BOSTON BIOMEDICAL

Dainippon Sumitomo Pharma Global Oncology

TITLE:	**A Phase II Clinical Study of BBI608 in Adult Patients with Advanced Colorectal Cancer**
PROTOCOL NUMBER:	**BBI608-224**
STUDY DRUG:	**BBI608**
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SYNOPSIS

Study Title:	**A Phase II Clinical Study of BBI608 in Adult Patients with Advanced Colorectal Cancer**
Study Number:	BBI608-224
Study Phase:	Phase II
Study Drug	BBI608, a novel investigational small molecule anticancer drug that targets cancer stem cells.
Primary Objectives:	To determine the safety and preliminary anti-tumor activity of BBI608 when administered in combination with Cetuximab, or Panitumumab, or Capecitabine in adult patients with advanced colorectal cancer who have failed first and second line treatment. The primary endpoint will be disease control rate, defined as the proportion of patients with a documented complete response, partial response and stable disease (CR + PR + SD) based on RECIST. The secondary endpoints will include progression-free survival (PFS) and overall survival (OS).
Secondary Objectives:	To determine the pharmacokinetic profile of BBI608 and Cetuximab, or Panitumumab, or Capecitabine when administered in combination. To determine the pharmacodynamics (biomarkers) of BBI608. To evaluate tolerability of two dosing regimens of BBI608 by comparing administration of BBI608 at 240 mg twice daily starting dose and escalating to 480 mg twice daily after cycle 1 versus administration of BBI608 at 480 mg twice daily starting dose with dose adjustment permitted. To evaluate tolerability of BBI608 by comparing prophylactic administration of anti-diarrheal medication versus as needed administration of anti-diarrheal medication. The tolerability will be assessed using adverse event profile and achieved dose intensity.

Study Design:	This is an open label, multi-center, Phase II study of BBI608 administered in combination with either Cetuximab, or Panitumumab, or Capecitabine. A cycle will consist of daily and continuous oral administration of BBI608 for four weeks in combination with either Cetuximab, or Panitumumab, or Capecitabine. Arm A (Cetuximab) - BBI608 is administered at 480 mg po twice daily continuously and Cetuximab will be administered IV on day 5 at 400 mg/m^2 intravenous infusion over 120 minutes as the initial dose, then weekly at 250mg/m^2 over 60-minutes at subsequent cycles. Arm B (Panitumumab) - BBI608 is administered at 480 mg po twice daily continuously and Panitumumab will be administered IV on day 8 and 22 of each 28 day cycle at 6 mg/kg over 60 minutes. Arm C (Capecitabine) - BBI608 is administered at 480 mg po twice daily continuously and Capecitabine will be administered orally at 1000 mg/m^2 bid daily on days 8-21 every three weeks. 6 patients will be initially enrolled in each combination arm at a dose level of 480 mg twice daily (960 mg total daily dose) BBI608. If ≤ 1 out of 6 patients have an observable DLT in the combination regimen, then that combination arm may continue to accrue patients. If DLT occurs in ≥ 2 of the 6 patients in a combination arm, then the combination arm will closed and no new patients will be enrolled into this combination arm. A trial arm of BBI608 in combination with Cetuximab, or Panitumumab, or Capecitabine will continue to enroll patients only if no more than 1 out of 6 patients for each combination has an observable DLT. It is expected that approximately 60 to 80 CRC patients will be enrolled in the study. Pharmacokinetics (PK) will be performed in the first cycle for the initial 6 patients of each combination arm of the study. Pharmacodynamic assessments will be performed in patients with accessible tumors when tumor biopsy is possible. Evaluation of anti-tumor activity of BBI608 in combination with Cetuximab, or Panitumumab or Capecitabine will be performed at about 8 week intervals while patients remain on study. To evaluate the tolerability of BBI608, patients from each arm will be assigned to one of 4 sub-cohorts (with 6 patients in each sub-cohort IA, IB, IIA, IIB). The evaluation of tolerability will be done at select centers for the study arm that is continuing patient accrual and in which a total of 50 evaluable patients has not been reached. For each combination arm, patients assigned to sub-cohorts IA and IB will receive BBI608 at 240 mg po twice daily (480 mg total daily dose) starting on day 1 of cycle 1. Intra-patient dose escalation of BBI608 to 480 mg po twice daily (960 mg total daily dose) is allowed as tolerated. Patients assigned to sub-cohorts IIA and IIB will receive BBI608 at 480 mg po twice daily (960 mg total daily dose) starting on day 1 of cycle 1. In case of toxicity, dose adjustment of BBI608 is permitted, as recommended in dose modification guidelines. Additionally, patients assigned to sub-cohorts IA and IIA will receive prophylactic anti-diarrheal treatment starting the day prior to day 1 of cycle 1. Patients assigned to sub-cohorts IB and IIB will anti-diarrheal treatment on an as needed basis starting with development of diarrhea above baseline. For each combination regimen, patients will be sequentially assigned to each of the 4 sub-cohorts in the following order until 6 patients are accrued on each of the sub-cohorts: IA, IB, IIA, and IIB. If required, up to additional 20 patients may be assigned to selected sub-cohorts to validate the observations in initial 6 patients.

	Sub-cohort	**BBI608 Starting Dose Level**	**Anti-diarrheal Administration**
	IA	240 mg bid (480 total daily dose)[1]	Prophylactic
	IB	240 mg bid (480 total daily dose)[1]	As needed
	IIA	480 mg bid (960 total daily dose)[2]	Prophylactic
	IIB	480 mg bid (960 total daily dose)[2]	As needed
	[1]Intra-patient dose escalation of BBI608 to 480 mg po twice daily (960 mg total daily dose) is allowed as tolerated.. [2]In case of toxicity, dose adjustment of BBI608 is permitted.		
Study Population:	This study will enroll patients with advanced colorectal cancer. Patients must have received at least 2 lines of standard chemotherapy based on regimens containing 5-FU, irinotecan or oxaliplatin. Patients to be enrolled into the Cetuximab or Panitumumab combination arms must have colorectal cancer with wild-type K-Ras. Patient accrual will occur over a period of time dependent upon the enrollment rate of the study.		
Test Product, Dose, and Mode of Administration:	Patients in this trial will receive BBI608 orally at 480 mg twice daily (960 mg total daily dose). In each cycle BBI608 will be taken daily for 4 weeks (28 days). BBI608 will be administered twice daily, approximately one hour prior to or two hours after meals with the first dose given in the morning and doses separated by approximately 12 hours. For patients receiving Cetuximab, Cetuximab will be administered IV on day 5 at 400 mg/m^2 intravenous infusion over 120 minutes as the initial dose, then weekly at 250mg/m^2 over 60-minutes at subsequent cycles. For patients receiving Panitumumab, Panitumumab will be administered IV on days 8 and 22 of each 28 day cycle at 6 mg/kg over 60 minutes. For patients receiving Capecitabine therapy, Capecitabine will be administered orally at 1000 mg/m^2 bid daily on days 8-21 every three weeks. Cycles will be repeated until progression of disease, unacceptable toxicity, or another discontinuation criterion is met.		
Duration of Treatment:	For an individual patient, treatment with BBI608 and the combination drug will continue until unacceptable toxicity, disease progression (clinical or radiological) or another discontinuation criterion is met. It is expected that most patients will receive between one and four cycles of BBI608 for a treatment period of 4 to 16 weeks.		

Criteria for Determination of Dose-Limiting Toxicity:	A DLT is defined by the occurrence of any of the following toxicities possibly or probably related to BBI608, or BBI608 in combination with Cetuximab, or Panitumumab, or Capecitabine within 28 days of treatment: ● CTCAE (Common Terminology Criteria for Adverse Events) Grade 4 hematological toxicity. ● Grade 3 or 4 non-hematological toxicity, except Grade 3 or 4 nausea/vomiting/anorexia, diarrhea, or fatigue will be considered a DLT only if it persists more than three (3) days despite optimal medical management; and alopecia will not be considered a DLT. ● Any other toxicity that in the view of the Principal Investigator represents a clinically significant hazard to the patient. Whether a DLT has occurred will be assessed during the first 28 days of BBI608 therapy. DLT will be determined from adverse events, changes from baseline in physical examination findings and laboratory parameters. The incidence of adverse events and DLTs will be evaluated for each combination arm and for all patients. Adverse events considered related to Cetuximab, or Panitumumab or Capecitabine will not be considered DLTs for BBI608 in combination with Cetuximab, or Panitumumab or Capecitabine.
Pharmacokinetic and Pharmacodynamic Variables:	Pharmacokinetic variables to be determined include maximum plasma drug concentration (Cmax), volume of distribution, area under the time-concentration curve (AUC), distribution half-life, and terminal half-life. Blood samples for PK determination will be drawn on Days 5-12 and Days 26-33 for the Cetuximab arm, on Days 8-15 and 22-29 for the Panitumumab arm, and on Days 8-9 and Days 21-22 for the Capecitabine arm during the first cycle for the first 6 patients in each combination arm. Response (increase or decrease) of several biomarkers from biopsied tumors following BBI608 administration will be examined in patients with accessible tumors for biopsy. Tumor biopsies will be collected prior to the first dose of BBI608 and on day 8 of the first cycle for patients with accessible tumors and who have signed the optional tumor biopsy consent.

Statistical Methods:	In general, categorical variables will be summarized as the number and percentage of patients in each category. Continuous variables will be summarized by the mean, standard deviation, median, minimum, and maximum. The primary population for the efficacy evaluation will be the evaluable population, defined as patients who have been treated with at least 80% of 1 complete cycle of BBI608 and a combination drug. The primary endpoint will be disease control rate, defined as the proportion of patients with a documented complete response, partial response and stable disease (CR + PR + SD) based on RECIST. The secondary endpoints will include progression-free survival (PFS) and overall survival (OS). PFS will be calculated from the date of patient enrolment into the trial until documented disease progression based on RECIST. OS will be calculated from the date of patient enrolment into the trial until death. Patient sample size for each combination regimen is calculated based on a power of 80 and a one-sided alpha of 5% to detect a disease control rate improvement for Cetuximab and Panitumumab from about 30% (historical DCR for 3rd and 4th line colorectal cancer) to 60% and for Capecitabine from about 15% (historical DCR for 3rd line colorectal cancer) to 60%. Safety analyses will include adverse events, SAEs, laboratory tests, and physical examination changes from baseline. The safety population will include all patients who have received any amount of BBI608 and the combination drug. All patients receiving at least one daily dose of BBI608 will be considered evaluable for safety analyses. In addition to the evaluation and categorization of adverse events, listings of laboratory test results collected at baseline and during the study will be generated. Descriptive statistics summarizing the changes in those laboratory tests over time will be presented. Analysis and Sample size for sub-cohorts: Each sub-cohort will be analyzed for number of days of Grade II/III diarrhea and % treatment compliance achieved during the first two cycles. The % treatment compliance will be calculated as the number of capsules actually ingested over the number of capsules that should have been ingested per dose level multiplied by 100. The research questions addressed by sub-cohorts are hypothesis-generating and exploratory in nature. Initially 6 subjects will be assigned to each sub-cohort to assess the effect of intervention. Selected sub-cohorts will be extended to enroll up additional 20 patients. The decision of extending the enrollment will be based on clinical assessment without formal statistical testing. However, 20 patients will give 80% power to detect 20% difference in % treatment compliance between two arms and 57% power to detect the difference of 25% in the number of days of having Grade II/III diarrhea using two-sided Z test with pooled variance at type I error rate of 0.15. The control rates assumed are 70% for treatment compliance and 30% for diarrhea. The patients will be sequentially allocated to different sub-cohorts by the Sponsor to avoid section bias.

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse event
ALT	Alanine transaminase (SGPT)
ANOVA	Analysis of variance
AP	Alkaline phosphatase
AST	Aspartate transaminase (SGOT)
AUC	Area under the time-concentration curve
BSA	Body surface area
BUN	Blood urea nitrogen
CBC	Complete blood count
CDER	Center for Drug Evaluation and Research
C_{max}	Maximum plasma drug concentration
C_{min}	Minimum plasma drug concentration
CFR	Code of Federal Regulations
CI	Confidence interval
CR	Complete response
Cru	Complete response / unconfirmed
CRF	Case report form
CT	Computed tomography
CV	Coefficient of variation
CTCAE	Common terminology criteria for adverse events
DLT	Dose limiting toxicity
ECOG	Eastern Cooperative Oncology Group
ECG	Electrocardiogram
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GGT	Gamma glutamyl transferase
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice

Hct	Hematocrit
HED	Human equivalent dose
Hgb	Hemoglobin
HGF	Hepatocyte growth factor
HIPAA	Health Information Portability and Accountability Act
IC_{50}	Inhibitory concentration, 50%
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IND	Investigational New Drug
IRB	Institutional Review Board
LD	Longest diameter
LDH	Lactic dehydrogenase
MR	Minor response
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose
NCI	National Cancer Institute
NOAEL	No observable adverse effect level
NOEL	No observable effect level
ORR	Overall response rate
PD	Progressive disease
PK	Pharmacokinetic
PR	Partial response
qd	Once daily
RECIST	Response evaluation criteria in solid tumors
RP2D	Recommended Phase 2 dose
RBC	Red blood cell (count)
SAE	Serious adverse event
sCR	Stringent complete response

SD	Stable disease
SE	Standard error
SGOT	Serum glutamic oxaloacetic transaminase (AST)
SGPT	Serum glutamic pyruvic transaminase (ALT)
SPEP	Serum protein electrophoresis
T_{max}	Time to maximum plasma concentration
TNM Scale	Tumor node metastases scale
ULN	Upper limits of normal
UPEP	Urine protein electrophoresis
VGPR	Very good partial response
WBC	White blood cell (count)

1 PRECLINICAL SUMMARY AND STUDY RATIONALE

1.1 Scientific Background of BBI608

Recent studies have uncovered the presence of cancer stem cells (CSC, also called tumor initiating cells or cancer stem-like cells) which have self-renewal capability and are considered to be fundamentally responsible for malignant growth, relapse and metastasis. Importantly, CSCs are inherently resistant to conventional therapies. Therefore, a targeted agent with activity against cancer stem cells holds a great promise for cancer patients (Lobo, Shimono et al. 2007 [1]; Boman and Wicha, 2008 [2]; Please find a list of updated reviews on cancer stem cells in all major cancer types in the special issue of *Journal of Clinical Oncology* on cancer stem cells (J Clin Oncol. 2008 Jun 10;26(17)). [3]

BBI608 is the most advanced product candidate selected from our BBI6000 program designed by Boston Biomedical, Inc. (BBI) to target cancer stem cells. BBI608 is a small molecule that targets cancer by blocking self-renewal of and inducing apoptosis in cancer stem cells. While not a kinase inhibitor, BBI608 works by inhibiting the CSCP3 pathway.

