A Phase II Study of Hepatic Arterial Infusion (HAI) with Floxuridine (FUDR) and Dexamethasone (Dex) Combined with Systemic Gemcitabine and Oxaliplatin in Patients with Unresectable Intrahepatic Cholangiocarcinoma (ICC)

MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

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

OneMSK Sites	
Manhattan	All Protocol Activites
Basking Ridge	All Protocol Activites
Commack	All Protocol Activites
Monmouth	All Protocol Activites
Nassau	All Protocol Activites
Westchester	All Protocol Activites

Participating Institutions:	Site PI's Name	Site's Role

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

This is a phase II study of continuous HAI FUDR/Dex plus IV gemcitabine and oxaliplatin in patients with unresectable primary intrahepatic cholangiocarcinoma confined to the liver or associated with limited, resectable porta hepatis lymph node metastases. *Cohorts:* This protocol will enroll patients with intrahepatic cholangiocarcinoma treated with HAI FUDR/Dex plus IV gemcitabine and oxaliplatin or HAI FUDR/Dex plus IV gemcitabine alone and includes three cohorts. Cohort 1 will be comprised of patients who either have not received chemotherapy before for their liver disease, or responded to or had stable disease on prior chemotherapy. Cohort 2 will include patients who have failed systemic therapy. Cohort 3 will be for patients who cannot receive oxaliplatin because of neuropathy or allergic reactions, and will receive gemcitabine alone with HAI FUDR/Dex. Primary objective will be evaluated separately for the three cohorts. The protocol includes biological and radiographic correlative studies. The primary endpoint is 6-month progression free survival (PFS) for Cohort 1, 3-month PFS for Cohort 2, and response for Cohort 3.

Protocol Schema

Cycle Schema for Cycle 1	
Cycle 1 Day 1	Pump Therapy FUDR* + Dexamethasone (14 day infusion) Research blood*
Cycle 1 Day 15	Systemic Gemcitabine + Oxaliplatin or Gemcitabine alone** Pump Emptied Saline Research blood*
Cycle Schema for All Subsequent Cycles	
All subsequent Cycles on Day 1	Systemic Gemcitabine + Oxaliplatin or Gemcitabine alone** Pump Therapy FUDR* + Dexamethasone (14 day infusion) Research blood*
All subsequent cycles on Day 15	Systemic Gemcitabine + Oxaliplatin or Gemcitabine alone** Pump Emptied Saline
Months 1, 3, 6 and 9 post treatment start	DCE MRI
Months 3, 5 and 8 post treatment start	CT chest, abdomen and pelvis

*Research blood draws will be done on Cycle 1 Day 1, Cycle 1 Day 15, and Cycle 2 Day 1, and at various additional time points throughout treatment, one additional STRECK tube will be taken for cfDNA analysis during the patient's regularly scheduled blood draws.** Cohort 3 will receive Systemic Gemcitabine alone

2.0 OBJECTIVES AND SCIENTIFIC AIMS

Cohorts: This protocol will enroll patients with intrahepatic cholangiocarcinoma treated with HAI FUDR/Dex plus IV gemcitabine and oxaliplatin or HAI FUDR/Dex plus IV gemcitabine alone and will consist of three cohorts. Cohort 1 will be comprised of patients who either have not received chemotherapy before for their liver disease, or responded to or had stable disease on prior chemotherapy. Cohort 2 will include patients who have failed systemic therapy. Cohort 3 is for patients who have had prior oxaliplatin and have existing neuropathy or have allergic reactions and therefore will receive HAI FUDR/Dex with IV gemcitabine only. Primary objectives will be evaluated separately for the three cohorts.

Primary Objective for Cohort 1: The primary objective in Cohort 1 is to assess the 6-month progression free survival.

Primary Objective for Cohort 2: The primary objective in Cohort 2 is to assess the 3-month progression free survival.

Primary Objective for Cohort 3: The primary objective in Cohort 3 is to assess the response rate.

Secondary Objectives: Correlative objectives include an assessment of the clinical relevance of dynamic contrast enhanced (DCE)-MRI and diffusion weighted imaging (DWI) of intrahepatic cholangiocarcinoma before treatment and early during the course of treatment. This study will investigate DCE-MRI and DWI as potential imaging biomarkers and will measure tumor perfusion parameters and diffusion coefficients before initiating treatment and on follow-up MRI scans during treatment. Perfusion parameters (K^{trans} and IAUGC) and diffusion coefficients (ADC and ADC^{high}) at baseline, and early following treatment will be evaluated as prognostic factors for response and PFS. We are planning to analyze the secondary objectives combining data from both cohorts since there is no evidence that exposure to chemotherapy for liver disease (within the limits of inclusion/exclusion criteria) will alter the prognostic ability of DCE-MRI and DWI-MRI.

Other correlative aims are to analyze tumors for expression of CEACAM1 and CEACAM6, a potential biomarker of outcome and resistance to chemotherapy and to analyze the genomic profiles of intrahepatic cholangiocarcinoma and to correlate the findings with clinical and histopathological variables. Specifically, we hope to detect genetic mutations in biopsied tissue DNA as well as cell-free DNA (cfDNA) in plasma. We will analyze these secondary objectives separately, since exposure to chemotherapy has the potential to alter gene expression and alterations.

3.0 BACKGROUND AND RATIONALE

Primary intrahepatic cholangiocarcinoma (ICC) is the second most common primary hepatic malignancy. While ICC is much less common than hepatocellular carcinoma (HCC), its incidence and associated mortality are increasing. 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[1].

Complete resection is the most effective therapy for ICC, associated with 25-40% 5-year survival, but is not possible in many patients [2]. A report from MSKCC showed that locally advanced disease confined to the liver is the predominant cause of unresectability. Underlying hepatic parenchymal disease (i.e. chronic hepatitis, cirrhosis) is also a potentially treatment-limiting factor for some patients, although this is more common in HCC; however, its presence may preclude resection as a treatment option.

Patients with unresectable primary liver cancer have a median survival of less than 12 months. Multiple systemic regimens have been evaluated and shown to have limited efficacy. A recent study using systemic gemcitabine and cisplatin versus gemcitabine alone in advanced biliary tract cancer produced a median PFS of 8 and 5 months ($p<0.001$), respectively, with an overall survival difference of 3.6 months (11.7 versus 8.1 months, respectively, $p = 0.001$) [3]. Ablative approaches (radiofrequency ablation, microwave ablation, embolization) are often used in patients with unresectable HCC but their applicability in ICC is much more limited. The reason for this is that IHC are typically not hypervascular with respect to the surrounding liver, unlike HCC, making them less suitable for embolization; additionally, their large size at presentation precludes percutaneous ablative modalities.

Thus, for many patients with ICC, effective treatment options are limited. Liver-directed hepatic arterial 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. Experience with liver-directed chemotherapy in patients with biliary tract cancer is limited, although results of small series have been reported. Smith et al. reported 1 complete response (9%) and 6 partial responses (55%) out of 11 patients with cholangiocarcinoma and gallbladder carcinoma treated with bolus injection of hepatic artery 5FU and mitomycin C [4]. Using continuous HAI floxuridine (FUDR) through an implantable pump, Seeger et al. treated 3 patients with cholangiocarcinoma, 1 of whom had a complete pathological response [5].

Recently, the results of a phase II study, conducted at MSKCC (IRB#02-120) and evaluating the efficacy of HAI FUDR (with no systemic component) in patients with primary liver cancer the liver, were reported [6]. This study included 34 patients, 26 with ICC and 8 with HCC. The response rate was 47% and the median TTP was 7.4 months (CI 5.30-9.28), hepatic TTP of 10.1 months (CI 7.14-12.86) and overall survival of 29.5 months (CI 21.28-32.70). Patients with ICC appeared to derive more benefit, with a partial response rate of 54% (compared to 25% for HCC), although the number of HCC patients was much lower (Fig. 1). The therapy was well-tolerated, with only one grade 2 elevation is AST. The response rate and TTP observed in this study were higher than those reported for any systemic chemotherapeutic regimen, suggesting an important role for regional chemotherapeutic strategies in the treatment of unresectable ICC. However, since most initial treatment failures occurred in the liver, improving drug delivery to the liver appeared to be a means of enhancing the results of regional therapy [6].

Building on these initial results, a second study was initiated and completed at MSKCC (IRB#06-114), evaluating the efficacy of HAI FUDR combined with systemic bevacizumab in patients with liver-only disease. The rationale for this study was that bevacizumab would 'normalize' the tumor vasculature and improve FUDR delivery. Twenty-two patients were enrolled, 18 with ICC and 4 with HCC. The overall response rate was 32%, and the median TTP was 8.8 months, with a median follow-up time of 19 months (Fig. 2). While there was a modest improvement in TTP by adding bevacizumab, this came at a cost of significant toxicity, leading early termination of the study. Bevacizumab appeared to induce significant changes in tumor perfusion kinetics, as measured by dynamic contrast enhanced MRI, although the relationship of these changes to the observed increased toxicity is unclear [7].

Fig. 1

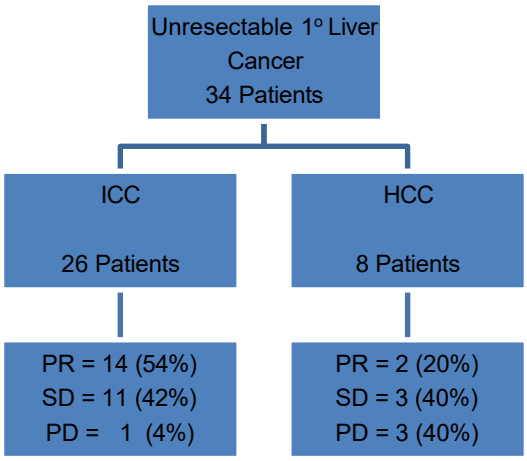
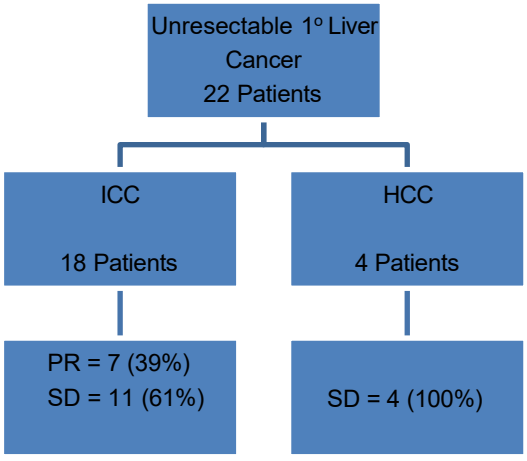


Fig. 2



The combined results of these two prospective trials demonstrated the potential utility of hepatic arterial chemotherapy for providing prolonged control of the hepatic disease in advanced ICC. It is apparent, however, that the addition of systemic therapy will be necessary if further improvements are to be made. The addition of such agents will potentially treat disease at

extrahepatic sites and may also enhance hepatic disease control. A recently published randomized study of over 400 patients has established gemcitabine, in combination with cisplatin, as the most active regimen of systemic cytotoxic agents [3]. Additionally, EGFR inhibitors have shown some promise in treating biliary tract cancers, particularly when used as combination therapy.

Currently, combined gemcitabine and cisplatin is the most effective systemic therapy for advanced biliary cancer, as discussed above. The current proposal will explore the utility of combining gemcitabine and oxaliplatin with HAI therapy. Each of these systemic agents has been effectively combined with HAI therapy, with acceptable toxicity. The combination of gemcitabine and oxaliplatin (GEMOX) has also been used pancreatic cancer protocols, with reasonable toxicity profile. Given the large experience using these agents in various combinations and their proven activity in biliary tract cancer, this treatment approach has a reasonable chance of improving disease control without significant increase in toxicity [8].

