biopsy in the Cleveland Clinic Department of Radiology. *If being treated at the Weill Cornell College of Medicine, patients will undergo biopsy in the Weill Cornell College of Medicine Department of Radiology.* Use of CT or US guidance for each biopsy will be per the discretion of the physician performing the biopsy and the same metastatic focus of tumor will be biopsied at baseline and 10-15 days following initiation of treatment. *If for whatever reason the same lesion cannot be biopsied, a second lesion may be biopsied but this must be noted and recorded.* Biopsies will be conducted according to standard practice. Three core biopsies will be obtained for each patient during each biopsy. Fine needle aspiration biopsies are NOT appropriate. Three RNA cryovials will be labeled with the date, patient initials, patients study number and vial number. All study personnel, particularly those handling RNA cryovials, must wear clean gloves. Each of the three core biopsies will be placed in its own cryovial. *One cryovial should contain approximately 1.5 ml sterile PBS for placement of a fresh specimen and then will be placed on wet ice (see section 10.4 for further details). The remaining two* ~~The three~~ cryovials will immediately be placed in dry ice or liquid nitrogen. *Biopsies collected at UHCMC will be* ~~and~~ transported to the Case Comprehensive Cancer Center, Translational Research *Shared Resources* ~~and Pharmacology Core Facility~~ for *the Wang lab to pick up* ~~processing~~. *Biopsies collected at TCI will be transported to the Cleveland Clinic Central Biorepository for the Wang lab to pick up. Biopsies collected at Weill Cornell College of Medicine will be transported to the Laboratory of Dr. Manish Shah for processing. The frozen* specimens will be processed individually with the second ~~and third~~ specimens being processed only if insufficient nucleic acid is recovered or if histologic review identifies the presence of necrotic tumor in the first specimen. The remaining fresh frozen specimens will be stored for later use. Platelets: Approximately 24ml of whole blood will be collected via peripheral venipuncture into *3, 10 ml yellow top ACD-A (Acid Citric Dextrose (ACD) Solution A) tubes* ~~dextrose/citrate~~ for analysis of glutaminase activity in platelets. Whole blood samples will be collected on wet ice and transported to the Translational Research *Share Resource* ~~and Pharmacology Core Facility~~ for processing and storage. Specimens will be collected at the time of the pre-treatment tissue biopsy as well as 10-15 days following initiation of treatment (obtained on the same day as the tissue biopsy).

		Handling of Specimens *at UHCMC* *The vial containing PBS will be placed on wet ice (>5 lb), and the other two cryovials will immediately be placed in dry ice (>5 lb). Personnel from the Case Comprehensive Cancer Center, Translational Research Shared Resource will then be contacted (see below) for transportation, tracking and storage. Upon acquisition, TRSR personnel will contact the Wang Lab to pick up the PBS containing vial for organoid culture and analyses as described in the Laboratory Manual and the frozen specimens for storing at -80°C.* ~~Specimens will be transported on either dry ice or liquid nitrogen (tumor biopsies) from the Interventional Radiology suite of either UHCMC or the TCI to the Translational Research and Pharmacology Core Facility (TRPC) for further processing. Whole blood will be transported on wet ice (platelets) from the Coleman Research Unit (UHCMC) or from either the outpatient clinic area or Clinical Research Unit (CRU) of the TCI, also to the TRPC. All specimens collected at TCI will be collected as outlined and will be delivered by courier to the TRPC. TRPC personnel will log collected specimens for each patient into the OnCore database using the assigned patient and specimen numbers assigned at the time of study enrollment and tissue acquisition respectively.~~ Translational Research *Shared Resource Contact Information (UH Seidman)* ~~and Pharmacology Core~~ ATTN: Erin Hohler
	10.1.4	University Hospitals *Cleveland* ~~Case~~ Medical Center ***Wang Lab Contact Information:*** *Yiqing Zhao* [redacted] *John Wang* [redacted] *Wolstein Research Building* [redacted] *2013 Cornell Road* *Cleveland, OH 44106* *10.1.4.2Handling of Specimens at TCI* *The fresh biopsy vial (containing PBS) will be placed on wet ice (>5 lb), and the other two cryovials will immediately be placed in dry ice (>5 lb). Personnel from the Cleveland Clinic Central Biorepository will then be contacted (see below) for transportation, tracking and storage. Upon acquisition, the Cleveland Clinic Central Biorepository personnel will contact the Wang Lab to pick up the PBS containing vial for organoid culture and analysis as described in the Laboratory Manual and the frozen specimens for storing at -80°C.*

Protocol Date	Section	Change
		The Cleveland Clinic Central Biorepository contact information *ATTN: Brad Skilton* *Cleveland Clinic Central Biorepository* *Department of Pathology—Tissue Procurement* [redacted] ***Wang Lab Contact Information:*** *Yiqing Zhao* [redacted] *John Wang* [redacted] *Wolstein Research Building* [redacted] *2013 Cornell Road* *Cleveland, OH 44106* *10.1.4.3Handling of Specimens at Weill Cornell College of Medicine* *The fresh biopsy vial (containing PBS) will be placed on wet ice (>5 lb), and the other two cryovials will immediately be placed in dry ice (>5 lb). Personnel from the Laboratory of Dr. Manish Shah will then be contacted (see below) for processing. The PBS containing vial will be cultured as organoids in the Shah lab. The frozen specimens will be shipped to the Wang laboratory according to procedures described in the Laboratory Manual.* ***Shah Lab Contact Information*** *ATTN: Kyle bocchino* *Laboratory of Dr. Manish Shah* [redacted] *510 East 70th Street* *Weill Cornell Medical College* *New York, New York 10065*

Protocol Date	Section	Change
	10.1.5	Analytical Laboratory Personnel in the ~~Tissue Resources Core Facility~~ *Wang lab* will process fresh frozen specimens. One core biopsy per patient will be divided on a metal plate that is kept cold with dry ice. Additional biopsies will be archived and used on an as needed basis (see section 10.1.3). Each biopsy will be sectioned into 5 sections. Light microscopy to confirm the histologic presence of viable cancer tissue will be conducted on peripheral sections (H&E staining). Tissue sections immediately adjacent to and internal to the microscopy confirmed sections will be used for subsequent pharmacodynamic analysis (glutaminase activity, DNA extraction, RNA extraction) (22). ***Wang Lab Contact Information:*** *Yiqing Zhao* [redacted] *John Wang* [redacted] *Wolstein Research Building* [redacted] *2013 Cornell Road* *Cleveland, OH 44106* [redacted] Histologically confirmed fresh frozen specimens will be used by the Translational Research *Shared Resource or the Wang Lab* ~~and Pharmacology Core~~ for conduct of the glutaminase assay. Translational Research *Shared Resource* ~~and Pharmacology Core~~ ATTN: Erin Hohler University Hospitals *Cleveland* ~~Case~~ Medical Center

	10.2	~~**10.2 UPP1 Gene Expression**~~ ~~The purpose of assessing the change in UPP1 gene expression is to determine if administration of CB-839 results in an upregulation of UPP1 gene expression in post-treatment biopsy specimens as compared to prior to CB-839 treatment. UPP1 gene expression will be assessed using RT-PCR.~~ ~~10.2.1 Background~~ ~~The mechanism of cytotoxicity of 5-fluorouracil has been ascribed to the mis-incorporation of fluoronucleotides into RNA and DNA and to the inhibition of the nucleotide synthetic enzyme thymidylate synthase (13). To interrogate the molecular mechanisms by which CB-839 enhances the tumor inhibitory effect of 5-fluorouracil, we noted that our gene expression analysis shows that glutamine deprivation up-regulates uridine phosphorylase 1 (UPP1) in PIK3CA mutant cells. UPP1 facilitates conversion of 5-FU to FdUTP and FUTP (14), which can be incorporated into DNA and RNA. It has been shown that UPP1 -/- embryonic stem cells are much more resistant to 5-FU (15), whereas overexpression of UPP1 enhances cytotoxicity of 5-FU (16). We examined UPP1 gene expression in the isogenic HCT116 PIK3CA mut/- and WT/- cell lines. CB-839 induced more UPP1 in PIK3CA mut/- cells than in the WT/- cells. Similar results were observed in the DLD1 PIK3CA mut/- and WT/- cell lines. We therefore hypothesize that in addition to blocking glutamine metabolism, CB-839 treatment also induces UPP1 gene expression and thus enhances 5-FU toxicity.~~ ~~10.2.2 Rationale for Analysis~~ ~~Demonstration of upregulation of UPP1 gene expression in tumor specimens following treatment with single agent CB-839 as compared to prior to initiation of treatment will provide a pharmacodynamic marker indicating that CB-839 up-regulates UPP1 via the inhibitory effect of CB-839 on glutamine metabolism in PIK3CA mutant colorectal cancers. See section 14.0 for statistical plan.~~ ~~10.2.3 Collection of Specimens~~ ~~During the phase II component of the study, the first 10 patients will undergo either CT or US guided biopsy of a metastatic focus both prior to the initiation of treatment and 10-15 days following initiation of treatment. The pre-treatment biopsy may be performed up to 14 days prior to the initiation of treatment.~~ ~~Tumor Biopsies: Patients will undergo biopsy in the University Hospitals Case Medical Center (UHCMC) Department of Radiology by study interventional radiologist, Dr. Dean Nakamoto. If being treated at the Taussig Cancer Institute (TCI) of the Cleveland Clinic, patients will undergo biopsy in the Cleveland Clinic Department of Radiology. Use of CT or US guidance for each biopsy will be per the discretion of the physician performing the biopsy and the same metastatic focus of tumor will be biopsied at baseline and 10-15 days following initiation of treatment. Biopsies will be conducted~~

Protocol Date	Section	Change
		~~according to standard practice. Three core biopsies will be obtained for each patient during each biopsy. Fine needle aspiration biopsies are NOT appropriate. Three RNA cryovials will be labeled with the date, patient initials, patients study number and vial number. All study personnel, particularly those handling RNA cryovials, must wear clean gloves. Each of the three core biopsies will be placed in its own cryovial. The three cryovials will immediately be placed in dry ice or liquid nitrogen and transported to the Case Comprehensive Cancer Center, Translational Research and Pharmacology Core Facility for processing Specimens will be processed individually with the second and third specimens being processed only if insufficient nucleic acid is recovered or if histologic review identifies the presence of necrotic tumor in the first specimen. The remaining fresh frozen specimens will be stored for later use.~~ ~~10.2.4 Handling of Specimens~~ ~~See section 10.1.4 for handling of specimens.~~ ~~10.2.5 Analytical Laboratory~~ ~~UPP1 gene expression will be conducted in the laboratory of Dr. Zhenghe Wang.~~ ~~Dr. Zhenghe Wang~~ ~~Wolstein Research Building~~ ~~Case Western Reserve University~~ ~~2103 Cornell Road, WRB [redacted]~~ ~~Cleveland, OH 44106~~ [redacted] ~~10.2.6 Methods~~ ~~Fresh frozen tumor tissue will be obtained, de identified, from the TRPC facility. Total RNAs will be isolated using the Qiagen RNeasy Kit and reverse transcription will be performed using the first strand synthesis kit from Invitrogen. UPP1 will be amplified using primer pairs AACAGAGCAGGCAGTGGATA and ATACGCCTGCTTGTCCTTCT.~~
	10.2.2	10.2.~~3.~~2 Rationale for Analysis Pharmacokinetic analysis of patients receiving CB-839 at each dose level will allow for correlations to be made between trough levels of CB-839 and ~~both~~ glutaminase activity level ~~and UPP1 gene expression~~.

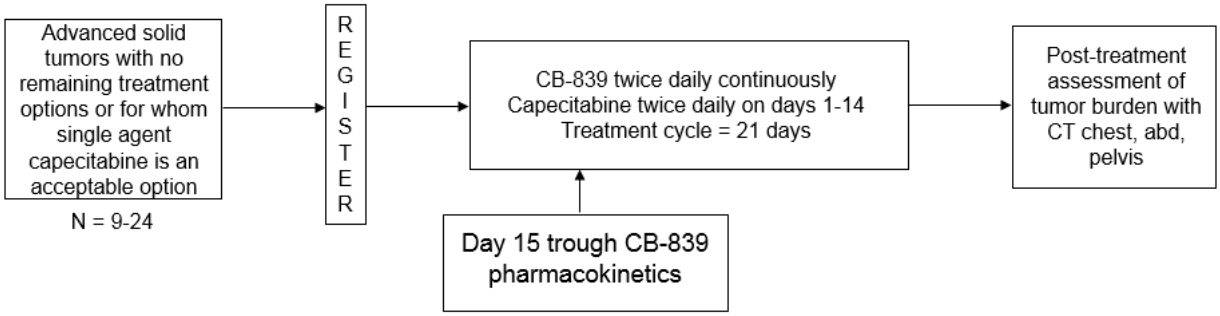
Protocol Date	Section	Change
	10.3	10.*3*~~4~~ **Additional Pharmacodynamic Analyses** An evaluation of ~~glutathione levels,~~ nucleotide *levels as well as a whole exome analysis and an RNA seq analysis* ~~and the incorporation of 5-fluorouracil into RNA and DNA~~ will be conducted on baseline and post-treatment tissue biopsies to determine the pharmacodynamic effect of CB-839 and capecitabine on PIK3CA mutant colorectal cancer. This will be conducted during the phase II portion of the trial. 10.*3.*4.1Background and Rationale ~~We have hypothesized that CB-839 treatment will induce UPP1 gene expression (section 10.2.1) in PIK3CA mutant colorectal cancers. UPP1 facilitates the conversion of 5-FU to FdUTP and FUTP (14), which can be incorporated into both RNA and DNA. We therefore hypothesize that in addition to blocking glutamine metabolism, CB-839 treatment will also induce UPP1 gene expression, subsequently leading to enhanced 5-FU toxicity and greater incorporation of 5-FU into RNA and DNA.~~ *Genome wide changes are anticipated to occur with the administration of CB-839 and capecitabine on both the DNA and RNA level. We will therefore use tumor tissue to determine changes occurring in DNA with pre- and post-treatment assessments of the genome via whole genome sequencing and also changes in tumor tissue at the expression level by performing pre- and post-treatment RNA seq analyses.* ~~Glutamine is converted to glutamate by glutaminase where glutamate can then act as a substrate for glutathione synthesis. Following administration of CB-839, we anticipate that glutaminase inhibition will deplete the glutamate pool, thereby decreasing the production of glutathione and therefore decreasing glutathione levels.~~

Protocol Date	Section	Change
		10.*3*.4.3 Analytical Laboratory ~~Glutathione levels will be measured in the laboratory of Dr. Zhenghe Wang.~~ ~~Dr. Zhenghe Wang~~ ~~Wolstein Research Building~~ ~~Case Western Reserve University~~ ~~2103 Cornell Road, WRB 3203~~ ~~Cleveland, OH 44106~~ ~~Phone:~~ [redacted] [redacted] [redacted] [redacted] Nucleotide levels ~~and the incorporation of 5-FU into RNA and DNA~~ will be measured in the ~~Translational Research and Pharmacology Core~~ *Proteomics Shared Resources* by Dr. Yan Xu *as outlined in the laboratory manual. Specimens will be processed for whole exome sequencing and RNA seq by the Wang Lab:* ***Wang Lab Contact Information:*** *Yiqing Zhao* [redacted] *John Wang* [redacted] *Wolstein Research Building* [redacted] *2013 Cornell Road* *Cleveland, OH 44106* ~~Specimens will be submitted from patient collection to:~~ ~~Translational Research and Pharmacology Core~~ ~~ATTN: Yan Xu~~ ~~University Hospitals Case Medical Center~~ ~~11100 Euclid Avenue~~ ~~Seidman Cancer Center,~~ [redacted] ~~Cleveland, OH 44106~~ [redacted] [redacted] [redacted]

Protocol Date	Section	Change
	10.4.4	Methods Please see the Laboratory Manual for methods on measurement of ~~glutathione levels, measurement of~~ nucleotide levels *whole exome sequencing and RNA seq* ~~and measurement of the incorporation of 5-FU into RNA and DNA.~~

STUDY SCHEMA—PHASE I

Phase I dose-escalation study of CB-839 and capecitabine in patients with advanced solid tumors and patients for whom single agent capecitabine is an acceptable treatment option



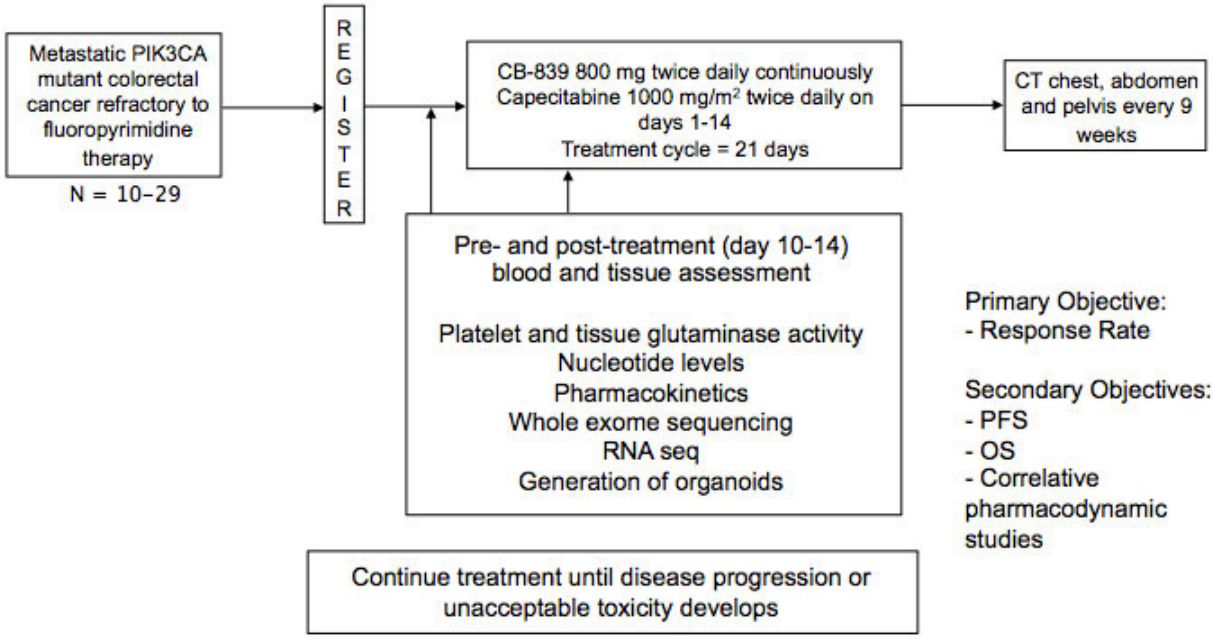
PHASE I DOSE ESCALATION STRATEGY

DOSE ESCALATION SCHEDULE		
Dose Level	CB-839 (mg) orally twice daily x 21 days	Capecitabine (mg/m^2) orally twice daily for 14/21 days
-1	400	500
1	400	750
2	600	750
3	600	1000
4	800	1000
3A	400	1000
3A to be conducted only if dose level 3 is too toxic		

Phase I: Patients will be treated with CB-839 by mouth twice daily for 21 days (continuous dosing) as well as capecitabine by mouth twice daily for 14/21 days. Doses will be determined per the phase I dose escalation schedule. Day 15 trough CB-839 pharmacokinetics will be obtained. Patients will undergo a disease assessment with CT imaging of the chest, abdomen and pelvis following 3 cycles (9 weeks) of treatment.