With our proprietary target discovery technology TPIV®, scientists at Boston Biomedical, Inc. have discovered that the CSCP3 pathway activity is critical for self-renewal and survival of cancer stem cells in human cancer. Blocking the CSCP3 pathway offers a novel and potentially highly effective strategy to target cancer stem cells as well as the bulk of the heterogeneous cancer cells, while sparing normal cells and normal adult stem cells

1.2 Preclinical Efficacy

Cancer stem cells are intrinsically resistant (more than 5 to 10 fold) to chemotherapeutic drugs. BBI608 has potent activity (~100 to 500 nM) against cancer stem cells *in vitro* and *in vivo* while sparing normal hematopoietic stem cells (IC_{50} not reached at 30 μM), offering a wide therapeutic window. The dependence on CSCP3 is conserved in various non-stem cancer cells. BBI608 has demonstrated *in vitro* efficacy in a broad spectrum of human cancer cell lines derived from both solid tumors and hematologic malignancies (IC_{50} ~100 nM to 500 nM).

BBI608 monotherapy has demonstrated potent anti-tumor activity *in vivo* in multiple murine xenograft models of human cancer, including liver, head and neck, breast, prostate, colon, gastric and pancreatic cancers, in the absence of adverse effects. BBI608 monotherapy has demonstrated anti-metastatic activity using an *in vivo* spontaneous metastasis mouse models. These data suggest broad potential of BBI608 for a wide variety of human cancers.

1.3 GLP Toxicology

GLP 28-day repeat dose toxicology studies were performed in both rats and dogs at doses of 10, 30 and 100 mg/kg/day by oral gavage.

In the rat study, 100 mg/kg was not tolerated by the male rats. Toxic observations include significant weight loss, soft feces and diarrhea, and decreased food consumption, which led to early sacrifice. These observations recovered in the remaining male rats within 14 days after termination of dosing. Female rats receiving 100 mg/kg/day showed weight loss during the first week of dosing, however weight gain recovered during the continued dosing phase without significant abnormal clinical observations.

In the rat study, abnormal laboratory findings (azotemia, hyponatremia, hypochloremia, hyperkalemia, polycythemia, neutrophilia, monocytosis, and lymphopenia) were observed primarily in moribund male rats dosed at 100 mg/kg. These findings are consistent with acute renal failure in the setting of dehydration and diarrhea. In rats dosed at 100 mg/kg for 28 days, there was mild decrease in sodium, chloride and albumin levels and mild elevation of white blood cells, red blood cells, neutrophils, lymphocytes and monocytes. No significant abnormal laboratory findings in recovery rats, suggesting these abnormal laboratory findings are reversible. There were no abnormal laboratory findings in rats dosed at 10 and 30 mg/kg.

In the rat study, histopathological findings were noted in the rats dosed at 100 mg/kg, primarily in mordibund male rats, including microscopic changes in the stomach and urinary bladder (focal or multifocal chronic ulceration, epithelial hyperplasia, chronic active inflammation or hemorrhagic changes), in lymphoid tissues (mild to marked lymphoid atrophy with mild to moderate lymphocyte apoptosis in thymus; mild to moderate lymphoid atrophy and mild to moderate mastocytosis in mesenteric lymph nodes; mild to moderate lymphoid atrophy in 2 moribund male rats of the 100 mg/kg group and some elevation of hemosiderosis in the spleen) and in adrenal glands (mild cortical vacuolation). These findings are considered non-specific changes related to the test vehicle in the setting of dehydration and diarrhea. There were no significant histopathological findings in recovery rats, suggesting these histopathological changes are reversible. There was no significant test vehicle related microscopic changes in rats dosed at 30 mg/kg and 10 mg/kg.

In the rat study, toxicokinetics showed that all dose groups in rats achieved BBI608 exposure well above the predicted exposure levels needed for efficacy with exposure lasting beyond 10 hours.

For the dog study, toxicity was observed in dogs receiving 100 mg/kg/day consisting of mild weight loss, as well as clinical observations including emesis, diarrhea, mucoid and soft feces. These adverse effects were reversible within 14 days in the setting of continued dosing. No treatment related clinical pathology, gross pathology or histopathological effects were observed at any dose level. All dose groups achieved plasma levels of BBI608 above the predicted levels needed for efficacy, with exposure lasting beyond 10 hours.

The no observable adverse effect level (NOAEL, based on clinical observation, laboratory tests, gross and histopathological changes) for rats administered BBI608 daily orally over 28 days was 30 mg/kg/day (human equivalent dose: 180 mg/m^2) and the NOAEL in dogs administered BBI608 daily orally over 28 days was 30 mg/kg/day (human equivalent dose: 600 mg/m^2).

1.4 Safety and Encouraging Signs of Antitumor Activity in Phase I

A phase 1 dose escalation study (BBI608-101) in adult patients with advanced cancer was initiated to determine the safety, tolerability, recommended phase 2 dose (RP2D), pharmacokinetics and preliminary anti-tumor activity of BBI608. A modified Simon accelerated titration scheme was used for dose escalation, with a cycle consisting of twice-daily oral administration of BBI608 for 4 weeks, and was repeated every 4 weeks (28 days) until progression of disease, unacceptable toxicity, or other discontinuation criteria were met.

The dose of BBI608 has been escalated from 20 mg to 2000 mg/day, and a MTD was not reached. Further dose escalation is limited by pill burden. Adverse events have been generally mild; the most common being grade 1-2 diarrhea and nausea. Grade 3 events included fatigue and transient diarrhea. BBI608 has exhibited favorable pharmacokinetics. The plasma concentration of BBI608 reached several folds above the IC_{50}. Prolonged stable disease beyond 12 weeks was observed in about 60% of evaluable patients, including patients with colon cancer, head and neck cancer, gastric cancer, ovarian cancer and breast cancer. Complete regression of a metastatic lesion to the kidney was also

observed in one colon cancer patient. Decrease in tumor markers (CEA and CA125) have been observed in colon cancer and ovarian cancer.

BBI608 has shown exciting signs of activity in colorectal cancer (CRC) patients in the phase I study where 22 CRC patients with k-Ras mutant or wild-type tumors have been enrolled. These patients were all end-stage patients who had failed standard treatments for CRC including FOLFOX, FOLFIRI, Avastin, EGFR therapy (if k-Ras wild-type) and experimental drugs. Thirteen (13) patients were evaluable with 8 (62%) showing SD or MR (27.6% regression) yielding a disease control rate (DCR=CR+PR+SD) of 62% compared to the historical control of about 10% for similar CRC patients on best supportive care. Progression free survival (PFS) was 13 weeks compared to the historical control of 7.7 weeks (hazard ratio = 0.382; 95% CI, 0.159 to 0.918; p=0.015) in CRC patients on best supportive care.[4,6]

In addition, a phase Ib/II trial of BBI608 in combination with paclitaxel in patients with advanced malignancies carried out under IND 100,887 has shown that BBI608 given at 500 mg bid (1000 mg total daily dose) in combination with weekly paclitaxel is well tolerated.

In summary, BBI608 has shown an excellent safety profile, favorable pharmacokinetics, and encouraging signs of antitumor activity.

2 STUDY OBJECTIVES

2.1 Primary Objectives

The primary objectives of the study are:

- To assess the safety and preliminary anti-tumor activity of BBI608 in adult patients with advanced colorectal cancer who have failed first and second line treatment, when BBI608 is administered in combination with Cetuximab, or Panitumumab, or Capecitabine. The primary endpoint will be disease control rate, defined as the proportion of patients with a documented complete response, partial response and stable disease (CR + PR + SD) based on RECIST. The secondary endpoints will include progression-free survival (PFS) and overall survival (OS).

2.2 Secondary Objectives

The secondary objectives of the study are:

- To determine the pharmacokinetic profile of BBI608 and Cetuximab, or Panitumumab, or Capecitabine when administered in combination.
- To determine the pharmacodynamics (biomarkers) of BBI608.
- To evaluate tolerability of two dosing regimens of BBI608 by comparing administration of BBI608 at 240 mg twice daily starting dose and escalating to 480 mg twice daily after cycle 1 versus administration of BBI608 at 480 mg twice daily starting dose with dose adjustment permitted.
- To evaluate tolerability of BBI608 by comparing prophylactic administration of anti-diarrheal medication versus as needed administration of anti-diarrheal medication. The tolerability will be assessed using adverse event profile and achieved dose intensity.

3 SELECTION OF STUDY POPULATION

This study will be conducted in patients with advanced metastatic colorectal cancer.

The study may be conducted at sites in Canada, the United States, and Europe

3.1 Inclusion Criteria

Each patient must meet the following criteria to be enrolled in this study.

1. Signed written informed consent must be obtained and documented according to International Conference on Harmonization (ICH), Good Clinical Practice (GCP), the local regulatory

requirements, and permission to use private health information in accordance with the Health Insurance Portability and Accountability Act (HIPPA) prior to study-specific screening procedures.

2. A histologically or cytologically confirmed colorectal cancer that is metastatic, unresectable, or recurrent.
3. Patients must have received at least 2 regimens containing 5-FU, oxaliplatin, or irinotecan.
4. Patients to be enrolled in the Cetuximab or Panitumumab combination arms must have colorectal cancer which is K-Ras wild-type.
5. ≥ 18 years of age.
6. Measurable disease as defined by Response Evaluation Criteria in Solid Tumors (RECIST 1.1, see Section 9).
7. Karnofsky performance Status ≥ 70% (Section 15).
8. Male or female patients of child-producing potential must agree to use contraception or avoidance of pregnancy measures during the study and for 30 days after the last BBI608 dose.
9. Females of childbearing potential must have a negative serum pregnancy test.
10. Aspartate transaminase (AST) and alanine transaminase (ALT) ≤2.5 × upper limit of normal (ULN), or ≤ 3.5 × ULN with metastatic liver disease.
11. Hemoglobin (Hgb) ≥ 10 g/dl.
12. Total bilirubin ≤ 1.5 × ULN.
13. Creatinine ≤ 1.5 × ULN or creatinine clearance > 60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal.
14. Absolute neutrophil count ≥ 1.5 x 10^9/L.
15. Platelets ≥ 100 x 10^9/L.
16. Life expectancy ≥ 3 months.

3.2 Exclusion Criteria

Patients who meet any of the following criteria will be excluded from the study.

1. Anti-cancer chemotherapy, radiotherapy, immunotherapy, or investigational agents within 7 days of first dose provided all treatment-related adverse events have resolved or have been deemed irreversible, with the exception for a single dose radiation up to 8 Gray (equal to 800 RAD) with palliative intent for pain control up to 7 days before beginning the administration of BBI608.
2. Surgery within 4 weeks prior to first dose.
3. Any known symptomatic brain metastases requiring steroids. Patients with treated brain metastases must be stable for 4 weeks after completion of that treatment, with image documentation required. Patients must have no clinical symptoms from brain metastases and must be either off steroids or on a stable dose of steroids for at least 2 weeks prior to protocol enrollment. Patients with known leptomeningeal metastases are excluded, even if treated.
4. Pregnant or breastfeeding

5. Significant gastrointestinal disorder(s), in the opinion of the Principal Investigator, (e.g., Crohn's disease, ulcerative colitis, extensive gastric and small intestine resection)
6. Unable or unwilling to swallow BBI608 capsules daily.
7. Uncontrolled intercurrent illness including, but not limited to ongoing or active infection, clinically significant non-healing or healing wounds, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, significant pulmonary disease (shortness of breath at rest or mild exertion), uncontrolled infection or psychiatric illness/social situations that would limit compliance with study requirements.

3.3 Number of Patients

It is expected that approximately 60-80 patients will be enrolled in the study. Patient accrual will occur over a period of time dependent upon the number of cohorts enrolled.

4 INVESTIGATIONAL PLAN

4.1 Overall Study Design

This is an open-label, multicenter Phase II study of oral BBI608 administered with Cetuximab, or Panitumumab, or Capecitabine to patients with advanced metastatic colorectal cancer. The study is based on a Simon 2-stage design that will examine the safety, tolerability and pharmacokinetics of BBI608 and Cetuximab, or Panitumumab, or Capecitabine, and assess the preliminary anti-tumor activity of BBI608 when administered in combination with Cetuximab, or Panitumumab, or Capecitabine in patients with advanced colorectal cancer.

BBI608 treatment will be at a dose level of 480 mg twice daily (960 mg total daily dose). In each cycle, BBI608 will be taken daily continuously for 4 weeks (28 days). BBI608 will be administered twice daily approximately one hour prior to or two hours after meals with the first dose given in the morning and approximately 12 hours between doses.

For patients receiving Cetuximab therapy, Cetuximab will be administered IV on day 5 of cycle 1 at 400 mg/m^2 intravenous infusion over 120 minutes as the initial dose, then weekly at 250mg/m^2 over 60-minutes at subsequent cycles.

For patients receiving Panitumumab therapy, Panitumumab will be administered IV on day 8, and 22 of each 28 day cycle at 6 mg/kg over 60 minutes.

