**IRB#: 06-114A(6)**

# A PHASE II STUDY OF HEPATIC ARTERIAL INFUSION WITH FLOXURIDINE AND DEXAMETHASONE IN COMBINATION WITH INTRAVENOUS BEVACIZUMAB (A MONOCLONAL ANTIBODY TO VASCULAR ENDOTHELIAL GROWTH FACTOR-A), IN PATIENTS WITH UNRESECTABLE PRIMARY HEPATIC MALIGNANCY

MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

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**Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.**

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## Table of Contents

## 1.0 PROTOCOL SUMMARY AND/OR SCHEMA

This phase II study evaluates the efficacy of regional chemotherapy plus Bevacizumab in patients with unresectable primary hepatic malignancy. Specifically, eligible patients with unresectable hepatocellular carcinoma (HCC) and intrahepatic or peripheral cholangiocarcinoma (ICC) will undergo hepatic artery pump placement for continuous infusion of floxuridine (FUDR) combined with systemic IV Bevacizumab. A recent phase II study at MSKCC evaluated hepatic arterial FUDR alone in this same group of patients and found an overall response rate of 39% with a median time to progression (TTP) of 7.3 months. At 1 year, 84.6% of patients were alive, with a median follow up of 8.8 months. The current protocol seeks to improve on these initial encouraging results by enhancing FUDR delivery to liver tumors.

Emerging data suggest that 'normalization' of tumor vasculature with subsequent improvement in oxygenation and delivery of cytotoxic agents is a major mechanism of action of Bevacizumab. We hypothesize that such changes in liver tumor vasculature will increase the response rate and prolong the time to progression in patients undergoing concomitant treatment with regional FUDR. We further speculate that the addition of Bevacizumab will result in measurable changes in tumor perfusion kinetics compared to baseline data with FUDR treatment alone. The protocol includes radiological and biological correlative studies.

All patients enrolled in the study will begin continuous HAI FUDR at 0.16 mg/kg/day. A total of 55 patients with unresectable HCC or ICC will be treated with HAI FUDR plus systemic Bevacizumab. All patients will receive HAI FUDR (0.16 mg/kg X pump volume / pump flow rate), Dexamethasone (Dex) (1 mg/day X pump volume / pump flow rate) and IV Bevacizumab 5 mg/kg every other week.

The primary objective of the study is to assess the median time to progression of HAI FUDR/Dex + Bevacizumab. The calculated sample size will provide 90% power to detect a 50% improvement in TTP using HAI FUDR + BEV, over that observed using HAI FUDR alone. Improved TTP will be considered indicative of improved efficacy and will serve as the baseline for a future comparative study.

The secondary objective is to further investigate the utility of dynamic contract enhanced MRI (DCE-MRI) for assessing initial tumor perfusion prior to treatment, monitoring changes in tumor perfusion during treatment, and correlating these findings with radiographic tumor response.

## 2.0 OBJECTIVES AND SCIENTIFIC AIMS

### 2.1 PRIMARY

To assess the median time to progression of continuous (HAI) of FUDR/Dex plus Bevacizumab in patients with unresectable hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC).

### 2.2 SECONDARY

To investigate further the utility of dynamic contrast enhanced MRI (DCE-MRI) for assessing initial tumor perfusion prior to treatment, monitoring changes in tumor perfusion during treatment and in correlating these findings with radiographic tumor response.

### 2.3 EXPLORATORY

2.3.1 To assess the expression pattern of VEGFR1, VEGFR2, and VEGFR3 and their cognate ligands (including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and P1GF), and correlate with patient progression and survival following therapy.

2.3.2 To assess the pro-angiogenic activity of peripheral blood before and during treatment.

2.3.3 To assess tumors for immunohistochemical markers of hypoxia (hypoxia inducible factor [HIF-1$\alpha$], carbonic anhydrase IX [CA IX], and the glucose transporters Glut-1 and Glut-3) for correlation with initial and treatment-related changes in perfusion and permeability, as determined by DCE-MRI.

## 3.0 BACKGROUND AND RATIONALE

### 3.1 INTRODUCTION

Hepatocellular carcinoma (HCC) and primary intrahepatic cholangiocarcinoma (ICC) account for nearly all primary hepatic malignancies. HCC is among the most common cancers in the world, responsible for nearly 1 million annual deaths. Previously considered uncommon in Western countries, HCC incidence and mortality have both increased substantially in the United States over the past several years. ICC is much less common than HCC but its incidence and associated mortality have likewise increased. A recent analysis of Surveillance, Epidemiology and End Results (SEER) data showed a 9% annual percentage increase in incidence of ICC and a 10-fold increase in ICC-related mortality since 1973.

Complete resection remains the most effective therapy for both tumors. Resection is associated with 25-40% 5-year survival but is not possible in many patients. In most reported series, one-third of patients or fewer with HCC are amenable to a potentially

curative resection. The rarity with which ICC is encountered makes it difficult to accurately assess overall resectability, since most studies only report patients submitted to operation. A report from MSKCC found a 62% resectability rate for ICC but did not include patients with unresectable disease at presentation. For both tumors, locally advanced disease confined to the liver is the predominant cause of unresectability. In addition, underlying hepatic parenchymal disease (ie, chronic hepatitis, cirrhosis) is also a potentially treatment-limiting factor in patients with HCC, less so for those with ICC.

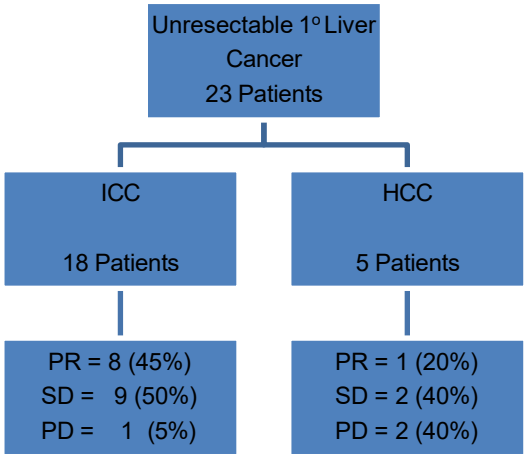
Patients with unresectable primary liver cancer (HCC or ICC) have a median survival of 12 months or less, and non-resectional therapies have had generally disappointing results. For both tumors, multiple systemic regimens have been evaluated and shown to have limited efficacy. Modest activity (response rate of approximately 20%) of irinotecan-based regimens has been reported for both HCC and cholangiocarcinoma, although no complete responses. More recent studies using gemcitabine and oxaliplatin have not resulted in much improvement in response rates. Ablative approaches (cryoablation, ethanol injection, radiofrequency ablation) are often used in patients with unresectable HCC but their impact on disease natural history is unclear. Furthermore, most patients present with large tumors (> 5 cm) or multifocal disease, neither of which can be effectively ablated. Hepatic artery embolization or chemoembolization may have some utility in patients with HCC but there are conflicting data regarding the survival benefit of this approach. In contrast to HCC, experience with ablative techniques for ICC is extremely limited. ICC are typically firm tumors, making them unsuitable for ethanol injection, and most patients present with very large lesions that are inappropriate for other ablative approaches.

Thus, for many patients with HCC and ICC, effective treatment options are limited. Liver directed chemotherapy (HAI), delivered through a surgically implanted hepatic artery infusion pump, has been evaluated in several small series and appears to have greater efficacy than systemic therapy alone. Although used predominantly in patients with hepatic colorectal metastases, HAI chemotherapy has been shown to have efficacy in patients with primary liver cancer. In an initial study from MSKCC, 10 patients with primary hepatic malignancy (8 HCC, 2 ICC) were treated with continuous intrahepatic FUDR (0.3mg/kg/day over 14 days, alternating with heparinized saline for 14 days) combined with Mitomycin C 10 mg/m$^2$ given through a side port injection every 4 weeks. Four of 9 evaluable patients had a partial response (> 50% tumor reduction) and 2 had a minor response (25 – 50% tumor reduction). The median overall survival was 14.5 months after initiation of HAI therapy. In patients without cirrhosis, median survival was 27 months, compared to 5.2 months for those with cirrhosis. Additionally, 2 of 3 patients previously treated with systemic chemotherapy had a partial response. In this study, the treatment was well tolerated, with only 1 patient incurring any significant liver related toxicity (biliary sclerosis after 18 months of treatment). Furthermore, there was no evidence from this initial experience that patients with cirrhosis were less tolerant of liver-directed therapy than were patients with normal parenchyma. More recently,

investigators at MD Anderson Cancer Center reported results of 25 patients with unresectable HCC treated with a variety of continuous infusion regimens. In four patients (16%), tumor response was sufficient to allow for subsequent resection or radiofrequency ablation. Pathological analysis of resected specimens showed no viable tumor in one case and >90% necrosis in another. As in the study from MSKCC, these investigators did not find significant toxicity associated with this treatment approach. Additionally, Clavien et al recently reported their results using liver-directed plus systemic chemotherapy in patients with unresectable HCC. Using intrahepatic FUDR (0.2mg/kg/day) combined with bolus injection of cisplatin and adriamycin, the authors found that 5 patients with initially unresectable disease subsequently underwent a complete resection.

Experience with liver-directed chemotherapy in patients with biliary tract cancer is more limited, although results of small series have been reported. Smith et al reported 1 complete response (11%) and 6 partial responses (55%) out of 11 patients with cholangiocarcinoma and gallbladder carcinoma treated with bolus injection of hepatic arterial 5-FU and mitomycin C. Using continuous HAI FUDR through an implantable pump, Seeger et al. treated 3 patients with cholangiocarcinoma, 1 of whom had a complete pathological response.

A recent phase II study at MSKCC (IRB# 02-120) evaluated the efficacy of HAI FUDR with no systemic component in patients with disease confined to the liver, 18 with ICC and 5 with HCC. The overall response rate was 39% and the median TTP was 7.3 months. Partial responses appeared to be more common with ICC than HCC (45% vs. 20%), although the number of HCC patients was much lower (Figure 1). To date, 12 patients have developed disease progression, 6 (50%) of which were initially within the liver. Furthermore, the therapy was well tolerated, with only one grade 2 elevation in AST. The response rate and TTP observed in this study are higher than those reported for any systemic chemotherapeutic regimen and suggest that regional chemotherapeutic strategies have a potentially important role in the treatment of ICC and HCC.




## 3.2 INTRAHEPATIC FUDR

Several studies have shown that hepatic parenchymal tumors larger than a few millimeters in diameter derive most of their blood supply from the hepatic artery. Direct infusion of chemotherapeutic agents into the hepatic arterial system therefore exposes the tumor to higher drug concentrations than can be achieved with systemic administration. Several studies have shown that tumor response rates are higher with HAI therapy than with systemic treatment. In a prospective study of patients with metastatic colorectal cancer, patients were randomized to receive intrahepatic FUDR versus systemic FUDR. The partial response rate in the intrahepatic arm was 50% compared to 20% in the systemic arm. Furthermore, in a subsequent study, approximately one-third of patients with hepatic metastases that progressed on systemic therapy responded to intrahepatic FUDR. In this study, hepatic toxicity was dose-limiting. The addition of dexamethasone (20mg total dose for 14/28 days) has been shown to reduce hepatic toxicity, allowing administration of higher doses of FUDR and resulting in a higher response rate.

Patients with 1º hepatic malignancies appear to tolerate intrahepatic FUDR well. In the initial study from MSKCC, the median duration of treatment was 11.2 months; one patient experienced a transient rise in bilirubin and one patient developed biliary sclerosis. Transient, reversible elevations of liver enzymes occurred in 9 patients. It should be noted that 4/10 patients had cirrhosis and 3/10 had serologic evidence of chronic hepatitis. It should further be noted that the dose of FUDR used in this study was 0.3mg/kg/day, nearly twice that used in the more recent phase II study (0.16mg/kg/day). In the latter study, there was only one grade 2 elevation of liver enzymes noted.

