VANDERBILT UNIVERSITY
MEDICAL CENTER

Phase I/II Study to Evaluate the Safety, Efficacy, and Novel PET/CT Imaging Biomarkers of CB-839 in Combination with Panitumumab and Irinotecan in Patients with Metastatic and Refractory RAS Wildtype Colorectal Cancer

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1. SYNOPSIS

Trial Title	Phase I/II Study to Evaluate the Safety, Efficacy, and Novel PET/CT Imaging Biomarkers of CB-839 in Combination with Panitumumab and Irinotecan in Patients with Metastatic and Refractory RAS Wildtype Colorectal Cancer.
Phase I Trial Design	This is a three-agent, open-label, non-randomized, Phase I dose escalation study of combinatorial oral CB-839 plus infusional panitumumab and irinotecan in patients with metastatic *RAS* wildtype colorectal cancer. The phase I portion was designed in order to collect toxicity data of the triplet combination, as this treatment regimen could go forward in clinical development.
Phase II Trial Design	This is a two-agent, open-label, non-randomized, Phase II dose expansion study of combinatorial oral CB-839 plus infusional panitumumab in patients with metastatic and refractory *RAS* wildtype colorectal cancer. The phase II portion is a hypothesis driven study to investigate the efficacy of adding a glutaminase 1 inhibitor to single agent anti-EGFR monoclonal antibody therapy.
Trial Sites	Vanderbilt University Medical Center
Study Drug and Mechanism	CB-839 is an oral small molecule inhibitor of glutaminase 1 under investigation for the treatment of various solid and hematologic malignancies, where alterations in glutamine metabolism may play a role in cancer cell growth and survival. Regulatory approval has not been sought and CB-839 is not approved or marketed in any country. Calithera will provide CB-839 to the site.
Standard of Care Drugs and Mechanisms	Panitumumab (VECTIBIX) is a recombinant, human IgG2 kappa monoclonal antibody that binds to the human epidermal growth factor receptor (EGFR). Binding of panitumumab to EGFR thus inhibits ligand-induced tyrosine phosphorylation of EGFR. Panitumumab is FDA-approved for the treatment of wild-type *KRAS* metastatic colorectal cancer (mCRC): In combination with FOLFOX for first-line treatment; and as monotherapy following disease progression after prior treatment with fluoropyrimidine, oxaliplatin, and irinotecan-containing chemotherapy. The site will obtain panitumumab from commercial supply. Irinotecan (CAMPTOSAR) is a topoisomerase inhibitor. Irinotecan is a derivative of camptothecin. Irinotecan and its active metabolite SN-38 bind to the topoisomerase I-DNA complex, thus inhibiting re-ligation of single-strand breaks induced by topoisomerase intended to relieve torsional strain during DNA replication. Irinotecan is FDA approved, in combination with 5-fluorouracil and leucovorin, for patients with metastatic carcinoma of the colon or rectum; and for patients with metastatic carcinoma of the colon or rectum whose disease has recurred or progressed following initial fluorouracil-based therapy. The site will obtain irinotecan as standard of care from commercial supply.

Treatment Schedule	Treatment will consist of Cycles lasting 4 weeks (28 days) each. All patients (both phases) are scheduled to receive oral CB-839 twice daily (BID) on a continuous dosing schedule. All patients (both phases) are scheduled to receive panitumumab as a 60 minute (if dose ≤ 1000 mg) or 90 minute (if dose > 1000 mg) intravenous infusion on Day 1 and Day 15 of each 28 day cycle. All patients in phase I only are scheduled to receive irinotecan as a 90-minute intravenous infusion on Day 1 and Day 15 of each 28 day cycle. On days when both panitumumab and irinotecan drugs are administered (i.e. scheduled for Days 1 and 15 of each cycle), the panitumumab infusion will be completed BEFORE initiation of the irinotecan infusion.
Objectives and Outcome Measures	**Objectives:** Primary Objective of Phase I: • Determine the safety and tolerability of CB-839 in combination with panitumumab and irinotecan. Exploratory Objective of Phase I (Optional Imaging Sub-study): • Correlate radiological features of pre- and post-treatment ^{11}C-Glutamine PET/CT and ^{18}F-FSPG PET/CT with clinical outcome. Primary Objective of Phase II: • Determine the efficacy of CB-839 in combination with panitumumab as measured by the response rate (RR). Secondary Objectives of Phase II: • Determine the disease control rate (DCR), progression-free survival (PFS), and overall survival (OS). • Perform the following correlative studies (in the phase 2 component): • Correlate radiological features of pre- and post-treatment ^{18}F-FSPG PET/CT with clinical outcome and biological correlates (tissue gene signature, exosomes). • Collect blood samples during each radiotracer injection to assess pharmacokinetics. • Collect pre-treatment biopsy tissue and prospectively correlate clinical outcome with a glutamine metabolism gene signature. • Quantify exosomal content in the plasma. Exploratory Objective of Phase II: • Development of patient-derived organoids from pre-treatment tissue biopsy **Endpoints:** Primary Endpoint of Phase I: • Maximum Tolerated Dose (MTD) and recommended phase 2 dose (RP2D) of CB-839 in combination with standard doses of panitumumab and irinotecan. Exploratory Endpoint for Phase I (Optional Imaging Sub-study): • Maximum Standardized Uptake Value (SUVmax) of ^{11}C-Glutamine and ^{18}F-FSPG uptake at pre-treatment and after one cycle of treatment.

	Primary Endpoint of Phase II: • Response Rate (RR) of CB-839 in combination with panitumumab. Secondary Endpoints of Phase II: • Disease control rate (DCR), Progression-free Survival (PFS), and Overall Survival (OS). • Maximum Standardized Uptake Value (SUV_{max}) of ^{18}F-FSPG uptake at pre-treatment and after one cycle of treatment. • Glutamine metabolism gene signature using qRT-PCR analysis and RNA-Seq. • Radiotracer pharmacokinetic modeling parameters • Plasma exosomal content at pre-treatment, after one cycle of treatment, and at disease progression. Exploratory Endpoint for Phase II: • Development of patient-derived organoid model
Main Inclusion and Exclusion Criteria	**Main inclusion criteria:** • Histologically or cytologically-confirmed diagnosis of metastatic *RAS* wildtype colorectal cancer (CRC). • ECOG performance status of 0 or 1. • In Dose Escalation, patients must have had at least one prior line of chemotherapy for advanced disease or progressed within 6 months of adjuvant therapy (prior chemotherapy and/or anti-EGFR therapy is permitted). • In Dose Expansion, patients must have received prior anti-EGFR therapy and achieved at least stable disease on at least one scan as their best response. • In Dose Expansion, patients must be willing to undergo a pre-treatment biopsy, and four research PET imaging techniques (^{11}C-Glutamine and ^{18}F-FSPG), two pre-treatment and two after one cycle of treatment. • Adequate organ function including: — Absolute neutrophil count (ANC) ≥ 1,500/μL — Platelets ≥ 100,000/μL — Serum albumin ≥ 3.0 g/dL — Serum creatinine ≤ 2 mg/dL, or calculated creatinine clearance > 50 mL/min (per the Cockcroft-Gault formula) — Total bilirubin ≤ 1.5 times upper limit of normal (ULN) — Aspartate transaminase (AST) and Alanine Aminotransferase (ALT) ≤ 5.0 x ULN. **Main exclusion criteria:** • Within 28 days before first dose of protocol-indicated treatment: — Anti-cancer treatment including chemotherapy, radiation, hormonal therapy, targeted therapy, immunotherapy, or biological therapy. — Major surgery requiring general anesthesia. (Note: within this time frame, placement of a central line or portacath is acceptable and does not exclude.) — Receipt of an investigational agent. • Within 14 days before first dose of protocol-indicated treatment: — Active uncontrolled infection. Patients with infection under active treatment and controlled with antibiotics initiated at least 14 days prior to initiation of protocol-indicated treatment are not excluded (e.g. urinary tract infection controlled with antibiotics).

<table>
<tr><td></td><td>

For Dose Escalation only: Known Grade 4 toxicity probably or definitely attributed to past irinotecan treatment.
Active inflammatory bowel disease, other bowel disease causing chronic diarrhea (defined as > 4 loose stools per day), or bowel obstruction.
History of interstitial pneumonitis or pulmonary fibrosis, or evidence of interstitial pneumonitis or pulmonary fibrosis on baseline chest CT scan.
Unable to receive oral medication.
CNS metastasis, unless asymptomatic or previously treated and stable; and no evidence of CNS progression for at least 30 days prior to initiating protocol-indicated treatment. Anticonvulsant and/or corticosteroid therapy will be allowed if patient is on a stable or decreasing dose of such treatment for at least 30 days prior to initiating protocol-indicated treatment.

</td></tr>
<tr><td>Overall Trial Design</td><td>
Phase I: Dose-Escalation:
Bayesian continual reassessment method with the following intended dose levels:
<table>
<tr><th>Dose Level</th><th>CB-839
Oral
continuous
BID</th><th>Panitumumab
I.V.
Days 1 and 15</th><th>Irinotecan
I.V.
Days 1 and 15</th></tr>
<tr><td>-1</td><td>400 mg</td><td>6 mg/kg</td><td>180 mg/m^2</td></tr>
<tr><td>1</td><td>600 mg</td><td>6 mg/kg</td><td>180 mg/m^2</td></tr>
<tr><td>2</td><td>800 mg</td><td>6 mg/kg</td><td>180 mg/m^2</td></tr>
</table>
1 cycle = 28 days

Patients will be treated in cohorts of 3. All toxicity outcomes must be observed before calculating the recommended dose level for the next cohort. Dose levels may not be skipped. Up to 12 patients may be enrolled since there are 2 working dose levels and a -1 dose level to be evaluated and a minimum of 6 patients are required to establish the MTD.

Phase II:
Patients enrolled will be treated at the MTD (or recommended phase 2 dose [RP2D]) of CB-839 determined from phase I. Enrollment will use a Simon's optimal 2-stage design to monitor efficacy (best overall response rate). If zero responses are observed in the first 10 patients, we will stop accrual for lack of efficacy. Otherwise we will continue accrual up to 29. If 4 or more patients achieve a response among 29 treated, we reject the null hypothesis ($p<0.05$) of low efficacy (RR ≤ 5%) and declare this regimen sufficiently active in this patient population to warrant further study in more definitive trials.
</td></tr>
<tr><td>Number of Patients</td><td>Approximately 40 total patients: About 9-12 eligible patients are anticipated during phase I, and up to 29 patients in phase II.</td></tr>
</table>

Planned Enrollment Period	Overall enrollment is anticipated to last approximately 24 months.
Study Assessments	See the Schedule of Assessments in Section 6.

2. BACKGROUND AND RATIONALE

2.1. Colorectal Cancer (CRC)

Colorectal cancer (CRC) is the third most common cancer in American men and women. The American Cancer Society estimates that over 134,000 new cases and approximately 49,000 deaths are expected to occur in 2016.[1,2] Most (70%-80%) newly diagnosed patients have localized disease that is amendable to curative resection,[3] where 5-year survival rates range from 93% for those with stage I disease to 44% in those patients with stage IIIC disease.[4] The remaining percentage of patients present with unresectable metastatic disease, where the 5-year survival rate for stage IV disease remains dismal at less than 10%.[4] Additionally, a significant proportion of patients with surgical resection have disease recurrence and eventually develop metastatic disease.[5]

5-fluorouracil (5-FU) based regimens are the standard frontline therapeutic options for those patients requiring systemic therapy, and 5-FU in combination with leucovorin and oxaliplatin or irinotecan has been shown to prolong overall survival.[3] Combining monoclonal antibodies that target the epidermal growth factor receptor (EGFR) with standard frontline therapies have also improved clinical outcomes in patients with RAS wildtype metastatic disease.[6,7] Despite the improvement in clinical benefit, patients will eventually develop resistance and therefore require additional therapeutic options.

2.2. EGFR Monoclonal Antibody Treatment Regimens

Panitumumab is a fully human, EGFR-directed monoclonal antibody that is clinically indicated for the treatment of patients with RAS wildtype metastatic CRC who have progressed on 5-FU based chemotherapy regimens. Panitumumab binds to the extracellular domain of EGFR thus inhibiting the activation of downstream signaling networks. As a monotherapy, panitumumab significantly improved progression-free survival (PFS) in patients with RAS wildtype metastatic CRC despite the fact that response rates were less than 10%.[8]

As a second-line therapy, panitumumab plus FOLFIRI achieved a median PFS of 6.5 months and a response rate of 23% in patients with RAS wildtype metastatic CRC.[9] In wildtype RAS patients that were refractory to standard chemotherapy, panitumumab plus irinotecan achieved an objective response rate of 29.2%.[10] Median progression-free and overall survivals were 5.5 and 9.7 months, respectively.[10] When compared to single agent irinotecan, panitumumab plus irinotecan significantly improved response rate from 12% to 34% in patients who had progressed on 5-FU based regimens; however, no difference in the primary endpoint of overall survival was observed.[11] These data suggest that a large proportion of patients do not respond to EGFR blockade therapies despite having wildtype RAS, and the patients that respond initially will progress in less than 6 months.

2.3. Glutamine Metabolism and Synergy with EGFR Blockade

It is well known that a hallmark of cancer is altered metabolism.[12] The first metabolic anomaly observed in cancer was the Warburg effect, which is characterized by an increase in glycolysis and lactate production regardless of oxygen availability.[13] More recently, alterations in glutamine metabolism have been proposed to play a critical role in cancer cell growth and survival.[14-16]

Mitochondrial glutaminase is the key enzyme in the conversion of glutamine to glutamate and is highly expressed in several cancer cell lines.[17-19] In CRC, Huang *et al*. observed that glutaminase expression was significantly increased in tumors compared to normal colonic tissue.[20] These authors also observed that inhibition of glutaminase suppressed cell growth and induced apoptosis in two human colorectal cell lines, thus suggesting that glutaminase may serve as a target for CRC therapy.

Furthermore, glutaminase is transcriptionally regulated by the proto-oncogene c-Myc,[21,22] which is a downstream effector of the EGFR/Ras/MEK/Erk signaling pathway; therefore, combining a glutaminase inhibitor with EGFR blockade might have a synergistic treatment effect. Indeed, a recent study observed a synergized inhibitory effect of erlotinib, a small molecule inhibitor of EGFR, in combination with a glutaminase inhibitor in human non-small cell lung cancer cells that were resistant to erlotinib.[23]

2.4. Glutaminase Inhibitor, CB-839

CB-839 is a potent and selective reversible inhibitor of glutaminase activity. It is an allosteric and noncompetitive inhibitor of both splice variants of the broadly expressed gene for glutaminase (gene symbol: GLS), but does not inhibit glutaminase-2 (gene symbol: GLS2), which is expressed predominantly in liver. Incubation of recombinant human glutaminase with CB-839 results in time-dependent and slowly reversible inhibition of glutaminase activity (IC50 = 34 nM with 1 hour pre-incubation). In mice bearing human tumors and treated with CB-839, there is a significant increase in tumor concentration of glutamine and a decrease in tumor glutamate and aspartate. Profound reductions in the cellular pools of several tricarboxylic acid (TCA) cycle intermediates, amino acids, glutathione, and other metabolic intermediates downstream of glutaminase have been observed in tumor cells *in vitro* and *in vivo* following CB-839 treatment.[24]

Glutaminase inhibition is associated with antiproliferative activity in a wide range of tumor cell lines. Pro-apoptotic activity has been observed in triple-negative breast cancer (TNBC), clear cell renal cell carcinoma (RCC), KRAS-mutant non-small cell lung cancer (NSCLC), mesothelioma, as well as hematologic tumor cell lines including multiple myeloma (MM) and non-Hodgkin's lymphoma (NHL). Twice-daily administration of CB-839 to immunocompromised mice bearing TNBC, RCC, NSCLC, and MM tumors has been shown to result in a significant reduction in tumor growth.

Synergistic activity in cell lines and additive or synergistic activity *in vivo* have been seen with CB-839 in combination with standard-of-care agents including immunomodulatory (IMiD) compounds in MM, paclitaxel in TNBC, tyrosine kinase and mTor inhibitors in RCC, and erlotinib in NSCLC. Following dosing with CB-839 in mice and rats, there is a dose-dependent suppression of systemic glutaminase activity reflected in an increase in plasma glutamine concentration. CB-839 shows additive or synergistic activity in immunocompetent mice with the immuno-oncology (I-O) antibodies anti-Programmed Death Protein-1 (anti-PD-1) and anti-Programmed Death-Ligand 1 (anti-PD-L1), possibly due to indirect stimulation of T-cell proliferation resulting from accumulation of glutamine in the tumor microenvironment.

2.4.1 Nonclinical Toxicity Studies

The toxicity of repeated oral daily doses of CB-839 was evaluated in rats in a 14-day range-finding and a 28-day GLP toxicity study using doses up to 500 mg/kg/day. A Severely Toxic Dose in 10% of rats (STD10) was not identified. Clinical pathology effects included modest changes in platelets (14-day non-GLP study only), serum electrolytes, decreased serum alkaline phosphatase, decreased prothrombin time, as

well as increased cholesterol and triglycerides – all of which were considered non-adverse and were fully reversible during a 14-day non-dosing recovery period. A modest increase in urine volume with a decrease in specific gravity was considered non-adverse. All of these changes were modest in magnitude but did reach statistical significance. Minimal hepatocellular hypertrophy was observed in high-dose male and female rats; this finding was fully reversible and not considered adverse. None of these findings in rats were found in marmoset monkeys.

The toxicity of repeated twice-daily oral doses of CB-839 was evaluated in marmoset monkeys up to 125 mg BID (250 mg/kg/day). The Highest Non-Severely Toxic Dose (HNSTD) was determined to be ≥ 250 mg/kg/day. Dosing of CB-839 at the mid and high doses produced sporadic but significant elevation in two liver enzymes, γ-glutamyl-transpeptidase (GGT) and glutamate dehydrogenase (GLDH) while other liver function parameters were not impacted (ALT, AST, bilirubin, alkaline phosphatase). The increases in liver enzymes correlated with minimal to slight bile duct hyperplasia in the same animals. This finding was not seen in rats where systemic CB-839 exposure was ~10-fold greater.

2.4.2. Phase 1 Clinical Trials

The safety and tolerability of CB-839, both as a monotherapy and in selected combinations, is currently being studied in three Phase 1 clinical studies, CX-839-001, CX-839-002, and CX-839-003:

- CX-839-001: A Phase 1 study evaluating the safety and tolerability of CB-839 in patients with solid tumors, either as monotherapy or in combination with paclitaxel and everolimus. (A total of 125 patients on CX-839-001 had adverse event data in the clinical database at the time of the available data cut. No patients were enrolled on the erlotinib combination as of the data cut used.)

- CX-839-002: A Phase 1 study evaluating the safety and tolerability of CB-839 in patients with hematological tumors (multiple myeloma and non-Hodgkin's lymphoma), either as monotherapy or in combination with pomalidomide and dexamethasone or with dexamethasone alone. (A total of 24 patients on CX-839-002 had adverse event data in the clinical database at the time of the data cut.)

- CX-839-003: A Phase 1 study evaluating the safety and tolerability of CB-839 in patients with hematological malignancies (acute myelogenous leukemia (AML), acute lymphocytic leukemia (ALL), or myelodysplastic syndrome (MDS) either as monotherapy or in combination with azacitidine. (A total of 31 patients on CX-839-003 had adverse event data in the clinical database at the time of the available data cut.)

As of the cut-off date for the clinical database of January 26, 2016, 147 subjects were treated with CB-839 monotherapy across the three studies.

CB-839 has been evaluated at a range of dose levels when administered three times daily in the fasting state (100-1000 mg TID) and when administered twice daily with food (600-1000 mg BID). Due to the improved pharmacokinetic parameters and safety profile in patients dosed with food, future development will focus on administration twice daily with food.

A total of 73 serious adverse events (SAEs) were recorded as of January 26, 2016.

Four of these events were considered at least possibly related to CB-839 treatment: increased creatinine, increased AST/ALT, seizure, and stomatitis.

To date, two DLT events (elevated creatinine, elevated LFTs) have been reported for the monotherapy. Although a maximum tolerated dose (MTD) has not been defined, 800 mg BID is the highest dose that is confirmed to be safe and well tolerated as monotherapy.

Expansion cohorts have been opened with the 600 mg orally BID with food on the basis of PK and PDn parameters that are consistent with substantial glutaminase inhibition and evidence of LFT elevations at higher doses in the TID cohorts. However, additional dose escalation is ongoing with the BID fed regimen, in order to better understand the safety profile of CB-839 when administered in this way. One expansion cohort has also been opened at the 800 mg BID dose level, which has been shown to be safe and tolerable.

Given the limited number of patients treated to date and the open label, nonrandomized nature of the studies to date, the safety profile of CB-839 has not been fully established. CB-839 has been well tolerated across all dose levels tested to date, with 6 patients (N = 147) discontinuing due to adverse events. The large majority of toxicities that have been observed to date have been Grades 1 and 2 and manageable with supportive care and/or interruption of study drug.

The primary treatment-related toxicities that have been observed to date include fatigue, gastrointestinal events (nausea, vomiting, diarrhea and constipation), photophobia and elevations in liver function tests.

The most frequent drug-related Grade 3/4 toxicity has been reversible elevations in liver function tests (AST, ALT, GGT, and/or total bilirubin) although the frequency of Grade 3/4 LFT elevations is substantially reduced with BID dosing with food as compared to the original TID regimen. The change to dosing CB-839 with food on a BID schedule has resulted in a significant reduction in Grade 3/4 elevations in liver function tests to a rate of ~2%. Treatment-related elevations in LFTs have generally been asymptomatic and rapidly reversible upon holding study treatment.

Photophobia and related ocular toxicities (e.g., photopsia) have been identified as CB-839-related events. In almost all cases, these events have been Grade 1 and generally do not have an impact on the patient's daily life, although one Grade 2 event required the patient rest for 1-2 hr in a dark room. These events appear to occur around Cmax (1-4 hours after dosing) and resolve with time. Anecdotal reports suggest that tachyphylaxis develops and these events tend to become less frequent over extended dosing. More severe cases can be managed with dose reduction and the use of sunglasses may be considered.

A single case of reversible Grade 3 renal insufficiency was observed at the 250 mg TID dose level, characterized by a Grade 3 elevation in creatinine that recurred upon rechallenge at a lower dose. Thirteen other events of elevated creatinine (all Grade 1-2) have been noted in patients receiving monotherapy CB-839 across the three ongoing studies (10%) at doses up to 1000 mg TID.

Two events of Grade 3 mucositis/stomatitis have been observed at the 600 mg BID dose

level, both in patients with AML. Three other Grade 1/2 stomatitis events have occurred with CB-839, two of which were AML patients receiving monotherapy and one event was in an RCC patient receiving CB-839 in combination with everolimus, for which stomatitis is a well-known adverse event. Given the absence of events in patients receiving monotherapy CB-839 in non-AML patients, it is not clear if mucositis is clearly CB-839 related at this time or if it is just associated with the underlying disease (AML) or concomitant medication (everolimus).

Overall, the safety and tolerability profile of CB-839 supports the continued development of CB-839 in patients with advanced solid and hematological malignancies.

2.4.3 Pharmacokinetics and Pharmacodynamics

Systemic exposure to CB-839 following oral administration is highly species dependent because of large cross-species differences in absorption and metabolism. Mice and rats had good oral exposure to CB-839. 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 low to modest likely due to poor absorption. Good oral exposure of CB-839 in humans was observed in most of the patients in the three ongoing CB-839 Phase 1 clinical studies with daily doses of 1.2 g (400 mg TID or 600 mg BID) or higher. Pharmacokinetic parameters were similar when CB-839 was dose 600 mg TID without food or 600 mg BID with food. Therefore, dosing regimen was changed to BID with food in order to improve convenience.

Following oral dosing to mice, CB-839 was widely distributed in a variety of tissues including heart, lung, spleen, muscle, and subcutaneously-implanted tumors; in contrast, brain had 20-fold lower CB-839 concentration than plasma. CB-839 also has high plasma protein binding across multiple species including humans.

In vitro studies indicated that CB-839 is neither an inhibitor of human CYP1A2, CYP2C8, CYP2C19, CYP2D6, and CYP3A4 nor an inducer of human CYP1A2, CYP2B6, and CYP3A4. CB-839 appears to be a moderate inhibitor of human CYP2C9 (~40-50% inhibition at 5 μM). The risk of drug-drug interactions due to inhibition of CYP enzymes appears to be low but concomitant administration of CYP2C9 substrates should be done with caution.

CB-839 is a weak base and, therefore, requires low pH conditions for optimal solubilization. As for other oral agents that are weak bases, concomitant treatment with proton pump inhibitors (PPIs) results in reduced exposure to CB-839. If possible, concomitant administration of CB-839 with PPIs should be avoided. Patients may be switched to histamine H2 receptor antagonists (H2RA) since, based on limited clinical data, they do not appear to have result in a significant reduction of CB-839 exposure. If H2RA therapy is insufficient, investigators are encouraged to reach out to the sponsor-investigator to discuss options.

Inhibition of glutaminase activity has been measured in tumors and in a wide range of tissues following CB-839 dosing. When the plasma concentration is maintained above 300 nM, maximal glutaminase inhibition is achieved and maintained in multiple tumor types. Therefore, in clinical studies our objective is to maintain a CB-839 trough level of > 300 nM in human plasma to ensure continuous maximal inhibition of glutaminase. An analysis of platelet glutaminase demonstrates a clear PK/PD relationship between CB-

839 exposure and glutaminase inhibition, with very strong inhibition at plasma exposures above 300 nM (>90% inhibition in most patients). In addition, clear evidence of glutaminase inhibition was demonstrated in tumors of all patients tested (n=5).

2.4.4 Preliminary Clinical Efficacy

Clinical outcome data for CX-839-001 is derived from the data cut (October 1, 2015) used for the most recent public presentation of the data, which was the AACR/NCI/EORTC Molecular Targets Meeting in November, 2015.

As of October 1, 2015, 50 patients receiving the BID fed regimen (out of a total of 66 enrolled) were evaluable for tumor response. Of those 50 evaluable patients, which included a variety of tumor types [renal cell carcinoma (RCC), triple negative breast cancer (TNBC), non-small cell lung cancer (NSCLC), and a variety of tumors with TCA-cycle alterations], 44% (22 pts) had a best overall response (BOR) of SD or better, including one RCC patient with a confirmed PR. Among the 16 RCC patients enrolled (9 clear cell, 4 papillary and 3 other), 15 patients were evaluable. Nine of the 15 evaluable pts had a BOR of SD or better, and the median time on study was 3.7 months.

Clinical outcome data for CX-839-002 is derived from the data cut (November 9, 2015) used for the most recent public presentation of the data, which was the American Society of Hematology (ASH) in December, 2015.

