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Full Title: Phase II trial of CAPOX+Bevacizumab+Trastuzumab for patients with HER2-positive metastatic esophagogastric cancer.

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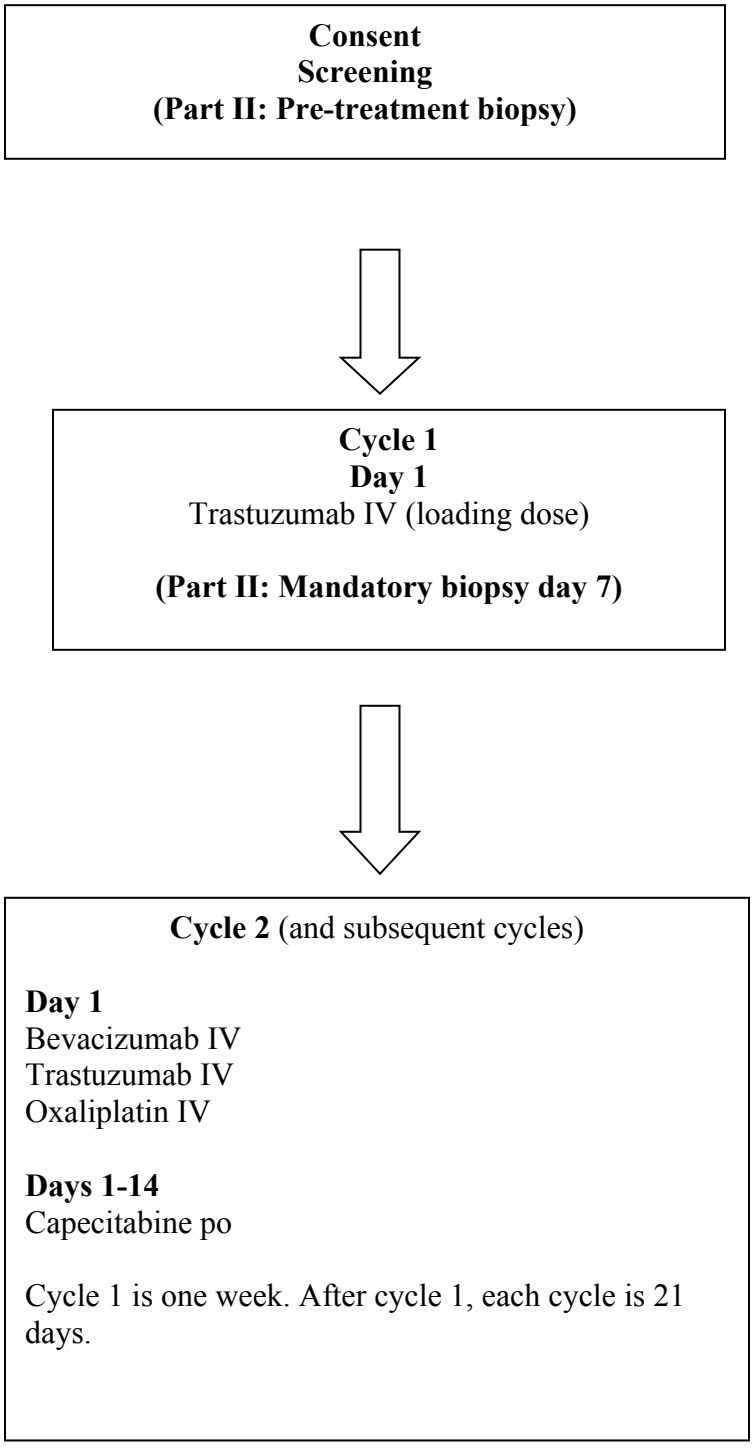
SCHEMA

A Phase II trial of CAPOX+Bevacizumab+Trastuzumab for patients with HER2-positive metastatic esophagogastric cancer.



1. OBJECTIVES

1.1 Study Design

This is a Phase II study of CAPOX (capecitabine and oxaliplatin) plus bevacizumab plus trastuzumab for patients with HER2-positive esophagogastric adenocarcinoma that is metastatic or unresectable. A total of 36 evaluable patients will be enrolled in this study.

The study will have a three stage design. Part I of this study will consist of two stages that will include the first 20 patients. Initially 5 patients will be enrolled, treated, and evaluated through the first two cycles of therapy. Following an interim safety analysis, an additional 15 patients will then be enrolled and will also be evaluated for efficacy, safety and tolerability. We have enrolled 16patients to date. The interim efficacy, safety and tolerability data have been reviewed. It is evident that this is a well tolerated regimen and the response rates are encouraging. We therefore will be moving to Part II (third stage of the study) which will now consist of 20 patients. All patients at DF/HCC sites will be required to have mandatory pre and post trastuzumab biopsy.

1.2 Primary Objectives

The primary objective of this study is to determine the major response rate of CAPOX plus bevacizumab plus trastuzumab for patients with HER2-positive metastatic or unresectable esophagogastric adenocarcinoma

1.3 Secondary Objectives

Determine toxicity profile

Determine the duration of response

Rate of median, overall and progression free survival

1.4 Correlative Science Objectives (Part II only): Utilizing tumor biopsy specimens

Determine signal transduction pathways by immunohistochemistry (IHC) with the following antibodies; pAKT, pERK, pEGFR, pPDGFR, and HER2.

Correlate pre-treatment pathway action with one week post trastuzumab treatment.

2. BACKGROUND

2.1 Investigational Agents

2.1.1 HER2/neu and Trastuzumab Clinical Experience

The HER2/*neu* oncogene lies on chromosome 17q, and encodes a transmembrane glycoprotein (p185) with homology to the epidermal growth factor receptor. HER2 over expression and/or amplification has been observed in breast, colon, bladder, ovarian, endometrial, lung, cervical, head and neck, esophageal and gastric carcinomas. In breast cancer, HER2 positivity is noted in 20-25% and may correlate with clinical outcome and has been shown to be a predictive factor for prognosis.

The overexpression/amplification of HER2/*neu* has been reported in 6-35% of esophagogastric cancers and is a significant negative prognostic factor. Overexpression of HER2 protein in gastric cancer using immunohistochemistry (IHC) was first described in 1986 (Sakai K, Mori S et al). In the 1990's, it was reported that 9-38% of gastric tumors where HER2-positive tumors using polyclonal antibodies directed against different domains of HER2 protein and restricting the evaluation to the staining of the cell membrane (Yonemura Y; Ishida T; Tokunaga, A). More recent studies which determine HER2 overexpression by IHC using monoclonal antibody (HercepTest™) and/or gene amplification by fluorescence in situ hybridization (FISH) have observed similar rates. To date, most studies have noted a higher incidence of positivity in the gastroesophageal junction than in other areas of the stomach.

The recently reported ToGA trial centrally tested tumor samples using validated detection methods; IHC and FISH, and provided valuable information specific to gastroesophageal junction and stomach cancer. This international, multicenter trial was the first large Phase III trial to provide prospective information on the incidence of HER2 positivity in advanced gastroesophageal junction- and gastric- cancer. The investigators noted that of 2,168 tumors tested, 460 were positive (IHC 3+ and/or FISH +). Of these, 20% of gastric cancer samples were positive and 34% of gastroesophageal junction samples were positive (Kang, et al, ASCO abstract 2008).

Because of its selective expression on tumor cells, HER2 is a potential target for anti tumor therapies. To facilitate the development of appropriate reagents for treating human cancer, a murine antibody against HER2 was "humanized", that is, engineered so that the conserved units of the antibody molecule were transcribed from human DNA sequences, while the specific anti-HER2 domain remained of mouse DNA origin. As a result of these modifications, the antibody is not immunogenic in the vast majority of people. The resulting antibody (Herceptin®; Trastuzumab; Genentech) has been studied both as a single agent and in combination therapy.

At least 3 clinical trials are exploring the addition of trastuzumab to chemotherapy in trials specifically for HER2-positive esophagogastric cancer. Cortes-Funes, et al are conducting a Phase II trial to investigate the efficacy and tolerability of trastuzumab

plus cisplatin in chemo-naive patients. The main inclusion criteria are: no prior chemotherapy, measurable and non resectable disease, ECOG < 2, LVEF ≥ 50%, and adequate organ function. Prior adjuvant treatment is allowed. Trastuzumab 8mg/kg (loading dose) and cisplatin 75mg/m2 on day one, followed by trastuzumab 6 mg/kg (maintenance dose) and cisplatin 75mg/m2 every 21 days. Preliminary results show a 35% response rate; no grade 4 toxicity has been documented.

Nicholas et al are conducting a multicenter Phase II study of trastuzumab, cisplatin and docetaxel in patients with metastatic gastric and gastroesophageal junction cancer with FISH+ or IHC 3+ for HER2 overexpression. They noted that 16% of tumors screened were HER2-positive. The treatment regimen for this trial consists of cisplatin 75mg/m2, docetaxel 75mg/m2, trastuzumab 8mg/kg (loading dose in cycle 1) and then 6mg/kg in subsequent cycles (21 day cycles). Grade 3 or 4 toxicities noted to date include peripheral neuropathy, abdominal cramping, neutropenia (one patient each). One patient died of an upper GI bleed. Responses were recorded in five patients: one CR, three PR, and one SD.

The study of trastuzumab added to standard chemotherapy in first-line human epidermal growth factor receptor 2 (HER2)-positive advanced gastric cancer, known as the ToGA trial was presented at the ASCO Annual Meeting in 2009 (van Cutsem, et al). Patients with HER2 positive tumors were randomized to receive trastuzumab and chemotherapy or chemotherapy alone every three weeks for 6 cycles. After 6 cycles of chemotherapy, patients on the trastuzumab arm continued receiving trastuzumab alone until disease progression. The primary objective of the study was to demonstrate superiority in overall survival of the trastuzumab arm, compared to standard chemotherapy alone. The chemotherapy regimen included capecitabine 1000mg/m2 po BID on days 1-14, or 5 F-U 800mg/m2/day as a continuous infusion on days 1-5 (physicians choice) and cisplatin 80mg/m2 on day 1 of a three week cycle. Trastuzumab was administered as an 8mg/kg loading dose followed by 6 mg/kg every three weeks until disease progression.

The ToGA trial demonstrated a 2.7 month improvement in overall survival (from 11.1 months to 13.8 months; p=0.0046) for patients receiving trastuzumab. Additionally, there was a 1.2 month improvement in progression-free survival and a 12.8% improvement in response rate for the patients receiving trastuzumab. Trastuzumab increased the response rate from 34.5% to 47.3%. Except for a slight increase in fatigue and diarrhea in the trastuzumab group, there was no significant increase in toxicity. Cardiac toxicity included asymptomatic decline in LVEF (4.6% of patients) and was not thought to be clinically significant. The investigators also noted that tumors exhibiting high (2+ or 3+) levels of HER2 expression experienced greater benefit; these patients had a median overall survival of 16 months. The investigators concluded that trastuzumab is the first biologic agent to show a survival benefit in patients with advanced gastric cancer and suggested that the combination of trastuzumab plus chemotherapy be added as a treatment option for patients with HER2-positive gastric cancer.

Trastuzumab is associated with the potential for left ventricular cardiac dysfunction and congestive heart failure (CHF). In the pivotal trial of trastuzumab for metastatic breast cancer, the rate of cardiac dysfunction was highest when given concurrently with doxorubicin, with a rate of New York Heart Association (NYHA) class III/IV CHF of 19%, versus 3% in the control group. The rate of NYHA class III/IV CHF was much lower (4%) in patients receiving trastuzumab with paclitaxel (Slamon et al., 2001). In an adjuvant study of carboplatin, docetaxel, and trastuzumab (BCIRG 006), the rate of grade 3 or 4 left ventricular cardiac dysfunction was 0.3% (4 events/1,056 patients) and no cardiac deaths were observed (Slamon et al., 2006).

Other reported adverse events include infusion reactions, characterized by fever, chills, nausea, vomiting, headache, dyspnea, hypotension, and rash. Trastuzumab may also exacerbate chemotherapy-induced neutropenia. Trastuzumab treatment can very rarely result in serious pulmonary toxicity.

2.1.2 Bevacizumab Clinical Experience

Bevacizumab has been evaluated in more than 5000 clinical subjects, having multiple different tumor types. In addition, data are available from 3,863 patients enrolled in two post-marketing studies in metastatic colorectal cancer (CRC). Approximately 130,000 patients have been exposed to bevacizumab as a marketed product or in clinical trials. The following discussion summarizes bevacizumab's safety profile and presents some of the efficacy results pertinent to this particular trial. Please refer to the bevacizumab Investigator Brochure for descriptions of all completed Phase I, II, and III trials reported to date.

In a large Phase III study (AVF2107g) in patients with metastatic colorectal cancer, the addition of bevacizumab, a monoclonal antibody directed against vascular endothelial growth factor (VEGF), to irinotecan/5-fluorouracil/leucovorin (IFL) chemotherapy resulted in a clinically and statistically significant increase in duration of survival, with a hazard ratio of death of 0.67 (median survival 15.6 vs. 20.3 months; $p < 0.001$). Similar increases were seen in progression-free survival (6.2 vs. 10.6 months; $p < 0.001$), overall response rate (35% vs. 45%; $p < 0.01$) and duration of response (7.1 vs. 10.4 months; $p < 0.01$) for the combination arm versus the chemotherapy only arm (bevacizumab Investigator Brochure, October 2005).

Based on the survival advantage demonstrated in Study AVF2107g, bevacizumab was designated for priority review and was approved on February 26, 2004 in the United States for first-line treatment in combination with IV 5-FU–based chemotherapy for subjects with metastatic colorectal cancer.

Additional data from Phase III trials in metastatic CRC (E3200), non–small cell lung cancer (NSCLC; E4599), and metastatic breast cancer (E2100) have also demonstrated clinical benefit from bevacizumab when added to chemotherapy. In Study E3200, the addition of bevacizumab to FOLFOX chemotherapy resulted in improved overall

survival compared with FOLFOX alone (13.0 vs. 10.8 months, respectively, HR = 0.75; $p < 0.01$) in a population of previously treated CRC patients.

There was also improved overall survival in first-line NSCLC patients (E4599) treated with carboplatin/paclitaxel + bevacizumab compared with chemotherapy alone (12.3 vs. 10.3 months, respectively; HR = 0.80; $p = 0.003$). The results from this trial were the basis for FDA approval of bevacizumab for use in combination with carboplatin + paclitaxel as first-line treatment of patients with unresectable, locally advanced, recurrent or metastatic, non-squamous NSCLC in October 2006.

Among patients with metastatic breast cancer treated in the first-line setting (E2100), the combination of bevacizumab with paclitaxel was associated with significantly longer PFS compared with paclitaxel alone (median, 11.8 vs. 5.9 months, HR for progression 0.60; $p<0.001$). The objective response rate was also significantly higher (36.9% vs. 21.2%, $p<0.001$) (Miller et al., 2007)

2.1.2.1 Safety Profile

In the initial Phase I and II clinical trials, four potential bevacizumab-associated safety signals were identified: hypertension, proteinuria, thromboembolic events, and hemorrhage. Additional completed Phase II and Phase III studies of bevacizumab as well as spontaneous reports have further defined the safety profile of this agent. Bevacizumab-associated adverse events identified in Phase III trials include congestive heart failure (CHF) (primarily in metastatic breast cancer), gastrointestinal perforations, wound healing complications, and arterial thromboembolic events (ATE). These and other safety signals are described in further detail as follows and in the bevacizumab Investigator Brochure.

Hypertension: An increased incidence of hypertension has been observed in patients treated with bevacizumab. Grade 4 and 5 hypertensive events are rare. Clinical sequelae of hypertension are rare but have included hypertensive crisis, hypertensive encephalopathy, and reversible posterior leukoencephalopathy syndrome (RPLS) (Ozcan et al., 2006; Glusker et al., 2006).

There is no information on the effect of bevacizumab in patients with uncontrolled hypertension at the time of initiating bevacizumab therapy. Therefore, caution should be exercised before initiating bevacizumab therapy in these patients. Monitoring of blood pressure is recommended during bevacizumab therapy. Optimal control of blood pressure according to standard public health guidelines is recommended for patients on treatment with or without bevacizumab.

Temporary interruption of bevacizumab therapy is recommended in patients with hypertension requiring medical therapy until adequate control is achieved. If hypertension cannot be controlled with medical therapy, bevacizumab therapy should be permanently discontinued. Bevacizumab should be permanently

discontinued in patients who develop hypertensive crisis or hypertensive encephalopathy.

Proteinuria: An increased incidence of proteinuria has been observed in patients treated with bevacizumab compared with control arm patients. In the bevacizumab-containing treatment arms of clinical trials (across all indications), the incidence of proteinuria (reported as an adverse event) was up to 38% (metastatic CRC Study AVF2192g). The severity of proteinuria has ranged from asymptomatic and transient events detected on routine dipstick urinalysis to nephrotic syndrome; the majority of proteinuria events have been grade 1. NCI-CTC Grade 3 proteinuria was reported in up to 3% of bevacizumab-treated patients, and Grade 4 in up to 1.4% of bevacizumab-treated patients. The proteinuria seen in bevacizumab clinical trials was not associated with renal impairment and rarely required permanent discontinuation of bevacizumab therapy. Bevacizumab should be discontinued in patients who develop Grade 4 proteinuria (nephrotic syndrome).

Patients with a history of hypertension may be at increased risk for the development of proteinuria when treated with bevacizumab. There is evidence from the dose-finding, Phase II trials (AVF0780g, AVF0809s, and AVF0757g) suggesting that Grade 1 proteinuria may be related to bevacizumab dose.

Proteinuria will be monitored by urine protein: creatinine (UPC) ratio approximately every 6 weeks (2 cycles).

Thromboembolic Events: Both venous and arterial thromboembolic (TE) events, ranging in severity from catheter-associated phlebitis to fatal, have been reported in patients treated with bevacizumab in the colorectal cancer trials and, to a lesser extent, in patients treated with bevacizumab in NSCLC and breast cancer trials.

Venous thromboembolism (including deep venous thrombosis, pulmonary embolism, and thrombophlebitis: In the Phase III pivotal trial in metastatic CRC, there was a slightly higher rate of venous TE events in patients treated with bevacizumab plus chemotherapy compared with chemotherapy alone (19% vs. 16%).

In Study AVF2107g, a Phase III, pivotal trial in metastatic CRC, VTE events, including deep venous thrombosis, pulmonary embolism, and thrombophlebitis, occurred in 15.2% of patients receiving chemotherapy alone and 16.6% of patients receiving chemotherapy + bevacizumab.

The incidence of NCI-CTC Grade ≥ 3 venous VTE events in one NSCLC trial (E4599) was higher in the bevacizumab-containing arm compared to the chemotherapy control arm (5.6% vs. 3.2%). One event (0.2%) was fatal in the bevacizumab-containing arm; not fatal events were reported in the carboplatin/paclitaxel arm (see bevacizumab Investigator Brochure). In metastatic

CRC clinical trials, the incidence of VTE events was similar in patients receiving chemotherapy + bevacizumab and those receiving the control chemotherapy alone.

In clinical trials across all indications the overall incidence of VTE events was 2.8%–17.3% in the bevacizumab-containing arms compared with 3.2%–15.6% in the chemotherapy control arms. The use of bevacizumab with chemotherapy does not substantially increase the risk of VTE event compared with chemotherapy alone. However, patients with metastatic CRC who receive bevacizumab and experienced a VTE event may be at higher risk for recurrence of VTE event.

Arterial Thromboembolic Events: An increased incidence of ATE events was observed in patients treated with bevacizumab compared with those receiving control treatment. ATE events include cerebrovascular accidents, myocardial infarction, transient ischemic attacks (TIAs), and other ATE events. In a pooled analysis of data from five randomized Phase II and III trials (mCRC [AVF2107g, AVF2192g, AVF0780g]; locally advanced or metastatic NSCLC [AVF0757g]; and metastatic breast cancer [AVF2119g]), the incidence rate of ATE events was 3.8% (37 of 963) in patients who received chemotherapy + bevacizumab compared with 1.7% (13 of 782) in patients treated with chemotherapy alone. ATE events led to a fatal outcome in 0.8% (8 of 963) of patients treated with chemotherapy + bevacizumab and 0.5% (4 of 782) of patients treated with chemotherapy alone. Cerebrovascular accidents (including TIAs) occurred in 2.3% of patients treated with chemotherapy + bevacizumab and 0.5% of patients treated with chemotherapy alone. Myocardial infarction occurred in 1.4% of patients treated with chemotherapy + bevacizumab compared with 0.7% of patients treated with chemotherapy alone (see the bevacizumab Investigator Brochure for additional details).

Aspirin is a standard therapy for primary and secondary prophylaxis of arterial thromboembolic events in patients at high risk of such events, and the use of aspirin ≤ 325 mg daily was allowed in the five randomized studies discussed above. Use of aspirin was assessed routinely as a baseline or concomitant medication in these trials, though safety analyses specifically regarding aspirin use were not preplanned. Due to the relatively small numbers of aspirin users and arterial thromboembolic events, retrospective analyses of the ability of aspirin to affect the risk of such events were inconclusive. However, similarly retrospective analyses suggested that the use of up to 325 mg of aspirin daily does not increase the risk of grade 1-2 or grade 3-4 bleeding events, and similar data with respect to metastatic colorectal cancer patients were presented at ASCO 2005 (Hambleton et al., 2005). Further analyses of the effects of concomitant use of bevacizumab and aspirin in colorectal and other tumor types are ongoing.

Gastrointestinal perforation: Patients with metastatic carcinoma may be at increased risk for the development of gastrointestinal perforation and fistula when treated with bevacizumab and chemotherapy. Bevacizumab should be permanently discontinued in patients who develop gastrointestinal perforation. A causal

association of intra-abdominal inflammatory processes and gastrointestinal perforation to bevacizumab treatment has not been established. Nevertheless, caution should be exercised when treating patients with intra-abdominal inflammatory processes with bevacizumab. Gastrointestinal perforation has been reported in other trials in non-colorectal cancer populations (e.g., ovarian, renal cell, pancreas, breast, and NSCLC) and may be higher in incidence in some tumor types.

Fistula: Bevacizumab use has been associated with serious cases of fistulae including events resulting in death. Fistulae in the GI tract are common (1%–10% incidence) in patients with metastatic CRC, but uncommon (0.1%–1%) or rare (0.01%–0.1%) in other indications. In addition, fistulae that involve areas of the body other than the GI tract (e.g., tracheoesophageal, bronchopleural, urogenital, biliary) have been reported uncommonly (0.1%–1%) in patients receiving bevacizumab in clinical studies and postmarketing reports. Events were reported at various timepoints during treatment, ranging from 1 week to > 1 year following initiation of bevacizumab, with most events occurring within the first 6 months of therapy.

Bevacizumab should be permanently discontinued in patients with tracheoesophageal fistulae or any Grade 4 fistula. Limited information is available on the continued use of bevacizumab in patients with other fistulae. In cases of internal fistula not arising in the GI tract, discontinuation of bevacizumab should be considered.

Wound healing complications: Wound healing complications such as wound dehiscence have been reported in patients receiving bevacizumab. In an analysis of pooled data from two trials in metastatic colorectal cancer, patients undergoing surgery 28-60 days before study treatment with 5-FU/LV plus bevacizumab did not appear to have an increased risk of wound healing complications compared to those treated with chemotherapy alone (Scappaticci et al., 2005). Surgery in patients currently receiving bevacizumab is not recommended. No definitive data are available to define a safe interval after bevacizumab exposure with respect to wound healing risk in patients receiving elective surgery; however, the estimated half life of bevacizumab is 21 days. Bevacizumab should be discontinued in patients with severe wound healing complications.

If patients receiving treatment with bevacizumab require elective major surgery, it is recommended that bevacizumab be held for 4–8 weeks prior to the surgical procedure. Patients undergoing a major surgical procedure should not begin or restart bevacizumab until 4 weeks after that procedure (in the case of high-risk procedures such as liver resection, thoracotomy, or neurosurgery, it is recommended that chemotherapy be restarted no earlier than 6 weeks and bevacizumab no earlier than 8 weeks after surgery).

Hemorrhage: Overall, Grade 3 and 4 bleeding events were observed in 4.0% of 1132 patients treated with bevacizumab in a pooled database from eight Phase I, II,

and III clinical trials in multiple tumor types (bevacizumab Investigator Brochure, October 2005). The hemorrhagic events that have been observed in bevacizumab clinical studies were predominantly tumor-associated hemorrhage (see below) and minor mucocutaneous hemorrhage.

Tumor-Associated Hemorrhage: Major or massive pulmonary hemorrhage or hemoptysis has been observed primarily in patients with NSCLC. Life-threatening and fatal hemoptysis was identified as a bevacizumab-related adverse event in NSCLC trials. These events occurred suddenly and presented as major or massive hemoptysis. Among the possible risk factors evaluated (include squamous cell histology, treatment with anti-rheumatic/anti-inflammatory drugs, treatment with anticoagulants, prior radiotherapy, bevacizumab therapy, previous medical history of atherosclerosis, central tumor location, and cavitation of tumors during therapy), the only variables that showed statistically significant correlations with bleeding were bevacizumab therapy and squamous cell histology.