## 3.3 SYSTEMIC BEVACIZUMAB

It is well established that tumor growth is critically dependent on the development of neovasculature for delivery of oxygen, nutrients, growth factors and other agents that promote proliferation. New blood vessel formation, or angiogenesis, is a complex process regulated by several pro- and anti-angiogenic factors. Growth of malignant solid tumors is associated with predominance of pro-angiogenic mediators, the so-called angiogenesis switch that promotes new blood vessel formation. This switch to a pro-angiogenic phenotype is a hallmark of malignant disease, and the extent to which it occurs is prognostic for some tumor types.

Of the known pro-angiogenic mediators, vascular endothelial growth factor (VEGF) is the most important. Binding of VEGF to its receptor(s) (VEGFR) activates several signaling pathways that promote multiple pro-angiogenic events, including survival and proliferation of endothelial cells, mobilization of endothelial progenitor cells from bone marrow, and increased vascular permeability. VEGF's ability to increase permeability of vascular beds led to its initial discovery (as vascular permeability factor) and results in deposition of several plasma proteins into the extra-vascular space that transform the normally anti-angiogenic stromal tissue into pro-angiogenic microenvironment. Over-expression of VEGF has been associated with poor prognosis in a number of different tumor types, and the VEGF/VEGFR pathway is now a major focus of anti-cancer research and drug development.

There are several secreted glycoproteins that comprise the VEGF family of angiogenic and lymphangiogenic growth factors: VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placenta growth factor (PlGF) -1 and -2. Of these proteins, VEGF-A (commonly referred to as VEGF) appears to be the most critical. The dominant isoform of VEGF-A is a 45-kDa homodimeric glycoprotein with a broad range of pro-angiogenic functions, both normal and pathologic. Alternative splicing of the VEGF-A gene gives rise to several mature isoforms, all of which are capable of binding to VEGFR (see below). The roles of VEGF-B, -C, -D and PlGF in tumor angiogenesis and vasculogenesis are less clear; VEGF-C and VEGF-D appear to be more important in lymphangiogenesis, although they may have some role in the development of new blood vessels in pathologic states. VEGF-E is a viral protein and not a mammalian VEGF homologue.

The VEGF ligands mediate their effects through 3 different VEGFR: VEGFR-1 (or Flt-1), VEGFR-2 (or Flt-1/KDR) and VEGFR-3 (or Flt-4). Expression of VEGFR is largely confined to endothelial cells, although VEGFR-1 and VEGFR-2 have been identified on some adult hematopoietic cells. Certain tumor cells have also been recently shown to express VEGFR, the significance of which is unclear. The various VEGF ligands have different receptor binding specificities: VEGF-A isoforms bind to VEGFR-1 and VEGFR-2; VEGF-B and PlGF bind only to VEGFR-1; VEGF-C and VEGF-D can bind to both VEGFR-2 and VEGFR-3. Most of the downstream angiogenesis- related events orchestrated by VEGF-A are mediated through VEGFR-2, and this interaction is among the most critical. VEGFR-1 plays an important role in fetal development but its role in

tumor angiogenesis is less clear. In adults, VEGFR-3 expression is limited to lymphatic endothelial cells, and through its interactions with VEGF-C and VEGF-D, mediates lymphangiogenesis. Increased expression of VEGF-C and VEGF-D, combined with activation of VEGFR-3, have been documented in several tumor types and are associated with lymph node metastases and may promote development of abnormal, tumor-related blood vessels. While tumor cells have the capacity of simultaneous expression of multiple VEGF ligands, VEGFR expression is more specific: VEGFR-2 is expressed by nearly all endothelial cells but expression of VEGFR-1 and VEGFR-3 is limited to distinct vascular beds.

VEGF expression is regulated by several different factors, including hypoxia (hypoxia inducible factor or HIF-1), several different cytokines and growth factors, oncogenes and tumor suppressor genes (including p53).

Substantial direct evidence for a role of VEGF in tumorigenesis has been provided by numerous studies showing that a murine anti-VEGF neutralizing antibody, alone or in combination with cytotoxic chemotherapy (see below), inhibits the growth of several different tumor types.

**Bevacizumab (rhuMAb VEGF)**

Bevacizumab is a recombinant humanized anti-VEGF-A monoclonal antibody composed of human IgG1 framework and antigen-binding complementarity-determining regions from a murine monoclonal antibody (rhuMAb VEGF A.4.6.1) that blocks binding of human VEGF-A to its receptors. Approximately 93% of the amino acid sequence, including most of the antibody framework, is derived from human IgG1.

Pre-clinical Studies:
This antibody has been shown to recognize all isoforms of VEGF. It fails to recognize other peptide growth factors tested (fibroblast growth factor, epidermal growth factor, hepatocyte growth factor, platelet-derived growth factor, and nerve growth factor). The binding affinity and specificity of rhuMAb VEGF (Bevacizumab) are essentially equivalent to those of its murine counterpart.

a. In vitro and in vivo VEGF studies:

A correlation has been noted between the degree of tumor vascularization and the level of VEGF mRNA expression. In virtually all specimens examined, VEGF mRNA is expressed in tumor cells but not in endothelial cells. In contrast, as previously noted, mRNAs for VEGFR are up-regulated in tumor-associated endothelial cells. These findings are consistent with the hypothesis that VEGF is primarily a paracrine mediator. Freeman et al. suggested that lymphocytes

infiltrating the tumor might constitute an additional source of VEGF that contributes to tumor angiogenesis

b. In vivo efficacy studies:

In agreement with the hypothesis that inhibition of neovascularization is the mechanism of tumor suppression, the density of blood vessels is significantly lower in sections of tumors from antibody-treated animals compared with control. These findings provided a direct demonstration that inhibition of endogenous VEGF can suppress the growth of established tumors in vivo. Furthermore, substantial evidence has emerged showing improved anti-tumor activity when anti-VEGF therapy is combined with other chemotherapeutic agents (see below).

**Clinical Experience**

Phase I/II Efficacy Results:
Antitumor activity has been demonstrated in multiple tumor types and with both single-agent Bevacizumab and combination therapy. Objective responses have been reported in a breast cancer trial (AVF0776g), including a complete response of supraclavicular nodal disease and partial responses of cervical node, liver, and skin-subcutaneous disease. In a NSCLC trial (AVF0757g), the data showed that Bevacizumab at a dose of 15 mg/kg every 3 weeks and in combination with Carboplatin/Paclitaxel chemotherapy, increased response rates and prolonged time to disease progression compared with chemotherapy alone.

In a separate trial of patients with colorectal cancer (AVF0780g), based on blinded assessments by an independent review facility, the hazard of experiencing disease progression was reduced by 57% in subjects receiving 5 mg/kg Bevacizumab plus 5-FU/Leucovorin alone ($p=0.005$). Improvements in response rate and median survival were also seen for subjects treated with 5 mg/kg Bevacizumab plus 5-FU/Leucovorin versus 5-FU/Leucovorin alone.

In a randomized, open-label, Phase III study (AVF2119g, metastatic breast cancer), the results of the primary efficacy analysis showed that treatment with the combination of Bevacizumab plus Capecitabine did not result in a statistically significant prolongation in progression-free survival compared with Capecitabine alone. There was a statistically significant increase in objective response rate (secondary endpoint); however, this did not result in an improvement in progression-free survival.

In a randomized, double-blind, placebo-controlled phase III study, AVF2107g, the addition of Bevacizumab (5mg/kg IV q2 weeks) to first line therapy for metastatic colorectal cancer (Irinotecan/5-Fluorouracil/Leucovorin (IFL)) resulted in a statistically and clinically significant improvement in median survival as

compared to the IFL + placebo control arm (15.6 vs 20.3 months, HR 0.65, p=0.00003). Improvements in median progression-free survival (6.2 vs 10.6 months, p=0.00001), objective response rate (34.7% vs 44.9%, p=0.0029) and response duration (7.1 vs 10.4 months, p=0.0014) were also achieved with the addition of Bevacizumab to IFL chemotherapy.

Safety of Bevacizumab:
In the initial Phase I and II clinical trials, four potential Bevacizumabd-associated safety signals were identified: hypertension, proteinuria, thromboembolic events, and hemorrhage. Additional completed Phase II and 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), gastrointestinal perforations, wound healing complications, and arterial thromboembolic events (ATE). These and other safety signals are described in further detail in section 11.2. For further information regarding safety and adverse event rates, please refer to the Bevacizumab investigator's brochure.

### 3.4 Rationale for HAI FUDR/Dex and Systemic Bevacizumab:

It was initially postulated that anti-angiogenic therapy would essentially starve tumors of blood, thereby preventing growth beyond that sustainable with a rudimentary vascular supply. While pre-clinical studies showed that anti-angiogenic therapy alone can cause substantial regression of solid tumors, the objective response rates of such an approach in human trials have been disappointingly low. Subsequent studies have shown that anti-angiogenic therapy combined with cytotoxic agents significantly enhances disease control and survival. A recent landmark report from Hurwitz et al (NEJM, 350;2335-42, 2004) showed that when IFL (irinotecan, 5-FU, leucovorin) chemotherapy was combined with Bevacizumab, progression-free survival increased from 6.2 months to 10.6 months and overall survival increased from 15.6 months to 20.3 months compare to IFL alone.

From this and other observations, the concept of vascular normalization through the use of anti-angiogenic agents has been put forward and has data support from human studies. The concept of enhanced efficacy as combination therapy would initially seem paradoxical, since anti-angiogenic agents would be expected to reduce rather than augment the delivery of other anti-tumor drugs. Although new blood vessel formation is critical for tumor growth, tumor vasculature is grossly abnormal, both structurally and functionally. Tumor vasculature lacks the organized arrangement of normal vascular beds – vessels are tortuous and dilated, the system lacks defined arterioles, venules and capillaries, and connections between blood vessels are often incomplete. The arrangement of endothelial cells is also irregular, with wide gaps in some areas, which contributes to hyperpermeability. In addition, the lymphatic system within tumors is also abnormal. All of these features contribute to interstitial hypertension and poor intra-

tumoral blood flow, which result in relative hypoxia and acidosis. In short, pathologic tumor vasculature creates a microenvironment that protects tumors from cytotoxic chemotherapy. Transient reversal of these abnormalities should therefore improve delivery of chemotherapeutic agents and make them more effective.

By combining Bevacizumab with HAI FUDR, we seek to improve control of the primary liver disease, extend the time to hepatic and extrahepatic progression, and improve patient survival. Increased expression of VEGF mRNA is common in both ICC and HCC, and there is some suggestion of correlation with survival. The current study is based on the hypothesis that the growth and progression of primary liver cancer are dependent on the factors that promote angiogenesis and lymphangiogenesis. As signaling molecules regulating these two processes are driven by three different tyrosine kinase receptors – namely VEGFR1, VEGFR2 and VEGFR3 – we propose to assess the expression pattern of these receptors and their cognate ligands including VEGF-A, VEGF-B, VEGF-C, VEGF-D and P1GF, in primary liver tumors. Since Bevacizumab selectively blocks the activity of VEGF-A, but not VEGF-C and VEGF-D or P1GF, these studies will provide important information as to whether inhibition of the VEGF-A/VEGFR-2 signaling pathway is sufficient to block tumor angiogenesis and lymphangiogenesis within the primary liver lesions.

### 3.5 Dynamic MRI

The emergence and increasing use of therapeutic agents targeting tumor vasculature has made it necessary to develop imaging modalities that assess changes in tumor perfusion in order to determine treatment efficacy. Many techniques have been used and reported for evaluating tumor angiogenesis. Radiographic techniques are based on the fact that angiogenesis increases both the perfusion and permeability of tumors with respect to the surrounding normal tissue.