STUDY SCHEMA—PHASE II

Phase II study of CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer that is refractory to front-line fluoropyrimidine based therapy



Phase II: Patients will be treated with CB-839 800 mg by mouth twice daily for 21 days (continuous dosing) as well as capecitabine 1000 mg/m^2 by mouth twice daily for 14/21 days as were determined to be safe doses during the phase I portion of the study. Pre- and post-treatment (post-treatment being day 10-15) blood samples will be obtained for assessment of glutaminase activity. Baseline tissue biopsies and a day 10-15 tissue biopsy will be obtained to assess tissue glutaminase activity and nucleotide levels as well as for whole exome sequencing, RNA seq and for the production of organoids. Blood and tissue specimens will be collected the same day. Post-15 day treatment pharmacokinetics will be obtained and correlated with other laboratory assessments. Patients will undergo a disease assessment with CT imaging of the chest, abdomen and pelvis following 3 cycles (9 weeks) of treatment.

PROTOCOL SUMMARY

Protocol Number/Title	CASE 1216: Phase I/II study of CB-839 and capecitabine in patients with advanced solid tumors and fluoropyrimidine resistant PIK3CA mutant colorectal cancer
Study Phase	Phase I/II
Brief Background/ Rationale	Colorectal cancer is the third most common cause of cancer and second highest cause of cancer related death in the United States. Limited treatment options are available and the median overall survival is just over two years. Approximately 15-30% of colorectal cancers harbor a mutation in PIK3CA. Recent preclinical data suggest that PIK3CA mutant colorectal cancers may be more dependent on glutamine for growth and inhibition of glutamine metabolism with CB-839 inhibits growth. Additionally, treatment with CB-839 induces UPP1 gene expression and may potentiate the activity of fluoropyrimidines. In the phase I portion of this study, we will evaluate the safety, toxicity and pharmacokinetics of CB-839 and capecitabine chemotherapy in patients with advanced solid tumors for whom there are no further treatment options or for whom capecitabine is an acceptable treatment option. In the phase II portion of the study, we will evaluate the antitumor activity and survival benefit of combination CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy, and will also explore the pharmacodynamics of this combination therapy in pre- and post-treatment blood and tissue specimens.
Primary Objective	<u>Phase I Primary Objective:</u> To determine the safety, tolerability and recommended phase II dose (RP2D) of combination CB-839 and capecitabine chemotherapy in patients with advanced solid tumors for whom there are no remaining treatment options or for whom single agent capecitabine is an acceptable therapy. <u>Phase II Primary Objective:</u> To determine the disease control rate of combination CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancers who are refractory to fluoropyrimidine based therapy.
Secondary Objectives	<u>Phase I Secondary Objectives:</u> To determine the dose-limiting toxicities and maximum tolerated dose of combination therapy with CB-839 and capecitabine in patients with advanced solid tumors for whom there are no remaining treatment options or for whom single agent capecitabine is an acceptable therapy.

	To determine the antitumor response as assessed by RECIST criteria of combination therapy with CB-839 and capecitabine in patients with advanced solid tumors for whom there are no remaining treatment options or for whom single agent capecitabine is an acceptable therapy. <u>Phase II Secondary Objectives:</u> To determine the progression free survival following treatment with CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer and are refractory to fluoropyrimidine based therapy. To determine the overall survival following treatment with CB-839 and capecitabine chemotherapy in patients who have metastatic PIK3CA mutant colorectal cancer and are refractory to fluoropyrimidine based therapy.
Correlative Objectives	<u>Phase II Correlative Objectives:</u> To determine the change in glutaminase activity level in tumor specimens and platelets following treatment with CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy. To determine the reduction in nucleotide levels in tumor specimens following treatment with CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy. To determine genomic changes in both DNA and RNA in tumor specimens following treatment with CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy. To determine the correlation between plasma trough levels of CB-839 and biomarkers of interest in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy and who have received treatment with CB-839 and capecitabine chemotherapy. To determine the correlation between clinical response to treatment and biomarkers of interest in patients with metastatic PIK3CA mutant colorectal cancer who are refractor to fluoropyrimidine based therapy and who have received treatment with CB-839 and capecitabine chemotherapy. To generate organoids from patient tissue biopsies obtained prior to and

	following treatment in patients treated with CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who are refractory of fluoropyrimidine based therapy.
Sample Size	Phase I: 9-24 patients Phase II: 18-40 patients Males and females permitted
Disease sites/ Conditions	Phase I: all solid tumors Phase II: metastatic colorectal cancer, PIK3CA mutant
Interventions	Phase I: Patients will receive CB-839 orally twice daily for 21 days (continuous administration) and capecitabine orally twice daily for 14/21 days. Dosing of CB-839 and capecitabine will occur according to the aforementioned dosing table. Day 15 trough CB-839 pharmacokinetics will be assessed. Patients will undergo a disease assessment with CT imaging of the chest, abdomen and pelvis following 3 cycles (9 weeks) of treatment.
	Phase II: Patients will be treated with CB-839 800 mg by mouth twice daily for 21 days (continuous dosing) as well as capecitabine 1000 mg/m^2 by mouth twice daily for 14/21 days as was determined during the phase I portion of the study. Pre-treatment and post-10-15 day treatment blood samples will be obtained for assessment of glutaminase activity (to be obtained the same day as the post-treatment biopsy). Day 15 blood samples will also be archived for as needed assessment of CB-839 pharmacokinetics. Baseline tissue biopsies and a day 10-15 tissue biopsy will be obtained to assess tissue glutaminase activity and nucleotide levels as well as for whole exome sequencing, RNA seq and for the production of organoids. Patients will be assessed with CT scans of the chest, abdomen and pelvis every 3 cycles (9 weeks). Treatment will continue until disease progression, until unacceptable toxicity develops or per patient/physician preference.

ABBREVIATIONS

5-FU	5-fluorouracil
AE	adverse event
ALT	alanine aminotransferase
ANC	absolute neutrophil count
aPTT	activated partial thromboplastin time
ASCO	American Society of Clinical Oncology
AST	aspartate aminotransferase
ATP	adenosine triphosphate
AUC	area under the curve
BID	twice a day
BUN	blood urea nitrogen
CBC	complete blood count
CCCC	Case Comprehensive Cancer Center
Cmax	maximum concentration
Cmin	minimum concentration
CNS	central nervous system
CR	complete response
CRC	colorectal cancer
CRU	Clinical Research Unit
CT	computerized tomography
CTCAE	Common Terminology Criteria for Adverse Events
DLT	dose limiting toxicity
DNA	deoxyribonucleic acid
DSMP	Data Safety Monitoring Plan
DSTC	Data Safety Toxicity Committee
DVT	deep vein thrombosis
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic case report form
EDTA	ethylenediaminetetraacetic acid
EGFR	epidermal growth factor receptor
FDA	Food and Drug Administration
FDG	fluorodeoxyglucose
FdUTP	5-fluoro-2'-deoxyuridine-5'-triphosphate
FH	fumarate-hydratase
FUTP	5-formyluridine-5'-triphosphate
GDH	glutamate dehydrogenase
GI50	dose of 50% growth inhibition
GIST	gastrointestinal stromal tumor
GLP	good laboratory practice
GLS	glutaminase
HIV	human immunodeficiency virus
ICH	International Conference on Harmonization
IDH	isocitrate dehydrogenase

IEC	Independent Ethics Committee
INR	international normalized ratio
IRB	Institutional Review Board
LC-MS	liquid chromatography-mass spectrometry
LFTs	liver function tests
MDS	myelodysplastic syndrome
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
NADPH	nicotinamide adenine dinucleotide phosphate
NCI	National Cancer Institute
PARP	poly ADP ribose polymerase
PBS	Phosphate buffered saline
PD	progressive disease
PE	pulmonary embolism
PET	positron emission tomography
PFS	progression free survival
PIK3CA	phosphatidylinositol-4,5-biphosphate 3-kinase, catalytic subunit alpha
PR	partial response
RECIST	Response Evaluation Criteria in Solid Tumors
RNA	ribonucleic acid
RP2D	recommended phase II dose
RT-PCR	reverse transcriptase polymerase chain reaction
SAE	serious adverse event
SD	stable disease
SDH	succinate dehydrogenase
TCA	tricarboxylic acid
TCI	Taussig Cancer Institute
TID	three times a day
TRSR	Translational Research Shared Resource
UHCMC	University Hospitals Cleveland Medical Center
ULN	upper limit of normal
UPP1	uridine phosphorylase 1
US	ultrasound
VEGF	vascular endothelial growth factor
WT	wild-type

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1.0 INTRODUCTION

1.1 Background of Colorectal Cancer

Colorectal cancer is the second most commonly diagnosed cancer within the United States with an estimated 132,700 new cases in 2015. It is also the third most common cause of cancer related death in both males and females with approximately 49,700 colorectal cancer related deaths occurring in 2015 (1). For patients with metastatic disease, combination therapy with cytotoxic chemotherapeutic agents and biologic agents are typically used. Cytotoxic therapies include fluoropyrimidines (5-fluorouracil (5-FU) or capecitabine, the oral prodrug of 5-FU), oxaliplatin and irinotecan while biologic agents include bevacizumab (a vascular endothelial growth factor inhibitor), cetuximab or panitumumab (epidermal growth factor receptor inhibitors) in patients harboring a ras mutation and regorafenib (a multi-target tyrosine kinase inhibitor also with inhibitory vascular endothelial growth factor pathway effects) (2). However, despite optimal utilization of all of these agents, median overall survival is still poor at approximately 2 years. Improved treatment strategies for these patients are therefore very much in need.

Multiple mutations have been identified in colorectal cancers including mutations in several ras genes, BRAF and PIK3CA. To date, targeted agents acting upon these mutations are only available to patients harboring a ras mutation (approximately 40% of patients) but no targeted therapy exists for patients with PIK3CA mutations, which are thought to be present in approximately 15-30% of colorectal cancers (3).

Role of glutamine in cancer

It has been well described that tumor cancer cells are dependent on glutamine for survival. Both the "Warburg effect" and "glutamine dependency" are well-known metabolic reprogramming events that occur in cancer cells (4). In normal cells, glucose is converted to acetyl-CoA, which enters the tricarboxylic acid (TCA) cycle and undergoes oxidative phosphorylation in the mitochondria (5). In cancer cells however, glucose is converted to lactate even in the presence of oxygen (the "Warburg effect") (5). It was previously thought that the Warburg effect is caused by impaired mitochondrial function in cancer cells however recent studies clearly demonstrate that most cancer cells retain functional mitochondria (6). Instead of using glucose, cancer cells utilize glutamine to replenish the TCA cycle (7). To enter the TCA cycle, glutamine is first deaminated by glutaminase (GLS) to generate glutamate. Glutamate is then converted to alpha-ketoglutarate (alpha-KG), a TCA cycle intermediate, by either an aminotransferase (e.g. GPTs and GOTs) or a glutamate dehydrogenase.

Glutamine metabolites are utilized to produce ATP, lipids and other macromolecules, thereby promoting tumor growth (4).

PIK3CA mutations render cancer cells dependent on glutamine

Most PIK3CA mutations are clustered in two hot spots: H1047R in the kinase domain and E545K in the helical domain (3). Preclinical work conducted by our group sought to demonstrate that PIK3CA mutations reprogram cell metabolism in colorectal cancers (CRCs). The CRC cell line HCT116 harbors a heterozygous H1047R mutation, whereas DLD1 CRC cells express a heterozygous E545K mutation. To interrogate how PIK3CA mutations impact cancer metabolism, we exploited isogenic cell lines with either the WT or mutant alleles of PIK3CA knocked out (gift from Dr. Bert Vogelstein) to test their sensitivity to glucose or glutamine deprivation. We termed the parental cells as Mut/WT, the WT PIK3CA allele knockout as Mut/-, and the mutant allele knockout as -/WT. As had been previously reported (8), the parent cells and knockout cells grew at a similar rate under normal conditions in the presence of glucose and glutamine. However, glutamine deprivation (-Gln) induced more apoptosis in PIK3CA Mut/- cells than PIK3CA -/WT cells as assayed by percentages of sub-GI cells, amounts of cleaved PARP and counting of viable cells. Similar results were observed with both HCT116 and DLD1 cell lines. In contrast, none of these cell lines showed differential sensitivity to glucose deprivation. Glutamine sensitivity tested in two additional cell lines with PIK3CA mutations—RKO with a PIK3CA H1047R mutation and HT29 with a PIK3CA P449T mutation—and two CRC cell lines with WT PIK3CA (SW480 and LOVO) generated similar results; glutamine deprivation induced significantly more apoptotic cells in the two PIK3CA mutant cell lines than in the two WT PIK3CA cell lines, suggesting that these results are generalizable. Prior studies have demonstrated that KRAS increases glutamine metabolism in pancreatic cancers (9). Both the HCT116 and DLD1 CRC cell lines harbor a heterozygous oncogenic KRAS mutation. Using isogenic HCT116 and DLD1 clones with either WT or mutant KRAS knockout (10), we showed that KRAS mutant and WT clones did not show differential sensitivity to glutamine deprivation. Overall, this suggests that mutant PIK3CA/p110α, but not mutant KRAS, renders CRC cells dependent on glutamine to grow and provides the first evidence that oncogenic PIK3CA mutations cause glutamine dependency in cancer cells.

1.2 CB-839

1.2.1 Preclinical Data

PIK3CA mutant CRC cells are more sensitive to a glutaminase inhibitor—CB-839

Glutaminases have been shown to be overexpressed in CRCs (11). CB-839 is a highly selective, reversible, allosteric inhibitor of glutaminase (12). We have shown that isogenic PIK3CA Mut/- cells are more sensitive to CB-839 than their WT counterparts (Fig 1). The GI50 (dose of 50% growth inhibition) of CB-839 of the PIK3CA Mut/- cell lines are 3-4 fold lower than the isogenic PIK3CA -/WT cell lines derived from either HCT116 or DLD1 CRC cell lines. In CRC xenografts treated with CB-839, CB-839 inhibited xenograft tumors with PIK3CA mutations. In contrast, xenografts with WT PIK3CA did

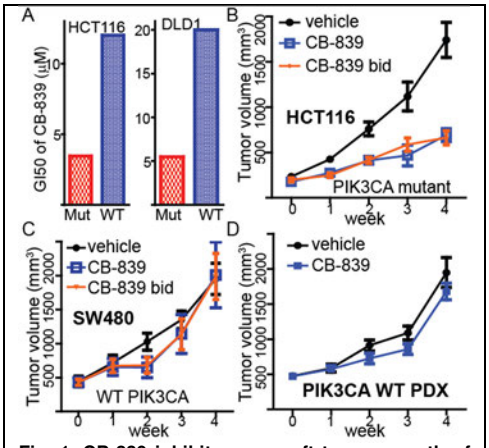

Fig. 1. CB-839 inhibits xenograft tumor growth of PIK3CA mutant, but not WT, CRCs. (A) PIK3CA mut/- (Mut) cells are more sensitive to CB-839 than isogenic PIK3CA -/WT cells. (**B**) to (**D**) Xenograft tumors were treated with vehicle, CB-839 (200 mg/kg) once or twice (bid) per day orally. (B) HCT116; (C) SW480; (D) A colon cancer patient-derived xenograft (PDX).

not respond to CB-839.

Combination CB-839 and 5-fluorouracil shrinks PIK3CA mutant xenograft tumors

Based on tissue culture data demonstrating that 5-fluorouracil enhanced cytotoxicity of CB-839, our group sought to determine if CB-839 alone or in combination with 5-fluorouracil inhibits xenograft tumor growth of a PIK3CA mutant colorectal cancer. A dose of 30mg/kg of 5-fluorouracil was tested after it was determined that 40mg/kg was the maximum tolerated dose in mice. Mice were treated with 200mg/kg CB-839 and 30mg/kg 5-fluorouracil combination therapy which induced tumor regression (Fig 2). In contrast, although CB-839 and 5-fluorouracil alone each inhibited tumor growth to various extents, neither induced tumor regression. Furthermore, combination of CB-839 and 5-fluorouracil also induced tumor regression of a patient derived xenograft. Overall, these results provide a strong rationale for conduct of a clinical trial assessing the combination of CB-839 and 5-fluorouracil in colorectal cancer with PIK3CA mutations.

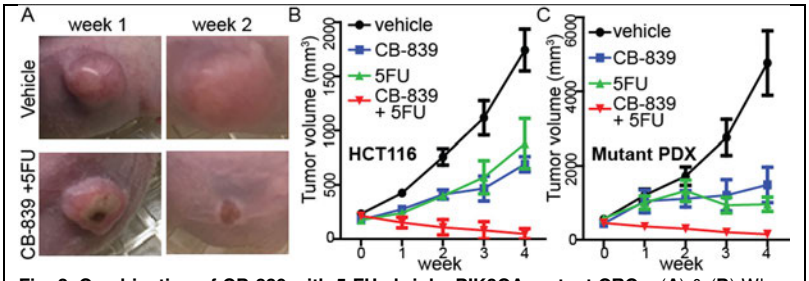

Fig. 2. Combination of CB-839 with 5-FU shrinks PIK3CA mutant CRCs. (**A**) & (**B**) When HCT116 PIK3CA mutant xenograft tumors reached ~200 mm^3 size, mice were treated daily with vehicle, CB-839 (200 mg/Kg, oral gavage), 5-FU (30 mg/kg, IP) or combination of CB-839 with 5-FU (5 mice/group). Representative images of a tumor regressing over time in the combination treatment group (A). Growth curves of tumors (B). (**C**) Combination of CB-839 with 5-FU shrinks a PIK3CA-mutant colon cancer patient-derived xenograft (PDX).