Of patients experiencing pulmonary hemorrhages requiring medical intervention, many had cavitation and/or necrosis of the tumor, either preexisting or developing during bevacizumab therapy. Patients developing lung cavitation on treatment should be assessed by the treating physician for risk-benefit.

In Study E4599, in which squamous cell carcinoma was excluded, the rate of any type of Grade ≥ 3 hemorrhage was 1.0% in the control arm (carboplatin and paclitaxel) versus 4.1% in the carboplatin and paclitaxel + bevacizumab arm (Sandler et al. 2006).

GI hemorrhages, including rectal bleeding and melena have been reported in patients with CRC, and have been assessed as tumor-associated hemorrhages.

Tumor-associated hemorrhages were also seen rarely in other tumor types and locations, including a case of CNS bleeding in a patient with hepatoma with occult CNS metastases and a patient who developed continuous oozing of blood from a thigh sarcoma with necrosis. A recent review examined the risk of CNS hemorrhage in association with anti-VEGF therapy. Among 669 patients treated on Phase I and II studies of bevacizumab that excluded brain metastases, only 1 case (0.2%) of CNS bleeding was reported (Carden et al, 2008). Bevacizumab has also been studied as treatment for refractory, high-grade gliomas.

Mucocutaneus Hemorrhage: Across all bevacizumab clinical trials, mucocutaneous hemorrhage has been seen in 20%-40% of patients treated with bevacizumab. These were most commonly NCI-CTC Grade 1 epistaxis that lasted less than 5 minutes, resolved without medical intervention and did not require any changes in bevacizumab treatment regimen.

There have also been less common events of minor mucocutaneous hemorrhage in other locations, such as gingival bleeding and vaginal bleeding.

Reversible Posterior Leukoencephalopathy Syndrome: There have been rare reports of bevacizumab-treated patients developing signs and symptoms that are consistent with RPLS, a rare neurological disorder that can present with the following signs and symptoms (among others): seizures, headache, altered mental status, visual disturbance, or cortical blindness, with or without associated hypertension. Brain imaging is mandatory to confirm the diagnosis of RPLS. In patients who develop RPLS, treatment of specific symptoms, including control of hypertension, is recommended along with discontinuation of bevacizumab. The safety of reinitiating bevacizumab therapy in patients previously experiencing RPLS is not known (Glusker et al. 2006; Ozcan et al. 2006).

Congestive heart failure: In clinical trials, CHF was observed in all cancer indications studied to date, but predominantly in patients with metastatic breast cancer. In the Phase III clinical trial of metastatic breast cancer (AVF2119g), 7 (3%) bevacizumab-treated patients experienced CHF, compared with two (1%) control arm patients. These events varied in severity from asymptomatic declines in left ventricular ejection fraction (LVEF) to symptomatic CHF requiring hospitalization and treatment. All the patients treated with bevacizumab were previously treated with anthracyclines (doxorubicin cumulative dose of 240–360 mg/m^2). Many of these patients also had prior radiotherapy to the left chest wall. Most of these patients showed improved symptoms and/or left ventricular function following appropriate medical therapy (Miller et al. 2005).

In a randomized, Phase III trial of patients with previously untreated metastatic breast cancer (E2100), the incidence of LVEF decrease (defined as NCI-CTC Grade 3 or 4) in the paclitaxel + bevacizumab arm was 0.3% versus 0% for the paclitaxel alone arm.

No information is available on patients with preexisting CHF of New York Heart Association (NYHA) Class II–IV at the time of initiating bevacizumab therapy, as these patients were excluded from clinical trials.

Prior anthracyclines exposure and/or prior radiotherapy to the chest wall may be possible risk factors for the development of CHF. Caution should be exercised before initiating bevacizumab therapy in patients with these risk factors.

A Phase II trial in patients with refractory acute myelogenous leukemia reported 5 cases of cardiac dysfunction (CHF or LVEF decrease to < 40%) among 48 patients treated with sequential cytarabine, mitoxantrone, and bevacizumab. All but 1 of these patients had significant prior exposure to anthracyclines as well (Karp et al. 2004).

Two additional studies investigated concurrent administration of anthracyclines and bevacizumab. In 21 patients with inflammatory breast cancer treated with neoadjuvant docetaxel, doxorubicin, and bevacizumab, no patients developed

clinically apparent CHF; however, patients had asymptomatic decreases in LVEF to < 40% (Wedam et al. 2004). In a small Phase II study in patients with soft tissue sarcoma, 2 of the 17 patients treated with bevacizumab and high-dose doxorubicin (75 mg/m^2) developed CHF (one Grade 3 event after a cumulative doxorubicin dose of 591 mg/m^2, one Grade 4 event after a cumulative doxorubicin dose of 420 mg/m^2); an additional 4 patients had asymptomatic decreases in LVEF (D'Adamo et al. 2004).

Other studies in patients with various tumor types and either a history of anthracycline exposure or concomitant use with bevacizumab are ongoing.

Patients receiving concomitant anthracyclines or with prior exposure to anthracyclines should have a baseline MUGA scans or echocardiograms (Echo's) with a normal LVEF.

Neutropenia: Increased rates of severe neutropenia, febrile neutropenia, or infection with severe neutropenia (including some fatalities) have been observed in patients treated with some myelotoxic chemotherapy regimens plus bevacizumab in comparison to chemotherapy alone (Sandler et al. 2006).

Additional Adverse Events: See the bevacizumab Investigator Brochure for additional details regarding the safety experience with bevacizumab.

2.1.3 Platinum and Fluorouracil Based Chemotherapy

In the United States, platinum and fluorouracil based regimens of chemotherapy are considered the standard of care for both locally advanced and metastatic esophagogastric cancers. These regimens include: CF, ECF, ECX, EOX, EOF, FOLFOX, CAPOX and have response rates of 40-67%. Among these, regimens that incorporate the combination of oxaliplatin and a fluropyrimidine have response rates of 38-56%, time to progression of 5.2-7.1 months, and overall survival of 7.1-11.4 months. Grade 3 and 4 toxicity has included neutropenia (5-63%), febrile neutropenia (2-11%), thrombocytopenia (2-13%), diarrhea (4-17%), emesis (2-13%), stomatitis (0-9%), asthenia (0-17%), and neurological toxicity (0-13%).

Mauer and colleagues (Ann Oncol, 2005) evaluated the FOLFOX regimen in 30 patients with esophageal cancer and 5 patients with gastric cancer. The majority of these patients had adenocarcinoma. 34 of these patients were evaluable for response; the overall response rate was 40% (95% confidence interval, 24-57%). The median duration of response was 4.6 months, median overall survival was 7.1 months, and the 1-year survival was 31%. Grade 3 and 4 toxicity was primarily neutropenia (63%), diarrhea (11%), fatigue (9%), and anorexia (6%). The authors concluded that there was significant anti-tumor activity and a favorable toxicity profile with this regimen for patients with esophagogastric cancer.

The REAL2 trial (Cunningham, NEJM 2008) was a large randomized trial that compared four different chemotherapy regimens in 1002 patients with advanced gastric cancer. The regimens were ECF (epirubicin, cisplatin and infusional 5-FU), ECX (epirubicin, cisplatin and capecitabine), EOF (epirubicin, oxaliplatin and infusional 5-FU) and EOX (epirubicin, oxaliplatin and capecitabine). The study demonstrated comparable outcomes with either infusional 5-FU or oral capecitabine and with either cisplatin or oxaliplatin. There was also a trend towards improved median overall survival for the EOX arm when compared to ECF arm: 11.2 months vs. 9.9 months, [HR 0.85, 95% CI (0.65-0.97)]. More recent clinical trials by Kang (ASCO 2006, Ann Oncol 2009) and Al-Batran (JCO 2008) also suggest that oxaliplatin and capecitabine are good substitutes for the more traditional cisplatin and infusional 5-FU.

In a Phase II trial by the NCCTG (North Central Cancer Treatment Group), Jatoi and colleagues studied CAPOX (capecitabine and oxaliplatin) in patients with metastatic adenocarcinoma of the esophagus, GE junction and gastric cardia (Jatoi, Ann Oncol, 2006). Oxaliplatin 130mg/m2/day 1 and capecitabine 1000mg/m2/bid po/days 1-14 were initially given to chemo-naïve patients. The investigators found it necessary to reduce the dose of capecitabine to 850mg/m2/bid/po on days 1-14 due to toxicity noted in the first 24 patients. Of the 43 patients evaluable for response, there was an overall response rate of 35% [95% confidence interval (CI) 23% to 50%]. Due to the findings of the forementioned study, we initiated 09-457 study with the recommended dose of oxaliplatin 130 mg/m2 every 3 weeks and capecitabine 850 mg/m2 po bid on days 1-14 of a 21-day cycle. The PI has discovered that 4 of the first 5 patients treated on this trial have required dose attenuation of capecitabine after the 3rd cycle due to diarrhea. Diarrhea is an expected toxicity of capecitabine, and this trend is reflective of the known median time to first occurrence of diarrhea being approximately 34 days. As a result of this, there will be a modification of the starting dose of capecitabine to 600mg/m2 po bid on days 1-14 for all subsequent patients enrolled to this trial.

Two recent studies have demonstrated that the addition of bevacizumab to chemotherapy regimens for metastatic esophagogastric cancer is well tolerated and can be administered safely. In both studies there also appears to be an improvement in response rate when compared to standard chemotherapy regimens (Shah, JCO 2005; Enzinger, ASCO 2008). Shah observed no significant increase in chemotherapy related toxicity. Possible bevacizumab related toxicity included a 28% incidence of Grade 3 hypertension, gastric perforation (6%), and myocardial infarction (2%). Grade 3 or 4 thromboembolic events occurred in 25% of patients. They concluded that bevacizumab could be added to chemotherapy safely and is active in the treatment of advanced gastric and GE junction adenocarcinoma. A Phase II study at Dana-Farber Harvard Cancer Center (Enzinger, ASCO 2008) of Docetaxel, Cisplatin, Irinotecan and Bevacizumab in Metastatic Esophageal and Gastric Cancer (TPCA), is currently under investigation. The study is now closed to enrollment and the data are being compiled. The palliative effect of this regimen appears to be significant, with many patients noting improvement or resolution of their dysphagia after one cycle of therapy. An EGD was performed on consenting patients after one cycle of therapy to perform visual tumor assessment and obtain biopsy sample of tumor for correlative science studies.

The procedure was well tolerated by all patients, with no toxicity or hemorrhagic events noted after the EGD was performed. Preliminary data suggests that this regimen can be safely administered to patients with advanced esophagogastric cancer and there are promising results in the first 32 patients that are evaluable. Partial responses are noted in 22 patients (69%). Survival data is being analyzed. Patients were required to take ASA 81 mg po daily (unless contraindicated due to allergy or other reasons). To date, there have been significantly less thromboembolic events (9%, Grade 3 or 4 events) than reported by Shah, et al. (25%). Only one patient is reported to have had a Grade 3 or 4 hemorrhagic event.

The combination of trastuzumab and bevacizumab has been studied in both Phase I and II clinical trials (Pegram, et al, 2004; Pegram, et al, 2006). The Phase 1 study indicated a potentially enhanced antitumor response and the PK data set indicates that co-administration of trastuzumab and bevacizumab does not alter the PK of either agent. The MTD was not reached on this study, as there were no Grade 3 or 4 adverse events noted. Grade 1 or 2 adverse events possibly or probably related to the combination included diarrhea, fatigue, and nausea. No cases of Grade 3 or 4 cardiac dysfunction were observed and the recommended Phase 2 dose was set as 10 mg/kg of bevacizumab every 14 days plus trastuzumab 4 mg/kg loading dose, then 2 mg/kg weekly.

Final results from the Phase 2 trial in first-line treatment of HER2- amplified advanced breast cancer was presented in a poster at the 32nd San Antonio Breast Cancer Symposium, on December 13th, 2009 (Hurvitz SA, Pegram, MD, et al: Poster 6094). In this trial, 50 patients were enrolled between 12/2004-4/2007. Based on results from phase 1 trial, the treatment consisted of Bevacizumab 10mg/kg every 2 weeks, and Trastuzumab 4mg/kg loading dose, followed by 2mg/kg weekly. Major inclusion criteria: women, aged 18-75 years, confirmed unresectable or metastatic breast cancer, confirmed HER2- amplification by FISH, ECOG: 0-2, normal left ventricular function by MUGA scan or echocardiogram. Major exclusion: more than 3 different organ sites of metastasis, prior chemotherapy, bevacizumab or trastuzumab in the metastatic setting, adjuvant trastuzumab was allowed if discontinued 1 year prior to study entry, uncontrolled hypertension, history of bleeding diathesis or coagulopathy, and proteinuria. Patients were treated with median of 6.25 cycles. Adverse events leading to withdrawal from the study were grade 3 proteinuria (n = 3), grade 2 recurrent proteinuria (n = 1), grade 2 decreased LVEF (n = 2), grade 4 LV dysfunction (n = 1), required surgery for T2 compression fracture (n = 1), grade 2 bevacizumab-related rectal vaginal fistula (n = 1), poor quality of life due to grade 2 epistaxis and grade 1 fatigue (n = 1), and death due to sepsis after surgery for bevacizumab-related gastric perforation (n = 1). The authors noted that hypertension was the most common adverse event with this combination, noted as a grade 3 event in 18 (36%) and as a grade 1 or 2 event in 12 additional patients. (**Of note, this is based on Version 2.0 of the CTCAE criteria, guidelines for documentation of hypertension have been changed in more recent versions. Most events considered grade 3 in version 2.0 would be grade 2 with the more current versions). One patient, who had previously received doxorubicin, experienced a grade 4 decrease in LVEF at the end of cycle 2. Fifteen additional patients had grade 1/2 treatment-related asymptomatic decreases in LVEF. No

significant correlation was found between prior anthracycline exposure and decrease in LVEF of any grade (Spearman correlation 0.11, *P* = .4) No significant correlation was found between hypertension events and decrease in LVEF (Spearman correlation 0.17, *P* = .22). Grade 3/4 treatment-related adverse events occurring in ≥2 patients were dyspnea (1 grade 4, 1 grade 3), grade 3 hypertension (n = 18), proteinuria (n = 4), headache (n = 2), and infusion reaction (n = 2). Treatment-related adverse events of any grade occurring in ≥10 patients were hypertension (n = 30), cardiac events (n = 19), headache (n = 17), epistaxis (n = 17), fatigue (n = 15), proteinuria (n = 12), elevated aspartate transaminases (AST) and/or alanine transaminase (ALT) levels (n = 12), fever/chills (n = 12), and diarrhea (n = 10). The authors noted objective responses in 24 patients (48%), including 2 CRs (WHO criteria). The authors concluded that despite the absence of chemotherapy, trastuzumab plus bevacizumab is clinically feasible and active as first line therapy for patients with locally advanced and unresectable metastatic breast cancer. Also presented at the 2009 San Antonio Breast Cancer symposium was an abstract (Falchook, G.S., Moulder, S, et al: Abstract 244) of a Phase 1 trial combining trastuzumab, lapatinib, and bevacizumab in HER2- positive breast cancer and other malignancies. Patients with advanced malignancy refractory to standard therapy were eligible. The treatment consisted of 3 week cycles with trastuzumab 6mg/kg loading dose, and 4mg/kg maintenance dose, lapatinib 750mg and bevacizumab 7.5mg/kg. Data on 24 treated patients was presented in this abstract. The most common drug related toxicities were hypertension (21%), fatigue (21%) and diarrhea (17%). No cardiomyopathy was observed in these patients. The authors noted that the combination was well tolerated and demonstrated preliminary evidence of clinical activity in advanced HER-2 positive breast cancer refractory to prior trastuzumab and lapatinib, suggesting that this combination of agents may overcome resistance to previous HER-2 therapy.

Additional studies combining bevacizumab, trastuzumab and chemotherapy agents have been presented at recent ASCO meetings. A multicenter trial of weekly nab-paclitaxel, carboplatin plus bevacizumab and trastuzumab in HER2- positive locally advanced breast cancer (Yardley, D.A., et al. ASCO 2009: Abstract: 527) reports data on 29 patients evaluable for toxicity. Treatment consisted of nab-paclitaxel 125mg/m2 on days 1, 8, 15 and carboplatin AUC 6 on day 1 every 28 days. In addition, trastuzumab 4mg/kg loading dose, followed by 2mg/kg weekly maintenance dose plus bevacizumab 5mg/kg weekly for 6 cycles followed by surgery. Post surgery trastuzumab and bevacizumab continued for 52 weeks. The authors reported no unexpected toxicity and evidence of LVEF dysfunction in 1 patient. Also presented at ASCO 2009 meeting (Smith, J. W., et al: ASCO2009, Abstract:580) was a trial of Epirubicin plus cyclophosphamide followed by docetaxel plus trastuzumab and bevacizumab as neoadjuvant therapy for HER2-positive breast cancer or as adjuvant therapy for HER2-positive stage III breast cancer. The purpose of this trial was to determine the cardiac safety profile of the agents. Preliminary data showed grade 2 LVEF dysfunction in 5 patients and grade 3 LVEF dysfunction in 2 patients, one of whom had symptomatic CHF. The authors concluded the regimen had an acceptable preliminary rate of cardiac toxicity.

There is one active, IRB approved Phase II study at DF/HCC that combines chemotherapy (carboplatin) with bevacizumab and trastuzumab for breast cancer patients with brain metastasis (09-224, Nancy Lin, MD). This Phase II study has enrolled 4 patients to date with all four of these patients receiving both bevacizumab and trastuzumab as well as carboplatin. Of the 4 patients, one was taken off study for progressive disease at the time of the first restaging, and 3 patients have had first restaging and are continuing on treatment. The patient that was taken off study had an incidental finding of a grade 3 IVC thrombus that was considered possibly related to the study medication. No significant cardiac toxicity has been reported (conversation with study staff).

A trial to evaluate the combination of oxaliplatin and trastuzumab in patients with metastatic breast cancer (Yardley, 2008 Breast Cancer Symposium) demonstrated tolerability of this combination with no significant increase in Grade 3 or 4 toxicity. The combination of CAPOX plus trastuzumab has not been reported in any cancer.

2.2 Study disease

In 2009, the American Cancer Society estimates that 37,600 Americans will be diagnosed with esophagogastric cancer and predicts there will be 25,100 deaths. The prognosis is related to the stage of disease at diagnosis. The majority of patients, however, present with metastatic or advanced disease. Symptoms such as dysphagia, GI bleeding or weight loss often do not develop until the disease is locally advanced or metastatic. Patients with locally advanced disease have a five-year survival of 20-30%. Despite recent chemotherapeutic advances, median survival for patients with metastatic disease remains less than 10 months.

The histopathology and natural history of esophageal and gastric adenocarcinoma appears to be quite similar and the two cancers respond similarly to chemotherapy. The goal of chemotherapy with esophagogastric cancer is most often to palliate symptoms and improve survival. The addition of targeted therapies to chemotherapy is being studied in esophagogastric cancer with the goal of improving response rates and overall survival.

2.3 Rationale

Based on the above data, we propose to conduct this Phase II study to determine the rate of response for patients with HER2-positive esophagogastric adenocarcinoma receiving CAPOX plus bevacizumab plus trastuzumab. Recent data presented at ASCO 2009 and the 2009 San Antonio Breast Cancer symposium have demonstrated safety and tolerability of the combination of bevacizumab and trastuzumab with chemotherapy. The ToGA study has made capecitabine/platinum + trastuzumab a recommended standard of care for HER2 positive esophagogastric cancers (van Cutsem, Proc ASCO 2009). The recently completed AVAGAST study (Protocol ID NCT00548548) may similarly elevate capecitabine/platinum + bevacizumab to another standard of care in this disease at the ASCO 2010 meeting. If the AVAGAST study is positive, medical oncologists will want to combine these two regimens in patients with HER2 positive tumors.

The REAL 2 study, the largest ever phase III study in advanced esophageal and gastric cancer, found that capecitabine may replace 5-FU and oxaliplatin may replace cisplatin in regimens used for the treatment of advanced esophageal and gastric cancer. The CAPOX regimen is a well established regimen, with known toxicity profile and is used for the treatment of a variety GI malignancies. Oral capecitabine allows for ease of administration, independence and patient convenience. The schedule of every-three-week oxaliplatin, bevacizumab and trastuzumab will allow us to recruit from a larger geographical area.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

Participants must meet the following criteria to be eligible to participate in the study:

3.1.1 Participants must have confirmed HER2-positive esophageal, GE junction or gastric adenocarcinoma (including undifferentiated and adenosquamous carcinoma) that is metastatic or unresectable. HER-2 testing will be performed using IHC or FISH. Any one of the following criteria would apply for this study:

- IHC: 3+
- IHC: 2+, with confirmation by FISH required
- FISH positive

3.1.2 All patients must have available tumor sample (either paraffin block or 15 freshly cut, unstained slides) prior to study entry.

Part II: All patients must have primary esophagogastric tumor in place or other tumor that is accessible for mandatory biopsy.

3.1.3 Participants must have measurable disease, defined in RECIST 1.1. Refer to section 10.

3.1.4 Age: ≥18 years.

3.1.5 Life expectancy of greater than 12 weeks.

3.1.6 ECOG performance status <2.

3.1.7 Organ and marrow function as defined below:

- Absolute neutrophil count ≥ 1,500/mcL
- Platelets ≥ 100,000/mcL
- Hemoglobin ≥ 9
- total bilirubin less than 1.5 X institutional upper limit of normal
- AST (SGOT) and ALT (SGPT) ≤ 3 X institutional upper limit of normal, or <5 X upper limit of normal if liver metastases are present

- Alkaline phosphatase <2.5X institutional upper limit of normal or <5X if liver metastases are present or <10X if bone metastases are present
- Serum creatinine less than 2.0mg/dL.
- eGFR greater than 50ml/min/1.73m2 (if patient is African-American, multiply results by 1.21)
- Urine Protein/Creatinine ratio less than 1

3.1.8 Women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) during study participation, and for 30 days from the date of the last study drug administration.

3.1.9 Ability to understand and the willingness to sign a written informed consent document.

3.1.10 Part II only: Participant agrees to undergo mandatory pre and post loading dose of trastuzumab biopsy for correlative science.

3.2 Exclusion Criteria

Participants who exhibit any of the following conditions at screening will not be eligible for admission into the study.

3.2.1 Prior therapy with any of the following: capecitabine, oxaliplatin, bevacizumab or trastuzumab is not allowed. May have received and completed adjuvant therapy at least 6 months prior to study entry or one prior therapy for metastatic disease as long as it did not include any of the above agents.

3.2.2 Chemotherapy within 4 weeks prior to entering the study or radiotherapy to greater than 25% of bone marrow within 4 weeks prior to entering the study.

3.2.3 Palliative radiation therapy to isolated bone metastasis within 2 weeks of initiating therapy.

3.2.4 Major surgery, open biopsy, significant traumatic injury within 4 weeks prior to study entry.

3.2.5 Minor surgery, including placement of vascular access device within 7 days prior to first dose of bevacizumab.

3.2.6 Residual toxicity from prior chemotherapy and/or radiation therapy of Grade 2 or greater.

3.2.7 Participants may not be receiving any concurrent investigational agents.

3.2.8 Active brain or other CNS metastasis by history or clinical examination.

3.2.9 History of allergic reactions attributed to compounds of similar chemical or biologic composition to capecitabine, bevacizumab or trastuzumab. No known allergy or hypersensitivity to Chinese hamster ovary, or any of the study agents. No known DPD deficiency.

3.2.10 Warfarin is prohibited; anticoagulation using low molecular weight heparin is allowed.

3.2.11 Uncontrolled, intercurrent illness including, but not limited to ongoing or active infection, history of congestive heart failure, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.12 Patients with history of other malignancy are not eligible except for the following circumstances:

Individuals with a history of other malignancies are eligible if they have been disease-free for at least 3 years and are deemed by the investigator to be at low risk for recurrence of that malignancy.

Individuals with the following cancers are eligible if diagnosed and treated with curative intent within the past 5 years: cervical cancer *in situ*, basal cell or squamous cell carcinoma of the skin.

3.2.13 Known HIV seropositivity, Hepatitis C, acute or chronic Hepatitis B or other serious active infection.

3.2.14 LVEF less than 50% as determined by MUGA scan or echocardiogram within 28 days prior to initiation of therapy.

3.2.15 Inadequately controlled hypertension, defined as BP greater than systolic 150 and/or diastolic of greater than 100 mmHg. Initiation of antihypertensive medication is recommended, and adequate control must be documented prior to C1D1.