One heavily pretreated patient with long-standing IgG kappa light chain myeloma receiving CB-839 in combination with pomalidomide and dexamethasone experienced a significant reduction in urine M-spike and Kappa light chain that did not meet the criteria for a PR (urine M-spike reduction of 86% fell short of the 90% required by IMWG criteria).

Clinical outcome data for CX-839-003 is derived from the data cut (November 9, 2015) used for the most recent public presentation of the data, which was the American Society of Hematology (ASH) in December, 2015.

As of November 9, 2015, 2015, 26 patients (24 AML, 1 ALL, 1 mixed lineage leukemia) were enrolled across a variety of dose levels and regimens. Ten patients received CB-839 on the BID with food regimen at the dose of 600 mg. Clear reductions in blast counts were achieved in 2 AML patients receiving the monotherapy. One patient was classified as a Complete Response with incomplete recovery of peripheral counts (CRi) and remains on study after >18 months. A second patient had a dramatic reduction in peripheral blast count (30% down to 3%) and a 33% reduction in bone marrow blast count (21% to 14%) over the first cycle of therapy but was removed from the study due to CNS progression of the disease. Nine patients have remained on treatment for ≥ 2 cycles of dosing without treatment failure. Efficacy data are not available for patients receiving the CB-839/azacitidine combination.

2.5. Rationale for the Combination Therapy

From our preliminary data, we observed synergy between the glutaminase inhibitor CB-839 and anti-EGFR monoclonal antibody therapy *in vitro* and *in vivo*. Compared to single agent exposure, CB-839 administered with cetuximab or panitumumab resulted in significantly decreased viability in multiple human CRC cell lines suggesting the combination regimen is

more efficacious than either agent alone (Figure 1A). In three-dimensional culture, CB-839 in combination with either cetuximab or panitumumab overcame resistance to EGFR monoclonal antibody treatment in a cetuximab-resistant model (Figure 1B). The *in vitro* results were recapitulated *in vivo* (Figure 1C).

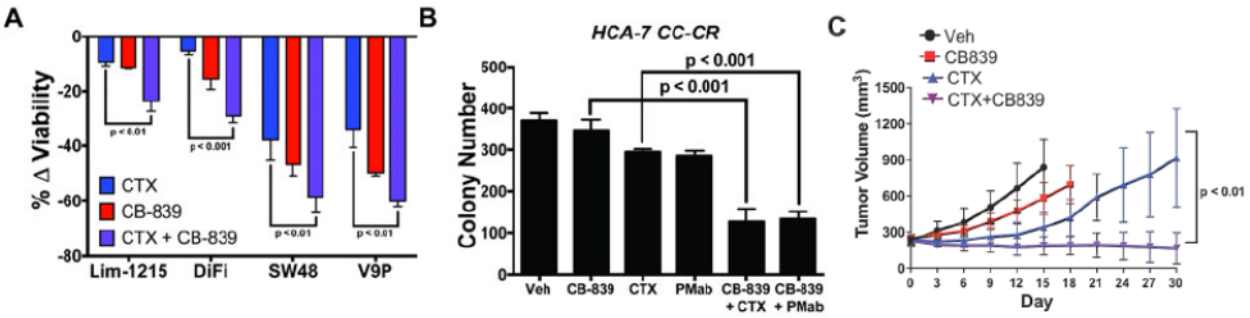


Figure 1. Combined GLS1/EGFR therapy results in cooperative efficacy *in vitro and in vivo*. (**A**) CRC cell lines propagated in 2D culture expressing WT *RAS* exhibit reduced viability with combined therapy compared to single agents relative to vehicle control. (**B**) Combination therapy using CTX or PMab results in decreased colonies in CTX-resistant 3D culture model (HCA-7 CC-CR). CTX = cetuximab; PMab = panitumumab. (**C**) *In vitro* results are recapitulated in mouse xenografts of the human CRC cell line SW48. Error bars represent ± std. dev

Based on the data presented above, we propose a Phase 1/2 clinical trial combining the glutaminase inhibitor CB-839 with an EGFR monoclonal antibody (i.e., panitumumab) in patients with metastatic and refractory *RAS* wildtype CRC. We note that our preliminary data also evaluated the EGFR monoclonal antibody cetuximab. However, given that panitumumab and cetuximab have the same efficacy in CRC[25], we will use panitumumab for this trial since cetuximab has an increased potential of inducing infusion-related reactions in the southeast.[26,27]

The primary objective of the phase I portion is to determine the maximum tolerated dose (MTD) of CB-839 in combination with panitumumab and irinotecan. The inclusion of irinotecan in dose escalation allows for the collection of toxicity data of the triplet combination, as this treatment regimen could go forward in clinical development. However, the hypothesis-driven (i.e., enhanced glutamine metabolism contributes to anti-EGFR therapy resistance) phase II will not include irinotecan. The primary objective of phase II is to evaluate the objective response rate. Secondary objectives include the characterization of potential noninvasive imaging and tissue- and blood-based biomarkers and their association with clinical benefit, as well as further defining the safety profile.

2.6. Rationale for Correlative Science

2.6.1 Rationale for ^{18}F-FSPG PET/CT

The development of noninvasive imaging techniques that can provide surrogate biomarkers of tumor response is an active area of research. Additionally, it is becoming significantly important to prospectively identify those patients that would derive clinical benefit from targeted therapies. A leading PET tracer that provides a surrogate measurement of glutamine metabolism under active investigation at the Vanderbilt Center for Molecular Probes (CMP) is (*S*)-4-(3-[18F]Fluoropropyl)-L-glutamic acid (^{18}F-FSPG,

originally BAY 94-9392, Piramal Imaging GmbH, Berlin, Germany) (Figure 2).

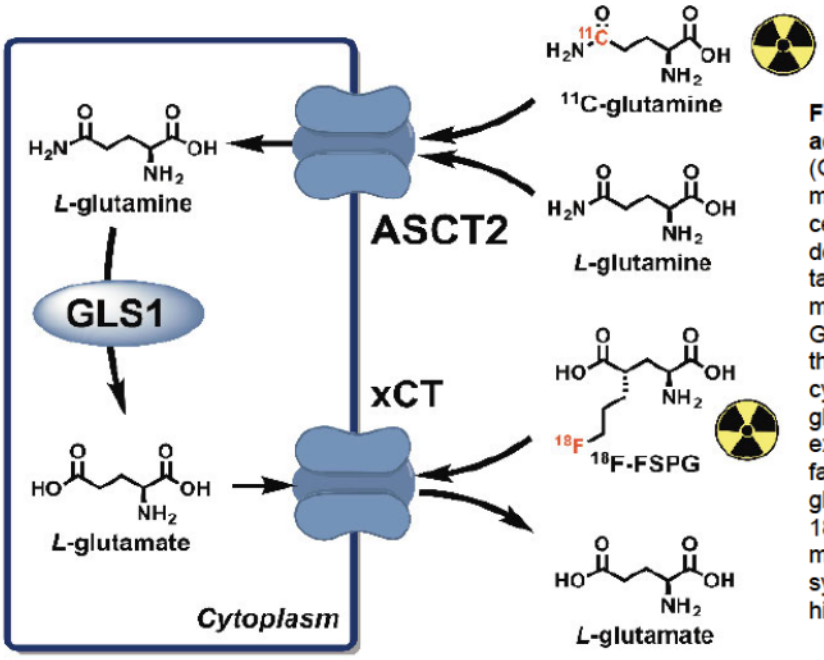


Figure 2. ^{11}C-Glutamine and ^{18}F-FSPG accumulation in cancer cells. Glutamine (Gln) and glutamate (Glu) are major metabolic and anabolic substrates of tumor cells, in parallel or even in lieu of, glucose-derived metabolites like pyruvate. Gln is taken up via the ASCT2 transporter and metabolized to Glu through the activity of GLS enzyme. Glu can then be shuttled to the TCA cycle or exported in exchange for cystine. The system xCT transporter, a glutamate-cystine exchanger, exports Glu in exchange for cystine in a rate-limiting fashion, thus providing precursors for glutathione (GSH) biosynthesis. Uniquely, 18F-FSPG, which functions as a cystine mimetic, is specifically taken up via the system xCT transporter, which is expressed highly in CRC.

^{18}F-FSPG is a glutamic acid that measures x_C- transporter activity.[28,29] Similar to cystine, extracellular ^{18}F-FSPG is exchanged through the x_C- transporter for intracellular glutamate and accumulates inside the cell (Figure 2). Therefore, if the intracellular concentration of glutamate is high, ^{18}F-FSPG uptake will also be high. Conversely, if the intracellular concentration of glutamate is decreased by, for example, a glutaminase inhibitor that inhibits the conversion of glutamine to glutamate, ^{18}F-FSPG uptake will also decrease.

All patients must consent to undergo two (one pre- and one post-therapy) ^{18}F-FSPG PET/CT imaging sessions. Radiologic features from each research PET imaging session will be prospectively correlated with clinical benefit, and could provide a longitudinal pharmacodynamic measurement of CB-839 activity *in vivo*. Additionally, we foresee this agent will be clinically useful to identify patients that are exceptional responders to treatments designed to alter glutamine metabolism.

2.6.2 Rationale for ^{11}C-Glutamine PET/CT

Another leading PET tracer that provides a surrogate measurement of glutamine metabolism under active investigation at the Vanderbilt Center for Molecular Probes (CMP) is carbon-11-labeled glutamine (^{11}C-Glutamine; Figure 2). To reflect the uptake of glutamine in tumors, we will utilize ^{11}C-Glutamine. This tracer is chemically and biologically identical to naturally occurring glutamine; *via* PET imaging, ^{11}C-Glutamine will enable quantitative assessment of glutamine flux in tumors. Imaging with ^{11}C-Glutamine will represent the first-in-human study of this tracer.

Patients that consent to ^{11}C-Glutamine will be continuously monitored for the safety,

biodistribution and metabolism characteristics of ^{11}C-Glutamine through the course of the study and it is anticipated that, after approximately 15 patients have received ^{11}C-Glutamine, we will have collected sufficient data to pursue an IND submission.

2.6.3 Tissue-Derived, Glutamine Metabolism Gene Signature

A pre-treatment research biopsy will be collected for qRT-PCR and RNA-Seq analyses of genes that are associated with glutamine metabolism. We anticipate that responders to combined CB-839/panitumumab therapy will be those patients whose glutamate content is glutamine-dependent. However, multiple redundant, glutamine-independent mechanisms exist within cancer cells to maintain intracellular glutamate levels. Therefore, we will carry out qRT-PCR and RNA-Seq on tumor biopsies collected at baseline. We anticipate that these data will provide mechanistic insight into patient responses observed in this trial. Our hypothesis is that patients whose tumors exhibit enhanced expression of non-glutamine-derived glutamate metabolism genes will exhibit poor response to CB-839.

2.6.4 Radiotracer Pharmacokinetic Modeling

A kinetic analysis of the dynamic time course of the PET radiotracer uptake can be used to quantify vascular delivery of the tracer to a specific tissue from its metabolic retention in the tissue. This facilitates a better understanding of the information contained in the PET imaging study and can lead to enhanced image-derived variables for consideration of disease diagnosis and prognosis.

These dynamic time courses can be fit to mathematical models in order to quantify specific parameters, called rate constants, that relate to perfusion of the tracer into a tissue, retention and/or metabolism of the tracer, and extravasation from the tissue back to plasma. These rates constants provide more information about the tracer kinetics

2.6.5 Circulating Exosomes

The investigation of the architecture and function of extracellular vesicles (EVs) is a rapidly growing field of interest. EVs have been shown to have an effect on a variety of cellular processes, including immune signaling, angiogenesis, and detoxification of bacterial products.[30-32] Recent data (summarized in a review article by Shifrin *et al.*[33]) suggests that EVs released from a "donor" cell contribute to normal physiological functions, such as developmental processes and normal morphogenesis, immune responses, bone formation, and cardiovascular and neuronal system function. A key research finding that first linked EVs to cancer was the discovery that membrane vesicles from metastatic cells induced lung metastasis.[34] Although the specific differences between EVs released from normal and cancer cells are not fully understood, recent research suggests that EVs facilitate horizontal transfer of cargo that can stimulate intracellular signaling pathways thus promoting tumor progression.[35-37]

The role of exosomes in the progression of CRC is an increasing area of research. Wang *et al.*, observed that exosomes secreted from a highly metastatic cell line increased the metastatic tumor burden and distribution of a CRC line that typically exhibits poor liver metastatic potential.[38] A study from Ragusa *et al.*, investigated changes in the cargo of exosomes during therapy, and observed that cetuximab significantly altered the cargo of cetuximab-responsive cells suggesting a cetuximab-linked effect.[39]

We have shown that exosomes purified from the conditioned medium of mutant KRAS

CRC cells contained higher levels of the EGFR ligand amphiregulin (AREG), and these exosomes increased the invasion potential of recipient cells.[40] Our analysis also detected levels of several other EGFR ligands, including heparin-binding EGF-like growth factor, and TGFα. Additionally, we have shown that exosomes can transfer mutant *KRAS* to cells with wild-type *KRAS* and that these mutant *KRAS*-containing exosomes enhanced wild-type *KRAS* cell growth.[37]

All patients enrolled on the Phase II portion of the study must consent to have three pharmacodynamic blood samples collected at pre-treatment, after one cycle of treatment, and at the end of therapy. As an exploratory objective, the concentration of specific molecular content (e.g., EGFR, AREG) found within exosomes will be correlated with clinical outcome.

3. OBJECTIVES AND ENDPOINTS

3.1. Objectives

Primary Objective of Phase I:

- Determine the safety and tolerability of CB-839 in combination with panitumumab and irinotecan.

Exploratory Objective of Phase I (Optional Imaging Sub-study):

- Correlate radiological features of pre- and post-treatment ^{11}C-Glutamine PET/CT and ^{18}F-FSPG PET/CT with clinical outcome.

Primary Objective of Phase II:

- Determine the efficacy of CB-839 in combination with panitumumab as measured by the response rate (RR) in patients with previously EGFR treated *RAS* wildtype colorectal adenocarcinoma.

Secondary Objectives of Phase II:

- Determine the disease control rate (DCR), progression-free survival (PFS), and overall survival (OS).
- Perform the following correlative studies (in the Phase II component):
    - Correlate radiological features of pre- and post-treatment ^{18}F-FSPG PET/CT with clinical outcome and biological correlates (tissue gene signature, exosomes)
    - Collect blood samples during each radiotracer injection to assess pharmacokinetics
    - Collect pre-treatment biopsy tissue and prospectively correlate clinical outcome with a glutamine metabolism gene signature
    - Quantify exosomal content in the plasma.

Exploratory Objective of Phase II:

- Development of patient-derived organoids from pre-treatment tissue biopsy

3.2. Endpoints

Primary Endpoint of Phase I:

- Maximum Tolerated Dose (MTD) and/or recommended phase 2 dose (RP2D) of CB-839 in combination with standard doses of panitumumab and irinotecan.

Exploratory Endpoint for Phase I (Optional Imaging Sub-study):

- Maximum Standardized Uptake Value (SUV_{max}) of ^{11}C-Glutamine and ^{18}F-FSPG uptake at pre-treatment and after one cycle of treatment.

Primary Endpoint of Phase II:

- Response Rate (RR).

Secondary Endpoints of Phase II:

- Disease control rate (DCR), Progression-free Survival (PFS), and Overall Survival (OS).
- Maximum Standardized Uptake Value (SUV_{max}) of ^{18}F-FSPG uptake at pre-treatment and after one cycle of treatment.
- Glutamine metabolism gene signature using qRT-PCR and RNA-Seq.
- Radiotracer pharmacokinetic modeling parameters.
- Plasma exosomal content at pre-treatment, after one cycle of treatment, and at disease progression.

Exploratory Endpoint for Phase II:

- Development of patient-derived organoid models

4. PATIENT SELECTION

Questions regarding patient eligibility must be addressed and resolved by the investigator in consultation with the sponsor-investigator or designee prior to enrollment.

4.1. Inclusion Criteria

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

1. Signed and dated written informed consent.
2. Male or female ≥ 18 years of age.
3. Histologically or cytologically-confirmed diagnosis of metastatic *KRAS* wildtype colorectal cancer (CRC).
4. Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1.
5. In Dose Escalation, patients must have had at least one prior line of chemotherapy for advanced disease or progressed within 6 months of adjuvant therapy (prior chemotherapy and/or anti-EGFR therapy is permitted).

6. In Dose Expansion, patients must have received prior anti-EGFR therapy and achieved at least stable disease on at least one scan as their best response.
7. In Dose Expansion, patients must be willing to undergo a pre-treatment biopsy, and two research PET imaging techniques (^{18}F-FSPG), one at pre-treatment and one after one cycle of treatment.
8. In Dose Expansion, at least one measurable lesion as defined by RECIST 1.1 which can be followed by CT or MRI.
9. Adequate organ function including:
    - Absolute neutrophil count (ANC) ≥ 1,500/μL
    - Platelets ≥ 100,000/μL
    - Serum albumin ≥ 3.0 g/dL
    - Serum creatinine ≤ 2 mg/dL, or calculated creatinine clearance > 50 mL/min (per the Cockcroft-Gault formula)
    - Total bilirubin ≤ 1.5 times upper limit of normal (ULN)
    - Aspartate transaminase (AST) and Alanine Aminotransferase (ALT) ≤ 5.0 x ULN.
10. Women of childbearing potential (WOCBP) must have a negative serum pregnancy test within 14 days prior to receiving first dose of protocol-indicated treatment; and additionally agree to use at least 2 methods of acceptable contraception (Appendix 4) or abstain from heterosexual intercourse from the time of signing consent, and until 2 months after patient's last dose of protocol-indicated treatment.

    WOCBP of childbearing potential are defined as those not surgically sterile or not post-menopausal (i.e. if a female patient has *not* had a bilateral tubal ligation, a bilateral oophorectomy, or a complete hysterectomy; or has *not* been amenorrheic for 12 months in the absence of an alternative medical cause, then patient will be considered a female of childbearing potential). Postmenopausal status in females under 55 years of age should be confirmed with a serum follicle-stimulating hormone (FSH) level within laboratory reference range for postmenopausal women.
11. Men able to father children who are sexually active with WOCBP must agree to use at least 2 methods of acceptable contraception (Appendix 4) from the time of signing consent and until 2 months after patient's last dose of protocol-indicated treatment.

    Men able to father children are defined as those who are not surgically sterile (i.e. patient has not had a vasectomy).

4.2. Exclusion Criteria

Patients meeting any of the following criteria will <u>not</u> be permitted to enter the trial:

1. Within <u>28 days</u> before first dose of protocol-indicated treatment:
    - Anti-cancer treatment including chemotherapy, radiation, hormonal therapy, targeted therapy, immunotherapy, or biological therapy.
    - Major surgery requiring general anesthesia. (Note: within this time frame, placement of a central line or portacath is acceptable and does not exclude.)
    - Receipt of an investigational agent.

2. Within 14 days before first dose of protocol-indicated treatment:
   - Active uncontrolled infection. Patients with infection under active treatment and controlled with antibiotics initiated at least 14 days prior to initiation of protocol-indicated treatment are not excluded (e.g. urinary tract infection controlled with antibiotics).

3. *Dose Escalation Only.* Known Grade 4 toxicity probably or definitely attributed to past irinotecan treatment.

4. Active inflammatory bowel disease, other bowel disease causing chronic diarrhea (defined as > 4 loose stools per day), or bowel obstruction.

5. History of interstitial pneumonitis or pulmonary fibrosis, or evidence of interstitial pneumonitis or pulmonary fibrosis on baseline chest CT scan.

6. Unable to receive oral medication.

7. CNS metastasis, unless asymptomatic or previously treated and stable; and no evidence of CNS progression for at least 30 days prior to initiating protocol-indicated treatment. Anticonvulsant and/or corticosteroid therapy will be allowed if patient is on a stable or decreasing dose of such treatment for at least 30 days prior to initiating protocol-indicated treatment.

8. Patient is pregnant or breastfeeding.

9. Current or previous malignant disease (other than colorectal cancer) within the last 5 years; with the exception of the following if considered curatively treated: non-melanoma skin cancer(s), carcinoma in situ of the cervix, and ductal carcinoma in situ. Subjects with another active malignancy requiring concurrent anti-cancer intervention are excluded. (Note the following does not exclude: effectively treated malignancy that has been in remission for more than 5 years and is considered to be cured AND no additional anti-cancer therapy is ongoing and required during the study period.)

10. Known positive test for Human Immunodeficiency Virus (HIV), Acquired Immunodeficiency Syndrome (AIDS), Hepatitis A, Hepatitis B, Hepatitis C, or Cytomegalovirus (CMV).

11. Known psychiatric condition, social circumstance, or other medical condition reasonably judged by the patient's study physician to unacceptably increase the risk of study participation; or to prohibit the understanding or rendering of informed consent or anticipated compliance with scheduled visits, treatment schedule, laboratory tests and other study requirements.

4.3. Inclusion of Underrepresented Populations

Women and men of all races and ethnic groups are eligible for this trial. There is no bias towards gender, age, or race in the clinical trial outlined.

4.4. Number of Patients and Replacement of Patients Who Discontinue Early

Approximately 40 patients (about 6-12 in Dose Escalation and up to 29 in Dose Expansion) evaluable for response are anticipated to enroll in this study at Vanderbilt University Medical Center.
In general, it is intended that patients will be treated until confirmed disease progression or intolerable toxicity. The criteria for patient discontinuation are listed in Section 10.

If a patient discontinues study treatment for reasons clearly not related to study treatment, after completing less than 75% of scheduled oral doses of CB-839 and/or less than 2 planned infusions of panitumumab and 2 planned infusions of irinotecan (in phase I) over the first 28 days after initiating protocol-indicated treatment on Cycle 1, Day 1, then that patient will be considered not evaluable for DLT (phase I) or response (phase II) to study treatment and may be replaced with a new patient.

4.5. Optional Imaging Sub-Study for Phase I (Maximum of 9 patients)

Patients accrued to the Phase I Dose Escalation portion of this study may optionally participate in an imaging sub-study. Patients must sign the optional consent for participation in this sub-study and be willing to undergo four research PET imaging techniques (11C-Glutamine and 18F-FSPG), two pre-treatment and two after one cycle of treatment. Please refer to Table 6.1 for details regarding the timing of relevant assessments.

5. ENROLLMENT PROCEDURES

5.1. Guidelines for VICC

The Vanderbilt-Ingram Cancer Center (VICC) Coordinating Center will coordinate enrollment in the study.

Prior to registration, a copy of the IRB approval at the site will be kept on file at the Vanderbilt-Ingram Cancer Center (VICC) Coordinating Center. Eligible participants will be entered on study centrally at the VICC Coordinating Center. All sites should email the Coordinating Center at [redacted] to verify slot availability prior to enrollment.

All patients MUST be registered with the Vanderbilt-Ingram Cancer Center (VICC) prior to the start of study procedures such as tissue acquisition. Registration can only be conducted during the business hours of 8AM – 5PM Central Time, Monday through Friday.

1) All sites must email the VICC CTSR Coordinating Center at [redacted] to notify of upcoming registration and ensure slot availability. The following information should be included in this email:

    - Study number.
    - Patient initials.
    - Disease type.

- Anticipated consent date.
- Anticipated start date.

2) If a subject ID number is required prior to patient enrollment (i.e. at screening due to sample collection requirement), the site must submit the following documents with their email notification to the Coordinating Center:

    - Copy of the patient's signed and dated Informed Consent including documentation of the consent process.
    - HIPAA authorization form (if separate from the main consent form).
    - VICC Patient Enrollment Form.

    The Coordinating Center will then provide a subject ID number via email.

3) Email the following documents to the Coordinating Center for eligibility review and patient enrollment [redacted]

    - Copy of the patient's signed and dated Informed Consent, including documentation of the consent process.
    - HIPAA authorization form (if separate from the main consent form).
    - VICC Patient Enrollment Form.
    - Eligibility supporting documents such as pathology reports, laboratory tests, etc. *or* EMR access. Note: all source documents should be de-identified and screening/subject ID number added prior to sending.
    - Tissue Block Registration Form
    - Signed and completed Eligibility Checklist. **To be eligible for registration to the study, the participant must meet each inclusion and exclusion criterion listed in the eligibility checklist.**

Note: VICC Coordinating Center requires 3 business days to review all documents and confirm eligibility. registrations will only be accepted with prior notice and discussion with the Lead Institution. Please email the CTO if enrollment is needed sooner at [redacted].

Upon satisfactory review of eligibility documents submitted, the Coordinating Center will approve enrollment and issue a subject ID number if one was not issued at screening. Once registration/enrollment confirmation from Coordinating Center is received, proceed with protocol procedures.

Please contact the assigned Study Contact with any questions regarding this process. You can also reach out to your assigned CRA once the study is activated.

The VICC Coordinating Center will assign Subject ID numbers to all patients whose eligibility has been confirmed. Only patients deemed eligible will be registered to the study. Sequence/study ID numbers will not be re-used if a patient screen fails. Following registration, eligible participants should begin the study consistent with the protocol no later than 28 days after registration/enrollment by the VICC Coordinating Center. If a participant does not begin the study following registration within the allowed time period, the participant's registration on the study will be canceled. The Study Contact will be notified of cancellations as soon as possible. Patients being re-screened will need to consent to repeated procedures. As such, the Coordinating Center will require a new, signed Informed Consent document.

Issues that would cause treatment delays should be discussed with the sponsor-investigator.

As is generally accepted, standard of care procedures performed prior to consent, but within the protocol defined screening window for each assessment, can be used for study purposes. All research-only procedures must be performed after patient consent.

5.2. Screen-Failures

A patient found not eligible for the trial after giving informed consent is considered a screening-failure. The enrollment form must be completed and sent to the sponsor-investigator or designee to confirm the outcome of the screening process.

Re-screening of a patient is allowed. A new enrollment packet would need to be submitted for such cases.