The rationale for using three cohorts is that many patients will be referred after starting systemic chemotherapy, which could impact the efficacy of regional FUDR. Currently, gemcitabine combined with a platin agent (oxaliplatin or cis-platin) is the most active systemic regimen for biliary cancer. Based on our prior experience with hepatic colorectal metastases, patients that have failed first line systemic therapy have a reduced (although still substantial) response rate to regional FUDR. Such differences are likely to be seen also in patients with IHC, and separate analyses for these cohorts are, therefore, reasonable in order to assess the efficacy of the intervention.

3.1 Gemcitabine

Gemcitabine is an antineoplastic agent that is structurally related to cytarabine. It is a pyrimidine analogue that is cell-cycle specific. Gemcitabine is cytotoxic to cells undergoing DNA synthesis (S-phase) and also blocks the progression of cells through the G1/S-phase boundary. Gemcitabine was approved for the treatment of pancreatic cancer based on the results of a randomized control trial that demonstrated improvement of disease-related symptoms and survival when compared to 5-FU based therapy. Single-agent gemcitabine for the treatment of biliary tumors is associated with a response rate of 22.6%, tumor control rate of 65%, and a median TTP of 5.5 months and medical overall survival of 8 months according to a pooled analysis of 2810 patients. The standard dosing for gemcitabine is 1000 mg/m ^{2} over 30 minutes, administered weekly. Most common (>5%) reported serious adverse events were fever (7.3%), pain (6.8%), asthenia (6%), abdominal pain (5.5%), and dyspnea (5%) [9]. Discontinuations due to adverse events in 2140 patients were at a rate of 4.6%. Nausea and vomiting occurred in less than 4% of patients [10]. A toxicity overview on 790 patients treated with single-agent gemcitabine has been completed. The analysis included patients enrolled in 18 completed clinical trials in different tumor types. In terms of laboratory toxicity, grade 3/4 adverse events presented with the following incidence: anemia 6.4/0.9%, leucopenia 8.1/0.5%, neutropenia 18.7/5.7%, thrombocytopenia 3.7/1%, elevated ALT 7.4/1.8%, elevated AST 5.7/1.4%, elevated alkaline phosphatase 4.5/2/1%, elevated bilirubin 1/0.5%, elevated creatinine 0.1/0%, proteinuria 0.5/0%, and hematuria 1.3/0%. Symptomatic toxicity was available for 439 patients. Grade 3/4 adverse events included: nausea and vomiting 19.7/0.9% (all patients treated with

prophylactic antiemetics scored at least as grade 3 regardless of actual toxicity experienced); alopecia 0.5/0%; dyspnea 1.6/0.2%; cutaneous 0.2/0%; mucositis 0.2/0%; allergic 0.2/0%; infection 0.9/0%, and somnolence 0.5/0%. Moderate to severe flu-like syndrome presented in 5.4/1.5% of patients, respectively. Moderate to severe peripheral edema presented in 12.1 and 2.6% of patients respectively. Rare but clinically significant side effects include hemolytic uremic syndrome and/or renal failure, liver failure and severe pulmonary toxicity [11].

3.2 Oxaliplatin

Oxaliplatin (trans-1-1,2-diaminocyclohexane oxalatoplatinum), is an antineoplastic platinum derivative with a 1,2-diaminocyclohexane [DACH] carrier ligand. Platinum compounds in general are thought to exert their cytotoxic effects through the formation of DNA adducts that block both DNA transcription and replication which results in cell death and the induction of apoptosis in actively dividing cells. The precise mechanism of action of oxaliplatin is unknown. DACH-platinum adducts formed by oxaliplatin appear to be more effective in inhibiting DNA synthesis than cis-diamine-platinum adducts formed by cisplatin and carboplatin. The superior potency of oxaliplatin over cisplatin *in vitro* may be related to the bulky DACH carrier group which can prevent binding to the mismatch repair complex and makes replicative bypass past the site of DNA damage more difficult [12]. Oxaliplatin has been used in conjunction with gemcitabine and cetuximab for patients with unresectable advanced or metastatic biliary tract cancer. The study was conducted with 30 patients of which 63% had an objective response, 10% achieved a complete response, and 53% had a partial response. No patients underwent a potentially curative secondary resection after response. Grade 3 toxicities were experienced by patients; skin rash (13%), peripheral neuropathy (13%), thrombocytopenia (10%), nausea (3%), diarrhea (3%), and neutropenia (3%) [13].

3.3 Rationale for HAI FUDR/Dex and Gemcitabine + Oxaliplatin

The proposed study seeks to build on the encouraging results observed with HAI FUDR in the first two studies that resulted in median survival rates of 29 and 30 months, respectively. The systemic combination of gemcitabine and oxaliplatin has recently been shown have activity against biliary tract cancer and to improve disease control in patients with unresectable disease. A study conducted at MSKCC used oxaliplatin and CPT-11 combined with HAI therapy for patients with unresectable metastatic colorectal carcinoma to the liver and proved to be tolerable with excellent survival results. The response rate was 92%, and 47% of patients attained sufficient reduction in tumor burden to allow a complete resection. The median survival for chemotherapy naïve patients in this study was 50.8 months and 35 months for patients previously treated [14].

The purpose of the present study is to increase treatment efficacy in patients with intrahepatic cholangiocarcinoma by adding systemic gemcitabine and oxaliplatin to HAI therapy. The rationale for the phase II study design is that we have ample toxicity data in patients with unresectable intrahepatic cholangiocarcinoma treated with regional and systemic chemotherapy; this is in contrast to the adjuvant setting, where toxicity data does not exist.

3.4 Functional MR Imaging

3.4.1 Background

Tumor response to chemotherapy has traditionally been assessed by measurements of tumor size. More recently, functional imaging techniques that can assess biological parameters, such as vascularity or metabolism, have been explored to better predict tumor response either before or early after initiation of therapy. Functional MR imaging, including dynamic contrast enhanced (DCE)-MRI and Diffusion Weighted Imaging (DWI), can help assess the biological effects of therapy before changes in tumor size occur. Functional imaging can potentially 1) improve pre-treatment prediction of tumor response, 2) predict response early after initiating therapy, and 3) monitor tumor biology once size has stabilized.

Clinical MR examinations of the liver routinely include the use of gadolinium (Gd) based contrast agents to assess tumor vascularity and improve tumor conspicuity. Dynamic contrast enhanced (DCE)-MRI can be incorporated into routine liver MRI to quantitatively evaluate the passage and distribution of Gd contrast agents from the circulation to tumors over time. Quantitative perfusion parameters obtained from DCE-MRI reflect the rate of exchange of Gd and include K^{trans} - a volume transfer constant between blood plasma or vascular space (VS) and extracellular extravascular space (EES), kep - the rate constant between EES and VS, and ve - the fractional vascular volume. These parameters can be measured at baseline and compared on follow-up post treatment scans. Since perfusion parameters are derived from DCE-MRI, this technique was initially applied for evaluation of antiangiogenic therapy, but its use has expanded to other cytotoxic agents.

In a prior phase II trial at MSKCC for unresectable ICC or HCC using HAI with FUDR and dexamethasone, pre treatment and post-treatment changes in tumor perfusion characteristics measured by DCE-MRI correlated with treatment outcome [6]. Limited data is available on the use of DCE-MRI to assess response in cholangiocarcinoma or other tumors to gemcitabine or oxaliplatin. For example, DCE-MRI was applied in a feasibility study that included malignant pleural mesothelioma treated with gemcitabine [15]. Perfusion CT can generate perfusion parameters similar to DCE-MRI, and has been applied to monitor the response of HCC to bevacizumab in combination with gemcitabine and oxaliplatin [16]. Perfusion CT has also been used in the evaluation of pancreatic cancer to concurrent gemcitabine based chemotherapy with radiation therapy [17]. This study showed a higher pre-treatment K^{trans} parameter in responders versus non-responders. Thus, perfusion parameters obtained with DCE-MRI or perfusion CT show promise for evaluation of treatment response to chemotherapeutic agents used in this study.

Diffusion weighted imaging (DWI) is also emerging as a functional imaging biomarker to help predict and monitor tumor response to therapy [18] [19] [20]. Using DWI, one can measure the apparent diffusion coefficients (ADC) of tumors, which reflect water diffusivity within tumors, an indirect measure of tumor cellularity at baseline, and necrosis following treatment. For example, in primary rectal tumors [21] [22] and colorectal liver metastases [23] [24], pretreatment ADC predict response to chemoradiation and chemotherapy respectively. A study using DWI to monitor hepatocellular carcinoma response to sorafenib is also promising [25]. As an indirect measure of tumor cellularity and necrosis, DWI is ideally suited as a biomarker to assess early

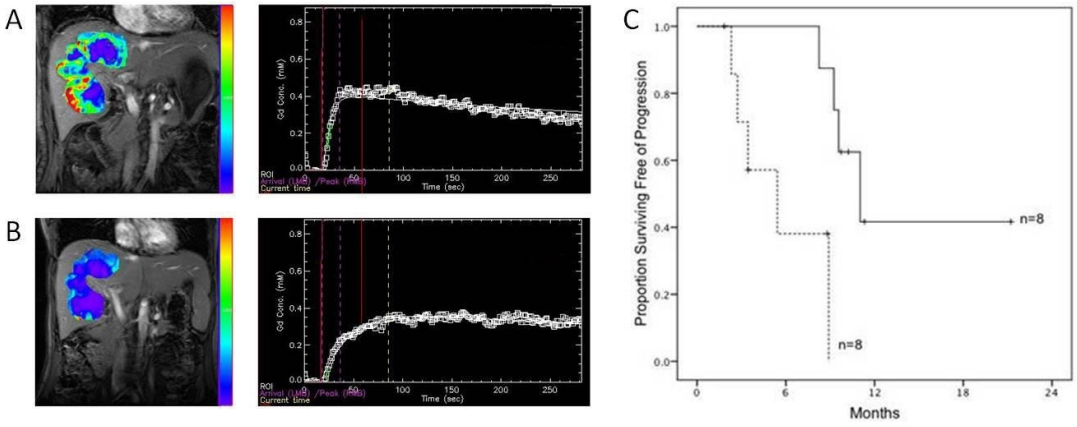
therapy response for combination chemotherapy, where the effects of individual therapies may be impossible to separate.

Limited data is available on the use of DWI to assess the chemotherapeutic agents used in the current proposal. In a study of patients with advanced pancreatic cancer treated with gemcitabine, ADC measurements prior to and during treatment was lower in patients with stable disease versus those with disease progression at six month follow-up [26]. In a study of 12 patients with colorectal metastases treated with hepatic arterial infusion pump using 5-FU, percentage changes in ADC at day 9 of treatment correlated with tumor size changes at 3 months [27].