Standard bi-dimensional tumor measurements may underestimate response to agents that target the tumor vasculature. Conventional MRI is useful for assessing tumor size and burden. Signal intensity changes may be assessed, but are not reliable for demonstrating possible responses to treatment. T1 weighted images alone cannot differentiate viable tumor from nonviable tissue or edema. Furthermore, standard T2 weighted images cannot adequately distinguish tumor from necrosis and frequently overestimate lesion boundaries due to the presence of edema and hemorrhage. While tumor shrinkage is useful and is the gold standard for response assessment, tumor measurements alone may underestimate tumor necrosis and do not consider changes in tumor vascularity. By contrast, DCE-MRI can document changes in tumor perfusion kinetics.

DCE-MRI has been used to study a variety of tumors, including sarcoma, breast, brain and colorectal tumors. Injection of gadopentetate dimeglumine (Gd-DTPA) intravenously is followed by the agent passing from the intravascular space to the

interstitial space at a rate that depends on perfusion and tissue permeability. This perfusion and tissue permeability within tumors can be visualized and measured using dynamic contrast-enhanced MRI. The same area of the tumor can be imaged before and several times after the administration of contrast medium, prior to and following a therapeutic intervention. In this way, it is possible to depict the change in MRI signal intensity due to the perfusion of the contrast medium over time. In malignancy, the number of blood vessels and the trans-endothelial permeability of the vessels are often higher than in normal tissue

DCE-MRI has been shown to be useful for detecting viable tumor and differentiating it from necrosis in patients undergoing chemotherapy (Verstraete et al., Dyke et al.). Necrotic tumor, viable tumor, normal hepatic parenchyma, and blood vessels display distinct time intensity curves in dynamic contrast enhanced studies. Determining tumor necrotic fraction on static MRI during or after treatment is severely limited. On the other hand, the rapid acquisition sequences used in the dynamic studies may provide more information on vascular uptake than either static MRI or static post contrast images. Dynamic contrast enhanced images have the potential to provide quantitative estimates of necrotic fraction through an analysis of the entire time course of contrast agent uptake.

More recent studies have suggested that dynamic contrast enhanced MRI (DCE-MRI) can be used to assess tumor oxygen levels, which in turn correlate with response to treatment and outcome. In studies on HCC, MRI findings have been shown to correlate with the degree of VEGF expression, which is also correlated with the degree of tumor hypoxia. Additionally, in a recent study of 7 patients with HCC treated with the anti-angiogenic drug thalidomide, changes in the time-intensity curve-related parameters from tumor regions of interest correlated with response to therapy. Additionally, in a recent MSKCC phase II study of HAI FUDR in patients with unresectable primary liver cancer, changes in the tumor perfusion kinetics on the first post-treatment DCE-MRI (2 months) correlated with response as measured conventionally. In this study, the post-treatment changes in tumor perfusion, as measured by the time intensity curve slope (TIC) at an early (2 months) time point, were a significantly better predictor of treatment response compared to bi-dimensional tumor measurements at the same time point. In addition, the pre-treatment baseline (BL) slope values were found to be >300 (% increase/min) in all responders, and below this empirical value in non-responders. Similar trends were observed with the estimated compartmental model parameter $AK_{ep}$

In the present study, unidimensional tumor measurements on standard MRI sequences will be used to assess response to therapy. The purpose of the dynamic component is to further assess for changes in tumor perfusion during treatment. Standard tumor measurements on conventional imaging may underestimate treatment response, and the dynamic component may offer a more complete assessment in that regard. In addition, early changes in tumor perfusion may signify a greater likelihood of a response as

determined by reduction in tumor volume, which typically take longer to manifest. Data from the dynamic MRI will be correlated with conventionally measured tumor response, as determined on standard MRI sequences. Data from the dynamic portion of the MRI will be used experimentally, as described above, and will not be used to make clinical decisions.

There is very little data derived from direct measurements of tumor perfusion changes during or after anti-angiogenic therapy. Most human studies use surrogate markers to detect improvement in the perfusion characteristics, such as increased $pO_2$ and reduction in interstitial pressure. A recent study using DCE-MRI to monitor anti-angiogenesis treatment (PTK/ZK) of liver metastases demonstrated measurable differences in tumor perfusion kinetics as early as 2 days after the first dose. It is uncertain, however, how tumor perfusion and permeability, as measured by DCE-MRI will be altered by a treatment regimen that combines angiogenesis inhibition with direct cytotoxicity. In order to eliminate the risk of post-operative bleeding complications, Bevacizumab will not be given until 4 weeks after pump placement (see section 9.0, *Treatment Intervention* below). As in the previous protocol, patients will begin HAI FUDR at two weeks after pump placement, followed by pump emptying and refilling with heparinized saline for two weeks. It is during this latter time period that systemic Bevacizumab will begin. Patients will undergo an initial postoperative baseline DCE-MRI followed by a repeat study after the first two weeks of FUDR alone, and a third study following the initial treatment with Bevacizumab alone. In this way, we will obtain DCE-MRI data regarding changes in tumor perfusion kinetics as a result of antiangiogenesis treatment alone. We expect that DCE-MRI studies at this early time will reveal changes in tumor permeability and overall perfusion, indicating treatment effect of Bevacizumab. Subsequent MRI scans will be obtained at two-month intervals. We expect that the addition of Bevacizumab will improve the overall delivery of HAI FUDR to liver tumors, thereby resulting in greater tumor killing, and that this will ultimately be reflected in later DCE-MRI studies as greater reduction in tumor perfusion compared to our baseline data with HAI FUDR alone.

### 3.6 Tumor Hypoxia

Tumor oxygenation is well recognized as an important determinant of treatment response and survival. For a number of different solid malignancies, hypoxia correlates with a more malignant phenotype, greater metastatic potential and resistance to chemotherapy and radiation therapy. As discussed above, the pro-angiogenic malignant phenotype is characterized by grossly abnormal vasculature, leading to poor tumor perfusion, hypoxia and acidosis.

Hypoxic conditions regulate expression of several different genes with diverse functions that are important in promoting tumor cell survival. These include VEGF, hypoxia inducible transcription factor (HIF-1), carbonic anhydrase-IX (CA IX) and the glucose

transporters Glut-1 and Glut-3, among others. HIF-1 is a central regulatory gene that controls expression of many downstream target genes with diverse function, including glucose transporters, angiogenesis and transmembrane carbonic anhydrases. CA IX, Glut-1 and Glut-3 are both regulated through the HIF-1 pathway and are more specific endogenous hypoxia-associated markers. CA IX is one of several CA's that catalyzes the conversion of $C0_2$ to carbonic acid and help maintain normal intracellular pH under hypoxic conditions. Glut-1 and Glut-3 mediate cellular glucose uptake, thereby facilitating anaerobic glycolysis. Increased expression of both proteins has been correlated with poor outcome for several different human cancers. CA IX, in particular, appears to be among the most sensitive of the hypoxic markers. HIF-1$\alpha$, the nuclear protein product of the HIF-1 gene, has also been shown to be a useful intrinsic marker of hypoxia with prognostic relevance.

Direct measurements of tumor oxygen levels have been shown to correlate with immunohistochemical markers of hypoxia; however there is limited data pertaining to the results of DCE-MRI. We expect that these studies will yield important data that will not only improve our understanding of the biology of primary liver cancers but that will also improve our ability to interpret the DCE-MRI assessment of tumor perfusion and permeability.

## 4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

### 4.1 Design

A total of 55 patients whose liver lesions have been deemed unresectable will be treated with HAI FUDR plus systemic Bevacizumab. Unidimensional tumor measurements on standard MRI sequences will be used to assess response to therapy. Patients will remain on study as long as they show continued response as defined in section 13.0, and do not experience significant toxicity as defined in Sections 11.0 and 14.0.

### 4.2 Intervention

All patients enrolled in the study will receive HAI FUDR (0.16 mg/kg X pump volume / pump flow rate), Dexamethasone (1 mg/m$^2$/day) and IV Bevacizumab at 5mg/kg. Chemotherapy with HAI FUDR/Dex will commence no sooner than 14 days post surgical placement of HAI pump; patients will receive their first treatment with Bevacizumab no sooner than 28 days post surgical placement of HAI pump.

| **Cycle Schema q4 weeks** | | | |
|---|---|---|---|
| **Day 1** | | Day 15 | |
| Systemic chemotherapy<br>IV Bevacizumab* | | Systemic chemotherapy<br>IV Bevacizumab | |
| **HAI with FUDR+Dexamethasone**<br>(continuous 14-day infusion) | | Pump emptied and refilled with heparinized saline (recycle with FUDR in 2 weeks) | |

* The exception will be Cycle 1, Day 1, on which the patient will receive **only** HAI with FUDR/Dex. Systemic treatment with Bevacizumab will commence no sooner than Cycle 1, Day 15, and patient will receive Bevacizumab every 2 weeks thereafter.

The following doses will be used:

| ***FUDR***<br>*(mg/kg x pump vol / flow rate) over 14 days* | ***Dexamethasone***<br>*(mg) Given concurrent with pump* | ***Bevacizumab***<br>*(mg/kg) IV, over 10 min* |
|---|---|---|
| 0.16 | 25 | 5 |

## 5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

### 5.1 FUDR

5.1.1 Floxuridine (FUDR) is an antimetabolite that blocks the methylation of deoxyuridylic acid interfering with the synthesis of DNA. It is also incorporated into RNA and interferes with its function. The drug is metabolized in the liver.

5.1.2 FUDR is commercially available from Roche and Adria Laboratories in 500 mg/10 cc ampules. It is stable (protected from light) and is a colorless aqueous solution. Store at room temperature.

5.1.3 Toxicities associated with the intrahepatic administration of FUDR include biliary sclerosis, chemical hepatitis, gastric ulcers.

## 5.2 Dexamethasone

5.2.1 Dexamethasone is an adrenocortical steroid, used for chronic inflammation, neoplastic and autoimmune diseases; used in HAI treatment as an agent to prevent liver damage.

5.2.2 Common potential side effects include anxiety, mood alteration/lability, hyperglycemia, insomnia, peripheral edema, myopathy (with chronic use), acne, hirsutism.

## 5.3 Bevacizumab (rhuMab VEGF)

Description:

Bevacizumab is a clear to slightly opalescent, colorless to pale brown, sterile liquid concentrate for solution for intravenous (IV) infusion. Bevacizumab may be supplied in 5-cc (100 mg), 20-cc (400 mg), and 50-cc (1000 mg) glass vials containing 4 mL, 16 mL, or 40 mL of Bevacizumab, respectively (all at 25 mg/mL). Vials contain Bevacizumab with phosphate, trehalose, polysorbate 20, and Sterile Water for Injection (SWFI), USP. Vials contain no preservative and are suitable for single use only.

The monoclonal antibody (Bevacizumab) being administered is intended for use only in clinical trials. It is expected to be very similar in safety and activity to the commercially marketed drug (Avastin), but it is possible that some differences exist.

For further details and molecule characterization, see the Bevacizumab Investigator Brochure.

Administration:

Bevacizumab will be administered as per MSKCC guidelines.

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.

Bevacizumab Dose Modification and 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 toxicities associated or possibly associated with Bevacizumab treatment should be managed according to standard medical practice. Bevacizumab has a terminal half-life of 2 to 3 weeks; 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.

Infusion Reaction:

Infusion of Bevacizumab should be interrupted for subjects who develop dyspnea or clinically significant hypotension. Subjects who experience a NCI CTCAE v. 3.0 Grade 3 or 4 allergic reaction / hypersensitivity, adult respiratory distress syndrome, or bronchospasm (regardless of grade) will be discontinued from Bevacizumab treatment.