6. SCHEDULE OF ASSESSMENTS

6.1. Study Calendar

Protocol Activities *1 Cycle = 4 weeks (28 days)*	Screening (Day -14 to Day -1)	CYCLE 1			CYCLES ≥ 2		Post-Treatment	
		Day 1	Day 15	Day 28[22]	Day 1 (± 1 day)[14]	Day 15 (± 1 day)[14]	EOT (≤ 14d after last treatment)[15]	Follow-Up (28d + 7d after last study dose)[17]
Assessments & Observations								
Consent, Baseline Characteristics & Eligibility[1]	X							
Physical and Skin Examination[2]	X	X[9]	X		X	X	X	X
ECOG Performance Status	X	X	X		X	X	X	X
Vital Signs	X	X	X		X	X	X	X
Height	X							
Weight[3]	X	X[9]	X		X	X	X	X
Review of Con Meds and Adverse Events[4]	X	X	X		X	X	X	X
Study Drug Compliance (Dosing Diary)		X	X	X	X		X	
DLT Evaluation (dose-escalation only)[5]		X	X	X	X			
Laboratory Assessments								
Hematology and Blood Chemistry[6]	X	X[9]	X		X	X	X	X
Urinalysis	X							
Pregnancy Test[7]	X	X		X			X	
Disease Assessments								
CT or MRI Scan[8]	X				X		X[16]	X[16]
^{11}C-Glutamine PET/CT (Optional in Phase I only; not performed in Phase II)[20]	X[19]			X[13]				
^{18}F-FSPG PET/CT (Optional in Phase I)[20]	X[19]			X[13]				
Survival Follow-Up								X[18]
Biospecimen Collections								
Research Tumor Biopsy (Phase II Only)	X							
Pharmacokinetic (PK) Blood (Optional in Phase I)[10]	X			X				
Pharmacodynamic (PD) Blood (Phase II Only)[10]		X		X			X	
Treatment								
CB-839 oral administration[21]		Continuous oral twice daily dosing[23]						
Panitumumab infusion[11]		X	X		X	X		
Irinotecan infusion (Phase I Only)[12]		X	X		X	X		

Notes:

1. Informed consent must be obtained before any study-specific screening assessments are performed. Screening assessments are to be performed within 14 days prior to Day 1 of Cycle 1 unless otherwise noted (e.g. baseline CT or MRI scan acceptable ≤ 28 days prior to initiating protocol-indicated treatment). Assessments performed as standard of care within the screening window may be used for screening. Baseline characteristics include but are not limited to: demographics, medical and surgical history, extent of disease, prior anti-cancer treatment, tumor histology, and KRAS tumor mutation status.

2. A comprehensive physical examination will be done at Screening; and at the End-of-Treatment or 28-day Follow-Up visit. At other visits, a targeted physical examination is acceptable (including assessment of lungs, heart, abdomen, skin, and any other body areas deemed appropriate by the clinician) unless a comprehensive physical examination is clinically indicated. Skin will be examined regularly to monitor for possible dermatologic toxicity to panitumumab. A guided skin evaluation by the patient's study oncologist or qualified designee (i.e. physician assistant or nurse practitioner) is acceptable. A comprehensive dermatologic examination should be performed if clinically indicated, and an appropriate clinical expert such as a dermatologist may be consulted as deemed necessary by the patient's study physician.

3. Weight at Screening; prior to treatment infusion (scheduled for Days 1 and 15 of each cycle); and at EOT and Follow-up visits. (Note: On Cycle 1, Day 1, body weight need not be repeated if already completed ≤ 7 calendar days prior to first dose of protocol-indicated treatment.)

4. Review and capture of all concomitant medications will be performed as indicated. Concomitant medications include prescription medications and over-the-counter preparations used by a patient within at least 14 days prior to first dose of protocol-defined treatment and continuing through at least the 28-day Follow-Up study visit. After signing informed consent, adverse events will be collected as detailed in protocol Section 12. All adverse events will be followed from initiation of study treatment for Phase I patients. For Phase II patients, adverse events will be followed from the time of the first research scan. Adverse events for all patients in both phases will be followed through at least 28 days after a patient's final protocol-directed treatment with CB-839 + panitumumab ± irinotecan (whichever occurs last) or until initiation of another anti-cancer therapy – whichever occurs first.

5. For dose-escalation only: Dedicated surveillance and expedited reporting of Dose Limiting Toxicity (DLT) are required for 28 days after initiating protocol-indicated treatment. On Cycle 2, Day 1, satisfactory completion of the physical exam and the safety labs is required to help detect evidence of past or present DLT as having occurred during the first 28 days of protocol-indicated treatment. If intolerable delayed toxicity attributable (per sponsor-investigator judgement) to protocol treatment is detected past Day 28, then the 28-Day DLT window may possibly be extended. See protocol Section 10.1 for definition of DLTs.

6. Hematology includes white blood cell count with differential, hemoglobin, hematocrit, and platelet count. Blood Chemistry to include sodium, potassium, chloride, bicarbonate or carbon dioxide, blood urea nitrogen (BUN), creatinine, glucose, total bilirubin, AST, ALT, alkaline phosphatase (ALP), calcium, albumin, magnesium, phosphorus and total protein.

7. For females of childbearing potential: Serum pregnancy test required during Screening; and either serum or urine pregnancy test required at End-of-Treatment. Females of childbearing potential are defined as those not surgically sterile or not post-menopausal (i.e. if a female patient has *not* had a bilateral tubal ligation, a bilateral oophorectomy, or a complete hysterectomy; or has *not* been amenorrheic for 12 months in the absence of an alternative medical cause, then patient will be considered a female of childbearing potential). Postmenopausal status in females under 55 years of age should be confirmed with a serum follicle-stimulating hormone (FSH) level within laboratory reference range for postmenopausal women (if a patient's postmenopausal status is considered for childbearing potential and study-required contraception). A serum pregnancy test is also required within 24 hours of each research PET/CT visit.

8. Baseline evaluation of disease status by CT or MRI within 28 days prior to initiating protocol-indicated treatment on Cycle 1, Day 1. Baseline and subsequent scans to include imaging of the chest, abdomen and pelvis. *Re-scanning to occur every 8 weeks* (± 7 days) after initiating study treatment on Cycle 1, Day 1. The ± 7 day scan window is intended to facilitate flexibility in scheduling re-scans *during Week 4 of each EVEN cycle* (i.e. Cycles 2, 4, 6, etc.), prior to scheduled infusion of panitumumab/irinotecan on Day 1 of every new ODD cycle (i.e. Cycles 3, 5, 7, etc.). Scanning on the same day as infusion with panitumumab/irinotecan is allowed, provided scan results receive appropriate RECIST review prior to initiating panitumumab/irinotecan in a new cycle of study treatment (e.g. first re-scan is discouraged, but permitted on Cycle 3, Day 1, prior to panitumumab/irinotecan infusion later that same day).

9. On Cycle 1, Day 1: The physical exam need not be repeated if already completed ≤ 7 calendar days prior to first dose of protocol-indicated treatment; and safety laboratories (i.e. Hematology and Blood Chemistry) need not be repeated if already completed ≤ 3 calendar days prior to first dose of protocol-indicated treatment.

10. Pharmacokinetic blood samples for research will be collected during each radiotracer injection (^{18}F-FSPG and ^{11}C-Glutamine (if applicable)) in order to perform pharmacokinetic modeling (only for those patients enrolled in the optional Phase I imaging sub-study and all patients enrolled in Phase II). Details regarding PK collection and handling are provided in the lab manual. Pharmacodynamic (PD) blood samples for research to be obtained on Cycle 1, Day 1 (collected once prior to initiating protocol-indicated treatment) for all patients enrolled in Phase II; Pre-Dose on Cycle 1, Day 28 (or alternatively and ideally obtained during Week 4 of Cycle 1, on same-day as ^{18}F-FSPG PET/CT scan, prior to morning oral dose of CB-839); and at End-of-Treatment.

11. All patients are scheduled to receive panitumumab as a 60 minute (if dose ≤ 1000 mg) or 90 minute (if dose > 1000 mg) intravenous infusion on Day 1 and Day 15 of each 28 day cycle. (Note: If an initial 60 minute infusion is tolerated, subsequent panitumumab infusions of ≤ 1000 mg may be administered over 30 to 60 minutes.) The site will obtain panitumumab as standard of care from commercial supply.

12. All patients in dose escalation are scheduled to receive irinotecan as a 90-minute intravenous infusion on Day 1 and Day 15 of each 28 day cycle. The site will obtain irinotecan as standard of care from commercial supply. Note: Panitumumab and irinotecan are to be administered as separate infusions. On days when both drugs are administered (i.e. scheduled for Days 1 and 15 of each cycle), the panitumumab infusion will be completed BEFORE initiation of the irinotecan infusion.

13. ^{11}C-Glutamine PET/CT and ^{18}F-FSPG PET/CT scan for patients enrolled in optional imaging sub-study for Phase I; ^{18}F-FSPG PET/CT is required for all patients enrolled to Phase II. Ideally completed on Cycle 1, Day 28, but for purpose of scheduling flexibility: Scans can be completed anytime during Week 4 of Cycle 1 (i.e. during Days 22-28 of Cycle 1), or alternatively as late as prior to administration of any protocol-directed treatment on Day 29 (i.e. prior to initiating intended treatment on Cycle 2, Day 1).

14. Beginning with Cycle 2, Day 15: In order to accommodate scheduling, visits and procedures may occur with flexibility of ± 1 day.

15. Reasonable effort should be made to complete End-of-Treatment (EOT) procedures on the day it is decided a patient will no longer receive protocol-indicated treatment. These procedures must be completed subsequent to and not later than 14 days after investigator decision to permanently discontinue protocol-indicated treatment with CB-839 + panitumumab ± irinotecan (whichever treatment occurs last) and prior to any subsequent anti-cancer therapy.

16. At End-of-Treatment, CT/MRI required only if the previous CT/MRI was done > 28 days before. At the 28 day Follow-Up visit, CT/MRI scan required only if disease progression not already documented by CT/MRI done at or before the prior EOT visit. If a patient discontinues the study for reason other than progressive disease confirmed by CT or MRI (e.g. adverse event), then CT or MRI scans of the chest, abdomen and pelvis must be continued every 8 weeks (± 7 days) until disease progression is confirmed by imaging.

17. A Follow-up clinic visit is to be completed 28 days (+7 days) after patient's final protocol-indicated treatment with CB-839, panitumumab, or irinotecan (whichever occurs last). Documented attempt(s) should be made for patient return to the study clinic. It will not be considered a protocol deviation if the patient is physically unable to return for the follow-up visit; such circumstance should be recorded in the study documents, and as much of the follow-up information as possible should be obtained via feasible patient contact and from local and outside facilities.

18. Each patient will be followed for survival every 3 months (± 14 days) after patient's final protocol-indicated treatment with CB-839, panitumumab, or irinotecan (whichever occurs last) until death, end of the study, until patient withdraws consent, or for a maximum of 1 year after the patient's final protocol-indicated treatment – whichever comes first. Survival contact can be made via clinic visit, chart review, obituary or similar observation (e.g. Social Security death index), or by telephone.

19. The screening ^{18}F-FSPG PET/CT scan should be completed within 7 days of C1D1.

20. Patients will be contacted by phone to determine whether they experienced any adverse events within the 24 hours following the each research PET exam. Patients will be contacted no sooner than 24 hours after and no later than 48 hours following the time of tracer injection.

21. If scans are performed on day 1 of the cycle, patients should be instructed not to take their morning dose of CB-839 until after the scans are performed. For CT/MRI scans evaluating disease status, this would apply to scans performed on Day 1 of each odd cycle prior to initiating study treatment. For research PET/CTs, this would apply to scans performed pre-dose on Cycle 2 Day 1. Please see footnotes 8 and 13 for more details regarding the timing of these scans.
22. For patients enrolled in the Phase I portion of the study, C1D28 and C2D1 may be the same visit.
23. On Day 1 of each cycle, subjects may only take a single evening dose of CB-839 if drug is not dispensed in time for the morning dose.

6.2. Screening Visit Assessments

Prior to performing any study-based procedures, patient informed consent must be obtained.

The following procedures must be completed ≤ 28 days prior to a patient's first dose of protocol-indicated treatment:

- Baseline evaluation of disease status by CT or MRI to include imaging of the chest, abdomen and pelvis

The following procedures must be completed ≤ 14 days prior to a patient's first dose of protocol-indicated treatment:

- Medical history and Demographics
- Comprehensive Physical exam, including assessment of the skin, conducted by a physician or qualified designee (i.e. physician assistant or nurse practitioner)
- ECOG Performance Status
- Height
- Weight
- Concomitant medication (prescription and over-the-counter drugs taken at least 14 days prior to intended Cycle 1, Day 1 dosing) and Adverse Event review
- Complete Blood Count (CBC) with differential (including white blood cell count with differential, hemoglobin, hematocrit, and platelet count)
- Blood Chemistry (including sodium, potassium, chloride, bicarbonate or carbon dioxide, blood urea nitrogen (BUN), creatinine, glucose, total bilirubin, AST, ALT, alkaline phosphatase (ALP), calcium, albumin, magnesium, phosphorus and total protein
- Macroscopic urinalysis (microscopic analysis only if clinically indicated)
- Serum Pregnancy test in women of childbearing potential (as defined in Sections 6.1 and 7.9)
- An image-guided pre-treatment tissue biopsy will be collected for biomarker research for all patients enrolled in Phase II

The following procedures must be completed ≤ 7 days prior to a patient's first dose of protocol-indicated treatment:

- ^{18}F-FSPG PET/CT scan (only for those patients enrolled in the optional Phase I imaging sub-study and all patients enrolled in Phase II)
- ^{11}C-Glutamine PET/CT scan (only for those patients enrolled in the optional Phase I imaging sub-study)
- Pharmacokinetic (PK) blood samples will be collected during ^{18}F-FSPG PET/CT scans (only for those patients enrolled in the optional Phase I imaging sub-study and all patients enrolled in Phase II). Details regarding PK collection and handling are provided in the lab manual.
- Serum pregnancy test must be performed within 24 hours prior to scheduled research scans

6.3. Cycle 1, Day 1 Assessments & Observations

On Cycle 1, Day 1, the following procedures must be completed, unless previously completed ≤ 7 calendar days prior to a patient's first dose of protocol-indicated treatment:

- Targeted physical exam by physician or qualified designee (e.g. physician assistant or nurse practitioner), including assessment of the lungs, heart, abdomen, skin, and any other body area deemed appropriate by the clinician; or comprehensive physical exam if clinically indicated
- Weight

On Cycle 1, Day 1, the following procedures must be completed, unless previously completed ≤ 3 calendar days prior to a patient's first dose of protocol-indicated treatment:

- Complete Blood Count (CBC) with differential (including white blood cell count with differential, hemoglobin, hematocrit, and platelet count)
- Blood Chemistry (including sodium, potassium, chloride, bicarbonate or carbon dioxide, blood urea nitrogen (BUN), creatinine, glucose, total bilirubin, AST, ALT, alkaline phosphatase (ALP), calcium, albumin, magnesium, phosphorus and total protein

On Cycle 1, Day 1, the following procedures will be completed:

- ECOG performance status
- Concomitant medication and Adverse Event review
- Education & review of study dosing diary
- Surveillance for and expedited reporting of Dose Limiting Toxicity (DLT) – see Section 10.1 for DLT definition
- Pharmacodynamic (PD) blood samples (Pre-Dose; collected once prior to initiating protocol-indicated treatment) for those patients enrolled in Phase II
- Initiate twice daily (BID) oral CB-839
- Panitumumab infusion
- Irinotecan infusion (For phase I patients only)

6.4. Cycle 1, Day 15 Assessments

- Targeted physical exam (including assessment of the lungs, heart, abdomen, and skin) or comprehensive physical exam if clinically indicated
- ECOG Performance Status
- Weight
- Concomitant medication and Adverse Event review
- Review of study dosing diary
- Surveillance for and expedited reporting of Dose Limiting Toxicity (DLT) – see Section 10.1 for DLT definition
- Complete Blood Count (CBC) with differential (including white blood cell count with differential, hemoglobin, hematocrit, and platelet count)
- Blood Chemistry (including sodium, potassium, chloride, bicarbonate or carbon dioxide, blood urea nitrogen (BUN), creatinine, glucose, total bilirubin, AST, ALT, alkaline phosphatase (ALP), calcium, albumin, magnesium, phosphorus and total protein
- Continue twice daily (BID) oral CB-839
- Panitumumab infusion
- Irinotecan infusion (For Phase I patients only)

6.5. Cycle 1, Day 28 Assessments

- Surveillance for and expedited reporting of any detected Dose Limiting Toxicity (DLT) – see Section 10.1 for DLT definition
- ^{18}F-FSPG PET/CT scan (only for those patients enrolled in the optional Phase I imaging sub-study and all patients enrolled in Phase II). Ideally completed on Cycle 1, Day 28, but for purpose of scheduling flexibility: Scans can be completed anytime during Week 4 of Cycle 1 (i.e. during Days 22-28 of Cycle 1)
- Pharmacokinetic (PK) blood samples will be collected during ^{18}F-FSPG PET/CT scan (only for those patients enrolled in the optional Phase I imaging sub-study and all patients enrolled in Phase II). Details regarding PK collection and handling are provided in the lab manual.
- Serum pregnancy test must be performed within 24 hours prior to scheduled research scans
- Pharmacodynamic (PD) blood samples (Pre-Dose; collected once prior to oral dose of morning CB-839) for those patients enrolled in Phase II. Note: PD blood samples to be ideally drawn on same-day as the research PET/CT scans
- Review of study dosing diary
- Continue twice daily (BID) oral CB-839

6.6. Additional Cycles, Day 1 Assessments

In the absence of delayed dosing (e.g. due to an adverse event), every reasonable effort should be made to remain on a consistent schedule of 4 Week (28-day) cycles; but for purpose of accommodating holidays, scheduling limitations, etc., subsequent protocol activities beginning with Cycle 2, Day 15 may occur with permissible scheduling flexibility of up to every ± 1 day. (Note: In order to properly evaluate the DLT period, the Cycle 2, Day 1 visit should occur 28 days after Cycle 1, Day 1.)

For patients that continue beyond Cycle 1, the following assessments will occur on Day 1 of each new cycle:

- Targeted physical exam (including assessment of the lungs, heart, abdomen, and skin) or comprehensive physical exam if clinically indicated
- ECOG Performance Status
- Weight
- Concomitant medication and Adverse Event review
- Review of study dosing diary
- Cycle 2, Day 1: Surveillance for and expedited reporting of Dose Limiting Toxicity (DLT) – see Section 10.1 for DLT definition
- Complete Blood Count (CBC) with differential (including white blood cell count with differential, hemoglobin, hematocrit, and platelet count)
- Blood Chemistry (including sodium, potassium, chloride, bicarbonate or carbon dioxide, blood urea nitrogen (BUN), creatinine, glucose, total bilirubin, AST, ALT, alkaline phosphatase (ALP), calcium, albumin, magnesium, phosphorus and total protein
- Continue twice daily (BID) oral CB-839
- Panitumumab infusion
- Irinotecan infusion (For phase I patients only)

6.7. Additional Cycles, Day 15 Assessments

- Targeted physical exam (including assessment of the lungs, heart, abdomen, and skin) or comprehensive physical exam if clinically indicated
- ECOG Performance Status
- Weight
- Concomitant medication and Adverse Event review
- Complete Blood Count (CBC) with differential (including white blood cell count with differential, hemoglobin, hematocrit, and platelet count)
- Blood Chemistry (including sodium, potassium, chloride, bicarbonate or carbon dioxide, blood urea nitrogen (BUN), creatinine, glucose, total bilirubin, AST, ALT, alkaline phosphatase (ALP), calcium, albumin, magnesium, phosphorus and total protein
- Continue twice daily (BID) oral CB-839
- Panitumumab infusion
- Irinotecan infusion (For phase I patients only)

6.8. Additional Cycles: Re-Scanning on Week 4 of Even Cycles

Re-scanning to occur every 8 weeks (± 7 days) after initiating study treatment on Cycle 1, Day 1. (The ± 7 day scan window is intended to facilitate flexibility in scheduling re-scans during **Week 4 of each EVEN cycle (i.e. Cycles 2, 4, 6, etc.). This can be completed prior to scheduled treatment with CB-839/panitumumab/irinotecan on Day 1 of every new ODD cycle (i.e. Cycles 3, 5, 7, etc.). If the scan is to be performed on Day 1 of the odd cycle, patients should be instructed not to take their morning CB-839 dose until after the scan.** Scanning on the same day as initiating a new treatment cycle is allowed provided scan results receive appropriate RECIST review prior to treatment later in the day (e.g. first re-scan is discouraged but permitted on Cycle 3, Day 1, prior to CB-839/panitumumab/irinotecan treatment later that same day). Additional disease evaluations or increased scan frequency may be performed according to the medical judgment of the patient's study physician.

6.9. End-of-Treatment / Withdrawal Assessments

Reasonable effort should be made to complete End-of-Treatment (EOT) / Withdrawal procedures on the day it is decided that a patient will no longer receive protocol-indicated treatment.

The following EOT procedures must be completed subsequent to and not later than 14 days after investigator decision to permanently discontinue protocol-indicated treatment with CB-839 / panitumumab ± irinotecan (whichever treatment occurs last) and prior to any subsequent anti-cancer therapy:

- Comprehensive Physical Exam conducted by a physician; or a Targeted Physical Exam (including assessment of the lungs, heart, abdomen, and any other body area deemed necessary by the clinician) conducted by a physician or qualified designee (e.g. physician assistant or nurse practitioner). If a Targeted Physical Exam is done at EOT, then a Comprehensive Physical Exam must be performed by a physician at the 28-Day Follow-Up Visit
- ECOG Performance Status
- Weight
- Concomitant medication and Adverse Event review
- Review of study dosing diary
- Complete Blood Count (CBC) with differential (including white blood cell count with differential, hemoglobin, hematocrit, and platelet count)

- Blood Chemistry (including sodium, potassium, chloride, bicarbonate or carbon dioxide, blood urea nitrogen (BUN), creatinine, glucose, total bilirubin, AST, ALT, alkaline phosphatase (ALP), calcium, albumin, magnesium, phosphorus and total protein
- Serum or urine pregnancy test in women of childbearing potential (as defined in Sections 6.1 and 7.9)
- Disease evaluation by CT/MRI (only if the previous CT/MRI were done > 28 days before).
- Pharmacodynamic (PD) blood sample (only for those patients enrolled in Phase II)

6.10. 28-Day Follow-Up Visit Assessments

Documented attempt(s) should be made for patient return to the study clinic. It will not be considered a protocol deviation if the patient is physically unable to return for the follow-up visit; such circumstance should be recorded in the study documents, and as much of the follow-up information as possible should be obtained via feasible patient contact and from local and outside facilities.

A Follow-up clinic visit is to be completed 28 days (+7 days) after patient's final protocol-indicated treatment with CB-839 + panitumumab ± irinotecan (whichever occurs last), in order to undergo the following assessments:

- Comprehensive Physical Exam conducted by a physician – if comprehensive exam not previously performed by a physician at the EOT visit; otherwise a Targeted Physical Exam (including assessment of the lungs, heart, abdomen and any other body area deemed appropriate) conducted by a physician or other qualified designee (e.g. nurse practitioner).
- ECOG Performance Status.
- Weight.
- Concomitant medication and Adverse Event review.
- Complete Blood Count (CBC) with differential (including white blood cell count with differential, hemoglobin, hematocrit, and platelet count).
- Blood Chemistry (including sodium, potassium, chloride, bicarbonate or carbon dioxide, blood urea nitrogen (BUN), creatinine, glucose, total bilirubin, AST, ALT, alkaline phosphatase (ALP), calcium, albumin, magnesium, phosphorus and total protein.

If a patient discontinues the study for reason other than progressive disease confirmed by CT or MRI (e.g. adverse event), then CT or MRI scans of the chest and abdomen (and any additional sites of known or suspected disease) must be continued not later than every 8 weeks (± 7 days) until disease progression is confirmed by imaging.

6.11. Survival Follow-Up

Each patient will be followed for survival every 3 months (± 14 days) after patient's final protocol-indicated treatment with CB-839 + panitumumab ± irinotecan (whichever occurs last) until death, end of the study, until patient withdraws consent, or for a maximum of 1 year after the patient's final protocol-indicated treatment – whichever comes first. Survival contact can be made via clinic visit, chart review, obituary or similar observation (e.g. Social Security death index), or by telephone.

7. STUDY PROCEDURES

7.1. Informed Consent

Informed consent must be obtained before any study-specific screening assessments are performed. Screening assessments are to be performed within 14 days prior to Day 1 of Cycle 1 unless otherwise noted (e.g. baseline CT or MRI scan allowed up to 28 days prior to initiating Cycle 1, Day 1 treatment). Assessments performed as standard of care within the screening window may be used for screening. Baseline characteristics include but are not limited to: demographics, medical and surgical history, extent of disease, prior anti-cancer treatment, tumor histology, and *RAS* mutation status.

7.2. Physical Examination

A comprehensive physical exam will be done at Screening; and at the End-of-Treatment or 28-day Follow-Up visit.

At other visits indicated in Section 6, a targeted physical examination may be done including assessment of the lungs, heart, abdomen and any other body area deemed appropriate by the treating physician or qualified designee (e.g. nurse practitioner), unless a comprehensive physical examination is deemed necessary by the clinician.

Screening and either EOT or Follow-Up exams must be done by a physician; at other time points, physical exams may be conducted by either a physician or other qualified designee (e.g. physician assistant or nurse practitioner).

Skin will be examined regularly to monitor for possible dermatologic toxicity to panitumumab. A guided dermatologic evaluation may be done by the patient's study oncologist or qualified designee (i.e. physician assistant or nurse practitioner). A comprehensive dermatologic examination should be performed if clinically indicated, and an appropriate clinical expert such as a dermatologist may be consulted as deemed necessary by the patient's study physician.

7.3. Height and Weight

Height only during screening. Weight at Screening; prior to treatment infusion (scheduled for Days 1 and 15 of each cycle); and at EOT and Follow-up visits

7.4. Review of Concomitant Medications and Adverse Events

Review and capture of all concomitant medications will be performed at each visit as indicated in Section 6.1. Concomitant medications include prescription medications and over-the-counter preparations used by a patient within at least 14 days prior to first dose of protocol-defined treatment and continuing through at least the 28-day Follow-Up study visit. After signing the informed consent, adverse events will be collected as detailed in protocol Section 12. All adverse events will be recorded at least until 28 days after a patient's final protocol-directed treatment with CB-839 or panitumumab or irinotecan (whichever occurs last) or until initiation of another anti-cancer therapy – whichever occurs first.

7.5. Review of CB-839 Dosing Diary

Patients should be educated on the completion of the CB-839 dosing diary prior to initiating dosing on Cycle 1 Day 1. Subsequent review of CB-839 dosing diary for completion and dosing compliance should be completed as indicated on the Schedule of Assessments table in section 6.

7.6. Surveillance and Expedited Reporting of Dose Limiting Toxicity (DLT)

For dose-escalation only: Dedicated surveillance and expedited reporting of Dose Limiting Toxicity (DLT) are required for 28 days after initiating protocol-indicated treatment. On Cycle 2, Day 1, satisfactory completion of the physical exam and the safety labs is required to help detect evidence of past or present DLT as having occurred during the first 28 days of protocol-indicated treatment. If intolerable delayed toxicity attributable (per sponsor-investigator judgement) to protocol treatment is detected past Day 28, then the 28-Day DLT window may possibly be extended. See protocol Section 10.1 for definition of DLT and toxicities which if experienced during the first 28 days after initiating protocol-indicated treatment shall be considered dose limiting.