For patients receiving Capecitabine therapy, Capecitabine will be administered orally at 1000 mg/m^2 bid daily on days 8-21 every three weeks.

Cycles will be repeated until progression of disease, unacceptable toxicity, or another discontinuation criterion is met. In the case of toxicity, adjustment is permitted. If administration of Cetuximab, or Panitumumab, or Capecitabine is discontinued due to Cetuximab, or Panitumumab, or Capecitabine-related toxicity, administration of BBI608 will be continued until disease progression if tolerated.

Initially, 6 patients will be enrolled in each combination arm at a dose level of 480 mg twice daily (960 mg total daily dose) BBI608. If $\leq$ 1 out of 6 patients has an observable DLT in the combination regimen, then that arm of the study will continue to enroll patients at 480 mg po twice daily (960 mg total daily dose) of BBI608 following the Simon 2-stage design (see Table 1). If

DLT occurs in ≥ 2 of the 6 patients in a combination arm, then that arm of the study will be closed and no further patients will be enrolled. A given arm of the study of BBI608 in combination with Cetuximab, or Panitumumab, or Capecitabine will continue to enroll patients if no more than 1 out of 6 patients in the combination arm has an observable DLT.

Number of Subjects with DLT at 480 mg bid BBI608	DLT Decision Rule
≤1 out of 6	Continue patient enrollment in combination arm
≥ 2 out of 6	Close combination arm of the study

The following number of colorectal cancer patients will be enrolled based on a two-stage Simon design with an alpha of 5% and a power of 80% to detect a disease control rate improvement for Cetuximab and Panitumumab from about 30% (historical DCR for 3rd and 4th line colorectal cancer) to 60%, and a disease control rate improvement for Capecitabine from about 15% (historical DCR for 3rd line colorectal cancer) to 60%.

Table 1 Simon two-stage design for Phase II

	Patient number		
Combo Therapy	**Stage 1**	**Stage 2**	**Total***
Cetuximab	8	16	24
Panitumumab	8	16	24
Capecitabine	6	12	18

*If any of the three arms reach a DCR of 60% or more at the end of Simon Stage 2, a one-time option to expand accrual to a total sample size of up to 50 patients may be implemented for each arm.

Table 2 Disease Control Rate – Third Line

	Monotherapy (Historical)	Combination * (Historical)	Combination with BBI608 (Expected)
Cetuximab	30-40% [4,5]	~ 63% [10-15]	60% or more
Panitumumab	30-40% [6-8]	~ 68% [16]	60% or more
Capecitabine	~ 15% [9]	~ 45% [17,18]	60% or more

* Cetuximab + Irinotecan
Panitumumab + Irinotecan
Capecitabine + Mitomycin C

For the Cetuximab and Panitumumab combination cohorts, if the disease control rate in stage 1 reaches 3 of 8 or above, additional patients will be enrolled into the stage 2 cohort. For the

Capecitabine combination cohort, if the disease control rate in stage 1 reaches 2 of 6 or above, additional patients will be enrolled into the stage 2 cohort.

To evaluate the tolerability of BBI608, patients from each arm will be assigned to one of 4 sub-cohorts (with 6 patients in each sub-cohort IA, IB, IIA, IIB). The evaluation of tolerability will be done at select centers for the study arm that is continuing patient accrual and in which a total of 50 evaluable patients has not been reached. For each combination arm, patients assigned to sub-cohorts IA and IB will receive BBI608 at 240 mg po twice daily (480 mg total daily dose) starting on day 1 of cycle 1. Intra-patient dose escalation of BBI608 to 480 mg po twice daily (960 mg total daily dose) is allowed as tolerated. Patients assigned to sub-cohorts IIA and IIB will receive BBI608 at 480 mg po twice daily (960 mg total daily dose) starting on day 1 of cycle 1. In case of toxicity, dose adjustment of BBI608 is permitted, as recommended in dose modification guidelines. Additionally, patients assigned to sub-cohorts IA and IIA will receive prophylactic anti-diarrheal treatment starting the day prior to day 1 of cycle 1. Patients assigned to sub-cohorts IB and IIB will anti-diarrheal treatment on an as needed basis starting with development of diarrhea above baseline. For each combination regimen, patients will be sequentially assigned to each of the 4 sub-cohorts in the following order until 6 patients are accrued on each of the sub-cohorts: IA, IB, IIA, and IIB. If required, up to additional 20 patients may be assigned to selected sub-cohorts to validate the observations in initial 6 patients.

Table 3 Tolerability Analysis of BBI608

Sub-cohort	BBI608 Starting Dose Level	Anti-diarrheal Administration
IA	240 mg bid (480 total daily dose)[1]	Prophylactic
IB	240 mg bid (480 total daily dose)[1]	As needed
IIA	480 mg bid (960 total daily dose)[2]	Prophylactic
IIB	480 mg bid (960 total daily dose)[2]	As needed

[1]Intra-patient dose escalation of BBI608 to 480 mg po twice daily (960 mg total daily dose) is allowed as tolerated.
[2]In case of toxicity, dose adjustment of BBI608 is permitted.

4.2 Rationale for Study Design

Considerable data has been collected from the Phase I monotherapy study of BBI608 (Protocol BBI608-101) conducted in advanced oncology patients who have failed multiple previous regimens. Dose escalation for BBI608 has been successfully conducted from a total daily dose of 20 mg to 2000 mg total daily dose. Further dose escalation is limited by pill burden. A MTD was not reached. In addition, a phase Ib/II trial of BBI608 in combination with paclitaxel in patients with advanced malignancies carried out under IND 100,887 has shown that BBI608 given at 500 mg bid (1000 mg total daily dose) in combination with weekly paclitaxel is well tolerated.

Combination of cancer stem cell therapeutics with chemotherapy or a targeted agent would allow simultaneous inhibition of cancer stem cells as well as non-stem regular cancer cells.

Based on the activity seen in colorectal cancer patients in the phase I monotherapy trial, and the excellent safety profile of BBI608, combination of BBI608 with Cetuximab, or Panitumumab or Capecitabine provides a good option for these patients.

4.3 Selection of Dose

The dose escalation of BBI608 in the BBI608-101 Phase I study has reached a total daily dose of 2000 mg without dose-limiting toxicity. No MTD was reached and further dose escalation above 2000 mg total daily dose was limited by pill burden. Adverse events observed in the BBI608-101 phase I trial have been generally mild with the most common being: diarrhea, nausea, and fatigue. Grade 3 events include: fatigue or transient diarrhea. No bone marrow suppression or neuropathy was observed.

In the BBI608-224 study, administration of BBI608 will be begin at a dose of 480 mg twice daily with a total daily dose of 960 mg, in combination with Cetuximab, or Panitumumab, or Capecitabine with exception of patients enrolled into the IA and IB combination arm sub-cohorts, for whom administration of BBI608 will begin at a dose of 240 mg twice daily with a total daily dose of 480 mg in cycle 1. Intra-patient dose escalation of BBI608 to 480 mg po twice daily (960 mg total daily dose) is allowed as tolerated.

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4.4 Risk Benefit Assessment

Cancer stem cell inhibitors offer an exciting approach to the treatment of advanced malignancies. Preclinical studies of BBI608 indicate that the drug is active against a wide range of solid tumors. Encouraging signs of antitumor activity have been observed in the BBI608-101 phase I trial.

Combination of cancer stem cell therapeutics with chemotherapy or targeted agents would allow enhanced simultaneous inhibition of cancer stem cells as well as non-stem regular cancer cells.

BBI608 has been well tolerated in phase I trials. A MTD was not reached in the BBI608-101 Phase I study with doses escalated from 20 mg up to 1000 mg bid (2000 mg total daily dose). Further dose escalation above 2000 mg/day was limited by pill burden.

Adverse events observed in the BBI608-101 phase I trial have been generally mild with the most common being: diarrhea, nausea, and fatigue. Grade 3 events include: fatigue or transient diarrhea. There is no clinically significant overlap between adverse events observed for BBI608 and the adverse effects of Cetuximab, Panitumumab, or Capecitabine.

The sponsor believes that the proposed study is acceptable in the context of its risks and benefits.

4.5 Dose-Limiting Toxicity

Dose-limiting toxicity is defined by the occurrence of any of the following toxicities possibly or probably related to BBI608 in combination with Cetuximab, or Panitumumab or Capecitabine within 28 days of treatment. DLT will be scored during the 28 days of BBI608 treatment.

- Grade 4 hematological toxicity
- Grade 3 or 4 non-hematological toxicity, except:
  - Grade 3 or 4 nausea/vomiting/anorexia, diarrhea and fatigue will be considered a DLT only if it persists more than three (3) days despite optimal medical management
  - Alopecia will not be considered a DLT

Adverse events considered related to Cetuximab, or Panitumumab or Capecitabine will not be considered DLTs.

4.6 Study Duration

Patients will receive treatment with BBI608 and Cetuximab, or Panitumumab, or Capecitabine until progression of disease, unacceptable toxicity or another of the discontinuation criteria is documented (see Section 5.6). It is expected that most patients will receive between one and four cycles of BBI608 and Cetuximab, or Panitumumab, or Capecitabine for a treatment period of 4 to 16 weeks.

5 STUDY VISITS

Study Visits will consist of a Pre-Study Evaluation, during which the patient is evaluated to determine suitability for entry into the study, Cycle 1 Evaluations which will be performed on Weeks 1, 2, 3 and 4 of the first cycle only for Cetuximab, on Weeks 2 and 4 the first cycle only for Panitumumab, and on Weeks 2 and 3 the first cycle only for Capecitabine, evaluations in Cycle 2 and beyond, which will be performed in Week 1 or 2 of each cycle, and an End-of-Study Evaluation. (See Section 14 for a schedule of Assessments)

Following the Pre-Study Evaluation and a determination by a Principal Investigator that the patient meets all inclusion/exclusion criteria and signs the informed consent, the patient will be considered entered into the study.

5.1 Informed Consent

Patients who agree to participate will sign the approved informed consent and will be provided a copy of the signed document.

5.2 Pre-Study Evaluations (Baseline) **(1 week ±3 days prior to the first dose of BBI608*)**

After written informed consent is obtained according to ICH-GCP and local regulations, the patient will be evaluated for inclusion and exclusion criteria according to the eligibility criteria listed in Section 3.

The following will be evaluated and documented within seven days prior to first dose of BBI608:

- Medical history
- Physical examination

- Karnofsky performance status (see Section 15)
- Vital signs (weight, temperature, blood pressure, height, respiration and pulse)
- Hematology (see Section 6.3)
- Blood chemistry (see Section 6.3)
- Liver function tests (see Section 6.3)
- Electrolytes (see Section 6.3)
- Urinalysis (see Section 6.3)
- Serum pregnancy test (if applicable)
- Tumor markers if applicable
- Tumor biopsy, if applicable (see Section 6.5.1)
- 12-lead electrocardiogram (ECG)
- Tumor measurement and staging[computed tomography (CT) or magnetic resonance imaging (MRI) acceptable] *

*All CT and MRI scans can be used as the baseline assessment if they were performed within three weeks prior to the first scheduled dose of BBI608.

Archival tissue samples from a prior biopsy or surgery should be collected from all patients enrolled in the clinical trial if they are available (See Section 17: Appendix E).

5.3 On-Study Assessments

5.3.1 Cetuximab Arm

5.3.1.1 Week 1 (Cycle 1, Day 1)

- Dispense BBI608 (four day supply)
- 12-lead electrocardiogram (2 hours after first BBI608 dose)

5.3.1.2 Week 1 (Cycle 1, Day 5)

- Karnofsky performance status
- Vital signs (weight, pulse)
- Hematology (see Section 6.3)
- Blood chemistry (see Section 6.3)
- Liver function tests (see Section 6.3)
- Electrolytes (see Section 6.3)
- Urinalysis (see Section 6.3)
- Assess AEs
- Record concomitant medication
- Cetuximab, IV infusion starting about 3 hours after morning dose of BBI608
- The second dose of BBI608 will be given approximately 12 hours after the first dose.
- Blood samples for pharmacokinetics, (for only the first 6 patients enrolled in this arm).
- Dispense BBI608 (one week supply)

Blood samples for pharmacokinetics will be collected immediately prior to the first dose of BBI608 and will continue through 171 hours after administration of the first dose of BBI608. Specific details for the collection of blood samples for pharmacokinetics can be located in Sections 6.4 and Section 16.

5.3.1.3 Week 2 (Cycle 1, Day 8)

- Tumor biopsy, if applicable (see Section 6.5.1)

5.3.1.4 Week 2 (Cycle 1, Day 12)

The following assessments will be made during this visit:

- Karnofsky performance status
- Vital signs (weight, pulse)
- Hematology (see Section 6.3)
- Blood chemistry (see Section 6.3)
- Liver function tests (see Section 6.3)
- Electrolytes (see Section 6.3)
- Assess AEs
- Record concomitant medication
- Cetuximab IV infusion, starting at about 3 hours after morning dose of BBI608 Dispense BBI608 (one week supply)

5.3.1.5 Week 3 (Cycle 1, Day 19)

The following assessments will be made during this visit:

- Karnofsky performance status
- Vital signs (weight, pulse)
- Hematology (see Section 6.3)
- Blood chemistry (see Section 6.3)
- Liver function tests (see Section 6.3)
- Electrolytes (see Section 6.3)
- Urinalysis (see Section 6.3)
- Assess AEs
- Record concomitant medication
- Cetuximab IV infusion, starting at about 3 hours after morning dose of BBI608
- Dispense BBI608 (one week supply)

5.3.1.6 Week 4 (Cycle 1, Day 26)

The following assessments will be made during this visit:

- Karnofsky performance status
- Vital signs (weight, pulse)
- Hematology (see Section 6.3)
- Blood chemistry (see Section 6.3)
- Liver function tests (see Section 6.3)
- Electrolytes (see Section 6.3)
- Urinalysis (see Section 6.3)
- Assess AEs
- Record concomitant medication
- Cetuximab IV infusion, starting at about 3 hours after morning dose of BBI608
- Blood samples for pharmacokinetics, (for only the first six patients enrolled in this arm.)
- The second dose of BBI608 will be given about approximately 12 hours after the first dose.
- Dispense BBI608 (one week supply)

Blood samples for pharmacokinetics will be collected immediately prior to the first dose of BBI608 and will continue through 171 hours after administration of the first dose of BBI608. Specific details for the collection of blood samples for pharmacokinetics can be located in Sections 6.4 and Section 16.