3.2.16 History of prior hypertensive crisis or hypertensive encephalopathy.

3.2.17 History of any arterial thrombosis, CVA, TIA, MI or unstable angina in past6 months.

3.2.18 Evidence of bleeding diathesis or coagulopathy (other than deep venous thrombosis, portal vein thrombosis, treated pulmonary embolism, atrial fibrillation). Patients on therapeutic anticoagulation with low molecular weight heparin may be enrolled provided they have been clinically stable on anticoagulation for at least 2 weeks prior to initiation of bevacizumab therapy.

3.2.19 Serious, unhealed wounds, bone fractures or skin ulcers.

3.2.20 Pregnant or breast feeding. (Women of child bearing potential must have negative urine or serum pregnancy test within 14 days prior to start of therapy)

3.2.21 >Grade 1 peripheral neuropathy at baseline.

3.2.22 Lack of physical integrity of the upper gastrointestinal tract or malabsorption syndrome

4. REGISTRATION PROCEDURES

4.1. General Guidelines for DF/HCC and DF/PCC Institutions

Institutions will register eligible participants with the DF/HCC Quality Assurance Office for Clinical Trials (QACT) central registration system. Registration must occur prior to the initiation of therapy. Any participant not registered to the protocol before treatment begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the QACT protocol-specific eligibility checklist.

Following registration, participants may begin protocol treatment. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study may be canceled. Notify the QACT Registrar of registration cancellations as soon as possible.

4.2 Registration Process for DF/HCC and DF/PCC Institutions

The QACT registration staff is accessible on Monday through Friday; from 8:00 AM to 5:00 PM Eastern Standard Time. In emergency situations when a participant must begin treatment during off-hours or holidays, call the QACT registration line at 617-632-3761 and follow the instructions for registering participants after hours.

The registration procedures are as follows:

1. Obtain written informed consent from the participant prior to the performance of any study related procedures or assessments.

2. Complete the QACT protocol-specific eligibility checklist using the eligibility assessment documented in the participant's medical and/or research record. **To be eligible for registration to the protocol, the participant must meet all inclusion and exclusion criterion as described in the protocol and reflected on the eligibility checklist.**

Reminder: Confirm eligibility for ancillary studies at the same time as eligibility for the treatment study. Registration to both treatment and ancillary studies will not be completed if eligibility requirements are not met for all studies.

3. Fax the eligibility checklist and all pages of the consent form to the QACT at 617-632-2295.

4. The QACT Registrar will (a) review the eligibility checklist, (b) register the participant on the protocol, and (c) randomize the participant when applicable.

5. An email confirmation of the registration and/or randomization will be sent to the Overall PI, study coordinator(s) from the Lead Site, treating investigator and registering person immediately following the registration and/or randomization.

4.3 General Guidelines for Other Participating Sites

Eligible participants will be entered on study centrally at the Dana-Farber Cancer Institute by the Study Coordinator. All sites should call the Study Coordinator [617-632-6316] to verify slot availabilities. Following registration, participants should begin protocol treatment within 5 days. Issues that would cause treatment delays should be discussed with the Overall PI. If a participant does not receive protocol therapy following registration, the participant's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

4.4 Registration Process for Other Participating Institutions

To register a participant, the following documents should be completed by the research nurse or data manager and faxed [Fax # 617-582-7988] or e-mailed [Katharine_Straw@dfci.harvard. edu] to the DFCI Study Coordinator:

- Copy of pathology report confirming cancer diagnosis, laboratory results (CBC with differential and platelets, comprehensive chemistry panel, PT/PTT/INR, urinalysis and urine protein/creatinine ratio, pregnancy test, if applicable, baseline CT/MRI report, MUGA/Echocardiogram report, EKG)
- Signed participant consent form
- HIPAA authorization form
- Eligibility Checklist

The research nurse or data manager at the participating site will then call [617-632-6316] or e-mail [katharine_straw@dfci.harvard.edu] the DFCI Study Coordinator to verify eligibility. To complete the registration process, the Coordinator will:

- register the participant on the protocol with the QACT
- fax or e-mail the participant study number, and if applicable the dose treatment level to the participating site
- call the research nurse or data manager at the participating site and verbally confirm registration

NOTE: Registration and randomization with the QACT can only be conducted during the business hours of 8:00 AM and 5:00 PM Eastern Standard Time Monday through Friday. Same day treatment registrations will only be accepted with prior notice and discussion with the DF/HCC Lead Institution.

5. TREATMENT PLAN

Treatment will be administered on an outpatient basis or if necessary, inpatient (for DF/HCC patients at BWH or MGH). Expected toxicities and potential risks as well as dose modifications for CAPOX+bevacizumab+trastuzumab are described in Section 6 (Expected Toxicities and Dosing Delays/Dose Modifications).

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

Premedication for trastuzumab and bevacizumab will not be required. However, premedication is recommended if there has been an infusion reaction in a previous cycle.

Table 1: Treatment plan

Agent	Dose	Route	Schedule	Cycle
Trastuzumab	4 mg/kg Loading dose	IV	Day 1	**Cycle 1 Only** (cycle 1 is one week)
Bevacizumab	7.5mg/kg	IV	Day 1	**Cycle 2** (and all subsequent cycles) Each cycle is 21 days
Trastuzumab	6mg/kg	IV	Day 1 Administer after bevacizumab	
Oxaliplatin	130mg/m2	IV	Day 1 Administer after trastuzumab	
Capecitabine*	1200* mg/m2	PO *	Days 1-14 in divided dose Administer after oxaliplatin	

*calculate dose for 1200mg/m2, then round to nearest multiple of 500mg tablets, then give in divided dose daily. If an odd number of pills, the patient should take the extra tablet in the evening. Examples:

- 1200mg/m2 x BSA of 2.0=2400mg Round to 2500 mg in divided dose, take 2 tablets in am and take 3 tablets in pm.
- 1200mg/m2 x BSA of 1.7=2040 mg Round to 2000 mg in divided dose, take 2 tablets in am and 2 tablets in pm

5.1 Pre-treatment Criteria

5.1.1 Cycle 1, Day 1

- Screening labs must meet eligibility criteria and must have been done within 14 days of C1D1. Do not have to be repeated on C1D1.
- Screening labs will include: CBC with differential, Platelets, comprehensive chemistry panel, PT/PTT/INR, U/A and Urine protein/creatinine ratio (UPCR)
- Pregnancy test is required within 14 day for women of child bearing potential (urine or serum)
- EKG within 28 days
- MUGA scan or echocardiogram within 28 days
- ECOG 0-1. **No significant change in ECOG performance status in past two weeks.**
- CT scan of chest, abdomen and pelvis within 28 days.
- No evidence of active bleeding from tumor in past 2 weeks
- No clinically significant concurrent illness or infection (determined by treating investigator).
- **Pre treatment mandatory biopsy within 2 weeks of loading dose of trastuzumab.**

5.1.2 Cycle 1 Day 7

- **Mandatory biop**sy of tumor for correlative science.

  This **must** be done on day 7. **Cycle 1 loading dose of trastuzumab must be given on a Tuesday, Wednesday, Thursday or Friday as biopsy procedures**

are performed Monday – Friday (please refer to Section 9., Study Calendar).

5.1.3 **Cycle 2, Day 1 and all Subsequent Cycles** (refer to study calendar for required study tests).

- ANC ≥1000 /mcL
- Platelet count ≥75,000 /mcL
- Recovery of clinically significant study drug related non-hematological toxicity to grade 1 or less. Exceptions as noted in table 4 (page 50) and table 7 (page 56) are allowed.
- **Part I**: Cycle 2 will begin 1 week after loading dose of trastuzumab
- **Part II** only: Tumor biopsy must have been completed C1D7. (Cycle 2 will begin 9-11 days after loading dose of trastuzumab. This will allow a minimum of 2 days rest post biopsy). Approval by PI: Dr Peter Enzinger will be required to continue patient on study if this biopsy was not performed.
- ECOG: ≤ 2

5.2 Agent Administration

Please refer to table 2 (pg 32)

- Calculation of BSA will be per institutional policy. Weight will be documented on day 1 of each cycle and BSA will be recalculated.
- Vital signs: temperature, pulse, BP, respiratory rate
    - At each visit, pre dose
    - C1D1: one hour after trastuzumab has infused
- Bevacizumab will be administered first, followed by trastuzumab. Oxaliplatin will be administered after trastuzumab.
- Pre Hydration is not required for any of the study agents below.
- Pre medication for hypersensitivity or possible allergic reaction is not required prior to the first dose of any of the study agents.
- Pre medication with anti-emetics are recommended prior to oxaliplatin infusion.
- Oxaliplatin has vesicant and irritant properties and may cause pain if given peripherally. Central venous access is recommended but not required.

- Capecitabine tablets may be crushed if necessary. We recommended that only the patient do this due to risk of exposure to the chemotherapy when crushing tablets.

Table 2: Agent administration

Agent		Route	Schedule	Cycle 1
Trastuzumab	**Loading dose** Do not administer I.V. push or by rapid bolus. Do not administer with D_5W *** give patient prescription for capecitabine to fill at local pharmacy**	**IV**	**Cycle 1 Day 1** Administer over approximately 90 minutes **Observe for one hour post infusion** For infusion related adverse reaction refer to section 5.2.1 **Vital signs prior to infusion and after one hour of observation post infusion**	**(Part II: Cycle 1 Day 7** EGD/biopsy) (note: cycle 1 is 7 days)
Agent		**Route**	**Schedule**	**Cycle 2 and subsequent cycles**
Bevacizumab	Dilute prescribed dose of bevacizumab in NS. Do not mix with dextrose-containing solutions	**IV**	**Day 1** Administer at approximately 0.5mg/kg/minute For infusion related adverse reaction refer to section 5.2.2	**Part I: Cycle 2 begins 1 week post loading dose of trastuzumab** **Part II: Cycle 2 will begin 9-11 days post loading dose of trastuzumab.** Each cycle is 21 days
Trastuzumab	Do not administer I.V. push or by rapid bolus. Do not administer with D_5W.	**IV**	**Day 1** If loading dose was tolerated, administer over approximately 30 minutes. Administer after bevacizumab. If infusion reaction, refer to section 5.2.1 for guidelines for subsequent administration.	

Oxaliplatin	Mix in D5W	**IV**	**Day 1** Administer over approximately 2 hours Lengthen infusion time to 6 hours if ACUTE laryngopharyngeal dysesthesia or hypersensitivity noted in any cycle For infusion related adverse reaction refer to section 5.2.3 Administer after bevacizumab and trastuzumab Oxaliplatin has vesicant and irritant properties.	**Cycle 2** (and all subsequent cycles) **Part I: Cycle 2 begins 1 week post loading dose of trastuzumab** **Part II: Cycle 2 will begin 9-11 days post loading dose of trastuzumab.** Each cycle is 21 days
Capecitabine	Round to the nearest multiple of whole 500 mg tablets and divide as equally as possible. If there is odd number of tablets, the patient should take the extra tablet with the evening dose. Instruct patient to take each dose with 8 oz. of H2O. Patient diary is in appendix	**PO** **BID**	**Days 1-14** Administer **30 minutes after a meal,** with H2O approximately 12 hours apart. **Day 1**: if start time is delayed to afternoon, one dose will be taken for that day and the final dose for cycle will be taken on day 15 in the am. Missed or vomited doses are not to be made up	

5.2.1 Trastuzumab Administration

Trastuzumab will be administered as a loading dose on Cycle 1 Day 1. The initial loading dose infusion of trastuzumab will be administered over approximately 90 minutes. There will be a 60-minute observation period after the infusion is complete. Vital signs will be checked after 60 minutes of observation.

During the first infusion with trastuzumab, a symptom complex consisting of chills and/or fever is observed commonly in patients. Other signs and/or symptoms may include nausea, vomiting, pain, rigors, headache, cough, dizziness, rash, and asthenia. These symptoms are usually mild to moderate in severity, and occur infrequently with subsequent trastuzumab infusion. If the initial infusion is well tolerated (no fever, chills, or rigors with or post infusion), all subsequent infusions may be given over 30 minutes. If adverse reaction is noted with any infusion, subsequent infusions will be administered over 90 minutes.

Administer medications including but not limited to acetaminophen, meperidine (Demerol®), diphenhydramine, steroids, and ranitidine or equivalent H2 blocker except for cimetidine due to possible interaction with capecitabine. If the 90 minute infusion is well tolerated, may reduce infusion time to 30-minutes for subsequent infusions. Pre medication with acetaminophen may be given in subsequent cycles if necessary. Trastuzumab will be administered after bevacizumab and before oxaliplatin in cycle 2 and all subsequent cycles.

5.2.2 Bevacizumab Administration

The first dose of bevacizumab will be administered on cycle 2 day 1. Cycle 2 should start one week after the loading dose of trastuzumab. Premedication will not be required prior to first dose.

Bevacizumab will be infused at a rate of approximately 0.5 mg/kg/minute. For infusion related adverse reaction, the infusion will be interrupted until recovery and necessary support has been administered (i.e., diphenhydramine, steroids, or ranitidine). **For any Grade 3 or 4 (CTCAE version 4.0) allergic reaction or anaphylaxis, or any grade of bronchospasm, bevacizumab will be permanently discontinued (may remain on study and receive other agents at the discretion of the treating investigator).**

Upon recovery of adverse reaction, the infusion will be resumed at the slower rate, and administered over 30+/-10 minutes. If this is well tolerated, the next infusion will be administered following pre-medication (i.e., diphenhydramine, steroids, ranitidine) over 30+/-10 minutes. At the discretion of the treating investigator, and if no further evidence of adverse reaction with premedication, all subsequent infusions may be administered at the starting rate of approximately 0.5mg/kg/minute.

5.2.3 Oxaliplatin Administration

Acute laryngopharyngeal dysesthesia (LPD), is the subjective loss of sensation of breathing without any objective evidence of respiratory distress (i.e., laryngospasm, bronchospasm or hypoxia) and been observed in patients during oxaliplatin administration. It may be cold induced or related to rate of infusion and may occur with any infusion. LPD should be distinguished from an acute hypersensitivity or allergic reaction. It is managed initially by suspending the infusion, while supportive measures are carried out. Vital signs including pulse oximetry should be monitored. If pulse oximetry is normal, the use of a benzodiazepine or other anti anxiety medication or measures are prescribed as necessary. Upon recovery, the infusion may be resumed at a reduced rate for the remainder of the infusion and should be administered over 6 hours. All subsequent infusions will be administered over 6 hours. Patients will be instructed to avoid cold liquids or exposure to cold for 24-48 hours post infusion.

Oxaliplatin hypersensitivity reaction is most commonly seen after multiple doses of treatment. For any (CTCAE version 4.0) Grade 3 or 4 hypersensitivity or anaphylaxis, oxaliplatin will be permanently discontinued. For Grade 1 or 2 hypersensitivity reactions, the infusion will be suspended and supportive measures will be applied. Treatment may resume at reduced delivery rate, following complete resolution of symptoms (bronchospasm, hypotension, etc). Infuse over 6 hours for all subsequent infusions. Premedication with corticosteroids, diphenhydramine, and ranitidine is recommended 30-60 minutes prior to all subsequent infusions. The doses of which may be determined by the treating investigator. Oxaliplatin should be permanently discontinued if premedication, or prolonged administration measures fail to prevent oxaliplatin related hypersensitivity.

5.3 Other Modality(ies) or Procedures:

Mandatory biopsies of tumor (Part II only):

In Part II of the study, there will be two mandatory biopsies of tumor for patients enrolled at DF/HCC, the cost of which will be covered by the study. The first will be performed within 2 weeks of loading dose of trastuzumab. The second will be performed on Cycle 1 Day 7. The treating investigator will determine the most appropriate site to biopsy. These biopsies will be performed for correlative science studies; therefore, we do not intend to send these samples for processing in the clinical pathology laboratory, unless there is a concern about any findings during the biopsy procedure as determined by the MD performing the biopsy. If a patient is unwilling or unable (i.e. biopsy is too difficult to obtain) to undergo the required biopsy, the treating investigator may request a biopsy waiver, which will be granted at the discretion of the Principal Investigator.

Esophagogastric duodenoscopy (EGD) is the preferred modality to obtain the biopsy if the primary tumor is in place. EGD is usually performed on an outpatient basis and performed under conscious sedation. Visualization of the tumor and surrounding area should be noted in the final report. Specific pre and post endoscopy instructions will be given to the patient by the endoscopy lab performing the procedure. The procedure is generally well tolerated; however, the risks associated include possible esophageal or gastric perforation, bleeding or

trauma at tumor or biopsy site, adverse reaction to the local anesthetic or medication which can lead to bradycardia, apnea, or laryngospasm.

Other possible sites for biopsy include liver or lymph node.

The biopsies will be performed at either Brigham and Women's Hospital or Massachusetts General Hospital.

5.4 General Concomitant Medication and Supportive Care Guidelines

- Diarrhea should be managed aggressively with the use of loperamide and other anti diarrheal regimens as determined by the treating investigator. IV hydration and other supportive measures should be used as needed. Patients with neutropenia and diarrhea should be considered for use of prophylactic antibiotics, such as oral quinolones.

- Anti nausea medications including 5-HT3 antagonists (i.e., ondansetron) with corticosteroids (i.e. dexamethasone) are highly recommended prior to oxaliplatin administration. The dose and regimen are to be determined by the treating investigator. Prochlorperazine may also be used as needed. If necessary, supportive measures such as IV hydration may be used.

- Cold induced acute laryngopharyngeal dysesthesia (LPD) or peripheral parestheias/dysesthesias are associated with oxaliplatin administration. Patients should be instructed to avoid ice and cold liquids during infusion of oxaliplatin and for 24-48 hours post infusion. LPD should be distinguished from an acute hypersensitivity or allergic reaction. Refer to section 5.2.3 for management of LPD and hypersensitivity.

- Erythropoietin growth factors will be allowed as needed.

- The use of leukocyte growth factors is not allowed to avoid dose delay or reduction. If clinically indicated for febrile neutropenia, ASCO guidelines should be followed.

- Sunscreens and daily use of skin moisturizers are highly recommended while taking capecitabine. The use of fragrance free, hypoallergenic and non irritating products is recommended.

- As a result of the potential for interaction with other concomitantly administered drugs through the cytochrome P450 system, the case report form must capture the concurrent use of all other drugs or over-the-counter medications.

- Capecitabine has known interaction with phenytoin and warfarin. The use of warfarin is not allowed on this protocol. The use of phenytoin should be used with caution; levels should be monitored closely and dose may need to be reduced. No formal drug-drug interaction studies between capecitabine and other CYP2C9 substrates have been conducted; therefore care should be exercised when co administration is medically necessary.

- Oxaliplatin has vesicant and irritant properties. There have been reported cases of extravasation induced necrosis to skin and surrounding tissues. Cool compress may be used for immediate management of extravasation, with consideration of potential for peripheral neuropathy exacerbated by cold. Warm compresses may avoid peripheral neuropathic discomfort, however, while possibly increasing drug removal through local vasodilation may increase cellular uptake and injury.

5.5 Duration of Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse events, treatment may continue until one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s)
- Participant decides to withdraw from the study
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the opinion of the treating investigator.

5.6 Duration of Follow Up

Participants will be followed until resolution of any treatment related Grade 3-4 toxicity after removal from study or until death, whichever occurs first. Participants removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

5.7 Criteria for Removal from Study

Participants will be removed from study when any of the criteria listed in Section 5.5 applies. The reason for study removal and the date the participant was removed must be documented in the study-specific case report form (CRF). Alternative care options will be discussed with the participant.

In the event of unusual or life-threatening complications, participating investigators must immediately notify the Principal Investigator Dr. Peter C. Enzinger, via phone 617.632.6855 or email at peter_enzinger@dfci.harvard.edu.

6. EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

There will be no dose modifications for bevacizumab or trastuzumab.

NCI Common Terminology Criteria for Adverse Events (CTCAE v4.0) will be used for all toxicity assessment.

All adverse events experienced by participants will be collected from the time of the first dose of study treatment, through the study and until the final study visit. Participants continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

6.1 Anticipated Toxicities

6.1.1 Trastuzumab

- Cardiomyopathy: [U.S. Boxed Warning]: Trastuzumab is associated with symptomatic and asymptomatic reductions in left ventricular ejection fraction (LVEF) and severe heart failure (HF) and may result in mural thrombus formation and stroke, and even cardiac death; discontinue for cardiomyopathy. Evaluate LVEF in all patients prior to and during treatments. Extreme caution should be used in patients with pre-existing cardiac disease or dysfunction. Concomitant administration of anthracyclines and prior exposure to anthracyclines or radiation therapy significantly increases the risk of cardiomyopathy; other potential risk factors include advanced age, high or low body mass index, smoking, diabetes, and hyper/hypothyroidism. Discontinuation should be strongly considered in patients who develop a clinically significant reduction in LVEF during therapy; treatment with HF medications (e.g., ACE inhibitors, beta-blockers) should be initiated. Cardiomyopathy due to trastuzumab is generally reversible over a period of 1-3 months after discontinuation. (When LVEF returns to baseline, reinitiation may be considered if indicated.) Trastuzumab is also associated with arrhythmias and hypertension.

- Infusion reactions: [U.S. Boxed Warning]: Infusion reactions (including fatalities) have been associated with use; discontinue for anaphylaxis or angioedema. Most reactions occur during or within 24 hours of the first infusion; interrupt infusion for dyspnea or significant hypotension. Retreatment of patients who experienced severe hypersensitivity reactions has been attempted (with premedication). Some patients tolerated retreatment, while others experienced a second severe reaction.

- Pulmonary toxicity: [U.S. Boxed Warning]: May cause serious pulmonary toxicity (dyspnea, hypoxia, interstitial pneumonitis, pulmonary infiltrates, pleural effusion, noncardiogenic pulmonary edema, pulmonary insufficiency, acute respiratory distress syndrome [ARDS], and/or pulmonary fibrosis); discontinue for ARDS or interstitial pneumonitis. Use caution in patients with pre-existing pulmonary disease or patients with extensive pulmonary tumor involvement; these patient populations may have more severe toxicity. Pulmonary events may occur during or within 24 hours of administration; delayed reactions have occurred.

Concurrent drug therapy issues:

- Chemotherapy: When used in combination with myelosuppressive chemotherapy, trastuzumab may increase the incidence of neutropenia (moderate-to-severe) and febrile neutropenia.

Table 3 Expected Toxicities Associated with Trastuzumab by Body System

Body System	>10%	1-10%	<1%
Cardiovascular	• LVEF Decreased (4%-22%)	• Peripheral edema (5%-10%) • Edema (8%) • CHF (2% -7%; severe: <1%) • Tachycardia (5%) • Hypertension (4%) • Arrhythmia (3%) • Palpitation (3%)	• Cardiac arrest • Cardiomyopathy • Hypotension • Ventricular dysfunction • Pericardial effusion
Central Nervous System	• Pain (47%) • Fever (6%-36%) • Chills (5%-32%) • Headache (10%-26%) • Insomnia (14%) • Dizziness (4%-13%)	• Depression (6%)	• Seizure • Stroke • Syncope • Hydrocephalus • Amblyopia • Confusion • Deafness • Mania
Dermatologic	• Rash (4%-22%)	• Acne (2%) • Nail disorder (2%) • Pruritus (2%)	• Skin ulcers • Angioedema • Cellulitis
Gastrointestinal/ Hepatic	• Nausea (6%-33%) • Diarrhea (7%-25%) • Vomiting (4%-23%) • Abdominal pain (2%-22%) • Anorexia (14%)	• Constipation (2%) • Dyspepsia (2%)	• Colitis • Hepatic failure • Hepatitis • Pancreatitis • Esophageal ulcer • Gastroenteritis • Ileus • Intestinal obstruction • Ascites • Stomatitis
Genitourinary/ Renal		• Urinary tract infection (3%-5%)	• Oligohydramnios
Renal			• Hydronephrosis • Renal failure • Pyelonephritis • Nephrotic syndrome • Glomerulonephritis (membranous, focal and fibrillary) • Glomerulopathy • Glomerulosclerosis • Hemorrhagic cystitis
Neuromuscular & Skeletal	• Weakness (4%-42%) • Back pain (5%-22%)	• Paresthesia (2%-9%) • Bone pain (3%-7%)	• Bone necrosis • Myopathy

		• Arthralgia (6%-8%) • Myalgia (4%) • Muscle spasm (3%) • Peripheral neuritis (2%) • Neuropathy (1%)	• Pathological fracture
Respiratory	• Cough (5%-26%) • Dyspnea (3%-22%) • Rhinitis (2%-14%) • Pharyngitis (12%)	• Sinusitis (2%-9%) • Nasopharyngitis (8%) • Upper respiratory infection (3%) • Epistaxis (2%) • Pharyngolaryngeal pain (2%)	• Acute respiratory distress syndrome (ARDS) • Apnea • Asthma • Ataxia • Bronchospasm • Respiratory failure • Hematemesis • Hypoxia • Pleural effusion • Pneumonitis • Pneumothorax • Pulmonary edema (noncardiogenic) • Pulmonary fibrosis • Pulmonary hypertension • Pulmonary infiltrate • Respiratory distress • Interstitial pneumonitis • Paroxysmal nocturnal dyspnea • Laryngitis
Hematologic		• Anemia (4%) • Leukopenia (3%)	• Coagulopathy • Pancytopenia • Leukemia (acute) • Neutropenia
Miscellaneous/ Other	• Infusion reaction (21%-40% chills and fever most common; severe: 1%) • Infection (20%)	• Flu-like syndrome (2%-10%) • Accidental injury (6%) • Influenza (4%) • Allergic reaction (3%) • Herpes simplex (2%)	• Anaphylaxis • Anaphylactoid reaction • Hemorrhage • Hypersensitivity • Herpes zoster • Radiation injury • Hypercalcemia • Hypothyroidism • Lymphangitis • Mural thrombosis • Sepsis • Shock • Thyroiditis (autoimmune) • Vascular thrombosis • Volume overload

6.1.2 Bevacizumab:

Boxed warnings:

- Gastrointestinal perforation: See "Concerns related to adverse effects" below.
- Hemorrhage: See "Concerns related to adverse effects" below.
- Wound dehiscence: See "Concerns related to adverse effects" below.