The infusion should be slowed to 50% or less or interrupted for subjects who experience any infusion-associated symptoms not specified above. When the subject's symptoms have completely resolved, the infusion may be continued at no more than 50% of the rate prior to the reaction and increased in 50% increments every 30 minutes if well tolerated. Infusions may be restarted at the full rate during the next cycle.

Adverse events requiring delays or permanent discontinuation of Bevacizumab are listed in the Table in section 12.3

Toxicity: Toxicities associated with the intravenous administration of Bevacizumab include hypertension, hemorrhage, thromboembolic events, proteinuria, and diarrhea. These are described in detail under section 11.2.

## 6.0 CRITERIA FOR SUBJECT ELIGIBILITY

### 6.1 Subject Inclusion Criteria

- Histologically confirmed and radiographically measurable hepatocellular carcinoma (HCC) or intrahepatic cholangiocarcinoma (also variously reported as peripheral cholangiocarcinoma, cholangiolar carcinoma or cholangiocellular carcinoma) (ICC) with no clinical or radiographic evidence of extrahepatic disease. Confirmation of the diagnosis must be made at MSKCC. If the patient has a liver mass that is

radiographically consistent with HCC and a serum alpha fetoprotein (AFP) > 400 ng/dl, then biopsies will be performed at the time of pump placement. Radiographically measurable disease are lesions for which 2 dimensional measurements can be made on a cross sectional image. Minimum lesion size is 2 cm in greatest diameter as per RECIST criteria.

- Patients with suspected ICC will undergo radiographic evaluation to exclude the possibility of metastatic liver disease. For patients who have undergone pre- or postoperative biopsies that definitively diagnose ICC, the diagnostic studies may be modified at the discretion of the Principal Investigator.

- Mixed HCC/ICC is an uncommon variant of primary liver cancer with histologic features of both HCC and ICC. With respect to patient demographics and clinical behavior, mixed tumors are very similar to ICC. Patients suspected of having mixed tumors will be eligible and will be exempt from the extensive radiographic evaluation; they will have biopsies performed at the time of pump placement.

- Patients with hepatocellular carcinoma who are not eligible for first-line MSKCC protocols for hepatocellular carcinoma will be eligible for this protocol if they have no clinical or radiographic evidence of extrahepatic disease.

- Patients with HCC or ICC undergoing exploration for a possible curative resection but found to have unresectable disease confined to the liver will be eligible.

- <70% liver involvement by cancer.

- Disease must be considered unresectable at the time of preoperative evaluation.

- Patients who have failed ablative therapy will be eligible.

- Patients who have failed prior systemic chemotherapy will be eligible.

- KPS $\geq$ 60% and be considered candidates for general anesthesia and hepatic artery pump placement.

- Patients with chronic hepatitis and/or cirrhosis are eligible but must be Child-Pugh class A.

- Preoperative laboratory values, within 14 days of registration:
  - Serum albumin >2.5 g/dl
  - Total serum bilirubin <1.8 mg/dl
  - WBC >3500 cells/$mm^3$
  - Platelet count >100,000/$mm^3$

- International normalized ratio (INR) < 1.5 (in patients not on coumadin therapy)

- Patients discovered to have ≥ 2+ proteinuria at baseline will undergo a 24-hour urine collection, which must be an adequate collection and must demonstrate < 1g of protein/24 hours.

- Age ≥ 18 years.

- Patients must be able to understand and sign informed consent.

## 6.2 Subject Exclusion Criteria

- Prior treatment with FUDR.
- Prior external beam radiation therapy to the liver.
- Diagnosis of sclerosing cholangitis.
- Diagnosis of Gilbert's disease.
- Clinical ascites.
- Hepatic encephalopathy.
- Current, recent (with 4 weeks of the first infusion of this study), or planned participation in an experimental drug study other than a Genentech-sponsored Bevacizumab cancer study.
- Life expectancy of less than 12 weeks.
- Inability to comply with study and/or followup procedures
- Inadequately controlled hypertension (defined as systolic blood pressure >150 and/or diastolic blood pressure > 100 mmHg on antihypertensive medications).
- Any prior history of hypertensive crisis or hypertensive encephalopathy.
- New York Heart Association (NYHA) Grade II or greater congestive heart failure.
- Patients with known CNS disease.
- Significant vascular disease (e.g. aortic aneurysm, aortic dissection), or symptomatic peripheral vascular disease.

- History of abdominal fistula, gastrointestinal perforation, or intra-abdominal abscess within 6 months prior to study enrollment.

- History of allergic reactions attributed to compounds of similar chemical or biologic composition to Bevacizumab.

- Serious or non-healing active wound, ulcer, or bone fracture.

- Major surgical procedure, open biopsy or significant traumatic injury within 28 days prior to first treatment with Bevacizumab, or anticipation of need for major surgical procedure during the course of the study (surgery performed to place pump will not exclude patient from protocol; first treatment with Bevacizumab will take place no sooner than 28 days after surgery).

- Core biopsy, or other minor surgical procedure (excluding placement of a vascular access device) within 7 days prior to treatment start.

- Current or recent use of a thrombolytic agent.

- Patients must have adequate blood coagulation parameters: PT such that international normalized ratio (INR) is < 1.5 (or an in-range INR, usually between 2 and 3, if a patient is on a stable dose of therapeutic warfarin), and a PTT < 1.5 times the institution upper limit of normal. Patients not meeting this criteria will be ineligible for study.

- Chronic daily treatment with aspirin (> 325 mg/d) or nonsteroidal anti-inflammatory medications known to inhibit the platelet function.

- Presence of bleeding diathesis or coagulopathy.

- Proteinuria at screening as demonstrated by either

  - Urine protein: creatinine (UPC) ratio $\geq$ 1.0 at screening OR
  - Urine dipstick for proteinuria $\geq$ 2+ (patients discovered to have $\geq$2+ proteinuria on dipstick analysis at baseline should undergo a 24 hour urine collection and must demonstrate $\leq$1g of protein in 24 hours to be eligible).

- Patients with history of stroke, transient ischemic attack, unstable angina, or myocardial infarction MI) within 6 months.

- Presence of central nervous system metastases.

- Patients who have radiographic evidence of esophageal varices or history of variceal hemorrhage.
- Patients with occlusion of the main portal vein or of the right and left portal branches.
- Patients that have concurrent malignancies (except localized basal cell or squamous cell skin cancers).
- Patients with active infection.
- Female patients who are pregnant or lactating.

## 7.0 RECRUITMENT PLAN

All patients meeting the eligibility requirements will be considered for enrollment regardless of sex, race, or religion. Patients will be accrued from the Hepatopancreatobiliary, Gastrointestinal, and Gastric/Mixed Tumor services, and from both the Department of Surgery and Department of Medicine. Eligibility criteria may not be waived by the investigator. Discussions regarding protocol enrollment and patient eligibility will begin with any of the investigators named on the protocol. Patients will be made aware of the protocol, its specific aims and objectives, and the potential risks and benefits the patients may incur. Patients will be required to read, agree to, and sign an IRB-approved informed consent form prior to registration on this trial. Patients will be consented prior to surgery. Registration will consist of two steps: Step 1 will allow the procurement of preoperative blood samples and tissue at surgery and will take place preoperatively; Step 2 will register patient fully in order to receive chemotherapy treatment. Only patients registered to both Step 1 and 2 will be treated per protocol. There will be no financial compensation for patients enrolling on this protocol.

## 8.0 PRETREATMENT EVALUATION

Prior to treatment start, patients will undergo the following procedures, in order:

1. CT angiogram (direct, MRI or CT angiography)to determine arterial structures; this may be done at any time prior to surgery
2. Surgery to undergo cholecystectomy, place hepatic arterial infusion pump, and to obtain tissue biopsy
3. Perfusion flow study (Tc-99 MAA)
4. Baseline MRI scan of the chest, abdomen and pelvis

The following tests are required of ALL patients:

- CT or MRI of the chest/abdomen within 6 weeks of registration.
- Hepatitis serology within 52 weeks of registration (if never previously tested or if previously negative for hepatitis B or C).

The following tests are required of all patients with a presumptive diagnosis of ICC:

- Colonoscopy within 2 years of registration (exemption may be made for patients who have biopsy-proven ICC).
- Bilateral mammography (females only) within 24 weeks of registration.

In addition, the following evaluations will be required at the indicated times:

| | Within 14 days of Registration | Within 14 days of surgery | Post surgery, within 21 days of Cycle 1 | Post surgery, within 48 hours of Cycle 1 |
|---|---|---|---|---|
| EKG | X | X[1] | | |
| HX, PE, BP | X | X | | |
| Pregnancy test (females of child-bearing potential) | X | | | |
| DCE-MRI Abdomen[2] | | | X | |
| Tumor Size measurements[2] | | | X | |
| KPS[3] | X | | | X |
| Ht / Wt[4] | X | | | X |
| PT | X | | | |
| CBC with diff/plts | X | | | X |
| Albumin | X | | | X |
| Total bilirubin | X | | | X |
| BUN, creatinine | X | | | X |
| Alk Phos, SGOT, LDH | X | | | X |
| CEA, AFP, CA 19-9[5] | X | | | X |
| Urinalysis | X | | | X |
| Serum electrolytes | | | | X |
| CT angiogram or Celiac axis & SMA angiogram[6] | X [5] | | | |
| Tc-99m MAA hepatic artery | | | | |

| | | | | |
|---|---|---|---|---|
| catheter injection | | | X | |

1 Required within 7 days of operation

2 Baseline MRI kinetic study/scan will be obtained on all patients prior to starting therapy and will be used for tumor measurement(s) and to assess disease response or progression during and after therapy. Exception will be made for any patient who cannot undergo MRI; in that case, CT scanning will be accepted.

3 KPS will be determined prior to surgery and prior to initiating chemotherapy. KPS will also be evaluated on day 1 of each cycle while undergoing therapy.

4 Height will only be assessed at initial visit; weight will be assessed at every MD visit.

5 Tumor markers will be measured as part of the patient's standard bloodwork prior to initiating chemotherapy, and will be monitored at the attending MD's discretion during treatment.

6 Either direct angiography, MR angiography, or CT angiography are acceptable, and can be performed at any time prior to surgery.

## 9.0 TREATMENT/INTERVENTION PLAN

### 9.1 Administration:

Chemotherapy will be administered on a 4-week cycle basis. Pump therapy with FUDR and Dex plus heparinized saline to make 30cc will be administered on Day 1 of each cycle. The pump will be emptied and re-filled with heparin and normal saline on Day 15. Bevacizumab (5mg/kg) will be administered on Days 1 and 15 of each cycle, however initial treatment with Bevacizumab will not take place until Day 15 of Cycle 1. Treatment recycles on Day 29.

On Day 15 of cycle 1, patients will receive their initial treatment with Bevacizumab, administered by IV, administered per MSKCC guidelines.

### 9.2 Dose Calculation:

For the first cycle, doses of FUDR and Dex will be calculated based on the predetermined flow rate provided by the pump manufacturer. Thereafter, doses will be adjusted (lowered, if necessary, but never increased) based on actual observed flow rate. The pump will be filled with FUDR, Dexamethasone, heparin and saline.

**FUDR Calculation:**

FUDR: $\frac{0.16 \text{ mg/kg} \times \text{pump volume}}{\text{pump flow rate}}$

Dexamethasone: $\frac{1 \text{ mg/day} \times \text{pump volume}}{}$

pump flow rate

**Overweight patients:**

If a patient is 25% above ideal weight, dose of FUDR and systemic chemotherapy will be calculated as follows:

Ideal Body weight (kg):

Males: 50kg + (2.3 x height in inches above 5 ft)
(ie: for a patient who is 5'10", use 10)
Females: 45.5 kg + (2.3 x height in inches above 5 ft)
*Example:* Male who is 100 kg and 5'10"
Ideal Body Weight is: 50 + (2.3 x 10) = 73 kg
Therefore, 100 + 73 = 173 ÷ 2 = 86.5 would be the Ideal Average Weight to use.