7.7. Complete Blood Count (CBC) with Differential

Hematology includes white blood cell count with differential, hemoglobin, hematocrit, and platelet count. In the event of hematologic toxicity, the evaluation frequency may be increased per investigator discretion to include additional evaluations between scheduled assessments, as clinically indicated.

7.8. Blood Chemistry

Local blood chemistry results to include sodium, potassium, chloride, bicarbonate or carbon dioxide, blood urea nitrogen (BUN), creatinine, glucose, total bilirubin, AST, ALT, alkaline phosphatase (ALP), calcium, albumin, magnesium, phosphorus and total protein.

7.9. Pregnancy Test

Serum pregnancy test for females of childbearing potential required during Screening, and within 24 hours prior to each research PET/CT visit. Subsequent pregnancy tests – either serum or urine – to be done if clinically indicated.

Females of childbearing potential are defined as those not surgically sterile or not post-menopausal (i.e. if a female patient has not had a bilateral tubal ligation, a bilateral oophorectomy, or a complete hysterectomy; or has not been amenorrheic for 12 months in the absence of an alternative medical cause, then patient will be considered a female of childbearing potential).

Postmenopausal status in females under 55 years of age should be confirmed with a serum follicle-stimulating hormone (FSH) level within laboratory reference range for postmenopausal women (if a patient's postmenopausal status is considered for childbearing potential and study-required contraception).

7.10. Disease Assessment by CT/MRI

Baseline evaluation of disease status by CT or MRI within 28 days prior to initiating first treatment on Cycle 1, Day 1. Baseline and subsequent scans to include imaging of the chest, abdomen and pelvis.

Re-scanning to occur every 8 weeks (± 7 days) after initiating study treatment on Cycle 1, Day 1.
The ± 7 day scan window is intended to facilitate flexibility in scheduling re-scans during Week 4 of each EVEN cycle (i.e. Cycles 2, 4, 6, etc.), prior to scheduled infusion of panitumumab / irinotecan on Day 1 of every new ODD cycle (i.e. Cycles 3, 5, 7, etc.).

Scanning on the same day as infusion with panitumumab/irinotecan is allowed, provided scan results receive appropriate RECIST review prior to initiating panitumumab/irinotecan in a new cycle of study treatment (e.g. first re-scan is discouraged, but permitted on Cycle 3, Day 1, prior to panitumumab/irinotecan infusion later that same day).
Additional disease evaluations or increased scan frequency may be performed according to the medical judgment of the patient's study physician.

At End-of-Treatment, CT/MRI required only if the previous CT/MRI was done > 28 days before. At the 28-day Follow-Up visit, CT/MRI scan required only if disease progression not already documented by CT/MRI done at or before the prior EOT visit.

If a patient discontinues the study for reason other than progressive disease confirmed by CT or MRI (e.g. adverse event), then CT or MRI scans of the chest, abdomen and must be continued every 8 weeks (± 7 days) until disease progression is confirmed for up to 1 year.

7.11. ^{18}F-FSPG PET/CT scan

^{18}F-FSPG PET/CT scans are required for all patients enrolled in the optional Phase I imaging sub-study and all patient enrolled in Phase II. ^{18}F-FSPG PET/CT scans and standard of care CT scans may be performed on the same day.

7.11.1 ^{18}F-FSPG Radiochemistry

^{18}F-FSPG is produced by the Vanderbilt Center for Molecular Probes Radiochemistry Core Laboratory in accordance with the Chemistry, Manufacturing, and Control (CMC) section of Vanderbilt-held IND124202. Tracers meet all USP <823> requirements for sterile, injectable PET radiopharmaceuticals. Production of ^{18}F-FSPG employed radiolabeling of the protected precursor di-tert-butyl-(2S,4S)-2-(3-((naphthalen-2-ylsulfonyl)oxy)propyl)-4-(tritylamino) pentane-dioate) (PI-020) with cyclotron-generated 18F-fluoride in the presence of K^+-$K_{2.2.2}$/K_2CO_3. After acidic deprotection, neutralization, and aqueous dilution, the tracer is purified over SPE cartridges and finally formulated for intravenous injection by passing the solution through a sterile filter. The synthesis is performed within an automated synthesis module (GE MX Reaction Module) in a lead-shielded hot cell. The production methods, sterile filtration, and formulation allow for the production of a sterile and pyrogen-free solution ready for injection. A small aliquot is removed for analysis to confirm the quality of the final product solution.

7.11.2 ^{18}F-FSPG PET/CT Data Acquisition

For ^{18}F-FSPG PET/CT, a dynamic study will be conducted. Prior to injection the patient will be allowed to void the bladder, then positioned onto the PET scanner table. PET scan data acquisition will be initiated simultaneously with intravenous injection of 300 MBq ± 10% of ^{18}F-FSPG injected over 30 sec. Dynamic emission images will be acquired using six 1-min scans, six 2-min scans, six 5-min scans, and six 10-min scans for a total scan duration of 108 min scanning time, as tolerated by the patient. Venous blood samples will be collected following injection for use in pharmacokinetic modeling. A whole body PET scan (2 min per bed position, 20 bed positions ~40 min), analogous to the routine VUMC oncology whole body (vertex of skull to mid-thighs) PET scan protocol, will then be conducted.

Prior to each PET image exam, a brief, low-energy, whole-body transmission CT scan without contrast will be collected. This CT allows correction of PET images for attenuation and anatomic localization. Following an initial scout, the acquisition parameters for the transmission CT include: 120 KVp, 10 mA, Plane = 0 (AP view, no tilt); 1-47 axial

slices/bed position. Reconstructed slice thickness is 3.75 mm with a total exposure time ~50 sec. One whole-body transmission CT scan without contrast will be acquired for each ^{18}F-FSPG PET/CT exam (pre- and post-treatment), for a total of 2.

7.11.3 ^{18}F-FSPG Dose Rationale

The radioactive target dose of 5.10 mSv (males) and 6.54 mSv (females) for ^{18}F-FSPG were calculated based on our previous experience and the literature. [41,42] A dose of 300 MBq (8.1 mCi) for ^{18}F-FSPG studies was chosen in accordance with a typical radioactive dose of ^{18}F-FDG used for oncological diagnostics (350 MBq for an adult). This dose is expected to provide suitable imaging contrast of the target structures.

7.12. ^{11}C-Glutamine PET/CT scan

^{11}C-Glutamine PET/CT scans are optional for patients enrolled in phase I. ^{11}C-Glutamine scans and standard of care CT scans may be performed on the same day.

Patients will be continuously monitored for the safety, biodistribution and metabolism characteristics of ^{11}C-Glutamine through the course of the study and it is anticipated that, after approximately 15 patients have received ^{11}C-Glutamine, we will have collected sufficient data to pursue an IND submission.

7.12.1 ^{11}C-Glutamine Radiochemistry

[11C]Carbon dioxide will be produced by bombardment of N2 gas in the presence of a small percentage of O2 with high energy protons to induce the 14N(p,a) 11C nuclear reaction. Following the irradiation, the target gas will be processed with a GE Procab module. Briefly, the [11C]CO2 will be trapped on activated molecular sieves, then mixed with a nickel catalyst and reacted at 400°C in the presence of hydrogen gas to provide [11C]CH4. [11C]CH4 will then be reacted with ammonia over a platinum catalyst at 950°C. The [11C]HCN produced will be transferred to an automated synthesis module and bubbled into a solution containing 18-crown- 6 (18-C-6) and CsHCO3 in N,N-dimethylformamide at room temperature. To this reaction mixture will then be added (2S)-2-[(tertbutoxycarbonyl)amino]-4-iodobutanoate, followed by heating at 90°C for a period 8 minutes. The reaction mixture will then be diluted with H2O and purified via solid-phase extraction (SPE). The radioactivity will then be eluted from the SPE cartridge into a second reactor using pure acetonitrile. The intermediate will then be azeotropically dried by iterative additions and evaporations of acetonitrile. Once completely dry, a solution of trifluoroacetic acid/sulfuric acid will then be added to the reactor, and the reaction mixture will be heated at 90°C for 5 min. Following dilution of the product with H2O, the mixture will then be loaded onto an Ag11-A8 resin packed column, drained, and then eluted with additional H2O to give the desired ^{11}C-Glutamine.

Table 1: Number of subjects to be studied with each proposed radiotracer	
Radiotracer	Total Number of patients to be studied

^{18}F-FSPG PET/CT	29
^{11}C-Glutamine PET/CT (optional for phase I)*	29
*Note: An IND will be sought after 15 participants have been imaged with ^{11}C-Glutamine	

7.13. Pharmacokinetic (PK) and Pharmacodynamic (PD) Tissue and Blood Samples

An image-guided pre-treatment tissue biopsy will be collected for biomarker research for all patients enrolled in Phase II.

Pharmacokinetic (PK) blood samples for research will be obtained during each radiotracer injection in order to perform pharmacokinetic modeling. Please see the lab manual for details regarding PK collection and handling.

Pharmacodynamic (PD) blood samples for biomarker research will be obtained on Cycle 1, Day 1 (prior to initiating protocol-defined treatment), once on Cycle 1, Day 28 (prior to CB-839 + panitumumab ± irinotecan), and at the End-of-Treatment visit for all patients enrolled in Phase II.

7.14. Survival Follow-Up

Each patient will be followed for survival every 3 months (± 14 days) after patient's final protocol-indicated treatment with CB-839 + panitumumab ± irinotecan until death, end of the study, until patient withdraws consent, or for a maximum of 1 year after the patient's final protocol-indicated treatment – whichever comes first.

Survival contact can be made via clinic visit, chart review, obituary or similar observation (e.g. Social Security death index), or by telephone.

7.15. Handling of Biological Samples

All biological samples to be analyzed locally will be collected and handled according to local institutional practices. All biological samples to be analyzed centrally will be collected and handled according to a provided laboratory manual. Retention time for biologic specimens will be specified in the laboratory manual.

7.16. Specimen Banking

Any leftover study tissue or blood samples may be stored for future research studies. The subjects will consent to the future use of samples in the consent form for the study. All future use as part of residual or repository specimens collected in this trial for purposes not prospectively defined will require review and approval by the Institutional Review Board according to its established policies, whether the specimens are stored in a central site or at a local institution or in a virtual repository.

8. PROTOCOL-INDICATED TREATMENT

8.1. Dose Level Summary

This is a phase I/II, single-institutional trial that will evaluate safety, tolerability, and efficacy of the glutaminase inhibitor CB-839 in combination with panitumumab with/without irinotecan in patients with metastatic and refractory RAS wildtype CRC.

The primary objective of the phase I component is to determine the MTD and/or RP2D of CB-839 in combination with panitumumab and irinotecan. Dose-escalation in Phase I will follow Bayesian continual reassessment method. The dose-escalation schema is presented in Table 2.

TABLE 2: Dose Escalation Summary

Dose Level	**CB-839** Oral continuous BID	**Panitumumab** I.V. Days 1 and 15	**Irinotecan** I.V. Days 1 and 15
-1	400 mg	6 mg/kg	180 mg/m^2
1	600 mg	6 mg/kg	180 mg/m^2
2	800 mg	6 mg/kg	180 mg/m^2

1 cycle = 28 days

Patients will be treated in cohorts of 3. All toxicity outcomes must be observed before calculating the recommended dose level for the next cohort. Dose levels may not be skipped. Up to 12 patients may be enrolled since there are 2 working dose levels and a -1 dose level to be evaluated and a minimum of 6 patients are required to establish the MTD.

8.2. Oral CB-839

200 mg capsules or tablets of CB-839 will be provided by Calithera.

Patients are scheduled to take an oral dose of CB-839 twice each day (BID), starting at Dose Level 1 in early-enrolling patients, consisting of 600 mg in the morning and 600 mg in the evening (1200 mg total daily dose of CB-839), continuously, until development of progressive disease or unacceptable adverse event.

CB-839 should be taken with food, at approximately the same time each morning and evening, about 12 hours (± 2 hours) apart.

Missed doses of CB-839 can be made up during the same day as soon as the patient remembers. However, if the previous dose was taken < 6 hours in the past, or if the next scheduled dose is due < 6 hours in the future, then the missed dose should be skipped. Patients with emesis must not take a replacement dose.

Each patient receiving CB-839 will be treated at the assigned dose, unless dose-reduction is necessary as specified in Section 10. There will be no intra-patient dose-escalation of CB-839.

8.3. Panitumumab Infusion

The site will obtain panitumumab as standard of care from commercial supply.

On Day 1 and Day 15 of each 28-day cycle, each patient is scheduled to receive an infusion of panitumumab at a dose of 6 mg/kg:

- On Day 1 of each cycle, the dose amount required to prepare the Cycle x, Day 1 panitumumab infusion will be based on the patient's current Day 1 body weight in kilograms.

  (Note: On Cycle 1, Day 1, for intended purpose of accommodating preparation and execution of initiating treatment orders, body weight need not be repeated if already completed ≤ 7 calendar days prior to first dose of protocol-indicated treatment.)

- Subsequently, within a cycle, the panitumumab dose (scheduled for Day 15 of each cycle) should be recalculated prior to dosing if the subject's weight on the day of infusion differs by > ± 10% from the weight used to calculate the previously administered dose of panitumumab.

- All doses should be rounded up (or down) to the nearest milligram.

- **All patients are scheduled to receive panitumumab as a 60 minute (if dose ≤ 1000 mg) or 90 minute (if dose > 1000 mg) intravenous infusion on Day 1 and Day 15 of each 28-day cycle.**

  (Note: If an initial 60 minute infusion of panitumumab is tolerated, subsequent panitumumab infusions of ≤ 1000 mg may be administered over 30 to 60 minutes.)

- Every effort should be made to target infusion timings to be as close to scheduled duration as possible. But given the variability of infusion pumps, time windows of ± 10 minutes for the duration of scheduled infusions are permitted. (Additionally, prolongation of infusion duration for the purpose of managing suspected or actual adverse event such as infusion reaction will not be considered a protocol deviation.)
- Panitumumab will be prepared and administered according to the product label:[43]
- Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. Although panitumumab should be colorless, the solution may contain a small amount of visible translucent-to-white, amorphous, proteinaceous, panitumumab particulates (which will be removed by filtration; see below). Do not shake. Do not administer panitumumab if discoloration is observed.
- Using aseptic technique, withdraw the necessary amount of panitumumab for a dose of 6 mg/kg.
- Dilute to a total volume of 100 mL with 0.9% sodium chloride injection, USP. Doses higher than 1000 mg should be diluted to 150 mL with 0.9% sodium chloride injection, USP. Do not exceed a final concentration of 10 mg/mL.
- Mix diluted solution by gentle inversion. Do not shake.
- Administer using a low-protein-binding 0.2 µm or 0.22 µm in-line filter.
- Panitumumab must be administered via infusion pump.
  - Flush line before and after panitumumab administration with 0.9% sodium chloride injection, USP, to avoid mixing with other drug products or intravenous solutions. Do not mix panitumumab with, or administer as an infusion with, other medicinal products. Do not add other medications to solutions containing panitumumab.
  - Infuse doses of 1000 mg or lower over 60 minutes through a peripheral intravenous line or indwelling intravenous catheter. If the first infusion is tolerated, administer

subsequent infusions over 30 to 60 minutes. Administer doses higher than 1000 mg over 90 minutes.

- Use the diluted infusion solution of panitumumab within 6 hours of preparation if stored at room temperature, or within 24 hours of dilution if stored at 2° to 8°C (36° to 46°F). DO NOT FREEZE.
- Discard any unused portion remaining in the vial.

For all panitumumab infusions, a complete dosing history will be recorded, i.e.:

- Total dose and volume administered
- Start and stop time of infusion
- Infusion interruption or termination and reason for such actions.

Each patient receiving panitumumab will be treated at the assigned dose, unless dose-reduction is necessary as specified in Section 10. There will be no intra-patient dose-escalation of panitumumab.

8.4. Premedication for Irinotecan Infusion (Phase I Only)

Consistent with the irinotecan product label[44] in combination with any overriding established institutional practice, it is recommended that patients receive premedication with antiemetic agents prior to administration of irinotecan:

- In clinical studies (of the weekly dosage schedule), the majority of patients received 10 mg of dexamethasone given in conjunction with another type of antiemetic agent, such as a 5-HT^3 blocker (e.g., ondansetron or granisetron).
- Antiemetic agents should be given on the day of treatment, starting at least 30 minutes before administration of irinotecan.
- Physicians should also consider providing patients with an antiemetic regimen (e.g., prochlorperazine) for subsequent use as needed.
- Prophylactic or therapeutic administration of atropine should be considered in patients experiencing cholinergic symptoms.

8.5. Irinotecan Infusion (Phase I Only)

The site will obtain irinotecan as standard of care from commercial supply.

Panitumumab and irinotecan are to be administered as separate infusions. **On days when both drugs are administered (i.e. scheduled for Days 1 and 15 of each cycle), the panitumumab infusion will be completed BEFORE initiation of the irinotecan infusion.**

On Day 1 and Day 15 of each 28-day cycle, each patient is scheduled to receive an infusion of irinotecan at a dose of 180 mg/m^2:

- On Day 1 of each cycle, the dose amount required to prepare the Cycle x, Day 1 panitumumab infusion will be based on the patient's body surface area (BSA), calculated using the patient's current Day 1 body weight in kilograms.

(Note: On Cycle 1, Day 1, body weight need not be repeated if already completed ≤ 7 calendar days prior to first dose of protocol-indicated treatment.)

- Subsequently, within a cycle, the irinotecan dose (scheduled for Day 15 of each cycle) should be recalculated prior to dosing if the subject's weight on the day of infusion differs by > ± 10% from the weight used to calculate the previously administered dose of irinotecan.
- All doses should be rounded up (or down) to the nearest milligram.
- **All patients are scheduled to receive irinotecan as a 90 minute intravenous infusion on Day 1 and Day 15 of each 28-day cycle.**
- Every effort should be made to target infusion timings to be as close to scheduled duration as possible. But given the variability of infusion pumps, time windows of ± 10 minutes for the duration of scheduled infusions are permitted. (Additionally, prolongation of infusion duration for the purpose of managing suspected or actual adverse event such as infusion reaction will not be considered a protocol deviation.)
- Irinotecan will be prepared and administered according to the product label:[44]
- Inspect vial contents for particulate matter and discoloration and repeat inspection when drug product is withdrawn from vial into syringe.
- Irinotecan injection is intended for single use only and any unused portion should be discarded.
- Irinotecan injection must be diluted prior to infusion. Irinotecan should be diluted in 5% Dextrose Injection, USP, (preferred) or 0.9% Sodium Chloride Injection, USP, to a final concentration range of 0.12 mg/mL to 2.8 mg/mL. Other drugs should not be added to the infusion solution.
- The solution is physically and chemically stable for up to 24 hours at room temperature and in ambient fluorescent lighting. Solutions diluted in 5% Dextrose Injection, USP, and stored at refrigerated temperatures (approximately 2° to 8°C, 36° to 46°F), and protected from light are physically and chemically stable for 48 hours. Refrigeration of admixtures using 0.9% Sodium Chloride Injection, USP, is not recommended due to a low and sporadic incidence of visible particulates. Freezing irinotecan and admixtures of irinotecan may result in precipitation of the drug and should be avoided.
- The irinotecan injection solution should be used immediately after reconstitution as it contains no antibacterial preservative. Because of possible microbial contamination during dilution, it is advisable to use the admixture prepared with 5% Dextrose Injection, USP, within 24 hours if refrigerated (2° to 8°C, 36° to 46°F). In the case of admixtures prepared with 5% Dextrose Injection, USP, or Sodium Chloride Injection, USP, the solutions should be used within 4 hours if kept at room temperature. If reconstitution and dilution are performed under strict aseptic conditions (e.g., on Laminar Air Flow bench), irinotecan Injection solution should be used (infusion completed) within 12 hours at room temperature or 24 hours if refrigerated (2° to 8°C, 36° to 46°F).
- For all irinotecan infusions, a complete dosing history will be recorded, i.e.:
    - Total dose and volume administered
    - Start and stop time of infusion
    - Infusion interruption or termination and reason for such actions.

Each patient receiving irinotecan will be treated at the assigned dose, unless dose-reduction is necessary as specified in Section 10. There will be no intra-patient dose-escalation of irinotecan.

8.6. Discontinuation of Protocol-Indicated Treatment

All patients will initiate the study via receipt of CB-839 in combination with panitumumab + irinotecan (phase I) or panitumumab only (phase II). Until confirmed disease progression, it is hoped that CB-839 in combination with either panitumumab + irinotecan or only panitumumab will be well tolerated as a combinatorial therapy:

- CB-839 + panitumumab + irinotecan (phase I)
- CB-839 + panitumumab (phase II)

Therefore:

- **A patient must permanently discontinue the study if unable to tolerate CB-839.** (Within the study, a patient will not receive panitumumab + irinotecan as an exclusive 'double-agent' therapy.)
- **Panitumumab and irinotecan treatments are severable; each may be permanently discontinued independently**. (Panitumumab may be discontinued before irinotecan is discontinued, or irinotecan may be discontinued before panitumumab is discontinued.)
- **A patient must permanently discontinue the study if unable to tolerate CB-839 in combination with at least either panitumumab or irinotecan.** (A patient cannot receive just CB-839 as a "single-agent". CB-839 treatment must be received in combination with panitumumab and/or irinotecan: As evaluated within the structure of this study, adequate data supporting the use of CB-839 in the patient population under investigation is not detected.)

The visit schedule for the treatment period will apply until CB-839 treatment in combination with panitumumab ± irinotecan has been permanently discontinued. Once this has occurred, an End-of-Treatment (EOT) visit will be performed within 14 days after the decision of permanent discontinuation, prior to any subsequent anti-cancer therapy.

After the EOT visit, the patient will continue to be followed until 28 days (+7 days) after the last dose of protocol-indicated treatment, at which time the Follow-Up visit must be completed.

Reasons for permanent discontinuation of a patient's protocol-indicated treatment include any of the following:

- Inability to tolerate CB-839 in combination with at least either panitumumab or irinotecan
- Patient withdraws consent to participate
- Occurrence of an AE considered by the investigator to require treatment discontinuation
- Toxicity requiring discontinuation as outlined in Section 10
- Progressive Disease (PD), verified by CT/MRI according to RECIST v1.1
- Treatment failure not meeting the criteria for PD, but considered by the investigator to require treatment discontinuation (e.g. clinical progression)
- Requirement for a significant surgical procedure

Note: Patients requiring a minor surgical procedure (e.g. port placement, skin abscess drainage) may continue at the investigator's discretion following discussion with the sponsor-investigator or designee. A brief interruption in therapy may be considered.

- An intercurrent illness which, in the opinion of the investigator, would prevent completion of trial-related evaluations
- The investigator judges it necessary due to medical reasons (e.g. significant deterioration in performance status)
- Required use of a prohibited concomitant medication, as defined in Section 9. Note: subject to discussion with and approval by the sponsor-investigator, inadvertent isolated receipt of a prohibited concomitant medication (e.g. incidental to acute management of an adverse event) does not require permanent discontinuation.
- The patient becomes pregnant during treatment

  (Cases of pregnancy that occur during maternal or paternal exposures to study treatment should be reported. Data on fetal outcome and breast-feeding may be collected for regulatory reporting and drug safety evaluation.)
- Significant deviation from the protocol or eligibility criteria. Such patients will be considered protocol violations and may be discontinued from treatment after discussion with the sponsor-investigator.
- Noncompliance with trial procedures may require discontinuation after discussion with the sponsor-investigator
- Termination of the trial by the sponsor-investigator

8.7. Duration of Follow-Up

In general, it is intended that patients will be treated until disease progression or intolerable toxicity. Criteria for patient discontinuation include those listed in Section 10.3. Patients should be assessed when it is decided the patient will no longer receive protocol-indicated treatment; and assessed again 28 days (+ 7 days) after patient's final protocol-indicated treatment with CB-839 / panitumumab / irinotecan.

Subsequently, each patient will be followed for survival every 3 months (± 14 days) after patient's final protocol-indicated treatment until death, end of the study, until patient withdraws consent, or for a maximum of 1 year after the patient's final protocol-indicated treatment – whichever comes first. Contact can be made via clinic visit, chart review, obituary or similar observation (e.g. Social Security death index), or by telephone.

8.8. Withdrawal from Study

Patients may withdraw from the study at any time at their own request, or they may be withdrawn at any time at the discretion of the investigator or sponsor for safety, or behavioral reasons; or the inability of the subject to comply with the protocol-required schedule of study visits or procedures, or an inability to maintain voluntary informed consent. The EOT and the Follow-Up visits should be performed to the extent possible and the Investigator should ensure any SAE is followed as described in Section 12.

Reasons for withdrawal from the study might include but are not limited to any of the following:

- The patient withdraws consent to participate in treatment, follow-up, or survival monitoring.
- The Investigator judges it necessary due to medical reasons.
- Subject is lost to follow-up.
- A maximum of 1 year of survival monitoring elapses after patient's last protocol-indicated dose.
- Study is terminated for any reason.

9. CONCOMITANT TREATMENT

9.1. Supportive Care Guidelines

All concomitant (non-oncological) therapies with the exception of vitamins, appetizers, or nutrient supplements, starting or changing must be recorded in the eCRF during the screening and treatment period, starting from the date of signature of informed consent, and ending at the EOT visit. After the EOT visit, only concomitant therapy indicated for treatment of an AE has to be reported. Trade name, indication, dose and dates of administration will be documented.

Patients may receive their current concomitant medication and any medication considered necessary for the welfare of the patient during trial, except as otherwise restricted or prohibited per protocol (e.g. if con med is restricted by Section 4 Inclusion/Exclusion Criteria, or Section 9.2).

- The more common adverse reactions (seen in ≥ 10% of patients) of CB-839 include: fatigue, abnormal liver function tests (alanine aminotransferase, aspartate transaminase, and alkaline phosphatase), and nausea.
- The less common adverse reactions (5-10%) of CB-839 include: anemia, vomiting, discomfort in the eyes due to light exposure and sensitivity to light, and decreased appetite.
- The most common adverse reactions (≥ 20%) of panitumumab as monotherapy are: skin rash with variable presentations, paronychia, fatigue, nausea, and diarrhea.
- The most common adverse reactions (≥ 20%) in clinical trials of panitumumab in combination with FOLFOX chemotherapy are: diarrhea, stomatitis, mucosal inflammation, asthenia, paronychia, anorexia, hypomagnesemia, hypokalemia, rash, acneiform dermatitis, pruritus, and dry skin.
- Common adverse reactions (≥30%) observed in single agent therapy clinical studies of irinotecan are: nausea, vomiting, abdominal pain, diarrhea, constipation, anorexia, neutropenia, leukopenia (including lymphocytopenia), anemia, asthenia, fever, body weight decreasing, alopecia.
- Common adverse reactions (≥30%) observed in combination therapy clinical studies of irinotecan are: nausea, vomiting, abdominal pain, diarrhea, constipation, anorexia, mucositis, neutropenia, leukopenia (including lymphocytopenia), anemia, thrombocytopenia, asthenia, pain, fever, infection, abnormal bilirubin, alopecia.