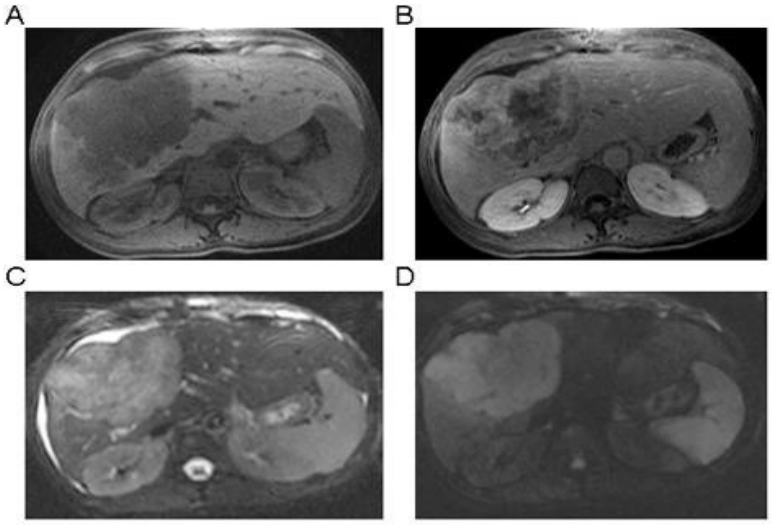
The development of functional MR imaging biomarkers for intrahepatic cholangiocarcinoma would represent an important clinical advance and requires further investigation. The combination of both DCE-MRI and DWI will offer a multiparameteric approach to assessing tumor response. The studies in this specific aim will provide more data regarding the utility of DCE-MRI and DWI as functional imaging biomarkers for cholangiocarcinoma before and during treatment.

3.4.2 Preliminary Data

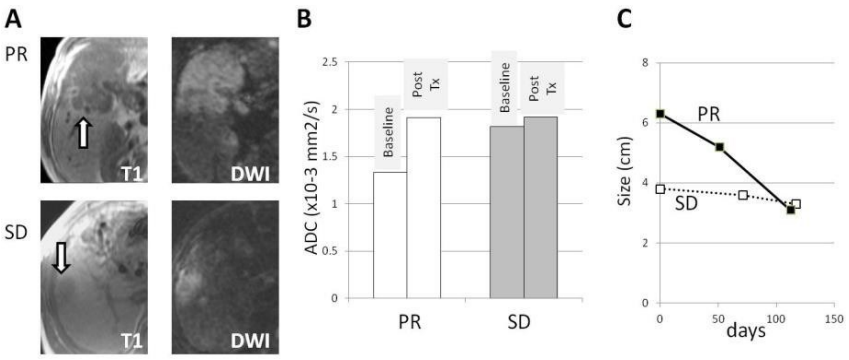
There is growing consensus [28] on which perfusion parameters should be used during the application of DCE-MRI: the transfer constant, **K^{trans}**, describing the passage of Gd from the vascular space to the extravascular, extracellular space [29], and the blood normalized Initial Area Under the Gd Curve (**IAUGC**) [30]. In our prior phase II trial at MSKCC for unresectable ICC or HCC using HAI FUDR, post-treatment changes in K^{trans} and IAUGC were measurable by DCE-MRI and correlated with treatment outcome Pre treatment (A) and early post-treatment (B) imaging of a large right intrahepatic cholangiocarcinoma demonstrate treatment induced changes in tumor perfusion characteristics. Left panels show coronal images through the liver, with a color overlay of the tumor K^{trans}, generated from DCE-MRI data. Right panels show corresponding gadolinium concentration curves as a function of time. Pre-treatment (A), the tumor shows overall high K^{trans}, predominantly at the periphery, with corres-ponding rapid uptake of Gd after contrast administration. Post HAI FUDR treatment (B), the tumor shows decreased K^{trans} and correspond slower uptake of Gd. Differences in median progression free survival (C) were seen in patients dichotomized by changes in IAUGC ($p=0.004$), with the solid line corresponding to patients dichotomized to those greater than (solid line) or less than (dotted line) a median reduction in IAUGC of 25%.



During our prior studies with intrahepatic cholangiocarcinoma, limited diffusion weighted imaging data were collected, as this functional imaging technique was recently incorporated on our clinical MR examinations. ICCs are generally large liver tumors with low signal on T1 weighted imaging (Panel A) with heterogeneous enhancement (Panel B). With their large size and high signal to noise ratio on T2 weighted imaging, they are ideally suited for DWI, even at high b values (Panels C, b = 0, Panel D, b = 400 s/mm^2).

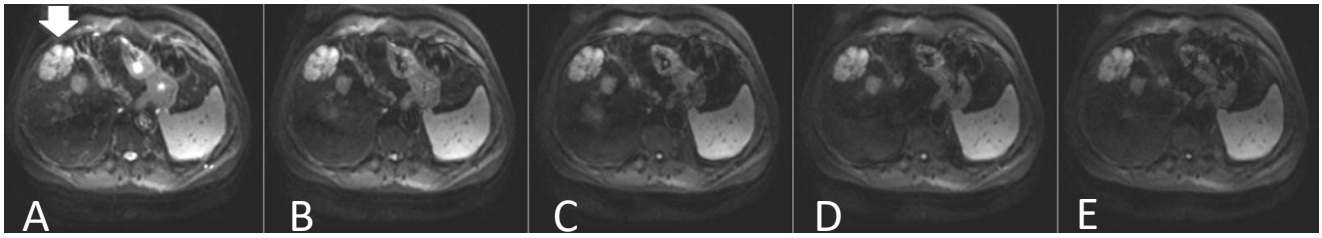


We have preliminary data showing the potential of DWI to predict response to HAI FUDR following treatment, before significant changes in size occur. In a patient who demonstrated a partial response based on anatomic criteria (Panel A, B, C - PR), a corresponding early increase in tumor ADC was seen on the first post-treatment MRI 8 weeks following treatment. An increase in ADC is expected with the breakdown of cellular integrity, freeing the motion of water molecules, which can be measured with DWI. In contrast, a patient whose tumor was stable in size during treatment (Panel A, B, C - SD), the tumor ADC remained stable as well.



Apparent diffusion coefficients (ADC) can be calculated from a DWI sequence using a single b value. However, the use of a single b value in DWI cannot separate water motion in the microcirculation (perfusion fraction) from water motion within the extravascular space. Water diffusion in these compartments can be separated using DWI with multiple b values, which is now possible with recent advances in clinical MR scanners and is preferable for calculations of ADC as a tumor biomarker based on a 2009 consensus statement [18]. With multiple high b values, DWI can also provide measurements of perfusion independent tissue diffusion coefficients. DWI and DCE-MRI can thus provide complimentary information on the tumor microenvironment, as they measure different properties of the tumor during the course of treatment: tumor cellular integrity and microvascular properties, respectively.

We have preliminary results below showing the feasibility of multi b value DWI in the liver performed during a routine clinical examination. For example, in a patient with metastatic neuroendocrine tumor, we can obtain high signal to noise at multiple b value in a liver metastasis (DWI sequence obtained at Panel A, b = 0 s/mm ^{2}; B, b = 50 s/mm2; C, b = 250 s/mm^2; D, b = 350, and E, b = 500 s/mm^2. White arrow: liver metastasis). From these images, perfusion independent diffusion coefficients can be obtained [31].



3.5 CEACAM6 and CEACAM1 as Biomarkers of Outcome and Resistance to Therapy

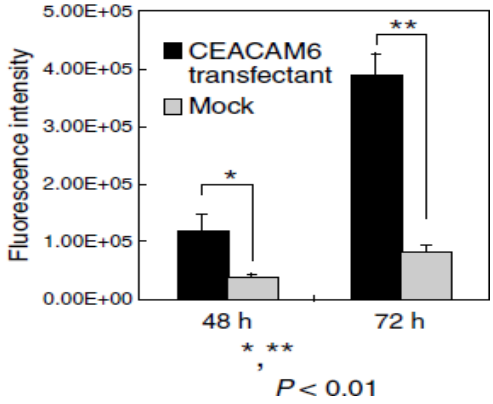
3.5.1 CEACAM6

CEACAM6 is a glycosylphosphatidylinositol (GPI)-linked immunoglobulin member of the CEA family. It is overexpressed in a variety of gastrointestinal malignancies and associated with poor prognosis in pancreatic and colorectal carcinoma [32, 33]. Cellular invasion, resistance to anoikis, apoptosis induced by inadequate cell-substrate adhesion, and chemoresistance to gemcitabine are all upregulated by CEACAM6 in models of pancreatic cancer [34] [35] [36]. The role of CEACAM6 expression in human biliary tract cancer has been studied to a limited degree. CEACAM6 promotes IGF-1-mediated cellular invasiveness [37], and appears to have

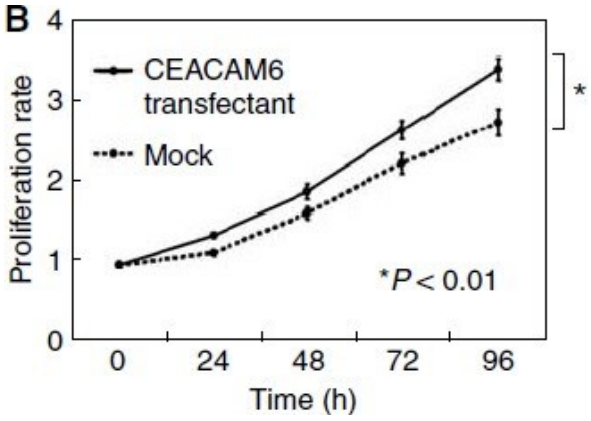
an important role in the pathogenesis of biliary tract cancer. In a small study [38] of 23 patients with intrahepatic cholangiocarcinoma, intense CEACAM6 immunohistochemical staining was detected on the cell membrane of tumor tissues compared to normal hepatic parenchyma. Furthermore, CEACAM6 mRNA signal was higher in tumor compared to normal tissues, and the level of expression correlated directly with lymph node metastases, more advanced stage and a worse disease-free interval. Additionally, cholangiocarcinoma cells (HuCC-T1) transfected with CEACAM6 were more invasive in Matrigel and more proliferative by MTT assay (panels A and B, below). Additionally, CEACAM6-transfected cholangiocarcinoma cells (HuCC-T1) were more resistant to Gemcitabine (panel C).

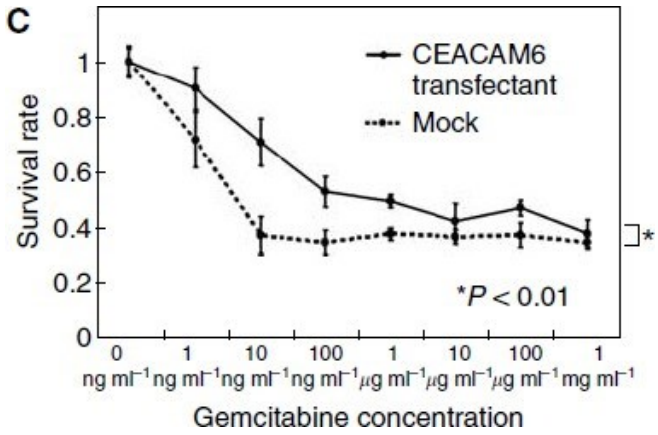
A recent study of patients with biliary tract adenocarcinoma showed that CECAM6 over-expression was associated with significantly worse survival (MSKCC, unpublished data, see panel D).