5.3.2 **Panitumumab Arm**

5.3.2.1 Week 1 (Cycle 1, Day 1)

- Dispense BBI608 (1 week supply)
- 12-lead electrocardiogram (2 hours after first BBI608 dose)

5.3.2.2 Week 2 (Cycle 1, Day 8)

- Karnofsky performance status
- Vital signs (weight, pulse)
- Hematology (see Section 6.3)
- Blood chemistry (see Section 6.3)
- Liver function tests (see Section 6.3)
- Electrolytes (see Section 6.3)
- Urinalysis (see Section 6.3)
- Assess AEs
- Tumor biopsy, if applicable (see Section 6.5.1)
- Record concomitant medication
- Panitumumab, IV infusion starting about 3 hours after morning dose of BBI608
- The second dose of BBI608 will be given approximately 12 hours after the first dose.
- Blood samples for pharmacokinetics, (for only the first six patients enrolled in this arm.)
- Dispense BBI608 (two week supply)

Blood samples for pharmacokinetics will be collected immediately prior to the first dose of BBI608 and will continue through 171 hours after administration of the first dose of BBI608. Specific details for the collection of blood samples for pharmacokinetics can be located in Sections 6.4 and Section 16.

5.3.2.3 Week 4 (Cycle 1, Day 22)

The following assessments will be made during this visit:

- Karnofsky performance status
- Vital signs (weight, pulse)
- Hematology (see Section 6.3)
- Blood chemistry (see Section 6.3)
- Liver function tests (see Section 6.3)
- Electrolytes (see Section 6.3)
- Urinalysis (see Section 6.3)
- Assess AEs
- Record concomitant medication
- Panitumumab IV infusion, starting at about 3 hours after morning dose of BBI608
- The second dose of BBI608 will be given approximately 12 hours after the first dose.

- Blood samples for pharmacokinetics, (for only the first six patients enrolled in this arm.)
- Dispense BBI608 (two week supply)

Blood samples for pharmacokinetics will be collected immediately prior to the first dose of BBI608 and will continue through 171 hours after administration of the first dose of BBI608. Specific details for the collection of blood samples for pharmacokinetics can be located in Sections 6.4 and Section 16.

5.3.3 **Capecitabine Arm**

5.3.3.1 Week 1 (Cycle 1, Day 1)

- Dispense BBI608 (one week supply)
- 12-lead electrocardiogram (2 hours after first BBI608 dose)

5.3.3.2 Week 2 (Cycle 1, Day 8)

- Karnofsky performance status
- Vital signs (weight, pulse)
- Hematology (see Section 6.3)
- Blood chemistry (see Section 6.3)
- Liver function tests (see Section 6.3)
- Electrolytes (see Section 6.3)
- Urinalysis (see Section 6.3)
- Assess AEs
- Tumor biopsy, if applicable (see Section 6.5.1)
- Record concomitant medication
- The second dose of BBI608 will be given approximately 12 hours after the first dose.
- Blood samples for pharmacokinetics, (for only the first six patients enrolled in this arm.)
- Dispense BBI608 (one week supply)
- Dispense Capecitabine (two week supply)

Blood samples for pharmacokinetics will be collected immediately prior to the first dose of BBI608 and will continue through 24 hours after administration of the first dose of BBI608. Specific details for the collection of blood samples for pharmacokinetics can be located in Sections 6.4 and Section 16.

5.3.3.3 Week 3 (Cycle 1, Day 15)

The following assessments will be made during this visit:

- Karnofsky performance status
- Vital signs (weight, pulse)
- Hematology (see Section 6.3)
- Blood chemistry (see Section 6.3)
- Liver function tests (see Section 6.3)
- Electrolytes (see Section 6.3)
- Urinalysis (see Section 6.3)
- Assess AEs
- Record concomitant medication

- Dispense BBI608 (1 week supply)

5.3.3.4 Week 4 (Cycle 1, Day 21)

The following assessments will be made during this visit:

- Karnofsky performance status
- Vital signs (weight, pulse)
- Hematology (see Section 6.3)
- Blood chemistry (see Section 6.3)
- Liver function tests (see Section 6.3)
- Electrolytes (see Section 6.3)
- Urinalysis (see Section 6.3)
- Assess AEs
- Record concomitant medication
- The second dose of BBI608 will be given approximately 12 hours after the first dose.
- Blood samples for pharmacokinetics, (for only the first six patients enrolled in this arm.)
- Dispense BBI608 (1 week supply)

Blood samples for pharmacokinetics will be collected immediately prior to the first dose of BBI608 and will continue through 24 hours after administration of the first dose of BBI608. Specific details for the collection of blood samples for pharmacokinetics can be located in Sections 6.4 and Section 16.

5.3.3.5 Monthly Evaluations (Cycle 2 and beyond, all arms)

5.3.3.6 Week 1 or 2 (Day 1 – Capecitabine: Day 5 – Cetuximab; Day 8 - Panitumumab)

Patients will have the following assessments:

- Physical examination
- Karnofsky performance status
- Vital signs (weight, temperature, blood pressure, respiration and pulse)
- Hematology (see Section 6.3)
- Blood chemistry (see Section 6.3)
- Liver function tests (see Section 6.3)
- Electrolytes (see Section 6.3)
- Urinalysis (see Section 6.3)
- Tumor markers, if applicable
- Assess adverse events (AE)
- Record concomitant medication
- Cetuximab or Panitumumab IV infusion starting about 3 hours after morning dose of BBI608 (for Cetuximab and Panitumumab arms respectively)
- Dispense BBI608 (28 day supply)
- Dispense Capecitabine (sufficient for Cycle)

5.4 Tumor Evaluation Visits

Disease status and tumor response will be assessed in eight-week intervals up to one year after starting therapy (calculated from the first dose of BBI608) or as clinically indicated. Standard

imaging studies should be performed according to institutional procedures. Tumor response for solid tumors will be evaluated using the guidelines for Response Evaluation Criteria in Solid Tumors (RECIST 1.1) outlined in Section 9.

Once progression of disease during therapy is documented, patients must be taken off treatment and may receive any therapy as determined by their treating physician.

5.5 End of Study Evaluation

All patients will be followed for a minimum of 30 days after the last dose of BBI608. If a patient is removed from the study due to drug-related adverse events, the patient will be followed until resolution of any drug-related AE occurring during the study or within 30 days of the last BBI608 dose, or for 30 days, whichever is longer. In the presence of toxic effects, follow-up visits will be required every four weeks until all study related toxicities have resolved to baseline (or < Common Terminology Criteria for Adverse Events [CTCAE] Grade 1), stabilized or are deemed irreversible.

The following assessments will be made during the end of study visit:

- Physical examination
- Karnofsky performance status
- Vital signs (weight, temperature, blood pressure, respiration and pulse)
- Hematology (see Section 6.3)
- Blood chemistry (see Section 6.3)
- Liver function tests (see Section 6.3)
- Electrolytes (see Section 6.3)
- Urinalysis (see Section 6.3)
- 12-lead ECG
- Tumor marker, if applicable
- Tumor biopsy, if applicable (see Section 6.5.1)
- Tumor measurement and staging
- Assess adverse events
- Record concomitant medications

5.6 Discontinuation from Study

Patients will be removed from the study at any time if they meet any of the following criteria:

- Documented radiologic or clinical progression of disease
- Noncompliance with any part of the study, as evaluated by the Principal Investigator and Medical Monitor
- Withdrawal of consent
- Lost to Follow-up
- Death
- Clinically unacceptable toxicities despite optimal treatment.

6 STUDY PROCEDURES

6.1 Medical History

Medical history will include but not limited to the following:

- Demography: date of birth, sex, ethnic origin, height, and weight
- Clinically significant prior diagnoses, surgeries, and current medications
- Prior cancer history, current cancer diagnosis, tumor stage at time of diagnosis and screening, previous chemotherapy, including dates and duration of treatment, level of response per RECIST and duration of response, previous radiation therapy, including anatomic site, dose and date of treatment
- Other patient characteristics will be summarized as appropriate

6.2 Physical Examination

Complete physical examination including height, weight, blood pressure, heart rate, respiratory rate, temperature (oral, axillary or tympanic) and Karnofsky performance status (Section 15).

6.3 Clinical Laboratory Tests

Safety laboratory determinations will include hematology, blood chemistry, and urinalyses. All laboratory tests required during the study must be obtained at a primary laboratory designated by the Principal Investigator.

- Hematology: CBC including hemoglobin, hematocrit, white blood cell count with 5-part differential, red blood cell, platelet.
- Blood chemistry: HCO_2, calcium, phosphorus, magnesium, albumin, glucose, and serum creatinine
- Liver function tests: AST, ALT, lactate dehydrogenase (LDH), alkaline phosphatase, total and direct bilirubin, uric acid, total protein and blood urea nitrogen (BUN)
- Electrolytes: sodium, potassium, and chloride
- Routine urinalysis: dipstick and microscopy including protein, specific gravity, glucose and blood
- Serum pregnancy test for female patients of childbearing potential.

6.4 Pharmacokinetic Assessments

Blood samples for the PK of BBI608 and Cetuximab, or Panitumumab, or Capecitabine will be collected from the first 6 patients enrolled in each combination arm. Approximately 30 blood samples for PK will be collected for each patient. Collection, storage, and shipping of PK samples will be performed as described in Appendix D.

6.5 Pharmacodynamic Assessments

Pre-clinical studies conducted BBI have identified several biomarkers in tumor tissues whose levels either increase or decrease upon exposure to BBI608. Tumor biopsies will be collected as described below provided the patient has accessible tumor and has signed the optional tumor biopsy consent. The goal of the proposed biomarker study is to examine the response of biomarkers in patients treated with BBI608. For tumor biopsies, tumor samples will be obtained before and after dosing in selected subjects. Subject tumor samples will be processed for determination of pharmacodynamic markers of in malignant tissue by histopathology and flow cytometry.

6.5.1 Tumor Biopsy

Patients who are identified by the Principal Investigator as having a lesion, which could be biopsied with a minimally invasive procedure, will be asked to sign an additional consent. Tumor samples should be collected at baseline and at 4 h after administration of the BBI608 morning dose on Day 8. Collection, storage, and shipping of tissue samples will be performed as described in Appendix E.

7 TREATMENT

7.1 BBI608

BBI608 capsules will be supplied to the pharmacy at the clinical sites. BBI608 will be labeled as an investigational agent, limited by federal law. The pharmacist will dispense an appropriate number of each strength capsule to the Principal Investigator for in-clinic dosing. The appropriate quantity of capsules will be dispensed to the patient depending on the combination regimen.

For the Cetuximab arm, the appropriate number of capsules will be dispensed for four days of daily dosing on day 1, 1 week of daily dosing on day 5, day 12, day 19, and day 26 during cycle 1, and for 1 month of daily dosing for cycle 2 and beyond at the prescribed dose, including two extra doses.

For the Panitumumab arm, the appropriate number of capsules will be dispensed for eight days of daily dosing on day 1, 2 weeks of daily dosing on day 8, and 2 weeks of daily dosing on day 21 during cycle 1, and for 1 month of daily dosing for cycle 2 and beyond at the prescribed dose, including two extra doses.

For the Capecitabine arm, the appropriate number of capsules will be dispensed for 1 week of daily dosing on day 1, 1 week of daily dosing on day 8, 1 week of daily dosing on day 15, 2 weeks of daily dosing on day 21, and for 1 month of daily dosing for cycle 2 and beyond at the prescribed dose, including two extra doses. These instructions must appear on the label for the container in which capsules are delivered to the patient.

Cetuximab, Panitumumab, and Capecitabine will be handled as described in the Product Labels approved by US FDA or Health Canada (see **Appendix F**).

7.1.1 Investigational Product Accountability

BBI will provide all BBI608 required for completion of this study. The recipient will acknowledge receipt of the drug indicating shipment content and condition. Damaged supplies will be replaced. Until dispensed to the patients, the study drug will be stored at room temperature, in a secure locked area, accessible to authorized personnel only.

Accurate records of all BBI608 dispensed from and returned to the study site are to be maintained. The study site must supply a copy of their drug destruction policy to BBI before authorization for destruction will be granted. Product accountability will be monitored throughout the study. Upon completion or termination of the study, and after inventory by a BBI monitor or designated representative, all unopened drug is to be returned to BBI or designee in the original containers.

7.1.2 BBI608 Administration

- BBI608 will be administered daily for 28 consecutive days. BBI608 will be administered twice daily, approximately one hour prior to or two hours after meals, with the first dose given in the morning.

7.1.3 Cetuximab, Panitumumab, or Capecitabine Administration

- Detailed instructions for the preparation, premedication, and administration of Cetuximab, Panitumumab, or Capecitabine are provided in the Product Labels approved by Health Canada or US FDA.

- Cetuximab will be administered via IV on Days 5, 12, 19 and 26 of a 28 day cycle, about 3 hours after the first daily dose of BBI608. Panitumumab will be administered via IV on Days 8, and 22 of a 28 day cycle, about 3 hours after the first daily dose of BBI608. Capecitabine will be administered orally twice-daily for two weeks out of three weeks. The first dose of Capecitabine should be taken about 3 hours after the first daily dose of BBI608 on day 8 of each 3 week cycle of Capecitabine.

7.1.4 **Diarrhea Management**

For each combination arm, treatment for BBI608-related diarrhea will be instituted on a prophylactic or on an as needed basis depending on sub-cohort assignment.