Concerns related to adverse effects:

- Fistula formation (nongastrointestinal): Nongastrointestinal fistula formation (including tracheoesophageal, bronchopleural, biliary, vaginal, and bladder fistulas) has been observed, most commonly within the first 6 months of treatment. Permanently discontinue in patients who develop internal organ fistulas.
- Gastrointestinal perforation: **[U.S. Boxed Warning]: Gastrointestinal perforation, fistula (including gastrointestinal, enterocutaneous, esophageal, duodenal, and rectal fistulas), and intra-abdominal abscess have been reported in patients receiving bevacizumab for colorectal cancer and other cancers (not related to treatment duration).** Most cases occur within 50 days of treatment initiation; may be fatal in some cases; monitor patients for signs/symptoms (e.g., fever, abdominal pain with constipation and/or nausea/vomiting). Permanently discontinue in patients who develop these complications.
- Hemorrhage: **[U.S. Boxed Warning]: Severe or fatal hemorrhage, including hemoptysis, gastrointestinal bleeding, central nervous system hemorrhage, epistaxis, and vaginal bleeding have been observed. Avoid use in patients with serious hemorrhage or recent hemoptysis (≥2.5 mL blood).** Serious pulmonary hemorrhage has been reported in patients receiving bevacizumab (primarily in patients with nonsmall cell lung cancer with squamous cell histology [not an FDA-approved indication]). Intracranial hemorrhage, including cases of Grade 3 or 4 hemorrhage has occurred in patients with previously treated glioblastoma. Treatment discontinuation is recommended in all patients with intracranial bleeding or other serious hemorrhage. Use with caution in patients at risk for thrombocytopenia.
- Hypertension: May cause and/or worsen hypertension significantly; use caution in patients with pre-existing hypertension and monitor BP closely in all patients. Permanent discontinuation is recommended in patients who experience a hypertensive crisis or encephalopathy. Temporarily discontinue in patients who develop uncontrolled hypertension.
- Infusion reactions: Infusion reactions (e.g., hypertension, hypertensive crisis, wheezing, oxygen desaturation, hypersensitivity, chest pain, rigors, headache, and diaphoresis) may occur with the first infusion (uncommon). Interrupt therapy in patients experiencing severe infusion reactions; there are no data to address reinstitution of therapy in patients who experience severe infusion reactions.
- Nephrotic syndrome/proteinuria: Proteinuria and/or nephrotic syndrome have been associated with use; risks may be increased in patients with history of hypertension.

Thrombotic microangiopathy has been associated with bevacizumab-induced proteinuria. Withhold treatment for ≥2 g proteinuria/24 hours and resume when proteinuria is <2 g/24 hours; discontinue in patients with nephrotic syndrome.

- Reversible posterior leukoencephalopathy syndrome (RPLS): Cases of reversible posterior leukoencephalopathy syndrome (RPLS) have been reported. Symptoms (which include headache, seizure, confusion, lethargy, blindness and/or other vision, or neurologic disturbances) may occur from 16 hours to 1 year after treatment initiation. Resolution of symptoms usually occurs within days after discontinuation; however, neurologic sequelae may remain. RPLS may be associated with hypertension; discontinue therapy and begin management of hypertension, if present.
- Thromboembolism: Bevacizumab is associated with an increased risk for arterial thromboembolic events (ATE), including stroke, MI, TIA, and angina, when used in combination with chemotherapy. History of ATE or ≥65 years of age may present an even greater risk; permanently discontinue with serious ATE; the safety of treatment reinitiation after ATE has not been studied. Although patients with cancer are at risk for venous thromboembolism (VTE), a meta-analysis of 15 controlled trials has demonstrated an increased risk for VTE in patients who received bevacizumab (Nalluri, 2008).
- Wound dehiscence: **[U.S. Boxed Warning]: Wound dehiscence/wound healing complications have been reported in patients (not related to treatment duration);** monitor patients for signs/symptoms of improper wound healing. Permanently discontinue in patients who develop these complications. The appropriate intervals between administration of bevacizumab and surgical procedures to avoid impairment in wound healing has not been established. Therapy should not be initiated within 28 days of major surgery and only following complete healing of the incision. Bevacizumab should be discontinued at least 28 days prior to elective surgery.
- The incidental finding of pneumatosis intestinalis has been found in two patients in the first stage of this trial on routine restaging CT scans. This resolved without intervention in both patients and is thought to be probably associated with bevacizumab.

Disease-related concerns:

- Cardiovascular disease: The risk for heart failure (HF), including left ventricular dysfunction, is higher in patients receiving bevacizumab plus chemotherapy when compared to chemotherapy alone. Use with caution in patients with cardiovascular disease; patients with significant recent cardiovascular disease were excluded from clinical trials. The safety of therapy resumption or continuation in patients with cardiac dysfunction has not been studied.

Percentages reported as monotherapy and as part of combination chemotherapy regimens. Some studies only reported hematologic toxicities Grades ≥4 and nonhematologic toxicities Grades ≥3.

Table 4: Frequency of Toxicities Associated with Bevacizumab Monotherapy by Body System

Body System	>10%	1%-10%	<1%
Cardiovascular	• Hypertension (23%-67%; Grades 3/4: 5%-18%) • Thromboembolic event (≤21%; Grades 3/4: 15%; venous thrombus/ embolus: 8%; Grades 3/4: 5%-7%; arterial thrombosis 6%; Grades 3/4: 3%), hypotension (7%-15%)	• DVT (6%-9%; Grades 3/4: 9%) • Syncope (Grades 3/4: 3%) • Intra-abdominal venous thrombosis (Grades 3/4: 3%), cardio-/cerebrovascular arterial thrombotic event (2%-4%) • CHF (with prior anthracycline therapy: 4%; Grades 3/4: 2%) • Left ventricular dysfunction (Grades 3/4: 1%)	• Angina • Hypertensive crises • Myocardial infarction
Central Nervous System	•Pain (31%-62%) •Headache (26%-37%; Grades 3/4: 2%-4%) •Dizziness (19%-26%) •Fatigue (≤45%; Grades 3/4: 4%-19%) •Sensory neuropathy (Grades 3/4: 1%-17%; in combination with paclitaxel: 24%)	• Confusion (1%-6%) • Abnormal gait (1%-5%) • CNS hemorrhage (1%-5%; Grades 3/4: 1%) • Reversible posterior leukoencephalopathy syndrome ([RPLS] ≤1%)	• Cerebral infarction • Transient ischemic attack • Subarachnoid hemorrhage • Hemorrhagic stroke • Hypertensive encephalopathy
Dermatologic	• Alopecia (6%-32%) • Dry skin (7%-20%) • Exfoliative dermatitis (3%-19%) • Skin discoloration (2%-16%)	• Nail disorder (2%-8%) • Skin ulcer (≤6%) • Rash desquamation (Grades 3/4: 3%) • Wound dehiscence (1%-6%)	• Wound healing complications • Polyserositis • Nasal septum perforation • Enterocutaneous fistula
Endocrine & Metabolic	• Hypokalemia (12%-16%)	• Dehydration (Grades 3/4: 3%-10%) • Hyponatremia (Grades 3/4: 4%)	
Gastrointestinal	• Abdominal pain (50%-61%; Grades 3/4: 8%) • Vomiting (47%-52%; Grades 3/4: 6%-11%) • Anorexia (35%-	• Xerostomia (4%-7%) • Colitis (1%-6%), ileus (Grades 3/4: 4%-5%) • Gingival bleeding (2%) • Fistula (1%)	• Intestinal necrosis • Intestinal obstruction • Fistula (biliary, duodenal, esophageal, gastrointestinal, rectal)

	43%) • Constipation (29%-40%) • Diarrhea (Grades 3/4: 1%-34%) • Stomatitis (25%-32%) • Gastrointestinal hemorrhage (19%-24%) • Dyspepsia (17%-24%) • Taste disorder (14%-21%) • Flatulence (11%-19%) • Weight loss (9%-16%) • Nausea (Grades 3/4: 4%-12%)	• Gastrointestinal perforation (≤4%) • Intra-abdominal abscess (1%)	
Hematologic	• Hemorrhage (≤40%; Grades 3/4: 1%-5%) • Leukopenia (Grades 3/4: 37%) • Neutropenia (Grade 4: 6%-27%)	• Neutropenic fever/infection (5%; Grades 3 and/or 4: 4% -5%) • Thrombocytopenia (5%)	• Microangiopathic hemolytic anemia (when used in combination with sunitinib) • Pancytopenia
Hepatic		• Bilirubinemia (1%-6%)	
Neuromuscular & skeletal	• Weakness (57%-74%) • Myalgia (8%-15%)	• Bone pain (Grades 3/4: 4%) • Neuropathy (other than sensory: Grades 3/4: 1%-5%)	
Ocular	• Tearing increased (6%-18%)		• Eye inflammation • Vision blurred • Toxic anterior segment syndrome (TASS) • Endophthalmitis fistula
Renal	• Proteinuria (4% to 36%; Grade 3: ≤21%; Grades 3/4: 1%-3%)		• Renal failure • Renal thrombotic microangiopathy • Nephrotic syndrome • Ureteral stricture • Bladder fistula
Genitourinary		• Polyuria/urgency (3%-6%) • Vaginal hemorrhage (4%)	• Vaginal fistula
Respiratory	• Upper respiratory infection (40%-	• Voice alteration (6%-9%)	• Pulmonary hypertension

	47%) • Epistaxis (16%-35%) • Dyspnea (25%-26%)	• Pneumonitis/pulmonary infiltrates (Grades 3/4: 5%) • Hemoptysis (nonsquamous histology 2%)	• Pulmonary embolism • Pulmonary hemorrhage • Bronchopleural fistula
Miscellaneous	• Infection (≤55%; serious: 9% to 14%; pneumonia, catheter, or wound infections)	• Infusion reactions (<3%)	• Anaphylaxis • Sepsis • Hypersensitivity • Mesenteric venous occlusion • Anastomotic ulceration • Tracheoesophageal fistula

6.1.3 Oxaliplatin

- Anaphylaxis: **[U.S. Boxed Warning]: Anaphylactic/anaphylactoid reactions may occur within minutes of oxaliplatin administration; symptoms may be managed with epinephrine, corticosteroids, and antihistamines.** Grade 3 or 4 hypersensitivity has been observed. Allergic reactions may occur with any cycle and may include bronchospasm (rare), erythema, hypotension (rare), pruritus, rash, and/or urticaria.
- Neuropathy: Two different types of peripheral sensory neuropathy may occur: First, an acute (within first 2 days), reversible (resolves within 14 days), with primarily peripheral symptoms that are often exacerbated by cold (may include pharyngolaryngeal dysesthesia); avoid mucositis prophylaxis with ice chips during oxaliplatin infusion; may recur with subsequent doses. Secondly, a more persistent (>14 days) presentation that often interferes with daily activities (e.g., writing, buttoning, swallowing), these symptoms may improve in some patients upon discontinuing treatment.
- Hepatotoxicity: Hepatotoxicity (including rare cases of hepatitis and hepatic failure) has been reported. Liver biopsy has revealed peliosis, nodular regenerative hyperplasia, sinusoidal alterations, perisinusoidal fibrosis, and veno-occlusive lesions. The presence of hepatic vascular disorders (including veno-occlusive disease) should be considered, especially in individuals developing portal hypertension or who present with increased liver function tests.
- Pulmonary fibrosis: May cause pulmonary fibrosis; withhold treatment for unexplained pulmonary symptoms (e.g., crackles, dyspnea, nonproductive cough, pulmonary infiltrates) until interstitial lung disease or pulmonary fibrosis are excluded.

Table 5: Frequency of Toxicities Associated with Oxaliplatin Monotherapy by Body System

Body System	>10%	1%-10%	<1%
Cardiovascular		• Edema (10%) • Chest pain (5%) • Peripheral edema (5%) • Flushing (3%) • Thromboembolism (2%)	• Hypertension
Central Nervous System	• Fatigue (61%) • Fever (25%) • Pain (14%) • Headache (13%) • Insomnia (11%)	• Dizziness (7%)	• Aphonia • Ataxia • Cranial nerve palsies • Deep tendon reflex loss • Deafness • Dysarthria • Fasiculations • Gait abnormal • Intracerebral bleeding • Lhermittes' sign • Myoclonus • Seizure • Trigeminal neuralgia
Dermatologic		• Rash (5%), alopecia (3%), hand-foot syndrome (1%)	
Endocrine & Metabolic		• Dehydration (5%) • Hypokalemia (3%)	• Hypomagnesaemia • Metabolic acidosis
Gastrointestinal	• Nausea (64%) • Diarrhea (46%) • Vomiting (37%) • Abdominal Pain (31%) • Constipation (31%) • Anorexia (20%) • Stomatitis (14%)	• Dyspepsia (7%) • Taste perversion (5%) • Flatulence (3%) • Mucositis (2%) • Gastroesophageal reflux (1%) • Dysphagia (acute 1% - 2%)	• Colitis • Ileus • Intestinal obstruction • Pancreatitis • Rectal hemorrhage
Genitourinary		• Dysuria (1%)	
Hematologic	• Anemia (64%; Grades 3/4: 1%) • Thrombocytopenia (30%; Grades 3/4 3%) • Leukopenia (13%)	• Neutropenia (7%)	• Hemolysis • Hemolytic anemia (immuno-allergic) • Hemolytic uremia syndrome • Hemorrhage • INR increased • Neutropenic fever • Neutropenic sepsis • Prothrombin time increased • Thrombocytopenia (immuno-allergic)
Hepatic	• AST increased (54%; Grades 3/4: 4%)		• Hepatic failure • Hepatitis • Hepatotoxicity

	• ALT increased (36%; grades 3/4: 1%) • Total bilirubin increased (13%; grades 3/4: 5%)		• Nodular regenerative hyperplasia • Peliosis • Veno-occlusive liver disease (sinusoidal obstruction syndrome and perisinusoidal fibrosis)
Neuromuscular & Skeletal	• Peripheral neuropathy (may be dose limiting; 76%-92%; acute 65%; grades 3/4: 5%; persistent 43%; grades 3/4: 3%) • Back pain (11%)	• Rigors (9%) • Arthralgia (7%)	• Muscle spasm • Rhabdomyolysis
Ocular		• Abnormal lacrimation (1%)	• Diplopia • Optic neuritis • Ptosis • Visual disturbance (acuity decreased, field disturbance, transient loss)
Renal		• Serum creatinine increased (5%-10%)	• Acute renal failure • Hematuria • Interstitial nephritis (acute) • Tubular necrosis (acute)
Respiratory	• Dyspnea (13%) • Cough (11%)	• URI (7%) • Rhinitis (6%) • Epistaxis (2%) • Pharyngitis (2%) • Pharyngolaryngeal dysesthesia (grades 3/4: 1%-2%)	• Dysphonia • Eosinophilic pneumonia • Hypoxia • Interstitial lung diseases
Miscellaneous		• Injection site reaction (9%; redness/swelling/pain) • Allergic reactions (3%) • Hypersensitivity (includes urticaria, pruritus, facial flushing, shortness of breath, bronchospasm, diaphoresis, hypotension, syncope: grades 3/4: 2%-3%) • Hiccup (2%)	• Alkaline phosphatase increased • Anaphylactic/ anaphylactoid reactions • Anaphylactic shock • Angioedema • Extravasation (including necrosis) • Sepsis

Disease-related concerns:

- Renal impairment: Use with caution in patients with renal impairment; increased toxicity may occur

6.1.4 Capecitabine:

Concerns related to adverse effects:

- Cardiotoxicity: There has been cardiotoxicity associated with fluorinated pyrimidine therapy, including myocardial infarction, angina, dysrhythmias, cardiogenic shock, sudden death, ECG changes, and cardiomyopathy. These adverse events may be more common in patients with a history of coronary artery disease.
- Diarrhea: Can cause severe diarrhea; median time to first occurrence is 34 days; subsequent doses should be reduced after grade 3 or 4 diarrhea or recurrence of grade 2 diarrhea.
- Hand-and-foot syndrome: May cause hand-and-foot syndrome (palmar-plantar erythrodysesthesia or chemotherapy-induced acral erythema) is characterized by numbness, dysesthesia/paresthesia, tingling, painless or painful swelling, erythema, desquamation, blistering, and severe pain. If grade 2 or 3 hand-and-foot syndrome occurs, interrupt administration of capecitabine until the event resolves or decreases in intensity to grade 1. Following grade 3 hand-and-foot syndrome, decrease subsequent doses of therapy.
- Necrotizing enterocolitis (typhlitis): Has been reported.

Disease-related concerns:

- Bone marrow suppression: Use with caution in patients with bone marrow suppression.
- Dihydropyrimidine dehydrogenase (DPD) deficiency: Rare and unexpected severe toxicity (stomatitis, diarrhea, neutropenia, neurotoxicity) may be attributed to Dihydropyrimidine dehydrogenase (DPD) deficiency.
- Hepatic impairment: Use with caution in patients with hepatic impairment.
- Renal impairment: Use with caution in patients with renal impairment; reduce dose with moderate impairment and carefully monitor and reduce subsequent dose (with any grade 2 or higher adverse effect) with mild-to-moderate impairment.

Concurrent drug therapy issues:

- Alkylating therapy: Use with caution in patients who have received alkylating therapy.
- Fluorouracil/leucovorin (FU/LV): In patients with colorectal cancer, treatment with capecitabine immediately following 6 weeks of FU/LV therapy has been associated with an increased incidence of grade ≥3 toxicity, when compared to patients receiving the reverse sequence, capecitabine (two 3-week courses) followed by FU/LV (Hennig, 2008).
- Warfarin: **[U.S. Boxed Warning]: Capecitabine may increase the anticoagulant effects of warfarin; monitor closely.**

Table 6: Frequency of Toxicities Associated with Capecitabine Monotherapy by Body System

Body System	>10%	5%-10%	<5%
Cardiovascular	• Edema (9%-15%)	• Venous thrombosis (8%) • Chest pain (6%)	• Angina • Atrial fibrillation • Bradycardia • Cardiac arrest • Cardiac failure • Cardiomyopathy • Dysrhythmia • ECG changes • Hyper-/hypotension • MI • Myocardial ischemia • Myocarditis • Pericardial effusion • Tachycardia • Ventricular extrasystoles
Central Nervous System	• Fatigue (16%-42%) • Fever (7%-18%) • Pain (12%)	• Headache (5%-10%) • Lethargy (10%) • Dizziness (6%-8%) • Insomnia (7%-8%) • Mood alteration (5%) • Depression (5%)	• Ataxia • Cerebral vascular accident • Confusion • Dysarthria • Encephalopathy • Impaired balance • Irritability • Loss of consciousness • Sedation • Tremor • Vertigo
Dermatologic	• Palmar-plantar erythrodysesthesia (hand-and-foot syndrome) (54%-60%; Grade 3: 11%-17%; may be dose limiting) • Dermatitis (27%-37%)	• Nail disorder (7%) • Rash (7%) • Skin discoloration (7%) • Alopecia (6%) • Erythema (6%)	• Ecchymosis • Thrombocytopenic purpura • Pruritus • Radiation recall syndrome • Skin ulceration
Endocrine & Metabolic		• Dehydration (7%)	• Cachexia • Diaphoresis • Hypokalemia • Hypomagnesemia • Hypertriglyceridemia • Thirst • Weight gain
Gastrointestinal	• Diarrhea (47%-57%; may be dose limiting; Grade 3: 12%-13%; Grade 4: 2%-3%) • Nausea (34%-53%) • Vomiting (15%-37%) • Abdominal pain (7%-35%) • Stomatitis (22%-	• Motility disorder (10%) • Oral discomfort (10%) • Dyspepsia (6%-8%) • Upper GI inflammatory disorders (colorectal cancer: 8%) • Hemorrhage (6%)	• Abdominal Distension • Appetite increased • Ascites • Colitis • Duodenitis • Dysphagia • Esophagitis • Gastric ulcer • Gastritis • Gastroenteritis

	25%) • Appetite decreased (26%) • Anorexia (9%-23%) • Constipation (9%-15%)	• Ileus (6%) • Taste perversion (colorectal cancer: 6%)	• Ileus • Intestinal obstruction (~1%) • Necrotizing enterocolitis (typhlitis) • Proctalgia • Toxic megacolon
Hematologic	• Lymphopenia (94%; grade 4: 14%) • Anemia (72%-80%; grade 4: <1%-1%) • Neutropenia (2%-26%; grade 4: 2%) • Thrombocytopenia (24%; grade 4: 1%)		• Coagulation disorder • Idiopathic thrombocytopenia purpura • Leukopenia • Pancytopenia
Hepatic	• Bilirubin increased (22%-48%; grades 3/4: 11%-23%)		• Cholestasis • Hepatic fibrosis • Hepatitis
Neuromuscular & skeletal	• Paresthesia (21%)	• Back pain (10%) • Weakness (10%) • Neuropathy (10%) • Myalgia (9%) • Arthralgia (8%) • Limb pain (6%)	• Arthritis • Bone pain • Joint stiffness
Ocular	• Eye irritation (13%-15%)	• Abnormal vision (colorectal cancer: 5%) • Conjunctivitis (5%)	• Keratoconjunctivitis
Renal			• Nocturia • Renal impairment
Respiratory	• Dyspnea (14%)	• Cough (7%)	• Asthma • Bronchitis • Bronchopneumonia • Bronchospasm • Epistaxis • Hematemesis • Hemoptysis • Hoarseness • Laryngitis • Pneumonia • Pulmonary embolism • Respiratory distress • Sore throat
Miscellaneous		• Viral infection (colorectal cancer: 5%)	• Deep vein thrombosis • Fibrosis • Fungal infection • Hot flushes • Hypersensitivity • Infection • Influenza-like illness • Lymphedema • Oral candidiasis • Photosensitivity reaction • Sepsis • Thrombophlebitis

Postmarketing and/or case reports: Fingerprint distortion, hepatic failure, lacrimal duct stenosis, multifocal leukoencephalopathy.

A list of the adverse events and potential risks associated with the agents administered in this study appear below and will determine whether dose delays and modifications will be made or whether the event requires expedited reporting **in addition** to routine reporting.

6.2 Toxicity Management

[For management of adverse infusion reaction refer to section 5.2.1 (trastuzumab), 5.2.2 (bevacizumab), and 5.2.3 (oxaliplatin)].

6.2.1 Trastuzumab Toxicity Management

There are no dose reductions for trastuzumab. If adverse events occur that require holding trastuzumab, the dose will remain the same once treatment resumes.

LVEF is to be determined by echocardiogram or MUGA scan. The same method of evaluation should be used for an individual throughout the study.

If trastuzumab is held for any reason, bevacizumab, oxaliplatin and capecitabine may be given if parameters are met for administration of these agents. Once the criteria to resume trastuzumab is met, it will be given on day 1 of the next cycle.

Table 7: Trastuzumab: Guidelines for LVEF

LVEF	Check prior to C4D1 and then every 4 cycles
≥50%	Administer trastuzumab
< 50 %	Hold trastuzumab Recheck MUGA or echocardiogram every 3 weeks May resume at current dose when LVEF returns to ≥ 50% If LVEF remains < 50% for greater than 12 weeks, may discontinue trastuzumab permanently.

6.2.2 Bevacizumab Toxicity Management

There are no reductions in the bevacizumab dose. If adverse events occur that require holding bevacizumab, the dose will remain the same once treatment resumes.

Any toxicity associated or possibly associated with bevacizumab treatment should be managed according to standard medical practice. Discontinuation of bevacizumab will have no immediate therapeutic effect. Bevacizumab has a terminal half-life of 21 days; therefore, its discontinuation results in slow elimination over several months. There is no available antidote for bevacizumab.

Subjects should be assessed clinically for toxicity prior to, during, and after each infusion. If unmanageable toxicity occurs because of bevacizumab at any time during the study, treatment with bevacizumab should be discontinued.

Regardless of the reason for holding bevacizumab, the maximum allowable length of treatment interruption is 9 weeks. Bevacizumab will be permanently discontinued if held for greater than 9 weeks.