A

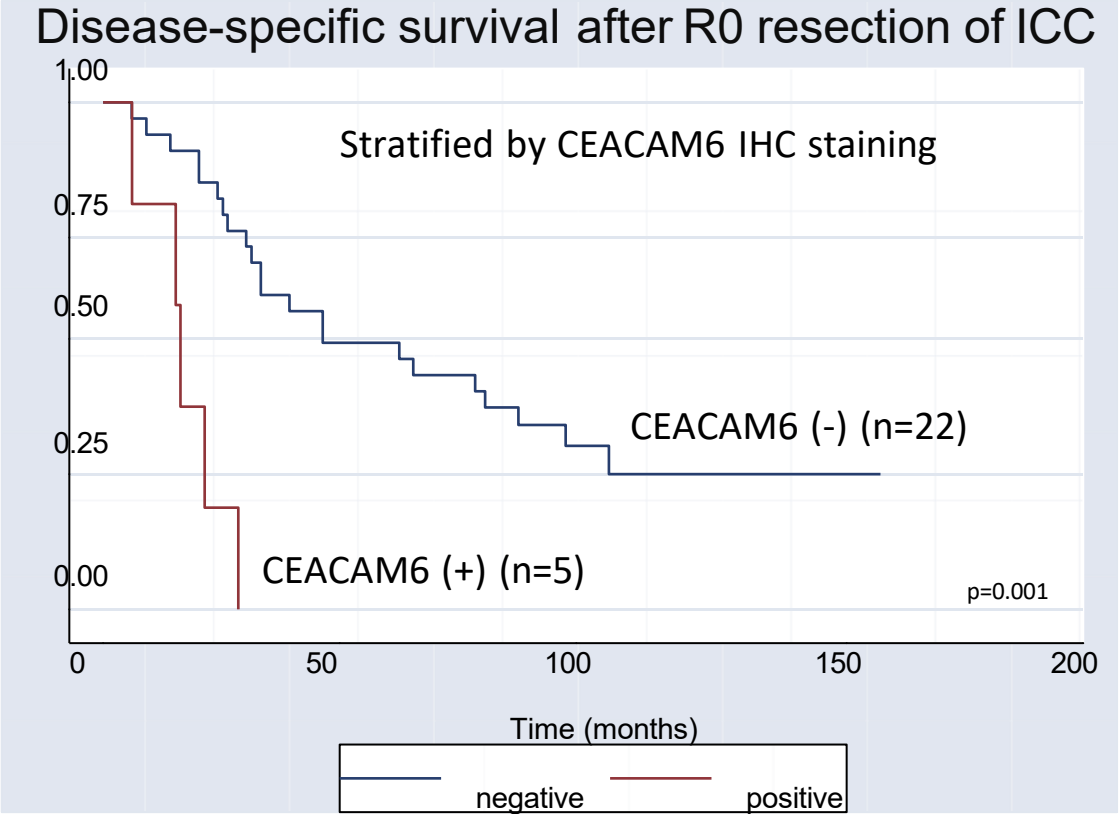


B





D



3.5.2 CEACAM1

CEACAM1 is another glycosylphosphatidylinositol (GPI)-linked immunoglobulin member of the CEA family. CEACAM1 is a negative regulator of tumor cell growth. Its expression is down-regulated with increasing histologic grade in a number of malignancies. Well differentiated hepatocellular carcinoma (HCC) cells show diffuse CEACAM1 expression on immunohistochemistry, whereas poorly differentiated tumor cells lose CEACAM1 expression. A study from Japan showed a loss of CEACAM1 expression was significantly associated with large tumor burden, portal vein invasion, and satellite nodules and affected survival adversely.

Of the 139 patients, 66% with Edmondson-Steiner grades III or IV tumors had loss of CEACAM1 expression, compared to only to 5% with grades I or II tumors. This study suggests that loss of CEACAM1 expression is associated with aggressive tumor biology and a poor prognosis for patients with HCC[39]. By contrast, CEACAM1 expression is increased in lung cancer and melanoma, and higher levels of CEACAM1 expression in these two tumor types is associated with more aggressive disease[40, 41]. CEACAM1 expression in cholangiocarcinoma has not been investigated; however, in a study of pancreatic carcinoma, CEACAM1 expression was upregulated in 87% of patients with early, non-invasive malignant lesions (carcinoma *in situ* or pancreatic intra-epithelial neoplasia-3 (PanIN-3)[42], suggesting that change in expression pattern may represent an early step in pathogenesis. Furthermore, serum levels of CEACAM1 was a more reliable marker of disease than the current standard CA19-9 and may therefore have a role as a biomarker.

The correlative studies involving CEACAM6 and CEACAM1 are exploratory in nature and may be hypothesis generating. While preliminary data with CEACAM6 in a small cohort of patients with cholangiocarcinoma are provocative, the sample size was limited and the analysis was performed retrospectively from resected specimens; the results, therefore, cannot be extrapolated to the larger subgroup of patients with unresectable disease. Data on CEACAM1 in cholangiocarcinoma, by contrast, is non-existent.

3.6 Genomic Profiling of Intrahepatic Cholangiocarcinoma

Recent years have seen a tremendous increase in our understanding the genetic basis of several solid tumors, many of which have influenced treatment. Intrahepatic cholangiocarcinoma represents a notable exception in this regard, however. While several studies have documented a number of selected genetic alterations in intrahepatic cholangiocarcinoma, comprehensive analyses are lacking. Thus, the full range of genetic alterations is poorly understood. A recent study in prostate cancer identified unique, complex chains of balanced rearrangements within or adjacent to known cancer genes [43]. In the present study, a similar approach of paired-end, massively parallel sequencing on tumor and matched normal genomic DNA will be used to fully explore the genomic alterations in intrahepatic cholangiocarcinoma. Correlations will be made with clinical and histopathological variables, including tumor grade, lymphovascular invasion, multifocality, lymph node metastases, and therapeutic response.

Cell-free DNA (cfDNA) in peripheral blood samples is considered a potentially useful tool for noninvasive cancer diagnosis, a possible surrogate for tumor genotyping and clinical outcomes predictor in multiple solid tumors. For cholangiocarcinoma in particular, obtaining adequate biopsy material for genotyping may be challenging and potentially expose the patient to complications given the anatomic location of tumor. Thus, analysis of cfDNA would be preferable as it could be used for patients in whom a biopsy is not feasible, lends itself to multiple repetitions, and allows for real-time monitoring of disease progression or response during therapy. cfDNA detection has reported in 40–80% of patients with metastatic breast cancer and circulating tumor and counts above a cut-off value of 5 cells per 7.5 ml of blood was showed to correlate with tumor burden and poor clinical outcomes. Furthermore, dynamic changes in cfDNA counts during therapy have been reported to reflect treatment response.

Recent publications have demonstrated an association of acquired drug resistance and specific cfDNA alterations in patients with advanced ovarian, lung, and breast cancers. Currently, there is no published data on cfDNA detection or quantification in cholangiocarcinoma. Peripheral blood sampling would not only be less invasive and an easier method with regards to detection and treatment response, but also possibly more informative than standard radiologic imaging as radiologic assessment in these tumors is especially challenging due to central necrosis. In a prospective multicenter trial, persistent high levels of cfDNA were shown to be predictive of worse clinical outcome despite radiological therapy response.

The published genomic studies on intrahepatic cholangiocarcinoma are few and suffer from inadequate sample size and corruption from inclusion of tumors arising from other areas of the biliary tree. A recent multi-center study suggested that genetic profiles may stratify patients in to risk groups; however, as with prior studies with CEACAM6, this analysis was retrospectively performed on resected specimens, and the relevance of the findings for the much larger cohort of patients with advanced, unresectable disease is not clear.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

A total of 86 patients (37 patients in Cohort 1, 39 patients in Cohort 2, and ≤ 10 patients in Cohort 3) with cholangiocarcinoma whose liver lesions have been deemed to be unresectable will be treated with HAI FUDR/Dex plus systemic gemcitabine and oxaliplatin (Cohorts 1 and 2) or HAI FUDR/Dex plus systemic gemcitabine alone (Cohort 3). Patients with limited, resectable nodal disease will be eligible. Unidimensional tumor measurements on standard MRI sequences will be used to assess response to therapy at months 1, 3, 6 and 9, following guidelines from Response Evaluation Criteria in Solid Tumors (RECIST) 1.1. Patients will remain on study as long as they show continued response as defined in section 12.0, and do not experience significant toxicity as defined in Section 11.0.

Cycle Schema for Cycle 1	
Cycle 1 Day 1***	***Cycle 1 Day 15***
Pump Therapy* FUDR + Dexamethasone (14 day infusion)	**Pump Emptied** Saline **Systemic Therapy**** Gemcitabine + Oxaliplatin or Gemcitabine alone

Cycle Schema for All Subsequent Cycles	
All subsequent Cycles on Day 1	***All subsequent Cycles on Day 15***

Pump Therapy*	**Pump Emptied**
FUDR + Dexamethasone (14 day infusion)	Saline
Systemic Therapy**	**Systemic Therapy****
Gemcitabine + Oxaliplatin or Gemcitabine alone	Gemcitabine + Oxaliplatin or Gemcitabine alone

**The following pump dose will be used:*

FUDR [0.12 mg/kg * kg * 30] / pump flow rate

Dexamethasone [1 mg/day * 30] / pump flow rate

*** The following systemic doses will be used:*

Gemcitabine 800 mg/m^2 IV over approximately 30-minutes, every two weeks

Oxaliplatin 85 mg/m^2 IV over approximately 120-minutes, every two weeks

NOTE: Patients on Cohort 3 will receive Gemcitabine 1000 mg/m^2 IV over approximately 30-minutes, every two weeks

**** On Cycle 1 Day 1 systemic chemotherapy will be held*

If clinically appropriate, patients will have pump emptied and filled with glycerol instead of heparin saline. Glycerol will last for 6 to 8 weeks; within that time, patient does not have to have pump filled with heparin and saline. If patients are allergic to heparin, they will receive Fondaparinux in the pump instead along with saline.

4.2 Intervention

- All patients receive HAI FUDR ([0.12 mg/kg * kg * 30] / pump flow rate) and dexamethasone ([1 mg/day * 30] / pump flow rate) on Day 1 of each cycle. Chemotherapy with HAI FUDR/Dex will commence no sooner than 14 days post surgical placement of HAI pump.
- Patients in Cohort 1 and Cohort 2 will receive Gemcitabine (800 mg/m^2 IV over 30 minutes) and Oxaliplatin (85 mg/ m^2 IV over 120 minutes) and patients in Cohort 3 will receive Gemcitabine (1000 mg/m^2 IV over 30 minutes) alone on Days 1 and 15 of each cycle, however initiation with systemic chemotherapy will not take place until 4 weeks post-surgery for pump placement, so the first doses of systemic chemotherapy will be given on Cycle 1, Day 15, and then every 2 weeks thereafter.
- Clinical MRI examinations of the abdomen are obtained at baseline following surgery and approximately 4 weeks after initiation of HAI FUDR. Subsequently, the patient will undergo MRI approximately at months 3, 6 and 9 thereafter. A CT of chest, abdomen and pelvis will also be obtained approximately at months 3, 5, and 8 thereafter as part of routine clinical

care. A CT triphasic scan of the chest, abdomen, and pelvis can also be used at the discretion of the treating physician. A window of +/- 2 weeks for scans is allowed in order to accommodate patient schedules.

4.3 Correlative Studies

4.3.1 Liver and Tumor Biopsy, Blood Collection

- Permission from patients entering the study will be obtained to take normal and tumor liver biopsies at the time of surgery. These samples are voluntary and optional. Blood samples (2-10mL red top tubes with no additive) will be taken before the start of FUDR treatment on Cycle 1 Day 1, after the first cycle of FUDR treatment on Cycle 1 Day 15, and after the first cycle of treatment on Cycle 2 Day 1 for patients at MSKCC. At various time points throughout treatment, one additional STRECK tube will be taken for cfDNA analysis.