Patients assigned to sub-cohorts IA and IIA will receive prophylactic anti-diarrheal medication starting the day prior to day 1 of cycle 1. With development of intolerable grade 2 diarrhea or greater, prophylactic anti-diarrheal regiment may be adjusted as needed. Patients assigned to sub-cohorts IB and IIB will receive anti-diarrheal medication on an as needed basis starting with development of BBI608-related intolerable grade 2 or above diarrhea. Patients in all 4 sub-cohorts who develop sub-optimal diarrhea control while on protocol therapy will have adjustment of their anti-diarrheal regiment as needed.

The following treatments are suggested for diarrhea and accompanying abdominal cramping management:

Diarrhea & Abdominal Cramping Management
Diphenoxylate/atropine *(Lomotil)* 5 mg QID until control (max 20 mg/day); then maintain at 5 mg BID **Loperamide** *(Imodium/Gastrostop)* 4mg followed by 2 mg Q 4 hours or after each unformed stool (max 16 mg/day); then maintain at 2-4 mg BID
Systemic opioids (e.g. hydromorphone *[Dilaudid],* codeine) have been found effective in reducing abdominal pain and diarrhea
Hyoscine butyl *(Buscopan),* or hyoscyamine *(Levsin)* can also be used for abdominal cramps

7.1.5 Body Surface Area Calculation

The calculation of the dose of Cetuximab or Panitumumab will be based on the patient's body surface area (BSA). The BSA will be calculated before each cycle, based on the actual height and weight of the patient. The calculated dose will be adjusted downward to the nearest whole milligram.

7.2 Dose Modifications

7.2.1 Cetuximab

Infusion Reactions: Reduce the infusion rate by 50% for NCI CTC Grade 1 or 2 and non-serious NCI

CTC Grades 3–4 infusion reactions.

Immediately and permanently discontinue Cetuximab for severe (grade 3 or 4) infusion reactions requiring medical intervention and/or hospitalization.

Dermatological toxicity: If a patient experiences severe acneiform rash (grade 3 or 4), Cetuximab treatment adjustment should be made according to Table 3.

Table 3. Cetuximab Dose Modification Guidelines for Rash

Severe Acneiform Rash	Cetuximab	Outcome	Cetuximab Dose Modification
1st occurrence	Delay infusion 1 to 2 weeks	Improvement	Continue at 250 mg/m^2
		No improvement	Discontinue Cetuximab
2nd occurrence	Delay infusion 1 to 2 weeks	Improvement	Continue at 200 mg/m^2
		No improvement	Discontinue Cetuximab
3rd occurrence	Delay infusion 1 to 2 weeks	Improvement	Continue at 150 mg/m^2
		No improvement	Discontinue Cetuximab
4th occurrence	Discontinue Cetuximab		

7.2.2 Panitumumab

Infusion Reactions: Reduce the infusion rate by 50% for NCI CTC Grade 1 or 2 and non-serious NCI CTC Grades 3–4 infusion reactions.

Immediately and permanently discontinue Panitumumab for severe (grade 3 or 4) infusion reactions requiring medical intervention and/or hospitalization.

Dermatological toxicity: If a patient experiences dermatological toxicities (grade 3 or 4), Panitumumab treatment should be withheld until the toxicities have improved (≤ grade 2). Once improved, reinstate Panitumumab administration at 50% of the original dose. If toxicities do not worsen, escalate each additional dose of Panitumumab by 25% increments of the original dose until the recommended starting dose is reached. If toxicity dose not resolve to at least grade 2 after withholding 1 or 2 doses of Panitumumab, or if toxicity worsens or becomes intolerable at 50% of the original dose level, the use of Panitumumab should be permanently discontinued.

7.2.3 Capecitabine

Hematological:

ANC ($x10^9$/L)		Platelets ($x10^9$/L)	1st Event Dose	2nd Event Dose	3rd Event Dose	4th Event Dose
≥ 1.5	and	≥ 75	100%	100%	100%	100%
1-1.49	or	50-74.9	delay* then 100%	delay* then 75%	delay* then 50%	discontinue
0.5 - 099	or	25 - 49.9	delay* then 75%	delay* then 50%	discontinue	discontinue
< 0.5	or	< 25	discontinue or delay* then 50%	discontinue	discontinue	discontinue

* delay until ANC ≥ 1.5x10^9/L and platelets ≥ 75 x10^9/L

Hand-Foot Reaction

Grade	1st Event Dose	2nd Event Dose	3rd Event Dose	4th Event Dose
1	100%	100%	100%	100%
2	delay* then 100%	delay* then 75%	delay* then 50%	discontinue
3	delay* then 75%	delay* then 50%	discontinue	discontinue

* delay until until resolved to grade 0-1

Other Non-Hematological Toxicity

Grade	1st Event Dose	2nd Event Dose	3rd Event Dose	4th Event Dose
1	100%	100%	100%	100%
2	delay* then 100%	delay* then 75%	delay* then 50%	discontinue
3	delay* then 75%	delay* then 50%	discontinue	discontinue
4	discontinue or delay* then 50%	discontinue	discontinue	discontinue

* delay until until resolved to grade 0-1

7.2.4 BBI608

For any BBI608-related intolerable grade 3 or intolerable grade 2 adverse event, persisting despite optimized medical management, a dose holiday of 1-3 days followed by dose modification is recommended. Dose modification may include reduction of the total daily dose and/or in the number of doses taken per day. Investigators should discuss dose modifications with the medical monitor for the sponsor. Patients may up-titrate dose to full dose as tolerated.

If a toxicity is thought by the Investigator to be related to both BBI608 and the combination drug (Cetuximab, Panitumumab or Capecitabine), then the dose modification rules for both agents should be followed.

7.3 Treatment Compliance

A patient is considered compliant with the study protocol when study medication is administered at a compliance level of greater than 80%.

BBI608 compliance will be calculated using the following equation:

(Number of capsules actually ingested /number of capsules that should have been ingested per dose level) x 100= % compliance

Capecitabine compliance will be calculated using the following equation:

(Number of capsules actually ingested /number of capsules that should have been ingested per dose level) x 100= % compliance

Cetuximab or Panitumumab compliance will be calculated using the following equation:

(Number of treatments performed /number of treatments that should have been performed per dose level) x 100= % compliance

7.4 Blinding

This is an open label study. Neither the patient nor the investigator and site staff will be blinded to the treatment administered.

7.5 Prior Treatment

Reasonable efforts will be made to determine all relevant prior treatments received by the patient within four weeks of the first BBI608 dose. All relevant information must be recorded on the appropriate patient's case report form (CRF). All surgical procedure history, prior chemotherapy and radiation therapy must be recorded on the appropriate CRF.

7.6 Concomitant Medication

7.6.1 Permitted Treatment

All information regarding concomitant treatments (medications or procedures) must be recorded on the patient’s CRF (including the name of the medication or procedure and duration of treatment).

Palliative and supportive care for disease-related symptoms will be offered to all patients in this study. Patients may receive palliative radiation therapy.

BBI608 was shown *in vitro* to inhibit individual CYP P450 isoforms 1A2, 2D6, 2C19, 3A4, and 2C9 with IC_{50}'s of 0.25 μM, 0.25 μM, 2.5 μM, 5 μM, and 0.5 μM respectively. Since *in vitro*, BBI608 has shown the ability to inhibit these CYP P450 isoforms, concomitant use of agents that are substrates of these CYP P450 enzymes should be avoided unless deemed medically necessary by the primary investigator. Examples of commonly prescribed agents that are metabolized by these CYP P450 enzymes are:

NSAIDS: such as ibuprofen, naproxen
Proton pump inhibitors: such as lansoprazole, omeprazole
Oral hypoglycemic medications: sulfonylureas
Beta-blockers: metoprolol, carvedilol
Calcium channel blockers: amlodipine, diltiazem, nefedipine, verapamil
Antidepressants: paroxetine, imipramine, amitriptyline
Anti-epileptics: such as phenytoin, phenobarbitone Anti-
psychotics: such as haloperidol, risperidone
Antibiotics: such as clarithromycin, erythromycin
HMG CoA reductase inhibitor: atorvastatin, lovastatin, simvastatin
Anesthetics: such as halothane, enflurane;
HIV antivirals: such as saquinavir, indinavir, ritonavir
Immunomodulators (immunosuppressives): such as cyclosporine, tacrolimus
Steroids: such as hydrocortisone, estrodiol

BBI608 is metabolized by the CYP P450 isoform 1A2. Therefore, concomitant use of drugs which inhibit CYP1A2 should be avoided unless deemed medically necessary by the primary investigator. Known drugs that inhibit CYP 1A2 include ciprofloxacin and other fluoroquinolones, Fluvoxamine Verapamil, amiodarone, interferon, methoxsalen, enoxacin, mexiletine, and ticlopidine

In addition, the following treatments are allowed:

- Standard therapies for concurrent medical conditions
- Epoetin alfa (Epogen®, Procrit®)
- Hematopoietic growth factors, including filgrastim (Neupogen®), or other granulocyte colony stimulating factors (G-CSF), are permitted following documented and clinically significant neutropenia after the patient has completed at least one cycle of treatment with BBI608
- Prophylactic antiemetics may be administered according to standard practice
- Megestrol acetate (Megace®)
- Dethamethasone
- Diphenhydramine
- Cimetidine
- Ranitidine

7.6.2 Prohibited Treatment

- Any other concurrent chemotherapy, non-palliative radiotherapy, hormonal therapy, or immunotherapy
- Other investigational agents
- Immunosuppressive therapies, including systemic corticosteroids (except when used intermittently in an antiemetic regimen or when prescribed at a non-immunosuppressive dose for treatment of fatigue and low-appetite)

8 SAFETY ASSESSMENTS

8.1 Adverse Events

8.1.1 Assessments

The Investigator is responsible for monitoring the safety of patients who have enrolled in the study. All AEs considered to be related to BBI608 occurring after any administration of the study drug will be followed until the event resolves. AEs will be evaluated using the National Cancer Institute (NCI) CTCAE, Version 4.0, published 28 May 2009.

Investigators are required to document all AEs occurring during the clinical trial, commencing with the first dose of BBI608 and including the protocol-defined post-treatment follow-up period (21 Code of Federal Regulations [CFR] §312.64[b]) on designated CRF pages. AEs occurring following the signature of the informed consent, but prior to the first dose of study drug will not be reported as AEs. It is also important to record all AEs that result in permanent discontinuation of the investigational product being studied, whether serious or non-serious.

Serious adverse events (SAEs), as defined below, must be reported to Boston Biomedical Inc. or its representative within 24 hours of knowledge of their occurrence.

8.1.2 Definitions

An AE is defined as any untoward medical occurrence in a patient or clinical investigational patient administered a pharmaceutical product that does not necessarily have to have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Laboratory data are to be collected as stipulated in this protocol, and toxicity trends will be analyzed utilizing objective toxicity criteria. Clinical syndromes associated with laboratory abnormalities are to be recorded as appropriate (e.g., diabetes mellitus instead of hyperglycemia).

Progression of disease is considered an efficacy outcome parameter and should not be captured as an AE. A non-serious AE is any untoward medical occurrence that does not meet any of the criteria for SAEs as defined below.

Patients should be instructed to report any AE that they experience to the investigator. Investigators should assess the patient for AEs at each visit. AEs occurring during the clinical trial and the follow-up period should be recorded on the appropriate AE CRF. To capture the most potentially relevant safety information during a clinical trial, it is important that investigators record accurate AE terms on CRFs.

Wherever possible, a specific disease or syndrome rather than individual associated signs and symptoms should be identified by the investigator and recorded on the CRF. However, if an observed or reported sign or symptom is not considered a component of a specific disease or syndrome by the investigator, it should be recorded as a separate AE on the CRF.

8.2 Serious Adverse Events

8.2.1 Definitions

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

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization , except for events that are clearly disease related
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect

Important medical events may be considered an SAE based upon appropriate medical judgment.

Under this protocol, scheduled hospitalizations or elective surgical procedures will not be considered SAEs. Prolongation of a scheduled hospitalization can be considered an SAE. Complications associated with scheduled procedures are considered an AE.

8.2.2 Reporting Serious Adverse Events

Any SAE, including death, due to any cause that occurs during this investigation, whether or not related to the administration of study drug, must be reported to the Sponsor immediately (not to exceed 24 hours) by telephone or facsimile. The reaction must be completely described on the CRF and SAE report form.

Primary Medical Monitor Contact information:
[redacted]
[redacted]

9 ASSESSMENT OF ANTI-TUMOR ACTIVITY IN SOLID TUMORS

The following definitions and criteria (from Response Evaluation Criteria in Solid Tumors [RECIST 1.1, Eisenhauer et al. 2009] [19]) should be used for the baseline evaluations of existing disease, and for the ongoing evaluation of tumor responses.

Measurable disease - the presence of at least one measurable lesion.

Measurable lesions - lesions that can be accurately measured in at least one dimension with longest diameter ≥10 mm using conventional techniques or ≥10 mm with spiral CT scan. Malignant lymph nodes must be ≥15 mm in the shortest diameter.

Non-measurable lesions - all other lesions, including small lesions (longest diameter <10 mm with conventional techniques, <10 mm with spiral CT scan or ≥10 mm to <15 mm in short axis), i.e., bone lesions, ascites, pleural/pericardial effusion, cystic lesions and also abdominal masses that are not confirmed and followed by imaging techniques.

- All measurements should be taken and recorded in metric notation, using a ruler or calipers.
- 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.
- Lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). For the case of skin lesions, documentation by color photography, and a measurement with a ruler to estimate the size of the lesion, is recommended.

9.1 Methods of Measurement

- CT and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. Conventional CT and MRI should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm.
- All imaging methods should be performed according to institutional standards with each patient having consistency of methods beginning from baseline through the course of the study.