Combination of CB-839 and 5-fluorouracil depletes cellular dTTP levels

Given that glutamine is a precursor for nucleotide synthesis and that 5-FU is a thymidylate synthase inhibitor, we set to determine how the drug combination impacts cellular dTTP levels. As shown in Fig. 3, either drug alone significantly reduced amounts of dTTP, whereas the combination of CB-839 and 5-FU nearly completely depleted cellular dTTP. These data suggest that CB-839 enhances the cytotoxicity of 5-FU by further depleting cellular dTTP.

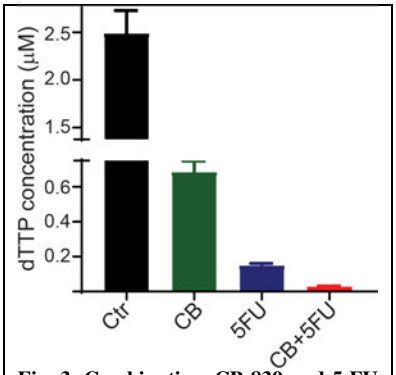

Fig. 3. Combination CB-839 and 5-FU depletes cellular dTTP levels. HCT116 cells were treated with the indicated drugs. Cellular dTTP amounts were measured by LC-MS/MS.

1.2.2 Clinical Data

A phase I study assessed CB-839 administered as a single agent in patients with metastatic solid tumors (17, 18), cumulative exposure to date is 125 patients (data cut 26 January 2016). Patients received CB-839 according to either a three times daily (TID) dosing schedule or a twice daily

dosing schedule (BID with food) during a 21-day treatment cycle. A standard 3+3 dose escalation strategy was employed assessing dose levels ranging from 100-800 mg TID (n=32), and 600-800 mg BID with food (n=27). Treatment was well tolerated with 16% of patients across all trials experiencing a grade 3/4 treatment related adverse event. One dose limiting toxicity occurred (grade 3 creatinine in a patient with known diabetes). The majority of adverse events were related to asymptomatic, reversible elevations in liver function tests (LFTs). Twice daily dosing with food resulted in a lower frequency (1.5%) of grade 3 treatment-related LFT abnormalities than TID dosing and hence has been selected as the dosing schedule to pursue in subsequent studies (18). Pharmacodynamic analyses (see section 1.2.3) have demonstrated that 600 mg BID results in continuous strong glutaminase inhibition in most patients and is a sufficient dose for further study. Expansion cohorts assessing single agent activity of CB-839 600 mg BID have been enrolled in triple negative breast cancer, KRAS-mutant non-small cell lung cancer, renal cell carcinoma, mesothelioma, fumarate-hydratase (FH)-deficient solid tumors, succinate dehydrogenase (SDH)-deficient GIST, succinate dehydrogenase (SDH)-deficient non-GIST and isocitrate dehydrogenase (IDH)-mutant solid tumors and have confirmed the adverse event profile and recommended phase 2 dose (18). Monotherapy dosing at 800 mg BID has also been shown to be safe and well tolerated and is being further explored in the clinic—this dose could eventually become the preferred recommended phase 2 dose. Two-drug combination studies with CB-839 and either paclitaxel, erlotinib, everolimus or docetaxel are planned.

Two phase I studies of CB-839 administered as a single agent were also conducted in patients with hematologic malignancies: relapsed and/or treatment refractory multiple myeloma or non-Hodgkins lymphoma (CX-839-02) and relapsed and/or treatment refractory leukemia and IDH-mutated myelodysplastic syndrome (CX-839-003) were also conducted (19). Totals of 14 and 18 patients received CB-839 in CX-839-002 and CX-839-003, respectively according to the same treatment strategy employed in the aforementioned solid tumor study. Treatment was similarly tolerated with 21% and 14% of patients respectively experiencing a grade 3/4 adverse event thought to be related to CB-839. The majority of the adverse events were due to asymptomatic, reversible LFT abnormalities or hematological adverse events. Given the larger experience in the solid tumor phase I study, BID dosing was selected for subsequent studies hematologic malignancy studies. Expansion cohorts assessing single agent activity of CB-639 600 mg BID are ongoing in acute myeloid leukemia (IDH wild-type), acute lymphoid leukemia, acute myeloid leukemia (IDH1 or IDH2 mutant) and myelodysplastic syndrome (IDH1 or IDH2 mutant).

1.2.3 Clinical Pharmacokinetics and Pharmacodynamics

Pharmacokinetic sampling was performed on Cycle 1, Day 1 (C1D1), C1D15 and D1 of each subsequent treatment cycle in each of the conducted phase I studies (17-19). A dose dependent relationship was observed with CB-839 exposure increasing with dose. The half-life of CB-839 was determined to be 4 hours. Additionally, Cmin fell below a target concentration of 200 ng/mL in 46% of patients receiving 400 mg or higher TID regimens while a large majority of patients receiving BID dosing with 600 mg maintained a Cmin greater than the 200 ng/mL target concentration. Additionally, an assessment of the effect of food on the PK profile of CB-839 demonstrated a modest positive food effect and delayed absorption, which resulted in similar Cmax and Cmin for the 600 mg TID and 600 mg BID fed regimens. Given the favorable adverse event profile observed with the 600 mg BID fed regimen, this is the favored dosing regimen.

Pharmacodynamics of CB-839 was also assessed in both studies. Glutaminase activity was measured in circulating platelets as well as tumor biopsy specimens. Blood samples collected at baseline and four hours following CB-839 treatment were assessed for glutaminase inhibition and demonstrated a dose dependent relationship. At a CB-839 treatment dose of 600 mg BID, greater than 90% glutaminase inhibition should be observed even at Cmin for most patients. An evaluation of glutaminase activity assessed approximately 4 hours after the C1D15 CB-839 dose within five (5) tumor specimens also demonstrated clear inhibition of glutaminase activity and suggested a dose-response relationship (18).

Based on both pharmacokinetics and pharmacodynamics, CB-839 600 mg BID or 800 mg BID for a 21-day treatment cycle, to be repeated every 21 days has been determined as the single agent dose for pursuit in further clinical trials.

1.3 Capecitabine

1.3.1 Clinical Data

Capecitabine is an oral fluoropyrimidine and is a prodrug of 5-fluorouracil. Per the National Comprehensive Cancer Network guidelines (2), capecitabine and 5-FU are considered equivalent and either may be used as frontline treatment for colorectal cancer, typically in combination with another chemotherapeutic agent (oxaliplatin or irinotecan) and a biologic agent (either an EGFR inhibitor or a VEGF inhibitor). It may also be used as a single agent as a maintenance therapy. Ultimately patients develop resistance to fluoropyrimidine therapy and hence disease progression. However, based on our preclinical data outlined in section 1.2.1, we hypothesize that co-administration of CB-839 with capecitabine will overcome fluoropyrimidine resistance, particularly in PIK3CA mutant colorectal cancers, and therefore capecitabine will be used as the cytotoxic backbone for this study.

1.4 Rationale for clinical and correlative studies

It has been demonstrated that CB-839, via targeting glutaminase, inhibits xenograft growth in 2 of 2 PIK3CA mutant colon cancer test xenograft models but does not inhibit growth in PIK3CA WT models, supporting that there is an addiction of PIK3CA mutant colorectal cancers to glutamine metabolism. Furthermore, CB-839 plus 5-FU shrinks 2 of 2 PIK3CA mutant xenografts that both grow through maximally tolerated doses of 5-FU alone. Based on these findings, we propose to test responses of the CB-839 and fluoropyrimidine combination in fluoropyrimidine resistant human patients. Based on our preclinical mechanistic studies described above, we propose a phase I clinical trial to determine the maximum tolerated dose (MTD), recommended phase 2 dose (RP2D) and dose limiting toxicities of CB-839 plus capecitabine in patients with advanced solid tumors. The data derived from this trial will be used for a subsequent phase II clinical trial where we will estimate the antitumor activity and pharmacodynamic effects of CB-839 and capecitabine in patients with PIK3CA mutant colorectal cancers that are resistant to fluoropyrimidine therapy. We hypothesize that CB-839 in combination with capecitabine will overcome fluoropyrimidine resistance and that the addition of capecitabine to CB-839 will result in the inhibition of glutaminase in tumors and platelets and the reduction in nucleotide levels in tumors. Genomic abnormalities on the DNA and RNA level will also be explored via a whole exome sequencing

analysis and an RNA seq analysis.

As of January 2018, the phase I portion of the study has been fully accrued. A total of 16 patients were treated on the study and we were able to assess all of the four planned dose levels. No dose limiting toxicities were observed and we have therefore determined that the recommended phase II dose be CB-839 800 mg by mouth twice daily continuously and capecitabine 1000 mg/m^2 orally twice daily on days 1-14 of a 21 day treatment cycle.

2.0 OBJECTIVES

The overall objectives for this study are to 1) determine the recommended phase II dose (RP2D) of CB-839 and capecitabine in patients with advanced solid tumors who have no further treatment options or patients for whom single agent capecitabine is an acceptable treatment regimen and 2) to determine the antitumor activity of CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy. The phase I portion of this study will also determine the dose-limiting toxicities of this treatment regimen as well as the pharmacokinetics. In the phase II portion of this study, the pharmacodynamic effects of CB-839 and capecitabine will be evaluated using multiple biomarker studies including glutaminase activity level and nucleotide levels as well as a whole exome sequencing analysis and an RNA seq analysis. We hypothesize that we will observe a reduction in glutaminase activity in both tumors and platelets and a reduction in nucleotide levels following treatment. The whole exome and RNA seq analyses will be exploratory. The generation of organoids from tumor biopsy specimens both prior to and following treatment will also be exploratory and will be used for further potential studies as additional preclinical information becomes available

2.1 Primary Objective

2.1.1 Phase I: To determine the safety, tolerability and recommended phase II dose (RP2D) of combination CB-839 and capecitabine chemotherapy in patients with advanced solid tumors for whom there are no remaining treatment options or for whom single agent capecitabine is an acceptable therapy.

2.1.2 Phase II: To determine the disease control rate of combination CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancers who are refractory to fluoropyrimidine based therapy.

2.2 Secondary Objectives

2.2.1 <u>Phase I</u>

To determine the dose-limiting toxicities and maximum tolerated dose of combination therapy with CB-839 and capecitabine in patients with advanced solid tumors for whom there are no remaining treatment options or for whom single agent capecitabine is an acceptable therapy.

To determine the antitumor response as assessed by RECIST criteria of combination therapy with CB-839 and capecitabine in patients with advanced solid tumors for whom there are no remaining treatment options or for whom single agent capecitabine is an acceptable therapy.

2.2.2 Phase II

To determine the progression free survival following treatment with CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer and are refractory to fluoropyrimidine based therapy.

To determine the overall survival following treatment with CB-839 and capecitabine chemotherapy in patients who have metastatic PIK3CA mutant colorectal cancer and are refractory to fluoropyrimidine based therapy.

2.3 Correlative Objective(s)

2.3.1 Phase II

To determine the change in glutaminase activity level in tumor specimens and platelets following treatment with CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy.

To determine the reduction in nucleotide levels in tumor specimens following treatment with CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy.

To determine genomic changes in both DNA and RNA in tumor specimens following treatment with CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy.

To determine the correlation between plasma trough levels of CB-839 and biomarkers of interest in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy and who have received treatment with CB-839 and capecitabine chemotherapy.

To determine the correlation between clinical response to treatment and biomarkers of interest in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy and who have received treatment with CB-839 and capecitabine chemotherapy.

To generate organoids from patient tissue biopsies obtained prior to and following treatment in patients treated with CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who are refractory of fluoropyrimidine based therapy.

3.0 STUDY DESIGN

3.1 Study design including dose escalation / cohorts for phase I component

This is a standard 3+3 phase I dose escalation trial to determine the maximum tolerated dose and dose limiting toxicities of combination therapy with CB-839 and capecitabine chemotherapy in patients with advanced solid tumors with no remaining treatment options or for whom single agent capecitabine is an acceptable therapy. Patients will receive combination therapy with CB-839 and capecitabine for 21 day cycles and will remain on treatment until either disease progression, development of unacceptable toxicity or discontinuation per patient/physician preference. Patients will receive CB-839 orally twice daily for 21 days (continuous administration) and capecitabine orally twice daily for 14/21 days. Dosing of CB-839 and capecitabine will occur according to the following dosing table. Day 15 trough CB-839 levels will be obtained for pharmacokinetic assessment. Patients will undergo a CT of the chest, abdomen and pelvis every 3 cycles (9 weeks). A minimum of three patients will be assessed at each dose level until the maximum tolerated dose has been determined. Please see section 6.1 for details.

DOSE ESCALATION SCHEDULE		
Dose Level	CB-839 (mg) orally twice daily x 21 days	Capecitabine (mg/m^2) orally twice daily for 14/21 days
-1	400	500
1	400	750
2	600	750
3	600	1000
4	800	1000
3A	400	1000
3A to be conducted only if dose level 3 is too toxic		

3.2 Study design for phase II component

The phase II portion of the study is a single arm design, assessing the disease control rate of combination therapy with CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who have developed fluoropyrimidine resistance. Patients will receive combination therapy with CB-839 and capecitabine for 21 day cycles and will remain on treatment until either disease progression, development of unacceptable toxicity or discontinuation per patient/physician preference. Patients will receive CB-839 800 mg orally twice daily for 21 days (continuous administration) and capecitabine 1000 mg/m^2 orally twice daily for 14/21 days as was determined during the phase I portion of the study. Patients will undergo blood and tissue sampling at baseline and 10-15 days post-treatment for assessment of glutaminase activity. Tissue specimens will also be assessed for changes in nucleotide levels as well as for a whole exome analysis, an RNA seq analysis and for the generation of organoids. Patients will undergo a CT of the chest, abdomen and pelvis following 3 cycles of therapy (every 9 weeks).

3.2 Number of Patients

A total of 9-24 patients will be enrolled in the phase I portion of this trial and a total of 18-40 patients will be enrolled in the phase II portion of this trial.

3.3 Replacement of Patients

3.3.1 Phase I: If a patient is withdrawn from the study for any reason other than a dose limiting toxicity prior to completing the first 21 days of combination therapy with CB-839 and capecitabine, a replacement patient will be enrolled and will be assigned to the same dose level.

Unless the patient has had their study drug held for an adverse event that may herald a DLT, if a patient does not take at least 75% of the planned doses of each of the two agents (CB-839 and capecitabine), the patient will be replaced because he/she has not taken enough drug to confirm safety at that dose level.

3.3.2 Phase II: If a patient does not take at least 75% of the planned doses of each of the two agents (CB-839 and capecitabine) prior to the first disease assessment (9 weeks), the patient will be replaced because he/she has not taken enough drug to assess efficacy.

3.4 Expected Duration of Treatment and Patient Participation

Patients will receive treatment with combination CB-839 and capecitabine in 21 day treatment cycles. Patients will continue to receive treatment until they develop disease progression, unacceptable toxicity or per the preference of the patient or treating physician. Patients will be followed for toxicity for a minimum of 30 days upon discontinuation of treatment. If treatment related toxicities have not resolved to at least a grade 1 by 30 days following discontinuation of treatment, patients will be followed for treatment related toxicities until grade 1 toxicity or better.

4.0 PATIENT SELECTION

Each of the criteria in the sections that follow must be met in order for a patient to be considered eligible for this study. Use the eligibility criteria to confirm a patient's eligibility.

Patient's Name _____

Medical Record # _____

Research Nurse / Study Coordinator Signature:
_____

Date _____

Treating Physician [Print] _____

Treating Physician Signature: _____

Date _____

4.1 Phase I Inclusion Criteria

Patients must meet all of the following inclusion criteria to be eligible for enrollment:

___ 4.1.1 Patients must have an advanced solid tumor malignancy with no remaining standard treatment options or for whom single agent capecitabine is an acceptable therapy. Patients with colorectal cancer must have progressed on at least one line of fluoropyrimidine containing therapy. Receipt of either oxaliplatin or irinotecan in combination with a fluoropyrimidine is required in the front line setting for all colorectal cancer patients unless either of these agents are otherwise contraindicated in the opinion of the treating physician. Prior regorafenib or TAS-102 therapy is not required.

___ 4.1.2 Patients must be >18 years of age. Because no dosing or adverse event data are currently available on the use of CB-839 either as a single agent or in combination with capecitabine in patient's ≤18 years of age, children are excluded from this study.

___ 4.1.3 Patients must have an ECOG performance status of 0-1 (See Appendix 1).

___ 4.1.4 Patients must have normal organ and marrow function as defined below:

- Hemoglobin ≥ 9.0 g/dl
- Leukocytes ≥ 3,000/mcL
- Absolute neutrophil count ≥ 1,500/mcL
- Platelet count ≥ 100,000/mcL
- Serum creatinine ≤ 1.5 X institutional upper limit of normal
- Total bilirubin ≤ 1.5 mg/dL
- AST (SGOT) ≤ 2.5 X institutional upper limit of normal

- ALT (SGPT) ≤ 2.5 x institutional upper limit of normal

___ 4.1.5 Patients must be able to swallow pills.

___ 4.1.6 Patients must have the ability to understand and the willingness to sign a written informed consent document.

___ 4.1.7 Female patients of childbearing potential must have a negative serum or urine pregnancy test within 3 days prior to the first dose of study drug and agree to use dual methods of contraception during the study and for a minimum of 3 months following the last dose of study drug. Post-menopausal females (>45 years old and without menses for >1 year) and surgically sterilized females are exempt from these requirements. Male patients must use an effective barrier method of contraception during the study and for a minimum of 3 months following the last dose of study drug if sexually active with a female of childbearing potential.