Heparin: 30,000 units total dose

Normal saline: quantity sufficient to make total reservoir volume of 30 ml.

If no dose modification due to toxicity is required, the dosages given above (adjusted for changes in weight and pump flow rate) will be repeated on Day 1 of Cycle 2 and all subsequent cycles.

**9.3 Pump Empty**

On Day 15 of each cycle, the pump will be emptied and then filled with 30,000 units of heparin in normal saline (q.s. 30cc) for 14 days.

**9.4 Hematologic Criteria**

Patients must meet all hematologic and blood chemistry criteria outlined in Section 6.0 before beginning the first cycle of therapy. For subsequent cycles, patients must meet the following criteria:

| | |
|---|---|
| WBC | $\geq$ 2500 µL |
| ANC | $\geq$ 1500 µL |
| Platelet count | $\geq$ 75,000 µL |
| Creatinine | $\leq$ 1.8 mg/dL |
| Bilirubin | < 1.5 mg/dL |

If counts are outside these levels on date of scheduled treatment, therapy will be delayed one week.

Parameters for treatment with FUDR via intrahepatic pump are outlined in Section 12.

**9.5 Treatment Duration**:

9.5.1 Patients will receive treatment until such time as progression is noted, or the

patient develops unacceptable toxicity, or there is a change in diagnosis (see section 14.0, “Criteria for Removal from Study”).
9.5.2 If FUDR is discontinued due to patient progression, then patient will be discontinued on study drug (Bevacizumab) as well
9.5.3 If FUDR is held for reasons other than progression, patient will continue on Bevacizumab at the attending physician’s discretion.
9.5.4 If Bevacizumab is discontinued due to toxicity (sections 11.0, 12.3), the patient will continue to receive FUDR as long as patient continues to respond, and at the discretion of the attending physician.

## 10.0 EVALUATION DURING TREATMENT/INTERVENTION

| | *At time of surgery* | *Day 1* each Cycle* | *q2 weeks* | *q8 weeks* | *q 12 weeks* |
|---|---|---|---|---|---|
| HX,PE, BP | | X | X | | |
| Tox assessment | | X | X | | |
| Weight | | X | | | |
| KPS | | X | | | |
| CBC, Plts | | X | X | | |
| BUN, Creat | | X | X | | |
| Bili, SGOT | | X | X | | |
| Alk phos, LDH | | X | X | | |
| Electrolytes | | X | | | |
| Tumor markers[1] | | X | | | |
| Urinalysis | | X | | | |
| EKG[4] | | | | | X |
| Chest x-ray/CT chest[5] | | | | | X |
| Dynamic MRI abdomen[2] | | | | X | |
| Tc-99m MAA hepatic artery catheter injection | | | | | |
| | | | | | |
| **Plasma levels[3]/VEGF **** | **X** | **X** | | | |
| **Tumor tissue levels / VEGF**** | **X** | | | | |

** Or within 48 hours prior to Day 1*

*** Tissue and blood samples will be stored preparatory to analysis.*

[1]Tumor markers (CEA, AFP, CA19-9) will be drawn if elevated before treatment, or at the discretion of the attending physician.

[2]All patients will undergo DCE MRI after initial treatment with FUDR, and again after initial treatment with Bevacizumab. DCE MRI will take place every 2 cycles thereafter, allowing for scheduling conflicts; a variance in the timing will not be considered a protocol deviation. The timing of the DCE MRI may be advanced at the discretion of the attending physician, in the interest of patient safety.

[3]Patients will have one lavender-top tube (7 or 10cc) and 1 8cc BD CPT tube of whole blood

drawn on Day 1 of each cycle.
[4]EKG will be done every 12 weeks, allowing for scheduling conflicts; a variance in the timing will not be considered a protocol deviation. Other EKGs performed as deemed clinically necessary, at the discretion of the attending MD.
[5] CT of the pelvis may be added at the discretion of the attending MD.

While being treated with protocol therapy, patients will be seen on or prior (within 48 hours) to the first day of each cycle by their medical oncologist.

**Specific Monitoring:**

Hypertension will be monitored through routine evaluation of blood pressure prior to each Bevacizumab treatment. Optimal control of blood pressure according to standard public health guidelines is recommended for patients on treatment with or without Bevacizumab.

Proteinuria will be monitored by urine protein:creatinine (UPC) ratio or dipstick at least every 4 weeks, not to exceed 6 weeks.

If patients on 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/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).

## 11.0 TOXICITIES/SIDE EFFECTS

All toxicities will be rated as per the NCI Common Toxicity Criteria except neurosensory and hepatic enzyme toxicities related to intrahepatic pump therapy (see section 12.5 FUDR Dose Modifications and Table I).

### 11.1 Toxicity Related to Regional Chemotherapy:

11.1.1 FUDR: gastritis, gastroduodenal ulcers, chemical hepatitis, biliary sclerosis with jaundice, pruritus, diarrhea.

11.1.2 Dex: anxiety, mood alteration/lability, hyperglycemia, insomnia, peripheral edema, myopathy (with chronic use), acne, hirsutism, sodium retention, fluid retention, hypertension, development of cushingoid state, secondary adrenocortical and pituitary hypo-responsiveness, decreased carbohydrate tolerance, manifestations of latent diabetes.

### 11.2 Toxicity Related to Bevacizumab:

In addition to fever, generalized aches, headache, rash, fatigue, shortness of breath, reversible liver function elevation and allergic reaction, the following toxicities may be seen:

Hypertension: Hypertension has been commonly seen in Bevacizumab clinical trials to date and oral medications have been used to manage the hypertension when indicated. 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). RPLS may include signs and symptoms of headache, altered mental function, seizures, and visual disturbances/cortical blindness and requires treatment, which should include control of hypertension, management of specific symptoms, and discontinuation of Bevacizumab.

Bleeding: In a lung cancer trial, there were six life-threatening bleeding events among 66 subjects. There were no life-threatening hemorrhagic events in completed Phase III trials in breast and colorectal cancers. Minor mucocutaneous bleeding (epistaxis) has also been seen in all Bevacizumab clinical trials at a rate of approximately 20-40%.

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. In the phase II pivotal trial in metastatic CRC, there was a slightly higher rate of venous TE events that was not statistically significant in patients treated with Bevacizumab plus chemotherapy compared with chemotherapy alone. There was also a higher rate of arterial TE events (3% vs. 1%) such as myocardial infarction, transient ischemia attack, cerebrovascular accident/stroke and angina/unstable angina. A pooled analysis of the rate of arterial TE events from 5 randomized studies (1745 patients) showed that treatment with chemotherapy plus Bevacizumab increased the risk of having an arterial TE event compared with chemotherapy alone. Furthermore, subjects with certain baseline characteristics (age > 65 years and/or a history of a prior arterial TE event) may be a higher risk of experiencing such an event.

Proteinuria: Proteinuria has been commonly seen in Bevacizumab clinical trials to date. 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 or 2. Rare events of nephrotic syndrome have occurred, and Bevacizumab should be discontinued in patients with nephrotic syndrome.

Gastrointestinal Perforation: Bevacizumab should be permanently discontinued in patients who develop gastrointestinal perforation. A causal association of intra-abdominal inflammatory process and gastrointestinal perforation to Bevacizumab 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, and breast) and may be higher in incidence in some tumor types.

CHF: CHF has been reported in Bevacizumab clinical trials and may be increased in incidence in patients with prior exposure to anthracyclines or prior irradiation to the chest wall. Patients receiving anthracyclines or with prior exposure to anthracyclines should have a baseline MUGA or ECHO with a normal ejection fraction.

Wound Healing: 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 5FU/LV plus Bevacizumab did not appear to have an increased risk of wound healing complications compared to those treated with chemotherapy alone. 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 20 days. Bevacizumab should be discontinued in patients with severe wound healing complications.

### 11.3 Toxicity Related to the Pump and Catheters

11.3.1 Infection, hepatic artery thrombosis, pump malfunction, catheter occlusion, intra-abdominal bleed.

## 12.0 DOSE MODIFICATIONS

### 12.1 FUDR Dose Modifications :

Elevations in SGOT, alkaline phosphatase, and serum bilirubin are common during treatment with intrahepatic FUDR and have been managed adequately by modifying the dose or delaying treatment, as reported in previous studies. Elevations in these laboratory values will be managed as outlined in the table below.

Percentages listed under “FUDR Dose” refer to percentage of last dose of FUDR administered; however, changes in flow rate and weight must be considered. Therefore, the modified dose will be calculated using the indicated percentage of the desired dose multiplied by patient’s weight and pump volume, then divided by current pump flow rate. For example, if a 75 kg patient required a 50% dose reduction, and the pump had an actual flow rate of 1.6 ml/day, then the FUDR dose would be calculated as follows:

.16mg/kg/day X dose attenuation [.50] X 75kg X 30ml = 131.3 mg FUDR

pump flow rate [1.6 ml/day]

**FUDR DOSE MODIFICATION TABLE**:

| | Reference Value* | % FUDR dose |
|---|---|---|
| **SGOT** *(at pump emptying or day of planned retreatment, whichever is higher)* | 0 to < 2 x reference value | 100% |
| | 2 to < 3 x reference value | 80% |
| | 3 to < 4 x reference value | 50% |
| | > 4 x reference value | Hold |
| **ALK PHOS** *(at pump emptying or day of planned retreatment, whichever is higher)* | 0 to < 1.2 x reference value | 100% |
| | 1.2 to < 1.5 x reference value | 50% |
| | > 1.5 x reference value | Hold |
| **TOT BILI** *(at pump emptying or day of planned retreatment, whichever is higher)* | 0 to < 1.2 x reference value | 100% |
| | 1.2 to < 1.5 x reference value | 50% |
| | > 1.5 x reference value | Hold |
| If SGOT > 4X reference value, alkaline phosphatase > 1.5X reference value, total bilirubin > 1.5X reference value, then treatment will be held and will not be reinstituted until values come down to more normal levels, as indicated in section "Resuming Treatment After Temporary Discontinuation". | | |

"Reference value" is the value obtained on the day the patient received last FUDR dose. To determine if an FUDR dose modification is necessary, compare reference value to either the value obtained on the day the pump was emptied or on day of planned pump filling, whichever is higher.

If a patient's Alkaline Phosphatase or T bili shows a continual rise from Day 1 of treatment, then the Day 1 value will be used as the reference value for that patient when determining whether to hold treatment, and time of re-treatment after hold.

### 12.2 Resuming Treatment After Temporary Discontinuation:

| REASON FOR TREATMENT DELAY | Chemotherapy resumed when value has returned to: | % FUDR dose |
|---|---|---|
| SGOT elevation | 3 X reference value | 25% of last dose |
| Alkaline Phosphatase elevation | 1.2 X reference value | 25% of last dose |
| Total bilirubin elevation | 1.2 X reference value | 25% of last dose |

12.2.1 Biliary Toxicity: If patient develops a total bilirubin ≥ 3.0 mg/dl, the pump should be emptied and Dex 25 mg plus heparin 30,000 u and saline 30 cc placed in the pump q 14 days. Once there is no longer evidence of toxicity, Dex dose should be tapered in increments of 5 mg every 14 days. Tapering will continue unless enzymes increase. FUDR should be permanently discontinued unless there is evidence of disease progression (increasing CEA, worsening CT scan, worsening clinical status) AND bilirubin has returned to ≤ 1.5 mg/dl. In this case, FUDR can be restarted as follows: Use 25% of the last FUDR dose given with Dex, heparin and saline in the pump for 7 days. Pump should be emptied after 7 days, and patients given a 3-week rest period. This treatment and treatment schedule should continue as long as bilirubin remains ≤ 1.5 mg/dl and liver enzyme values do not increase.