- All red top tubes (no additive) samples collected at MSKCC will be processed by the following protocol:
    - Allow blood to clot for 30-60 minutes at room temperature.
    - Spin at 4°C 1800g x 15min.
    - Transfer serum immediately to cryotube without disturbing the layers and freeze in -80°C.
  - Samples collected at MSKCC will be processed by Department of Surgery at Memorial Sloan-Kettering Cancer Center and subsequently stored at -70°C in a hepatopancreatobiliary freezer (HPB) freezer in Zuckerman.
- cfDNA extraction from STRECK tubes will be performed with a QIAsymphony SP system (QIAGEN) in collaboration with the Department of Laboratory Medicine. cfDNA will be quantified with a a Fragment Analyzer (Advanced Analytical) using a High Sensitivity Genomic DNA Analysis Kit. In addition, for patients with specific somatic mutations previously observed in the primary tumor via IMPACT assay (KRAS, NRAS, IDH, BAP1, FGR2, MEK1, EGFR, ERBB2), cfDNA will be extracted from stored serum samples and further analyzed by the CMO under the supervision of Dr. Berger. Specifically, we will utilize the expertise of CMO for evaluating the cfDNAs by:
    - 1) validating the mutations identified by MSK-IMPACT of tumor samples, using Digital PCR platform
    - 2) evaluating the concordance between tumor and cfDNA samples and correlate with treatment response as well as MRI imaging using IMPACT and/or custom targeted panels.
    - For further details, please see appendix B.

- All samples should be labeled with the unique patient study identification number provided at registration, sample time point, and the date of the specimen.

4.3.2 CEACAM6 Expression (tumor)

- Analysis for CEACAM6 expression will be performed at MSKCC according to a standard protocol that the investigators have used previously. Tissue biopsies (tumor and adjacent

non-neoplastic liver) from patients on this study will be obtained at the time of pump placement. The paraffin sections will be examined by a pathologist and the corresponding blocks will be marked and a precision manual tissue microarrayer will be used to procure triplicate 0.6mm cores that will be embedded into a recipient block. Microarray hematoxylin and eosin-stained 5mm sections will be cut and examined for integrity of cores.

- Immunohistochemistry will be performed at MSKCC by deparaffinizing, rehydrating and pretreating tissue on the slide as mandated by antibody protocols. Endogenous peroxidase activity will be quenched with 3% H_20_2 and bovine serum albumin used to minimize non-specific binding. Primary mouse anti-CEACAM6 Ab (By114, Santa Cruz Biotechnology) will be diluted 1:100 and applied to the TMA followed by a biotinylated anti-mouse secondary antibody at 1:500 (Vector). After washing with phosphate-buffered saline, a peroxidase-conjugated streptavidin diluted 1:500 (Dako) will be placed on the TMA for one hour. Diaminobenzidine will be used as the chromogen and hematoxylin as the nuclear counterstain. Appropriate positive controls will be used to optimize antibodies prior to detection. Stained sections from the TMA will be evaluated by a dedicated gastrointestinal pathologist (D Klimstra, MD), blinded to clinical data and graded for intensity on a scare of 0 – 4 (4=most intense) as previously described. Non-neoplastic tissue will serve as the internal control for the CEACAM6 antibody to exclude non-specific binding. Only neoplastic biliary tissue will be scored. After evaluation of raw data, results will be dichotomized into positive and negative for CEACAM6 and correlated with histopathological variables and clinical outcome (ie, therapeutic response and progression free survival).

4.3.3 CEACAM1 Expression (serum and tumor)

- CEACAM1 expression in serum will be measured at MSK prior to treatment and after the first treatment cycle. Ten ml of blood will be obtained at the indicated times and processed to obtain serum, which will be stored at -80^{o}C. A sandwich ELISA system will be used (KPL, Gaithersberg, MD). Affinity purified CEACAM1 monoclonal antibody (4D1/C2, EMD Millipore, Billerica, MA) will be coated to 96 well ELISA plastes at a concentration of 1 µg/mL overnight. The wells will then filled with five microliters of patients' sera and 95 µL of blocking buffer for one hour. After washing 3 times, a pan-CEA antibody, which recognizes CEA, CEACAM1, and nonspecific cross-reacting antigen, will be applied for another hour followed by washing. A horseradish peroxidase-labeled antibody that reacts with the pan-CEA antibody will then be added and incubated for 1 hour and substrate added after 5 washes. The plate will be read with a plate reader at 405 nm [39].

- Immunohistochemical analysis of tumor and adjacent non-neoplastic liver will be performed at MSK as described above for CEACAM6. The monoclonal antibody used for these studies will be 4D1/C2 (EMD Millipore, Billerica, MA), which is specific for CEACAM1 and will be used as previously described [39]. Only neoplastic biliary tissue will be scored. After evaluation of raw data, results will be dichotomized into positive and negative for CEACAM1 and correlated with histopathological variables and clinical outcome (ie, therapeutic response and progression free survival).

4.3.4 Clinical relevance of treatment-related changes in tumor perfusion parameters and diffusion coefficients

- MRI will be the primary imaging modality used to assess disease response. Standard T1 weighted sequences will be used for tumor size measurement, which will determine the primary outcome and will be calculated by a radiologist. Since MRI scans will be used throughout the study, reliable and reproducible bi-dimensional tumor measurements will be obtained. In addition, as part of each scan, DWI and DCE-MRI will be obtained to measure parameters related to cellular water diffusivity and tumor perfusion. The rationale for these studies is to determine if MRI can be used to assess the efficacy of drug therapy and as a predictor or early indicator of response.

4.3.4.1 Data Acquisition

- DCE-MRI with DWI studies will be performed on 1.5 Tesla clinical MR scanners (GE Medical System). A DWI sequence is routinely performed during contrast enhanced MRI of the abdomen, which also include pre contrast axial 2D T1 weighted gradient echo, axial and coronal T2 weighted fast spin echo, as well as fat suppressed axial 3D T1 weighted gradient echo sequences before and following intravenous administration of gadolinium contrast, using gadobenate dimeglumine (Gd-BOPTA, MultiHance, Bracco Diagnostics, Inc., Princeton, NJ) at a standard concentration of 0.1 mmol/kg.

- Our DCE-MRI examinations will be acquired using guidelines proposed by the RSNA QIBA (Radiologic Society of North America Quantitative Imaging Biomarker Alliance) Dynamic Contrast Enhanced Magnetic Resonance Imaging Technical Committee (http://qibawiki.rsna.org/images/7/7b/DCEMRIProfile_v1_6-20111213.pdf). These guidelines outline parameters for image acquisition, which are similar to those from our prior DCE-MRI studies [6, 7], with the following modifications: acquisition of a variable flip angle T1 weighted (T1w) sequence for T1 mapping prior to contrast administration, and use of 3D volumetric instead of a 2D single slice T1w gradient echo sequence for our dynamic imaging. The 3D T1w sequence will be obtained in a coronal oblique orientation and include the tumor and abdominal aorta (for measuring vascular input function) and to avoid through plane flow artifact [39]. As in our prior studies, patients will be instructed to hold their breath for the initial portion of the dynamic series, followed by shallow breathing.

- DCE-MRI will be performed following injection of contrast at a rate of 2 mL/s, with a delay of 6 s, followed by 20 mL of saline flush using an automatic injector. A series of images will be acquired over a time period of 4-5 min with a temporal resolution of approximately 8 s. Oblique coronal images covering the tumor and including the abdominal aorta will be performed under the guidance of pre-contrast T1 and T2 weighted images. The nominal parameters for the DCE-MRI, 3D spoiled gradient echo sequence (SPGR) are: TE = 1 ms, TR = 4 ms, flip angle 30 degrees, slice thickness 5 mm (with zero-filling), matrix size = 256 x 128, 12 slices, FOV = 320 x 320 mm, bandwidth = 23.8 kHz, NEx = 1.0, and number of phases = 30. A 3D SPGR sequence with the same parameters above will be performed for

T1 mapping of the liver, using multiple flip angles (e.g. FA = 2, 5, 10, 15, 20, 25, 30 degrees), in the same acquisition plane.

- Diffusion weighted images will be acquired before administration of intravenous contrast using a standard fat suppressed single-shot echoplanar imaging sequence. For DWI, 2D axial slices through the liver covering the tumor will be acquired, with the following nominal parameters: TR 3000 ms, TE minimum 70 ms, 128 kHz bandwidth, 40 x 40 cm field of view, 128 x 128 matrix, with b values of 0, 50, 250, 350 and 500 s/mm^2.

4.3.4.2 Data Analysis.

Once the DCE-MRI data is acquired, it will be analyzed according to the guidelines from the RSNA QIBA DCE-MRI Technical Committee. After generating a T1 map and applying motion correction to the dynamic data, we will convert the DCE-MRI signal intensity data to gadolinium concentration and create a vascular input function. A region of interest will be drawn by the radiologist to guide the calculation of the tumor parameter K^{trans}. In addition, as suggested by the RSNA QIBA DCE-MRI Technical Committee, we will calculate the IAUGC. Comparisons will be made between the pre- and post-treatment perfusion parameters, K^{trans} and IAUGC. The baseline measurements as well as post-treatment changes in these parameters will be correlated to tumor response on follow-up MRI. The tumor measurements will be performed by a reference radiologist using anatomic pre-contrast T1w images at every follow-up MRI, following guidelines from RECIST 1.1.

Once the DWI data is acquired, they will be analyzed according to guidelines from a consensus panel meeting published in 2009 [18]. ADC maps will be created using data from all b values, and regions of interests (ROIs) will be drawn by a radiologist to generate tumor ADC values. In addition, to avoid perfusion/microcirculation effects at lower b values, ADC^{high} maps will be generated from DWI data limited to high b values (i.e., b = 250, 350, and 500 s/mm^2). ROIs will be similarly drawn over the tumor for calculation of tumor ADC^{high}. ADC maps will be constructed based on the monoexponential model of diffusion, according to Eq. [A-1] on a voxel-wise basis, using Matlab software (version 7.1, Mathworks, Natick, MA).

$$S(b) = S(0) \cdot [\exp(-b \cdot ADC)] \qquad [A\text{-}1]$$

where $S(b)$ and $S(0)$ are signal intensities of each voxel with and without diffusion weighing, respectively, and b is the diffusion-sensitizing factor (b-value).

4.3.4.3 Correlation of DCE-MRI, DWI and Clinical Data

Comparisons will be made between the pre and post-treatment perfusion parameters and diffusion coefficients. The initial and post-treatment changes in these parameters will also be correlated to disease response, as assessed by subsequent tumor size measurements.

With our proposed treatment plan, MR examinations with DCE-MRI and DWI will be performed once prior to the initiation of pump therapy to establish a baseline examination. Post treatment MRIs will be scheduled approximately at one month following initiation of therapy and at months 3, 6 and 9 thereafter. Comparisons will be made between the pre- and post-treatment perfusion parameters (K^{trans}, IAUGC) and diffusion coefficients (ADC and ADC^{high}). The baseline

measurements as well as post-treatment changes in these parameters will be correlated to disease response, as assessed by RECIST 1.1. These parameters will also be correlated to progression free survival and overall survival. We will use both ROI analysis with mean parameter values as well as quantile analysis to see which measure is more reproducible and correlates better with disease response. This protocol will yield important information in the ability of DCE-MRI and DWI to predict and monitor treatment response in intrahepatic cholangiocarcinoma.

The possibility that the first post treatment DCE-MRI and DWI will show no significant difference in diffusion coefficients must be considered. This may be related to the timing of the first post-treatment MRI at day 28, if the maximal changes occur earlier during the course of therapy. If necessary, we will change the first post-treatment MRI to day 21, if we fail to see changes in these parameters after the first five patients.