4.2 Phase II Inclusion Criteria

Patients must meet all of the following inclusion criteria to be eligible for enrollment:

___ 4.2.1 Patients must have histologically or cytologically confirmed, metastatic colorectal cancer. PIK3CA mutant status must be confirmed by tumor sequencing conducted in a CLIA certified lab. Genetic sequencing performed on tissue specimens or circulating DNA from peripheral blood samples are allowed. Abnormalities in PIK3CA considered to be variants of unknown significance (VUS) will not be eligible.

___ 4.2.2 Patients must have measureable disease according to RECIST 1.1 criteria that is amenable to biopsy and be willing to undergo pre- and post-treatment tumor biopsies. Lesions to be biopsied do not have to be those used for measurement.

___ 4.2.3 Patients must have received and progressed on fluoropyrimidine or fluoropyrimidine based therapy. Patients must have a history of disease progression during treatment with fluoropyrimidine, or within 3 months of a dose of a fluoropyrimidine-containing regimen. Patients who have only progressed during treatment holiday of 3 months or greater will not be eligible. Receipt of either oxaliplatin or irinotecan in combination with a fluoropyrimidine is required in the front line setting unless either of these agents are otherwise contraindicated in the opinion of the treating physician, in which case a fluoropyrimidine only may be used. Prior regorafenib or TAS-102 therapy is not required.

___ 4.2.4 Patients must be >18 years of age.

___ 4.2.5 Patients must have an ECOG performance status of 0-1 (See Appendix 1).

___ 4.2.6 Patients must have normal organ and marrow function as defined below:
- Hemoglobin ≥ 9.0 g/dl
- Leukocytes ≥ 3,000/mcL

- Absolute neutrophil count ≥ 1,500/mcL
- Platelet count ≥ 100,000/mcL
- Serum creatinine ≤ 1X institutional upper limit of normal
- Total bilirubin ≤ 1.5 mg/dL
- AST (SGOT) ≤ 2.5 X institutional upper limit of normal
- ALT (SGPT) ≤ 2.5 X institutional upper limit of normal

___ 4.2.7 Patients must be able to swallow pills.

___ 4.2.8 Patients must have the ability to understand and the willingness to sign a written informed consent document.

___ 4.2.9 Female patients of childbearing potential must have a negative serum or urine pregnancy test within 3 days prior to the first dose of study drug and agree to use dual methods of contraception during the study and for a minimum of 3 months following the last dose of study drug. Post-menopausal females (>45 years old and without menses for >1 year) and surgically sterilized females are exempt from these requirements. Male patients must use an effective barrier method of contraception during the study and for a minimum of 3 months following the last dose of study drug if sexually active with a female of childbearing potential.

4.3 Phase I and II Exclusion Criteria

The presence of any of the following will exclude a patient from study enrollment.

___ 4.3.1 Patients with ongoing toxicities > grade 1 according to NCI CTCAE Version 4.0 (excluding alopecia) due to prior anti-cancer therapy.

___ 4.3.2 Patients receiving any other investigational agents or whom have received recent treatment for colorectal cancer (radiation within the previous two weeks, chemotherapy or investigational therapy within the previous four weeks).

___ 4.3.3 Patients with untreated brain metastases/CNS disease will be excluded due to their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.

___ 4.3.4 Patients with a history of allergic reactions attributed to or intolerance to compounds of similar chemical or biologic composition to either CB-839 or capecitabine. If capecitabine has been received previously, must have tolerated at least an equivalent dose to the dose to be administered at their assigned dose level.

___ 4.3.5 Patients who are unable to swallow pills or who have undergone surgery that prohibits the absorption of pills in the stomach.

___ 4.3.6 Patients with uncontrolled intercurrent illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure (i.e., NYHA Class II), unstable

angina pectoris or myocardial infarction within prior 6 months, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

4.3.7 Patients who are pregnant or breastfeeding will be excluded from the study due to the potential teratogenic or abortifacient effects that may results from CB-839 and/or capecitabine. Because there is an unknown, but potential risk for adverse events in nursing infants secondary to treatment of the mother with CB-839 and/or capecitabine, breastfeeding should be discontinued if the mother is treated with CB-839 and/or capecitabine. These potential risks may also apply to other agents used in this study.

4.3.8 Patients known to be HIV positive who are not receiving anti-retroviral therapy will be excluded due to the marrow suppressive therapy involved in administration of the study treatment.

4.4 Inclusion of Women and Minorities

Men, women and members of all races and ethnic groups are eligible for this trial.

5.0 REGISTRATION

All patients who have been consented are to be registered in the OnCore™ Database. For those patients who are consented, but not enrolled, the reason for exclusion must be recorded.

All patients will be registered through University Hospitals Cleveland Medical Center/Seidman Cancer Center and will be provided a study number by contacting the study coordinator.

6.0 TREATMENT PLAN

6.1 Treatment Regimen Overview

6.1.1 Phase I: Patients will receive combination therapy with CB-839 and capecitabine for 21 day cycles and will remain on treatment until either disease progression, development of unacceptable toxicity or discontinuation per patient/physician preference. Patients will receive CB-839 orally twice daily for 21 days (continuous administration) and capecitabine orally twice daily for the first 14/21 days.

A standard 3+3 dose escalation schema will be used to conduct this phase I study. Patients will be treated in cohorts of 3 patients starting with dose level 1, with escalating doses of both CB-839 and capecitabine. Escalation will continue through all dose levels until the MTD has been determined. The dose escalation strategy is as follows:

DOSE ESCALATION SCHEDULE		
Dose Level	CB-839 (mg) orally twice daily x 21 days	Capecitabine (mg/m^2) orally twice daily for 14/21 days
-1	400	500
1	400	750

2	600	750
3	600	1000
4	800	1000
3A	400	1000
3A to be conducted only if dose level 3 is too toxic		

6.1.2 Phase II: Patients will receive combination therapy with CB-839 and capecitabine for 21 day cycles and will remain on treatment until either disease progression, development of unacceptable toxicity or discontinuation per patient/physician preference. Patients will receive CB-839 800 mg orally twice daily for 21 days (continuous administration) and capecitabine 1000 mg/m^2 orally twice daily for the first 14/21 days as was determined during the phase I portion of the study. Additionally, given that there are ongoing studies, the safety profile will continue to be monitored in these studies and the RP2D could be adjusted based on emerging safety and tolerability data.

6.2 General Agent Administration Guidelines

As capecitabine involves a weight based administration, dosing will be based on actual body weight. Dose changes (beyond cycle 1) should be made only if there is a > 10% change in body weight as calculated by the Mosteller formula.

As CB-839 is administered as a fixed dose, the CB-839 dose does not need to be altered due to changes in body weight.

CB-839 will be supplied as 200 mg tablets. Capecitabine will be supplied as 500 mg tablets depending on the dose required. Capecitabine dose should be rounded to the closest 500 mg and the total dose should not exceed the calculated dose by more than 5%. If an odd number of 500 mg tablets are needed, the dose may be split between the morning and evening doses (example: if 2500 mg are required for the total daily dose, 1500 mg may be administered in the morning and 1000 mg may be administered in the evening).

Dose of up to 5% higher than the calculated BSA-based dose of capecitabine may be given when rounding down would result in significant under-dosing as determined by the treating physician. Any doses higher than the calculated BSA-based dose should be reviewed by the study PI. Total daily doses higher than 105% of the BSA-based dose should not be given.

As an example: a patient with a BSA of 1.97 would have a total calculated daily dose of capecitabine of 3940 mg but may receive 4000 mg total. In this case the dispensed (rounded) dose would be 1.5% higher than the BSA-calculated dose. If instead the dose was rounded down the patient would receive 3500 mg daily which would be 11% lower than the calculated dose. The lower dose should be used if the higher dose is more than 5% above the calculated dose.
Dose reductions for toxicity should be considered similarly with up to 80% of initial dose being allowed at the -1 dose level and up to 55% of initial dose being allowed at the -2 dose level (see section 7.2 below).

Both CB-839 and capecitabine pills should be swallowed whole and may not be crushed.

A missed or vomited dose of either CB-839 or capecitabine should not be replaced. The patient should be instructed to take the next scheduled dose at the regularly scheduled time.

Treatment will continue until development of progressive disease (as defined by section 12.4) or unacceptable toxicity.

Appropriate dose modifications for CB-839 and capecitabine are described in Section 7.0

Reported adverse events and potential risks of CB-839 and capecitabine as well as reporting requirements for adverse events are described in Section 8.0.

No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

6.2.1 CB-839 Administration

CB-839 will be given orally twice daily in combination with capecitabine chemotherapy during a 21 day treatment cycle. Administration of CB-839 will be continuous throughout the 21 day treatment cycle. During the phase I component of the study, the dose of CB-839 administered will be dictated by the dose escalation table. During the phase II component of the study, the dose of CB-839 administered will be 800 mg by mouth twice daily as was determined during the phase I portion of the study.

CB-839 should be taken with food—the first dose of the day should be taken with breakfast and the second dose of the day should be taken with dinner.

6.2.2 Capecitabine Administration

Patients will receive capecitabine chemotherapy orally by mouth twice daily for the first 14 days of each 21 day treatment cycle. During the phase I component of the study, the dose of capecitabine administered will be dictated by the dose escalation table. During the phase II component of the study, the dose of capecitabine administered will be 1000 mg/m^2 twice daily on days 1-14 of a 21 day treatment cycle as was determined during the phase I component of the study.

Capecitabine tablets should be taken whole with water within 30 minutes following a meal. Doses will be taken approximately 12 hours apart at the end of a meal.

6.3 Phase I Dose Escalation

Dose escalation will proceed within each cohort according to the following scheme.

DOSE ESCALATION SCHEDULE		
Dose Level	CB-839 (mg) orally twice	Capecitabine (mg/m^2) orally

	daily x 21 days	twice daily for 14/21 days
-1	400	500
1	400	750
2	600	750
3	600	1000
4	800	1000
3A	400	1000
3A to be conducted only if dose level 3 is too toxic		

For each cohort, 3 patients will be entered sequentially to each dose level. Dose-limiting toxicity (DLT) is defined in section 6.4.

- If none of the 3 patients at a dose level experience dose limiting toxicity (DLT) during the first cycle (21 day treatment period), new patients may be entered at the next dose level.
- If 1/3 patients experiences a DLT during the first cycle, up to 3 more patients will be treated at the same dose level.
- If 2 or more experience a DLT during the first cycle, no further patients are started at that dose and the MTD is the highest dose level in which <2 (of 6) patients develop a DLT.
- For the dose level deemed the MTD, 6 patients will be treated at this dose level, even if a DLT has not been observed.
- If at any time 2 or more DLTs are seen in 3 or 6 patients an any given dose level, the preceding dose level will be declared the MTD (Maximum Tolerated Dose) and recommended phase II dose. MTD is defined as the highest dose level at which < 33% of 6 patients experience a DLT. If 1 DLT is seen out of 6 patients at a given dose level, new patients may be entered at the next dose level.
- If dose level -1 exceeds the MTD definition, then the study will be suspended and additional dose levels will be considered.

For Dose Level -2, -1, 1 and 2

Number of Observed DLTs	Action
0/3	Escalate next 3 patients to next dose level
1/3	Add 3 more patients to current dose level
≤ 1/6	Escalate next 3 patients to next dose level
≥ 2/3	Suspend accrual, next lowest dose level is deemed the MTD
≥ 2/6	Suspend accrual, next lowest dose level is deemed the MTD

If the final dose level is achieved, a total of 6 patients must be treated at this dose level even if no DLTs are observed.

6.4 Definition of Dose-Limiting Toxicity

Patients must receive at least 75% of the planned CB-839 and capecitabine administrations in the

first treatment cycle to be considered evaluable for DLT, unless the patient experiences a DLT or has the study drug held for an AE that may herald a DLT. Patients who discontinue the study prior to receiving the requisite study treatment administrations for reasons that include, but are not limited to, clinical/radiographic progression, voluntary withdrawal, or complications that the Principal Investigator considers secondary to the patient's malignancy will not be considered evaluable for DLT and will be replaced.

A DLT is defined as any AE that cannot be determined to be unrelated to study treatment, occurs within the first treatment cycle (i.e., Cycle 1), and that meets at least one of the non-hematologic or hematologic criteria below:

Non-Hematologic DLT:

- ≥ Grade 3 non-hematologic toxicity according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4 *except the following*:
    - Nausea, vomiting, or diarrhea lasting < 48 hours and controlled by optimal antiemetic/antidiarrheal therapy
    - Grade 3 hyperglycemia lasting < 72 hours with standard anti-diabetic therapy
    - Clinical laboratory abnormalities that are reversible to ≤ Grade 1 or baseline status within 72 hours with outpatient care and/or monitoring, or that are considered not clinically significant by the Principal Investigator
- Grade 4 hyperglycemia (>500 mg/dL; 27.8 mmol/L; life threatening consequences)
- Grade 4 hypoglycemia (<30 mg/dL; <1.7 mmol/L; life threatening consequences)
- Grade 4 AST (>20.0 x the upper limit of normal)
- Grade 4 ALT (>20.0 x the upper limit of normal)
- Grade 3 AST or ALT elevation (>5.0 – 20.0 x the upper limit of normal; >5 x the upper limit of normal for >2 weeks) with concomitant Grade 2 increase in total bilirubin (>1.5 – 3.0 x the upper limit of normal)

Events involving elevation in the AST, ALT or bilirubin with a clear alternative explanation (such as progressive disease within the liver) can be exempt from being considered a DLT-defining event.

Hematologic DLT:

- Grade 4 neutropenia [absolute neutrophil count (ANC) < 0.5 x 10^9/L]
- Grade 3 febrile neutropenia (ANC < 1.0 x 10^9/L with a fever ≥ 38.3 degrees C)
- Grade 4 thrombocytopenia (< 25.0 x 10^9/L)
- Grade ≥ 3 thrombocytopenia associated with Grade ≥ 3 bleeding

In addition, any other AE that is felt to be treatment-limiting in the medical opinion of the Principal Investigator may be considered a DLT.

Management and dose modifications are outlined in Section 7.

6.5 General Concomitant Medications and Supportive Care Guidelines

All supportive measures consistent with optimal patient care will be given throughout the study.

Anxiolytics, anti-emetics, analgesics and antibiotics may be provided at the discretion of the treating physician.

6.5.1 Use of Imodium and/or Lomotil should be implemented immediately to prevent dehydration should diarrhea develop.

6.5.2 The use of growth factors (filgrastim) is permitted and should be administered in accordance with ASCO guidelines (20). Growth factors are not permitted, however, in the first treatment cycle of the phase I portion of the study unless the patient experiences a hematologic DLT.

6.5.3 The use of erythropoietin stimulating agents is NOT permitted. Anemia may be managed with blood transfusions as indicated.

6.5.4 Caution should be taken when capecitabine or CB-839 is co-administered drugs that are highly dependent on CYP2C9 for metabolism.

6.5.5 Prophylactic anticoagulation will be allowed during therapy provided the activity of the agent used is reflected in either INR or aPTT and that those parameters remain as follows: INR < 1.5 or aPTT within normal limits. Anticoagulation for therapeutic use of DVT or PE is allowed. Caution should be taken in patients receiving Coumadin (which is permitted). Coumadin levels should be monitored and the dose of Coumadin may need to be reduced.

6.5.6 Caution should be taken in patients receiving phenytoin. Phenytoin levels should be monitored and the phenytoin dose may need to be reduced.

6.5.7 Proton pump inhibitors are not permitted while on study due to their interference with CB-839 that results in lower CB-839 drug exposure levels. H2 blockers may be used as an acceptable alternative. **In the event that a patient is not able to tolerate an H2-blocker as an alternative, please contact the study principal investigator.** Preliminary PK data generated in concurrent phase I studies suggest that concomitant use of agents that increase gastric pH (e.g., proton pump inhibitors, H2-receptor antagonists, antacids, etc.) may reduce absorption of CB-839, resulting in decreased systemic exposure. **Note:** these agents are NOT CONTRAINDICATED but rather discontinuation is recommended whenever possible. Based on emerging PK data from other CB-839 studies, the PI may allow concomitant use of a PPI with modified guidance regarding the administration of CB-839 (e.g., administer CB-839 with an acidic beverage or dietary supplement). In the event that a patient needs to resume proton pump inhibitor therapy, a PK sampling pre-dose and at 0.5, 1, 2 and 4 hours are required at a point 2-4 weeks after initiation of PPI therapy.

6.5.8 Emollients and/or urea based creams are recommended for use at the first sign of palmar-plantar erythrodysesthesia syndrome (hand and foot rash) and should be used liberally before making a dose modification based on this AE.

6.6 Criteria for Removal from Study

In the absence of treatment delays due to adverse events, treatment may continue until one of the

following criteria applies:

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- The investigator considers it, for safety reasons, to be in the best interest of the patient
- Unacceptable treatment related toxicity, NCI CTCAE version 4, grade 3 or 4 that fails to recover to baseline or < grade 3 in the absence of treatment within 4 weeks
- Patient decision to withdraw from treatment (partial consent) or from the study (full consent)
- Pregnancy during the course of the study for a child-bearing participant
- Death
- Sponsor reserves the right to temporarily suspend or prematurely discontinue the study

The date and reason for discontinuation must be documented. Every effort should be made to complete the appropriate assessments.

6.7 Duration of Follow Up

Patients will be followed for toxicity for 30 days after treatment has been discontinued (or longer if treatment related toxicities have not resolved to at least a grade 1) or until death, whichever occurs first.

The clinical course of each adverse event will 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 will necessitate follow-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 will be recorded and reported immediately.

Patients will be followed every 3 months for up to 2 years from study drug discontinuation.

7.0 DOSE DELAYS/DOSE MODIFICATIONS

Once a patient undergoes a dose modification, neither CB-839 nor capecitabine may be re-escalated in that patient.

Dose reductions may be implemented for either one agent or both agents depending on the treating physician's assessment of causality.

Patients requiring > 2 dose modification will be taken off protocol therapy.

Patients must receive at least 75% of the total assigned CB-839 and capecitabine doses in order to be evaluable for a DLT in the phase I portion of the study. Missed doses must be for reasons other

than toxicity.