Dermatologic adverse events (AEs) of the EGFR inhibitor panitumumab include rash, acne, dermatitis acneiform, and dry skin. General recommendations for prophylaxis are summarized below in Table 2:

TABLE 3: General Recommendations for Dermatologic Prophylaxis while receiving Panitumumab

Personal hygiene	• Use of gentle soaps and shampoos for the body, e.g. pH5 neutral bath and shower formulations and tepid water. • Use of very mild shampoos for hair wash. • Only clean and smooth towels are recommended because of potential risk of infection. The skin should be patted dry after a shower, whereas rubbing the skin dry should be avoided. • Fine cotton clothes should be worn instead of synthetic material. • Shaving has to be done very carefully. • Manicure, i.e. cutting of nails, should be done straight across until the nails no longer extend over the fingers or toes. Cuticles are not allowed to be trimmed because this procedure increases the risk of nail bed infections
Sun protection	• Sunscreen should be applied daily to exposed skin areas regardless of season. Hypoallergenic sunscreen with a high SPF (at least SPF30, PABA free, UVA/UVB protection), preferably broad spectrum containing zinc oxide or titanium dioxide are recommended • Patients should be encouraged to consequently stay out of the sun. • Protective clothing for sun protection and wearing a hat should be recommended.
Moisturizer treatment	• It is important to moisturize the skin as soon as anti-EGFR therapy is started. • Hypoallergenic moisturizing creams, ointments and emollients should be used once daily to smooth the skin and to prevent and alleviate skin dryness. • Note: avoid greasy creams (e.g. petrolatum, soft paraffin, mineral oil based) and topical acne medications
Prevention of paronychia	• Patients should keep their hands dry and out of water if ever possible. • They should avoid friction and pressure on the nail fold as well as picking or manipulating the nail. • Topical application of petrolatum is recommended around the nails due to its lubricant and smoothing effect on the skin.

Patients may receive antibiotics (minocycline, doxycycline or trimethoprim/sulfamthoxazole) per institutional guidelines as preventives of skin toxicity.

Patients should be advised to avoid any foods known to aggravate diarrhea.

Prior to initiation of irinotecan, patients should be informed that they may experience diarrhea, and consideration should be given to providing patients prophylactic anti-diarrheal medication.

A possible example of a prophylactic loperamide regimen against irinotecan-related diarrhea is the following:

- Loperamide 4mg with the first dose of irinotecan, followed by 2mg every 4 hours for the first 3 days.
- After the first 3 days, take loperamide 2mg every 6-8 hours until the end of the first cycle of therapy whether the patient is experiencing diarrhea or not.

- Beyond the first cycle it may be continued at the discretion of the treating physician.
- Loperamide should be held if constipation develops.

9.2. Prohibited and/or Restricted Medications and Therapies

Other than CB-839, panitumumab and irinotecan, additional use of therapeutic anti-cancer treatment and/or standard chemo-, immunotherapy, hormone treatment (with the exception of megestrol acetate and use of anti-androgens and/or gonadorelin analogues for treatment of prostate cancer), or radiotherapy (other than palliative radiotherapy for symptom control) is not allowed concomitantly with the administration of study treatment.

Except for erythropoietin or darbepoetin alpha (Aranesp®), use of growth factors (i.e., G-CSF, GM-CSF, etc.) is not permitted in the first treatment cycle unless the patient experiences a hematologic DLT.

Concomitant use of proton pump inhibitors (PPIs) should be avoided, if at all possible, due to significantly reduced exposure to CB-839. (CB-839 is a weak base and, therefore, requires low pH conditions for optimal solubilization.) If PPI therapy is required, Investigators must discuss the strategy to dosing in combination with PPIs.

Antagonists of the H2 histamine receptor (e.g., ranitidine, famotidine, etc.) may be substituted for PPIs.

For patients unable to discontinue PPI therapy, or in cases that require restarting PPI therapy while on study, administration of CB-839 with an acidic beverage (e.g., orange juice) or supplement (e.g., citric acid) may be an option. If an acidic beverage/supplement is approved by the sponsor-investigator to be administered along with the CB-839 dose, it should be recorded on the appropriate eCRF, including the identity of the beverage/supplement, dosage, route, and start and stop dates of administration.

CB-839 is a moderate inhibitor of CYP2C9, therefore concomitant medications that are metabolized by CYP2C9 should either be given with caution (including closely monitoring for signs of toxicity or altered efficacy) or substituted for a non-CYP2C9 substrate.

Although CB-839 is not expected to inhibit CYP2C9 at the exposure levels planned, **caution is warranted when administering CB-839 to patients taking drugs that are highly dependent on CYP2C9 for metabolism and have a narrow therapeutic index**. A list of medications that are CYP2C9 substrates is provided below:

CYP2C9 Substrates with a narrow therapeutic index*

• S-Warfarin (anticoagulant)

• Phenytoin (antiepileptic)

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

http://www.fda.gov/drugs/developmentapprovalprocess/developmentresources/druginteractionslabeling/ucm093664.htm

Other CYP2C9 Substrates

- NSAIDs (analgesic, antipyretic, anti-inflammatory)

  - celecoxib
  - lornoxicam
  - diclofenac
  - ibuprofen
  - naproxen
  - ketoprofen
  - piroxicam
  - meloxicam
  - suprofen
- fluvastatin (statin)
- sulfonylureas (antidiabetic)
  - glipizide
  - glibenclamide
  - glimepiride
  - tolbutamide
  - glyburide
- irbesartanlosartan
- sildenafil (in erectile dysfunction)
- terbinafine (antifungal)
- amitriptyline (tricyclic antidepressant)
- fluoxetine (SSRI antidepressant)
- nateglinide (antidiabetic)
- rosiglitazone (antidiabetic)
- tamoxifen (SERM)
- torasemide (loop diuretic) ketamine

Exposure to irinotecan or its active metabolite SN-38 is substantially reduced in adult and pediatric patients concomitantly receiving the CYP3A4 enzyme-inducing anticonvulsants phenytoin, phenobarbital, carbamazepine, or St. John's wort. The appropriate starting dose for patients taking these or other strong inducers such as rifampin and rifabutin has not been defined.

Consider substituting non-enzyme inducing therapies at least 2 weeks prior to initiation of CAMPTOSAR therapy. **Do not administer strong CYP3A4 inducers with irinotecan unless there are no therapeutic alternatives.**

Irinotecan and its active metabolite, SN-38, are metabolized via the human cytochrome P450 3A4 isoenzyme (CYP3A4) and uridine diphosphate-glucuronosyl transferase 1A1 (UGT1A1), respectively. Patients receiving concomitant ketoconazole, a CYP3A4 and UGT1A1 inhibitor, have increased exposure to irinotecan hydrochloride and its active metabolite SN-38.

Co-administration of irinotecan with other inhibitors of CYP3A4 (e.g., clarithromycin, indinavir, itraconazole, lopinavir, nefazodone, nelfinavir, ritonavir, saquinavir, telaprevir, voriconazole) or UGT1A1 (e.g., atazanavir, gemfibrozil, indinavir) may increase systemic exposure to irinotecan or SN-38.

Discontinue strong CYP3A4 inhibitors at least 1 week prior to starting irinotecan therapy.

Do not administer strong CYP3A4 or UGT1A1 inhibitors with irinotecan unless there are no therapeutic alternatives.

9.3. Hematopoietic Growth Factors and Transfusional Support

- Packed red blood cell and platelet transfusions should be administered only if clinically indicated, and should be avoided during the first 28 days after initiation of protocol-indicated treatment.

- Patients who enter the study on stable doses of granulocyte colony-stimulating factor, granulocyte-macrophage colony stimulating factor, erythropoietin, or darbepoietin may continue such treatment (provided such treatment was initiated at least 14 days prior to starting study treatment), and patients may start such treatment drug during the study (at the discretion of the treating physician if clinically indicated) after the first 28 days of following initiation of protocol-indicated treatment.

9.4. Palliative Radiotherapy

Radiotherapy other than palliative radiotherapy for symptom control is not allowed concomitantly with the administration of protocol-indicated treatment.

After study enrollment, palliative radiotherapy may be given for bone pain or for other reasons, provided that the total dose delivered is in a palliative range according to institutional standards.

The irradiated area cannot be used for tumor response assessment.

During palliative radiotherapy, study treatment should be delayed and may be resumed once the patient has recovered from any radiation associated toxicity.

If a treatment cycle is interrupted for more than 28 days, the decision to continue will be made by the investigator in agreement with the sponsor-investigator. Continuous interruption of >28 days due to palliative radiotherapy will not be allowed.

10. ADVERSE EVENT MANAGEMENT

Patients should be instructed to notify their study team at the first occurrence of any adverse symptom. In addition to dose delays according to protocol guidance, investigators are encouraged to employ best supportive care according to local institutional clinical practice.

Recognizing that new knowledge will be acquired and unforeseen safety issues may arise in the course of the study, the management guidelines are not exhaustive and do not represent the full spectrum of care or treatment options described. The dose modification guidelines are not intended to replace clinical judgment or dictate care of individual patients.

In general, an adverse event related to study treatment that results in a dose delay should be present on the day of intended study treatment (i.e. a delayed toxicity that develops mid-cycle, but which is no longer present or has resolved to an acceptable grade on the day of intended dosing, generally does not require a delay in treatment – unless, for example, the occasion involves retrospective detection of a prior event of sufficient grade to, regardless of duration, require delay or discontinuation).

To prevent the development of more severe adverse events, treatment-related diarrhea, nausea and vomiting or rash should be managed early and proactively.

In the event of multiple toxicities, treatment delay should be based on the worst toxicity observed.

Even if study drug dosing is interrupted, tumor scans and other visits, assessments and procedures should continue per protocol (i.e. per timeline in place prior to the dose interruption).

Dose reductions for management of adverse event(s) within a given patient should be considered permanent: Once the dose of CB-839, panitumumab or irinotecan has been reduced in a given patient, all subsequent cycles in the same patient should be ordinarily administered at that dose level (unless additional dose reduction is required). Dose re-escalation may be discussed on a case-by-case basis with the Sponsor.

Thus, intrapatient dose re-escalation in a given patient will not ordinarily be allowed, unless there is discussion and agreement between the investigator and the sponsor-investigator (e.g. as part of an appropriate evaluation of the combinatorial setting in which, after strategic dose reduction of one or more particular agent, any potentially overlapping toxicity between CB-839, panitumumab and irinotecan is ultimately judged best attributed to one individual drug, but unlikely or not related to the other drugs).

10.1. Definition of Dose-Limiting Toxicity

When classified by the investigator as possibly, probably or definitely related to CB-839, or to the combination of CB-839 + panitumumab/irinotecan, any of the following events occurring during Cycle 1 (i.e. the first 28 days after first dose of CB-839) will be considered a dose-limiting toxicity:

1) **<u>Non-Hematologic DLT</u>:**
   - Any ≥ Grade 4 non-hematological toxicity
   - Grade ≥ 3 total bilirubin or hepatic transaminases (ALT [SGPT] or AST [SGOT]) with the following exceptions:
     - Grade 3 elevated ALT/AST lasting for ≤7 days and not associated with a more than 35% increase in abnormal direct bilirubin (i.e. bili is both >ULN and elevated >35% over baseline) will not be considered a DLT
     - For patients with a Grade 1 hepatic transaminase level at baseline (i.e. ALT or AST), the same respective hepatic transaminase level must increase to > 7.5 × ULN to be considered a DLT.

- For patients with a Grade 2 hepatic transaminase level at baseline (i.e. ALT or AST), the same respective hepatic transaminase level must increase to > 10 × ULN to be considered a DLT.

- Grade 3 other non-hematologic toxicity lasting > 3 days despite optimal supportive care with the exception of:
  - Grade 3 fatigue
  - Grade 3 symptomatic rash that resolves to ≤ Grade 1 within 3 weeks; or a Grade 3 asymptomatic rash
  - Grade 3 tumor flare (defined as local pain, irritation or rash localized at sites of known or suspected tumor)
  - A transient (resolves within 6 hours of onset) Grade 3 infusion-related AE
  - Grade 3/4 elevations in serum amylase and/or lipase lasting < 3 weeks that are not associated with clinical or radiological evidence of pancreatitis.
- Any clinically meaningful (per investigator judgment) Grade 3 non-hematologic laboratory value if:
  - Medical intervention (other than electrolyte repletion) is required to treat the patient, OR
  - The abnormality leads to hospitalization, OR
  - The abnormality persists for >1 week.

2) **<u>Hematologic DLT</u>:**

- Grade ≥ 3 febrile neutropenia [ANC < 1.0 x 10^9/L with either a single temperature of > 38.3 °C (101 °F), or a sustained temperature of ≥ 38 °C (100.4 °F) for more than 1 hour]
- Grade 4 neutropenia (ANC <0.5 x 10^9/L) lasting > 5 days
- Grade 4 anemia lasting > 5 days despite optimum management by transfusions
- Grade 4 thrombocytopenia (< 25.0 x 10^9/L) lasting ≥ 7 days
- Grade 4 thrombocytopenia (< 25.0 x 10^9/L) lasting < 7 days if associated with:
  - A bleeding event that requires an elective platelet transfusion, OR
  - A life-threatening bleeding event which results in urgent intervention and admission to an Intensive Care Unit
- Any Grade 5 hematologic or non-hematologic toxicity excluding death due to disease progression

3) **<u>DLT for inability to initiate Cycle 2 within 4 weeks of scheduled treatment</u>**

- Due to slow recovery from adverse event classified by the investigator as possibly, probably or definitely related to CB-839, or to the combination of CB-839 + panitumumab/irinotecan and failure to meet protocol-defined retreatment criteria.

Subjects who have experienced a DLT do not automatically require discontinuation of protocol-indicated treatment, unless the nature or severity of the DLT is also an adverse event that would require permanent discontinuation of study as elsewhere defined by the protocol. Rather, a patient who has experienced a DLT should be evaluated for consideration of an appropriate dose hold or dose modification which, if consistent with the protocol, would allow continued dosing under revised circumstance.

Dose reductions are not allowed during the DLT window for a patient to count in dose-escalation decisions. After Cycle 1, dose reductions or interruptions for adverse events may take place at any time as otherwise consistent with the protocol.

For purpose of ensuring appropriate opportunity to determine the maximum tolerated dose (MTD) of CB-839 in combination with panitumumab + irinotecan, a subject who is withdrawn from the study prior to

completing the DLT assessment window (i.e. first 28 days after initiating protocol-indicated therapy on Cycle 1, Day 1) for reasons other than a DLT will not be considered evaluable for DLT and will be replaced.

Additionally, if a patient discontinues study treatment for reasons clearly not related to study treatment (in the judgement of the investigator), after completing less than 75% of scheduled oral doses of CB-839 and/or less than 2 planned infusions of panitumumab and 2 planned infusions of irinotecan over the first 28 days after initiating protocol-indicated treatment on Cycle 1, Day 1, then that patient will be considered not evaluable for response to overall protocol-indicated treatment and may be replaced with a new patient.

10.2. Dose-Adjustments and Delays of CB-839

Given the limited number of patients treated to date and the open label, nonrandomized nature of the studies to date, the safety profile of CB-839 has not been fully established. An overall summary of the available safety and tolerability data was profiled above in section 2.4.2.

Overall, CB-839 has been well tolerated across all dose levels tested to date. The large majority of toxicities that have been observed to date have been Grades 1 and 2 and manageable with supportive care and/or interruption of study drug.

Careful application of the dose-escalating rules and close observation of the subjects should minimize the potential risk of dosing with CB-839.

Based on available data, adverse events that are most likely to be observed with CB-839 treatment are mild to moderate and include fatigue, gastrointestinal events (nausea, vomiting, anorexia), photophobia and elevated liver function tests.

Photophobia and related ocular toxicities (e.g., photopsia) have been identified as CB-839-related events. In almost all cases, these events have been Grade 1 and generally do not have an impact on the patient's daily life, although one Grade 2 event required the patient rest for 1-2 hr in a dark room. These events appear to occur around Cmax (1-4 hr after dosing) and resolve with time. Anecdotal reports suggest that tachyphylaxis develops and these events tend to become less frequent over extended dosing. More severe cases can be managed with dose reduction and the use of sunglasses may be considered.

The study personnel must be able to recognize and diagnose these potential adverse events and initiate prompt intervention. In particular, regular monitoring of liver function tests and renal function is recommended.

Upon withholding CB-839 for adverse event, CB-839 may be restarted when the associated adverse event has returned to ≤ Grade 1, or to the patient's baseline value. (The baseline value is defined as the most immediately known value in place prior to patient's first receipt of CB-839 on Cycle 1, Day 1.)

For adverse events deemed by the investigator to be clinically significant and as possibly, probably, or definitely related to CB-839 treatment:

- **Grade 1 or 2:** Manage with appropriate supportive care.
- **Grade 3 or 4:** Hold CB-839 and Dose Reduce (by 1 or more dose levels) upon resumption

Note the CB-839 dose levels are indicated in Section 8.1. Accordingly, **a patient unable to tolerate CB-839 at a minimum dose of 400mg BID would be unable to continue the study**.

In the event of any *unrelated* adverse events, the investigator may choose to interrupt CB-839 for up to 28 days, but no dose reduction should occur.

CB-839 treatment may be delayed for up to 4 weeks from the last dose. If CB-839 is interrupted for more than 28 days, written permission to continue CB-839 must be obtained from the sponsor-investigator subsequent to consultation with the investigator.

If CB-839 is held for toxicity, tolerated treatment with panitumumab and/or irinotecan should continue as scheduled until CB-839 is permanently discontinued – at which time the patient must also permanently discontinue the study (per Section 8.6: a patient must permanently discontinue the study if unable to tolerate CB-839; within the study, a patient will not receive panitumumab + irinotecan as an exclusive 'double-agent' therapy.)

10.3. Dose-Adjustments and Delays of Panitumumab

Throughout the study, panitumumab is scheduled to be administered every two weeks (i.e. on Days 1 and 15 of each 28-day cycle).

Panitumumab dose withholding, reduction, or re-escalation (see Section 10.3.3 and Figure 3) following a previous dose-reduction may be required for patients who experience adverse events felt by the investigator to be at least possibly related to panitumumab.

As needed for management of potential panitumumab-related toxicity, the dose reduction levels for panitumumab are listed in Table 3 below:

TABLE 4: Panitumumab Dose Reduction Levels

	Starting Dose	Dose Reduction Level -1	Dose Reduction Level -2
Percentage	100 %	80 %	60 %
Panitumumab Dose	6 mg/kg	4.8 mg/kg	3.6 mg/kg

(As needed for management of adverse event.)

10.3.1 Criteria for Withholding a Dose of Panitumumab

Panitumumab will be withheld if any of the following related toxicities occur:

Skin-related or nail-related toxicities:

- Symptomatic skin-related or nail-related toxicity requiring narcotics, systemic steroids, or felt to be intolerable by the subject
- Skin or nail infection requiring IV antibiotic or IV antifungal treatment
- Need for surgical debridement
- Any skin-related or nail-related serious adverse event.

Non-skin-related or Non-nail-related toxicities:

- Any Grade 3 or 4 toxicity (except alopecia) with the following exceptions:
  - Panitumumab will only be withheld for symptomatic hypomagnesemia and/or hypocalcemia that persists despite aggressive magnesium and/or calcium replacement
  - Panitumumab will only be withheld for Grade 3 or 4 nausea, vomiting, or diarrhea that persists despite maximum supportive care
  - Panitumumab will only be withheld for Grade ≥ 3 anemia or Grade 4 thrombocytopenia that cannot be managed by transfusion(s) or cytokine therapy.

10.3.2 Criteria for Re-treatment with Panitumumab

Panitumumab administration may recommence once the reason for withholding the dose of panitumumab has resolved. Panitumumab-related toxicity will be considered "resolved" if it improves to an extent that meets the following criteria for re-treatment with panitumumab:

Skin-related or nail-related toxicities:
Panitumumab administration may recommence once:

- The adverse event has improved to ≤ Grade 2 or returned to baseline, and
- The skin-related or nail-related toxicity is no longer intolerable to the subject, and
- Systemic steroids are not required, and
- IV antibiotic or IV antifungal treatment is not required.

Non-skin-related or Non-nail-related toxicities:
Panitumumab administration may recommence once the adverse event has improved to ≤ Grade 1 or returned to baseline.

10.3.3 Dose Modification Schedule for Panitumumab

Subjects should be assessed for panitumumab toxicity before each dose.

With the understanding that the patient's study physician may elect a more conservative course of action, subjects who have had their dose of panitumumab withheld for one or more of the reasons listed in Section 10.3.1, and then subsequently meet the criteria for re-treatment as listed in Section 10.3.2, will recommence panitumumab according to the following schedule and as outlined in Figure 3 (note: panitumumab-related toxicity will

be considered “resolved” if it improves to an extent that allows for re-treatment with panitumumab, as per Section 10.3.2):

- Subjects receiving either 100% or 80% of the starting dose of panitumumab are allowed to have up to 2 subsequent doses withheld for toxicity. (However, the second dose should only be withheld if the toxicity has not resolved by the time that the subsequent dose is due – for additional context, see the “double-dagger” footnote “‡” in Figure 3.)

- Subjects treated at the 100% dose level whose toxicity resolves after 1 dose of panitumumab is withheld should be re-started at the 100% dose level (recommended but not required). A reduction to the 80% dose is allowed as an alternative to re-challenge with the 100% dose.

- If toxicity recurs, subjects treated at the 100% dose or 80% dose should be re-started at the 80% dose or 60% dose, respectively, when the toxicity resolves after withholding 1 or 2 doses of panitumumab. Subjects treated at the 100% dose level whose toxicity resolves only after 2 subsequent doses of panitumumab are withheld should be re-started at the 80% dose level.

- Subjects treated at the 80% dose level whose toxicity resolves after withholding 1 or 2 doses of panitumumab should be re-started at the 60% dose level.

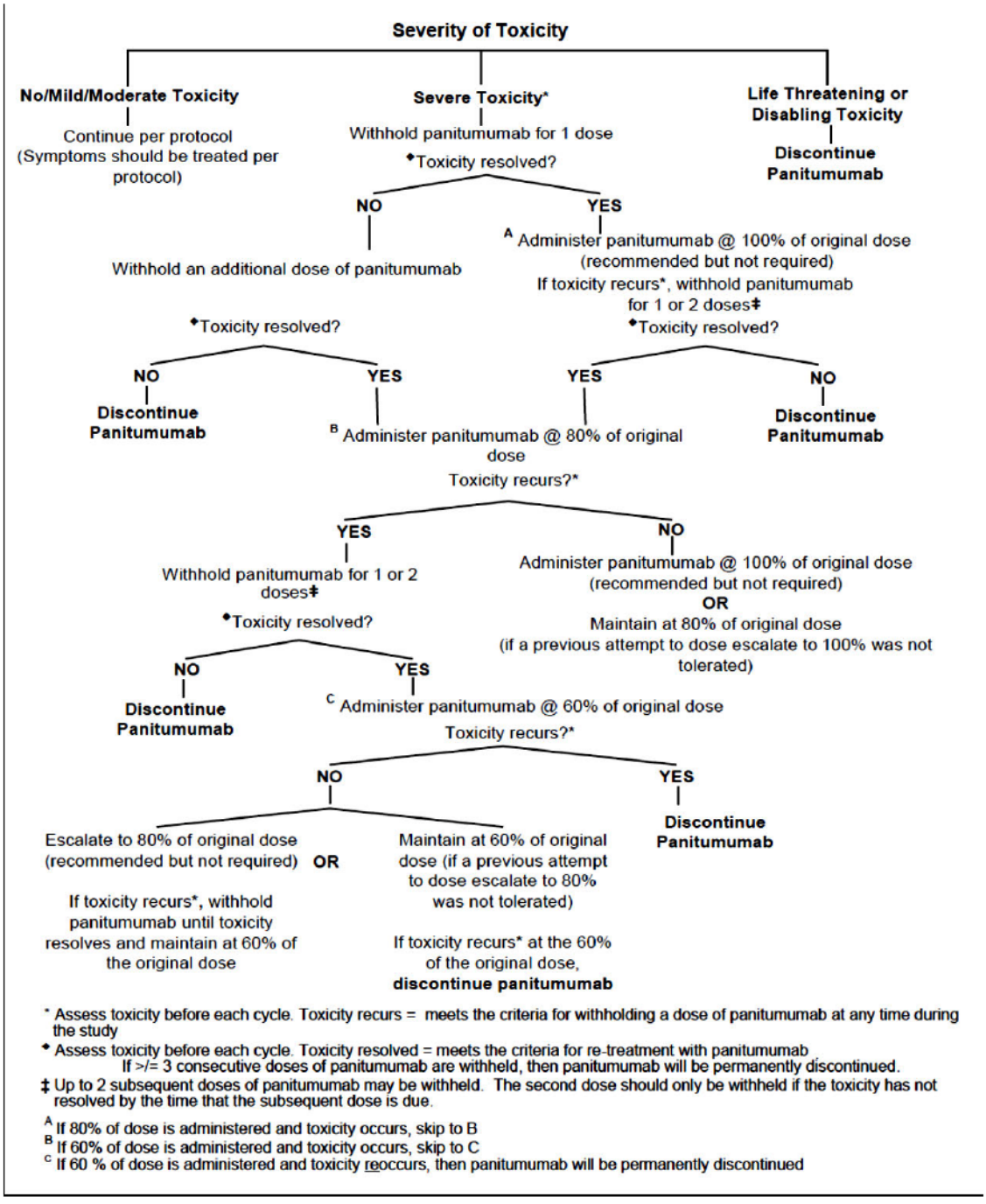
- Subjects who experience toxicity at the 60% dose level will not be re-treated with panitumumab.

It is recommended that panitumumab doses will be escalated in subjects whose toxicity resolves to the degree that meets the criteria for re-treatment with panitumumab (see Section 10.3.2). Dose escalations are recommended but not required. Dose escalations should occur in the following manner:

- Subjects treated at the 80% dose level whose toxicity does not recur should receive the 100% dose level at the next dose unless a previous attempt to re-escalate to the 100% dose level was not tolerated (re-initiation of the 80% dose is allowed as an alternative to dose escalation).