4.3.5 Genomic Profiling

The genomic profiles of cholangiocarcinomas using a next-generation sequencing platform. Specimens will be reviewed at MSKCC by Dr. Vakiani in the Department of Pathology to ensure greater than 50% tumor content and for histologic verification. Genomic DNA will be extracted from tumor biopsies by standard techniques and analyzed using an assay developed at MSKCC by Dr. Michael Berger (PhD, Department of Pathology); biopsies of non-tumor bearing liver from each patient will serve as controls. This assay uses targeted, massively parallel sequencing to analyze all exons of 230 genes selected for their known roles in cancer initiation or progression (examples include *APC, EGFR, KRAS, BRAF, TP53, NF1,* etc.). Massively parallel sequencing libraries (Illumina, San Diego, CA) will be generated according to the manufacturer's directions. Approximately 7,000 biotinylated RNA baits (Agilent, Santa Clara, CA) will be designed and synthesized corresponding to the coding sequence of the selected 230 genes. Genomic DNA will be subjected to solution-phase hybrid capture using the RNA baits, followed by massively parallel sequencing. To exclude germline single nucleotide polymorphisms, concurrent analysis of normal tissue DNA will be performed. Somatic alterations, including mutations, insertions and deletions, and copy number changes, detected by this assay will be correlated with clinical parameters [43].

One additional tube of blood will be collected in a cell-free DNA BCT (STRECK) at various time points throughout treatment, and this blood will be drawn with regular bloodwork (Please see Appendix B for more details). cfDNA extraction will be performed with a QIAsymphony SP system (QIAGEN) in collaboration with the Department of Laboratory Medicine. cfDNA will be quantified with a Fragment Analyzer (Advanced Analytical) using a High Sensitivity Genomic DNA Analysis Kit. Specifically, we will utilize the expertise of CMO for evaluating the cfDNAs by: 1) validating the mutations identified by MSK-IMPACT of tumor samples, using Digital PCR platform, 2) evaluating the concordance between tumor and cfDNA samples and correlate with treatment response as well as MRI imaging using IMPACT and/or custom targeted panels.

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 functions. The drug is metabolized in the liver.

5.1.2 FUDR is commercially available from Roche and Adria Laboratories in 500mg/10cc 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, hepatic enzyme elevation, 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, and hirsutism

5.3 Gemcitabine

5.3.1 Gemcitabine is a pyrimidine analogue of deoxycytidine in which the deoxyribose moiety contains 2 fluorine atoms at the 2'-position. The drug acts as an inhibitor to ribonucleotide reductase and inhibition of DNA synthesis may result in perturbations of deoxynucleotide pools and interference with DNA chain elongation. The drug is cell-cycle specific and blocks cells in the G1/S interface. Cytotoxicity is schedule-dependent and increases with duration of exposure. The drug is rapidly eliminated from plasma, owing mainly to deamination. Renal clearance of drug is less than 10% of parent drug.

5.3.2 The drug is supplied as either a 200mg or 1 gram lyophilized powder in a 50 mL sterile single vial for reconstitution.

5.3.3 The drug is administered via a freely-running intravenous catheter per institutional guidelines.

5.3.4 Common potential side effects include nausea, vomiting, alopecia, stomatitis, anorexia, fatigue, elevations of hepatic transaminases, reversible myelosuppression, rash, flu-like symptoms, edema, constipation, paresthesia, hypersensitivity reactions, phlebitis, proteinuria, hematuria, and rarely interstitial pneumonitis, ARDS and hemolytic uremic syndrome.

5.4 Oxaliplatin

5.4.1 Oxaliplatin functions as an antineoplastic agent by forming platinum-DNA adducts which, if not excised, will prevent further DNA synthesis and/or transcription and thereby lead to cell death.

5.4.2 The freeze-dried powder is reconstituted by adding 10 to 20 mL (for the 50-mg vials) or 20-40 mL (for the 100-mg vials) of water for injection or 5% glucose solution and then by diluting

in an infusion solution of 250 mL or 500 mL of 5% glucose solution. The reconstitution or final dilution must never be performed with a sodium chloride solution.

5.4.3 Do not combine with alkaline medications or media (such as basic solutions of 5FU, trometamol) which cause Oxaliplatin to degrade. Do not use for the preparation of administration needles or intravenous infusion sets containing aluminum items (risk of degradation of Oxaliplatin upon contact with aluminum),

5.4.4 The compound may be stored (in the form of freeze-dried powder for two years at room temperature protected from light. *Reconstituted solution:* In 5% glucose solution or water for injection in the original vial, the solution may be stored for 24 to 48 hours at +2° /+8° C.

5.4.5 Toxicities associated with intravenous administration of Oxaliplatin include neuropathy, paresthesias/dysesthesias, neutropenia, thrombocytopenia, and diarrhea.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

6.1 Subject Inclusion Criteria

- Age ≥ 21 years
- Histologically confirmed intrahepatic cholangiocarcinoma (also variously reported as peripheral cholangiocarcinoma, cholangiolar carcinoma or cholangiocellular carcinoma) (ICC). Confirmation of the diagnosis at MSKCC or at the enrolling institution must be obtained prior to initiation of protocol therapy.
- Clinical or radiographic evidence of metastatic disease to regional lymph nodes will be allowed, provided it is amenable to resection.
- Radiographically measurable disease. Measurable disease is defined as disease that can be assessed with 2-dimensional measurements on a cross-sectional imaging. Minimum lesion size is 2cm in greatest diameter as per RECIST criteria.
- Disease must be considered unresectable at the time of preoperative evaluation.
- Presence of less than 70% liver involvement by cancer.
- Patients may have failed ablative therapy
- Patient previously treated with systemic chemotherapy will be eligible
- KPS ≥ 60% and be considered candidates for general anesthesia, abdominal exploration and hepatic artery pump placement
- Patients with chronic hepatitis and/or cirrhosis are eligible, but must be Child-Pugh class A
- Patients must be able to read, understand and sign informed consent
- WBC ≥ 2,000 cells/mm^3
- Platelet count ≥ 75,000/mm^3
- Creatinine ≤ 1.8 **mg/dl**
- Total bilirubin < 1.5 **mg/dl**

6.2 Subject Exclusion Criteria

- Presence of distant metastatic disease. Patients will undergo radiographic evaluation to exclude the possibility of distant metastatic 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 MSKCC Principal Investigator. Clinical or radiographic evidence of metastatic disease to regional lymph nodes will be allowed, provided it is amenable to resection.

- Prior treatment with FUDR
- Prior external beam radiation therapy to the liver
- Diagnosis of sclerosing cholangitis
- Clinical evidence or portal hypertension (ascites, gastroesophageal varices, or portal vein thrombosis; surgically related ascites does not exclude the patient)
- Active infection
- Pregnant or lactating women
- History of other malignancy within the past 3 years (except non-melanoma skin cancer)
- Life expectancy less than 12 weeks
- Inability to comply with study and/or followup procedures
- History of peripheral neuropathy (Note: this does not apply to Cohort 3)

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 Oncology, and Gastric/Mixed Tumor services, and from both the MSKCC Department of Surgery and Department of Medicine, or similar departments at Ohio State University and University of Texas Southwestern Medical Center. 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 or consenting professionals list. 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 the patient fully (once confirmation of histologic eligibility has been established), in order to receive chemotherapy treatment. Only patients registered to both Step 1 and Step 2 will be treated per protocol. If patients are consented after surgery, this will not constitute ineligibility. There will be no financial compensation for patients enrolling on this protocol. Based on our experience with previous trials of this type, we expect an accrual time of approximately 2 – 3 years. There are no competing protocols.

8.0 PRETREATMENT EVALUATION

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

- CT angiogram (direct, MRI or CT angiography) to determine arterial structures; this may be done at any time prior to surgery
- Surgery to undergo cholecystectomy, place hepatic arterial infusion pump, and to obtain tissue biopsy
- Perfusion flow study (Tc-99 MAA) to ensure adequate flow from HAI pump to the liver

- Baseline diffusion-weighted MRI scan of the abdomen

The following tests are required of all patients:

- CT of the chest, abdomen and pelvis within 6 weeks of registration. Separate CT scans of the chest, abdomen, and pelvis are acceptable, provided they are done within 6 weeks of registration.
- Colonoscopy within 2 years of registration (exemption may be made for patients who have biopsy-proven ICC at the time of screening)
- Bilateral mammography (females only) within 1 year of screening. Patients who have undergone a mastectomy may have a mammogram performed on side unaffected.

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

	Within 14 days of surgery	Within 14 days of registration	Postop, within 21 days before Cycle 1	Postop, within 48 hours before Cycle 1
EKG	X			
HX, PE, BP	X	X		
Pregnancy test (females of child-bearing potential)		X		
DCE-MRI abdomen[1]			X	
Tumor size measurement			X	
KPS/ECOG[2]		X		X
Ht/Wt[3]		X		X
PT		X		
CBC w/ diff/plt		X		X
Albumin		X		X
Total bilirubin		X		X
BUN, creat		X		X
Alk Phos, AST/SGOT, LDH		X		X
CEA, AFP, CA19-9[4]		X		X
Urinalysis		X		X
Tc99-m MAA hepatic artery catheter injection			Post Op	

[1] Baseline MRI diffusion 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 progressions during and after therapy. Exception will be made for any patient who cannot undergo MRI; in that case CT scanning will be accepted.

[2] KPS/ECOG will be determined prior to surgery and prior to initiating chemotherapy. KPS/ECOG will also be evaluated on day 1 of each cycle while undergoing therapy.
[3] Height will only be assessed at initial visit; weight will be assessed at every MD visit.
[4] 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.
[5] Either direct arteriography, 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 normal saline to make 30cc will be administered on Day 1 of each cycle. The pump will be emptied and refilled with heparinized normal saline on Day 15 of each cycle. Cohort 1 and Cohort 2 will receive Gemcitabine (800 mg/m^2) and Oxaliplatin (85 mg/m^2) by IV infusion per institutional guidelines every 2 weeks, beginning with Day 15, Cycle 1. Cohort 3 will receive Gemcitabine (1000 mg/m^2) by IV infusion per institutional guidelines every 2 weeks, beginning with Day 15, Cycle 1.

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:

$$\text{FUDR: } \frac{0.12 \text{ mg/kg} * 30}{\text{Pump flow rate}}$$

$$\text{Dexamethasone: } \frac{1 \text{ mg/day} * 30}{\text{Pump flow rate}}$$

FUDR Dose Calculation for Overweight Patients:

If a patient is 35% above ideal weight, doses of FUDR will be calculated as follows:

Ideal Body Weight (kg):

Males: 50kg + (2.3 x height in inches above 5 ft)
(i.e. for a patient who is 5'10", use 10)
Females: 45.5kg + (2.3 x height in inches above 5 ft)

Example: Male who is 100kg and 5'10"
Ideal body weight is calculated thus: 50 + (2.3 x 10) = 73kg
Therefore, 100 + 73 = 173 ÷ 2 = 86.5, which is the "Ideal Average Weight" upon which dose calculations would be based.

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 doses given above (adjusted to changes in weight and pump flow rate) will be repeated on Day 1 of Cycle 2 and all subsequent cycles.

* If the PI feels the patient is an appropriate weight, the ideal average weight equation should not be used and patients can have the regular dose calculation of FUDR.

Note: Weight will not affect dose of systemic therapy.

9.3 Pump Empty

On Day 15 of each cycle, the pump will be emptied of residual fluid volume, and then re-filled with 30,000 units of heparin in normal saline (q.s. 30cc) and infused over the next 14 days.

Parameters for treatment with FUDR via intrahepatic pump are outlined in Section 11.0.

9.4 Treatment Duration

9.4.1 Patients will receive treatment until progression is noted, the patient develops unacceptable toxicity, or there is a change in diagnosis (see Section 13.0, “Criteria for Removal from Study”).