For patients in the phase I portion of the study, dose reductions of CB-839 and capecitabine will be permitted during the first cycle (21 days) only if a patient experiences a toxicity that results in a DLT. If a patient experiences a DLT, treatment continuation at a lower dose of CB-839 and/or capecitabine will be permitted as long as the toxicity has returned to ≤ Grade 1 or baseline within 14 days. Upon recovery, patients may restart at one CB-839/capecitabine dose level lower. Patients who do not recover within 14 days will not be eligible for resumption of treatment with CB-839 or capecitabine.

After cycle 1, dose reductions or interruptions for adverse events may take place at any time at the discretion of the Principal Investigator. Dose interruptions for grade 2 non-hematologic toxicity for up to 5 days can be implemented at the discretion of the treating physician to manage clinically significant toxicity. No dose reduction is required when resuming treatment. Dose interruptions of > 5 days should be discussed with the PI. Patients whose dose is interrupted for > 21 days for any reason must be withdrawn from the study unless prior approval from the PI has been obtained for study continuation.

Adequate medical management for reversible symptoms using supportive medications must be used prior to making a dose modification for these symptoms.

7.1 CB-839 Dose Modifications

CB-839 will be initiated at the dose per the assigned dose level and will be given in combination with capecitabine twice daily throughout the 21 day treatment cycle.

7.1.1 CB-839 Dose Modification Table

Starting CB-839 dose (mg) by mouth twice daily	**1st Dose Modification (mg) by mouth twice daily**	**2nd Dose Modification (mg) by mouth twice daily**
400	200	discontinue
600	400	200
800	600	400

7.1.2 Hematologic and Non-Hematologic Toxicities (except for liver function changes)

Toxicity Grade	**CB-839 Dose Changes During Current Treatment Period**	**Dose Adjustments for Resumption of Treatment**
2 (or, for baseline grade 2, worsening to grade 3)	Hold study drug and provide supportive care	Restart at the same dose level upon resolution to ≤ Grade 1 or baseline
3 or 4	Hold study drug and provide supportive care	Reduce to the next lower dose level upon resolution of ≤ Grade 1 or

		baseline If symptoms persist for > 14 days despite dose interruption, the patient must be withdrawn from the study

7.1.3 Abnormal liver function tests (AST, ALT, bilirubin)

Grade	CB-839 Modification
1	No change in dose
2	Reduce CB-839 to the next lower dose level
≥ 3	Hold CB-839 until ≤ Grade 1 and resume at the next lower dose level
≥ 3 after resumption of treatment	Hold CB-839 until ≤ Grade 1 and resume at the next lower dose level

7.1.4 Non-hematologic Toxicity

For other non-hematologic toxicities, dose reductions of CB-839 will be at the discretion of the investigator. A dose reduction of 200 mg must be performed with each dose reduction. A maximum of 2 dose reductions are permitted.

7.2 Capecitabine Dose Modifications

Capecitabine will be initiated at the dose per the assigned dose level and will be given twice daily for the first 14 days out of a 21 day treatment cycle.

Dose modifications should be made according to the following table:

7.2.1 Dose Modification Table

Toxicity Grade	During a Course of Therapy	Dose Adjustment for Next Cycle (% of starting dose)
Grade 1	Maintain dose level	Maintain dose level
Grade 2		
1st appearance	Interrupt until resolved to grade 0-1	100%
2nd appearance	Interrupt until resolved to grade 0-1	75%
3rd appearance	Interrupt until resolved to grade 0-1	50%
4th appearance	Discontinue treatment permanently	
Grade 3		
1st appearance	Interrupt until resolved to grade 0-1	75%
2nd appearance	Interrupt until resolved to grade 0-1	50%
3rd appearance	Discontinue treatment permanently	

7.2.2 Hematologic Toxicities

ANC (/mm3)		Platelets (/mm3)	Modification
< 1500/mm3	and/or	< 100,000/mm3	Hold until ANC ≥ 1500/mm3 and platelets are ≥ 100,000/mm3, resume per dose modification table
≥ 1500/mm3	and	≥ 100,000/mm3	No dose modification

7.2.3 Nausea and Vomiting

Adequate medical management for these conditions should be implemented before a dose modification is made for one of these symptoms

Grade	Modification
1	No change in dose
2	Hold until ≤ grade 1; resume at same dose level
3	Hold until ≤ 1; resume per dose modification table
4	Off protocol therapy

7.2.4 Mucositis, Diarrhea and Esophagitis

Adequate medical management for these conditions should be implemented before a dose modification is made for one of these symptoms

Grade	Toxicities/Symptoms	Modification
1	Mucositis, esophagitis or diarrhea	No dose modification
2	Diarrhea	No dose modification
2	Mucositis or esophagitis	Hold until ≤ grade 1 and resume per dose modification table
3	Diarrhea	Hold until ≤ grade 1; resume per dose modification table
3/4	Mucositis or esophagitis	Hold until ≤ grade 1; resume per dose modification table

7.2.5 Palmar-Plantar Erythrodysesthesia Syndrome (Hand and Foot Rash)

Grade	Modification
1	No dose modification
2	Hold until symptoms resolve to grade 0 or 1. Resume per dose modification table.
≥ 3	Hold until symptoms resolve to grade 0 or 1. Resume per dose modification table.

7.2.6 Hyperbilirubinemia

If grade 3 or 4 hyperbilirubinemia occurs that is thought to be related to capecitabine, capecitabine should be held until the bilirubin improves to ≤ 3.0 x institutional ULN. When ready, resume capecitabine according to the capecitabine dose modification table (Section 7.2.1) where each episode of grade 3 toxicity results in a dose modification. Discontinue treatment permanently for the third grade 3 incident. If the patient has already had a dose modification for another toxicity, the new dose level should be used as the baseline for making these adjustments.

For grade 4 toxicity, resume capecitabine at 50% of the original starting dose for the first incident and discontinue treatment permanently for any subsequent incident. If the patient has already had a dose modification for another toxicity, the new dose levels should be used as the baseline for making these adjustments.

7.2.7 Non-hematologic Toxicity

For other non-hematologic toxicities, dose reductions of capecitabine will be at the discretion of the investigator. Dose modification should be made according to the dose modification table. A maximum of 2 dose reductions are permitted.

8.0 ADVERSE EVENTS AND POTENTIAL RISKS

The following is a list of AEs (section 8.1) and the reporting requirements associated with observed AEs (sections 8.3 and 8.4).

The clinical course of each event will 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 will necessitate follow-up to determine the final outcome. Any serious adverse event that occurs after the study period is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately.

8.1 CB-839

The most commonly encountered toxicities include fatigue, nausea, vomiting, elevation in liver function tests (including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase), elevation in creatinine, constipation, oral pain and photophobia. These have generally been mild and reversible. Elevation in gamma-glutamyltransferase, bilirubin, anorexia, anemia and dizziness have been observed as adverse events in rare instances (<2%). It should be noted that the rate of GGT elevation is more frequent but these elevations are often considered not clinically significant.

See section 7.1 for management recommendations and dose modifications.

8.2 Capecitabine

Known side effects occurring in patients receiving capecitabine include:

Hematologic: anemia, neutropenia, thrombocytopenia

Cardiovascular: edema, venous thrombosis

Constitutional: fatigue, pyrexia, swelling in hands, feet or abdomen, pain, chest pain

Dermatologic: hand-foot syndrome, dermatitis, skin discoloration, alopecia

Gastrointestinal: diarrhea, nausea, vomiting, stomatitis, abdominal pain, gastro-intestinal motility disorder, constipation, taste disturbance, upper GI inflammatory disorders, gastrointestinal hemorrhage, ileus

Hepatic: hyperbilirubinemia

Infections: bacterial or viral

Metabolic: appetite decreased, dehydration

Musculoskeletal: back pain, arthralgia

Neurologic: peripheral sensory neuropathy, headache, dizziness, insomnia

Ocular: eye irritation, visual abnormalities

Psychiatric disorders: mood alteration, depression

Pulmonary: dyspnea, cough, pharyngeal disorder, epistaxis, sore throat

Vascular: venous thrombosis

For a comprehensive list of adverse events related to capecitabine, please refer to the FDA-approved package insert (21). See section 7.2 for management recommendations and dose modifications.

8.3 Definitions

8.3.1 Adverse Event

An **adverse event** (AE) is any unfavorable or unintended event, physical or psychological, associated with administration of a test article in a research study. The event can include abnormal laboratory findings, symptoms, or disease associated with the investigational agents or procedures associated with the research study. The event does not necessarily have to have a causal relationship with the investigational agents under study, any risk associated with the research, the research intervention, or the research assessments.

Adverse events may be the result of the interventions and interactions used in the research; the collection of identifiable private information in the research; an underlying disease, disorder, or condition of the patient; and/or other circumstances unrelated to the research or any underlying disease, disorder, or condition of the patient.

8.3.2 Serious Adverse Events

A **serious adverse event** (SAE) is any adverse experience occurring at any dose that results in any of the following outcomes:

- Results in **death.**
- Is a **life-threatening** adverse experience. The term life-threatening in the definition of serious refers to an adverse event in which the patient was at risk of death at the time of the event. It does not refer to an adverse event which hypothetically might have

caused death if it were more severe.

- Requires **inpatient hospitalization or prolongation of existing hospitalization**. Any adverse event leading to hospitalization or prolongation of hospitalization will be considered as Serious, UNLESS at least one of the following expectations is met:
    - The admission results in a hospital stay of less than 24 hours OR
    - The admission is pre-planned (e.g., elective or scheduled surgery arranged prior to the start of the study) OR
    - The admission is not associated with an adverse event (e.g., social hospitalization for purposes of respite care.

  However it should be noted that invasive treatment during any hospitalization may fulfill the criteria of "medically important" and as such may be reportable as a serious adverse event dependent on clinical judgment. In addition where local regulatory authorities specifically require a more stringent definition, the local regulation takes precedent.
- Results in **persistent or significant disability/incapacity**. The definition of disability is a substantial disruption of a person's ability to conduct normal life's functions.
- Is a **congenital anomaly/birth defect**.
- Is an **important medical event**. Important medical events that may not result death, be life-threatening, or require hospitalization may be considered a serious adverse experience when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood disease or disorders, or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse. The development of a new cancer is always considered an important medical event.
- Clear progression of neoplasia should not be reported as an AE or SAE. Findings that are clearly consistent with the expected progression of the underlying cancer should not be reported as an adverse event, and hospitalizations due to the progression of cancer do not necessarily qualify for an SAE. All deaths should be reported as SAEs regardless of the underlying cause. If there is any uncertainly about a finding being due solely to progression of neoplasia, the finding should be reported as an AE or SAE as appropriate.

8.3.3 Other Reportable Information

Certain information, although not considered an SAE, must be recorded, reported, and followed up as indicated for an SAE. This includes:

- A case involving a pregnancy exposure to a test article, unless the product is indicated for use during pregnancy e.g., prenatal vitamins. Information about use in pregnancy encompasses the entire course of pregnancy and delivery and perinatal and neonatal outcomes, even if there were no abnormal findings. If a pregnancy is confirmed, test article must be discontinued immediately. All reports of pregnancy must be followed for information about the course of the pregnancy and delivery, as well as the condition of the newborn. When the newborn is healthy, additional follow-up is not needed. Pregnancies occurring up to 6 months after completion of the study treatment must also be reported to the investigator.

- Overdose (e.g., a dose higher than that indicated in the protocol) with our without an AE.
- Abuse (e.g., use for nonclinical reasons) with or without an AE.
- Inadvertent or accidental exposure with or without an AE.

8.3.4 Adverse Event Evaluation

The investigator or designee is responsible for ensuring that all adverse events (both serious and non-serious) observed by the clinical team or reported by the patient which occur after the patient has signed the informed consent are fully recorded in the patient's medical records. Source documentation must be available to support all adverse events.

A laboratory test abnormality considered clinically relevant (e.g., causing the patient to withdraw from the study, requiring treatment or causing apparent clinical manifestations, result in a delay or dose modification of study treatment, or judged relevant by the investigator), should be reported as an adverse event.

The investigator or sub-investigator (treating physician if applicable) will provide the following for all adverse events (both serious and non-serious):

- Event term (as per CTCAE)
- Description of the event
- Date of onset and resolution
- **Expectedness of the toxicity**
- **Grade of toxicity**
- **Attribution of relatedness to the investigational agent- (this must be assigned by an investigator, sub-investigator, or treating physician)**
- Action taken as a result of the event, including but not limited to; no changes, dose interrupted, reduced, discontinued, etc. or action taken with regard to the event, i.e. no action, received medication or other intervention, etc.
- Outcome of event

Descriptions and **grading scales** found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4 will be utilized for AE reporting.

An expected adverse event is an event previously known or anticipated to result from participation in the research study or any underlying disease, disorder, or condition of the patient. The event is usually listed in the Investigator Brochure, consent form or research protocol.

An unexpected adverse event is an adverse event not previously known or anticipated to result from the research study or any underlying disease, disorder, or condition of the patient.
Attribution is the relationship between an adverse event or serious adverse event and the study drug. Attribution will be assigned as follows:

- Definite – The AE is <u>clearly related</u> to the study drug.
- Probable – The AE is <u>likely related</u> to the study drug.
- Possible – The AE <u>may be related</u> to the study drug.

- Unlikely – The AE is doubtfully related to the study drug.
- Unrelated – The AE is clearly NOT related to the study drug.

Attribution should be assigned for individual components of the treatment regimen.

8.4 SAE Report Form

SAEs will be recorded on the FDA Form 3500A (MedWatch) but should only be reported as instructed below. The electronic FDA SAE reporting forms should not be used.

8.5 Reporting Procedures for Serious Adverse Events

For the purposes of safety reporting, all adverse events will be reported that occur on or after the day the first dose of study drug is received through 30 days after the final dose of study drug. Adverse events, both serious and non-serious, and deaths that occur during this period will be recorded in the source documents. All SAEs should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es). Related AEs will be followed until resolution to baseline or grade 1 or stabilization.

8.5.1 SAE Reporting Requirements

- Participating investigators (all sites) must report all serious adverse events to the Lead Site Principal Investigator (e.g. Sponsor-Investigator) within **24 hours** of discovery or notification of the event. The participating investigator must also provide follow-up information on the SAE until final resolution. All SAEs should be reported to the study principal investigator:

  David Bajor, MD
  [redacted]

- The Lead Site Principal Investigator will review the SAE and report the event to the FDA, external collaborator(s), and IRB as applicable. Reporting of SAEs by the PI to the IRB will be conducted in accordance with the standard operational procedures and policies of the IRB and adequate documentation will be maintained to attest to the fact that the IRB was properly notified.

- It is the Sponsor-Investigator's responsibility (e.g. lead site PI) to ensure that ALL serious adverse events that are unexpected and suspected to be related (SUSAR) that occur on the study (e.g. ALL SAEs that occur at each enrolling institution) are reported to all participating sites. **Serious Unexpected and Suspected Adverse Reaction (SUSAR)** means an event which requires expedited reporting to the Regulatory Authority and is *suspected* (by the Sponsor-Investigator) to be related to the Study Drug, is *unexpected* (not listed in the Sponsor-Investigator's Brochure), and is *serious* (as defined in the Protocol section 8.3.2). SUSARs are expedited IND safety reports which are required to be reported to the relevant Regulatory Authorities within 7 days (fatal or life-threatening event) or 15

days (all other serious events), the detail for FDA reporting requirements is given below.

- Safety data will be reported in parallel to Calithera Bioscience's PharmacoVigilance agent according to the Safety Data Exchange Agreement at the following:

  US Toll Free Fax number: 1 (800) 727-8347
  eFax forwarded to calithera@primevigilance.com
  Email: calithera@primevigilance.com

Institutional Review Board Reporting Requirements:

- Investigative sites will report adverse events to their respective IRB according to the local IRB's policies and procedures in reporting adverse events.

FDA Reporting

The University Hospitals Principal Investigator, as holder of the IND, will be responsible for all communication with the FDA. In accordance with 21 CFR 312.32, the University Hospitals Principal Investigator is responsible for notifying the FDA of SAEs that are serious, unexpected (not listed in the Investigator Brochure) and judged to be related (i.e., possible, probably, definite) to the study agents. Events meeting the following criteria need to be submitted to the FDA as Expedited IND Safety Reports.

7 Calendar Day IND Safety Report

Any unexpected fatal or life-threatening suspected adverse event represent especially important safety information and, therefore, must be reported more rapidly to FDA (21 CFR 312.32(c)(2)). Any unexpected fatal or life-threatening suspected adverse event must be reported to the FDA no later than 7 calendar days after the University Hospitals Principal Investigator initial receipt of the information (21 CFR 312.32(c)(2)). University Hospitals Principal Investigator will complete a Medwatch Form FDA 3500A and notify the FDA by telephone or facsimile transmission. Such reports will be notified in parallel to Calithera Biosciences.

15 Calendar Day IND Safety Report

The timeframe for submitting an IND safety report to FDA and all participating investigators is no later than 15 calendar days after the University Hospitals Principal Investigator determines that the suspected adverse event or other information qualifies for reporting (21 CFR 312.23(c)(1)). This includes any serious, unexpected adverse events considered reasonably or possibly related to the investigational agent. University Hospitals Principal Investigator will complete a Medwatch Form FDA 3500A and notify the FDA by telephone or facsimile transmission. If FDA requests any additional data or information, the University Hospitals Principal Investigator must submit it to FDA as soon as possible, but no later than 15 calendar days after receiving the request (21 CFR 312.23(c)(1)(v)). Such reports will be notified in parallel to Calithera Biosciences.

Follow-up IND Safety Report

Any relevant additional information that the University Hospitals Principal Investigator obtains that pertains to a previously submitted IND safety report must be submitted to FDA as a Follow-up IND Safety Report without delay, as soon as the information is available (21 CFR 312.32(d)(2)). The University Hospitals Principal Investigator will maintain records of its efforts to obtain additional information.

8.6 SAEs and OnCore

- All SAEs will be entered into OnCore.
- A copy of the SAE form(s) submitted to the sponsor-investigator is also uploaded into OnCore.

8.7 Data Safety and Toxicity Committee

It is the responsibility of each site PI to ensure that ALL SAEs occurring on this trial (internal or external) are reported to the Case Comprehensive Cancer Center's Data and Safety Toxicity Committee. This submission is simultaneous with their submission to the sponsor and/or other regulatory bodies.