If bevacizumab is held for any reason, trastuzumab, oxaliplatin and capecitabine may be given if parameters are met for administration of these agents. Once the criteria to resume bevacizumab is met, it will be given on day 1 of the next cycle.

Table 8 (table 1 of 3)**:** **Bevacizumab Dose Management due to Adverse Events** **CTCAE Version: 4.0**	
Event	**Action to be taken**
Hypertension Grade 1 or 2 Grade 3 Grade 4 (including hypertensive encephalopathy)	 No action If not controlled to ≤160/100 within 3 weeks of starting antihypertensive medication, discontinue permanently Discontinue bevacizumab
Hemorrhage (non-pulmonary or non-CNS event) Grade 1 or 2 Grade 3 Grade 4	 No action Hold bevacizumab until all of the following are met: • Bleeding has resolved and hemoglobin is stable • There is no bleeding diathesis that would increase the risk of therapy • There is no anatomic or pathologic condition that significantly increases the risk of hemorrhagic recurrence If there is a second Grade 3 hemorrhagic event the bevacizumab will be discontinued permanently Discontinue bevacizumab
Hemorrhage (Pulmonary or CNS) Any grade	Hold bevacizumab until all of the following are met: • Bleeding has resolved and hemoglobin is stable • There is no bleeding diathesis that would increase the risk of therapy There is no anatomic or pathologic condition that significantly increases the risk of hemorrhagic recurrence (not including brain metastases) All CNS hemorrhage events must be reported directly to the PI within 24 hours of occurrence. If CNS hemorrhage, perform brain MRI prior to next planned cycle of therapy. If stable or resolved, dosing may proceed. If there is progression of CNS hemorrhage, the bevacizumab will be permanently discontinued.

Table 8 (table 2 of 3)**:** **Bevacizumab Dose Management due to Adverse Events** **CTCAE Version: 4.0**	
Event	**Action to be taken**
Venous Thrombosis including pulmonary embolism and vascular access devise related thrombosis Grade 1 or 2 Grade 3 or 4	 No action Anti-coagulate as clinically indicated with low molecular weight heparin (warfarin is not allowed on this study). Bevacizumab may be administered when anticoagulant dosing is stable, provided the patient has not had any Grade 3 or 4 hemorrhagic events while on anticoagulation
Arterial thromboembolic Event (new onset, worsening, or unstable angina, MI, TIA, CVA or any other arterial thromboembolic event) Any Grade	 Discontinue bevacizumab
Congestive Heart Failure Grade 1 or 2 Grade 3 Grade 4	 No action Hold bevacizumab until resolution of symptoms Discontinue bevacizumab
Proteinuria or Nephrotic Syndrome **Grade 1** 1+proteinuria; Urinary Protein <1.0 g/24 hours **Grade 2** 2+proteinuria; Urinary Protein ≥1.0- 3.4g/24 hours	**(UPCR is collected every other cycle, treatment decision will be based on results of UPCR)** Continue bevacizumab • UPCR ≤1.9 Continue bevacizumab • UPCR >1.9 Hold bevacizumab and obtain 24 hour urine sample for protein prior to next visit. • Resume bevacizumab when UPCR is ≤1.9 or 24 hour urine protein is < 2gms/24 hours • Hold for a maximum of 9 weeks. Discontinue bevacizumab if no improvement in 9 weeks.

Grade 3 Urinary Protein ≥3.5g/24 hours	Hold bevacizumab Obtain 24 hour urine sample for protein prior to next visit. • Resume bevacizumab when UPCR is ≤1.9 or 24 hour urine protein is < 2gms/24 hours • Hold for a maximum of 9 weeks. Discontinue bevacizumab if no improvement in 9 weeks. • Suggest nephrology consult
Nephrotic Syndrome	Discontinue bevacizumab Suggest nephrology consult
GI perforation	Discontinue bevacizumab
Fistula Any Grade	Discontinue bevacizumab

Table 8 (table 3 of 3)**:**
Bevacizumab Dose Management due to Adverse Events
CTCAE Version: 4.0

Event	**Action to be taken**
Bowel Obstruction Grade 1	Continue bevacizumab if intervention is not indicated
Grade 2	Hold bevacizumab. Resume at investigators discretion
Grade 3 or 4	Hold bevacizumab. If surgical intervention is indicated, may restart after full recovery at investigators discretion
Wound Dehiscence Any grade	Discontinue bevacizumab
Reversible Posterior Leukoencephalopathy Any Grade (confirmed by MRI)	Discontinue bevacizumab
Pneumatosis Intestinalis	Hold bevacizumab until no radiographic evidence of this. Resume at investigator's discretion
Other unspecified Bevacizumab related Adverse Events Grade 1 or 2	No action
Grade 3	Hold bevacizumab until recovery to ≤Grade 1 or baseline
Grade 4	Discontinue bevacizumab

6.3 Dose Modifications/Delays

There will be no dose reductions of bevacizumab and trastuzumab. Criteria for holding bevacizumab and trastuzumab are noted in table 3 (pg 51) and table 4 (pg 53).

Doses of capecitabine or oxaliplatin (chemotherapy agents) that have been reduced for treatment related toxicity are permanent and will not be re-escalated.

If it is necessary to hold any of the study agents, treatment may continue with other agents at the discretion of the treating investigator providing re-treatment parameters are met. A maximum of 3 dose reductions for each of the chemotherapy agents will be allowed.

Table 9: Dose Modification

Chemotherapy Agent	Starting dose	Dose level -1	Dose level -2	Dose level -3
Oxaliplatin	130mg/m2	25% reduction of starting dose	25% reduction of dose level -1	25% reduction of dose level -2
Capecitabine	1200mg/m2 in divided dose	↓ starting dose by one 500 mg tablet	↓ previous dose by one 500 mg tablet	↓ previous dose by one 500 mg tablet

6.3.1 Hematological Toxicity:

Table 10: Hematological Toxicity

Hematologic Toxicity on Day 1 of Cycle:		
ANC (Absolute Neutrophil Count)	**Platelets**	***Dose Modification (if both ANC and platelet toxicity, dose modification for the more severe toxicity will apply)***
$\geq$ 1,000/mm^3	$\geq$ 75 x 10^9/L	Maintain current dose
<1,000/mm^3	<75 x 10^9/L	HOLD all study agents When ANC $\geq$ 1,000/mm^3 and Plts $\geq$ 75 x 10^9/L, initiate a **new** cycle and reduce both capecitabine and oxaliplatin one dose level **If not recovered in 3 weeks, or if more than 3 dose reductions are required for this toxicity, the patient will be removed from the study.**

6.3.2 Febrile Neutropenia:

In the event of febrile neutropenia, defined for this protocol as temperature above 100.4 with ANC less than 1000/mm³, capecitabine and oxaliplatin will be held. The use of leukocyte growth factors may be indicated during this period and should be given as per ASCO guidelines at the discretion of the treating investigator. Prophylactic antibiotics may also be offered at the discretion of the treating investigator.

Upon recovery**, including resolution of fever or infection, and recovery of ANC to greater than 1000/mm³,** the dose modifications and recovery times noted in table (Hematologic Toxicity) will apply.

6.3.3 Non Hematological Dose Modifications

These criteria apply only to treatment-related, clinically relevant toxicity as determined by the investigator. There will be no dose modifications for grade 1 events.

Non hematological dose modifications will be based on the worst toxicity experienced at any time in the cycle. Refer to table 7 (pg 58).

Table 11: Non-Hematological Toxicity

CTCAE version 4.0	Non hematological toxicity Other than those noted elsewhere in this table	Diarrhea	Creatinine	Bilirubin (related to study drug and not biliary obstruction)	Palmar/plantar erythrodysesthesia syndrome (Hand /foot syndrome)	Sensory or motor peripheral neuropathy
Grade 2	Maintain dose if tolerable to patient If intolerable HOLD capecitabine and oxaliplatin until recovery to ≤grade 1 Reduce dose of most likely causative agent one dose level when treatment is resumed.	HOLD capecitabine until recovery to ≤grade 1 If diarrhea was not optimally treated, continue at current dose when treatment is resumed. If grade 2 diarrhea despite optimal treatment, reduce dose of capecitabine one dose level when treatment is resumed	HOLD capecitabine and oxaliplatin Upon recovery to normal limits or baseline resume at current dose level.	HOLD capecitabine until recovery to ≤grade 1 For first occurrence, continue at current dose when treatment is resumed. For any subsequent occurrence, reduce dose of capecitabine one dose level when treatment is resumed	HOLD capecitabine until recovery to ≤grade 1 Reduce dose of capecitabine one dose level when treatment is resumed	HOLD oxaliplatin until recovery to ≤grade 1 Resume at same dose if recovered by next cycle. Dose reduction at the investigators discretion.

Grade 3	HOLD capecitabine and oxaliplatin until recovery ≤grade 1. Reduce dose of most likely causative agent one dose level when treatment is resumed.	HOLD capecitabine until recovery to ≤grade 1 Reduce dose of capecitabine one dose level when treatment is resumed	HOLD capecitabine and oxaliplatin Upon recovery to normal limits or baseline resume at current dose level.	HOLD capecitabine until recovery to ≤grade 1 Reduce dose of capecitabine one dose level when treatment is resumed	HOLD capecitabine until recovery to ≤grade 1 Reduce dose of capecitabine one dose level when treatment is resumed	Permanently discontinue oxaliplatin
Grade 4	HOLD capecitabine and oxaliplatin until recovery ≤grade 1. Reduce dose of most likely causative agent by one to two dose levels when treatment is resumed	HOLD capecitabine until recovery to ≤grade 1 Reduce dose of capecitabine one to two dose levels when treatment is resumed	HOLD capecitabine and oxaliplatin Upon recovery to normal limits or baseline resume capecitabine and oxaliplatin at current dose.	HOLD capecitabine until recovery to ≤grade 1 Reduce dose of capecitabine one to two dose levels when treatment is resumed	HOLD capecitabine until recovery to ≤grade 1 Reduce dose of capecitabine one to two dose levels when treatment is resumed	Permanently discontinue oxaliplatin

7. DRUG FORMULATION

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

7.1 Ordering & Accountability:

Oxaliplatin and Capecitabine will be obtained from commercial supply. Bevacizumab and Trastuzumab will be supplied by Genentech and provided free of charge to patient.

Before an initial supply of Bevacizumab and Trastuzumab may be ordered from Genentech, up to two designated pharmacists responsible for requesting Bevacizumab and Trastuzumab must be identified using the Genentech BioOncology Avastin Approval Signatures for Drug Re-supply form and Genentech BioOncology Herceptin Approval Signatures for Drug Re-supply form. Fax the completed forms to Genentech and an email to notify that the fax has been sent using the following information:

Bevacizumab (Avastin) Drug Orders
Fax to: 650-745-0978
Avastin-gsur@gene.com

Trastuzumab (Herceptin) Drug Orders
Fax to: 650-360-6908
herceptin-gsur@gene.com

Bevacizumab and Trastuzumab will be ordered by the responsible pharmacist(s) using the Genentech BioOncology Avastin Drug Request Form and Genentech BioOncology Herceptin Drug Request Form. The pharmacist(s) will fax the completed form to Genentech and confirm the request by email using the following information:

Bevacizumab (Avastin) Drug Orders
Fax to: 650-745-0978
Avastin-gsur@gene.com

Trastuzumab (Herceptin) Drug Orders
Fax to: 650-360-6908
herceptin-gsur@gene.com

Bevacizumab and Trastuzumab will be shipped directly from Genentech. Please allow at least two weeks for drug to arrive on site. Drug deliveries are made only on Tuesday, Wednesday, Thursday and Friday.

The responsible pharmacist will follow institutional guidelines for receipt and product accountability of the study agents.

7.1.1 Destruction and Return:

At the end of the study, unused supplies of bevacizumab and trastuzumab will be either destroyed per institutional policy or returned to Genentech as directed.

7.2 Bevacizumab

Bevacizumab will be provided by Genentech. See Section 7.1 for instruction on requesting re-supply.

Bevacizumab is a recombinant, humanized monoclonal antibody which binds to, and neutralizes, vascular endothelial growth factor (VEGF), preventing its association with endothelial receptors, Flt-1 and KDR. VEGF binding initiates angiogenesis (endothelial proliferation and the formation of new blood vessels). The inhibition of microvascular growth is believed to retard the growth of all tissues (including metastatic tissue).

Pharmacodynamics/Kinetics

Distribution: V_d: 46 mL/kg

Half-life elimination: ~20 days (range: 11-50 days)

Excretion: Clearance: 2.75-5 mL/kg/day

Bevacizumab will be diluted in a total volume of 100mL of 0.9% Sodium Chloride Injection, USP. Administration will be as an IV infusion.

Bevacizumab Storage

Upon receipt of the study drug, vials are to be refrigerated at 2°C–8°C (36°F–46°F) and should remain refrigerated until just prior to use. DO NOT FREEZE. DO NOT SHAKE. Vials should be protected from light.

Opened vials must be used within 8 hours. VIALS ARE FOR SINGLE USE ONLY. Vials used for 1 subject may not be used for any other subject. Once study drug has been added to a bag of sterile saline, the solution must be administered within 8 hours.

7.3 Trastuzumab

Trastuzumab will be provided by Genentech. See Section 7.1 for instruction on requesting re-supply.

Trastuzumab is a monoclonal antibody which binds to the extracellular domain of the human epidermal growth factor receptor 2 protein (HER-2); it mediates antibody-dependent cellular cytotoxicity by inhibiting proliferation of cells which overexpress HER-2 protein.

Pharmacodynamics/Kinetics

Distribution: V_d: 44 mL/kg; not likely to cross the (intact) blood brain barrier (due to the large molecule size)

Half-life elimination: Weekly dosing: Mean: 6 days (range: 1-32 days); every 3 week regimen: Mean: 16 days (range: 11-23 days)

Trastuzumab intravenous powder for solution: 150mg. Refer to package insert and/or institutional guidance for preparation, dispensing instructions and storage conditions. The trastuzumab 150mg vial will be used as a single dose vial only. Please note that the 440mg vials of trastuzumab can be used until they are depleted.

7.4 Oxaliplatin

Oxaliplatin is commercially available, and will not be provided by study.

Oxaliplatin is a platinum derivative, an alkylating agent. Following intracellular hydrolysis, the platinum compound binds to DNA forming cross-links which inhibit DNA replication and transcription, resulting in cell death. Cytotoxicity is cell-cycle nonspecific.

Pharmacodynamics/Kinetics

Distribution: V_d: 440 L

Protein binding: >90% primarily albumin and gamma globulin (irreversible binding to platinum)

Metabolism: Nonenzymatic (rapid and extensive), forms active and inactive derivatives

Half-life elimination: Terminal: 391 hours

Excretion: Urine (~54%); feces (~2%)

Administration: I.V.

Administer as I.V. infusion over 2 hours. The infusion time may be lengthened to 6 hours if the evidence of hypersensitivity or laryngeal dysesthesia. Flush infusion line with D_5W prior to administration of any concomitant medication.

Storage

Store intact vials at room temperature of 25°C (77°F); excursions permitted to 15°C to 30°C (59°F to 86°F); do not freeze. Protect concentrated solution from light (store in original outer carton). According to the manufacturer, solutions diluted for infusion are stable up to 6 hours at room temperature of 20°C to 25°C (68°F to 77°F) or up to 24 hours under refrigeration at 2°C to 8°C (36°F to 46°F). Oxaliplatin solution diluted with D_5W to a final concentration of 0.7 mg/mL (polyolefin container) has been shown to retain >90% of it's original concentration for up to 30 days when stored at room temperature or refrigerated; artificial light did not affect the concentration (Andre, 2007). As this study did not examine sterility, refrigeration would be preferred to limit microbial growth. Solutions diluted for infusion do not require protection from light.

Reconstitution

Oxaliplatin is supplied in vials containing 50 mg, 100 mg or 200 mg of oxaliplatin as a sterile, preservative-free, aqueous solution at a concentration of 5 mg/ml. Water for Injection, USP is present as an inactive ingredient. Dilution in D_5W (250 or 500 mL) is required prior to administration.

Do not prepare using a chloride-containing solution such as NaCl, due to rapid conversion to monochloroplatinum, dichloroplatinum, and diaquoplatinum; all highly reactive in sodium chloride. Do not use needles or administration sets containing aluminum during preparation.

Oxaliplatin has vesicant and irritant properties. There have been reported cases of extravasation induced necrosis to skin and surrounding tissues. Cool compress may be used for immediate management of extravasation, with consideration of potential for peripheral neuropathic discomfort exacerbated by cold. Warm compresses may avoid peripheral neuropathic discomfort, however, while possibly increasing drug removal through local vasodilation may increase cellular uptake and injury.

7.5 Capecitabine

Capecitabine is available commercially and will not be supplied by the study. Treating investigator will write prescription, and give to patient during cycle 1. The patient will fill through either local or mail order pharmacy depending on insurance carrier. 150 MG or 500 MG tablets of capecitabine are commercially available; however, for this study only 500 mg tablets will be used.

Capecitabine is a prodrug of fluorouracil. It undergoes hydrolysis in the liver and tissues to form fluorouracil which is the active moiety. Fluorouracil is a fluorinated pyrimidine antimetabolite that inhibits thymidylate synthetase, blocking the methylation of deoxyuridylic acid to thymidylic acid, interfering with DNA, and to a lesser degree, RNA synthesis. Fluorouracil appears to be phase specific for the G_1 and S phases of the cell cycle.

Pharmacodynamics/Kinetics

Absorption: Rapid and extensive

Protein binding: <60%; ~35% to albumin

Metabolism:

Hepatic: Inactive metabolites: 5′-deoxy-5-fluorocytidine, 5′-deoxy-5-fluorouridine
Tissue: Active metabolite: Fluorouracil

Half-life elimination: 0.5-1 hour

Time to peak: 1.5 hours; Fluorouracil: 2 hours

Excretion: Urine (96%, 57% as α-fluoro-β-alanine); feces

Administration: Oral

Administered in 2 divided doses taken approximately 12 hours apart. Doses should be taken with water and within 30 minutes after a meal.

Dietary Considerations

Because current safety and efficacy data are based upon administration with food, it is recommended that capecitabine be administered with food. In all clinical trials, patients were instructed to take with water within 30 minutes after a meal.

Storage

Store at room temperature of 25°C (77°F); excursions permitted between 15°C and 30°C (59°F and 86°F).

8. CORRELATIVE/SPECIAL STUDIES

8.1 General

The purpose of the samples is to further understand the mechanism of the mode of action of trastuzumab and bevacizumab in esophageal and gastric cancer, and/or potentially identify a marker to monitor or predict treatment results. All subjects will sign the informed consent form for the correlative science portion of this study.

Samples for the correlative science portion of the study will be analyzed after the completion of the clinical portion of this study in order to better understand biologic correlates of the clinical activity of trastuzumab and bevacizumab in the subject population. The analysis will be performed at a laboratory designated by the principal investigator.

All samples (tumor, plasma and whole blood) will be labeled according to directions noted below.

Fresh biopsy samples may also be used for the generation of cell lines. Any remaining samples, such as unstained slides, tumor blocks, plasma, and whole blood will be retained at the testing laboratory until completion of the trial. All samples will be destroyed after a maximum of 10 years.

An additional tube of blood will be collected at baseline and will be stored for future studies of other factors that may prove useful at that time. Permission for future research will be included separately in the informed consent form.

The analysis of results will not be made available to either the subject or the treating physician; however, group results from this trial will be published.

Contact person at DFCI for correlative science labs:

Katharine Straw, CRC 617 632 6316 or Katharine_Straw@dfci.harvard.edu

8.2 Collection of tumor blocks/slides

For all patients (Part I and II), a sample of tumor from primary biopsy that was obtained at the time of diagnosis (either paraffin block or 15 freshly cut unstained slides) must be obtained from all participants. This sample must have been taken prior to first loading dose of trastuzumab.

The participating investigator will request the tumor block or slides during the screening period, they need to be en route, but do not have to be received by the site to begin treatment.

Patients in Part II of the study, must be willing to consent to two mandatory biopsies. The first sample will be obtained anytime within two weeks of loading dose of trastuzumab; the second sample will be obtained on Cycle 1 Day 7, one week after the loading dose of trastuzumab. We do not intend to send this sample for processing in the clinical pathology laboratory, unless there is a concern about any findings during this biopsy procedure as determined by the MD performing the biopsy.

8.2.1 Rationale

A key to the success of any targeted agent is to identify which patients benefit most from its use. Therefore, in addition to clinical response, we would propose correlative scientific studies to identify this population to inform future therapeutic initiatives.

All patients (Part I and Part II) will have blood samples drawn at designated time points throughout the study. In addition, mandatory biopsy of tumor will be obtained from patients in Part II of this study at two time points. The first will be obtained within one week prior to loading dose of trastuzumab, the second will be obtained 7 days post loading dose of trastuzumab.

8.3. Evaluation of signaling pathways

8.3.1 Rationale

It has been demonstrated in preclinical models that trastuzumab exerts its anti-proliferative effects through multiple mechanisms. A principle pathway that is attenuated by trastuzumab, leading to growth arrest, is the PI3K/AKT pathway (Fujita, T et al. Br J Cancer. 2006 Jan 30;94(2):247-52; Fabi, A et al. Oncology. 2010;78(2):141-9). Additionally, the status of this pathway in human patient samples (measured by PTEN status and other components of the PI3K pathway) has been shown to significantly correlate with multiple different clinical endpoints in breast cancer patients treated with trastuzumab. (Fujita. Br J Cancer. 2006 Jan 30;94(2):247-52; Singer, CF et al. Biochim Biophys Acta. 2008 Dec;1786(2):105-13; Fabi, A et al. Oncology. 2010;78(2):141-9;)

It has recently been shown that trastuzumab has activity in esophagogastric cancer. In the ToGA study, reported at ASCO in June of 2009 (van Cutsem. J Clin Oncol 27:18s, 2009 (suppl; abstr LBA4509), patients with HER2/neu positive tumors were randomized to cisplatin/fluorouracil (or cisplatin/capecitabine) with or without trastuzumab. Patients who received trastuzumab had a higher response rate (47% vs. 35%; p=0.0017) and a longer overall survival (13.8mos vs. 11.1 mos; p=0.0046)

To date the molecular mechanism of this response and survival benefit has not been well studied in esophagogastric cancer, nor has the ability of trastuzumab to inhibit the PI3K pathway in esophagogastric tumors been well explored. We hypothesize that the PI3K pathway, as in breast cancer, may be predictive of response to this agent.

Therefore, demonstrating the ability of trastuzumab to inhibit this pathway in patient samples will be informative in understanding its mechanism of action in these cancers.

The first 20 patients will NOT undergo biopsy for this study. These patients will demonstrate the safety and tolerability of this regimen. Once safety and tolerability has been established, the subsequent 16 patients will undergo a mandatory baseline research biopsy no more than 2 weeks before initiation of trastuzumab therapy and a second mandatory research biopsy on day 7 after their first trastuzumab treatment. Seven days is the maximum time that patients are typically willing to wait before initiating palliative chemotherapy and, based on pre-clinical data (Mohsin, SK et al. J Clin Oncol. 2005 Apr 10;23(11):2460-8; Dasari VR et al. PLoS One. 2010 Apr 26;5(4):e10350.), this period of time should be sufficient to document downregulation of signal transduction pathways. We believe that 16 pre- and post-treatment patient samples should be sufficient to detect a clinically meaningful inhibition of the PI3K pathway. A sample size of 16 matched samples achieves an 80% power to detect a difference of at least 20% using a binomial test, with one-sided type I error 5% and assuming the population proportion under the null hypothesis is at least 5%."

We will utilize Immunohistochemistry (IHC) for the following surrogates of PI3K activation in the tumors (phopho-AKT and phosphor-S6) and pretreatment biopsies will be assessed for PTEN status (which may indicate hyperactivation of the PI3K pathway). IHC will be performed using standard methods. In brief, samples will be immediately placed in formalin to minimize artifacts of devascularized tissue, such as loss of phosphorylation events. Importantly, the processing of the pre-treatment and post-treatment biopsies will be performed in an identical fashion allowing for more robust comparisons of treatment effect. Antigen retrieval of formalin fixed, paraffin embedded tissue will be performed in a standard citrate buffer and heat in a pressure cooker. The primary antibodies used in this study include PTEN (Cell Signalling#9559), phospho-Akt Ser-473 (Cell signaling# 3787), and phospho-S6 Ser235/236 (Cell Signaling#4857).

These antibodies have been well characterized and used in multiple tumor tissue types:

PTEN – Saal et al. Nat Genet. 2008 Jan;40(1):102-7
pAKT – Kinkade et al. J Clin Invest. 2008 Sept; 118(9):3051-3064.
pS6 - Iwenofu et al. Modern Pathology. 2008 Dec; 21:231-237.