9.4.2 If FUDR is permanently discontinued due to disease progression, then the patient will be discontinued from the study.

9.4.3 If FUDR is held for reasons other than progression, patient may continue on study drug(s) at the attending physician’s discretion.

9.4.4 If systemic therapy is discontinued due to toxicity (see section 11.0), the patient may continue to receive FUDR, at the discretion of the attending physician, provided there is evidence of response.

9.4.5 All patients who consent to this study will be followed yearly until their death either in person at a clinic visit or over the phone.

9.5 Treatment Schedule

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, provided they do not, in the opinion of the investigator, constitute a major safety or compliance issue. Such variations, assuming they do not occur with unreasonable frequency or regularity, will not be considered protocol violations. A window of +/- 2 weeks is allowed for systemic treatments. If the patient needs a scan early for medical reasons or for resectability determination, this will count as their protocol scan in the discretion of the investigator. A window of +/- 2 weeks is allowed for scans. In the event that the patient may be considered for resectability, systemic therapy and/or pump therapy may be held. If the patient does not undergo resection, they are still considered on protocol.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

	Day 1* each Cycle	Q2 weeks	1 month	3 months	5 months	6 months	8 months	9 months
HX, PE, BP	X	X						
Tox Assessment	X	X						
Weight	X							
KPS/ECOG	X							
CBC, Plts	X	X						
BUN, Creat	X	X						
Bili, AST/SGOT	X	X						
Alk Phos, LDH	X	X						
CT Chest, abdomen and pelvis				X	X		X	
DCE-MRI abdomen[1]			X	X		X		X
Research Blood Draw for CEACAM1 and CEACAM6[2]	X							
Research Blood Draw for cfDNA[3]	X	X						

* Or within 48 hours prior to Day 1

[1]All patients will undergo DCE-MRI with DWI approximately 4 weeks after treatment start of HAI FUDR, and subsequently approximately at months 3, 6 and 9.

[2]Research blood draws (optional) will be done prior to treatment on Cycle 1 Day 1, Cycle 1 Day 15, and Cycle 2 Day 1.

[3]cfDNA research blood draws (optional) of one STRECK tube will be drawn at various and random time points throughout treatment with regular blood work.

10.1 While being treated with protocol therapy, patients will be seen at or prior to the first day of each cycle by their medical oncologist. In cases approved by the investigator, patients can see local oncologists for systemic treatment (post cycle 1). Patients who discontinue FUDR due to hepatic toxicity and receive only systemic treatment may see their local oncologists for treatment but must follow up with medical oncologist for imaging.

10.2 The consent indicates that samples and genetic information collected may be shared with other qualified researchers and placed in online databases. An example of an online

database is the NIH dbGAP database, which is monitored by the National Institutes of Health, and may be made accessible to investigators approved by the U.S. government. Such information will not include identifying information such as name. It is also stated in the Research Authorization that research data (e.g. genomic sequence) may be shared with regulators. The requirements for submission of genotype/phenotype data into the NIH dbGAP or any other public database will be followed as per the IRB SOP for Genomic Data Sharing.

In the course of this research it is possible that some patients whose tumors are analyzed through investigational "next-generation" profiling in a research (non-CLIA) environment will be found to have somatic or germline mutations in genes that are known to be associated with an increased risk of cancer or other diseases. It will be stated in the consent that the participants will not receive any specific results from research tests. The consent will tell participants that if they wish to have genetic testing done for personal reasons than they should make an appointment with the MSK Clinical Genetics Service or Clinical Genetics Service at their site.

If in the course of this research a research finding is obtained that, in the opinion of the investigator, may be critical to the preventive care of the participant or their family, the investigator can communicate that finding to the MSK IRB Genomic Advisory Panel (GAP). The finding will be reviewed by the GAP to determine whether the incidental finding should be discussed with the participant. For MSK patients, in the event that the GAP determines that the finding should be discussed with the participant, and the participant has consented to be re-contacted, then the treating/consenting physician shall be contacted by the panel and asked to refer the participant to the Clinical Genetics Service for further discussion of the research finding.

The following information must be provided to GAP for review:

- Participant Name/MRN #
- Type of Biospecimen (tissue, blood, saliva)
- Incidental Finding
- Collection Protocol #
- Contact: ocrgapirb@mskcc.org

For non-MSK patients being treated at one of the participating institutions, in the event the GAP determines that the finding should be discussed with the participant, and the participant has consented to be re-contacted, the findings will be returned to the Site Principal Investigator. Site policies on returning these research findings to the patient should be followed.

We anticipate that other research assays may be incorporated into this protocol as technology evolves.

11.0 TOXICITIES/SIDE EFFECTS

All toxicities will be rated as per the NCI Common Toxicity Criteria (Version 4.0), except neurosensory and hepatic enzyme toxicities related to intrahepatic pump therapy (see FUDR Dose Modifications/Table 1).

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.

A number of measures will be taken to ensure the safety of patients participating in this trial. These measures are addressed through exclusion criteria (see Section 6.2) and routine monitoring as follows.

Patients enrolled in this study will be evaluated clinically and with standard laboratory tests before and at regular intervals during their participation in this study. Safety evaluations will consist of medical interviews, recording of adverse events, physical examinations, blood pressure, and laboratory measurements. Patients will be evaluated for adverse events (all grades), serious adverse events, and adverse events requiring study drug interruption or discontinuation at each study visit for the duration of their participation in the study.

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 Dexamethasone: 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 Gemcitabine

11.2.1 Flu-like syndrome: Flu-like syndrome is manifested by fever, fatigue, myalgias, headache, and cough.

11.2.2 Myelosuppression: Myelosuppression is the usual dose-limiting toxicity. Infusions longer than 1 hour are associated with increased myelosuppression.

11.2.3 Hepatotoxicity: Mild elevations in hepatic transaminase levels occur in as many as two-thirds of patients, but are reversible.

11.2.4 Dyspnea: Dyspnea occurs in 10-23% of patients and is usually associated with a drug-induced pneumonitis. More often, dyspnea is likely associated with the underlying malignancy.

11.2.5 Gastrointestinal: Nausea, vomiting, and anorexia are common but usually of mild to moderate severity. Stomatitis and diarrhea or constipation occur less often.

11.2.6 Nephrotoxicity: Proteinuria and hematuria are usually asymptomatic though they occur frequently. A serious hemolytic-uremic syndrome occurs in less than 1% of patients.

11.2.7 Nervous system: Parasthesias and peripheral neuropathies occur in 2-10% of patients.

11.2.8 Hypersensitivity reactions: Allergic reactions including bronchospasm have been reported in 4% of patients.

11.2.9 Dermatologic: Minimal alopecia (15%) and macular or maculopapular rashes have been reported.

11.3 Toxicity Related to Oxaliplatin

11.3.1 Neurosensory toxicity: Prior to each Oxaliplatin dose, the patient should be questioned for symptoms of neurosensory toxicity. The table below presents the scale, which should be used instead of the NCI Common Toxicity Criteria for assessing neurosensory toxicity.

Specific Neurosensory Toxicity Scale	
Grade 0	No symptoms
Grade 1	Paresthesias/dysesthesias of short duration with complete resolution prior to the next cycle. Does not interfere with function
Grade 2	Paresthesias/dysesthesias persisting between two cycles without functional impairment
Grade 3	Paresthesias/dysesthesias with functional impairment that also interfere with ADL

See Section 11.7 for Oxaliplatin dose reduction related to neurosensory toxicity

11.4 Toxicity Related to the Pump and Catheters

Infection, hepatic artery thrombosis, pump malfunction, catheter occlusion, intra-abdominal hemorrhage and pump pocket seroma are potential complications associated with placing the hepatic artery pump. The rate of pump failure is less than 1 percent. Patients will receive standard systemic therapy if the pump is unusable.

11.5 Dose Modifications

11.5.1 FUDR Dose Modifications

"Reference value" is defined as the value obtained on the first day of the most recent FUDR dose.

To determine if a FUDR dose modification is necessary, compare reference value to the either the value obtained on the day pump was emptied (e.g. day 14) or the value obtained on the day of planned pump filling (e.g. day 28), whichever is higher.
Percentages listed under "FUDR Dose" refer to percentage of last dose of FUDR administered.

TABLE I: FUDR DOSE MODIFICATION SCHEMA:

	Reference Value*	% FUDR dose
AST/SGOT *(at pump emptying or day of planned*	0 to < 2 x reference value	100%
	2 to < 3 x reference value	80%
	3 to < 4 x reference value	50%

retreatment, whichever is higher)	> 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 AST/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 "Recommencing FUDR Treatment After Hold".		

** If 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.*
*** Bilirubin elevation without any other elevations in Alk Phos or AST/SGOT may be due to dehydration and reductions should not be followed in this case.*

RECOMMENCING TREATMENT AFTER HOLD

Reason for treatment delay	Chemotherapy resumed when value has returned to:	% FUDR dose
AST/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

- If patient develops a total bilirubin $\geq$ 3.0 mg/dl, the pump should be emptied and Dexamethasone 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, Dexamethasone dose should be tapered in increments of 5 mg every 14 days. Tapering will continue unless enzymes increase. FUDR should be permanently discontinued unless the patient responded previously and there is evidence of disease progression (increasing CEA, worsening CT scan, worsening clinical status) while off therapy AND bilirubin has returned to $\leq$ 1.5 mg/dl. In this case, FUDR can be restarted as follows: Use 25% of the last FUDR dose given with Dexamethasone, 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 $\leq$ 1.5 mg/dl and liver enzyme values do not increase.

- 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.

- If the physician feels the patient cannot tolerate systemic therapy, they can hold therapy for one to two weeks. If the patient has elevated liver function tests, systemic therapy can be held and Decadron can be placed in the pump with heparin saline at the discretion of the MSKCC Principal Investigator.

- If patients have delays in treatments due to hospitalization or other reasons, they should proceed with treatment as scheduled.

11.5.2 Dose Modification Schedule

- Subjects should be assessed for toxicity before each dose. Dose modification should be performed according to the schedule described below.

11.5.3 Guidelines for Diarrhea Management

- Symptoms of diarrhea and/or abdominal cramping may occur at any time and should be managed according to standard institutional practice.
- Subjects should also be instructed to notify the investigator or nurse for the occurrence of bloody or black stools, symptoms of dehydration, fever, inability to take liquids by mouth, inability to control diarrhea (return to baseline) within 24 hours. Subjects with diarrhea should be evaluated frequently by a nurse or physician until resolution of diarrhea.
- Changes in electrolytes, even without BUN/urea and/or creatinine elevation, may reflect early physiologic consequences of treatment-induced gastrointestinal toxicity. Subjects with clinically significant electrolyte changes should be evaluated for dehydration and receive aggressive fluid and electrolyte replacement, if indicated.

Nonhematological Diarrhea Toxicities

Grade 1	Grade 2	Grade 3	Grade 4
Increase of < 4 stools per day over baseline; mild increase in ostomy output compared to baseline	Increase of 4 – 6 stools per day over basline; moderate increase in ostomy output compared to baseline	Increase of ≥ 7 stools per day over baseline; incontinence; hospitalization indicated; sever increase in ostomy output compared to baseline; limiting self care ADL	Life-threatening consequences; urgent intervention indicated

11.5.4 Proscribed Therapy During Study Period

- Subjects must be withdrawn from the study if they receive any other investigational agents, anti-EGFR targeting agents, experimental or approved anti-tumor therapies (e.g., bevacizumab), chemotherapy or radiotherapy (with the exception of use for pain control).
- Subjects should not schedule any elective surgeries (excluding central venous catheter placement) during their participation in the study, or until 7 days after their last administration of study treatment. If a subject undergoes any unexpected surgery during the course of the study, that subject must discontinue all study treatment immediately, and the treating physician should be notified as soon as possible. A subject may be allowed to

resume study treatment after each surgical case is reviewed by the study team and the investigator to determine the appropriateness of treatment resumption.