The sponsor-investigator is responsible for submitting an annual report to the DSTC as per CCCC Data and Safety Monitoring Plan.

8.8 Data and Safety Monitoring Plan (DSMP)

This protocol will adhere to the policies of the Case Comprehensive Cancer Center Data and Safety Monitoring Plan in accordance with NCI guidelines.

9.0 PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in Sections 8.1 and 8.2.

9.1 CB-839

Name of Agent: CB-839

Other Names: NA

Product description: CB-839 is provided as 200 mg opaque Swedish orange gelatin capsules or as tablets that are manufactured, packaged, and labeled according to current Good Manufacturing Practices (cGMP).

Storage requirements: CB-839 capsules should be stored as will be indicated on the study drug label—this should be in a clinical site pharmacy at controlled room temperature, defined as

between 20° and 25°C +/- 5°C (i.e. between 15° and 30°C (59° to 86°F). Stability studies being carried out on the phase I clinical supplies support storage for CB-839 capsules for at least 24 months at room temperature. CB-839 HCl tablets must be stored in a secure area with controlled access and separately from commercially available and other investigational drugs, preferably in a separate location. Any breach of investigational product storage conditions including temperature excursions outside the range 15° and 30°C must be reported to the Sponsor upon detection and the IP in question must be quarantined until the Sponsor authorizes usage or otherwise. Patients will be instructed to store medication according to storage conditions noted on the label, out of reach of children or other cohabitants.

Route of administration: CB-839 will be administered orally twice daily with food. The first dose of the day should be taken with breakfast and the second dose of the day should be taken with dinner.

Absorption, Distribution, Metabolism and Excretion of CB-839

Systemic exposure to CB-839 following oral administration is highly species dependent due to significant cross-species differences in absorption and metabolism. The oral bioavailability was good in mice and rates when CB-839 was dosed to animals as a solution; the systemic exposure increased less than dose-proportionally. On the other hand, the oral exposure and bioavailability in dogs and cynomolgus monkeys was low to modest likely due to extensive first-pass metabolism. The oral exposure and bioavailability in marmoset monkeys were also low to moderate likely due to poor absorption. Based on the *in vitro* and *in vivo* ADME data, good oral exposure of CB-839 in humans was predicted. Ongoing Phase I studies have demonstrated significant exposure following oral dosing.

Absorption: The apical-to-basolateral permeability of CB-839 in Caco-2 monolayers was determined to be 9.6 x 10^{-6} cm/sec and is predictive of good permeation through gut wall in humans. The aqueous solubility of CB-839 is pH-dependent, with high solubility under low pH and poor solubility at neutral pH. The oral bioavailability of 44-70% in mice and 66-157% in rats demonstrated good absorption of CB-839 in these species. Oral exposure of CB-839 in rats was affected by feeding status. Systemic exposure to CB-839 was higher in fasted rats than in fed rats following oral administration. However, in Phase I clinical trials most patients demonstrated better CB-839 exposures when taking the drug with food.

Distribution: High *in vitro* plasma protein binding of CB-839 was observed in mouse, rat, dog, and human plasma at 1 and 10 uM using an ultracentrifugation approach; there were no significant differences observed among species. The mean plasma protein binding was 98.0, 97.0, 97.9, and 99.1%, respectively. Following a single oral dose of 200 mg/kg to scid/bg mice, CB-839 was broadly distributed to systemic tissues including heart, lung, spleen, muscle, and subcutaneously-implanted tumors; brain had 20-fold lower CB-839 concentration than plasma.

Metabolism: In vitro metabolic stability of CB-839 was evaluated using cryopreserved hepatocytes derived from mice, rats, dogs, monkeys (cynomolgus, rhesus, and marmoset), and humans. *CB-839 was most stable in human hepatocytes*. In hepatocytes from marmoset

monkeys, CB-839 was also stable. Gender differences were observed only in rats; CB-839 was more stable in rat hepatocytes from females than males. CB-839 was considerably less stable in the other species tested. The *in vitro* clearance derived from the hepatocyte stability is well correlated with *in vivo* clearance in mice, rats, dogs, marmoset monkeys, and cynomolgus monkeys. The human clearance was predicted to be slow for CB-839 based upon hepatocyte studies *in vitro*. Two pathways of metabolic transformation of CB-839 have been identified *in vitro* in hepatocytes and *in vivo*; amide hydrolysis and, to a lesser extent, P450-mediated hydroxylation. There are considerable differences in the mechanism of metabolism across species. Amide hydrolysis appears to be the predominant pathway of metabolism of CB-839 in rat and marmoset monkey hepatocytes. Amide hydrolysis also contributes significantly to the metabolism of CB-839 in hepatocytes from mice, cynomolgus monkeys, and rhesus monkeys. The relative abundance of metabolites in hepatocytes is correlated with those observed *in vivo* for all species tested (mice, rats, dogs, marmoset monkeys, and cynomolgus monkeys). In human hepatocytes, metabolites resulting from both amide hydrolysis and hydroxylation of the thiadiazole and pyridazine rings were detected. However, all these metabolites have low abundances (<10%) in human hepatocyte incubations up to 4 hr and no metabolites unique to human have been observed. Based on the *in vitro* hepatocyte stability and metabolite profiling data, we chose rat and marmoset monkey as GLP toxicology species. Significant exposures of major human metabolites (the hydrolysis products of CB-839) were observed in the plasma samples from both CX-839 clinical Phase I trials and GLP toxicity studies.

Excretion: When CB-839 was dosed orally to bile duct-cannulated rats, thereby not allowing bile flow to the intestine, 0.3-1.2% of orally dosed CB-839 appeared in biliary excretion predominately in the first 2 hr after dosing. A 41-49% reduction in systemic exposure to CB-839 was seen relative to normal rats. Further studies are needed to better understand biliary excretion of CB-839 in rats.

Drug-drug interaction: Direct and time-dependent inhibition (TDI) of human cytochrome P450 (CYP) enzymes by CB-839 was evaluated in human liver microsomes. CB-839 was no an inhibitor for CYP1A2, CYP2B6, CPY2C8, CPY2C19, CYP2D6 and CYP3A. CB-839 appears to be a moderate inhibitor of CYP2C9 (~40-50% inhibition of 5 uM). Further studies are needed to understand the mechanism of inhibition. CB-839 was not an inducer of human CYP1A2, 2B6, or 3A4 in cryopreserved hepatocytes from three donors. CB-839 is a moderate substrate of efflux transporters with an efflux ratio of 6.2 in Caco-2 monolayers.

Drug Procurement: CB-839 will be supplied for this study by Calithera Biosciences and will be stored in the Investigational Pharmacy.

Packaging and labeling: Tablets and capsules are packaged in white plastic bottles with a label on the outside identifying the investigational product, capsule strength, storage conditions, investigational drug supplier, lot number and date of manufacture. Each bottle is sealed with a tamper-evident seal and a child-proof cap. Each bottle contains fifty (50) tablets or capsules.

Drug Accountability: Drug storage, dispensing and accountability will be managed by the University Hospitals Seidman Cancer Center Investigational Pharmacy according to standard protocol. Patients will be asked to keep a pill diary throughout the duration of treatment (Appendix II).

Drug Destruction: At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

Other Information: While receiving CB-839, the following nursing/patient implications apply:

- Monitor CBC, chemistries and liver function tests prior to drug administration.
- Symptom management of nausea, vomiting, etc. as needed.
- Drug should be administered with breakfast and dinner.

9.2 Capecitabine

Name of Agent: Capecitabine

Other Names: Xeloda

Product description: Capecitabine is supplied as biconvex, oblong film-coated pills, available as 500 mg pills (peach).

Storage requirements: Capecitabine pills should be stored at 25°C, with excursions permitted to 15 to 30˚C.

Route of administration: Capecitabine is taken orally and needs to be taken within 30 minutes after a meal.

Drug Procurement: Capecitabine is commercially available. The cost of this agent will be the patient's responsibility.

Drug Accountability: Patients will be asked to maintain a pill diary throughout the duration of the study (Appendix III).

Other Information: While receiving capecitabine therapy, the following nursing/patient implications apply:

- Monitor CBC and platelet count prior to drug administration.
- Symptom management of expected nausea, vomiting, diarrhea and hand-foot skin syndrome.
- Drug should be administered at least 12 hours (+/- 2 hours) apart.
- Capecitabine pills should be swallowed with water within 30 minutes after a meal.
- Capecitabine pills may not be crushed.

10.0 CORRELATIVE STUDIES

10.1 Glutaminase Activity Level

The purpose of assessing the change in glutaminase activity level is to determine if administration of CB-839 results in a decrease in glutaminase activity in post-treatment platelets and biopsy specimens as compared to prior to CB-839 treatment using a glutaminase inhibition assay.

10.1.1 Background

As is outlined in section 1.1, PIK3CA mutant cancer cells are dependent on glutamine for survival. To enter the TCA cycle, glutamine is first deaminated by glutaminase (GLS) to generate glutamate. Glutamate is then converted to alpha-ketoglutarate (alpha-KG), a TCA cycle intermediate, by either an aminotransferase (e.g. GPTs and GOTs) or a glutamate dehydrogenase. Glutamine metabolites are utilized to produce ATP, lipids and other macromolecules, thereby promoting tumor growth (4). CB-839 is a glutaminase inhibitor that we hypothesize will starve colorectal cancer tumors that are PIK3CA mutant of glutamine, thereby inhibiting growth. To assess if CB-839 is producing a pharmacodynamic effect in both platelets and tumors, glutaminase activity may be measured via a glutaminase inhibition assay as has been previously described (17,19).

10.1.2 Rationale for Analysis

Demonstration of glutaminase inhibition in both platelets and tumor specimens following treatment with single agent CB-839 as compared to prior to initiation of treatment will provide a pharmacodynamic marker indicating that CB-839 is reaching its intended target. If correlation is seen between platelets and tumor specimens in regard to level of inhibition, it may be necessary to assess platelets only in regard to glutaminase inhibition in subsequent studies. See section 14.4 for statistical plan.

10.1.3 Collection of Specimens

During the phase II component of the study, patients will undergo either CT or US guided biopsy of a metastatic focus both prior to the initiation of treatment and 10-15 days following initiation of treatment. The pre-treatment biopsy may be performed up to 14 days prior to the initiation of treatment. The pre-treatment biopsy must be done after the 4-week washout from previous systemic therapy.

Tumor Biopsies: Patients will undergo biopsy in the University Hospitals Cleveland Medical Center (UHCMC) Department of Radiology. If being treated at the Taussig Cancer Institute (TCI) of the Cleveland Clinic, patients will undergo biopsy in the Cleveland Clinic Department of Radiology. Use of CT or US guidance for each biopsy will be per the discretion of the physician performing the biopsy and the same metastatic focus of tumor will be biopsied at baseline and 10-15 days following initiation of treatment. If for whatever reason the same lesion cannot be biopsied, a second lesion may be biopsied but this must be noted and recorded. Biopsies will be conducted according to standard practice. Four core biopsies will be obtained for each patient during each biopsy. Fine needle aspiration biopsies are NOT appropriate. Three RNA cryovials

will be labeled with the date, patient initials, patients study number and vial number. All study personnel, particularly those handling RNA cryovials, must wear clean gloves. Each of the three core biopsies will be placed in its own cryovial. Two cryovials should contain approximately 1.5 ml sterile PBS for placement of a fresh specimen and then will be placed on wet ice (see section 10.4 for further details). The remaining two cryovials will immediately be placed in dry ice or liquid nitrogen. Biopsies collected at UHCMC will be transported to the Case Comprehensive Cancer Center, Translational Research Shared Resource for the Wang lab to pick up. Biopsies collected at TCI will be transported to the Cleveland Clinic Central Biorepository for the Wang lab to pick up. Biopsies collected at Weill Cornell College of Medicine will be transported to the Laboratory of Dr. Manish Shah for processing. The frozen specimens will be processed individually with the second specimen being processed only if insufficient nucleic acid is recovered or if histologic review identifies the presence of necrotic tumor in the first specimen. The remaining fresh frozen specimens will be stored for later use.

Platelets: Approximately 24ml of whole blood will be collected via peripheral venipuncture into 3, 10 ml yellow top ACD-A (Acid Citric Dextrose (ACD) Solution A) tubes for analysis of glutaminase activity in platelets. Whole blood samples will be collected on wet ice and transported to the Translational Research Shared Resource for processing and storage. Specimens will be collected at the time of the pre-treatment tissue biopsy as well as 10-15 days following initiation of treatment (obtained on the same day as the tissue biopsy).

10.1.4 Handling of Specimens

10.2.4.1 Handling of Specimens at UHCMC

The vials containing PBS will be placed on wet ice (>5 lb), and the other two cryovials will immediately be placed in dry ice (>5 lb). Personnel from the Case Comprehensive Cancer Center, Translational Research Shared Resource will then be contacted (see below) for transportation, tracking and storage. Upon acquisition, TRSR personnel will contact the Wang Lab to pick up the PBS containing vial for organoid culture and analyses as described in the Laboratory Manual and the frozen specimens for storing at -80°C.

Translational Research Shared Resource Contact Information (UH Seidman)
ATTN: Erin Hohler
University Hospitals Cleveland Medical Center
11100 Euclid Avenue
Seidman Cancer Center,
Cleveland, OH 44106

Wang Lab Contact Information:
Yiqing Zhao

Email: [redacted]

John Wang
[redacted]

Wolstein Research Building [redacted]
2013 Cornell Road
Cleveland, OH 44106

10.1.4.2 Handling of Specimens at TCI

The fresh biopsy vials (containing PBS) will be placed on wet ice (>5 lb), and the other two cryovials will immediately be placed in dry ice (>5 lb). Personnel from the Cleveland Clinic Central Biorepository will then be contacted (see below) for transportation, tracking and storage. Upon acquisition, the Cleveland Clinic Central Biorepository personnel will contact the Wang Lab to pick up the PBS containing vial for organoid culture and analysis as described in the Laboratory Manual and the frozen specimens for storing at -80°C.

The Cleveland Clinic Central Biorepository contact information
ATTN: Brad Skilton
Cleveland Clinic Central Biorepository
Department of Pathology—Tissue Procurement
[redacted]

Wang Lab Contact Information:
Yiqing Zhao
[redacted]

John Wang
[redacted]

Wolstein Research Building 3204
2013 Cornell Road
Cleveland, OH 44106

10.1.4.3 Handling of Specimens at Weill Cornell College of Medicine

The fresh biopsy vials (containing PBS) will be placed on wet ice (>5 lb), and the other two cryovials will immediately be placed in dry ice (>5 lb). Personnel from the Laboratory of Dr.

Manish Shah will then be contacted (see below) for processing. The PBS containing vial will be cultured as organoids in the Shah lab. The frozen specimens will be shipped to the Wang laboratory according to procedures described in the Laboratory Manual.

Shah Lab Contact Information

[redacted]
Laboratory of Dr. Manish Shah [redacted]
510 East 70th Street
Weill Cornell Medical College
New York, New York 10065

10.1.5 Analytical Laboratory

Personnel in the Wang Lab will process fresh frozen specimens. One core biopsy per patient will be divided on a metal plate that is kept cold with dry ice. Additional biopsies will be archived and used on an as needed basis (see section 10.1.3). Each biopsy will be sectioned into 5 sections. Light microscopy to confirm the histologic presence of viable cancer tissue will be conducted on peripheral sections (H&E staining). Tissue sections immediately adjacent to and internal to the microscopy confirmed sections will be used for subsequent pharmacodynamic analysis (glutaminase activity, nucleotide levels, DNA extraction, RNA extraction) (22).

Wang Lab Contact Information:

Yiqing Zhao
[redacted]

John Wang
[redacted]

Wolstein Research Building [redacted]
2013 Cornell Road
Cleveland, OH 44106

Histologically confirmed fresh frozen specimens will be used by the Translational Research Shared Resource or the Wang Lab for conduct of the glutaminase assay.

Translational Research Shared Resource
ATTN: Erin Hohler
University Hospitals Cleveland Medical Center
11100 Euclid Avenue
Seidman Cancer Center, [redacted]
Cleveland, OH 44106
[redacted]

[redacted]

10.1.6 Methods

Glutaminase activity level will be measured using a glutaminase inhibition assay in both solid tumors and platelets.

Tumor Biopsy Specimens: The glutaminase inhibition assay will be conducted during the phase II portion of the study and will require fresh frozen biopsy specimens. These will be obtained as described in section 10.1.3. Patients will undergo a pretreatment biopsy up to 14 days prior to the initiation of treatment, then will undergo a second biopsy on day 10-15 of treatment. *Only the post-treatment tumor biopsy will be used for the glutaminase activity assay.* Samples will be frozen in liquid nitrogen and transferred to the Translational Research and Pharmacology Core on dry ice. Specimens will be evaluated for glutaminase activity per the Glutaminase Activity Assay Protocol for Platelets and Tumor Biopsy Samples (methods provided in laboratory manual).

Platelets: The glutaminase inhibition assay will be conducted during the phase II portion of the study. 24ml of blood will be collected from each patient on day 10-15 of treatment and will be collected on the same day as the post-treatment tissue biopsy. Specimens will be evaluated for glutaminase activity per the Glutaminase Activity Assay Protocol for Platelets and Tumor Biopsy Samples (methods provided in laboratory manual). Processing of samples will be done for each of the sites as outlined in section 10.2.4 and per the laboratory manual.

10.2 Pharmacokinetic analysis of CB-839

The purpose of conducting a pharmacokinetic analysis of CB-839 is to determine if there is a correlation seen between plasma trough levels of CB-839 and both glutaminase activity levels (in tumors and platelets) and UPP1 gene expression.

10.2.1 Background

Pharmacokinetic sampling was performed on Cycle 1, Day 1 (C1D1), C1D15 and D1 of each subsequent treatment cycle in each of the previously conducted phase I studies (17,18). A dose dependent relationship was observed with CB-839 exposure increasing with dose. The half-life of CB-839 was determined to be 4 hours. Additional pharmacokinetic parameters (AUC, Cmax and Cmin) demonstrated greater variability with TID dosing than with BID dosing. Additionally, Cmin fell below a target concentration of 200 ng/mL in 46% of patients receiving 400 mg or higher TID regimens while all patients receiving BID dosing with 600 mg maintained a Cmin greater than the 200 ng/mL target concentration.