9.2 Baseline Documentation of "Target" and "Non-Target" Lesions

- All measurable lesions up to a maximum of five lesions total with a maximum of 2 lesions per organ, representative of all involved organs should be identified as ***target lesions*** and recorded and measured at baseline.
- Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinical assessments).
- A sum of the longest diameter (LD) for *all target lesions* will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as the reference by which to characterize the objective tumor response.
- All other lesions (or sites of disease) should be identified as ***non-target lesions*** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

9.3 Response Criteria

	Evaluation of target lesions
Complete Response (CR):	Disappearance of all target lesions Any pathological lymph nodes must have reduction in short axis of <10mm
Partial Response (PR):	At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD
Progressive Disease (PD):	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started. In addition to the increase of 20%, the sum must also demonstrate an absolute increase of at least 5mm. 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 LD since the treatment started

	Evaluation of non-target lesions
Complete Response (CR):	Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10mm short axis)
Stable Disease (SD):	Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above normal limits
Progressive Disease (PD):	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions

9.4 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence and is determined as indicated in the table below:

Target lesions	Non-Target lesions	Evaluation of 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
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

10 PLANNED STATISTICAL METHODS

10.1 General Considerations

Because of the nature of this study, no formal statistical analysis is planned. Evaluation of the data will consist primarily of summary displays (i.e., descriptive statistics and graphs).

10.2 Determination of Sample Size

The following number of colorectal cancer patients will be enrolled based on a two-stage Simon design with an alpha of 5% and a power of 80% to detect a disease control rate improvement for Cetuximab and Panitumumab from about 30% (historical DCR for 3rd and 4th line colorectal cancer) to 60%, and a disease control rate improvement for Capecitabine from about 15% (historical DCR for 3rd line colorectal cancer) to 60%

Disease Control Rate – Third Line

	Monotherapy (Historical)	Combination * (Historical)	Combination with BBI608 (Expected)
Cetuximab	30-40% [4,5]	~ 63% [10-15]	60% or more
Panitumumab	30-40% [6-8]	~ 68% [16]	60% or more
Capecitabine	~ 15% [9]	~ 45% [17,18]	60% or more

* Cetuximab + Irinotecan
Panitumumab + Irinotecan
Capecitabine + Mitomycin C

Simon two-stage design for Phase II

	Patient number		
Combo Therapy	**Stage 1**	**Stage 2**	**Total***
Cetuximab	8	16	24
Panitumumab	8	16	24
Capecitabine	6	12	18

*If any of the three arms reach a DCR of 60% or more at the end of Simon Stage 2, a one-time option to expand accrual to a total sample size of up to 50 patients may be implemented for each arm.

For the Cetuximab and Panitumumab combination cohorts, if the disease control rate in stage 1 reaches 3 of 8 or above, additional patients will be enrolled into the stage 2 cohort. For the Capecitabine combination cohort, if the disease control rate in stage 1 reaches 2 of 6 or above, additional patients will be enrolled into the stage 2 cohort.

10.3 Analysis Populations

All patients receiving at least one dose of BBI608 will be considered evaluable for safety analysis. Adverse event incidence rates will be described by the frequency of adverse events, categorized by NCI CTCAE. Listings of laboratory test results collected at baseline and during the study will be generated. Descriptive statistics summarizing the changes in those laboratory tests over time will be presented.

Patients who have received at least one cycle of study treatment and have had at least one disease assessment following the initiation of therapy will be considered evaluable for disease control rate and overall response. Progression-free survival and overall survival will be evaluated in the intent-to-treat population. Anti-tumor activity will be evaluated on an exploratory basis and will be summarized using descriptive statistics or graphics.

10.4 Efficacy

The primary population for the efficacy evaluation will be the evaluable population, defined as patients who have been treated with at least 80 % of 1 complete cycle of BBI608 and a combination drug. The primary efficacy outcome measure will be disease control rate, defined as the proportion of patients with a documented complete response, partial response and stable disease (CR + PR + SD) based on RECIST. In addition, progression-free survival (PFS) and overall survival (OS) will be obtained from intent-to-treat patients. PFS will be calculated from the date of patient enrolment into the trial until documented disease progression based on RECIST. OS will be calculated from the date of patient enrolment into the trial until death.

10.5 Demographics and Baseline Characteristics

Patient characteristics will include a complete summary of the following and will be recorded in the CRFs:

- Patient demographics
- Baseline disease characteristics
- Pre-existing conditions
- Prior therapies
- Concomitant medications and treatments

Other patient characteristics will be summarized as appropriate

10.6 Statistical Analysis of Pharmacokinetic Variables

Pharmacokinetics will only be performed on the first 6 patients enrolled in each combination arm of this study.

Timed blood sample collection for BBI608 and Cetuximab pharmacokinetic analysis will be performed on Days 5-11 and 26-32 after the first dose of Cycle 1 for the first 6 patients enrolled in the Cetuximab arm. Timed blood sample collection for BBI608 and Panitumumab pharmacokinetic analysis will be performed on Days 8-14 and 22-28 after the first dose of Cycle 1 for the first 6 patients enrolled in the Panitumumab arm. Timed blood sample collection for BBI608 and Capecitabine pharmacokinetic analysis will be performed on Days 8-9 and 21-22 after the first dose of Cycle 1 for the first 6 patients enrolled in the Capecitabine arm.

Bioanalytical analysis of patient samples will be conducted at a centralized laboratory using GLP-validated assays. Plasma concentrations will be summarized by descriptive statistics, including mean, standard deviation, coefficient of variation, minimum, maximum, and median**.**

Concentration profiles will be analyzed by noncompartmental and/or nonlinear least squares regression using WinNonLin. Pharmacokinetic parameters, including C_{max}, volume of distribution, distribution half-life, terminal half-life, and AUC will be evaluated.

10.7 Safety Analysis

Safety will be assessed by physical examination, and laboratory assessments. Adverse events will be graded according to the NCI CTCAE, version 4.0. The incidence of adverse events and DLTs will be evaluated for each combination arm, and for all patients combined. Patients will be followed for adverse events for at least 30 days after the last dose of BBI608, or until recovered from all related BBI608 adverse events.

10.8 Analysis and Sample size for sub-cohorts

Each sub-cohort will be analyzed for number of days of Grade II/III diarrhea and % treatment compliance achieved during the first two cycles. The % treatment compliance will be calculated as the number of capsules actually ingested over the number of capsules that should have been ingested per dose level multiplied by 100. The research questions addressed by sub-cohorts are hypothesis-generating and exploratory in nature. Initially 6 subjects will be assigned to each sub-cohort to assess the effect of intervention. Selected sub-cohorts will be extended to enroll up additional 20 patients. The decision of extending the enrollment will be based on clinical assessment without formal statistical testing. However, 20 patients will give 80% power to detect 20% difference in % treatment compliance between two arms and 57% power to detect the difference of 25% in the number of days of having Grade II/III diarrhea using two-sided Z test with pooled variance at type I error rate of 0.15. The control rates assumed are 70% for treatment compliance and 30% for diarrhea. The patients will be sequentially allocated to different sub-cohorts by the Sponsor to avoid section bias.

11 QUALITY CONTROL AND ASSURANCE

The study will be initiated and conducted under the Sponsorship of BBI. The clinical supplies of BBI608 and CRFs will be supplied by BBI or its representative. Representatives of BBI will monitor the study to verify study data, medical records, and CRFs in accordance with current ICH GCPs and other applicable regulations and guidelines.

11.1 Compliance with the Protocol

The Investigator will notify the Sponsor of any deviations from the protocol. Such contact with the Sponsor will be made as soon as possible to permit a decision as to whether or not the subject (for whom the deviation from the protocol was effected) is to continue in the study. The case records will describe the deviation from the protocol and state the grounds for it.

11.2 Registration and Enrollment

This is an open-label, non-randomized study. Boston Biomedical should be notified as soon as a subject qualifies for entry in the protocol. Subjects will be registered by faxing Boston Biomedical or their designee, within 7 days prior to the 1st drug administration. At that time the combination arm will be assigned. The subject will be enrolled into the study when the subject receives the 1st dose of study drug. Registration and enrollment forms and faxing instructions will be provided with the case report forms (CRFs). The site should maintain a log of all subjects who are screened but do not qualify for the study or who do not receive study drug. The reason for disqualification should be noted in the log.

11.3 Removal, Replacement, or Early Withdrawals of Subjects

If a subject exits the study prior to receiving four weeks of BBI608 or does not receive four weeks of BBI608 for a reason other than DLT, an additional subject may be recruited to replace the subject.

12 COMPLIANCE WITH GOOD CLINICAL PRACTICE, ETHICAL CONSIDERATIONS AND INFORMED CONSENT

12.1 Institutional Review Board

The protocol, any protocol modifications, informed consent form that will be used, and, if applicable, the permission to use private health information must be approved by the Investigator's IRB or Independent Ethics committee (IEC) (compliant with federal regulations 21 CFR 56) before the study is initiated. Documentation of this approval (i.e., a copy of the document showing IRB/IEC approval including the chairperson's signature and the date of approval) must be provided to Boston Biomedical or its designee, and made available during an inspection by the FDA, Health Canada or other regulatory agency inspectors. The Investigator will submit to Boston Biomedical:

- A list of the names, occupations, and affiliations of the members of the IRB
- Documentation that the IRB is duly constituted or a General Assurance Number
- No supplies will be shipped until the IRB has given written approval of the protocol and informed consent and Boston Biomedical has received copies of the approvals

It is the responsibility of the Investigator to:

- Submit to the IRB/IEC for review any advertisements that will be used to recruit subjects
- During the conduct of the study, submit progress reports to the IRB, if required, and request review of the study
- Report, in writing, to the IRB all SAEs that occurred during the study or SAEs reported in other studies using study drug, per local IRB regulations
- Inform the IRB of any changes in the protocol and obtain documented IRB approval of the changes
- Maintain a file of study-related information, including all correspondence with the IRB/IEC
- Within 3 months of study completion, provide the IRB with a final report on the study

12.2 Compliance with Good Clinical Practice and Ethical Considerations

This study must be conducted in compliance with IRB/IEC informed consent regulation and the ICH GCP Guidelines. In addition, all local regulatory requirements will be adhered to, in particular those which afford greater protection to the safety of the trial participants.

This study will be conducted according to the current revision of the Declaration of Helsinki (Revised Edinburgh, Scotland, 2000) and with local laws and regulations relevant to the use of new therapeutic agents in the country of conduct.

Before initiating a trial, the Investigator/institution should have written and dated approval/favorable opinion from the IRB/IEC for the trial protocol/amendment(s), written informed consent form, patient recruitment procedures (e.g., advertisements) and written information to be provided to patients.
Changes to the protocol will require written IRB/IEC approval/favorable opinion prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to patients.

12.3 Informed Consent and Permission to Use Private Health Information

The Investigator, or designee, is responsible for the content of the informed consent form, but the content must be submitted and approved by Boston Biomedical prior to submission to the IRB. Before the start of required study procedures, the Principal Investigator or associate must obtain informed consent from each study participant (or the subject's parent/guardian) in accordance with the US federal regulations (21 CFR Part 50) and ICH document "Guidance for Industry – E6 Good Clinical Practice: Consolidated Guidance" dated April 1996. It should also include any additional information required by local laws relating to institutional review.

Informed consent must be obtained from the subject before any screening activity, washout of medication, or treatment (that is not part of routine care) is undertaken. Informed consent will be obtained by discussing with the subject the purpose of the study, the risks and benefits, the study procedures, and any other information relevant to the subjects.

The subject or his/her legal representative will document their informed consent by signing the current version of the written, IRB-approved, informed consent form in the presence of a witness.

The person, who conducted the informed consent discussion with the subject and/or guardian, must also sign the informed consent form. The subject should be given a copy of the informed consent form with all of the appropriate signatures.

The Principal Investigator will ensure that a copy of the signed consent is kept with the Clinical Trial Master File.

The Investigator or designee must explain to the patient subject that for evaluation of study results, that subject's private health information obtained during the study may be shared with the study Sponsor, regulatory agencies, and ECs/IRBs, before enrolling that subject into the study. It is the Investigator's (or designee's) responsibility to obtain permission to use private health information per HIPAA from each subject, or if appropriate, the subject's parent or legal guardian.

13 STUDY MANAGEMENT

13.1 Amendments to the Protocol

Once the protocol has been approved by the IRB, the Investigator will not modify it without obtaining the prior concurrence of Boston Biomedical. In turn, Boston Biomedical will inform the Investigator in writing of any amendment to the protocol. The Investigator must submit the protocol modifications and any informed consent modifications to the IRB, and approval must be obtained before the modifications are implemented. Boston Biomedical will submit protocol modifications the FDA, and to Health Canada.

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13.2 Investigator Brochure and Information Materials

Before the study begins, the Investigator will receive an Investigator's Brochure describing all known contraindications, warnings, precautions, and adverse reactions associated with the administration of the study drug. If such information is revised while the study is in progress,

the brochure will be amended or revised, and Boston Biomedical will provide the most current version to the Investigator.

13.3 Pre-investigational Documents

Prior to the shipment of the study drug(s), the Investigator will supply Boston Biomedical with the following:

- A signed Investigator Clinical Research Agreement
- A completed Form FDA 1572 signed by the Investigator
- Current curricula vitae and copy of current medical license for the Principal Investigator and Sub-Investigators listed on Form FDA 1572
- A completed financial disclosure form for all personnel listed on Form FDA 1572
- Signed and dated protocol signature page by the Principal Investigator
- A copy of the approval for this protocol from the IRB listed on Form FDA 1572
- A copy of the approval for the informed consent from the IRB listed on Form FDA 1572
- A copy of the IRB-approved informed consent
- Evidence of laboratory certification and a list of laboratory normal ranges for all laboratories listed on Form FDA 1572
- A list of the IRB members (listed on Form FDA 1572) and the member occupations and affiliations; written verification that the IRB is duly constituted or the General Assurance Number

13.4 Drug Inventory Record

The Investigator, or a responsible party (research pharmacist or other) designated by the Investigator, must maintain an inventory record of drug received and dispensed. Boston Biomedical will provide forms to facilitate the inventory control. These forms must be used unless the Investigator has previously established a system that complies with FDA and/or Health Canada regulations and is approved by Boston Biomedical. The study drug must be dispensed only to the institutions(s) specified on form FDA 1572.