- **Subjects treated at the 60% dose level whose toxicity does not recur should receive the 80% dose at the next dose unless a previous attempt to re-escalate to the 80% dose level was not tolerated (re-initiation of the 60% dose is allowed as an alternative to dose escalation).**

FIGURE 4: Panitumumab Dose Modification Algorithm for Toxicity



* Assess toxicity before each cycle. Toxicity recurs = meets the criteria for withholding a dose of panitumumab at any time during the study

◆ Assess toxicity before each cycle. Toxicity resolved = meets the criteria for re-treatment with panitumumab
If >/= 3 consecutive doses of panitumumab are withheld, then panitumumab will be permanently discontinued.

‡ Up to 2 subsequent doses of panitumumab may be withheld. The second dose should only be withheld if the toxicity has not resolved by the time that the subsequent dose is due.

A If 80% of dose is administered and toxicity occurs, skip to B
B If 60% of dose is administered and toxicity occurs, skip to C
C If 60 % of dose is administered and toxicity reoccurs, then panitumumab will be permanently discontinued

As per Section 12.1, adverse events will be graded according to the NCI's Common Terminology Criteria for Adverse Events (NCI CTCAE), Version 4.03, dated June 14, 2010.

For purpose of managing potential panitumumab-related toxicity, adverse events graded according to CTCAE version 4.03 will be reconciled with guidance presented in Figure 3 according to Table 4:

TABLE 5: Reconciliation of AE Severity Grades in CTCAE v4.03 versus Figure 3

CTCAE v4.03 severity grade[a]	Equivalent to	Figure 3 severity
Grade 0, 1 or 2	=	No / Mild / Moderate
Grade 3	=	Severe
Grade 4	=	Life Threatening or Disabling

(As needed for purpose of managing potential panitumumab-related adverse event.)

[a] NCI's Common Terminology Criteria for Adverse Events (NCI CTCAE), Version 4.03, dated June 14, 2010.

Dose delays and or modifications for panitumumab are to be considered for skin reactions of Grade 3 that are symptomatic; or which are considered intolerable at any grade.

Exception for Grade 3 *asymptomatic* rash: Note that rash of Grade 3 by body surface area (BSA) but which is asymptomatic does not require panitumumab treatment delay (or dose reduction) if uninterrupted panitumumab treatment is medically acceptable per investigator discretion.

Note: If, during the course of managing a patient's panitumumab treatment, conflicting guidance were to be detected between the management guidance as presented by narrative in Section 10.3, versus the algorithm presented in Figure 3, the investigator may elect either course of action deemed most appropriate by the investigator.

If panitumumab is held for toxicity, tolerated treatment with CB-839 + irinotecan may continue as scheduled. Alternatively, if panitumumab is delayed, the investigator may choose to also delay administration of irinotecan.

Delays of panitumumab administration beyond 6 weeks of last administered dose of panitumumab are not allowed. **Subjects who miss 3 or more consecutive scheduled doses of panitumumab due to toxicity or who are unable to receive a dose of panitumumab within 6 weeks of having received their previous dose of panitumumab due to toxicity will be considered unable to tolerate panitumumab and will not be retreated with panitumumab.** Additional consideration may be made toward continuing treatment with CB-839 + irinotecan alone.

10.4. Dose-Adjustments and Delays of Irinotecan

As needed for management of potential irinotecan-related toxicity, the dose reduction levels for irinotecan are listed in Table 5 below:

TABLE 6: Irinotecan Dose Reduction Levels

	Starting Dose	Dose Reduction Level -1	Dose Reduction Level -2
Irinotecan Dose	180 mg/m^2	150 mg/m^2	120 mg/m^2

(As needed for management of adverse event.)

10.4.1 Dose Modification Schedule for Irinotecan

Throughout the study, irinotecan is scheduled to be administered every two weeks (i.e. on Days 1 and 15 of each 28-day cycle).

Irinotecan should not be administered until:

- Absolute neutrophil count has recovered to ≥ 1000 /mm^3, the
- Platelet count has recovered to ≥ 100,000 /mm^3, and
- Treatment-related diarrhea is fully resolved to (or controlled at) baseline level.

The treating physician may require that a patient return to baseline bowel function without requiring antidiarrheal medication for at least 24 hours before the next chemotherapy administration. (The baseline status is defined as the most immediately known status in place prior to initiation of the patient's first protocol-indicated irinotecan on Cycle 1, Day 1.)

Irinotecan treatment should be delayed 1 or 2 weeks, and if necessary up to 4 weeks, to allow for recovery from irinotecan-related toxicity.

Dose modifications for irinotecan therapy should be carried out according to Table 6 (with the understanding that the patient's study physician may elect a more conservative course of action):

TABLE 7: Recommended Dose Modifications for Irinotecan[a]

Toxicity NCI CTC Grade[b] (Value)	Recommended Dose Modifications for Irinotecan
Neutropenia	
1 (1500 to 1999/mm^3)	Maintain dose level
2 (1000 to 1499/mm^3)	Maintain dose level
3 (500 to 999/mm^3)	Omit dose until resolved to ≤ Grade 2, then ↓ 1 dose level
4 (< 500/mm^3)	Omit dose until resolved to ≤ Grade 2, then ↓ 2 dose levels
Febrile Neutropenia ANC < 1000 /mm^3 with a single temperature of > 38.3 °C (101 °F) or a sustained temperature of ≥ 38 °C (100.4 °F) for more than one hour	Omit dose until resolved, then ↓ 2 dose levels
Other hematologic toxicities	Dose modifications for leukopenia or thrombocytopenia during a cycle of therapy and at the start of subsequent cycles of therapy are also based on NCI toxicity criteria and are the same as recommended for neutropenia above.
Diarrhea	
1 (2-3 stools/day > pretx[c])	Delay dose until resolved to Baseline[c], then give same dose
2 (4-6 stools/day > pretx)	Omit dose until resolved to Baseline, then ↓ 1 dose level
3 (7-9 stools/day > pretx)	Omit dose until resolved to Baseline, then ↓ 1 dose level
4 (≥10 stools/day > pretx)	Omit dose until resolved to Baseline, then ↓ 2 dose levels
Other non-hematologic toxicities[d]	
1	Maintain dose level
2[e]	Omit dose until resolved to ≤ Grade 1, then ↓ 1 dose level
3	Omit dose until resolved to ≤ Grade 2, then ↓ 1 dose level
4	Omit dose until resolved to ≤ Grade 2, then ↓ 2 dose levels

[a] Due to adverse events deemed (by patient's study physician) to be possibly, probably, or definitely related to irinotecan. Note: Patient's study physician may elect a more conservative course of irinotecan management.

[b] NCI Common Terminology Criteria for Adverse Events (CTCAE, version 4.03).

[c] Pretreatment and Baseline defined as the most immediately known status prior to initiating protocol-indicated irinotecan on Cycle 1, Day 1.

[d] Unless otherwise elected by the investigator: Excludes alopecia, anorexia, asthenia, fatigue; as well as nausea or vomiting that are not controlled with optimal management.

[e] Except alopecia, alkaline phosphatase elevation, asymptomatic lipase elevation

If irinotecan is held for toxicity, tolerated treatment with CB-839 + panitumumab may continue as scheduled. Alternatively, if irinotecan is delayed, the investigator may choose to also delay administration of panitumumab.

Subjects who require persistent 1 or 2 week delays for resolution of recurring Grade 1 toxicities, may have their irinotecan dose reduced by 1 dose level to permit administration of irinotecan every 2 weeks, at the investigator's discretion.

If, despite optimal supportive care and a treatment interruption, diarrhea does not resolve to CTC AE Grade ≤ 1 or baseline within 28 days, treatment with irinotecan should be permanently discontinued. However, in the event that the patient is deriving obvious clinical benefit according to the investigator's judgment, further treatment with irinotecan will be decided in agreement between the sponsor-investigator and the investigator. **Otherwise, subjects who require more than 2 dose reductions of irinotecan, or miss 2 or more consecutive scheduled doses due to toxicity, or are unable to receive a dose of irinotecan within 4 weeks of having received their previous dose of irinotecan due to toxicity, will be considered unable to tolerate irinotecan and will not be retreated with irinotecan.** Additional consideration may be made toward continuing treatment with CB-839 + panitumumab alone.

10.5. Management of Dermatologic AEs Following Treatment with Panitumumab

Dermatologic adverse events (AEs) of the EGFR inhibitor panitumumab include rash, acne, dermatitis acneiform, and dry skin.

The prescribing information[43] for panitumumab (VECTIBIX) includes the following black-box warning: **"Dermatologic toxicities were reported in 90% of patients and were severe in 15% of patients receiving monotherapy."**

Thus, specific interventions should be reassessed at least after 2 weeks or at any worsening of symptoms, in which case the specific intervention should be adjusted and, depending on own clinical experience, early involvement of a dermatologist should be considered.

- General recommendations for prophylaxis are summarized above in Section 9.1.
- Grade-specific treatment recommendations are summarized below in Table 7.
- For dose adjustment of panitumumab, refer to Section 10.3 (including Figure 3).
- Note: a Grade 4 (i.e. life threatening or disabling) drug-related skin toxicity requires discontinuation of panitumumab (see above Section 10.3).

Dose delays and or modifications for panitumumab are to be considered for skin reactions of Grade 3 that are symptomatic; or which are considered intolerable at any grade.

Note that rash of Grade 3 by body surface area (BSA) but which is asymptomatic does not require treatment delay (or dose reduction) if uninterrupted treatment is medically acceptable per investigator discretion.

If a patient experiences Grade 4 skin reactions, treatment with panitumumab should be permanently discontinued.

TABLE 8: Treatment Recommendations for Panitumumab-Related Skin Reactions

Severity (CTCAE Grading)	Description	Specific intervention
ACNEIFORM RASH		
Grade 1 Mild	Papules and/or pustules covering <10% BSA, which may or may not be associated with symptoms of pruritus or tenderness	Consider topical antibiotics, e.g. clindamycin 2% or topical erythromycin 1% cream of metronidazole 0.75% or topical nadifloxacin 1%; Isolated scattered lesion: cream preferred Multiple scattered areas: lotion preferred
Grade 2 Moderate[1]	Papules and/or pustules covering 10 - 30% BSA, which may or may not be associated with symptoms of pruritus or tenderness; associated with psychosocial impact; limiting instrumental ADL	Topical treatment as for Grade 1 plus short term topical steroids, e.g. prednicarbate cream 0.02% plus an oral antibiotic (for at least 2 weeks) e.g. Doxycycline 100mg b.i.d. or Minocycline hydrochloride 100mg b.i.d
Grade 3 Severe	Papules and/or pustules covering >30% BSA, which may or may not be associated with symptoms of pruritus or tenderness; limiting self care ADL; associated with local superinfection with oral antibiotics indicated	Topical and systemic treatment as for Grade 2. Consider referral to dermatologist Consider systemic steroids
Grade 4 Life threatening	Papules and/or pustules covering any % BSA, which may or may not be associated with symptoms of pruritus or tenderness and are associated with extensive superinfection with IV antibiotics indicated; life threatening consequences	See Grade 3 Systemic steroids are recommended
EARLY AND LATE XEROTIC SKIN REACTIONS – PRURITUS		
Grade 1 Mild	Mild or localized	Topical polidocanol cream. Consider oral antihistamines, e.g. diphenhydramine, dimethindene, cetirizine, levocetirizine, desloratidine, fexofenadine or clemastine)
Grade 2 Moderate[1]	Intense or widespread	See Grade 1 plus oral antihistamines; Consider topical steroids, e.g. topical hydrocortisone
Grade 3 Severe	Intense or widespread and interfering with activities of daily living (ADL)	See Grade 2.

Severity (CTCAE Grading)	Description	Specific intervention
XEROSIS / DRY SKIN		
Grade 1 Mild	Mild or localized; topical intervention indicated	Soap-free shower gel and/or bath oil. Avoid alcoholic solutions and soaps. Urea- or glycerin-based moisturizer. In inflammatory lesions consider topical steroids (e.g. hydrocortisone cream).
Grade 2 Moderate[1]	Intense or widespread; intermittent; skin changes from scratching (e.g., edema, papulation, excoriations, lichenification, oozing/crusts); oral intervention indicated; limiting instrumental ADL	See Grade 1. In inflammatory lesions consider topical steroids (e.g. hydrocortisone cream).
Grade 3 Severe	Intense or widespread; constant; limiting self-care ADL or sleep; oral corticosteroid or immunosuppressive therapy indicated	See Grade 2. Topical steroids of higher potency (e.g. prednicarbate, mometasone furoate) Consider oral antibiotics
FISSURES		
Grade 1 Mild	Asymptomatic	Petroleum jelly, Vaseline® or Aquaphor for 30 minutes under plastic occlusion every night, followed by application of hydrocolloid dressing; antiseptic baths (e.g. potassium permanganate therapeutic baths, final concentration of 1:10,000, or povidone-iodine baths). Topical application of aqueous silver nitrate solutions to fissures
Grade 2 Moderate[1]	Symptomatic, not interfering with ADL	See Grade 1. Consider oral antibiotics.
Grade 3 Severe	Symptomatic, Interfering with ADL	See Grade 2.

[1] If Grade 2 rash persists for ≥ 7 days despite treatment and is poorly tolerated by the patient, the investigator may choose to
pause treatment up to 6 weeks followed by a reduction in the dose of panitumumab according to the dose reduction
scheme in Section 10.3.

10.6. Management of Diarrhea

The prescribing information[44] for irinotecan (CAMPTOSAR) includes the following black-box warning: **"Early and late forms of diarrhea can occur. Early diarrhea may be accompanied by cholinergic symptoms which may be prevented or ameliorated by atropine. Late diarrhea can be life threatening and should be treated promptly with loperamide. Monitor patients with diarrhea and give fluid and electrolytes as needed.**

Institute antibiotic therapy if patients develop ileus, fever, or severe neutropenia. Interrupt CAMPTOSAR and reduce subsequent doses if severe diarrhea occurs."

Prior to initiation of irinotecan, patients should be counseled on the appropriate use of loperamide. Investigators may wish to ensure patients are given a supply of loperamide to keep with them at all times, and additional investigator consideration may be given to initiation of a prophylactic anti-diarrheal regimen (similar to that outlined above in Section 9). Patients must be advised to drink an adequate amount of fluids to make up for the fluid lost through diarrhea. Additionally, please see Section 10.8, regarding management of electrolyte abnormalities, including hypomagnesemia.

As diarrhea may lead to dehydration and compel treatment modification or discontinuation, early management of diarrhea is essential (Table 8):

TABLE 9: Treatment Recommendations for Management of Diarrhea

Severity (CTCAE Grading)	Description	Specific intervention
Grade 1 Mild	Increase of < 4 stools per day over baseline; mild increase in ostomy output compared with baseline	Stop any laxatives, consider initiation of loperamide (if not already taking as prophylaxis); advise patient to drink at least 8-10 glasses of water of clear fluids per day.
Grade 2 Moderate	Increase of 4 - 6 stools per day over baseline; moderate increase in ostomy output compared to baseline	Continue loperamide; assess for dehydration and electrolyte imbalance; consider IV fluids and electrolyte replacement.
Grade 3 Severe	Increase of ≥ 7 stools per day over baseline; incontinence; hospitalization indicated; severe increase in ostomy output compared to baseline; limiting self-care ADL	See Grade 2; plus: an infectious process should be ruled out with stool cultures; aggressive iv fluid replacement; hospitalization to monitor progress; consider prophylactic antibiotics if patient is also neutropenic.
Grade 4 Life threatening	Life-threatening consequences (e.g. haemodynamic collapse)	See Grade 3.

10.7. Guidelines for the Management of Infusion Reactions

Infusion reactions/hypersensitivity reactions have been reported with panitumumab and irinotecan. An infusion-related reaction is defined as an adverse event occurring during and up to 1 hour after the end of a panitumumab or irinotecan infusion, which is assessed by the investigator to be at least possibly related to the infusion of panitumumab or irinotecan.

Symptoms of an infusion-related reaction may possibly include but are not limited to fever, chills, rigors, diaphoresis, and headache. According to established local procedures, monitor patients during and following an infusion for signs of hypersensitivity and infusion-related reactions with resuscitation equipment readily available.

Immediately and permanently discontinue respective treatment (i.e. panitumumab or irinotecan for Grade 3 or Grade 4 infusion-related reaction. For Grade 1 or Grade 2 infusion-related reaction, adjust dose per Table 7 below (in conjunction with any overriding more conservative local policies and procedures for the management of infusion-related reaction).

TABLE 10: Management Recommendations for Hypersensitivity/Infusion-Related Reactions (IRRs)

Toxicity Grade	Management Recommendations (Any Occurrence)
Grade 1 Mild transient reaction.	• **Decrease infusion rate by 50% for the duration of infusion.**[a] • Monitor closely for worsening of condition.
Grade 2 Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (e.g. antihistamines, NSAIDs, narcotics, iv fluids); prophylactic medications indicated for ≤ 24 h.	• **Stop the infusion.** • Administer symptomatic treatment (e.g. antihistamines, NSAIDs). • Use supportive treatment (e.g. bronchodilator, oxygen), if necessary. • **When the reaction has resolved to Grade 0 or 1, resume infusion at a 50% decreased infusion rate.**[a] • Monitor closely for worsening of condition. • If symptoms reoccur, stop the infusion, institute remedial therapy, monitor closely and evaluate whether the patient can continue the trial.
Grade 3 Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae.	• **Stop the infusion.** • Immediately and permanently discontinue treatment associated with the toxicity. • Administer symptomatic and supportive treatment (e.g. bronchodilator, antihistamine, glucocorticoid, iv fluid, oxygen) as needed. • **Withdraw the patient from treatment associated with the toxicity.**
Grade 4 Life-threatening consequences; urgent intervention indicated.	• **Stop the infusion.** • Immediately and permanently discontinue treatment associated with the toxicity. • Administer necessary life-support measures as needed. • **Withdraw the patient from treatment associated with the toxicity.**

[a] Once the infusion rate has been reduced for a Grade 1 or 2 hypersensitivity/infusion related reaction, it is recommended that the lower infusion rate be utilized for all subsequent infusions.

All Grade 3 or 4 infusion reactions will be evaluated as to whether or not the event is a DLT. All infusion reactions that are serious adverse events (per Section 12) must be rapidly reported as such via notification procedures described in Section 12.

10.8. Management of Electrolyte Abnormalities, Including Hypomagnesemia

Closely monitor serum electrolytes, including serum magnesium, potassium, and calcium on days of infusion and aggressively replenish when warranted. Monitor for hypomagnesemia, hypocalcemia, and hypokalemia during treatment and during study follow-up.

Withhold for Grade 3 or 4 electrolyte abnormalities if indicated by protocol or investigator judgment. Replete electrolytes as medically appropriate.

10.9. Management of Pulmonary Toxicity

Fatal and nonfatal cases of interstitial lung disease (ILD) (1%) and pulmonary fibrosis have been observed in patients treated with panitumumab. Pulmonary fibrosis occurred in less than 1% (2/1467) of patients enrolled in clinical studies of panitumumab. **In the event of acute onset or worsening of pulmonary symptoms, interrupt panitumumab therapy. Discontinue panitumumab therapy if interstitial lung disease (ILD) is confirmed.**

In patients with a history of interstitial pneumonitis or pulmonary fibrosis, or evidence of interstitial pneumonitis or pulmonary fibrosis, the benefits of therapy with panitumumab versus the risk of pulmonary complications must be carefully considered.

Interstitial Pulmonary Disease (IPD)-like events, including fatalities, have occurred in patients receiving irinotecan (in combination and as monotherapy). Risk factors include pre-existing lung disease, use of pneumotoxic drugs, radiation therapy, and colony stimulating factors.

Patients with risk factors should be closely monitored for respiratory symptoms before and during irinotecan therapy. In Japanese studies, a reticulonodular pattern on chest x-ray was observed in a small percentage of patients. **New or progressive, dyspnea, cough, and fever should prompt interruption of irinotecan, pending diagnostic evaluation. If interstitial pulmonary disease (IPD) is diagnosed, irinotecan should be discontinued and appropriate treatment instituted as needed.**

10.10. Management of Ocular Toxicity

Keratitis and ulcerative keratitis, known risk factors for corneal perforation, have been reported with panitumumab use. Monitor for evidence of keratitis or ulcerative keratitis. **Interrupt or discontinue panitumumab therapy for acute or worsening keratitis.**

Photophobia and related ocular toxicities (e.g., photopsia) have been identified as CB-839-related events. In almost all cases, these events have been Grade 1 and generally do not have an impact on the patient's daily life, although one Grade 2 event required the patient rest for 1-2 hr in a dark room. These events appear to occur around Cmax (1-4 hr after dosing) and resolve with time. Anecdotal reports suggest that tachyphylaxis develops and these events tend to become less frequent over extended dosing. More severe cases can be managed with dose reduction and the use of sunglasses may be considered.

11. DRUG FORMULATION, SUPPLY, AND STORAGE

11.1. Description of CB-839

CB-839 is an oral small molecule inhibitor of glutaminase 1 under investigation for the treatment of various solid and hematologic malignancies, where alterations in glutamine metabolism may play a role in cancer cell growth and survival.

11.2. Packaging of CB-839

Calithera will supply CB-839 to the site.
CB-839 is intended for oral administration, and typically available in unit strength of 200 mg capsules or tablets manufactured, packaged, and labeled according to current Good Manufacturing Practices (cGMP).

11.3. Handling and Storage of CB-839

CB-839 capsules and tablets meet specifications for appearance, identity, purity, strength, and dissolution. Stability studies being carried out on the Phase I clinical supplies support storage of CB-839 Capsules for at least 24 months at room temperature.

CB-839 HCl Capsules and 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.

Store CB-839 HCl capsules and tablets in the 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)). 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.

11.4. Description of Panitumumab

Panitumumab (VECTIBIX) is a recombinant, human IgG2 kappa monoclonal antibody that binds specifically to the human epidermal growth factor receptor (EGFR). Panitumumab binds to the ligand-binding domain 2 (L2 domain) of the human epidermal growth factor receptor (EGFR), thus blocking the tyrosine phosphorylation of EGFR induced by known ligands (e.g. EGF, TGF-α, amphiregulin, betacellulin, HB-EGF, and epiregulin).
Panitumumab is FDA-approved for the treatment of wild-type *KRAS* (exon 2 in codons 12 or 13) metastatic colorectal cancer (mCRC): In combination with FOLFOX for first-line treatment; and as monotherapy following disease progression after prior treatment with fluoropyrimidine, oxaliplatin, and irinotecan-containing chemotherapy.

11.5. Packaging of Panitumumab

The site will obtain panitumumab from commercial supply.
Panitumumab is typically available as a sterile, colorless, preservative-free solution containing 20 mg/mL panitumumab (Vectibix) in a single-use vial.

As one vial per carton, each 5 mL single-use vial contains 100 mg of panitumumab in 5 mL (20 mg/mL); each 10 mL single-use vial contains 200 mg of panitumumab in 10 mL (20 mg/mL); and each 20 mL single-use vial contains 400 mg of panitumumab in 20 mL (20 mg/mL).
Panitumumab is a sterile, colorless, pH 5.6 to 6.0 liquid for intravenous (IV) infusion, which may contain a small amount of visible translucent-to-white, amorphous, proteinaceous, panitumumab

particulates. Each single-use 5 mL vial contains 100 mg of panitumumab, 29 mg sodium chloride, 34 mg sodium acetate, and Water for Injection, USP. Each single-use 10 mL vial contains 200 mg of panitumumab, 58 mg sodium chloride, 68 mg sodium acetate, and Water for Injection, USP. Each single-use 20 mL vial contains 400 mg of panitumumab, 117 mg sodium chloride, 136 mg sodium acetate, and Water for Injection, USP.

11.6. Handling and Storage of Panitumumab

Store panitumumab vials in the original carton under refrigeration at 2° to 8°C (36° to 46°F) until time of use. Protect from direct sunlight. DO NOT FREEZE. Since panitumumab does not contain preservatives, any unused portion remaining in the vial must be discarded.
The diluted infusion solution of Vectibix should be used within 6 hours of preparation if stored at room temperature, or within 24 hours of dilution if stored at 2° to 8°C (36° to 46°F). DO NOT FREEZE. For preparation instructions, please see the product label.

11.7. Description of Irinotecan

Irinotecan (CAMPTOSAR) is a topoisomerase inhibitor. Irinotecan is a derivative of camptothecin. Camptothecins interact specifically with the enzyme topoisomerase I, which relieves torsional strain in DNA by inducing reversible single-strand breaks. Irinotecan and its active metabolite SN-38 bind to the topoisomerase I-DNA complex and prevent re-ligation of these single-strand breaks.
Irinotecan is FDA approved, in combination with 5-fluorouracil and leucovorin, for patients with metastatic carcinoma of the colon or rectum; and patients with metastatic carcinoma of the colon or rectum whose disease has recurred or progressed following initial fluorouracil-based therapy.

11.8. Packaging of Irinotecan

The site will obtain irinotecan from commercial supply.
Irinotecan is typically available in three single-dose sizes: 2, 5 and 15 mL-fill vials, respectively containing 40, 100 and 300 mg irinotecan hydrochloride.

11.9. Handling and Storage of Irinotecan

Irinotecan vials should be stored consistent with the product label[44] at controlled room temperature 15° to 30°C (59° to 86°F). Protect from light. Keep the vial in the carton until time of until time of use. For preparation instructions, please see the product label.

11.10. Drug Accountability and Compliance Check

The Investigator is responsible for ensuring accountability for CB-839, panitumumab and irinotecan, including maintenance of adequate drug accountability records.

Drug accountability records should include an appropriate inventory of CB-839, panitumumab and irinotecan including:

- Confirmation that CB-839 supplied by the study was delivered to the trial site
- Record of each dose of CB-839, panitumumab and irinotecan dispensed
- Return of unused CB-839 provided by the study to the sponsor-investigator or designee, or documentation of destruction at site (if drug destruction by the site is authorized by sponsor-investigator or designee).

Records should specify relevant dates, quantities, batch numbers, use-by dates and patient numbers, as applicable.

The Investigator, or designee, should maintain records that adequately document:
- That patients were provided the doses specified by the clinical trial protocol, and
- That all CB-839 provided by the study was fully reconciled

12. SAFETY REPORTING OF ADVERSE EVENTS

12.1. General

Safety assessments will consist of monitoring and appropriate reporting AEs and SAEs that are considered possibly, probably or definitely related to **the investigational treatment (i.e. to CB-839, or to the combination of CB-839 + panitumumab/irinotecan) and research ^{18}F-FSPG PET/CT scans**, all events of death, and any study-specific issue of concern.