Pharmacodynamics of CB-839 were also assessed in both previously conducted phase I studies. Glutaminase activity was measured in circulating platelets as well as tumor biopsy specimens. Blood samples collected at baseline and four hours following CB-839 treatment were assessed for glutaminase inhibition and demonstrated a dose dependent relationship. At a CB-839 treatment dose of 600 mg BID, greater than 90% glutaminase inhibition should be observed even at Cmin.

Glutaminase activity within tumor specimens also demonstrated a dose-response relationship.

10.2.2 Rationale for Analysis

Pharmacokinetic analysis of patients receiving CB-839 at each dose level will allow for correlations to be made between trough levels of CB-839 and glutaminase activity level.

10.3.3 Collection of Specimens

Approximately 2ml of blood will be collected via peripheral venipuncture into an EDTA tube (lavender top). This blood sample will be used to measure concentrations of CB-839 of each pharmacokinetic blood collection. The exact actual time of collection must be noted in the source documents and eCRFs.

For collecting specimens, the following protocol should be followed:

- Label a 3.0 mL K2EDTA vacutainer tube.
- Label 2 cryovials.
- Prepare an ice bucket.
- Draw blood into the 3.0 mL vacutainer tube. Ensure that tube is filled with a minimum of 2 mL of blood.
- Samples should be thoroughly mixed by completely and gently inverting the tube 8 times. If a complete 2 mL volume of blood was not obtained, please discard sample and re-collect with a fresh vacutainer.
- The whole blood samples should be placed on ice immediately after collection. Try not to wet the label.
- Plasma should be prepared within 30 minutes of collection by centrifuging the blood samples at 2000 x g for 10 minutes at 4˚C.
- Split the resultant plasma into two aliquots of approximately equal amounts (at least 0.3 mL) and transfer into the two labeled cryovials.
- Place each of the three samples immediately into the cryobox and store at -70˚C until shipment.

  Note: PK assay will be conducted at a central laboratory where the assay for CB-839 has been validated. Details are given below and in the laboratory manual:

  Intertek Pharmaceutical Services
  [redacted]
  10420 Wateridge Circle
  San Diego, CA 92121
  [redacted]

See section 14.0 for statistical plan.

10.2.4 Handling of Specimens

See section 10.1.4 for handling of specimens. PK specimens will be shipped to Intertek Pharmaceutical Services as outlined (see section 10.2.5).

10.2.5 Analytical Laboratory

Pharmacokinetic analysis will be carried out by central lab

Intertek Pharmaceutical Services
[redacted]
10420 Wateridge Circle
San Diego, CA 92121
[redacted]

10.2.6 Methods

All plasma samples will be analyzed for CB-839 by using a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method of appropriate specificity and sensitivity according to Good Laboratory Practices (GLPs).

10.3 **Additional Pharmacodynamic Analyses**

An evaluation of nucleotide levels as well as a whole exome analysis and an RNA seq analysis will be conducted on baseline and post-treatment tissue biopsies to determine the pharmacodynamic effect of CB-839 and capecitabine on PIK3CA mutant colorectal cancer. This will be conducted during the phase II portion of the trial.

10.3.1 Background and Rationale

Glutamine can be deaminated to generate ammonia as a nitrogen donor for nucleotide synthesis. Since CB-839 blocks glutamine deamination, it should impact nucleotide synthesis. On the other hand, the 5-FU metabolite, FdUMP, inhibits thymidylate synthase and blocks production of dTTP, thereby perturbing levels of other nucleotides through various feedback mechanisms. Taken together, these observations suggest that the combination of CB-839 with 5-FU may have additive or synergistic effects on cellular nucleotide pools. As such, we will determine nucleotide levels in pre- and post-treatment biopsy specimens to determine the impact of CB-839 and capecitabine on nucleotide levels.

Genome wide changes are anticipated to occur with the administration of CB-839 and capecitabine on both the DNA and RNA level. We will therefore use tumor tissue to determine changes occurring in DNA with pre- and post-treatment assessments of the genome via whole genome sequencing and also changes in tumor tissue at the expression level by performing pre- and post-treatment RNA seq analyses.

10.3.2 Collection and Handling of Specimens

See sections 10.1.3 and 10.1.4 for collection and handling of specimens.

10.3.3 Analytical Laboratory

Nucleotide levels will be measured in the Proteomics Shared Resource by Dr. Yan Xu as outlined in the laboratory manual.

Specimens will be processed for whole exome sequencing and RNA seq by the Wang Lab:

Wang Lab Contact Information:

Yiqing Zhao
[redacted]

John Wang
[redacted]

Wolstein Research Building [redacted]
2013 Cornell Road
Cleveland, OH 44106

10.3.4 Methods

Please see the Laboratory Manual for methods on measurement of nucleotide levels, whole exome sequencing and RNA seq.

10.4 Generation of Organoids

10.4.1 Background

The purpose of generating organoids from patient tissue specimens is to allow for a sustained source of genetic material upon which additional pharmacodynamic assessments may be performed. This could be either due to limited tissue being available to perform the planned assessments or for material to remain available for future assessments and additional research is conducted.

10.4.2 Rationale for Analysis

Given the limited material available for molecular analyses, the generation of organoids from patient tissue specimens is a way to generate additional molecular material that will allow for completion of the planned assessments.

10.4.3 Collection of Specimens

During the phase II component of the study, patients will undergo either CT or US guided biopsy of a metastatic focus both prior to the initiation of treatment and 10-15 days following initiation of treatment. The pre-treatment biopsy may be performed up to 14 days prior to the initiation of treatment.

Tumor Biopsies: Patients will undergo biopsy in the University Hospitals Cleveland Medical Center (UHCMC) Department of Radiology. If being treated at the Taussig Cancer Institute (TCI) of the Cleveland Clinic, patients will undergo biopsy in the Cleveland Clinic Department of Radiology. If being treated at the Weill Cornell College of Medicine, patients will undergo biopsy in the Weill Cornell College of Medicine Department of Radiology. Use of CT or US guidance for each biopsy will be per the discretion of the physician performing the biopsy and the same metastatic focus of tumor will be biopsied at baseline and 10-15 days following initiation of treatment. If for whatever reason the same lesion cannot be biopsied, a second lesion may be biopsied but this must be noted and recorded. Biopsies will be conducted according to standard practice. Three core biopsies will be obtained for each patient during each biopsy. Fine needle aspiration biopsies are NOT appropriate. Three RNA cryovials will be labeled with the date, patient initials, patients study number and vial number. All study personnel, particularly those handling RNA cryovials, must wear clean gloves. Each of the three core biopsies will be placed in its own cryovial. One cryovial, should contain approximately 1.5 ml of sterile PBS for placement of a fresh specimen and then will be placed on wet ice (see section 10.4 for further details). The remaining two cryovials will immediately be placed in dry ice or liquid nitrogen. Biopsies collected at UHCMC will be transported to the Case Comprehensive Cancer Center, Translational Research and Pharmacology Core Facility for the Wang lab to pick up. Biopsies collected at TCI will be transported to the Cleveland Clinic Central Biorepository for the Wang lab to pick up. Biopsies collected at Weill Cornell College of Medicine will be transported to the laboratory of Dr. Manish Shah for processing. The frozen specimens will be processed individually with the second specimen being processed only if insufficient nucleic acid is recovered or if histologic review identifies the presence of necrotic tumor in the first specimen. The remaining fresh frozen specimens will be stored for later use.

10.4.4 Handling of Specimens

10.4.4.1 Handling of Specimens at UHCMC

The vial containing PBS will be placed on wet ice (>5 lb). Personnel from the Case Comprehensive Cancer Center, Translational Research Shared Resource will then be contacted (see below) for transportation, tracking and storage. Upon acquisition, TRSR personnel will contact the Wang Lab to pick up the PBS containing vial for organoid culture and analyses as described in the Laboratory Manual.

Translational Research Shared Resource Contact Information (UH Seidman)
ATTN: Erin Hohler
University Hospitals Case Medical Center
11100 Euclid Avenue
Seidman Cancer Center, [redacted]

Cleveland, OH 44106
[redacted]

Wang Lab Contact Information:
Yiqing Zhao
[redacted]

John Wang
[redacted]

Wolstein Research Building [redacted]
2013 Cornell Road
Cleveland, OH 44106

10.4.4.2 Handling of Specimens at TCI

The vial containing PBS will be placed on wet ice (>5 lb). Personnel from the Cleveland Clinic Central Biorepository will then be contacted (see below) for transportation, tracking and storage. Upon acquisition, the Cleveland Clinic Central Biorepository personnel will contact the Wang Lab to pick up the PBS containing vial for organoid culture and analyses as described in the Laboratory Manual.

The Cleveland Clinic Central Biorepository contact information
ATTN: Brad Skilton
Cleveland Clinic Central Biorepository
[redacted]

Wang Lab Contact Information:
Yiqing Zhao
[redacted]

John Wang
[redacted]
Wolstein Research Building [redacted]

2013 Cornell Road
Cleveland, OH 44106

10.4.4.3 Handling of Specimens at Weill Cornell College of Medicine

The vial containing PBS will be placed on wet ice (>5 lb). Personnel from the Laboratory of Dr. Manish Shah for processing (see below). The PBS containing vial will be cultured as organoids in the Shah lab. The frozen specimens will be shipped to the Wang laboratory according to procedures described in the Laboratory Manual.

Shah Lab Contact Information
Attn: Kyle Bocchino
Laboratory of Dr. Manish Shah [redacted]
510 East 70th Street
Weill Cornell Medical College
New York, New York 10065

10.4.5 Analytical Laboratory

Fresh specimens at UHCMC and TCI will be processed in the laboratory of Zhenghe (John) Wang.

Wang Lab Contact Information:
Yiqing Zhao
[redacted]

John Wang
[redacted]

Wolstein Research Building [redacted]
2013 Cornell Road
Cleveland, OH 44106

Fresh specimens at Weill Cornell College of Medicine will be processed in the laboratory of Dr. Manish Shah.

Weill Cornell Medical College
Attn: Kyle Bocchino
Laboratory of Dr. Manish Shah [redacted]
510 East 70th Street
New Yor, NY 10065

10.4.6 Methods

The generation of organoids will be conducted during the phase II portion of the study. And will require fresh biopsies. These will be obtained as described in section 10.4.3. Patients will undergo a pretreatment biopsy up to 14 days prior to the initiation of treatment, then will undergo a second biopsy on day 10-15 of treatment. Samples will be placed fresh in sterile PBS and placed on wet ice and will be transferred to the laboratory of Dr. Zhenghe Wang for processing. Organoids will be generated as outlined in the laboratory manual.

11.0 STUDY PARAMETERS AND CALENDAR

11.1 Phase I Study Parameters

11.1.1 Screening Evaluation

Screening studies and evaluations will be used to determine the eligibility of each patient for study inclusion. All evaluations must be completed ≤ 21 days prior to administration of protocol therapy.

The following should be obtained at baseline:

- Informed consent
- Demographics
- Medical History
- Complete physical examination
- Height
- Weight
- Vital signs including temperature, heart rate, blood pressure and respiratory rate
- Concomitant medications including all over the counter medications and supplements (note that if patients are on a PPI, transition to an H2 blocker prior to treatment is required)
- ECOG performance status
- Baseline symptom/adverse event assessment
- Laboratory studies
    - Complete blood count (CBC) with differential
    - Comprehensive metabolic panel
    - Calculated creatinine clearance if creatinine and/or BUN are abnormal
    - Pregnancy test (if female of reproductive age)
- CT of the chest, abdomen and pelvis
- Baseline CT/US guided tumor biopsy

11.1.2 Treatment Period

CB-839 and Capecitabine, Cycle 1, Day 1

All assessments may be performed up to 24 hours prior to treatment.

- Physical examination
- Weight
- Vital signs including temperature, heart rate, blood pressure and respiratory rate
- Concomitant medications including over the counter medications and supplements
- ECOG performance status

- Symptom/adverse event assessment
- Laboratory studies
    - Complete blood count (CBC) with differential
    - Comprehensive metabolic panel
    - Calculated creatinine clearance if creatinine and/or BUN are abnormal
- Administration of CB-839 by mouth twice daily for a 21 day treatment cycle
- Administration of capecitabine by mouth twice daily for first 14 days of 21 day cycle

CB-839 and Capecitabine, Cycle 1, Day 8 and Day 15

Labs may be obtained +/- one day.

- Symptom/adverse event assessment
- Laboratory studies
    - Complete blood count (CBC) with differential
    - Comprehensive metabolic panel
    - Calculated creatinine clearance if creatinine and/or BUN are abnormal
    - Pharmacokinetic assessment prior to morning dose of CB-839 (day 15 only)

CB-839 and Capecitabine, Cycle 2 and beyond, Day 1

All assessments may be performed +/- one day

- Physical examination
- Weight
- Vital signs including temperature, heart rate, blood pressure and respiratory rate
- Concomitant medications including over the counter medications and supplements
- ECOG performance status
- Symptom/adverse event assessment
- Laboratory studies
    - Complete blood count (CBC) with differential
    - Comprehensive metabolic panel
    - Calculated creatinine clearance if creatinine and/or BUN are abnormal
- Administration of CB-839 by mouth twice daily for a 21 day treatment cycle
- Administration of capecitabine by mouth twice daily for first 14 days of 21 day cycle

CB-839 and Capecitabine, Cycle 3, sometime between days 15-21 of treatment cycle

- CT of the chest, abdomen and pelvis for restaging evaluation

Subsequent CT imaging of the chest, abdomen and pelvis should be performed on an every 9 week basis +/- one week for as long as patient is receiving study treatment.

11.2 Phase II Study Parameters

11.2.1 Screening Evaluation

Screening studies and evaluations will be used to determine the eligibility of each patient for study inclusion. All evaluations must be completed ≤ 21 days prior to administration of protocol therapy (with the exception of the PIK3CA status which may be obtained at any time).
The following should be obtained at baseline:

- Informed consent
- Demographics
- Medical History
- Complete physical examination
- Height
- Weight
- Vital signs including temperature, heart rate, blood pressure and respiratory rate
- Concomitant medications including all over the counter medications and supplements (note that if patients are on a PPI, transition to an H2 blocker prior to treatment is required)
- ECOG performance status
- Baseline symptom/adverse event assessment
- Laboratory studies
    - Complete blood count (CBC) with differential
    - Comprehensive metabolic panel
    - Calculated creatinine clearance if creatinine and/or BUN are abnormal
    - CEA (carcinoembryonic antigen) blood test
    - Pregnancy test (if female of reproductive age)
- CT of the chest, abdomen and pelvis
- Baseline CT/US guided tumor biopsy

11.2.2 Treatment Period

CB-839 and Capecitabine, Cycle 1, Day 1

All assessments other than tumor biopsy and pharmacokinetics may be performed up to 24 hours prior to treatment.

- Physical examination
- Weight
- Vital signs including temperature, heart rate, blood pressure and respiratory rate
- Concomitant medications including over the counter medications and supplements
- ECOG performance status
- Symptom/adverse event assessment
- Laboratory studies
    - Complete blood count (CBC) with differential
    - Comprehensive metabolic panel
    - Calculated creatinine clearance if creatinine and/or BUN are abnormal
- Administration of CB-839 by mouth twice daily for a 21 day treatment cycle
- Administration of capecitabine by mouth twice daily for first 14 days of 21 day cycle

CB-839 and Capecitabine, Cycle 1, Day 8 and Day 15

Labs may be obtained +/- one day.

- Symptom/adverse event assessment
- Laboratory studies
    - Complete blood count (CBC) with differential
    - Comprehensive metabolic panel
    - Calculated creatinine clearance if creatinine and/or BUN are abnormal

CB-839 and Capecitabine, Cycle 1, Day 10-15

- CT/US guided tumor biopsy
- Platelet glutaminase assay (to be obtained the same day as the tumor biopsy)
- Pharmacokinetic assessment prior to morning dose of CB-839 (day 15 only)

CB-839 and Capecitabine, Cycle 2 and beyond, Day 1

All assessments may be performed +/- one day

- Physical examination
- Weight
- Vital signs including temperature, heart rate, blood pressure and respiratory rate
- Concomitant medications including over the counter medications and supplements
- ECOG performance status
- Symptom/adverse event assessment
- Laboratory studies
    - Complete blood count (CBC) with differential
    - Comprehensive metabolic panel
    - Calculated creatinine clearance if creatinine and/or BUN are abnormal
    - CEA (carcinoembryonic antigen) blood test
- Administration of CB-839 by mouth twice daily for a 21 day treatment cycle
- Administration of capecitabine by mouth twice daily for first 14 days of 21 day cycle

CB-839 and Capecitabine, Cycle 3, sometime between days 15-21 of treatment cycle

- CT of the chest, abdomen and pelvis for restaging evaluation

Subsequent CT imaging of the chest, abdomen and pelvis should be performed on an every 9 week basis +/- one week for as long as patient is receiving study treatment

11.3 Calendar—Phase I Component

Study Days	Pre-Study[a]	CB-839 and cape, C1D1[b]	CB-839 and cape, C1D8	CB-839 and cape, C1D15	Cycle 2 and beyond, Day 1	Cycle 3, Day 14-21	Every 9 weeks	30 Day Follow-Up
REQUIRED ASSESSMENTS								
Informed Consent	X							
Demographics	X							
Medical History	X							
Height	X							
Weight	X	X			X			X
Vitals	X	X			X			X
Physical Examination	X	X			X			X
Concomitant Med Assessment	X	X			X			
ECOG PS	X	X			X			X
Symptom/Adverse Event Assessment	X	X	X	X	X			X
CBC with diff[c]	X	X	X	X	X			X
Serum comprehensive panel[f]	X	X	X	X	X			X
Pregnancy test (if applicable)	X							
DISEASE ASSESSMENT								
CT chest, abdomen and pelvis	X					X	X[e]	
		Tumor assessments are performed at baseline and every 9 weeks. Documentation (radiologic) must be provided for patient removed from study for progressive disease						
TREATMENT								
CB-839		X[g]			X			
Capecitabine		X[g]			X			
CORRELATIVE STUDIES								
Pharmacokinetics (CB-839)				X[d]				

[a] All evaluations must be completed ≤ 21 days prior to administration of protocol therapy.
[b] Cycle 1, Day 1 assessments do not need to be repeated if done within 7 days of initiation of treatment.
[c] CBC with differential and comprehensive panel for day 1 of the treatment cycle may be drawn up to 24 hours prior to treatment.
[d] Pharmacokinetic sample should be drawn just prior to the administration of the morning dose of CB-839 and capecitabine.
[e] Restaging CT scans may be performed +/- 7 days at all timepoints beyond the cycle 3 evaluation.
[f] Serum comprehensive panel should include sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, AST, ALT, alk phos, total protein, albumin, total bilirubin, direct bilirubin, magnesium and phosphorus.
[g] Treatment starts this day. CB-839 is to be taken twice daily continuously (21/21 days) and capecitabine is to be taken twice daily for 14/21 days).