12.2.2 Epigastric pain unresponsive to oral $H_2$ blocker use is suggestive of gastroduodenal irritation or ulcer. Severe pain should prompt workup with an upper gastrointestinal endoscopy. Serum amylase should be checked along with the routine blood (screening profile, creatinine, and CBC) in patients with abdominal pain. If an ulcer or gastroduodenitis is documented, therapy should be held for one month to allow healing. If abdominal pain is severe, the pump should be emptied of FUDR until results of workup are available.

12.2.3 Severe diarrhea (Grade 3 or 4) may result from extrahepatic perfusion of FUDR. If a patient develops diarrhea at the level of dose limiting toxicity, the pump will be emptied of FUDR and a repeat flow scan will be obtained to exclude the possibility of extrahepatic perfusion. If the flow scan suggests extrahepatic perfusion, then an arteriogram will be obtained through the side port of the pump and all mis-perfusing arteries will be embolized. Treatment will resume only after this is done and the diarrhea resolves. If the flow scan is normal, then the possibility of infectious diarrhea will be investigated. If such investigations are negative, then the diarrhea will be considered a dose-limiting toxicity (DLT). Treatment may resume with FUDR at 0.14mg/kg/day.

## 12.3 Bevacizumab Treatment Modifications

Drug treatment will be modified in the event of certain adverse events (as shown in the following table). Regardless of the reason for holding the drug treatment, the maximum allowable length of treatment interruption is 2 months (2 cycles).

**Bevacizumab Dose Modification Table**:

| Event | Action to be Taken |
|---|---|

| | |
|---|---|
| **Hypertension**<br>Grade 3<br><br><br>Grade 4 (including RPLS [confirmed by MRI] or hypotensive encephalopathy) | If not controlled to 150/100 mmHg with medication, discontinue Bevacizumab<br><br>Discontinue Bevacizumab |
| **Hemorrhage**<br>Grade ≥ 2 pulmonary or CNS hemorrhage<br>Grade 3 nonpulmonary and non-CNS hemorrhage<br><br><br><br><br><br><br>Grade 4 | Discontinue Bevacizumab<br><br>Subjects who are also receiving full-dose anticoagulation will be discontinued from receiving Bevacizumab<br>All other subjects will have study treatment held until all of the following criteria are met:<br>• The bleeding has resolved and hemoglobin is stable<br>• There is no bleeding diathesis that would increase the risk of therapy<br>• There is no anatomic or pathologic condition that significantly increases the risk of hemorrhage recurrence<br>Patients who experience a repeat Grade 3 hemorrhagic event will be discontinued from receiving Bevacizumab<br><br>Discontinue Bevacizumab |

| | |
|---|---|
| **Venous Thrombosis**<br>Grade 3/asymptomatic Grade 4 | Hold study drug treatment. If the planned duration of full-dose anticoagulation is < 2 weeks, study drug should be held until the full-dose anticoagulation period is over. If the planned duration of full-dose anticoagulation is > 2 weeks, drug may be resumed during the period of full-dose anticoagulation if <u>all</u> of the following criteria are met:<br>• The patient must have an in-range INR (usually between 2 and 3) on a stable dose of warfarin (or other anticoagulant) prior to restarting study drug treatment<br>• The patient must not have had a Grade 3 or 4 hemorrhagic event while on anticoagulation<br>• The patient must not have had evidence of tumor involving major blood vessels on any prior CT scan |
| Symptomatic Grade 4<br>Arterial Thromboembolic Event (Angina, MI, TIA, CVA, and any other arterial thromboembolic event | Discontinue Bevacizumab |
| Any Grade | Discontinue Bevacizumab |
| **Arterial Thromboembolic Event** (angina, myocardial infarction, transient ischemic attack, cerebrovascular accident, and any other arterial thromboembolic event)<br><br>Any Grade | Discontinue Bevacizumab |
| **Congestive Heart Failure** (Left ventricular systolic dysfunction)<br>Grade 3 | Hold Bevacizumab until resolution to Grade <u>≤</u> 1 |
| Grade 4 | Discontinue Bevacizumab |
| **Proteinuria**<br>Grade 3 (UPC > 3.5, urine collection > 3.5 g/24 hr, or dipstick 4+) | Hold Bevacizumab treatment until < Grade 2, as determined by either UPC ration <u>≤</u> 3.5, or 24 hr collection <u>≤</u> 3.5 g |
| Grade 4 (nephrotic syndrome) | Discontinue the patient from the study |
| **GI Perforation** | Discontinue Bevacizumab |

| | |
|---|---|
| **Bowel Obstruction** | |
| Grade 1 | Continue patient on study for partial obstruction NOT requiring medical intervention |
| Grade 2 | Hold Bevacizumab for partial obstruction requiring medical intervention. Patient may restart upon complete resolution. |
| Grade 3/4 | Hold Bevacizumab for complete obstruction. If surgery is necessary, patient may restart Bevacizumab after full recovery from surgery, and at investigator's discretion |
| **Wound dehiscence** (requiring medical or surgical therapy) | Discontinue Bevacizumab |
| **Other Unspecified Bevacizumab-Related Adverse Events** | |
| Grade 3 | Hold Bevacizumab until recovery to $\leq$ Grade 1 |
| Grade 4 | Discontinue Bevacizumab |

Patients who have an ongoing bevacizumab-related Grade 4 or serious adverse event at the time of discontinuation from study treatment will continue to be followed until resolution of the event or until the event is considered irreversible

### 12.4 General

If any patient on this protocol has a sudden change in condition, liver function tests will be checked immediately. The pump will be emptied of chemotherapy if there is any suspicion of drug-induced toxicity.

All reasonable efforts will be made to adhere to treatment and evaluation schedules, however variations to accommodate holidays, transportation issues, or patient's personal schedule will be permitted if these do not, in the opinion of the investigator, constitute a major safety or compliance issue. Additionally, evaluations (e.g. EKGs, scans) may be performed off-schedule if, in the estimation of the attending physician, patient condition so warrants. Such variations, assuming they do not occur with unreasonable frequency or regularity, will not be considered protocol violations.

## 13.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

The primary objective of this Phase II study will be to assess anti-tumor efficacy.

**13.1** Complete Response (CR): The disappearance of all target and non-target lesions. If necessary, persistent target or non-target lesions that appear to be residual scar

tissue may undergo biopsy and the best overall response may be classified as CR if no evidence of active cancer is found.

**13.2** Partial Response (PR): A 30% decrease in the sum of the longest diameter of target lesions, taking as reference the baseline sum longest diameter.

**13.3** Stable Disease (SD): Neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease, taking as reference the smallest sum longest diameter since the treatment start, or the persistence of one or more non-target lesions.

**13.4** Progressive Disease (PD): At least a 20% increase in the sum of the longest diameter of target lesions, taking as reference the smallest sum longest diameter recorded since the treatment start, or the appearance of one or more new lesions and/or unequivocal progression of non-target lesions.

## 14.0 CRITERIA FOR REMOVAL FROM STUDY

- If at any time the patient develops progressive disease he/she will be taken off study and referred for alternative therapy.
- If at any time the patient develops unacceptable toxicity he/she will be removed from study.
- If at any time the patient is found to be ineligible for the protocol as designated in the section on Criteria for Patient/Subject Eligibility (i.e., a change in diagnosis), the patient will be removed from the study.
- Patient elects to discontinue treatment.
- Changes in a patient's condition which render the patient unacceptable for further treatment in the judgment of the investigator.
- Grade 4 hypertension or reversible posterior leukoencephalopathy syndrome (RPLS)
- Nephrotic syndrome
- Grade $\geq$ 2 pulmonary or CNS hemorrhage; any Grade 4 hemorrhage
- Symptomatic Grade 4 venous thromboembolic event
- Any grade arterial thromboembolic event
- Grade 4 congestive heart failure

- Gastrointestinal perforation
- Wound dehiscence requiring medical or surgical intervention
- Inability of subject to comply with study requirements
- Determination by the investigator that it is no longer safe for the subject to continue therapy
- All Grade 4 events thought to be related to bevacizumab by the investigator

## 15.0 CORRELATIVE STUDIES

### 15.1 DYNAMIC CONTRAST-ENHANCED MRI

MRI will be the primary imaging modality used to assess disease response. Standard sequences will be used for unidimensional tumor measurement, which is a primary outcome determinant. In addition, as part of each scan, dynamic contrast-enhanced images will be obtained to measure parameters related to tumor perfusion (initial slope of the contrast uptake curve), and permeability ($Ak_{ep}$, see below).

**Data Acquisition**

DCE-MRI studies will be performed on a 1.5T GE (Milwaukee, WI) Signa LX scanner. Gadolinium (Gd-DTPA) will be administered at a concentration of 0.1 mmol/kg, resulting in a standard dose of 1mL gadopentetate dimeglumine per 10 kg of patient weight. A saline flush will be administered at the same flow rate with a volume of 20 cc following gadopentetate dimeglumine administration, to ensure complete mixing of the bolus upon delivery. A power injector delivery system (Medrad, Inc. Indianola, PA) will be used to provide accurate flow rates. Patients with a central venous silastic catheter will require a slower flow rate of 0.8-1.0 cc/s to prevent damage to the catheter. All other patients will receive contrast at a rate of 2 cc/s. All patients will receive contrast while at scan position in the magnet.

Dynamic contrast-enhanced images will be acquired using a fast multi-phase spoiled gradient echo sequence. Single slice images, passing through the center of the tumor and possibly covering aorta (whenever feasible) will be acquired with 9 ms repetition time (TR), a 2 ms echo time (TW), 30° flip angle, 15.63 kHz receive bandwidth, 20-24 cm (depending on patient size) field of view (FOV), and a 256 x 128 matrix, yielding a temporal resolution of ~1.0 seconds/image. This time resolution is sufficient to observe the initial uptake of Gd-DTPA into the region. The first 5 images obtained during the study will be pre-Gd-DTPA injection, to assure accurate baseline signal intensity. These data will also be used (in combination with the proton density image) to measure $T_{10}$. Dynamic imaging data will be obtained under shallow breathing for a total of 5 minutes to acquire 225 time points to characterize the time intensity curves (TIC).

**Data Analysis**

Following on-line reconstruction, data will be exported to a Sun Ultra 20 workstation for analysis. Software was been written to display and analyze the data using IDL 6.0 (Research Systems, Inc., Boulder CO). Time intensity curves will be analyzed for each voxel in the image. The initial uptake slope will use a 5-point sliding linear regression applied to the first 2 minutes of the time intensity curve. A baseline signal intensity (SI) value, $SI_{pre}$, will be calculated as the mean intensity of 3 points prior to injection. The percent increase/minute for each voxel is then calculated according to equation 1 (fig. 1):

**Fig. 1**

$$\frac{\%SI}{\min} = \frac{Slope}{SI_{pre}} \cdot 100$$

In addition, the data will be analyzed using a two-compartment model proposed by Hoffman, based on that of Brix , which incorporates rate constants of Gd-DTPA between the lesion to plasma compartments ($k_{ep}$) and elimination by the plasma ($k_{el}$). The plasma concentration is not directly measured since the clearance rate ($k_{el}$) can be estimated from the measured tissue curve. After a bolus injection ($\tau$ = bolus duration), if one assumes $k_{ep}\tau << 1$ and $k_{el}\ \tau << 1$, Hoffman's initial equation reduces to equation 2 (fig. 2) which has three fitted parameters: A (normalized amplitude), $k_{ep}$ ($min^{-1}$), and $k_{el}$ ($min^{-1}$):

**Fig 2:**

$$\frac{S(t)}{S_0} \approx 1 + A\frac{k_{ep}(e^{-k_{ep}t} - e^{-k_{el}t})}{k_{el} - k_{ep}}$$

.