Adverse event collection and reporting is a routine part of every clinical trial. Each adverse event will be graded according to the NCI's Common Terminology Criteria for Adverse Events (NCI CTCAE), Version 4.03, dated June 14, 2010, currently locatable via the following URL:

http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf

For events not listed in the CTCAE, severity will be designated as mild, moderate, severe or life threatening, or fatal which respectively correspond to Grades 1, 2, 3, 4, and 5 on the NCI CTCAE, with the following definitions:

- **Mild:** An event not resulting in disability or incapacity and which resolves without intervention;
- **Moderate:** An event not resulting in disability or incapacity but which requires intervention;
- **Severe:** An event resulting in temporary disability or incapacity and which requires intervention;
- **Life-threatening:** An event in which the patient was at risk of death at the time of the event;
- **Fatal:** An event that results in the death of the patient.

Information on all adverse events, whether serious or not, whether reported by the participant, directly observed, or detected by physical examination, laboratory test or other means, will be collected, recorded, followed and reported as described in the following sections.

Reporting period

Adverse events experienced by participants will be collected and reported from initiation of protocol-indicated treatment for Phase I patients. For Phase II patients, AEs will be collected and reported from the time of the first research scan. AEs will be tracked for all patients in both phases throughout the study through 28 days after the last dose of protocol-indicated treatment. Participants who experience an adverse event related to a study procedure and/or protocol-indicated treatment beyond 28 days will continue to be contacted by a member of the study team until the event is resolved, stabilized, or determined to be irreversible by the participating investigator.

Laboratory and vital sign abnormalities are to be recorded as Adverse Events only if they are medically relevant as judged by the investigator (e.g. symptomatic, requiring corrective treatment, leading to discontinuation and/or fulfilling a seriousness criterion). Unless otherwise specified by protocol or a patient's study physician, abnormal laboratory values of Grade 1 and Grade 2 will be deemed Not Clinically Significant (NCS) and are not required to be individually noted or recorded within the study data.

Baseline disease-related signs and symptoms which are initially recorded as medical history, will subsequently be recorded as adverse events during the trial if they worsen in severity or increase in frequency.

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. The investigator should notify the IRB and any other applicable regulatory agency of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

12.2. Risks Associated with CB-839

- The more common adverse reactions (seen in ≥ 10% of patients) of CB-839 include: fatigue, abnormal liver function tests (alanine aminotransferase, aspartate transaminase, and alkaline phosphatase), and nausea.
- The less common adverse reactions (5-10%) of CB-839 include: anemia, vomiting, discomfort in the eyes due to light exposure and sensitivity to light, and decreased appetite.
- A more detailed safety profile of CB-839 is provided in the investigator's brochure.[24]

12.3. Risks Associated with Panitumumab and Irinotecan

- The most common adverse reactions (≥ 20%) of panitumumab as monotherapy are: skin rash with variable presentations, paronychia, fatigue, nausea, and diarrhea.
- The most common adverse reactions (≥ 20%) in clinical trials of panitumumab in combination with FOLFOX chemotherapy are: diarrhea, stomatitis, mucosal inflammation, asthenia, paronychia, anorexia, hypomagnesemia, hypokalemia, rash, acneiform dermatitis, pruritus, and dry skin.
- Common adverse reactions (≥30%) observed in single agent therapy clinical studies of irinotecan are: nausea, vomiting, abdominal pain, diarrhea, constipation, anorexia, neutropenia, leukopenia (including lymphocytopenia), anemia, asthenia, fever, body weight decreasing, alopecia.
- Common adverse reactions (≥30%) observed in combination therapy clinical studies of irinotecan are: nausea, vomiting, abdominal pain, diarrhea, constipation, anorexia, mucositis, neutropenia, leukopenia (including lymphocytopenia), anemia, thrombocytopenia, asthenia, pain, fever, infection, abnormal bilirubin, alopecia.
- A more detailed safety profile of panitumumab and irinotecan are provided in the respective product labels, currently locatable online via following:

  Panitumumab (VECTIBIX):

  http://pi.amgen.com/united_states/vectibix/vectibix_pi.pdf

  Irinotecan (CAMPTOSAR):

  http://labeling.pfizer.com/ShowLabeling.aspx?format=PDF&id=533

12.4. Risks Associated with ^{18}F-FSPG PET/CT (optional for Phase I and required for Phase II) and ^{11}C-Glutamine PET/CT (optional for Phase I only)

- Both the PET and CT expose the subject to radiation.
- Patients should not be pregnant while on this study because the imaging agent being tested contains a small amount of radioactivity. This could affect an unborn baby.
- Risks and side effects related to the IV catheter may include discomforting pain at the site of injection, bleeding, and bruising.

12.5. Safety Parameters and Definitions

12.5.1 Adverse Event (AE)

An adverse event is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or any procedure specified in the protocol, even if the event is not considered to be related to the study.

Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

12.5.2 Serious adverse event (SAE)

A serious adverse event is an undesirable sign, symptom or medical condition which:

- is fatal or life-threatening;
- requires or prolongs inpatient hospitalization;
- results in persistent or significant disability/incapacity;
- constitutes a congenital anomaly or birth defect; or
- jeopardizes the participant and requires medical or surgical intervention to prevent one of the outcomes listed above.

Events **not** considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care

12.6. Assessment of Adverse Events

All AEs and SAEs, whether volunteered by the patient, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means, will be reported appropriately. Each reported AE or SAE will be described by its duration (i.e. start and end dates), regulatory seriousness criteria if applicable, suspected relationship to the investigational treatment (see following guidance), and actions taken.

To ensure consistency of AE and SAE causality assessments, investigators should apply the following general guidelines:

- **Yes**
  There is a reasonable causal relationship between the investigational treatment and the adverse event. There is a plausible temporal relationship between the onset of the AE and administration of the investigational treatment, and the AE cannot be readily explained by the patient's clinical state, intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response to the investigational treatment; and/or the AE abates or resolves upon discontinuation of the investigational treatment or dose reduction and, if applicable, reappears upon re-challenge.

- **No**
  There is no reasonable causal relationship between the investigational treatment administered and the adverse event. Evidence exists that the AE has an etiology other than the investigational treatment (e.g. pre-existing medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to administration of the investigational treatment.

12.6.1 Expectedness

- Expected: Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.
- Unexpected: An adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk

12.6.2 Attribution

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

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

12.7. Reporting Procedures

12.7.1 Specific Instructions for Recording Adverse Events

Investigators should use correct medical terminology/concepts when reporting AEs or SAEs. Avoid colloquialisms and abbreviations. All adverse events will be captured on the appropriate study-specific case report forms (CRFs).

12.7.2 Diagnosis versus Signs and Symptoms

If known at the time of reporting, a diagnosis should be reported rather than individual signs and symptoms (e.g. record only liver failure or hepatitis rather than jaundice, asterixis, and elevated

transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, it is acceptable to report the information that is currently available. If a diagnosis is subsequently established, it should be reported as follow-up information.

12.7.3 Deaths

All deaths that occur during the protocol-specified AE reporting period, regardless of attribution, will be reported to the appropriate parties. When recording a death, the event or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report "Unexplained Death." Deaths that occur during the protocol specified adverse event reporting period that are attributed by the investigator solely to progression of disease should be recorded only in the study CRF.

12.7.4 Pre-existing Medical Conditions

A pre-existing medical condition is one that is present at the start of the study. Such conditions should be reported as medical and surgical history. A pre-existing medical condition should be re-assessed throughout the trial and reported as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important to convey the concept that the pre-existing condition has changed by including applicable descriptors (e.g., "more frequent headaches").

12.7.5 Hospitalizations for Medical or Surgical Procedures

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE. If a patient is hospitalized to undergo a medical or surgical procedure as a result of an AE, the event responsible for the procedure, not the procedure itself, should be reported as the SAE. For example, if a patient is hospitalized to undergo coronary bypass surgery, record the heart condition that necessitated the bypass as the SAE.

Hospitalizations for the following reasons do not require reporting:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for pre-existing conditions,
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study, or
- Hospitalization or prolonged hospitalization for scheduled therapy of the target disease of the study.

12.7.6 Pregnancies in Female Patients

Female patients of childbearing potential will be instructed to immediately inform the investigator if they become pregnant during the study or within 2 months after the last dose of protocol-indicated treatment. Pregnancies should be reported by the site immediately (i.e., no more than 24 hours after learning of the pregnancy) using the Vanderbilt SAE form and submitted via fax or email. Unless otherwise agreed to and approved by the sponsor-investigator, investigator and the IRB, the patient should discontinue protocol-indicated treatment. The investigator or medical designee should counsel the patient, discussing the risks of the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy. Any SAEs associated with the pregnancy (e.g. an event in the fetus, an event in the mother during or after the pregnancy, or a congenital anomaly/birth defect in the child) should be reported.

12.7.7 Pregnancies in Female Partners of Male Patients

Male patients will be instructed through the informed consent form to immediately inform the investigator if their partner becomes pregnant during the study or within 2 months after completing protocol-indicated treatment. Male patients who received study treatment should not attempt to father a child until 2 months after stopping study treatment. Any pregnancies should be reported by the site immediately (i.e., no more than 24 hours after learning of the pregnancy) using the Vanderbilt SAE form and submitted via fax or email. Attempts should be made to collect and report details of the course and outcome of any pregnancy in the partner of a male patient exposed to study drug.
The pregnant partner may be asked to sign an Authorization for Use and Disclosure of Pregnancy Health Information to allow for follow-up on her pregnancy. Once the authorization has been signed, the will provide additional information on the course and outcome of the pregnancy. An investigator or medical designee who is contacted by the male patient or his pregnant partner may provide information on the risks of the pregnancy and the possible effects on the fetus, to support an informed decision in cooperation with the treating physician and/or obstetrician.

12.7.8 Post-Study Adverse Events

The investigator should expeditiously report any SAE occurring after a patient has completed or discontinued study participation if attributed to previous protocol-indicated CB-839 or CB-839 + panitumumab/irinotecan exposure. If the investigator should become aware of the development of cancer or a congenital anomaly in a subsequently conceived offspring of a female patient who participated in the study, this should be reported as an SAE.

12.7.9 Serious Adverse Events

All serious adverse events, regardless of causality to study drug, will be reported to the Principal Investigator and/or the Study Coordinator at each institution, and also to the Coordinating Center.

All serious adverse events must be reported to the Coordinating Center within 24 hours of the investigator becoming aware of the event. Events should be reported using the Vanderbilt SAE form, located in the packet of supplemental forms. This form must be fully completed and emailed (preferred), faxed, or scanned to:

Follow-up information must also be reported within 24 hours of receipt of the information by the investigator.

The Coordinating Center will disseminate information regarding serious adverse events to the participating sites as described in FDA guidance only in the case that the event(s) is/are unexpected, and is/are believed to be related (i.e., possibly, probably or definitely) to the study device/medication. The Coordinating Center will be responsible for reporting of events to the FDA and supporters, as appropriate (outlined below).

12.7.10 Institutional Review Board

All adverse events and serious adverse events will be reported to the IRB per current institutional standards. If an adverse event requires modification of the informed consent, these modifications will be provided to the IRB with the report of the adverse event. If an adverse event requires modification of the study protocol, these modifications will be provided to the IRB as soon as is possible.

12.7.11 Food and Drug Administration (FDA)

In this trial, unexpected serious adverse events believed to be definitely, probably, or possibly related to study treatment (as determined by the sponsor-investigator) will be reported to the FDA via MedWatch 3500A (available at https://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM048334.pdf. Submissions by the sponsor can be submitted via fax or email and must be addressed to Regulatory Project Manager in the FDA review division that has responsibility for review of the IND. The Coordinating Center will be responsible for correspondence regarding adverse events with the FDA for all participating sites.

12.7.12 Expedited 7 and 15 Day Reporting Requirements for IND

Events meeting the following criteria must be submitted to the FDA as expedited IND Safety Reports according to the following guidance and timelines:

7 Calendar Day Telephone or Fax Report

The sponsor-investigator is required to notify the FDA of any **fatal or life-threatening** AE that is **unexpected** and **suspected** by the sponsor-investigator to be possibly, probably or definitely related to protocol-indicated treatment. The sponsor-investigator must notify the FDA of such events as soon as possible but in no case later than 7 calendar days after the sponsor-investigator's initial receipt of the information.

15 Calendar Day Written Report

The sponsor-investigator is required to notify the FDA and all participating investigators, in a written IND Safety Report, of any **serious unexpected** adverse event **suspected** by the sponsor-investigator to be possibly, probably, or definitely related to protocol-indicated treatment. The sponsor-investigator must notify the FDA of such events no later than 15 calendar days after the sponsor-investigator's initial receipt of the information.

Written IND Safety reports should include an analysis of similar events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed by the sponsor-investigator with the IND concerning similar events should be analyzed and the significance of the new report in light of the previous, similar reports commented on.

Written IND safety reports with analysis of similar events are to be submitted to the FDA and all participating investigators as soon as possible but not later than 15 calendar days after the sponsor-investigator determines that the information qualifies for reporting.

The sponsor-investigator must submit each IND safety report in a narrative format or on FDA Form 3500A or in an electronic format that FDA can process, review, and archive. FDA will periodically issue guidance on how to provide the electronic submission (e.g., method of transmission, media, file formats, preparation and organization of files).

Each notification to FDA must bear prominent identification of its contents, i.e., "IND Safety Report," and must be transmitted to the review division in the Center for Drug Evaluation and Research (CDER) or in the Center for Biologics Evaluation and Research (CBER) that has responsibility for review of the IND.

Upon request from FDA, the sponsor-investigator must submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

Upon request from Calithera, copies of IND safety reports submitted to the FDA may also be submitted to Calithera Biosciences, to the attention of a point-of-contact specified by Calithera.

12.7.13 Safety data reporting to Calithera

All SAEs, regardless of causality, will be reported in parallel to Calithera Bioscience's PharmacoVigilance agent according to the Safety Data Exchange Agreement at the following:

- [redacted]

12.7.14 Reporting of Adverse Events related to Investigational PET/CT scans

The Principal Investigator (PI) and attending nuclear medicine physician(s) will monitor the study progress and safety of the participating patients and will monitor for serious symptoms or toxicities related to the investigational imaging on an ongoing basis. The PI or their designee will undertake reporting any significant adverse events to the IRB and FDA as required. Adverse events during each research PET scan visit will be tracked using a case report form. Adverse events following each PET scan visit will be tracked using the "Adverse Event Tracking for PET Scan Studies Utilizing FDA INDs 113638 (^{18}F-FLT), 120035 (^{18}F-Fallypride), 119446 (^{18}F-FES), 120704 (^{11}C-Acetate) and 124202 (^{18}F-FSPG)" Form. Patients will be contacted by phone to determine whether they experienced any adverse events within the 24 hours following the injection of the tracer. Patients will be contacted no sooner than 24 hours after and no later than 48 hours following the time of tracer injection. Adverse events related to the investigational imaging will be immediately reported (within three working days) to the IRB and FDA as required. These adverse events will be recorded in the database maintained by the PI or their designee.

Adverse events for the purposes of the investigational imaging in this trial are defined as any unexpected medical occurrence in a subject who receives ^{18}F-FSPG. The event does not necessarily have to be causally related to the PET radiopharmaceutical to qualify as an adverse event. An adverse event can be any unfavorable or unintended sign, symptom, or disease temporally associated with the injection of the radiopharmaceuticals, whether or not it is

considered related to the ^{11}C-Glutamine PET/CT (optional in Phase I only) or ^{18}F-FSPG PET/CT.

13. DATA SAFETY AND MONITORING

13.1. Data Management and Reporting

Data will be collected using a centralized electronic case report form called ON-line Clinical Oncology Research Environment (OnCore), currently locatable at:

[redacted]

OnCore is a highly secure, web based, cancer specific, and customizable system that provides fully integrative clinical data management and study administration capabilities developed in an ongoing collaborative effort with NCI designated Comprehensive Cancer Centers. The system is capable of storing basic protocol information (e.g. IRB approval dates, dates for annual renewals) and clinical trials research data, and it fully integrates study administration functionality including protocol tracking, patient registration, NCI reporting, review committee tracking, and SAE tracking, with clinical data management functionality including electronic case report forms (eCRF) design, clinical data capture, protocol and regulatory compliance monitoring.

OnCore allows the investigator to define specific protocol requirements and generate data collection forms. Creation of the data collection form is done with a single button click after the parameters of an individual protocol have been specified. OnCore permits specification of study protocols, management of patient enrollment, clinical data entry and viewing, and the generation of patient or study-specific reports based on time stamping. OnCore is embedded with a comprehensive domain repository of standard reference codes and forms to promote standardization. The sources for the repository include CDUS, CTC, CDEs from NCI, ICD, MedDRA and various best practices from contributing NCI-designated Comprehensive Cancer Centers. OnCore provides several reporting features specifically addressing NCI Summary 3 and Summary 4 and other reporting requirements. Data may also be exported in a format suitable for import into other database, spreadsheets or analysis systems (such as SPSS). This system will be used to manage all VICCC clinical trials data. OnCore is maintained and supported in the VICC Clinical and Research Informatics Resource.

Specified site members will submit all pertinent regulatory documents to designee(s) of the sponsor-investigator, who will store such items in a secure location.

The Principal Investigator or designee will inform Calithera as defined in any established Safety and Data Exchange Agreement (SDEA) of any serious adverse event, and will inform the Vanderbilt IRB in accordance with IRB policy. The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE or SAE, as provided in this protocol. During the study when there is a safety evaluation, the treating investigator or site staff will be responsible for detecting, documenting, and report AEs and SAEs, as detailed in the protocol. If any problem appropriate for review is identified related to the conduct of this research, the VICC Data Safety and Monitoring Committee (DSMC) will be formally asked to review the study and the situation that required DSMC intervention.

13.2. Meetings

This trial will be monitored by the VICC Gastrointestinal Oncology research team. The GI oncology research team is composed of Medical Oncologists, Research Nurses, Data Managers, and Regulatory Specialists. The team meets informally weekly and officially on a monthly basis to discuss all AEs/SAEs, accrual, compliance, safety issues, adherence to protocol, etc. pertaining to cancer studies conducted by the GI oncology team. This particular study will be thoroughly reviewed during these meetings.

13.3. Auditing and Monitoring

The Vanderbilt-Ingram Cancer Center (VICC) oversees patient safety and data monitoring for its investigator-initiated and NIH-NCI funded clinical trials through its Data and Safety Monitoring Committee (DSMC). The purpose of the DSMC is to ensure the efficient implementation and management of VICC Data and Safety Monitoring Plan (DSMP). The Committee maintains authority to intervene in the conduct of studies as necessary to ensure clinical research performed at VICC or by additional sites for VICC Investigator Initiated Trials, achieves the highest quality standards.

The VICC DSMC meets on a quarterly basis and ad hoc to discuss data and safety monitoring of clinical trials and to oversee the VICC DSMP. Internal audits for compliance with adverse event reporting, regulatory and study requirements, and data accuracy and completion are conducted according to the VICC DSMP according to study phase and risk. The committee reviews all serious adverse events (SAE) on Vanderbilt sponsored investigator-initiated studies on a quarterly basis and provides DSMC SAE review reports to the Vanderbilt IRB.

The trial additionally will be monitored by the VICC Multi-Institutional Coordinating Center. The actual frequency of monitoring will depend on the enrollment rate and performance of the site. Monitoring will be conducted through onsite and/or remote monitoring, teleconferences with the Investigator and site staff, and appropriate communications by mail, fax, email, or telephone. The purpose of monitoring is to ensure that the study is conducted in compliance with the protocol, standard operating procedures (SOPs), and other written instructions, and to ensure the quality and integrity of the data.

During scheduled monitoring visits, investigators and the investigational site staff must be available to discuss the progress of the trial, make necessary corrections to case report form entries, respond to data clarification requests, provide required regulatory documents, and respond to any other trial-related inquiries of the monitor.

In addition to the above, the FDA may review the conduct or results of the study at the investigational site.

13.4. Data Handling and Record Keeping

Electronic case report forms (eCRF) are required and must be completed for each included participant. The completed dataset should not be made available in any form to third parties, except for authorized representatives of appropriate Health/Regulatory Authorities, without written permission from Vanderbilt.

To enable evaluations and/or audits from health authorities and Vanderbilt, the investigator agrees to keep records including: The identity of all participants (sufficient information to link records; e.g., hospital records), all original signed informed consent forms, copies of all source documents, and detailed records of drug disposition. The records should be retained by the investigator in compliance with regulations.

During data entry, range and missing data checks will be performed online. The checks to be performed will be documented in the Data Monitoring Plan for the study. A summary report (QC Report) of these checks together with any queries resulting from manual review of the eCRFs will be generated for each site and transmitted to the site and the site monitor. Corrections will be made by the study site personnel. This will be done on an ongoing basis.

14. REGULATORY CONSIDERATIONS

14.1. Pre-Study Documentation

Prior to initiating the trial, the investigator will secure and provide to the Coordinating Center essential documents, including but not limited to:

- A signed FDA Form 1572
- A current curriculum vitae for the Principal Investigator and each sub-investigator listed on the FDA Form 1572
- A copy of the current medical license for the investigator
- A letter from the IRB stipulating approval of the protocol, the informed consent document and any other material provided to potential trial participants with information about the trial (e.g. advertisements)
- A copy of the IRB-approved informed consent document
- The current IRB membership list for the reviewing IRB
- A completed financial disclosure form for the investigator and all sub- investigators
- Current laboratory certification for the reference laboratory and curriculum vitae of the laboratory director
- A list of current laboratory normal values for the reference laboratory.

14.2. Protocol Review and Amendments

Information regarding study conduct and progress will be reported to the Institutional Review Board (IRB) per current institutional standards.

The trial will not be initiated until there is approval by the local IRB of the protocol, informed consent document and any other material used to inform the patient about the nature of the trial. The IRB should be duly constituted according to local regulatory requirements. The investigator will inform the IRB of the progress of the trial at least yearly.

Any changes to the protocol will be made in the form of a written amendment and must be approved by the sponsor-investigator and the local IRB prior to local implementation. All amendments will also be submitted as necessary to the FDA by the sponsor-investigator (or designee).

Departures from the protocol to eliminate an immediate hazard to a trial patient may be implemented by the investigator immediately. The investigator must then immediately inform

the local IRB; and the sponsor-investigator (or designee), who will communicate as appropriate with the FDA.

The sponsor-investigator (or designee) is responsible for the coordination and development of all protocol amendments, and will disseminate this information to the participating centers.

14.3. Informed Consent

The investigator (or designee) will explain to each subject the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject will be informed that participation in the study is voluntary, that s/he may withdraw from the study at any time, and that withdrawal of consent will not affect subsequent medical treatment or relationship with the treating physician(s) or institution. The informed consent will be given by means of a standard written statement, written in non-technical language, which will be IRB approved. The subject should read and consider the statement before signing and dating it, and will be given a copy of the document. No subject will enter the study or have study-specific procedures done before his/her informed consent has been obtained.

In accordance with the Health Information Portability and Accountability Act (HIPAA), the written informed consent document (or a separate document to be given in conjunction with the consent document) will include a subject authorization to release medical information to the study sponsor and supporting agencies and/or allow these bodies, a regulatory authority, or Institutional Review Board access to subjects' medical information that includes all hospital records relevant to the study, including subjects' medical history.

14.4. Ethics and GCP

This study will be carried out in compliance with the protocol and Good Clinical Practice, as described within:

1. ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.
2. US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).
3. Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).

The investigator agrees to adhere to the instructions and procedures described within the above and thereby to adhere to the principles of Good Clinical Practice with which the above conform.

14.5. Confidentiality

It is the responsibility of the investigator to ensure that the confidentiality of all patients participating in the trial and all of their medical information is maintained. Case report forms (CRFs) and other documents submitted to regulatory authorities must not contain the name of a trial patient. All patients in the trial will be identified by a unique identifier which will be used on all CRFs and any other material submitted to regulatory authorities. All case report forms and any identifying information must be kept in a secure location with access limited to the study staff directly participating in the trial.

14.6. Study Documentation

Each participating site is responsible for submitting copies of all relevant regulatory documentation to the Coordinating Center. The required documents include, but are not limited to the following: local IRB approvals (i.e., protocol, consent form, amendments, patient brochures and recruitment material, etc.), IRB membership rosters, summary of unanticipated problems or protocol deviations, and documentation of expertise of the investigators. The Coordinating Center will provide each participating site with a comprehensive list of the necessary documents. It is the responsibility of the participating sites to maintain copies of all documentation submitted to the Coordinating Center.

The requirements for data management, submissions, and monitoring are outlined below. The participating sites will submit all the research related information as applicable (source documents and research records – IRB approval documents, patient registration list, CRF info, toxicity assessments, tumor measurements / responses, etc.) to the Coordinating Center.

14.7. Study Termination

The sponsor-investigator reserves the right to terminate the study at any site and at any time. Reasons for study termination may include, but are not limited to, the following:

- Investigator non-compliance with the protocol, GCP or regulatory requirements
- Insufficient enrollment
- Safety concerns
- Decision by suppliers to modify or discontinue the availability, development or manufacture of protocol-indicated treatment.
- A request to discontinue the study by the IRB or FDA.

The sponsor-investigator will promptly notify investigators, the IRB and FDA if the study is terminated for any reason.

14.8. Records Retention

U.S. FDA regulations (21 CFR §312.62[c]) require that records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs, consent forms, laboratory test results, and medication inventory records, must be retained by each Principal Investigator for 2 years after marketing application approval. If no application is filed or if the application is not approved, these records must be kept until 2 years after the study is discontinued and the U.S. FDA and the applicable national and local health authorities are notified.

Following closure of the study, each participating center will maintain a copy of all site study records in a safe and secure location. The Coordinating Center will inform the investigator at each site at such time that the records may be destroyed.

15. STATISTICAL CONSIDERATIONS

In the phase I portion, it is anticipated that 2 dose escalation cohorts will be needed to reach the MTD or RP2D; therefore, approximately 9-12 eligible patients will be required to evaluate the safety and tolerability of CB-839 in combination with panitumumab and irinotecan.

We will use the Bayesian continual reassessment method (CRM) to conduct this phase I clinical trial.[45] Patients will be treated in cohorts of 3. All toxicity outcomes must be observed before calculating the recommended dose level for the next cohort. Dose levels may not be skipped. Up to 12 patients may be enrolled since there are 2 working dose levels and a -1 dose level to be evaluated and a minimum of 6 patients are required to establish the MTD. The probability of toxicity at the *i*th dose is assumed to follow $Pr(tox) = p_i^{\exp(\alpha)}$ where p_i is a constant and α is distributed N(0,2). Targeting a MTD of 30%, we assume *a priori* that p_i= 0.1, 0.2, and 0.3, for dose levels -1, 1 and 2, respectively. This CRM trial will stop if the probability that the estimated DLT rate of the lowest dose is excessive (> 0.3) is greater than 90%.