13.5 Disposition of Used and Unused Study Drug

Upon completion or termination of the study and after inventory by a Boston Biomedical monitor or designated representative, all unopened drug is to be returned to Boston Biomedical in the original containers. All used vials will be retained until released for destruction by the Boston Biomedical monitor. Unopened returned drug, with completed Boston Biomedical forms for return shipment, should be shipped as instructed by the Sponsor.

13.6 Study Records

Boston Biomedical will provide the Investigator with drug shipment records, CRFs designed to collect the data specified for each individual, and other forms as necessary.

The Investigator and/or institution is required to prepare and maintain these forms in accordance with federal regulations (set forth in the Statement of Investigator Form FDA-1572) and to sign, date, and return them to the Sponsor.

Upon the request of authorized Boston Biomedical or appropriate regulatory agency personnel, the Investigator will make available for inspection subject source documents, e.g., records of each subject who participates in this study. This information will be treated as confidential.

13.7 Record Retention

Records must be maintained for 25 years:

If the Investigator leaves the institution where the study was conducted, he/she agrees that the records will be retained and will not be destroyed without prior notification of Boston Biomedical.

Boston Biomedical will notify the Investigator when records are no longer required.

13.8 Subject Confidentiality

Every effort will be made to keep all subject identities confidential. All reports and communications submitted to the Sponsor will be identified only by the subject's initials and subject number. The identity of an individual subject may not be disclosed in any publication relating to this study.

In connection with this study, representatives of Health Canada, or other regulatory bodies outside of Canada, such as the United States Food and Drug Administration representatives of the local IRB may, in certain circumstances, review study source documentation including subject medical records.

13.9 Monitoring

In accordance with good clinical practices, the study will be monitored by Sponsor representatives. These representatives will have access to and will review source documents relating to this study, including subject medical records.

The status of drug storage, dispensing, and accountability will also be assessed during periodic visits.

At any time, each site may be audited either by Boston Biomedical personnel, or by a contractor acting on behalf of Boston Biomedical, or by a regulatory agency such as the FDA or Health Canada.

13.10 Case Report Form (CRF) Completion

A set of CRFs will be provided for each study subject. All forms must be filled out in non-erasable ink or typed. The Investigator will sign and date each CRF as indicated. Correction of data on a CRF will be made by crossing out the incorrect data in a manner that leaves the previous entry legible and writing the correct information next to the crossed out entry. "White-out" and erasures are not permitted. Each correction must be initialed and dated by the individual making the correction. After the CRFs have been collected by Boston Biomedical, all corrections will be made via a query resolution form, and no further corrections should be made on the site's copy of the CRF.

13.11 Final Site Report

The Principal Investigator or associate must notify the IRB when the study is closed and provide a final report to the IRB within 90 days of the last subject's completion of the study. A copy of this final report must also be provided to Boston Biomedical.

13.12 Final Study Report

At the conclusion of the study, after the data are analyzed, Boston Biomedical will prepare a final study report. A copy of this report will be provided to the Principal Investigator at each center.

The preparation of the final study report may be delegated to a contract research organization.

13.13 Use of Information

All personal information pertaining to subjects in this study and in any subsequent reports will be kept confidential. Subjects will be identified only by their initials and by a subject number. It is the responsibility of the Investigator to keep a subject listing for cross-referencing.

The Investigator understands that the information developed in the clinical study will be used by Boston Biomedical in connection with the development of the study drug. This information may be disclosed to other clinical investigators, the FDA, Health Canada and other government agencies.

All information disclosed to the Investigator(s) by Boston Biomedical for the purpose of having the Investigator(s) conduct the clinical trial described in this protocol or generated by the Investigator(s) as results in the clinical trial shall be treated by the Investigator(s) as strictly confidential. The Investigator(s) shall not use such information other than for the purpose of conducting the clinical trial and may not disclose such information to others, except when such disclosure is to colleagues and/or employees who reasonably require the information to assist in carrying out the clinical trial and who are bound by like obligations of confidentiality. Notwithstanding, the Investigator(s) may use or disclose to others any information which: (i) was known to the Investigator(s) prior to the date of its disclosure, (ii) is now, or becomes in the future, publicly available; or (iii) is lawfully disclosed to the Investigator(s) on a non-confidential basis by a third party who is not obligated to Boston Biomedical or any other party to retain such information in confidence.

13.14 Publication

Boston Biomedical acknowledges that the Investigator(s) have certain professional responsibilities to report to the scientific community on findings in clinical investigations they conduct. The Principal Investigator shall have the right to publish the results of research performed under this protocol, provided such publication does not disclose any Confidential Information or trade secrets of Boston Biomedical (other than the Clinical Data). If the Study is conducted as part of a multi-center protocol, Principal Investigator agrees not to independently publish their findings except as part of an overall multi-center publication, unless specifically approved in writing by Boston Biomedical. The Principal Investigator agrees to, prior to submitting a manuscript, abstract, or any other written or oral presentation describing the Data for publication or presentation, forward to Boston Biomedical a copy of the item to be submitted for publication or presentation. Upon reasonable request by Boston

Biomedical within 30 days of receipt, the Principal Investigator agrees to withhold such publication an additional 60 days to permit the preparation and filing of related patent applications. In addition, Boston Biomedical shall have the right to require the Principal Investigator to delete from any publication or presentation any Confidential Information (other than the Clinical Data) of Boston Biomedical's and to require that any publication or presentation concerning the Study acknowledge the Sponsor's support.

13.15 Research Outside the Terms of this Protocol

Boston Biomedical has a legal responsibility to report fully to the regulatory authorities all the results of administration of its investigational drugs.

No investigative procedures other than those described in this protocol shall be undertaken on subjects enrolled in this study (unless required for the care of the subject), without the agreement of the IRB/Ethics Committee and Boston Biomedical. The nature and results of any such procedures must be recorded and reported by a method agreed between Boston Biomedical and the Investigator. The consent of the subjects must be obtained before any such procedures are undertaken.

The investigative drug provided to the Investigator for use under this protocol may not be used for any other purpose, including another study, compassionate use, or personal use.

14 APPENDIX A: SCHEDULE OF ASSESSMENTS

BBI608 + Cetuximab

Tests & Procedures	Pre-Study Evaluation	Study Evaluations[1]										End of Study Visit
		Cycle 1						Cycle 2 & Beyond				
Week	0	1		2		3	4	1	2	3	4	
Day	0	1	5	8	12	19	26	5	12	19	26	≤ 30 days from last BBI608 dose
Window	-7 ± 3 days	± 1 day						± 1 day				
Medical history	X											
Physical examination	X							X				X
Serum pregnancy test[3]	X											
Karnofsky performance status	X		X		X	X	X	X				X
Vital signs, Weight	X		X		X	X	X	X				X
Hematology[2]	X		X		X	X	X	X				X
Blood chemistry[2]	X		X		X	X	X	X				X
Liver function tests[2]	X		X		X	X	X	X				X
Electrolytes[2]	X		X		X	X	X	X				X
Urinalysis[2]	X		X			X	X	X				X
12-Lead electrocardiogram	X	X										X
Tumor markers[3]	X							X				X
Tumor biopsy[3]	X			X								X
Pharmacokinetics (first 6 patients only)			X				X					
Tumor measurement & staging[4,5]	X[6]											X[7]
Concomitant medications	X		X		X	X	X	X				X
Adverse events[8]			X		X	X	X	X				X
Dispense BBI608		X	X		X	X	X	X				
Cetuximab infusion			X		X	X	X	X	X	X	X	

[1.] Calculated from the date of first BBI608 dose.
[2.] Refer to Section 6.3 for description of laboratory assessments.
[3.] If applicable
[4.] Including tumor measurements, following RECIST 1.1.
[5.] Prior to cycle 3 and every other cycle thereafter, assessments must be made utilizing the same imaging method as baseline
[6.] Unless CT/MRI has been performed within last three weeks
[7.] Unless CT/MRI has been performed within last four weeks.
[8.] Patients will be followed until resolution of any drug-related AE or SAE occurring during the study or within 30 days of last BBI608 administration.

BBI608 + Panitumumab

Tests & Procedures	Pre-Study Evaluation	Study Evaluations[1]								End of Study Visit
		Cycle 1				Cycle 2 & Beyond				
Week	0	1	2	3	4	1	2	3	4	
Day	0	1	8	15	22	1	8	15	22	≤ 30 days from last BBI608 dose
Window	-7 ± 3 days	± 1 day				± 1 day				
Medical history	X									
Physical examination	X						X			X
Serum pregnancy test[3]	X									
Karnofsky performance status	X		X		X		X			X
Vital signs, Weight	X		X		X		X			X
Hematology[2]	X		X		X		X			X
Blood chemistry[2]	X		X		X		X			X
Liver function tests[2]	X		X		X		X			X
Electrolytes[2]	X		X		X		X			X
Urinalysis[2]	X		X		X		X			X
12-Lead electrocardiogram	X	X								X
Tumor markers[3]	X						X			X
Tumor biopsy[3]	X		X							X
Pharmacokinetics (first 6 patients only)			X		X					
Tumor measurement & staging[4,5]	X[6]									X[7]
Concomitant medications	X		X		X		X			X
Adverse events[8]			X		X		X			X
Dispense BBI608		X	X		X		X			
Panitumumab infusion			X		X		X		X	

1. Calculated from the date of first BBI608 dose.
2. Refer to Section 6.3 for description of laboratory assessments.
3. If applicable
4. Including tumor measurements, following RECIST 1.1.
5. Prior to cycle 3 and every other cycle thereafter, assessments must be made utilizing the same imaging method as baseline
6. Unless CT/MRI has been performed within last three weeks.
7. Unless CT/MRI has been performed within last four weeks.
8. Patients will be followed until resolution of any drug-related AE or SAE occurring during the study or within 30 days of last BBI608 administration.

BBI608 + Capecitabine

Tests & Procedures	Pre-Study Evaluation	Study Evaluations[1]								End of Study Visit
		Cycle 1				Cycle 2 & Beyond				
Week	0	1	2	3	4	1	2	3	4	
Day	0	1	8	15	21	1	8	15	21	≤ 30 days from last BBI608 dose
Window	-7 ± 3 days	± 1 day				± 1 day				
Medical history	X									
Physical examination	X					X				X
Serum pregnancy test[3]	X									
Karnofsky performance status	X		X	X	X	X				X
Vital signs, Weight	X		X	X	X	X				X
Hematology[2]	X		X	X	X	X				X
Blood chemistry[2]	X		X	X	X	X				X
Liver function tests[2]	X		X	X	X	X				X
Electrolytes[2]	X		X	X	X	X				X
Urinalysis[2]	X		X	X	X	X				X
12-Lead electrocardiogram	X	X								X
Tumor markers[3]	X					X				X
Tumor biopsy[3]	X		X							X
Pharmacokinetics (first six patients only)			X		X					
Tumor measurement & staging[4,5]	X[6]									X[7]
Concomitant medications	X		X	X	X	X				X
Adverse events[8]			X	X	X	X				X
Dispense BBI608		X	X	X	X	X				
Dispense Capecitabine			X			X				

1. Calculated from the date of first BBI608 dose.
2. Refer to Section 6.3 for description of laboratory assessments.
3. If applicable
4. Including tumor measurements, following RECIST 1.1.
5. Prior to cycle 3 and every other cycle thereafter, assessments must be made utilizing the same imaging method as baseline
6. Unless CT/MRI has been performed within last three weeks.
7. Unless CT/MRI has been performed within last four weeks.
8. Patients will be followed until resolution of any drug-related AE or SAE occurring during the study or within 30 days of last BBI608 administration.

15 APPENDIX C: PERFORMANCE STATUS

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

16 APPENDIX D: PHARMACOKINETIC STUDIES

Instructions for Collecting and Processing Samples

Sampling to define the plasma pharmacokinetics of BBI608 and Cetuximab, or Panitumumab or Capecitabine will be performed in the first 6 patients enrolled in each combination arm of the study. For the Cetuximab arm, samples are taken on Day 5-12 and Day 26-33 of cycle 1. For the Panitumumab arm, samples are taken on Day 8-15 and Day 22-29 of cycle 1. For the Capecitabine arm, samples are taken on Day 8-9 and Day 21-22 of cycle 1. The sampling schedule and summary of procedures that are to be used to establish times, collect samples, and process specimens for storage prior to analysis, to insure the acquisition of accurate pharmacokinetic data, are described below. The sampling schedule has been devised to accommodate treatment on an outpatient basis. Dosing of the first dose of drug must be started before 10 a.m. on a Tuesday, Wednesday, or Thursday to facilitate the collection of the pharmacokinetic samples at the scheduled times during the regular operating hours of the outpatient clinics.

Before starting the BBI608 administration, place a large gauge peripheral catheter (e.g., 19 or 20 gauge angiocath straight set with T-connector, or similar IV access device) within a vein in the arm of the subject for the collection of pharmacokinetic blood samples. Patency of the sampling catheter should be maintained between blood draws using either a heparin lock (e.g., 10 U/mL in normal saline) or a slow drip of Normal Saline for Injection, USP (e.g., 10 mL/hr). Extraordinary caution should be taken to prevent hemolysis in the blood sample. Blood may be obtained directly by venipuncture on days when only a single pharmacokinetic blood specimen is scheduled for collection. When sampling through the peripheral catheter, begin to clear the catheter approximately 1 min before the specified sample time by withdrawing the lock solution and approximately 0.5 mL of blood into a syringe. Remove and properly dispose the syringe used to clear the catheter.