At short times after injection (small values of t), the right side of equation 2 reduces to $1+Ak_{ep}t$, thus the initial slope is proportional to $Ak_{ep}$. This product will be determined for each voxel in the ROI and placed in a histogram.

DCE-MRI scans will be obtained after hepatic artery pump placement but prior to treatment start (baseline). The first post-treatment scan will be obtained in the middle of cycle 1, immediately after completing the initial 14-day infusion of FUDR, but before initial treatment with Bevacizumab; the second post-treatment scan will be obtained at the end of cycle 1, after initial treatment with Bevacizumab only. DCE-MRI scans will be obtained at two month intervals thereafter. Comparisons will be made between the pre- and post-treatment slope of the contrast uptake curve (perfusion) and $Ak_{ep}$ (permeability). The initial and post-treatment changes in these parameters will also be correlated to disease response, as assessed on unidimensional tumor measurements, and immunohistochemical markers of tumor hypoxia (see below).

### 15.2 VEGF and VEGFR Assays

This clinical trial is based on the hypothesis that the growth and progression of primary liver cancer is dependent on the factors that support angiogenesis and lymphangiogenesis. As signaling molecules regulating these two processes are driven by three different tyrosine kinase receptors, namely VEGFR1 (hemangiogenesis), VEGFR2 (angiogenesis), and VEGFR3 (lymphangiogenesis), information regarding the expression pattern of these receptors and their cognate ligands (VEGF-A, -B, -C, -D, and P1GF) is essential in order to rationally interpret the results. Since bevacizumab selectively blocks the activity of VEGF-A, but not the other ligands, these studies will help clarify if inhibition of the VEGF-A/VEGFR2 signaling pathway is sufficient to block angiogenesis and

lymphangiogenesis in primary hepatic malignancy. It is anticipated that tumors primarily over-expressing VEGF-A/VEGFR2 will be more responsive to bevacizumab (anti-VEGF-A) therapy. On the other hand, tumors that over-express both VEGF-A/VEGFR2 and VEGF-C, -D/VEGFR3 signaling pathways may be less sensitive to bevacizumab, as angiogenesis may be promoted through these alternative mediators. Therefore, evaluating the spectrum of VEGF ligand and receptor expression will help clarify the mechanism or mechanisms through which primary hepatic malignancy maintains angiogenesis despite VEGF-A inhibition. As new agents emerge that target other members of the VEGF and VEGFR families, the results of these studies will help identify other potential sites of therapeutic intervention in primary liver cancer.

In parallel to these studies, VEGF-A, -B, -C, -D and PlGF levels in plasma and VEGFR-1, -2, and -3 expression on peripheral blood mononuclear cells will be measured. In addition, the number of circulating CD133+VEGFR2+ EPCs and CD34+VEGFR1+ pro-angiogenic HPC's will be quantified. These assays will be performed at baseline and on day 1 of each cycle during treatment. The proposed studies will determine if these surrogate markers have utility in assessing response to treatment with bevacizumab. Since no bio-marker reliably describes the angiogenic propensity of primary liver cancer or its response to anti-angiogenesis therapy, these data will provide insights regarding treatment-induced changes that are measurable in the peripheral blood.

Planned Experiments

The correlative studies in this section will be performed on biopsy samples of tumor and non-tumor bearing liver taken at operation (pre-treatment) and on peripheral blood taken before treatment and on day 1 of each cycle during treatment, as described above. The methods used in these experiments have been previously described and are currently in routine use.

*1. Tissue.* Biopsy samples of tumor and non-tumorous liver will be obtained at the time of operation, some of which will be frozen and some embedded in paraffin. Frozen cut sections of the tumor biopsy sample will be analyzed for the expression of VEGF-A, -B, -C, -D, PlGF, and VEGFR-1, -2, -3 using standard immunohistochemical techniques and commercially available antibodies (Santa Cruz Biotechnology), as previously described. Non-tumor involved liver from each patient will be simultaneously stained. Human umbilical vein endothelial cells (HUVEC) will be used as positive controls for VEGF receptors and leukemia cell lines (KG1a, KG1 and HL60) will be used as positive controls for VEGF ligands. Slides will be reviewed by a reference pathologist and scored for proportion of positively stained cells.[43]

*2. Peripheral Blood.* Samples of peripheral blood (~10 ml) will be collected and processed to separate plasma and circulating mononuclear cells. Plasma levels of VEGF-A, -B, -C, -D and PlGF will be measured in triplicate by ELISA using commercially available assay kits (R&D Systems), taking precautions to eliminate contribution by activated platelets. Both pro-VEGF-C and active VEGF-C will be quantified.[44]

A portion of the peripheral blood monocytes will be set aside for protein extraction and subsequent analysis of VEGF-A, -B, -C, -D, PlGF, and VEGFR-1, -2, -3 using Western blot and commercially available antibodies (see below). The remaining cells will be used for dual-color flow cytometry, according to well-established methods,[45-47] to determine the proportion of circulating CD133+VEGFR2+ EPCs and CD34+VEGFR1+ HPCs. The mononuclear cell fraction is separated with Ficoll-Hypaque centrifugation and CD34 purification is performed with a Clinimacs CD34-separation kit (Miltenyi Biotech) and the purity assessed by flow cytometry. CD34+ cells are then further analyzed for co-expression of CD133 and VEGFR2 by dual color flow cytometry using a PE-conjugated monoclonal anti-body to CD133 and FITC-conjugated monoclonal antibody to VEGFR2; separate analyses will be performed to identify CD34+VEGFR1+ cells. Assays will be performed in triplicate and expressed as proportions, which have been shown to be on the order of 10 -20%.[32, 50] In separate studies, freshly isolated CD34+ cells will be analyzed for their capacity to form late outgrowth vascular colonies, a feature that is characteristic of progenitor cells.[48-50] Approximately $10^5$ CD34+ cells are cultured in endothelial culture medium containing VEGF. Endothelial cell colonies are then quantified by co-staining with DiI-acetylated-LDL (DiI-Ac-LDL, PerImmune) and von Willebrand factor (vWf). DiI-Ac-LDL+vWf+ endothelial cell colonies formed after 14 days are scored as late outgrowth colonies. Analyses will be performed in triplicate and expressed as the percent of late outgrowth colonies/$10^5$ peripheral blood monocytes. Late outgrowth vascular colonies will also be harvested for protein extraction and analysis of VEGF and VEGFR expression by Western blot, as described below.

In separate studies, a small portion of plasma will also be assessed with an *in vitro* functional angiogenic assay using human umbilical vein endothelial cells (HUVEC-based angiogenic scale), modified from previously described methods.[51] Early passaged HUVECs will be maintained in enriched endothelial cell culture medium until approximately 70% confluent, and then changed to serum-free medium. Patient plasma will be added to the cells to final concentration of 2.5% (in triplicate). After 24 hours, angiogenic morphology will be examined under light microscopy and scored by 2 independent observers according to an angiogenic scale (Figure 2): 0 – well separated individual cells; 1 – cells begin to migrate and align; 2 – visible capillary tubes, no sprouting; 3 – sprouting of new capillary tubes; 4 – polygonal structures begin to form; 5 – presence of complex mesh-like structures. The HUVEC-based system provides a global assessment of the plasma angiogenic activity before and during treatment, and its simplicity makes it potentially ideal for use as a bio-marker for assessing treatment efficacy. To evaluate this further, changes in the HUVEC-score during treatment will be correlated with changes in the other circulating pro-angiogenic variables measured.

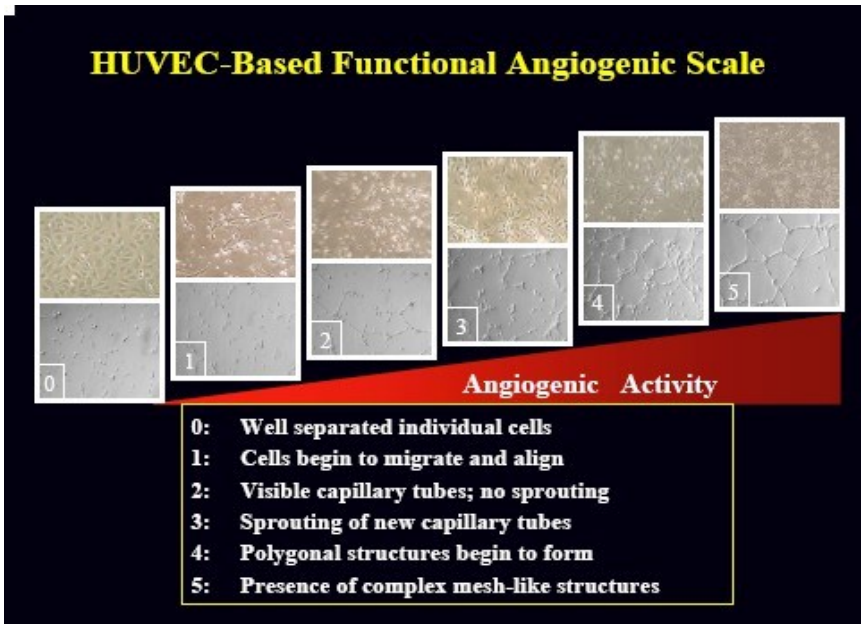


Figure 2. Summary of HUVEC-based functional angiogenic scale.

Protein extraction and Western blotting

Western blotting will be used to detect and validate the expression of VEGFs and VEGFRs on the peripheral blood mononuclear cells and late outgrowth colonies (CFU-EC) from CD133+VEGFR2+ EPCs and CD34+VEGFR1+ HPCs isolated from the peripheral blood cells. Endothelial colonies (CFU-EC) will be generated by incubating the peripheral blood mononuclear cells with VEGF; late outgrowth endothelial colonies and other hematopoietic mononuclear cells will be lysed in cold RIPA buffer in the presence of protease inhibitors. As controls, protein will also be extracted from HUVEC and leukemia cells. HUVECs will be used as positive controls for the expression of VEGFR-1, -2 and -3. Leukemia cell lines (KG1a, KG1 and HL60) will be used as positive controls for the expression of VEGF-A, -B, -C, -D and PlGF. After centrifugation to remove cell debris, supernatants (a total protein minimum of 500 ng) will be immunoprecipitated overnight at 4° C with protein G-agarose beads and an anti-phosphotyrosine antibody (Santa Cruz Biotechnology) to precipitate phosphorylated proteins or with a mouse antihuman VEGFs or VEGF receptors. Precipitated proteins/antibody/beads are then washed, re-suspended in loading buffer, and then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (7.5% gels) under reducing conditions. Proteins are subsequently blotted onto a nitrocellulose membrane following conventional protocols. Finally, blots will be blocked in 1% bovine serum albumin/phosphate-buffered salin-0.1%. Tween 20 for 1 hour at room temperature followed by incubation with primary and secondary antibodies. Antibodies used will include commercially available mouse antihuman VEGFs or VEGFRs, and a secondary peroxidase-labeled goat antimouse antibody. The ECL chemiluminescence detection system and ECL film will be used to visualize the presence of proteins on the nitrocellulose blots.