Table 10 contains MTD selection levels and operating characteristics (10,000 simulations) for various toxicity scenarios. For equitable comparisons, we assumed a maximum sample size of 12 for the CRM simulations since the expected total sample size was ~11 for the standard 3+3 algorithm. Here, STOP means that toxicity exceeded the targeted DLT rate of 30%. No patients are actually treated at a STOP level. Table 10 depicts the well-known comparative differences between the CRM and the standard 3+3. The 3+3 is memoryless and only the current dose level provides information for escalation or de-escalation. The BCRM, which uses all patient data at every escalation/de-escalation decision, finds the targeted MTD more often than the standard 3+3 algorithm. The 3+3 algorithm is inconclusive for finds the MTD at the -1 dose 26%, 19%, 21%, and 33% more frequently than BCRM. The BCRM does sample more frequently at higher than MTD dose levels. This effect can be dampened considerably by adjusting cohort size to 1 or 2 patients. For example, using a cohort size of 1, the proportion of 10,000 simulated trials choosing the MTD at a 40% DLT rate decreases 5% compared to using a cohort size of 3. Due to the low number of cohorts, methods incorporating escalation with overdose control (EWOC) have little effect on selection probabilities.

Table 11. Operating characteristics for Bayesian continual reassessment method assuming a maximum sample size of 12 patients compared to standard 3+3 algorithm

Scenario	Dose	True Pr(tox)	Probability Selected MTD		Expected number treated		Expected number DLT's	
			CRM	3+3	CRM	3+3	CRM	3+3
1	Stop	-	0	0.04	-	-	2.84	2.34
	-1	0.1	0.06	0.28	0.84	1.96		
	1	0.2	0.31	0.39	5.95	5.05		
	2	0.3	0.63	0.30	5.21	3.81		
2	Stop	-	0	0.001	-	-	2.39	1.92
	-1	0.05	0.01	0.20	0.39	1.28		
	1	0.15	0.20	0.36	5.33	4.85		
	2	0.25	0.79	0.43	6.27	4.46		
3	Stop	-	0	0.10	-	-	3.28	2.78
	-1	0.15	0.13	0.34	1.39	2.69		
	1	0.25	0.39	0.37	4.43	5.13		
	2	0.35	0.48	0.18	4.16	3.06		
4	Stop	-	0.01	0.20	-	-	3.71	3.21
	-1	0.2	0.24	0.37	2.12	3.41		
	1	0.3	0.43	0.32	6.53	5.11		
	2	0.4	0.33	0.11	3.31	2.41		

In phase II, we will test the hypothesis that the best objective response rate is at least 20% (p≤0.05). A 5% BOR rate would be considered low efficacy for this triple combination. A 20% response rate would be considered sufficiently active to warrant further study in more definitive trials. We will use Simon's optimal 2-stage design to monitor efficacy (RR) in this trial [12]. If no responses are experienced by the first 10 patients, we will stop accrual for lack of efficacy.

Otherwise we will continue accrual up to 29. If 4 or more patients experience BOR among 29 treated, we reject the null hypothesis ($p<0.05$) of low efficacy (BOR ≤ 5%) and declare this regimen sufficiently active in this patient population to warrant further study in more definitive trials. Assuming low efficacy, the probability of early termination is 60% with an expected sample size of 18.

Demographic information such as age, race, and sex, will be tabulated. Descriptive statistics, including means, standard deviations, and ranges for continuous parameters, as well as percentages and frequencies for categorical parameters, will be presented. Adverse medical events will be tabulated. NCI toxicity Grade 3 and Grade 4 laboratory abnormalities will be listed. All patients who received at least one cycle of the combination therapy will be included in the safety analyses.

Power Analysis:

Phase II: We assume a type I error of 5%. If the true RR with this triple agent therapy is 20% or greater, then 29 patients provide 80% power or more to declare this regimen sufficiently active to warrant further study.

Relationship between change in tumor size and radiotracer uptake: We will also evaluate the relationship between radiotracer uptake at baseline and change in tumor size at the time of objective response assessment using a standard linear regression analysis. The slope will describe the shape of the relationship between SUV_{max} and change in tumor size, while the coefficient of determination (R^2) describes the strength of the relationship between the two measures. A similar linear regression analysis will be conducted to quantify the relationship between the change in SUV_{max} as measured from baseline to after one cycle of therapy and change in tumor size.

All patients in Phase II will undergo two ^{18}F-FSPG imaging sessions at baseline and just prior to cycle 2 of therapy. Our preliminary data suggest that the ^{18}F-FSPG SUV_{max} (predictor) has a standard deviation of 2.04 (95% CI 1.42 to 3.58). From previous clinical trial data, the median (range) and mean (standard deviation) of the change in the sum of index lesions from patients treated with EGFR mAb therapy were 4.9 mm (-24 mm to 42 mm) and 6 mm (15.8 mm), respectively. Using the standard deviations (SDs) and their 95% confidence intervals for PET SUV_{max} (predictor) and change in tumor size, table 11 lists the true slope estimates that provide 80% power to reject the null hypothesis of no association between ^{18}F-FSPG imaging and tumor response. From the estimated SDs for PET SUV_{max} and change in tumor size, a reformulation of the coefficient of determination (R^2) gives the correlation associated with the slope estimates $r = \sqrt{\frac{1}{(\sigma/B*SX)^2+1}}$ where σ is the SD of the residuals, B is the slope and SX is the standard deviation for PET SUV_{max}. The estimated correlations for the scenarios in table 11 have a mean (± standard deviation) of 0.475 ± 0.0002, which represent biologically and clinically meaningful associations between early changes in PET SUV_{max} and tumor size.

Because ^{11}C-Glutamine is a novel tracer, we will use the same power and sample size analyses as 18F-FSPG.

Table 12. True slope estimates required to achieve 80% power to reject the null hypothesis of no association between baseline PET SUV_{max} and change in the sum of index lesions.

SD for ^{18}F-FSPG SUV_{max}	SD for Change in Tumor Size at Best Response		
	12	16	24
1.42	4.01	5.35	8.03
2	2.85	3.80	5.70

3.58	1.59	2.12	3.18

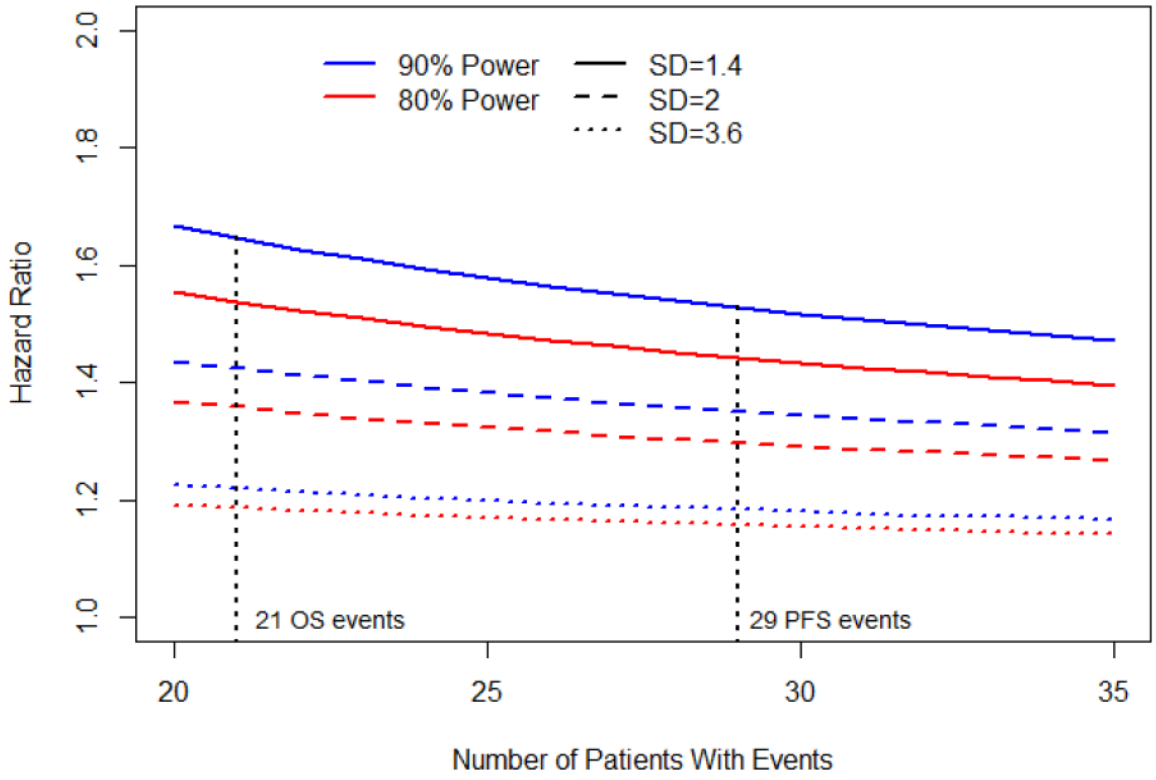
A similar linear regression will be conducted to predict change in tumor size by the change in PET SUV_{max} measured just prior to cycle 2. Using a standard deviation of 2 from table 11, we can estimate the SD of the difference in PET SUV_{max} as sqrt(2V(1-r)) where V is the assumed common variance (for a single SD of 2, the V =4) of the two measurements and *r* is the correlation between them. For a correlation set of 0.3, 0.5, and 0.7, the SD's of the difference in PET SUV_{max} are 2.36, 2, and 1.54. The data in table 12 reflect and average (SD) correlation between change in tumor size and ΔSUV_{max} of 0.475±0.0003.

Table 13. True slope estimates required to achieve 80% power to reject the null hypothesis of no association between change in PET SUV_{max} just prior to cycle 2 and change in the sum of index lesions.

SD for ^{18}F-FSPG SUV_{max}	SD for Change in Tumor Size at Best Response		
	12	16	24
1.54	3.70	4.93	7.4
2	2.85	3.80	5.70
2.37	2.40	3.21	4.81

Time-to-event. We expect median progression-free and overall survival of approximately 1.9 and 6.4 months, respectively. Power to reject a HR=1 for a single predictor in the Cox regression model depends on the number of events (progressions or deaths), the variability of the predictor, and the true HR [14]. Assuming exponential survival, we estimate that 29 and 21 patients will have progressed or expired by 12 months among the 29 patients in the phase I/II trial. We will use Cox proportional hazards model to estimate the association between PET SUV_{max} and PFS and OS. Preliminary data suggest ^{18}F-FSPG SUV_{max} has a standard deviation of 2.04 (95% CI 1.42 to 3.58). Figure 4 illustrates the hazard ratios necessary to reject the null hypothesis that HR=1 versus the alternative that the HR≠1 assuming SD estimates for PET SUV_{max}, a type I error of 5% and 80% or 90% power. For PFS and OS, we expect to have at least 90% power to reject the null hypothesis that the HR=1 if the for a SD PET SUV_{max} of 1.54 and the true hazard ratios are 1.65 and 1.53 or larger, respectively.

Figure 5: True hazard ratios (HRs) scenarios necessary to reject (p<0.05) a null hypothesis with 80% and 90% power.



Pharmacokinetic Modeling.

The ^{18}F-FSPG PET/CT data will be analyzed using pharmacokinetic modeling. Three-dimensional regions of interest will be manually drawn using ASIPro software over tumor and the abdominal aorta (to estimate the arterial input function, [AIF]) of dynamically acquired PET images. Venous samples will be collected over the course of scanning to measure ^{18}F-FSPG plasma concentrations, confirm blood pool radioactivity, evaluate metabolism, and to calibrate image-derived input functions. We will utilize the PMOD software package to assist in the estimation of various model parameters (e.g., influx (K_1), efflux (k_2)) in order to calculate tracer flux:

$$K_{flux} = (K_1 * k_3) / (k_2 + k_3).$$

All pharmacokinetic modeling endpoints are focused on estimation; thus, no formal hypothesis testing will be performed. All point estimates of outcome measures will be accompanied by 95% (e.g. based on standard Gaussian methods or bootstrap methods, as appropriate) confidence intervals. Categorical variables will be summarized in frequency tables. Continuous variables will be summarized using the minimum, 25th, 50th, and 75th percentiles and the maximum value.

Gene expression signatures

Patients from this protocol will provide pre-treatment tissue for collection of tumor RNA and qRT-PCR analysis of genes that regulate intracellular Gln metabolism. We hypothesize that qRT-PCR based gene expression is associated with change in tumor size, progression-free (PFS) and overall survival (OS).

Power Analysis:
PFS/OS. Estimated median PFS and OS in this patient population is 1.9 and 6.4 months, respectively, with an estimated 29 and 21 patients progressing or expired by 12 months. In preliminary data, the average standard deviation (SD) and bootstrapped (1000 replications) 95% confidence intervals of the SD for the deltaCt values of 84 genes was 2 cycles (1.85 to 2.14 cycles). Twenty-nine patients provide at least 90% power to reject the null hypothesis that the HR=1, assuming the qRT-PCR SD is 2 for true hazard ratios are 1.35 and 1.42 or larger, respectively.

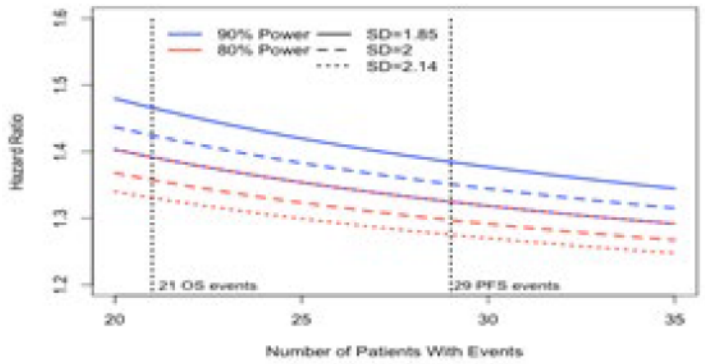


Figure 6: True hazard ratios (HRs) scenarios necessary to reject ($p<0.05$) a null hypothesis with 80% and 90% power.

Change in Tumor Size. Among 20 patients treated with mAb therapy, the mean SD of the change in the sum of index lesions was 6 mm (15.8 mm). Using the SD and their 95% confidence intervals for qRT-PCR (predictor; data provided above) and change in tumor size among 29 patients, table 2 lists the true slope estimates that provide 80% power to reject the null hypothesis of no association between qRT-PCR and tumor response. Given our preliminary data, 29 patients provide 80% power to detect ($p<0.05$) a 3.8 mm change in tumor volume per deltaCt cycle.

Table 14. True slope estimates (mm/cycle) required to achieve 80% power to reject the null hypothesis of no association between baseline qRT-PCR and change in the sum of index lesions.

SD for qRT-PCR	SD for Change in Tumor Size at Best Response		
	12	16	24
1.85	3.08	4.11	6.16

2	2.85	3.80	5.70
2.14	2.66	3.55	5.33

Patients from this protocol will also provide tissue for modelling individual gene expression (e.g., log(FPKM) using RNA-seq. Following extensive processing and quality control, we will model individual gene expression (e.g., log(FPKM) separately against ^{11}C-Glutamine and ^{18}F-FSPG PET as continuous dependent variables with standard linear regression with a FDR selection criteria of 5%.[46-48] This gene signature will be confirmed using feature selection via the elastic net with 4-fold cross validation.[49] This gene signature will be further annotated using knowledge base pathway analysis.[50] Due to the relatively smaller sample size, we will further summarize the gene signature by generating a compound score based on the univariate regression analysis considering one gene at a time on change in tumor size (pFDR < 0.05) as a cutoff point for selection of important features.[51] For the *i*th patient, the compound score (prediction index) for the gene signature is:

$$c_i = \sum_{j=1}^{k} (\text{sign of } \beta_j)\, w_j x_{j,i}\,.^{52}$$

Here w_j is the Wald statistic and β_j is the coefficient from linear regression analysis for the set of j=1 to k selected significant features. These summary prediction indices will again be used to predict change in tumor size. For that analysis, we will determine the statistical significance of the compound score using a permutation test (10,000 permutations).[53] Further, we will carry out the same method using the Cox (proportional hazards) regression model (i.e., $h_i(t) = h_0(t)\ \exp(\psi\ c_i)$) for PFS. Finally, we will conduct internal validation and model calibration using Efron's optimism bootstrap (b=500). Standard goodness-of-fit parameters (e.g. coefficient of determination) will be adjusted for over-optimism and predicted values adjusted to evaluate calibration plots that elucidate the predictive power of the gene expression model in future datasets.

Exosomal endpoints

Circulating exosomes will be isolated from whole blood samples to explore the potential role of vesicle trafficking in mediating response and resistance to the investigational therapy.

Whole blood is drawn into buffered citrate tubes. For port draws, first draw tubes should be discarded as per standard clinical practice and can be tubes of any kind. The tubes and blood should be kept at ambient/room temperature until processing which should within two hours of blood draw. The whole blood is spun in a centrifuge for 20 min at 3,000 × *g* at room

temperature. The resulting crude plasma is aspirated from the top to a new tube. The crude plasma is spun again for 20 min at 3,000 × *g* to pellet any remaining platelets. The final plasma preparation is carefully aspirated off into new tubes. The plasma will then be used immediately for exosome isolation or stored at -80°C for future analysis.

To isolate exosomes, plasma is diluted 10-20 fold with ice-cold phosphate buffered saline (PBS) and centrifuged for 30 min at 15,000 × *g,* 4°C, to pellet and remove larger, non-exosomal extracellular vesicles. The resulting supernatant is passed through a 0.22 µm PES membrane filter to remove any contaminating larger vesicles that might remain. To pellet exosomes, the supernatant is centrifuged at 120,000 × *g,* 4°C. The resulting crude plasma exosome pellet is re-suspended in ice-cold PBS and washed by centrifugation at 120,000 × *g*, 4°C. This wash and centrifugation step is repeated one more time to improve removal of non-exosomal particles and proteins. The final exosome pellet is processed immediately or stored for future use.

Whole blood samples will be collected at three time points during the study for all 29 patients enrolled in phase II: baseline, after cycle 1, and at the end of study/treatment. Expression levels of total EGFR, activated EGFR, and total amphiregulin (AREG), along with the proportion of exosomes containing total EGFR (percent positive EGFR/CD9), exosomes that have active EGFR (percent positive active EGFR/CD9), and exosomes carrying AREG (percent positive AREG/CD9) will be measured at each time point.

Exosomal endpoints are focused on estimation; thus, no formal hypothesis testing will be performed. All point estimates of outcome measures will be accompanied by 95% (e.g. based on standard Gaussian methods or bootstrap methods, as appropriate) confidence intervals. Categorical variables will be summarized in frequency tables. Continuous variables will be summarized using the minimum, 25th, 50th, and 75th percentiles and the maximum value.

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APPENDIX 1

Cockcroft-Gault Formula

The following formula may be used for estimated creatinine clearance rate (eC_{CR}) using Cockcroft-Gault formula. The use of on-line calculators or formulas which are institution standards for eC_{CR} and differ slightly may also be used. The calculations and results must be filed in the patient's chart.

When serum creatinine is measured in mg/dL;

$$eC_{CR} = \frac{(140 - \text{Age}) \cdot \text{Mass (in kilograms)} \cdot [0.85 \; if \; Female]}{72 \cdot \text{Serum Creatinine (in mg/dL)}}$$

When serum creatinine is measured in µmol/L;

$$eC_{CR} = \frac{(140 - \text{Age}) \cdot \text{Mass (in kilograms)} \cdot \text{Constant}}{\text{Serum Creatinine (in µmol/L)}}$$

Where *Constant* is *1.23* for men and *1.04* for women.

APPENDIX 2

ECOG Performance Status

Grade	ECOG Performance Status
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities, Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled, Cannot carry on any self-care Totally confined to bed or chair
5	Dead

APPENDIX 3
Response Evaluation Criteria In Solid Tumors (RECIST v1.1)[54]

Measurability of tumor at baseline

Definitions
At baseline, tumor lesions/lymph nodes will be categorized measurable or non-measurable as follows:

Measurable:

Tumor lesions: Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

- 10 mm by CT scan (CT scan slice thickness no greater than 5 mm)
- 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable)
- 20 mm by chest X-ray

Malignant lymph nodes: To be considered pathologically enlarged *and* measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed. See also notes below on "Baseline documentation of target and non-target lesions" for information on lymph node measurement.

Non-measurable:

All other lesions, including small lesions (longest diameter < 10 mm or pathological lymph nodes with 10 to < 15 mm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

Special considerations regarding lesion measurability:

Bone lesions, cystic lesions, and lesions previously treated with local therapy require particular comment:

Bone lesions

- Bone scan, PET scan or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions
- Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be considered as measurable lesions if the soft tissue component meets the definition of measurability described above
- Blastic bone lesions are non-measurable

Cystic lesions

- Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts
- "Cystic lesions" thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions

Lesions with prior local treatment

- Tumor lesions situated in a previously irradiated area, or other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion. Study protocols should detail the conditions under which such lesions would be considered measurable

Specifications by methods of measurements

Measurement of lesions

All measurements should be recorded in metric notation, using calipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

Method of assessment

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging based evaluation should always be done rather than 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 measurable when they are superficial and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is suggested. As noted above, when lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study

- Chest X-ray: Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung
- CT, MRI: CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. When CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g., for body scans)
- Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement

- Endoscopy, laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, they can be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following CR or surgical resection is an endpoint
- Tumor markers: Tumor markers alone cannot be used to assess objective tumor response
- Cytology, histology: These techniques can be used to differentiate between PR and CR in rare cases if required by protocol (for example, residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain). When effusions are known to be a potential adverse effect of treatment (e.g., with certain taxane compounds or angiogenesis inhibitors), the cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment can be considered if the measurable tumor has met criteria for response or SD in order to differentiate between response (or SD) and progressive disease

Tumor response evaluation

To assess objective response or future progression, it is necessary to estimate the overall tumor burden at baseline and use this as a comparator for subsequent measurements. Only patients with measurable disease at baseline should be included in protocols where objective tumor response is the primary endpoint. Measurable disease is defined by the presence of at least one measurable lesion. Response criteria are listed in Tables 13-16.

TABLE 15: Response Criteria for Evaluation of *TARGET* Lesions

	Evaluation of Target Lesions
Complete Response (CR)	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm
Partial Response (PR)	At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum 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

TABLE 16: Response Criteria for Evaluation of *NON-TARGET* Lesions

	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)
Progressive Disease (PD)	Unequivocal progression of existing non-target lesions. (Note: the appearance of one or more new lesions is also considered progression)
Non-CR/Non-PD	Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits

Evaluation of Best Overall Response

It is assumed that at each protocol specified time point, a response assessment occurs. Table 12 provides a summary of the overall response status calculation at each time point for patients who have measurable disease at baseline.

TABLE 17: Overall Response Status for Patients with Baseline Measurable Disease

Target Lesions	Non-target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Abbreviation: CR, complete response; NE, non-evaluable; PD, progressive disease; PR, partial response; SD, stable disease

The best overall response is determined once all the data for the patient is known.

Best response determination in trials where confirmation of CR or PR IS NOT required:
Best response in these trials is defined as the best response across all time points (for example, a patient who has SD at first assessment, PR at second assessment, and PD on last assessment has a best overall response of PR). When SD is believed to be best response, it must also meet the protocol specified minimum time from baseline. If the minimum time is not met when SD is otherwise the best time point response, the patient's best response depends on the subsequent assessments. For example, a patient who has SD at first assessment, PD at second and does not meet minimum duration for SD, will have a best response of PD. The same patient lost to follow-up after the first SD assessment would be considered unevaluable.

Best response determination in trials where confirmation of CR or PR IS required:
Complete or partial responses may be claimed only if the criteria for each are met at a subsequent time point as specified in the protocol (generally 4 weeks later). In this circumstance, the best overall response can be interpreted as shown in Table 13.

TABLE 18: Best Overall Response when Confirmation of CR and PR Required

Overall Response First Time Point	Overall Response Subsequent Time Point	Best Overall Response
CR	CR	CR
CR	PR	SD, PD or PR[1]
CR	SD	SD provided minimum criteria for SD duration met, otherwise PD
CR	PD	SD provided minimum criteria for SD duration met, otherwise PD
CR	NE	SD provided minimum criteria for SD duration met, otherwise NE
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD provided minimum criteria for SD duration met, otherwise PD
PR	NE	SD provided minimum criteria for SD duration met, otherwise NE
NE	NE	NE

Abbreviation: CR, complete response; NE, non-evaluable; PD, progressive disease; PR, partial response; SD, stable disease

1) If a CR is truly met at first time point, then any disease seen at a subsequent time point, even disease meeting PR criteria relative to baseline, makes the disease PD at that point (since disease must have reappeared after CR). Best response would depend on whether minimum duration for SD was met. However, sometimes 'CR' may be claimed when subsequent scans suggest small lesions were likely still present and in fact the patient had PR, not CR at the first time point. Under these circumstances, the original CR should be changed to PR and the best response is PR.

APPENDIX 4
Acceptable Contraception

Female patients of childbearing potential and male patients able to father children who have female partners of childbearing potential must agree to use at least 2 methods of acceptable contraception from the time of signing consent, and for at least 2 months after study participant's final dose of protocol-indicated treatment.

Females of childbearing potential are defined as those who are not surgically sterile or post-menopausal (i.e. patient has not had a bilateral tubal ligation, a bilateral oophorectomy, or a complete hysterectomy; or has not been amenorrheic for 12 months without an alternative medical cause). Postmenopausal status in females under 55 years of age should be confirmed with a serum follicle-stimulating hormone (FSH) level within laboratory reference range for postmenopausal women.

Male patients able to father children are defined as those who are not surgically sterile (i.e. patient has not had a vasectomy).

A study physician or clinical designee shall counsel female patients of childbearing potential and male patients able to father children who have female partners of childbearing potential, regarding the importance of pregnancy prevention, the implications of an unexpected pregnancy, and the use of acceptable contraception. At a minimum, applicable subjects must agree to use at least 2 methods of acceptable contraception, as listed below:

TABLE 19: Acceptable Methods of Contraception

Barrier Methods	Intrauterine Device Methods	Hormonal Methods
• Male or female condom plus spermicide • Cervical cap plus spermicide • Diaphragm plus spermicide	• Copper T (e.g. Paragard®) • Levonorgesterel-releasing intrauterine system (e.g. Mirena®) – also considered a hormonal method	• Implants • Hormone shot or injection • Combined pill (estrogen + progestin) • Minipill (progestin only) • Patch

Complete abstinence defined as complete avoidance of heterosexual intercourse, when consistent with the patient's preferred and established lifestyle, is an acceptable form of contraception for purposes of the study. Periodic abstinence (e.g. calendar, ovulation, symptothermal, and post-ovulation methods) and withdrawal are not acceptable forms of contraception in this study. In the event the subject chooses to forego complete abstinence, acceptable methods contraception must be discussed with the study physician or clinical designee.