A battery-powered digital timer/stopwatch programmed to operate continuously as a 24-hr clock will be used to accurately monitor drug administration and sample collection times. The same timer must be allowed to run without interruption until the last blood specimen has been obtained from the subject during the first cycle of therapy. Timer readings will be noted at the precise time that the administration is started as well as at the beginning and ending times of the blood sample collection intervals. Readings of the digital timer must be directly recorded on a copy of the appropriate Pharmacokinetic Dosing and Blood Collection Time Form.

Please note that blood and plasma must be protected from direct exposure to light and all sample processing procedures are to be performed in a room with indirect lighting. The volume of blood collected for each pharmacokinetic sample will be 3 mL. This volume has shown to be adequate for the pharmacokinetic assay. Samples are to be collected in plastic Vacutainer plasma collection tubes. Immediately wrap the tube with aluminum foil to protect it from exposure to light. Promptly mix the plasma collection tube by gently inverting 6-times, then place it on wet ice, and centrifuge (1,100-1,300 x g, 10 min, 4°C)

within 5-10 min after collection. Separate the plasma from the blood cells using a pipette and transfer approximately equal volumes into three 2 mL self-standing opaque amber polypropylene microcentrifuge cryogenic tubes with external threads. Affix a pre-printed label (protocol number, subject initials, subject number, sample collection date and time) to the cryotube, oriented lengthwise toward the upper part of the tube. Hand-written information on sample tube labels is absolutely prohibited. Completely cover the label with protective cryogenic freezer tape. Place the tube on crushed dry-ice until stored in a freezer maintained at ≤ -70°C. Any deviation from the sample collection time needs to be documented in the study subjects' case report forms and in their study binder.

The total volume of blood collected for pharmacokinetic studies, 100-120 mL, represents < 5% of the total blood volume for a 60 kg subject, during a 28-day treatment cycle. As pharmacokinetic data becomes available during the course of the trial, it may be necessary to modify the number of samples or the times at which they are collected to more accurately define the plasma concentration-time profile of the drug. However, the cumulative volume of blood collected for pharmacokinetic sampling will not exceed 240 mL, or approximately 5% of the total volume of a 60 kg subject during the first 4 weeks of study treatment.

Computer files for dose administration and sample collection time forms and specimen tube labels will be placed in a subdirectory located on a secure network server. All members of the clinical, laboratory, and administrative staff involved with phase II studies will be given read-only access to this subdirectory. The files are placed under the following subdirectories: PK_Time_Forms; PK_Tube_Labels. Files for the PK time forms are printed directly from the file stored on the network server for each subject study. Since changes may be periodically made to these forms, they cannot be copied onto user personal computers and staff members are instructed not to make photocopies of blank forms. The PK_Tube_Label files are templates, and the files are copied to user computers and the limited information pertaining to each subject studied, typically the subject entry no., is added by editing the computer file, then printed onto adhesive-backed labels. Hand-written information on sample tube labels is absolutely prohibited. There are separate sets of labels for blood collection vials and sample storage tubes. Blood collection vials are prelabeled.

Time points will be determined as the difference between the midpoint of the blood collection interval and starting time of dose administration. Concentration-time profiles of BBI608 and Cetuximab, or Panitumumab or Capecitabine will be analyzed by noncompartmental methods and/or nonlinear least squares regression using WinNonlin (Scientific Consulting, Inc.). Pharmacokinetic parameters and variables will be calculated according to standard equations.

Pharmacokinetic Sample Collection (Cetuximab Arm)

Day 5-12 in CYCLE 1 ONLY

Cycle No.	Procedure	Sample No.	Desired Time
1	Draw blood sample	PK-01	-5 min
	Start BBI608 administration		0 min
	Draw blood sample	PK-02	30 min
	Draw blood sample	PK-03	60 min
	Draw blood sample	PK-04	120 min
	Draw blood sample	PK-05	175 min
	Start Cetuximab infusion		180 min
	Draw blood sample	PK-06	240 min
	Draw blood sample	PK-07	300 min
	Draw blood sample	PK-08	360 min
	Draw blood sample	PK-09	420 min
	Draw blood sample	PK-10	480 min
	Draw blood sample	PK-11	540 min
	Draw blood sample	PK-12	24 hour
	Draw blood sample	PK-13	27 hour
	Draw blood sample	PK-14	99 hour
	Draw blood sample	PK-15	171 hour

Day 26-33 in CYCLE 1 ONLY

Cycle No.	Procedure	Sample No.	Desired Time
1	Draw blood sample	PK-01	-5 min
	Start BBI608 administration		0 min
	Draw blood sample	PK-02	30 min
	Draw blood sample	PK-03	60 min
	Draw blood sample	PK-04	120 min
	Draw blood sample	PK-05	175 min
	Start Cetuximab infusion		180 min
	Draw blood sample	PK-06	240 min
	Draw blood sample	PK-07	300 min
	Draw blood sample	PK-08	360 min
	Draw blood sample	PK-09	420 min
	Draw blood sample	PK-10	480 min
	Draw blood sample	PK-11	540 min
	Draw blood sample	PK-12	24 hour

	Draw blood sample	PK-13	27 hour
	Draw blood sample	PK-14	99 hour
	Draw blood sample	PK-15	171 hour

Pharmacokinetic Sample Collection (Panitumumab Arm)

Day 8-15 in CYCLE 1 ONLY

Cycle No.	Procedure	Sample No.	Desired Time
1	Draw blood sample	PK-01	-5 min
	Start BBI608 administration		0 min
	Draw blood sample	PK-02	30 min
	Draw blood sample	PK-03	60 min
	Draw blood sample	PK-04	120 min
	Draw blood sample	PK-05	175 min
	Start Panitumumab infusion		180 min
	Draw blood sample	PK-06	240 min
	Draw blood sample	PK-07	300 min
	Draw blood sample	PK-08	360 min
	Draw blood sample	PK-09	420 min
	Draw blood sample	PK-10	480 min
	Draw blood sample	PK-11	540 min
	Draw blood sample	PK-12	24 hour
	Draw blood sample	PK-13	27 hour
	Draw blood sample	PK-14	99 hour
	Draw blood sample	PK-15	171 hour

Day 22-29 in CYCLE 1 ONLY

Cycle No.	Procedure	Sample No.	Desired Time
1	Draw blood sample	PK-01	-5 min
	Start BBI608 administration		0 min
	Draw blood sample	PK-02	30 min
	Draw blood sample	PK-03	60 min
	Draw blood sample	PK-04	120 min
	Draw blood sample	PK-05	175 min
	Start Panitumumab infusion		180 min
	Draw blood sample	PK-06	240 min

	Draw blood sample	PK-07	300 min
	Draw blood sample	PK-08	360 min
	Draw blood sample	PK-09	420 min
	Draw blood sample	PK-10	480 min
	Draw blood sample	PK-11	540 min
	Draw blood sample	PK-12	24 hour
	Draw blood sample	PK-13	27 hour
	Draw blood sample	PK-14	99 hour
	Draw blood sample	PK-15	171 hour

Pharmacokinetic Sample Collection (Capecitabine Arm)

Day 8-9 in CYCLE 1 ONLY

Cycle No.	Procedure	Sample No.	Desired Time
1	Draw blood sample	PK-01	-5 min
	Start BBI608 administration		0 min
	Draw blood sample	PK-02	30 min
	Draw blood sample	PK-03	60 min
	Draw blood sample	PK-04	120 min
	Start Capecitabine administration		175 min
	Draw blood sample	PK-05	180 min
	Draw blood sample	PK-06	240 min
	Draw blood sample	PK-07	300 min
	Draw blood sample	PK-08	360 min
	Draw blood sample	PK-09	420 min
	Draw blood sample	PK-10	480 min
	Draw blood sample	PK-11	540 min
	Draw blood sample	PK-12	600 min
	Draw blood sample	PK-13	660 min
	Draw blood sample	PK-14	24 hour

Pharmacokinetic Sample Collection (Capecitabine)

Day 21-22 in CYCLE 1 ONLY

Cycle No.	Procedure	Sample No.	Desired Time
1	Draw blood sample	PK-01	-5 min

	Start BBI608 administration		0 min
	Draw blood sample	PK-02	30 min
	Draw blood sample	PK-03	60 min
	Draw blood sample	PK-04	120 min
	Start Capecitabine administration		175 min
	Draw blood sample	PK-05	180 min
	Draw blood sample	PK-06	240 min
	Draw blood sample	PK-07	300 min
	Draw blood sample	PK-08	360 min
	Draw blood sample	PK-09	420 min
	Draw blood sample	PK-10	480 min
	Draw blood sample	PK-11	540 min
	Draw blood sample	PK-12	600 min
	Draw blood sample	PK-13	660 min
	Draw blood sample	PK-14	24 hour

17 APPENDIX E: TUMOR BIOPSIES

Instructions for Collecting, Processing and Shipping Samples

Archival tissues:

Archival tissue samples should be collected from all patients enrolled in the clinical trial if they are available. Submission of paraffin embedded tissue blocks to Boston Biomedical is preferred. However, if tumor blocks are not available, 20 positively charged, unstained tissue slides at 5 micron thickness should be sent to Boston Biomedical. Archive tissue labels should be coded with the protocol number, study site number, the patient's initials and the patient number. Paraffin embedded tissue blocks or prepared tissue slides should be shipped by courier to Boston Biomedical at the address listed below.

Fresh Tumor Biopsies:

Patients who are identified by the principal investigator as having a lesion, which could be biopsied with a minimally invasive technique, will be asked to sign an additional consent. Tumor biopsy samples should be collected at baseline and 4 hours after administration of BBI608 on Day 8 of cycle 1. Tumor biopsies will be collected for immunohistochemitry-based analysis of the target and its downstream genes and for the analysis of the effect of BBI608 on cancer stem cells. These samples will be used for research purposes only. Tumor biopsies for analysis should be collected by core biopsy or minimally invasive procedures. Tumor specimen samples should be processed sterilely into three parts: fixed, frozen, and fresh.

Fixed Tumor Biopsies for Immunohistochemistry

The sample should be processed to yield paraffin embedded tissues by the hospital pathology department using their standard operating procedures. The samples should be labeled and coded with the protocol number, study site number, the patient's initials, the patient number and the sample collection date and time. The paraffin embedded tissue blocks should be shipped to Boston Biomedical at the address listed below.

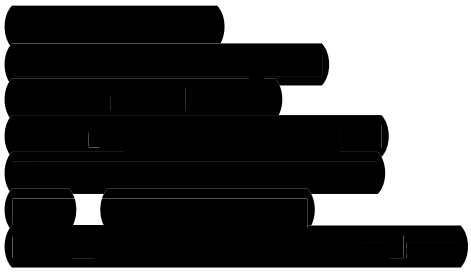
Fresh and Frozen Tumor Biopsy for cancer stem cell assays

Tumor biopsies for the analysis of the effect of BBI608 on cancer stem cells should be collected by core biopsy or similar minimally invasive procedures. Once the biopsy tissue is obtained, half of the biopsy tissue should be immediately and sterilely placed into CSC transport media provided by Boston Biomedical, stored and shipped at 4ºC. Overnight shipment should be arranged on the same day as the biopsy is performed. A label should be affixed to all sample tubes containing with the protocol number, study site number, the patient's initials, the patient number and the sample collection date and time. The other half should be immediately snap-frozen in liquid nitrogen, and then transferred to a

polypropylene microcentrifuge cryogenic tube. The tissue sample should then be stored at ≤ -70ºC until being shipped to Boston Biomedical on dry ice to the address listed below.

Shipping information:

Please ship all tumor samples to the following address:



On the day that specimens are sent to Boston Biomedical, please contact Boston Biomedical by phone, fax or email to notify what is being sent and when the shipment is expected to arrive.

18 APPENDIX F: CETUXIMAB, PANITUMUMAB AND CAPECITABINE PRODUCT LABELS

19 SPONSOR SIGNATURE

Study Title: **A Phase II Clinical Study of BBI608 in Adult Patients with Advanced Colorectal Cancer**

Study Number: BBI608-224

This clinical study protocol is subject to critical review and has been approved by the Sponsor. The following personnel contributed to writing and/or approving this protocol:

Signed: [redacted] Date: [redacted]

20 INVESTIGATOR'S SIGNATURE

Study Title: **A Phase II Clinical Study of BBI608 in Adult Patients with Advanced Colorectal Cancer**

Study Number: BBI608-224

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

Printed Name:_____

Signature:_____ Date: _____

21 REFERENCES

1. Lobo et al, (2007) Annu Rev Cell Dev Biol 23:675-699.
2. Boman and Wicha, (2008) J Clin Oncol. 26:2795-2799.
3. Please find a list of updated reviews on cancer stem cells in all major cancer types in the June 10 special issue of Journal of Clinical Oncology on cancer stem cells (J Clin Oncol. 2008 Jun 10;26(17)).
4. Jonker et al, New Engl J Med. (2007) 357:2040-2048
5. Lenz et al, J Clin Oncol. (2006) 24:4914-21.
6. Van Cutsem et al, J Clin Oncol. (2007) 25:1658-1664.
7. Van Cutsem et al, Annal Oncol. (2008) 19:92-98.
8. Hecht et al, Cancer (2007) 110:980-988.
9. Gubanski et al, Acta Oncol. (2005) 44:236-239.
10. Spindler et al, BMC Cancer (2011) 11:107.
11. Gebbia et al, Clin Colorectal Cancer (2006) 5:422-428.
12. Vincenzi et al, Br J Cancer (2006) 94:792-797.
13. Pfeiffer et al, Acta Oncol (2007) 46:697-701.
14. Sobero et al, J Clin Oncol. (2008) 26:2311-2319.
15. Garm Spindler et al, Ann Oncol. (2009) 20 :879-884.
16. Chibaudel et al, J Clin Oncol. (2011) 29:suppl; abstr 3573.
17. Chong et al, Br J Cancer (2005) 93:510-514.
18. Lim et al, Cancer Chemother Pharmacol. (2005) 56:10-14.
19. Eisenhauer et al. Eur J Cancer (2009) 45:228-47.