Secondary antibodies conjugated to HRP and the chromogenic substrate DAB, used for visualization of the antigen. Adjacent sections will be stained with H&E to validate the presence of tumor. Staining will be scored and quantitated using an iCys Laser Scanning Cytometer, through an ongoing collaboration with pathologist Dr. Gerry Chu.

8.4. Sample Management of tumor blocks and slides.

8.4.1 Biopsy Specimens (Part II) and preparation of slides from paraffin-embedded tumor blocks (Part I and Part II)

Part II: Fresh Biopsy specimens:
Two samples of tumor will be obtained during each biopsy procedure. One sample will be immediately placed in formalin, a second sample will be snap frozen according to instructions below.

The CRC will be responsible for collecting and delivery of this sample to the laboratory of Dr Alec Kimmelman. Every effort should be made to obtain tissue that contains cells representative of the tumor. Each specimen should contain sufficient non-necrotic tumor tissue.

Instructions for Formalin Specimen:

1. Pre-label one large cryovial (at least 3 ml) with protocol # (09-457), study subject # and date of collection
2. Fill each cryovial about halfway with Formalin
3. At time of biopsy collection, immediately place tumor specimen in cryovials, make sure specimen is immersed in formalin.
4. Deliver specimen as soon as possible to laboratory of Dr Alec Kimmelman, where specimen will be processed into paraffin block.

Instructions for Frozen Specimen:

1. Pre-label plastic block cryomold with protocol # (09-457), study subject #, and date of collection.
2. Place a few drops of OCT tissue compound into the cryomolds.
3. At the time of biopsy, immediately place tumor sample in cryomold, cover sample completely with OCT, wrap in aluminum foil, and place on dry ice.
4. Deliver specimen as soon as possible to the laboratory of Dr Alec Kimmelman for further processing.

Part I and Part II: Preparation of slides from paraffin-embedded tumor biopsies:

A minimum of ten and preferably fifteen unstained slides will be prepared as follows from the paraffin-embedded tumor (unstained and 1 stained with hematoxylin and eosin).

- Cut sections at 5 microns.
- Apply sections to positively-charged (also called “plus”) glass slides.
- Apply 1 section per slide.
- A ***minimum*** of 10 unstained slides are required.
- One hematoxylin and eosin-stained slide from the tumor block used for diagnosis should also be sent.
- Label the glass slides with the protocol # (09-457), study subject #, and date of collection.

- Do not treat the cut section in the oven before storage in the refrigerator.
- Store slides in a refrigerator at 4°C, and protect from light until shipped.
- Labeling of slides:

All slides/ tumor blocks must be labeled with:

1. Protocol Number: 09-457
2. Unique subject identifier: (initials of patient and study subject #)
3. Collection date.

Every effort should be made to obtain slides or blocks that contain cells representative of the tumor. The specimen should contain sufficient non-necrotic tumor tissue. In addition to the slides or block, one hematoxylin/eosin stained slide must be collected from the block that was used for diagnosis. The paraffin block could be a biopsy, or be part of a larger sample obtained at surgery. The minimum size paraffin block should be 3 mm x 3 mm of tumor tissue.

8.5 Blood sample management

Samples will be drawn by an experienced phlebotomist or nurse. Blood samples may be drawn either peripherally or via a central line.

Each site will be responsible for ordering their own laboratory supplies and shipping materials for the specimens. Shipping costs and supplies will be covered by the protocol budget. Specimens will be stored at the site until arrangements have been made to store at DF/HCC.

8.5.1 Plasma specimens:

Approximately 5-7 ml of blood for plasma-based studies will be collected in EDTA (purple top) tubes.

Blood samples will be drawn as follows:

- Within 2 weeks prior to initiation of therapy (may be done on C1D1)
- Day 1 cycle 2, 0-24 hours prior to initiation of therapy.
- Day 1 cycle 4, 0-24 hours prior to initiation of therapy
- Every 2 cycles thereafter, on Day 1, 0-24 hours prior to initiation of therapy
- End of study

Instructions for plasma samples:

Invert tube several times to assure complete mixing of anticoagulant (EDTA) and immediately centrifuge in a clinical centrifuge at 3,000 r.p.m. for 10 minutes at room temperature. Plasma should be aspirated without disturbing cells, aliquoted in 3 cryogenic vials, and frozen at –70C.

All cryovials will be labeled as follows:

1. Protocol Number: 09-457
2. Unique subject identifier (initials of patient and study subject #)
3. Collection date
4. Tube # (there should be 3 cryovials)/ contents (plasma)

Instruction for Whole Blood sample:

An additional tube of whole venous blood will be collected at baseline and will be stored for future studies of other factors that may prove useful at that time. Venous blood will be collected into a 6 ml purple-top EDTA tube. After collection of the sample, the tube should be gently inverted to ensure mixing with the anticoagulant. Whole blood should be transferred from EDTA tube into 2 labeled cryotubes and frozen at –80C.

The cryovials will be labeled as follows:

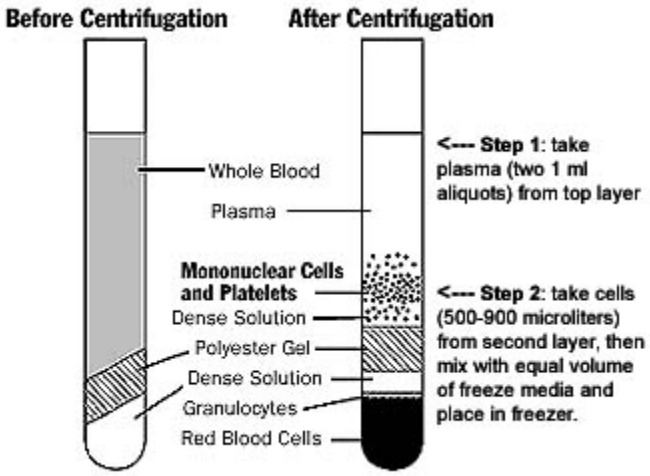
1. Protocol Number: 09-457
2. Unique subject identifier (initials of the patient and subject study #)
3. Collection date
4. Tube # (there should be 2 cryovials) / contents (whole blood)

8.5.2 Sample Management – CECs: Obtained at the following time points:

- Within 2 weeks of initiation of therapy (may be done on C1D1)
- Day 1 cycle 2, 0-24 hours prior to initiation of therapy.
- Day 1 cycle 4, 0-24 hours prior to initiation of therapy
- Every 2 cycles thereafter, on Day 1, 0-24 hours prior to initiation of therapy
- End of Study

Sample Management – Handling Instructions for CEC specimens

1. Approximately 7 cc of venous blood will be collected into a BD Vacutainer CPT™ tube with Sodium Citrate (Becton Dickinson product #362761). The tube should be gently inverted several times to ensure mixing with the anticoagulant.
2. Within two hours the CPT tube should be centrifuged at room temperature in a horizontal rotor for 25 minutes at 1600g. After centrifugation, the mononuclear cells will be visible in a whitish layer just under the plasma (refer to picture below).
3. 500-900 µL aliquot should be taken from the mononuclear layer (we are not collecting plasma from this tube) and transferred to a cryotube (designated tube #1/mononuclear), to which an equal volume of freezing media (RPMI-1640 media with 20% dimethyl sulfoxide) should be added.
4. Tube #1 should immediately be placed in a –80C degree freezer. All cryotubes should be labeled as noted above.


Before Centrifugation
After Centrifugation
Whole Blood
Plasma
Mononuclear Cells and Platelets
Dense Solution
Polyester Gel
Dense Solution
Granulocytes
Red Blood Cells
<--- Step 1: take plasma (two 1 ml aliquots) from top layer
<--- Step 2: take cells (500-900 microliters) from second layer, then mix with equal volume of freeze media and place in freezer.

9. CALENDAR

Screening Assessments will be performed within 14 days of C1D1 unless otherwise noted below. Informed consent may be within 28 days of study entry[1] **Screening Assessments**		**After cycle one, each cycle is 21 days +/- 1 day**				
		C1D1[2]	**C1D7**	**C2D1[3] and C3D1**	**C4D1 (and all subsequent cycles)**	**Off study**
Informed consent	X					
History	X					
Physical exam	X	X		X	X	X
Height/weight/BSA		X		X	X	
Concomitant medications	X	X		X	X	X
ECOG performance status	X	X		X	X	X
Toxicity Assessment		X	X	X	X	X
CBC with differential	X			X	X	X
Comprehensive metabolic panel	X			X	X	X
PT/PTT/INR	X					
U/A	X					
Urine Protein/Creatinine ratio (UPCR)	X				X[4]	
CT scan of chest, abdomen, pelvis	X[5]				X[6]	
MUGA scan or echocardiogram	X[7]				X[8]	
EKG	X[9]					
Urine or serum pregnancy test[10]	X					
Biopsy of tumor (Part II only)	X[11]		X[12]			
Study bloods		X[13]		X[14]	X[15]	X
Trastuzumab loading dose		X				
Bevacizumab/Trastuzumab/oxaliplatin administration				X	X	
Capecitabine days 1-14		X[16]		X	X	

[1] All labs must be done within 14 days of start and do not need to be repeated on day 1
[2] Part II study participants: C1D1 must be on Tuesday, Wednesday, Thursday or Friday
[3] Part I: C2D1 will begin one week after loading dose of trastuzumab; Part II: C2D1 will be 9-11 days after loading dose of trastuzumab in Part II.
[4] UPCR every other cycle. Not required if Bevacizumab discontinued.
[5] Screening CT scan of chest, abdomen and pelvis within 28 days of C1D1
[6]First restaging scans within 1 week of C4D1 and then every 2 cycles until C24D1. Scans after cycle 24 will be every 3 cycles.
[7] MUGA scan or Echocardiogram within 28 days of C1D1. Please use the same modality each time.
[8] MUGA or Echocardiogram to be performed anytime prior to cycle 4, then prior to every 4th cycle while on study (i.e. prior to Cycle 8, Cycle 12, etc.)
[9] EKG within 28 days
[10] For women of childbearing potential
[11] Tumor block or 15 freshly cut unstained slides are required for eligibility and are to be requested for delivery to study team, however do not have to be on site to begin cycle 1
[12] Part II DF/HCC study participants: Cycle 1 Day 7 Biopsy
[13] Study blood with screening labs (or just prior to C1D1 infusion)
[14] Study blood only C2D1
[15] Study bloodC4D1; then every other cycle; and Off study
[16] Give patient prescription for capecitabine to be filled at local pharmacy prior to C2D1

10. MEASUREMENT OF EFFECT.

Response will be assessed using RECIST version 1.1 criteria

10.1 Antitumor Effect– Solid Tumors

For the purposes of this study, participants should be evaluated for response after the first 3 cycles and then every 2 cycles (6 weeks) until cycle 24, after which participants should be evaluated after every 3 cycles.

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST 1.1) (Eisenhauer et al, 2009) guideline. Changes in the diameter (unidimensional measurement) of the tumor lesions are used in the RECIST 1.1 criteria

10.1.1 Definitions

Evaluable for toxicity. All participants who have received all intravenous study agents in cycle 1 and cycle 2 and at least one dose of capecitabine during cycle 2 will be evaluable for toxicity.

Evaluable for objective response. Only those participants who have measurable disease present at baseline, have received at least two cycles of therapy (all IV therapy and at least one dose of capecitabine), and have had their disease re-evaluated will be considered evaluable for response. These participants will have their response classified according to the definitions stated below.

10.1.2 Disease Parameters

Measurable disease. Measurable disease is the presence of at least one (1) lesion that can be accurately measured in at least one dimension with longest diameter ≥20 millimeters (mm) using conventional techniques (CT, MRI, x-ray) or ≥10 mm with spiral CT scan. Measurable lesions must be at least 2 times the slice thickness in mm. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

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

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥ 10 to < 15mm short axis, are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses identified by physical exam that are

not measurable by reproducible imaging techniques, and cystic lesions are all considered non-measurable.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Lesions must be accurately measured in 1 dimension with a minimum size of 10 mm by CT or MRI (slice thickness no greater than 5 mm), 20 mm by *chest* x-ray. Nodes must have a short axis ≥ 15 mm. The short axis should be included in the sum of the lesions in the calculation of response. Nodes that shrink to < 10 mm are considered normal. Target lesions should be selected on the basis of their size, be representative of all the involved organs, and should be lesions that can be followed with reproducible repeated measurements.

Lytic bone lesions or mixed lytic-blastic lesions, *with identifiable soft tissue components*, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be considered target lesions if the *soft tissue component* meets the definition of measurability as defined above. Cystic lesions thought to represent cystic metastases can be considered as target lesions. However, if non-cystic lesions are present, these are preferred for selection as target lesions. Lesions in previously irradiated areas or areas subject to other loco-regional therapy are usually not considered measurable unless there has been demonstrated progression of that lesion.

Non-target lesions. All other lesions, including small lesions < 10 mm or pathological lymph nodes measuring ≥ 10 mm to < 15 mm in short axis, as well as truly non-measurable lesions, which include leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses identified by physical exam that are not measurable by reproducible imaging technique

10.1.3 Methods for Evaluation of Measurable Disease

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

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

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

Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung; however, CT is preferable.

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

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

FDG PET and PET/CT. The acquisition of FDG PET and FDG PET/CT scans should follow the NCI Guidelines for using FDG PET as an indicator of therapeutic response (L.K. Shankar, J.M. Hoffman, S. Bacharach, M.M. Graham, J. Karp, A.A. Lammertsma, S. Larson, D.A. Mankoff, B.A. Siegel, A. Van den Abbeele, J. Yap, D. Sullivan. Consensus recommendations for the use of 18F-FDG PET as an indicator of therapeutic response in patients in National Cancer Institute Trials. J Nucl Med, 47(6):901-903, 2006). Patients should avoid strenuous exercise and be on a low carbohydrate diet for 24 hours prior to the scan. Patients should fast for 4 hours or longer prior to the FDG injection and should have a serum glucose of less than 200 mg/dL at the time of FDG injection. A 10-20 mCi dose of FDG should be injected for typical adult patients. For longitudinal studies with multiple scans, particular attention should be paid to ensure consistent patient preparation and acquisition parameters between the follow-up scan and the baseline scan. When designing a study where PET scans are going to be utilized as one of the modalities to evaluate efficacy, it is important to consult with physicians in nuclear medicine in designing the appropriate criteria to be utilized.

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

Tumor markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a subject to be considered in complete clinical response. Specific additional criteria for standardized usage of prostate-specific antigen (PSA) and CA-125 response in support of clinical trials are being developed.

Cytology/Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

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

10.1.4 Response Criteria

10.1.4.1 Evaluation of Target Lesions

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

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

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study with at least a 5 mm absolute increase in the sum of all lesions. The appearance of one or more new lesions* denotes disease progression.

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

Unknown (UN): Assessment of target lesions cannot be made due to insufficient or unevaluable data. In this case, a concise explanation must be given.

Note: If tumor response data is missing for target lesions, the overall assessment must be UN unless there is new disease that would result in an overall assessment of PD. However, if there is missing or unevaluable data for non-target lesions, but data is available for all target lesions, the overall response for that time point will be assigned based on the sum LD of all target lesions. Additionally, the assessment of CR cannot be made if there is missing or unevaluable data for non-target lesions. In this case, the overall assessment would be PR.

***Definition of New Lesion**: The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (ex: new bone lesions may be healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size,

etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

10.1.4.2 Evaluation of Non-Target Lesions

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

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

Incomplete Response/Stable Disease (SD): Persistence of one or more non-target lesions and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions* (new lesions must be > slice thickness) and/or unequivocal progression of existing non-target lesions.

Overall level of substantial worsening that merits discontinuation of therapy. A useful test that can be applied when assessing non-targets for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease.

Unknown (UN): Assessment of non-target lesions cannot be made due to insufficient or unevaluable data. In this case, a concise explanation must be given.

***Definition of New Lesion**: The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (ex: new bone lesions may be healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size, etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

10.1.4.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment

started). The participant's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response for when Confirmation is
CR	CR	No	CR	≥4 wks confirmation
CR	Non-CR/Non-PD	No	PR	≥4 wks confirmation
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/Not evaluated	No	PR	
SD	Non-CR/Non-PD/Not evaluated	No	SD	Documented at least once ≥4 wks from baseline
PD	Any	Yes or No	PD	No prior SD, PR or CR
Any	PD*	Yes or No	PD	
Any	Any	Yes	PD	

* In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

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

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD
Not all evaluated	No	Not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

Non-CR/non-PD is preferred over stable disease for non-target disease since SD is increasingly used an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised.

10.1.5 Duration of Response

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

Duration of overall complete response: The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

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

10.1.6 Progression-Free Survival

Progression-Free Survival (PFS) is defined as the duration of time from start of treatment to time of objective disease progression.

Response Review

Dana-Farber Harvard Cancer Center Tumor Metrics Imaging Core (TIMC) will conduct central review of all radiological assessments for this study.

11. ADVERSE EVENT REPORTING REQUIREMENTS

11.1 General

In the event of any adverse event the first concern will be for the safety of the patient.

Adverse event collection and reporting is a routine part of every clinical trial. This study will use the descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0) that is available at http://ctep.cancer.gov/reporting//ctc.html.

Information on all adverse events, whether reported by the participant, directly observed, or detected by physical examination, laboratory test or other means, will be collected, recorded, followed and reported as described in the following sections.

Adverse events experienced by participants will be collected and reported from initiation of study medication, throughout the study, and within 30 days of the last dose of study medication. Participants who experience an ongoing adverse event or related to a study procedures and/or study medication beyond 30 days will continue to be contacted by a member of the study team until the event is resolved, stabilized, or determined to be irreversible by the participating investigator.

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

11.2 Definitions

11.2.1 Adverse Event (AE)

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

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

11.2.2 Serious adverse event (SAE)

An SAE is any sign, symptom or medical condition that emerges during capecitabine, oxaliplatin, bevacizumab or trastuzumab treatment or post-treatment during the follow up period that (1) was not present at the start of treatment or is not a chronic condition that is part of the patient's medical history or (2) was present at the start of treatment or was part of the patients history but worsened in severity and/or frequency during therapy, and meets any of the following criteria:

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

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

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

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

11.2.3 Expectedness

Adverse events can be 'Expected' or 'Unexpected.'

11.2.3.1 Expected adverse event

Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.

Refer to Section 6.1 for a listing of expected adverse events associated with the study agent(s).

11.2.3.2 Unexpected adverse event

For the purposes of this study, an adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk.

11.2.4 Attribution

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

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

11.3 Recording Adverse Events

Adverse event information will be obtained at each contact with the participant. All adverse events will be recorded on the appropriate study-specific case report forms (CRFs).

11.4 Reporting Adverse Events

Investigators **must** report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

For multi-site trials where a DF/HCC investigator is serving as the principal investigator, each participating investigator **must** abide by the reporting requirements set by the DF/HCC. This applies to any medical event equivalent to an unexpected grade 2 or 3 with a possible, probable or definite attribution, grade 4 toxicities, and grade 5 (death) regardless of study phase or attribution.

Additionally, other investigative sites will also report SAEs to their respective IRB according to the local IRB policies and procedures in reporting adverse events. The study must be conducted in compliance with FDA regulations, local safety reporting requirements, and reporting requirements of the principal investigator.

11.4.1 SAE Reporting to Genentech Drug Safety

Med Watch 3500a Forms
http://www.fda.gov/medwatch/REPORT/Mfg.htm[for drugs/biologics]

All SAEs must be reported to Genentech Drug Safety within 24 hours of learning of the event. Reporting will be done on the Med Watch 3500a Form and should include the following information:

- Appropriate patient demographic information
- Suspect products information
- Treatment regimen (dosing frequency, combination therapy)
- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome, if known
- Supportive laboratory results and diagnostics
- Relevant history/preexisting medical conditions
- Concomitant medications
- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication per protocol section 11.2.4.

Med Watch 3500a Forms must be faxed to:

Genentech Drug Safety:
(650) 225-4682 or (650) 225-5288

Copies of SAE submission to Genentech Drug Safety must be simultaneously copied to the study sponsor by fax or email:

Peter C. Enzinger, MD
Fax: 617-582-7988
Peter_enzinger@dfci.harvard.edu

Follow-up information:
Within 24-48 hours, the participating investigator must provide follow-up information on the SAE. Follow-up information should describe whether the event has resolved or continues, if and how the event was treated, and whether the participant will continue or discontinue study participation.

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original Med Watch 3500a report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original Med Watch 3500a form
- Summarizing new information and faxing it with a cover letter including subject identifiers (i.e. D.O.B. initial, subject number), protocol description and number, if assigned, suspect drug, brief adverse event description, and notation that additional or follow-up information is being submitted (The subject identifiers are important so that the new information is added to the correct initial report)

Occasionally Genentech may contact the reporter for additional information, clarification, or current status of the subject for whom and adverse event was reported.

11.4.2 Notification by Investigator to Overall PI & DFCI IRB

All adverse events that occur after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment that meet the criteria outlined in the DFCI IRB Adverse Event Reporting Policy (outlined below) must be reported to the DF/HCC IRB using the DFCI Adverse Event Reporting Form.

The DFCI IRB requires the following Adverse Events (AE) be reported for all subjects enrolled and actively participating in the trial or when the AE occurs within 30 days of the last study intervention (e.g. drug administration):

- **Grade 2 (moderate) and Grade 3 (severe) Events** – Only events that are Unexpected and Possibly, Probably or Definitely Related / Associated with the Intervention.
- **ALL Grade 4 (life threatening or disabling) Events** – Unless expected AND specifically listed in protocol as not requiring reporting.
- **ALL Grade 5 (fatal) Events**

  Note: If the participant is in long term follow up, report the death at the time of continuing review.

Investigative sites within DF/HCC and DF/PCC will notify the Overall PI within 1 business day of the event or within 1 business day of learning of the event. Sites within DF/HCC and DF/PCC will report SAEs directly to the DFCI Office for Human

Research Studies (OHRS) per the DFCI IRB reporting policy. A copy of the submission must be sent to the Overall PI.

Other participating sites must report each serious adverse event to the DF/HCC Principal Investigator within 1 business day of learning of the occurrence. In the event that the participating investigator does not become aware of the serious adverse event immediately (e.g., participant sought treatment elsewhere), the participating investigator is to report the event within 1 business day after learning of it and document the time of his or her first awareness of the adverse event. Report serious adverse events by email or facsimile to:

Peter C. Enzinger, MD
Fax: 617-582-7988
Peter_enzinger@dfci.harvard.edu

Within the following 24-48 hours, the participating investigator must provide follow-up information on the serious adverse event. Follow-up information should describe whether the event has resolved or continues, if and how the event was treated, and whether the participant will continue or discontinue study participation.

11.4.3 Non-Serious Adverse Event Reporting Requirements

Non-serious adverse events will be reported to the Principal Investigator on the toxicity Case Report Forms.

11.5 Hospital Risk Management Notification by Investigator

The participating investigator will report to the Principal Investigator and to local Risk Management any subject safety reports or sentinel events that require reporting according to institutional policy.

12. DATA AND SAFETY MONITORING

12.1 Data Reporting

12.1.1 Method

The QACT will collect, manage, and monitor data for this study.

12.1.2 Data Submission

The schedule for completion and submission of case report forms (paper or electronic) to the QACT is as follows:

Form	**Submission Timeline**
Eligibility Checklist	Complete prior to registration with QACT
On Study Form	Within 14 days of registration
Baseline Assessment Form	Within 14 days of registration
Treatment Form	Within 10 days of the last day of the cycle
Adverse Event Report Form	Within 10 days of the last day of the cycle
Response Assessment Form	Within 10 days of the completion of the cycle required for response evaluation
Off Treatment/Off Study Form	Within 14 days of completing treatment or being taken off study for any reason
Follow up/Survival Form	Within 14 days of the protocol defined follow up visit date or call

12.2 Safety Meetings

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this trial. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Principal Investigator and study team.

The DSMC will meet quarterly and/or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days for Phase I or II protocols; Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Monitoring

Involvement in this study as a participating investigator implies acceptance of potential audits or inspections, including source data verification, by representatives designated by the Principal Investigator or DF/HCC. The purpose of these audits or inspections is to examine study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policy, and any applicable regulatory requirements.

All data will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue during protocol performance and completion. Please refer to Section 5.0 of the Dana-Farber/ Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (Appendix D) for further detail.

12.4 Multicenter Guidelines

This protocol will adhere to the policies and requirements of the DF/HCC Multi-Center Data and Safety Monitoring Plan. The specific responsibilities of the Overall PI, Coordinating Center, and Participating Institutions and the procedures for auditing are presented in Appendix D.

- The Overall PI/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports to all participating institutions for submission to their individual IRBs for action as required.

- Mechanisms will be in place to ensure quality assurance, protocol compliance, and adverse event reporting at each site.

Except in very unusual circumstances, each participating institution will order the study agent(s) directly from supplier. A participating site may order the agent(s) only after the initial IRB approval for the site has been forwarded to the Coordinating Center.

13. REGULATORY CONSIDERATIONS

13.1 Protocol Review and Amendments

This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB governing each study location.

Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. The Principal Investigator (or Protocol Chair) will disseminate protocol amendment information to all participating investigators.

All decisions of the IRB concerning the conduct of the study must be made in writing.

13.2 Informed Consent

All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form

must be signed and dated by the participant or the participant's legally authorized representative, and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

13.3 Ethics

This study is to be conducted according to the following considerations, which represent good and sound research practice:

- US Code of Federal Regulations (CFR) governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki
    - Title 21 Part 50 – Protection of Human Subjects www.access.gpo.gov/nara/cfr/waisidx_02/21cfr50_02.html
    - Title 21 Part 54 – Financial Disclosure by Clinical Investigators www.access.gpo.gov/nara/cfr/waisidx_02/21cfr54_02.html
    - Title 21 Part 56 – Institutional Review Boards www.access.gpo.gov/nara/cfr/waisidx_02/21cfr56_02.html
    - Title 21 Part 312 – Investigational New Drug Application www.access.gpo.gov/nara/cfr/waisidx_02/21cfr312_02.html
- State laws
- Institutional research policies and procedures www.dfhcc.harvard.edu/clinical-research-support/clinical-research-operations-cro/policies-and-procedures

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB according to the local reporting policy.

13.4 Study Documentation

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research participant. This information enables the study to be fully documented and the study data to be subsequently verified.

Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

13.5 Records Retention

All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

14. STATISTICAL CONSIDERATIONS

14.1 Statistical Design

This study will have a 3 stage design. Part I of the study will have two stages and will enroll the first 20 patients. Part II will enroll 16 additional patients if criteria for study continuation is met.

To be considered evaluable for this study, (for both toxicity and response), patients must have received all of the intravenous study agents in cycle 1 and 2 and at least one dose of capecitabine in cycle 2. Any patient that does not complete this period of evaluation will be replaced.

In the first stage of Part I of the study, 5 patients will be enrolled and evaluated through 2 cycles of therapy. Safety and tolerability criteria are defined below.

Safety and tolerability for the first 5 patients will be defined as:

1. No clinically significant, treatment related grade 3-5toxicity occurring in 4 or more patients.

If 4 or more patients have grade 3-5 toxicity, the study will be put on hold and the treatment will be either modified with IRB approval or will close. If the study is modified for safety and tolerability, upon reopening, 5 new patients will be enrolled and evaluated using the same toxicity criteria noted above.

This is based on the following calculation: Let X denote the number of subjects experiencing grade 3-5 toxicity. Let p denote the true underlying rate for this safety endpoint.

The table below gives the probability of stopping associated with this stopping rule when p=p0:

p0	P(X >/= 4 | p = p0)
0.50	0.19
0.55	0.26
0.60	0.34
0.65	0.43
0.70	0.53
0.75	0.63
0.80	0.74
0.85	0.84

Once the PI has determined that the regimen is safe and tolerable, an additional 15 patients will be enrolled for further evaluation of safety, tolerability and response to complete Part I of the study.

Safety and tolerability for the first 20 patients will be defined as:

1. No clinically significant, treatment related grade 3-5 toxicity occurring in 13 or more patients.

Let X denote the number of subjects experiencing grade 3-5 toxicity. Let p denote the true underlying rate for this safety endpoint.

Based on 20 subjects, the trial would stop if 13 or more out of these 20 subjects experience clinically significant, treatment related grade 3-5 toxicity.

The table below gives the probability of stopping associated with this stopping rule when p = p0:

p0	P(X >/= 13 | p = p0)
0.50	0.13
0.55	0.25
0.60	0.42
0.65	0.60
0.70	0.77
0.75	0.90
0.80	0.97
0.85	0.99

If the true grade 3-5 toxicity rate is 75%, we would have 90% chance to stop the trial based on the first 20 subjects. We consider a 75% rate or higher for this safety endpoint to be unacceptable because in the recent randomized CALGB 80403/ECOG 1206 trial, reported at ASCO 2010 in Chicago, the Grade 3-5 toxicity for the 3 treatment arms was 75%, 79%, and 86%, respectively.

Further support for this toxicity threshold is given in the only trial that has led to FDA 4approval of a chemotherapeutic agent for esophagogastric cancer. In the V325 study (van Cutsem. J Clin Oncol 2006), the grade 3-4 non-hematologic toxicity rate was 81.4% for the DCF arm that led to approval of docetaxel in esophagogastric cancer. Furthermore, this DCF regimen had a 82.3% grade 3-4 neutropenia rate (and lesser rates of anemia and thrombocytopenia).

Efficacy for the first 20 patients will be defined as:

If $\leq$6 responses are noted in the initial group of 20 evaluable patients (early termination probability 0.61), the trial will be terminated. If there are $\geq$ 7 patients with a major response, another 16 patients will be accrued to a total of 36 evaluable patients.

This regimen will be considered promising if 15 or more evaluable patients have a major response by RECIST 1.1 criteria in the final analysis. This is based on a hypothetical

historical response rate of less than or equal to 30% for the CAPOX combination versus the new response rate of at least 50% in this combination plus bevacizumab and trastuzumab, using a type I error of 10% (one sided) and power of 86%.

For correlative science, we believe that 16 pre-and post-treatment samples should be sufficient to detect a clinically meaningful inhibition of the P13K pathway. A sample size of 16 matched samples achieves an 80% power to detect a difference of at least 20% using a binomial test, with one-sided type I error 5% and assuming the population proportion under the null hypothesis is at most 5%.

Update:

As of 4.4.13, 16 patients have met eligibility requirements and have received study drug. Of these 16 patients, all are evaluable for toxicity and 15 are evaluable for efficacy (1 too early). There have been no grade 5 toxicities. Only one patient has had grade 4 toxicity (hypertriglyceridemia, a rare but documented toxicity of capecitabine). Clinically relevant grade 3 toxicities have occurred in only 5 patients. These include diarrhea: 5pts; dehydration: 2pts; GI disorder, other: 1pt; hypokalemia: 2pts; hypophosphatemia: 1pt; and thromboembolic event: 1pt. Additionally, 2 patients have had grade 3 lymphopenia. Thus, 10 of 16 patients have had no clinically relevant grade 3-5 toxicity. This exceeds the requirement that a minimum of 7 patients out of 20 patients must have no clinically significant, treatment related grade 3-5 toxicity to move forward with Part II of the study.

Of 15 patients evaluable for efficacy, 11 have had a confirmed PR and 1 has had a confirmed CR by RECIST version 1.1 for a major response rate of 80%. This is double the efficacy requirement of > 6 of 20 patients with major response to move forward with Part II of the study.

We have thus fulfilled the toxicity and efficacy criteria for Part I of the study and would like to move forward now with Part II. The reason that this is important is that patients meeting the HER2/neu eligibility requirements are fewer than previously expected. We would like to start requiring biopsies of the DF/HCC patients immediately (rather than waiting until patient #20) since we may otherwise not enroll sufficient biopsy eligible patients to reach the 16 paired biopsies that we have proposed.

14.2 Endpoints

14.2.1 Primary endpoint

To determine the response rate (based on RECIST version 1.1) for the combination of CAPOX + bevacizumab + trastuzumab in patients with metastatic or unresectable esophagogastric cancer.

14.2.2 Secondary endpoints

To determine the safety and tolerability of the CAPOX + bevacizumab + trastuzumab combination in patients with metastatic or unresectable esophagogastric cancer.

To determine the overall survival for the combination of CAPOX + bevacizumab + trastuzumab in patients with unresectable esophagogastric cancer.

To determine the time to progression for the combination of CAPOX + bevacizumab + trastuzumab in patients with unresectable esophagogastric cancer.

14.2.3 Exploratory analyses:

Using a baseline biopsy and day 7 biopsy, we will seek to determine if certain signal transduction pathways (using the following antibodies: pAKT, pERK, pEGFR, pMET, pPDGFR, HER2) in esophagogastric cancers are up regulated or down regulated after a single dose of trastuzumab.

To correlate expression of serum VEGF with response and survival in metastatic esophageal and gastric patients treated with CAPOX+ bevacizumab+ trastuzumab.

14.3 Sample Size/Accrual Rate

Due to the restrictive enrollment criteria, we expect only 1-2 patients to be enrolled per month. Thus, we expect to reach sufficient enrollment for the primary endpoint in approximately 1 year (20 evaluable patients as defined in 14.0). Patients who are not evaluable as defined in 14.0 will be replaced.

14.4 Analysis of Secondary Endpoints

Overall survival and time to progression will include all evaluable patients, will be measured from the date of enrollment, and will be calculated using the method of Kaplan and Meier.

14.5 Reporting and Exclusions

14.5.1 Evaluation of toxicity

To be considered evaluable, patients must have received all intravenous study agents in cycle 1 and cycle 2 and at least one dose of capecitabine during cycle 2.

14.5.2 Evaluation of response

All participants who received all intravenous study agents in cycle 1 and cycle 2 and at least one dose of capecitabine during cycle 2 will be assessed for response to treatment, even if there are major protocol treatment deviations or if they are determined to be ineligible. Each participant will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.

15. PUBLICATION PLAN

A full report of the outcomes should be made public no later than three (3) years after the end of data collection.

16. REFERENCES /BIBLIOGRAPHY

American Cancer Society: Cancer Reference Information 5/28/2009

Barnholtz-Sloan JS, Sloan AE, Davis FG, et al: Incidence proportions of brain metastases in patients diagnosed (1973 to 2001) in the Metropolitan Detroit Cancer Surveillance System. J Clin Oncol 22:2865-72, 2004

Batchelor TT, Sorensen AG, DiTomaso E, et al: AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. Cancer Cell 11:83-95, 2007

Bendell JC, Domchek SM, Burstein HJ, et al: Central nervous system metastases in women who receive trastuzumab-based therapy for metastatic breast carcinoma. Cancer 97:2972-7, 2003

Bullitt E, Ewend MG, Aylward S, et al: Abnormal vessel tortuosity as a marker of treatment response of malignant gliomas: preliminary report. Technol Cancer Res Treat 3:577-84, 2004

Bullitt E, Zeng D, Gerig G, et al: Vessel tortuosity and brain tumor malignancy: a blinded study. Acad Radiol 12:1232-40, 2005

Bullitt E, Lin NU, Smith JK, et al. Blood vessel morphologic changes depicted with MR angiography during treatment of brain metastases: a feasibility study. Radiology 2007; 245:824-30.

Carden CP, Larkin JMG, Rosenthal MA. What is the risk of intracranial bleeding during anti-VEGF therapy? Neuro-Oncology 2008; 10:624-30.

Carey LA, Mayer E, Marcom PK, et al. TBCRC 001: EGFR inhibition with cetuximab in metastatic triple negative (basal-like) breast cancer. Breast Cancer Res Treat 2007; 106: abstract 307

Cocconi G, Lottici R, Bisagni G, et al: Combination therapy with platinum and etoposide of brain metastases from breast carcinoma. Cancer Invest 8:327-34, 1990

Cortes-Funes H, Rivera F, Ales I, et al. Phase II trastuzumab and cisplatin in patients with advanced gastric cancer with Her2/neu overexpression/amplification. J Clin Oncol 2007; 15; 18S (Abstr 1105)

D'Adamo DR, Anderson S, Albritton K, et al. Cardiac toxicity in a phase II study of doxorubicin and bevacizumab for patients with metastatic soft-tissue sarcomas. J Clin Oncol 2004; 22 Suppl 14S:9012.

Enzinger, P.C., et al., Irinotecan and cisplatin in upper gastrointestinal malignancies. Oncology (Williston Park), 1998. 12 (8 Suppl 6): p. 110-3.

Enzinger, PC, et al. ESMO Congress Abstract Presentation of Phase II trial of TPCA for patients with metastatic Esophagogastric Cancer: 2008. Updated

Falchook S, Moulder J, Wheler J.J., Naing A, Hong D,Ng C, Jackson Y, Jiang R, Kurzrock R,;

Phase 1 trial of combination of trastuzumab, lapatinib, and bevacizumab in HER-2 positive breast cancer and other malignancies. 32nd Breast Cancer Symposium; December 2009; Abstract:244

Franciosi V, Cocconi G, Michiara M, et al: Front-line chemotherapy with cisplatin and etoposide for patients with brain metastases from breast carcinoma, nonsmall cell lung carcinoma, or malignant melanoma: a prospective study. Cancer 85:1599-605, 1999

Fujita T, et al: PTEN activity could be a predictive marker of trastuzumab efficacay in the treatment of ErbB2-overexpressing breast cancer. British Journal of Cancer (2006) 94, 247-252

Fujimoto-Ouchi K, Sekiguchi F, et al: Antitumor activity of trastuzumab in combination with chemotherapy in human gastric cancer xenograft models. Cancer Chemother Pharmacol. 2007 May; 59(6): 795-805. Epub 2006 Oct 10

Fulford LG, Reis-Filho JS, Ryder K, Jones C, Gillett CE, Hanby A, et al. Basal-like grade III invasive ductal carcinoma of the breast: patterns of metastasis and long-term survival. Breast Cancer Res 2007; 9(1): R4.

Glusker P, Recht L, Lane B. Reversible posterior leukoencephalopathy syndrome and bevacizumab [letter]. New Engl J Med 2006; 354:980–1.

Gravalos C, Marquez A, Garcia-Carbonero R et al. Correlation between Her2/neu overexpression/amplification and clinicopathological parameters in advanced gastric cancer patients: a prospective study 2007 Gastrointestinal Cancers Symposium. 130 (Abstr 4053)

Gravalos C, Jimeno A. HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target. Annals of Oncology 19: 1523-1529, 2008. Published on line 25 April 2008.

Groen HJ, Smit EF, Haaxma-Reiche H, et al: Carboplatin as second line treatment for recurrent or progressive brain metastases from small cell lung cancer. Eur J Cancer 29A:1696-9, 1993

Hambleton J, Skillings JR, Kabbinavar F, et al. Safety of low-dose aspirin (ASA) in a pooled analysis of 3 randomized, controlled trials (RCTs) of bevacizumab (BV) with chemotherapy (CT) in patients (pts) with metastatic colorectal cancer (mCRC). J Clin Oncol 2005; 23 Suppl 16S:3554.

Hurvitz SA, Pegram, MD, Lin L-S, Chan DS,Allen HJ, et al. Final results of Phase II trial evaluating trastuzumab and bevacizumab as first line treatment of HER-2 amplified advanced breast cancer. 32nd Annual Breast Cancer Symposium ; December 2009. Poster 6094.

Ishida T, Tsujisaki M, Hanzawa Y et al. Significance of erbB_2 gene product as a target molecule for cancer therapy. Scand J Immunol 1994:39:459-466

Jatoi A, Murphy B, Foster N, Nikcevich D, et al. Oxaliplatin and capecitabine in patients with metastatic adeocarcinoma of the esophagus, gastroesophageal junction and gastric cardia:

a phase II study from the North Central Cancer Treatment Group (NCCTG). Annals of Oncology 2006 17 (1):29-34; doi:10.1093/annonc/mdj063

Kang Y, Bang Y, Lordick, S et al. Incidence of gastric and gastroesophageal cancer in the ToGA trial: Correlation with HER2 positivity. ASCO 2008 Gastrointestinal Cancers Symposium. Abstract 11.

Kim LS, Huang S, Lu W, et al. Vascular endothelial growth factor expression promotes the growth of breast cancer brain metastases in nude mice. Clin Exp Metastasis 2004; 21:107-18.

Kim SY, Kim HP, Kim YJ, et al: Trastuzumab inhibits the growth of human gastric cancer cell lines with HER2 amplification synergistically with cisplatin. Int J Oncol. 2008 Jan;32 (1):89-95

Lin NU, Claus E, Sohl J, et al. Sites of distant recurrence and clinical outcomes in patients with metastatic triple-negative breast cancer: high incidence of central nervous system metastases. Cancer 2008;epub ahead of print.

Martin M, Diaz-Rubio E, Casado A, et al. Carboplatin: an active drug in metastatic breast cancer. J Clin Oncol 1992; 10:433-47.

Matsui,Y, Inomata M, Tojigamori, M, et al: Suppression of tumor growth in human gastric cancer with HER2 overexpression by an anti-HER2 antibody murine model. Int J Oncol. 2005 Sep;27 (3):681-5

Mauer A M, Kraut E, Krauss, A et al. Phase II trial of oxaliplatin, leukovorin and fluorouracin I patients with advanced carcinoma of the esophagus. Annals of Oncology 2005 16 (8): 1320-1325;doi:10.1093/annonc/mdi249

Miller KD, Chap LI, Holmes FA, et al. Randomized Phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer. J Clin Oncol 2005;23:792–9.

Miller K, Wang M, Gralow J, et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med 2007; 357:2666-76.

National Cancer Institute: A study of bevacizumab in combination with capecitabine and cisplatin as first-line therapy in patients with advanced gastric cancer (AVAGAST). Protocol ID NCT00548548. http://clinicaltrials.gov/ct2/results?term=NCT00548548

Nicholas, G, Cripps C, Au h-P et al. Early results of a trial of trastuzumab and cisplatin and docetaxel for the treatment of metastatic gastric cancer overexpressing Her-2. ESMO 2006; 17 (supl 9):3; 16 (Abstr 1105)

Norden AD, Young GS, Setayesh K, et al. Bevacizumab for recurrent malignant gliomas: efficacy, toxicity, and patterns of recurrence. Neurology 2008; 70:779-87.

O'Brien MER, Talbot DC, Smith IE. Carboplatin in the treatment of advanced breast cancer: a phase II study using a pharmacokinetic ally guided dose schedule. J Clin Oncol 1993; 11:2112-17.

Ozcan C, Wong SJ, Hari P. Reversible posterior leukoencephalopathy syndrome and bevacizumab [letter]. N Engl J Med 2006; 354:981–2.

Pegram MD, et al: Phase I combined biological therapy of breast cancer using two humanized monoclonal antibodies directed against HER-2 proto-oncogene and vascular endothelial growth factor (VEGF). Breast Cancer Res Treat 2004; 88 (Suppl 1):S124 (abstr 3039).

Pegram MD, et al: Phase II combined biological therapy targeting the HER-2 proto-oncogene and the vascular endothelial growth factor using trastuzumab (T) and bevacizumab (B) as first-line treatment of HER2-amplified breast cancer. Breast Cancer Res Treat 2006; 100 (Suppl 1):S28 (abstr 301).

Pestalozzi BC, Brignoli S. Trastuzumab in CSF. *J Clin Oncol* 2000;18(11):2349-51.

Pope WB, Lai A, Nghiemphu P, et al. MRI in patients with high-grade gliomas treated with bevacizumab and chemotherapy. Neurology 2006; 66:1258-60.

Robert N, Leyland-Jones B, Asmar L, et al. Randomized phase III study of trastuzumab, paclitaxel, and carboplatin compared with trastuzumab and paclitaxel in women with HER2-overexpressing metastatic breast cancer. J Clin Oncol 2006; 24:2786-92.

Raefsky E, Castillo R, Lahiry A, Thompson D.S., Hanson S, Meng D, Knauer V,. Trieu N, Desai D.A., Yardley D.A. Phase II study of neoadjuvant bev and trasz administered with nab-paclitaxel and carboplatin in HER-2 locally advanced breast cancer; 2008 ASCO Annual meeting: Abstract:627

Sandler A, Gray R, Perry MC, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. New Engl J Med 2006; 355(24):2542–50.

Saltz L, Clarke S, Diaz-Rubio, E et al. Bevacizumab in combination with oxaliplatin based chemotherapy as a first line therapy in metastatic colorectal cancer: A randomized Phase III study. Journal of Clinical Oncology, Vol 26, No 12 (April 20), 2008: pp.2013-2019

Sakai K, Mori S, Kawamoto T et al. Expression of epidermal growth factor receptors on normal human gastric epithelia and gastric carcinomas. J Natl Cancer Inst 1986: 77:1047-52

Scappaticci FA, Fehrenbacher L, Cartwright T, et al. Surgical wound healing complications in metastatic colorectal cancer patients treated with bevacizumab. J Surg Oncol 2005; 91:173–80.

Shah, M, et al: A Multicenter Phase II study of Irinotecan, cisplatin and Bevacizumab in patients with metastatic Gastric or GEJ adenocarcinoma. J Clin Oncol 24: 5201-5206

Singer CF, Kostler WJ, Hudelist G: Predicting the efficacy of trastuzumab-based therapy in breast cancer: current standards and future strategies. Biochim Biophys Acta, 2008 Dec;1786 (2):105-13. Epub 2008 Mar 4

Slamon DJ, et al: Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987; 235(4785):177-82.

Slamon DJ, et al: Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989; 244(4905):707-12.

Slamon DJ, et al: Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001; 344(11):783-92.

Slamon DJ, Eiermann W, Robert N, et al. Phase III trial comparing AC-T with AC-TH and with TCH in the adjuvant treatment of HER2 positive early breast cancer patients: second interim efficacy analysis. San Antonio Breast Ca Symp, 2006

Song Y, Huang J, Wang JW: Relationship between HER2/neu gene amplification and protein expression and prognosis in patients with advanced gastric carcinoma: (Article in Chinese) Ai Zheng.2010 Jan;29 (1): 76-81

Smith JW, Buyse M, Rastogi C, Geyer C, Jacobs S, et al. Epiriubicin plus cyclophosphamide followed by docetaxel plus trast and bev as neoadjuvant therapy for HER-2 positive locally advance breast cancer or as adjuvant therapy for stage III breast cancer: A phase II trial of the NSABP foundation research Group.; 2009 ASCO Meeting; Abstract:580

Stewart DJ, Mikhael NZ, Nair RC, et al. Platinum concentrations in human autopsy tumor samples. Am J Clin Oncol 1988;11:152-8.

Tokunaga A, Onda M, Okuda T et al. Clinical significance of epidermal growth factor (EGF), EGF receptor and c-erb-b2 in human gastric cancer. Cancer 1995; 75:1418-1425

Tsukada Y, Fouad A, Pickren JW, et al: Central nervous system metastasis from breast carcinoma. Autopsy study. Cancer 52:2349-54, 1983

Van Cutsem E, Kang Y, Chung, L, et al. Efficacy results from the ToGA trail: A phase III study of trastuzumab added to standard chemotherapy in first-line human epidermal growth factor receptor 2 (HER2)-positive advanced gastric cancer. ASCO 2009: J Clin Oncol 27:18s, 2009 (suppl:abstr LBA 4509)

Vlasveld LT, Beynen JH, Boogerd W, et al: Complete remission of brain metastases of ovarian cancer following high-dose carboplatin: a case report and pharmacokinetic study. Cancer Chemother Pharmacol 25:382-3, 1990

Vogel CL, et al: Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. J Clin Oncol 2002; 20(3):719-26.

Von Minckwitz G, Vogel P, Schmidt M, et al. Trastuzumab treatment beyond progression in patients with HER2-positive metastatic breast cancer – the TBP study (BDB26/GIB 3-05). Breast Cancer Res Treat 2007; 106(Suppl 1): abstract 4056

Vredenburgh JJ, Desjardins A, Herndon JE, et al. Bevacizumab plus irinotecan in recurrent glioblastoma multiforme. J Clin Oncol 2007;25:4722-29.

Wedam SB, Low JA, Yang X, et al. A pilot study to evaluate response and angiogenesis after treatment with bevacizumab in patients with inflammatory breast cancer. J Clin Oncol 2004; 22 Suppl 14S:578.

Yardley D.A., Raefsky E, Castillo A, et al: Results of a multicenter pilot study of weekly nab-paclitaxel, carboplatin with bevacizumab and trastuzumab as neoadjuvant therapy in HER-2 postitive locally advanced breast cancer with SPARC. 2009 ASCO annual meeting; Abstract: 527

Yonemura Y, Ninomiya I, Yamaguchi A et al. Evaluation of innomreactivity for erb-2 protein as a marker of poor short term prognosis in gastric cancer. Cancer Res 1991; 51:1034-1038cv

17. APPENDICES

Appendix A: Patient Diary

09-457
Patient initials_____ Cycle_____

MRN_____

Take capecitabine (xeloda) tablets twice a day 30 minutes after eating. Take with at least 8 oz of water. Try to take 12 hours apart. If you vomit or miss a dose, please note it on diary and do not make up the dose.
***If day 1 start is delayed until the afternoon, take only one dose on day 1 and take the final dose on day 15 in the morning.
The use of a daily moisturizer to hands, feet and face are recommended. Please use sunscreen when going outdoors.

Your dose will be _____tablets in the morning and _____tablets in the evening

Day/Date	**Capecitabine** *Check box and note time taken below*: **AM**	**PM**	**Comments**
1	□ __:__	□ __:__	
2	□ __:__	□ __:__	
3	□ __:__	□ __:__	
4	□ __:__	□ __:__	
5	□ __:__	□ __:__	
6	□ __:__	□ __:__	
7	□ __:__	□ __:__	
8	□ __:__	□ __:__	
9	□ __:__	□ __:__	
10	□ __:__	□ __:__	
11	□ __:__	□ __:__	
12	□ __:__	□ __:__	
13	□ __:__	□ __:__	
14	□ __:__	□ __:__	
15	*** □ __:__		****only if day 1 start was delayed*
16			
17			
18			
19			
20			
21			

Patient Signature: _____Date: _____

Research Team Signature: _____ Date: _____

Appendix B:

Performance Status Criteria:

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Description	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.