### 15.3 Analysis of Tumor Hypoxia

Paraffin-embedded biopsy specimens, obtained during operation and processed as above, will be used for these studies. Immunohistochemical staining will be overseen by Dr. Jinru Shia of the Department of Pathology, MSKCC, and will be performed through the Immunohistochemical Core Facility. Tumor and tumor-free liver specimens will be stained for HIF-1α, CA IX, Glut-1, Glut-3, and Ki-67 using well-characterized monoclonal antibodies and appropriate positive and negative controls. The slides will be evaluated and graded for both intensity and percentage of stained cells. Ki-67 will be scored as a continuous variable. For all other antibodies, <10% staining will be considered negative, while ≥10% staining will be considered positive. Correlations will be made between the immunohistochemical markers of hypoxia and pre- and post-treatment DCE-MRI measurements of tumor perfusion and permeability.

## 16.0 BIOSTATISTICS

The primary objective of this study is to assess the efficacy of continuous hepatic arterial infusion (HAI) of FUDR plus systemic bevacizumab in patients with unresectable HCC and ICC. The primary endpoint is median time to progression (TTP), as measured from time of pump placement to date of first documented progression. As explained in section 3.0, the median TTP was approximately 7 months in the investigator's Phase II study of HAI FUDR. We hypothesize that addition of systemic bevacizumab will result in a 50% improvement. A total of 48 events will give us 90% power to detect the hypothesized improvement, therefore we will enroll 55 patients, allowing for 15% censoring. This calculation assumes a Type I error of 5%, a one-sided test, and a median follow-up time of 2 years. It also assumes that survival times are exponentially distributed. This last assumption is for planning purposes only, and actual analysis will employ non-parametric methods for estimating the survival distribution.

The secondary objective is to assess dynamic MRI for measuring changes in tumor perfusion parameters during treatment. The measurements that will be obtained from this MRI scan are explained in detail above. Initial and post-treatment changes related to tumor perfusion (slope) and permeability ($Ak_{ep}$) will be compared and correlated with tumor response (as determined on unidimensional measurements) using two-sample tests and logistic regression.

The tertiary objectives include evaluating the expression patterns of VEGFR-1, -2, and –3 and their cognate ligands (VEGF-A, -B, -C, -D, and P1GF). These findings will be correlated to disease progression and survival using proportional hazards regression and Kaplan-Meier methods. Correlations will also be made between initial and post-treatment changes in tumor perfusion kinetics (as measured by DCE-MRI) and the tumor expression patterns of VEGF ligands and VEGF receptors. In addition, robust regression methods will be used to assess the relationship between baseline tissue and plasma levels of VEGF ligands and changes in the pre-

and post-treatment levels of VEGF ligands, VEGFR+ peripheral blood mononuclear cells, the proportion of circulating CD133+VEGFR2+ EPCs and CD34+VEGFR1+ HPCs, and HUVEC-based functional angiogenic scale. Correlation between treatment-related changes in the HUVEC scale and changes in other variables measured on peripheral blood and plasma samples will be assessed. Markers of tumor hypoxia (hypoxia inducible factor [HIF-1α, carbonic anhydrase IX [CA IX], and the glucose transporters Glut-1 and Glut-3, and microvessel density [CD31]) will be assessed on pre-treatment biopsies on a dichotomous scale. These data will be correlated with treatment efficacy and initial and post-treatment changes in tumor perfusion kinetics using logistic regression (for binary data such as treatment response) or linear regression (for continuous data such as tumor perfusion kinetics) analyses.

## 17.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

### 17.1 Subject Registration

The following person(s) can obtain informed consent:

William Jarnagin, MD
Nancy Kemeny, MD
Yuman Fong, MD
Peter Allen, MD
Ghassan Abou-Alfa, MD
Ki-Young Chung, MD
David D'Adamo, MD
David Ilson, MD

David Kelsen, MD
Mary Keohan, MD
Robert Maki, MD
Diane Reidy, MD
Eileen O'Reilly, MD
Leonard B. Saltz, MD
Gary Schwartz, MD
Neil Segal, MD
Manish Shah, MD
Archie Tse, MD
Leslie Blumgart, MD
Michael D'Angelica, MD
Ronald DeMatteo, MD

Confirm in the electronic medical record that the patient has received the Notice of

Privacy Practice. This must be obtained before the eligibility confirmation and obtaining of the research informed consent.

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject eligibility.

Obtain written informed consent, by following procedures defined in section entitled Informed Consent Procedures.

Patients must be registered to Step One, pending registration to Step Two before undergoing surgery in order to obtain liver tissue biopsy and research bloodwork. After pump placement, before treatment start, the Research Study Assistant will proceed with Step 2 of the registration process, thus allowing the patient to commence protocol therapy.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at (646) 735-8000. The PPR fax numbers are (646) 735-0008 and (646) 735-0003. Registrations can be phoned in or faxed. The completed signature page of the informed consent form, the completed signature page of the Research Authorization and a completed Eligibility Checklist must be faxed to PPR.

| | |
|---|---|
| Registering Individual | [Last, First Name] |
| Notice of Privacy Status | [Yes, No, N/A] |
| Research Authorization | [Date] |
| MSKCC IRB Protocol # | |
| Attending of Record (if applicable) | [Last, First Name] |
| Consenting Professional | [Last, First Name] |
| Informed Consent Date | |
| Participant's Full Name | [Last, First Name] |
| Participant's MRN | |

### 17.2 Randomization

This is a non-randomized study.

## 18.0 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team.

The data collected for this study will be entered into a secure database. Source documentation will be available to support the computerized patient record.

### 18.1 Quality Assurance

Weekly registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extext and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action

Random-sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year, more frequently if indicated.

### 18.2 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled "Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials" which can be found at: http://cancertrials.nci.nih.gov/researchers/dsm/index.html. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at: *http://mskweb2.mskcc.org/irb/index.htm*

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Center's Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) Will be addressed and the monitoring procedures will be established at the time of protocol activation.

## 19.0 PROTECTION OF HUMAN SUBJECTS

### 19.1 Risks

Treatment-related risks, including those related to pump placement and chemotherapy,

were discussed in Sections 5.0 Hepatic Artery Infusion Pump Background, 12.0 Toxicities/Side Effects and 13.0 Dose Modification.

### 19.2 Potential Benefits

Liver-directed chemotherapy for primary hepatic malignancy may increase the likelihood of tumor response and may extend life.

### 19.3 Possible Toxicities/Side-Effects

Treatment-related toxicity was discussed in Sections 5.0 and 11.0. Toxicity related to hepatic artery pump placement was discussed in Section 11.0.

### 19.4 Costs

Patients will be charged for physician visits, and routine laboratory and radiologic studies required for monitoring their condition. Patients will not be charged for protocol-related correlative study charges, which include the MRI imaging processing quantification, and analysis charge and image processing for the nuclear flow scans, and tissue and blood processing charges for laboratory correlative studies.

### 19.5 Alternatives

Patients eligible for this protocol are not candidates for resection. By virtue of their disease extent and relatively well-preserved hepatic function, these patients would not be considered for hepatic transplantation according to the current guidelines. These patients will have been treated with and failed at least one systemic regimen. The alternatives to the current protocol include systemic chemotherapy of a different type, either with standard or investigational agents, or ablative therapy. None of these options has been shown to improve survival. In addition, many patients will not be eligible for or will have failed ablative treatments. All of these issues will be discussed with the patients.

### 19.6 Patient Safety

All patients will be monitored in the outpatient clinic and chemotherapy unit, both of which are staffed by physicians and nurses familiar with clinical trials. In case of an adverse reaction, trained staff is available to provide immediate medical care. In the evenings and on weekends, medical attention is available through the Urgent Care Center, which is staffed at all times. Also, the principal investigators or their designees are available at all times.

### 19.7 Risk/Benefit Ratio

The treatment proposed in this study is aimed at patients with very limited, or no, effective alternatives. The investigators hope that the proposed treatment will be beneficial but this cannot be guaranteed. However, the investigators believe that the proposed treatment is based on sound scientific principles and draws on extensive experience from prior human studies. Every precaution has been and will be taken to ensure patient safety.

### 19.8 Inclusion of Children in Research

This protocol/project does not include children because the number of children is limited and because the majority are already accessed by a nationwide pediatric cancer research network. This statement is based on exclusion 4b of the NIH Policy and Guidelines on the Inclusion of Children as Participants in Research Involving Human Subjects.

### 19.9 Privacy

It is the responsibility of the Research Staff to ensure that protocol patients have received the Center's Notice of Privacy Practices. If the subject has not already done so, MSK personnel must try to obtain acknowledgment before the patient participates in this study.

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board.

### 19.10 Serious Adverse Event (SAE) Reporting

**All SAEs must be entered into the CRDB SAE form page.**

Any SAE must be reported to the IRB as soon as possible but no later than 5 calendar days. The IRB required a Clinical Research Database (CRDB) AE report to be delivered to the Institutional SAE Manager (307 East 63rd Street, 1st Floor) containing the following information:

Fields populated from the CRDB:

- Subject's name (generate the report with only initials if it will be sent outside of MSKCC)
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following information:
    - An explanation of how the AE was handled

- A description of the subject's condition
- Indication if the subject remains on the study
- If an amendment will need to be made to the protocol and/or consent form

The PI's signature and the date it was signed are required on the completed report.

### 19.11 Reporting of SAEs to Genentech, Inc.

All SAE reports must also be forwarded as soon as possible to:

Genentech Drug Safety
Fax: (650) 225-4682 or (650) 225-4683

For questions related to safety reporting, contact:

Genentech Drug Safety
Tel: 1-888-835-2555

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

**Genentech Adverse Event Reporting Definitions**

A serious treatment emergent adverse event (STEAE) is any sign, symptom or medical condition that emerges during Bevacizumab treatment or during a post-treatment follow-up period that (1) was not present at the start of Bevacizumab treatment and is not a chronic condition that is part of the patient's medical history, or (2) was present at the start of Bevacizumab treatment or as part of the patient's medical history but worsened in severity and/or frequency during therapy, AND that meets any of the following regulatory serious criteria:

- Results in death
- Is life-threatening
- Requires or prolongs inpatient hospitalization
- Is disabling
- Is a congenital anomaly/birth defect
- Is medically significant or requires medical or surgical intervention to prevent one of the outcomes listed above

Assessing Causality:

Investigators are required to assess whether there is a reasonable possibility that Bevacizumab caused or contributed to an adverse event. The following general guidance may be used.

*Yes:* if the temporal relationship of the clinical event to Bevacizumab administration makes a causal relationship possible, and other drugs, therapeutic interventions or underlying conditions do not provide a sufficient explanation for the observed event.

*No:* if the temporal relationship of the clinical event to Bevacizumab administration makes a causal relationship unlikely, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the observed event.

## 20.0 INFORMED CONSENT PROCEDURES

Consenting professionals are listed in section 17.0. Physicians qualified to conduct the informed consent process must be certified in the protection of human subjects for research. Before protocol-specified procedures are carried out, investigators or their staff will explain full details of the protocol and study procedures as well as the risks involved to the patients prior to their inclusion in the trial. Patients will also be informed that they are free to withdraw from the study at any time. All patients must sign an IRB-approved consent form indicating their consent to participate. This consent form will meet the requirements of the code of Federal regulations, the Institutional Review Board of this Center. The consent form will include the following:

- The nature of the objectives, potential toxicities and benefits of the intended study.
- The length of therapy and the likely follow-up required
- Alternatives to the proposed therapy. This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care.
- The name of the investigator(s) responsible for the protocol.
- The right of the patient to accept or refuse treatment and to withdraw from participation in this study.

The original signed consent forms will become a part of the patient's research file, stored in the electronic medical record. Each patient will receive a copy of the signed consent form.

### 20.1 Research Authorization

Procedures for obtaining Research Authorization: Before any protocol-specific procedures are carried out, investigators and/or designated staff will fully explain the details of the protocol, study procedures, and the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must sign the Research Authorization component of the informed consent form. The Research Authorization requires a separate set of signatures from the patient. The

original signed documents will become part of the patient's medical record, and each patient will receive a copy of the signed documents.

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