11.4 Calendar—Phase II Component

Study Days	Pre-Study[a]	Pre-treatment	CB-839 and cape, C1D1[b]	CB-839 and cape, C1D8	CB-839 and cape, C1D15	Cycle 2 and beyond, Day 1	Cycle 3, Day 14-21	Every 9 weeks	30 Day Follow-Up
REQUIRED ASSESSMENTS									
Informed Consent	X								
Demographics	X								
Medical History	X								
Height	X								
Weight	X		X			X			X
Vitals	X		X			X			X
Physical Examination	X		X			X			X
Concomitant Med Assessment	X		X			X			
ECOG PS	X		X			X			X
Symptom/Adverse Event Assessment	X		X	X	X	X			X
CBC with diff[d]	X		X	X	X	X			X
Serum comprehensive panel[d]	X		X	X	X	X			X
CEA (carcinoembryonic antigen) blood test	X					X			
Pregnancy test (if applicable)	X								
DISEASE ASSESSMENT									
Tumor measurements		Tumor assessments are performed at baseline and every 9 weeks. Documentation (radiologic) must be provided for patient removed from study for progressive disease.							
CT chest, abdomen and pelvis	X						X	X[g]	
MISC ITEMS									
Tumor biopsy		X[c]			X[e]				
TREATMENT									
CB-839			X[h]			X			
Capecitabine			X[h]			X			
CORRELATIVE STUDIES									
Pharmacodynamic studies		X[c]			X[c]				
Pharmacokinetics (CB-839)					X[f]				

[a] All evaluations must be completed ≤ 21 days prior to administration of protocol therapy.

[b] Cycle 1, Day 1 assessments do not need to be repeated if done within 7 days of initiation of treatment.

[c] Tumor biopsy may be done up to 14 days prior to initiation of treatment. Please allow 28 days washout from prior systemic therapy prior to pre-treatment biopsy. Platelet glutaminase assay should be drawn the same day as performance of the tissue biopsy. Glutaminase activity will only be assessed on the post-treatment blood and tissue specimens.

[d] CBC with differential and comprehensive panel for day 1 of the treatment cycle may be drawn up to 24 hours prior to treatment. Serum comprehensive panel should include sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, AST, ALT, alk phos, total protein, albumin, total bilirubin, direct bilirubin, magnesium and phosphorus.

[e] Post-treatment tissue biopsy may be performed day 10-15. Platelet glutaminase assay should be drawn the same day as performance of the tissue biopsy.

[f] Pharmacokinetic sample should be drawn just prior to the administration of the morning dose of CB-839 and capecitabine.

[g] Restaging CT scans may be performed +/- 7 days at all timepoints beyond the cycle 3 evaluation.

[h] Treatment starts this day. CB-839 is to be taken twice daily continuously (21/21 days) and capecitabine is to be taken twice daily for 14/21 days).

12.0 MEASUREMENT OF EFFECT

Although response is not the primary endpoint of the phase I portion of this trial, patients will be assessed by standard criteria. Response rate is the primary endpoint for the phase II portion of the trial. For the purposes of this study, patients should be re-evaluated every 9 weeks in addition to undergoing a baseline scan.

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) (23). 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.

12.1 Definitions

Evaluable for toxicity: All patients who receive at least one dose of study drug (either CB-839 or capecitabine) will be included in the analysis of safety regardless of the duration of treatment.

Evaluable for objective response: Only those patients who have measureable 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 measureable 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.

12.2 Disease Parameters

Measurable Disease: Measureable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter for non-nodal lesions and short axis for nodal lesions 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). Tumor lesions that are situated in a previously irradiated area will not be considered measureable.

Malignant lymph nodes: To be considered pathologically enlarged and measureable, 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 followup, only the short axis will be measured and followed.

Non-measureable 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-measureable 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-measureable.

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 measureable 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 measureable 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 lesion) 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 measureable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurement of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout followup.

12.3 Methods for Evaluation of Measureable 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 treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during followup. 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.

Clinical lesions: Clinical lesions will only be considered measureable 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.

Chest x-ray: Lesions on chest x-ray are acceptable as measureable lesions when they are clearly

defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI: This guideline has defined measurability of lesion 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 measureable 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 followup 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-holding techniques, if possible.

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 routine or serially performed. *PET may not be used for making determinations regarding response to treatment.*

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.

Endoscopy, Laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathologic response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

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.

Cytology, Histology: These techniques can be used to differentiated between partial responses

(PR) and complete responses (CR) in rare cases (e.g. residual lesions in tumor types, such as germ cell tumors where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

12.4 Response Criteria

Evaluation of Target Lesions

Response	**Evaluation of Target Lesions**
Complete Response (CR)	Disappearance of all target lesions. Any pathologic 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 diameters of target lesions, taking as reference the baseline sum of diameters.
Progressive Disease (PD)	At least a 20% increase in the sum of 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 progression.
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.

Evaluation of Non-Target lesions

Response	**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 [Incomplete response/Stable Disease (SD)]	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 PI).

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.

For Patients with Measureable Disease (e.g. Target Disease)

Target Lesions	**Non-Target Lesions**	**New Lesions***	**Best Overall Response**	**Remarks**
CR	CR	No	CR	
CR	Non-CR/Non-PD***	No	PR	
CR	Not evaluated	No	PR	
PR	Non-PD***/not evaluated	No	PR	
SD	Non-PD***/not evaluated	No	SD	Documented at least once ≥ 8 wks. from study entry
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.
** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.
***PD in non-target lesions should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase. Please refer to the Evaluation of Non-Target Lesions – Progressive Disease (Section 12.2) for further

explanation.

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.

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.

12.5 Progression Free Survival

Progression-free survival (PFS) is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

13.0 DATA REPORTING / REGULATORY CONSIDERATIONS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 8.0 (Adverse Events: List and Reporting Requirements).

13.1 Data Reporting

The OnCore™ Database will be utilized, as required by the Case Comprehensive Cancer Center, to provide data collection for both accrual entry and trial data management. OnCore™ is a Clinical Trials Management System housed on secure servers maintained at Case Western Reserve University. OnCore™. Access to data through OnCore™ is restricted by user accounts and assigned roles. Once logged into the OnCore™ system with a user ID and password, OnCore™ defines roles for each user which limits access to appropriate data. User information and password can be obtained by contacting the OnCore™ Administrator at OnCore-registration@case.edu.

OnCore™ is designed with the capability for study setup, activation, tracking, reporting, data monitoring and review, and eligibility verification. This study will utilize electronic Case Report Form completion in the OnCore™ database. A calendar of events and required forms are available in OnCore™.

13.2 Regulatory Considerations

The study will be conducted in compliance with ICH guidelines and with all applicable federal (including 21 CFR parts 56 & 50), state or local laws.

13.2.1 Written Informed consent

Provision of written informed consent must be obtained prior to any study-related procedures. The Principal Investigator will ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study as well as the patient's financial responsibility. Patients must also be notified that they are free to discontinue from the study at any time. The patient should be given the opportunity to ask questions and be allowed time to consider the information provided.

The original, signed written Informed Consent Form must be kept with the Research Chart in conformance with the institution's standard operating procedures. A copy of the signed written Informed Consent Form must be given to the patient. Additionally, documentation of the consenting process should be located in the research chart.

13.2.2 Patient Data Protection

In accordance with the Health Information Portability and Accountability Act (HIPAA), a patient must sign an authorization to release medical information to the sponsor and/or allow the sponsor, a regulatory authority, or Institutional Review Board access to patient's medical information that includes all hospital records relevant to the study, including patients' medical history.

13.2.3 Retention of records

The Principal Investigator of The Case Comprehensive Cancer Center supervises the retention of all documentation of adverse events, records of study drug receipt and dispensation, and all IRB correspondence for as long as needed to comply with local, national and international regulations. No records will be destroyed until the Principal Investigator confirms destruction is permitted.

13.2.4 Audits and inspections

Authorized representatives of the sponsor, sponsor's collaborators, a regulatory authority, an Independent Ethics Committee (IEC) or an Institutional Review Board (IRB) may visit the site to perform audits or inspections, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonization (ICH), and any applicable regulatory requirements. For multi-center studies, participating sites must inform the sponsor-investigator of pending audits.

14.0 STATISTICAL CONSIDERATIONS

14.1 Study Design/Endpoints

14.1.1 Definition of Primary Endpoint

Phase I: Safety, tolerability and recommended phase II dose (RP2D) of combination therapy with CB-839 and capecitabine chemotherapy in patients with no remaining treatment options or patients for whom single agent capecitabine is an acceptable therapy.

Phase II: : Progression free survival (PFS) on combination CB-839 and capecitabine as determined by clinical assessment and RECIST criteria in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy. Progression free survival is defined as the time from randomization to documented progression or death without progression.

14.1.2 Definition of Secondary Endpoints

Phase I: Dose-limiting toxicities as assessed by CTCAE version 4 of combination CB-839 and capecitabine in patients with advanced solid tumors with no remaining treatment options or patients for whom single agent capecitabine is an acceptable therapy.

Phase I: Response rate as assessed by RECIST criteria of combination CB-839 and capecitabine chemotherapy in patients with advanced solid tumors with no remaining treatment options or patients for whom single agent capecitabine is an acceptable therapy.

Phase II: Response rate as assessed by RECIST criteria of combination CB-839 and capecitabine chemotherapy in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy.

Phase II: Overall survival of patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy following treatment with CB-839 and capecitabine chemotherapy. Overall survival is defined as the time from randomization to death from any cause.

14.1.3 Definition of Correlative Endpoints

Phase I: Post-CB-839 pharmacokinetics as assessed by CB-839 drug levels following combination therapy with CB-839 and capecitabine in patients with advanced solid tumors for whom there are no further treatment options or patients for whom single agent capecitabine is an acceptable treatment option.

Phase II: Change in glutaminase activity level as assessed by a glutaminase inhibition assay in both colorectal tumor specimens as well as platelets from patients whom have metastatic PIK3CA mutant colorectal cancer and are refractory to fluoropyrimidine based therapy and who have received treatment with CB-839 and capecitabine chemotherapy.

Phase II: Reduction in nucleotide levels as assessed by HPLC in tumor specimens of patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy and who have received treatment with CB-839 and capecitabine chemotherapy.

Phase II: Change in cellular genome as assessed by whole exome sequencing of tumor specimens of patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy and who have received treatment with CB-839 and

capecitabine chemotherapy.
Phase II: Change in cellular gene expression as assessed by RNA seq of tumor specimens of patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy and who have received treatment with CB-839 and capecitabine chemotherapy.

Phase II: Correlation between plasma trough levels of CB-839 and biomarkers of interest in both tumors and platelets in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy and who have received treatment with CB-839 and capecitabine chemotherapy.

Phase II: Correlation between clinical response to treatment and biomarkers of interest in patients with metastatic PIK3CA mutant colorectal cancer who are refractory to fluoropyrimidine based therapy and who have received treatment with CB-839 and capecitabine chemotherapy.

14.2 Analytic Plan for Primary Objectives

The phase I component of this study is a dose-escalation trial of CB-839 and capecitabine in patients with advanced solid tumors for whom there are no remaining treatment options or for whom capecitabine is an acceptable treatment. It has been designed to define the recommended phase II dose of CB-839 and capecitabine. A traditional 3+3 dose escalation design will be adopted. Nine to twenty-four patients are expected to be enrolled, depending on the number of dose escalations and assuming that a total of 6 patients will be treated at the final recommended phase II dose level. Patients who complete the first 21 day treatment cycle of CB-839 and capecitabine chemotherapy will be included in the analysis. Patients who do not complete the first 21 day treatment cycle or who fail to receive at least 75% of the doses of both CB-839 and capecitabine for reasons unrelated to toxicity will not be included and must be replaced.

In the phase II component of this study, the primary endpoint is progression free survival rate at 6 months with a null hypothesis of 18% vs. a target of 36%. Employing a 2-stage design, and assuming 80% power and a 0.05 significance level, 40 total patients will be required with 18 in the first stage and the probability of early stopping if the null hypothesis is correct (<3/18 patients with stable disease at 6 months) will be 0.589. If at least 3 patients in 18 has stable disease or better at 6 months, an additional 22 patients will be treated (total of 40 patients). If antitumor activity is identified in the PIK3CA mutant population, we will consider amending the phase 2 trial to assess an additional cohort of patients with wild-type PIK3CA. The statistical considerations will follow the same rules as in the original mutant cohort. Response rate will be determined using RECIST criteria.

14.3 Analytic Plan for Secondary Objectives

All toxicities will be summarized as the percentage of patients experiencing each type and grade of event according to dose level. Patients who receive at least one dose of treatment will be included in the analysis.

In the phase I portion of the study, response rate will be summarized using frequencies and proportions by dose level. Response to treatment will be determined using RECIST criteria.

14.4 Analytic Plan for Correlative Objectives

All correlative studies will be performed in biospecimens from the intent to treat population of 18-40 patients from the phase II portion of the study. Pre- and post-treatment tumor samples from the same patients will be used for assessments, except glutaminase activity, which will be performed on post-treatment samples only (tumor samples and platelet samples). All correlative continuous measurements will be summarized using mean +/- SEM, range and median; all categorical measurements will be summarized using frequencies and proportions. Change in these measurements in tumors before and after therapy will be assessed using paired Wilcoxon tests. Unpaired Wilcoxon tests will be used for assessment of glutaminase actively in post-treatment samples only. Assuming a maximum of 40 patients (2-sided test with 0.05 significance level and 80% power), for the paired T-test we can detect normalized differences of 4.5 to 18.2% for standard deviations of the difference between pairs ranging from 0.10 to 0.40. Correlations of plasma trough levels and platelet glutaminase activity will be calculated using Spearman correlation coefficients. Assuming a maximum of 40 patients, 2-sided test with 0.05 significance level, we have >98% power to detect a Spearman correlation coefficient of 0.60 or higher.

Whole exome sequencing and RNA sequencing will be performed on pre and post treatment tissue samples and compared for differences for all patients (n=40 patients maximum). All of these analyses are exploratory, hence no power calculations are shown.

14.5 Sample Size Justification

The phase I component of the trial will be conducted using a standard 3+3 dose escalation design. As there are four dose levels to be assessed (and two minus dose levels if needed), a total of 9-24 patients will be needed to complete the study.

In the phase II portion of the study, disease control rate is the primary endpoint and the study will be conducted using a Simon two-stage design. As outlined in section 14.2, a minimum of 18 and a maximum of 40 patients will be required.

14.6 Accrual Rate Estimate

Accrual for this study will occur at the University Hospitals Seidman Cancer Center and the Cleveland Clinic Taussig Cancer Institute, both of the Case Comprehensive Cancer Center. For the phase I portion of the trial, we anticipate that it will take approximately 6-7 months to complete this portion of the trial if all doses levels need to be evaluated. For the phase II portion of the study, between the two institutions, 719 colorectal cancer patients are seen annually. We estimate that approximately 30% of our patients will have measureable, metastatic disease and that 15% of patients will have a PIK3CA mutation, resulting in 32 eligible patients per year. Based on our proposed sample size of 18-40 patients, we anticipate that it will take approximately 18-40 months to accrue to the phase II portion of the trial.

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APPENDIX I

PERFORMANCE STATUS CRITERIA

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

APPENDIX II

PATIENT PILL DIARY FOR TWICE DAILY DOSING OF CB-839 (WHILE TAKING WITH CAPECITABINE)

Patient Name _____ **Patient Study ID** _____ **Todays date** ___/___/___
Drug _____ **Cycle #:**_____

INSTRUCTIONS FOR THE PATIENT:

1. Complete one form every 3 weeks (one treatment cycle).
2. You will take CB-839 tablets twice each day about 12 hours apart. Each dose should be taken with food—the first dose of the day should be taken with breakfast, the second dose of the day should be taken with dinner.
   Morning dose: take ___ 200 mg capsules
   Evening dose: take ___ 200 mg capsules
3. Record the date, the number of capsules that you took, and what time you took them.
4. If you have any comments or notice any side effects, please record them in the "Comments" column.
5. Please bring this form and your bottle(s) of CB-839 to your physician when you return for each appointment.
6. Please sign your name at the bottom of the diary.

Day	Date	Time of morning dose	# of tablets taken			Time of evening dose	# of tablets taken			Comments
			200 mg				200 mg			
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										
16										
17										
18										
19										
20										
21										

Patient's Signature: _____ **Date:** _____

APPENDIX III

PATIENT PILL DIARY FOR TWICE DAILY DOSING OF CAPECITABINE (WHILE TAKING WITH CB-839)

Patient Name _____ **Patient Study ID** _____ **Todays date** ___/___/___
Drug _____ **Cycle #:**_____

INSTRUCTIONS FOR THE PATIENT:

1. Complete one form every 3 weeks (one treatment cycle).
2. You will take capecitabine tablets twice each day about 12 hours apart. Each dose should be taken within 30 minutes of eating breakfast and within 30 minutes of eating dinner.
   Morning dose: take ___ 500 mg tablets
   Evening dose: take ___ 500 mg tablets
3. Record the date, the number of tablets that you took, and what time you took them.
4. If you have any comments or notice any side effects, please record them in the "Comments" column.
5. Please bring this form and your bottle(s) of capecitabine to your physician when you return for each appointment.
6. Please sign your name at the bottom of the diary.

Day	Date	Time of morning dose	# of tablets taken			Time of evening dose	# of tablets taken			Comments
			500 mg				500 mg			
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
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16										
17										
18										
19										
20										
21										

Patient's Signature: _____ **Date:** _____