Appendix C: Urine Protein/Creatinine Ratio (UPCR):

Procedure for Obtaining Urine Protein / Creatinine Ratio (UPCR)

- Obtain at least 4 ml of a random urine sample (does not have to be a 24 hour urine)
- Determine protein concentration (mg/dL)
- Determine creatinine concentration (mg/dL)
- Divide results of protein concentration by creatinine concentration.
- (Urine protein / creatinine ratio = protein concentration (mg /dL) / creatinine concentration (mg /dL))

DFCI IRB Protocol #: 09-457

APPENDIX D

Dana-Farber/Harvard Cancer Center
Multi-Center Data and Safety Monitoring Plan

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1.0 INTRODUCTION

The Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (DF/HCC DSMP) outlines the procedures for conducting a DF/HCC Multi-Center research protocol. The DF/HCC DSMP should serve as a reference for any sites external to DF/HCC that will be participating in the research protocol.

1.1 Purpose

To establish standards that will ensure that a Dana-Farber/Harvard Cancer Center Multi-Center protocol will comply with Federal Regulations, Health Insurance Portability and Accountability Act (HIPAA) requirements and applicable DF/HCC Standard Operating Procedures, and Cancer Therapy Evaluation Program (CTEP) Multi-Center Guidelines.

1.2 Multi-Center Data and Safety Monitoring Plan Definitions

DF/HCC Multi-center Protocol: A research protocol in which one or more outside institutions are collaborating with Dana-Farber/Harvard Cancer Center where a DF/HCC investigator is the sponsor. DF/HCC includes Dana-Farber/Partners Cancer Care (DF/PCC) Network Clinical Trial Affiliates.

Lead Institution: One of the Dana-Farber/Harvard Cancer Center consortium members (Dana-Farber Cancer Institute (DFCI), Massachusetts General Hospital (MGH), Beth Israel Deaconess Medical Center (BIDMC), Children's Hospital Boston (CHB), Brigham and Women's Hospital (BWH)) responsible for the coordination, development, submission, and approval of a protocol as well as its subsequent amendments per the DFCI IRB and applicable regulatory guidelines (CTEP, Food and Drug Administration (FDA), Office of Biotechnology Activities (OBA) etc.). The Lead Institution is typically the home of the DF/HCC Sponsor. The Lead Institution also typically serves as the Coordinating Center for the DF/HCC Multi-Center Protocol.

DF/HCC Sponsor: The person sponsoring the submitted Multi-Center protocol. Within DF/HCC, this person is the Overall Principal Investigator who takes responsibility for initiation, management and conduct of the protocol at all research locations. In applicable protocols, the DF/HCC Sponsor will serve as the single liaison with any regulatory agencies (i.e. CTEP Protocol and Information Office (PIO), FDA, OBA etc.). The DF/HCC Sponsor has ultimate authority over the protocol and is responsible for the conduct of the study at DF/HCC and all Participating Institutions. In most cases the DF/HCC Sponsor is the same person as the DF/HCC Principal Investigator; however, both roles can be filled by two different people. For CTEP Protocols this person is called the Protocol Chair.

Participating Institution: An institution that is outside the DF/HCC and DF/PCC consortium that is collaborating with DF/HCC on a protocol where the sponsor is a DF/HCC Investigator. The Participating Institution acknowledges the DF/HCC Sponsor as having the ultimate authority and responsibility for the overall conduct of the study.

Coordinating Center: The entity (i.e. Lead Institution, Medical Monitor, Contract Research

Organization (CRO), etc) that provides administrative support to the DF/HCC Sponsor in order that he/she may fulfill the responsibilities outlined in the protocol document and DSMP, and as specified in applicable regulatory guidelines (i.e. CTEP Multi-Center Guidelines). In general, the Lead Institution is the Coordinating Center for the DF/HCC Multi-Center Protocol. Should the DF/HCC Sponsor decide to use a CRO, the CRO will be deemed the Coordinating Center.

DF/HCC Quality Assurance Office for Clinical Trials: A unit within DF/HCC developed to computerize and manage data, and to provide a Quality Control and Quality Assurance function for DF/HCC trials.

2.0 GENERAL ROLES AND RESPONSIBILITIES

For DF/HCC Multi-Center Protocols, the DF/HCC Sponsor, the Coordinating Center, and the Participating Institutions are expected to adhere to the following general responsibilities:

2.1 DF/HCC Sponsor

The DF/HCC Sponsor, Peter C. Enzinger, MD will accept responsibility for all aspects of conducting a DF/HCC Multi-Center protocol which includes but is not limited to:

- Oversee the coordination, development, submission, and approval of the protocol as well as subsequent amendments.
- Ensure that the investigators, study team members, and Participating Institutions are qualified and appropriately resourced to conduct the protocol.
- Submit the Multi-Center Data and Safety Monitoring Plan as an appendix to the protocol.
- Assure all Participating Institutions are using the correct version of the protocol.
- Ensure that each participating investigator and study team receives adequate protocol training and/or a Site Initiation Visit prior to enrolling participants and throughout trial's conduct as needed.
- Ensure the protocol will be provided to each participating site in a language understandable to all site personnel when English is not the primary language.
- Monitor progress and overall conduct of the study at all Participating Institutions.
- Ensure all DFCI Institutional Review Board (IRB), DF/HCC and other applicable (i.e. CTEP, FDA, OBA) reporting requirements are met.
- Review data and maintain timely submission of data for study analysis.
- Act as the single liaison with CTEP/PIO Office (CTEP trials) or FDA (investigator-held IND trials) or OBA (gene therapy trials), as applicable.
- Ensure compliance with all requirements as set forth in the Code of Federal Regulations, applicable DF/HCC requirements, HIPAA requirements, and the approved protocol.
- Commit to the provision that the protocol will not be rewritten or modified by anyone other than the DF/HCC Sponsor.
- Identify and qualify Participating Institutions and obtain accrual commitments prior to extending the protocol to that site.

2.2 Coordinating Center

The Coordinating Center, Dana-Farber Cancer Institute, will assume the following general responsibilities:

- Assist in protocol development
- Maintain copies of Federal Wide Assurance and Institutional Review Board (IRB) approvals from all Participating Institutions.
- Maintain CTEP, FDA or OBA correspondence, as applicable.
- Maintain updated roster of participants.
- Verify eligibility.
- Verify response.
- Oversee the data collection process from Participating Institutions.
- Maintain documentation of Serious Adverse Event (SAE) reports submitted by Participating Institutions and submit to DF/HCC Sponsor for timely review.
- Distribute adverse events reported to the DF/HCC Sponsor that fall under the DFCI IRB Adverse Event Reporting Policy to all participating investigators.
- Provide Participating Institutions with information regarding DF/HCC requirements that they will be expected to comply with.
- Monitor Participating Institutions either by on-site or virtual monitoring.
- Maintain Regulatory documents of all Participating Institutions.
- Conduct regular communications with all Participating Institutions (conference calls, emails, etc).
- Maintain documentation of all communications.
- Ensure that each Participating Institution has the appropriate assurance on file with the Office of Human Research Protection (OHRP).

2.3 DF/HCC Quality Assurance Office for Clinical Trials (QACT)

In addition to the Coordinating Center, the DF/HCC QACT provides the following support services to assist the DF/HCC Sponsor:

- Develop protocol specific case report forms (CRF/eCRFS).
- QA/QC data of protocol specific CRFs.
- Provide a central participant registration, which includes review of consent and eligibility.
- Provide auditing services (funding and QACT approval required).

2.4 Participating Institution

Each Participating Institution is expected to comply with all applicable Federal Regulations and DF/HCC requirements, the protocol and HIPAA requirements. All Participating Institutions will provide a list of personnel assigned to the role for oversight of data management at their site to the Coordinating Center.

The general responsibilities for each Participating Institution are as follows:

- Commit to the accrual of participants to the protocol.

- Submit protocol and/or amendments to their local IRB.
- Maintain a regulatory binder in accordance with DF/HCC requirements.
- Provide the Coordinating Center with regulatory documents as requested.
- Participate in protocol training prior to enrolling participants and throughout the trial as needed (i.e. teleconferences).
- Update Coordinating Center with research staff changes on a timely basis.
- Register participants through the Coordinating Center.
- Submit source documents, research records, and CRFs per protocol specific submission guidelines to the Coordinating Center.
- Submit Serious Adverse Event (SAE) reports to local IRB per local requirements and to the Coordinating Center, in accordance with DF/HCC requirements. For CTEP trials, submit SAE reports directly to CTEP and provide copies to the Coordinating Center
- Submit protocol deviations and violations to local IRB per local requirements and to the DF/HCC Sponsor in accordance with DF/HCC requirements.
- Secure and store investigational agents and/or other protocol mandated drugs per federal guidelines and protocol requirements.
- Have office space, office equipment, and internet access that meet HIPAA standards.
- For protocols using investigational agents, the Participating Institution will order their own investigational agents regardless of the supplier (i.e. National Cancer Institute (NCI), pharmaceutical company). [This is the preferred method of ordering investigational agent. Some pharmaceutical companies may require the Coordinating Center to order study drug for external sites. Contact pharmacy to confirm this is feasible. Revise as necessary.]

3.0 DF/HCC REQUIREMENTS FOR MULTI-CENTER PROTOCOLS

The following section will clarify DF/HCC Requirements and further detail the expectations for participating in a DF/HCC Multi-Center protocol.

3.1 Protocol Distribution

The Coordinating Center will distribute the final DFCI IRB approved protocol and any subsequent amended protocols to all Participating Institutions.

3.2 Protocol Revisions and Closures

The Participating Institutions will receive notification of protocol revisions and closures from the Coordinating Center. It is the individual Participating Institution's responsibility to notify its IRB of these revisions.

- **Non life-threatening revisions:** Participating Institutions will receive written notification of protocol revisions regarding non life-threatening events from the Coordinating Center. Non-life-threatening protocol revisions must be IRB approved and implemented within 90 days from receipt of the notification.

- **Revisions for life-threatening causes:** Participating Institutions will receive immediate

notification from the Coordinating Center concerning protocol revisions required to protect lives with follow-up by fax, mail, e-mail, etc. Life-threatening protocol revisions will be implemented immediately followed by IRB request for approval.

- **Protocol closures and temporary holds:** Participating Institutions will receive notification of protocol closures and temporary holds from the Coordinating Center. Closures and holds will be effective immediately. In addition, the Coordinating Center, will update the Participating Institutions on an ongoing basis about protocol accrual data so that they will be aware of imminent protocol closures.

3.3 Informed Consent Requirements

The DF/HCC approved informed consent document will serve as a template for the informed consent for Participating Institutions. The Participating Institution consent form must follow the consent template as closely as possible and should adhere to specifications outlined in the DF/HCC Guidance Document on Model Consent Language for PI-Initiated Multi-Center Protocols. This document will be provided separately to each Participating Institution.

Participating Institutions are to send their version of the informed consent document and HIPAA authorization, if a separate document, to the Coordinating Center for review and approval prior to submission to their local IRB. The approved consent form must also be submitted to the Coordinating Center after approval by the local IRB.

The Principal Investigator (PI) at each Participating Institution will identify the physician members of the study team who will be obtaining consent and signing the consent form for therapeutic protocols. **Participating institutions must follow the DF/HCC requirement that only attending physicians obtain informed consent and re-consent to interventional trials (i.e. drug and/or device trials)**.

3.4 IRB Documentation

The following must be on file with the Coordinating Center:

- Approval letter of the Participating Institution's IRB
- Copy of the Informed Consent Form approved by the Participating Institution’s IRB
- Participating IRB’s approval for all amendments

It is the Participating Institution's responsibility to notify its IRB of protocol amendments. Participating Institutions will have 90 days from receipt to provide the Coordinating Center their IRB approval for amendments to a protocol.

3.5 IRB Re-Approval

Verification of IRB re-approval from the Participating Institutions is required in order to continue research activities. There is no grace period for continuing approvals.

The Coordinating Center will not register participants if a re-approval letter is not received from the Participating Institution on or before the anniversary of the previous approval date.

3.6 Participant Confidentiality and Authorization Statement

In 1996, congress passed the first federal law covering the privacy of health information known as the Health Insurance Portability and Accountability Act (HIPAA). Any information, related to the physical or mental health of an individual is called Protected Health Information (PHI). HIPAA outlines how and under what circumstances PHI can be used or disclosed.

In order for covered entities to use or disclose protected health information during the course of a study, the study participant must sign an Authorization. This Authorization may or may not be separate from the informed consent document. The Coordinating Center, with the approval from the DFCI IRB and if applicable NCI/CTEP, will provide a consent template, which covered entities (Participating Institutions) must use.

The DF/HCC Sponsor will use all efforts to limit its use of protected health information in its trials. However, because of the nature of these trials, certain protected health information must be collected per NCI requirements. These are the primary reasons why DF/HCC has chosen to use Authorizations, signed by the participant in the trial, rather than limited data sets with data use agreements.

3.6.1 DF/HCC Multi-Center Protocol Confidentiality

All documents, investigative reports, or information relating to the participant are strictly confidential. Whenever reasonably feasible, any participant specific reports (i.e. Pathology Reports, MRI Reports, Operative Reports, etc.) submitted to the Coordinating Center must have the participant's full name & social security number "blacked out" and the assigned DF/HCC QACT case number (as described below) and DF/HCC protocol number written in (with the exception of the signed informed consent document). Participant initials may only be included or retained for cross verification of identification

3.7 DF/HCC Multi-Center Protocol Registration Policy

Refer to protocol section 4.0 for protocol registration procedures.

3.7.1 Initiation of Therapy

Participants must be registered with the DF/HCC QACT and according to local institution guidelines before receiving treatment. Treatment may not be initiated until the Participating Institution receives a faxed or e-mailed copy of the participant's registration confirmation memo from the Coordinating Center. Therapy must be initiated per protocol guidelines. The DF/HCC Sponsor and DFCI IRB must be notified of any exceptions to this policy.

3.7.2 Eligibility Exceptions

The DF/HCC QACT will make no exceptions to the eligibility requirements for a protocol without DFCI IRB approval. The DF/HCC QACT requires each institution to fully comply with this requirement.

3.7.3 Verification of Registration, Dose Levels, and Arm Designation

A registration confirmation memo for participants registered to DF/HCC Multi-Center Protocol will be faxed or emailed to the registering institution within one business day of the registration. Treatment may not be initiated until the site receives a faxed or e-mailed copy of the registration confirmation memo and once the participant has been registered according to local requirements.

3.8 DF/HCC Protocol Case Number

Once eligibility has been established and the participant successfully registered, the participant is assigned a five digit protocol case number. This number is unique to the participant on this trial and must be used for QACT CRF/eCRF completion and correspondence, and correspondence with the Coordinating Center.

3.9 Protocol Deviations, Exceptions and Violations

Federal Regulations require an IRB to review proposed changes in a research activity to ensure that researchers do not initiate changes in approved research without IRB review and approval, except when necessary to eliminate apparent immediate hazards to the participant. DF/HCC requires all departures from the defined procedures set forth in the IRB approved protocol to be reported to the DF/HCC Sponsor, who in turn is responsible for reporting to the DFCI IRB.

For reporting purposes, DF/HCC uses the terms “violation”, “deviation” and “exception” to describe derivations from a protocol. All Participating Institutions must adhere to these requirements for reporting to the DF/HCC Sponsor and will follow their institutional policy for reporting to their local IRB.

3.9.1 Definitions

Protocol Deviation: Any departure from the defined procedures set forth in the IRB-approved protocol which is *prospectively approved* prior to its implementation.

Protocol Exception: Any protocol deviation that relates to the eligibility criteria, e.g. enrollment of a participant who does not meet all inclusion/exclusion criteria.

Protocol Violation: Any protocol deviation that was not *prospectively approved* by the IRB prior to its initiation or implementation.

3.9.2 Reporting Procedures

DF/HCC Sponsor: is responsible for ensuring that clear documentation is available in the medical record and/or regulatory documents to describe all protocol exceptions, deviations and violations. The DF/HCC Sponsor will also be responsible for ensuring that all protocol violations/deviations are promptly reported per DFCI IRB guidelines.

Participating Institutions: Protocol deviations require prospective approval from the DFCI IRB. The Participating Institution must submit the deviation request to the Coordinating Center who will then submit the deviation request to the DFCI IRB. Upon DFCI IRB approval the deviation is submitted to the Participating Institution IRB, per institutional policy. A copy of the Participating Institution's IRB report and determination will be forwarded to the Coordinating Center within 10 business days after the original submission.

All protocol violations must be sent to the Coordinating Center in a timely manner.

Coordinating Center: Upon receipt of the violation/deviation report from the Participating Institution, the Coordinating Center will submit the report to the DF/HCC Sponsor for review. Subsequently, the Participating Institution's IRB violation/deviation report will be submitted to the DFCI IRB for review per DFCI IRB reporting guidelines. DF/HCC will forward all violation reports to CTEP via an internal DF/HCC process, as applicable.

3.10 Safety Assessments and Toxicity Monitoring

The study teams at all participating institutions are responsible for protecting the safety, rights and well-being of study participants. Recording and reporting of adverse events that occur during the course of a study help ensure the continuing safety of study participants.

All participants receiving investigational agents and/or other protocol mandated treatment will be evaluated for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical examination findings, and spontaneous reports of adverse events reported by participants. All toxicities encountered during the study will be evaluated according to the NCI criteria specified in the protocol. Life-threatening toxicities must be reported immediately to the DF/HCC Sponsor via the Coordinating Center. Protocols using CTEP supplied agents must also report these toxicities via the AdEERS system.

Additional safety assessments and toxicity monitoring will be outlined in the protocol.

3.10.1 Guidelines for Reporting Serious Adverse Events

Guidelines for reporting Adverse Events (AEs) and Serious Adverse Events (SAEs) are detailed in protocol section 11.4.

Participating Institutions must report the AEs to the DF/HCC Sponsor and the Coordinating Center following the DFCI IRB SAE Reporting Requirements.

The Coordinating Center will maintain documentation of all Participating Institution Serious Adverse Event reports and be responsible for communicating to all participating investigators, any observations reportable under the DFCI IRB Reporting Requirements. The Participating Institutions will review and submit to their IRB according to their institutional policies and procedures.

3.10.2 Guidelines for Processing IND Safety Reports

FDA regulations require sponsors of clinical studies to notify the FDA and all participating investigators of any adverse experience associated with the use of the investigational agent that is both serious and unexpected. The DF/HCC Sponsor will review all IND Safety Reports and ensure that all IND Safety Reports are distributed to the Participating Institutions. The Participating Investigators are to review, send a copy to their IRB according to their local IRB's policies and procedures, and file a copy with their regulatory documents.

3.11 Data Management

The DF/HCC QACT develops a set of electronic case report forms (CRF/eCRFs), for use with the protocol. These forms are designed to collect data for each study. The DF/HCC QACT provides a web based training for eCRF users. Refer to protocol section 12.1.2 for the schedule for completion and submission of case report forms (paper or electronic) to the QACT.

3.11.1 Data Forms Review

When data forms arrive at the DF/HCC QACT, they are reviewed for completeness, protocol treatment compliance, adverse events (toxicities) and response. Data submissions are monitored for timeliness and completeness of submission. Participating Institutions are notified of their data submission delinquencies in accordance with the following:

Incomplete or Questionable Data

If study forms are received with missing or questionable data, the submitting institution will receive a written or electronic query from the DF/HCC QACT Data Analyst or study monitor. Responses to all queries should be completed and submitted within 14 calendar days. Responses may be returned on the written query or on an amended paper case report form, or in the case of electronic queries, within the electronic data capture (eDC) system. In the case of a written query for data submitted on a paper case report form, the query must be attached to the specific data being re-submitted in response.

Missing Forms

If study forms are not submitted on schedule, the Participating Institution will receive a Missing Form Report from the Coordinating Center noting the missing forms. These reports are compiled by the DF/HCC QACT and distributed a minimum of four times a year.

4.0 REQUISITIONING INVESTIGATIONAL DRUG

The ordering of investigational agent is specified in protocol section 7.1.

5.0 MONITORING: QUALITY CONTROL

The quality control process for a clinical trial requires verification of protocol compliance and data accuracy. The Coordinating Center, with the aid of the QACT provides quality control oversight for the protocol.

5.1 Ongoing Monitoring of Protocol Compliance

The Participating Institutions will be required to submit participant source documents to the Coordinating Center for monitoring. Participating Institution will also be subject to at least one on-site monitoring conducted by the Coordinating Center's Clinical Trial Specialist.

The Coordinating Center will implement ongoing monitoring activities to ensure that Participating Institutions are complying with regulatory and protocol requirements, data quality, and participant safety. Monitoring will occur before the clinical phase of the protocol begins, continue during protocol performance and through study completion. Additional monitoring practices may include but are not limited to; source verification, review and analysis of the following: eligibility requirements of all participants, informed consent procedures, adverse events and all associated documentation, study drug administration/treatment, regulatory files, protocol deviations, pharmacy records, response assessments, and data management.

The DF/HCC Lead Institution will maintain regular and ongoing communication to Participating Institutions about study related information.

On-Site Monitoring will occur at least once per non DF/HCC site. Initial site visit to occur after first participant is enrolled or after study has been activated for 9 months, whichever comes first. Additional on-site monitoring may occur at the request of the DF/HCC Sponsor. Participating Institutions will be required to provide access to participants' complete medical record and source documents for source documentation verification and Site Regulatory Binder.

Virtual Monitoring will take place for verification of informed consent and eligibility, study visit source documents and CRF, investigational product accountability, CRF review for missing data and query resolution, AEs, SAEs, and protocol endpoints unless on-site monitoring is requested by the DF/HCC Sponsor. Participating Institutions will be required to forward de-identified copies of participants' medical record and source documents to the Coordinating Center.

All data submitted to the DF/HCC QACT will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. The Coordinating Center and if applicable, QACT Data Analysts assigned to the protocol, will perform the ongoing protocol data compliance monitoring.

5.2 Evaluation of Participating Institution Performance

5.2.1 Monitoring Reports

The DF/HCC Sponsor will review all monitoring reports for on-site and virtual monitoring of Participating Institutions to ensure protocol compliance and ability to fulfill responsibilities of participating in the study. The DF/HCC Sponsor may increase the monitoring activities at Participating Institutions that are unable to comply with the protocol, DF/HCC Sponsor requirements or federal and local regulations. Participating Institutions may also be subject to an audit as determined by the DF/HCC Sponsor.

6.0 AUDITING: QUALITY ASSURANCE

Auditing is a method of Quality Assurance. Its main focus is to measure whether standards and procedures were followed. Auditing is the systematic and independent examination of all trial related activities and documents. Audits determine if evaluated activities were appropriately conducted and whether data was generated, recorded and analyzed, and accurately reported per the protocol, Standard Operating Procedures (SOPs), and the Code of Federal Regulations (CFR).

6.1 DF/HCC Sponsored Trials

Auditing of this protocol will take place as determined by DFCI QACT. One on-site audit will be scheduled by the QACT, assuming at least three participants have been treated on protocol at the site. Approximately 3-4 participants would be audited at the site over a 2 day period. If violations which impact participant safety or the integrity of the study are found, more participant records may be audited.

6.2 Participating Institution

It is the Participating Institution's responsibility to notify the Coordinating Center of all scheduled audit dates (internal or NCI) and re-audit dates (if applicable), which involve this protocol. All institutions will forward a copy of final audit and/or re-audit reports and corrective action plans (if applicable) to the Coordinating Center, within 12 weeks after the audit date.

6.3 DF/HCC Sponsor and Coordinating Center

The DF/HCC Sponsor will review all final audit reports and corrective action plans if applicable. The Coordinating Center, must forward these reports to the DF/HCC QACT per DF/HCC policy for review by the DF/HCC Audit Committee. Based upon the audit assessments the DF/HCC Audit Committee could accept or conditionally accept the audit rating and final report.

Conditional approval could require the DF/HCC Sponsor to implement recommendations or require further follow-up. For unacceptable audits, the DF/HCC Audit Committee would forward the final audit report and corrective action plan to the DFCI IRB as applicable.

6.4 Sub-Standard Performance

The DF/HCC Sponsor, DFCI IRB and the NCI for CTEP trials, is charged with considering the totality of an institution's performance in considering institutional participation in the protocol.

6.4.1 Corrective Actions

Participating Institutions that fail to meet the performance goals of accrual, submission of timely accurate data, adherence to protocol requirements, and compliance with state and federal regulations, will be recommended for a six-month probation period. Such institutions must respond with a corrective action plan and must demonstrate during the probation period that deficiencies have been corrected, as evidenced by the improved performance measures. Participating Institutions that fail to demonstrate significant improvement will be considered by the DF/HCC Sponsor for revocation of participation.