For grade 3-4 non-hematological and grade 4 hematological complications and for febrile neutropenia, 25% dose reduction of BOTH drugs will be made. Consideration of future prophylactic use of colony stimulating factor (G-CSF, GM-CSF) is recommended for subsequent treatment cycles.

11.6 Hematologic Criteria for Gemcitabine/Oxaliplatin

Patients must meet all hematologic criteria outlined in Section 6.0 before beginning the systemic therapy. Pump therapy can start regardless of this bloodwork. For subsequent systemic therapy, patients must meet the following criteria for the full doses of systemic therapy:

- WBC $\geq$2000 cells/mm^3
- ANC $\geq$ 1000 cells/mm^3
- Platelets $\geq$ 75,000/mm^3
- Creatinine $\leq$ 1.8 mg/dL
- Bilirubin $\leq$ 1.5 mg/dL

If counts are outside these levels on date of scheduled treatment, therapy will be delayed by no less than one week. Bloodwork should be monitored at subsequent cycles, and if necessary, doses should be reduced according to Table 3. Patients will continue on reduced dose and can be reduced further according to the treating investigator. Once patient has been reduced they do not need further reduction unless the treating investigator feels the patient cannot tolerate therapy without further reduction.

Hematological Toxicities

	Grade 3	Grade 4
Platelet count decreased	<50 – 25 mm^3	<25 mm^3
Neutrophils decreased	<1000 – 500 mm^3	< 500 mm^3

Table 3a. Cohort 1 and Cohort 2 Gemcitabine and Oxaliplatin Dose Modifications:

	Dose Modifications for Nonhematological Toxicities	
Toxicity Grade	Gemcitabine	Oxaliplatin
1-2	Treat symptomatically	
3-4	Reduce dose by 25% to 600 mg/m^2 for first occurrence and subsequently to 400 mg/m^2 for second occurrence	Reduce dose by 25% to 64 mg/m^2

	Dose Modifications for Hematological Toxicities	
Toxicity Grade	Gemcitabine	Oxaliplatin
3	Reduce by 25%	Reduce by 25%
4	Reduce dose by 40%	Reduce dose by 40%

Table 3b. Cohort 3 Gemcitabine Dose Modifications:

	Dose Modifications for Nonhematological Toxicities
Toxicity Grade	Gemcitabine
1-2	Treat symptomatically
3-4	Reduce dose by 25% to 750 mg/m^2 for first occurrence and subsequently to 500 mg/m^2 for second occurrence

	Dose Modifications for Hematological Toxicities
Toxicity Grade	Gemcitabine
3	Reduce by 25%
4	Reduce dose by 40%

In the discretion of the investigator, patients can be held 1 week and resume at reduced dose as per Table 3.

Laboratory evaluation of renal and hepatic function, including transaminases and serum creatinine, should be performed prior to initiation of therapy and periodically thereafter. Gemzar should be administered with caution in patients with evidence of significant renal or hepatic impairment as there is insufficient information from clinical studies to allow clear dose recommendation for these patient populations.

Patients treated with Gem who complete an entire cycle of therapy may have the dose for subsequent cycles increased by 25%, provided that the absolute granulocyte count (AGC) and platelet nadirs exceed 1500 x 106/L and 100,000 x 106/L, respectively, and if non-hematologic toxicity has not been greater than WHO Grade 1. If patients tolerate the subsequent course of Gemzar at the increased dose, the dose for the next cycle can be further increased by 20%, provided again that the AGC and platelet nadirs exceed 1500 x 106/L and 100,000 x 106/L, respectively, and that non-hematologic toxicity has not been greater than WHO Grade 1.

11.6.2 Oxaliplatin Dose Modification for Neurological Toxicities

Symptom	**< 7 Days**	**Persistent for 2 weeks**
Dysesthesias with cold	No change	No change
Paresthesias	No change	Reduce 25%
Paresthesias with numbness	1st time: reduce 25% 2nd time: reduce 25%	Stop
Paresthesias with functional impairment	Stop	Stop

Note: If pseudo laryngopharyngeal dysesthesia occurs, the next dose of Oxaliplatin should be administered as a six-hour infusion. Subsequent Oxaliplatin infusions should be administered as six-hour infusions, or shorter as tolerated.
Patients who experience grade 3 or 4 toxicity may, at the discretion of the investigator, continue treatment at a lower dosage level once toxicities have fully resolved (Grade 0).

At the discretion of the investigator, patients may start with Oxaliplatin and, if necessary, discontinue the drug (because of neuropathy or other oxaliplatin related toxicities) and continue with systemic Gemcitabine alone. This will not remove the patient from protocol and is intended to avoid significant ongoing neuropathy.

11.7 MRI toxicities/side effects

MRI is a proven safe technique that has no radiation risk. There are no known risks or adverse effects resulting directly from exposure to magnetic fields and radiofrequency waves used in this study.

Likely

- Claustrophobia
- Discomfort repetitive noises that may be bothersome

The MRI scanners are equipped with a warning button held by the patients in their hands and upon a call by the patient, the scan will be stopped and the patient will be taken out of the MR I scanner.

Side effects or risks associated with contrast agent (Gadolinium)

Less likely

- Discomfort from the intravenous (IV) line inserted into your hand or arm to give you contrast

Rare

- Sweating
- Skin rashes
- Itching
- Hives
- Fatal complications include irritation of the blood vessels and blood clots
- Nephrogenic systemic fibrosis (scarring of skin, joints, eyes, or internal organs)

The reaction can be mild, moderate or severe. The Department of Radiology at MSKCC has procedures for the identification and management of contrast agent reaction and these procedures will be followed during this study.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

The primary objective of this Phase II study will be to assess 6-month PFS. Treatment evaluation will be done using RECIST (version 1.1)

12.1 For the purposes of this study, patients will undergo a baseline DCE-MRI with DWI, one approximately 4 weeks after HAI FUDR treatment start, and then they should be reevaluated

at months 3, 6 and 9. Patients will also be reevaluated at months 3, 5 and 8 with a CT of the chest, abdomen and pelvis. Determination of progression will be made according to the RECIST (version 1.1).

12.2 Definitions of Measureable and Non-Measureable Disease

12.2.1 Measurable disease is defined as at least one lesion whose longest diameter can be accurately measured as ≥ 2.0 cm with chest X-ray or as ≥ 1.0 cm with CT scan, CT component of a PET/CT, or MRI. Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules, palpable lymph nodes). Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

12.2.2 Nodes with a short axis of ≥ 1.5 cm by CT are considered measureable and assessable as target lesions. Only the short axis measurement should be included in the sum of lesions in calculation of tumor response. Nodes that shrink to < 1.0 cm short axis are considered normal.

12.2.3 All other lesions (or sites of disease), including small lesions (longest diameter < 2.0 cm with chest X-ray or as < 1.0 cm with CT scan, CT component of a PET/CT, or MRI) are considered non-measurable disease. Bone lesions leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

12.3 Guidelines for Evaluation of Measurable Disease

12.3.1 Measurement Methods: All measurements should be recorded in metric notation (i.e., decimal fractions of centimeters) using a ruler or calipers. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. For patients having only lesions measuring at least 1 cm to less than 2 cm must use spiral CT imaging for both pre- and post-treatment tumor assessments. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used at the same evaluation to assess the antitumor effect of a treatment.

12.3.2 Acceptable imaging modalities for measurable disease: CT scan (conventional and spiral), MRI, chest x-ray, and physical examination.

- Conventional CT and MRI should be performed with cuts of 1.0 cm or less in slice thickness contiguously.
- Spiral CT must be performed using a 5 mm contiguous reconstruction algorithm. This specification applies to tumors of the chest, abdomen, and pelvis, while head and neck tumors and those of the extremities require specific procedures.
- The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or

stable disease (an effusion may be a side effect of the treatment) and progressive disease.

- Cytologic and histologic techniques can be used to differentiate between PR and CR in rare cases.
- A lesion identified on a follow-up study in an anatomical location that was not scanned at baseline is considered a new lesion and will indicate disease progression.

12.4 Measurement of Effect

12.4.1 Target Lesions

All measurable lesions (as defined in Section 12.2.1) up to a maximum of 5 lesions representative of all involved organs should be identified as target lesions and recorded and measured at baseline. In this protocol, the unresectable intrahepatic cholangiocarcinoma will typically be the only target lesion measured, along with periportal lymph nodes if applicable. If the protocol specified studies are performed, and there are fewer than 5 lesions identified (as there often will be), there is no reason to perform additional studies beyond those specified in the protocol to discover new lesions.

Baseline Sum of Diameters (BSD): A sum of the diameters [longest for non-nodal target lesions (see 12.2.1), short axis for target lymph nodes (see 12.2.2)] for all target lesions will be calculated and reported as the baseline sum of diameters. The BSD will be used as reference to further characterize any objective tumor response in the measurable dimension of the disease.

Post-Baseline Sum of the Diameters (PBSD): A sum of the diameters [longest for non-nodal target lesions (see 12.2.1), short axis for target lymph nodes (see 12.2.2)] for all target lesions will be calculated and reported as the post-baseline sum of diameters. If the radiologist is able to provide an actual measure for the target lesion, that should be recorded, even if it is below 0.5 cm. If the target lesion is believed to be present and is faintly seen but too small to measure, a default value of 0.5 cm should be assigned. If it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0 cm.

The minimum sum of the diameters (MSD) is the minimum of the BSD and the PBSD.

12.4.2 Non-Target Lesions

All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required, and these lesions should be followed in accord with 12.4.3.3.

12.4.3 Response Criteria

12.4.3.1 All identified sites of disease must be followed on re-evaluation. Specifically, a change in objective status to either a PR or CR cannot be done without rechecking all identified sites (i.e., target and non-target lesions) of pre-existing disease.

12.4.3.2 Evaluation of Target Lesions

- Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 1.0 cm.
- Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.
- Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 0.5 cm. The appearance of one or more new lesions is also considered progression.

12.4.3.3 Evaluation of Non-Target Lesions

- Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (< 1.0 cm short axis).
- Stable Disease (SD): Persistence of one or more non-target lesions.
- Progressive Disease (PD): At least one new malignant lesion or unequivocal progression of existing non-target lesions.

12.4.4 Overall objective status

The overall objective status for an evaluation is determined by combining the patient's status on target lesions, non-target lesions, and new disease as defined in the following table.

Target Lesions	Non-Target Lesions	New Lesions	Overall Objective Status
CR	CR	No	CR
CR	SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

12.4.5 Residual Disease: In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends upon this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

12.4.6 Symptomatic Deterioration: Patients with global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time, and not either related to study treatment or other medical conditions, should be reported as PD due to "symptomatic deterioration." Every discontinuation of treatment due to symptomatic deterioration:

- Weight loss > 10% of body weight.
- Worsening of tumor-related symptoms.
- Decline in performance status of > 1 level on ECOG scale.

13.0 CRITERIA FOR REMOVAL FROM STUDY

1. If, at any time, progressive disease is identified, the patient will be taken off study and referred for alternative therapy.
2. If, at any time, unacceptable toxicity is identified, the patient will be removed from study.
3. If, at any time, protocol ineligibility is identified, 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.
4. Patient elects to discontinue treatment.
5. Changes in a patient's condition which render the patient unacceptable for further treatment in the judgment of the investigator.
6. Inability of subject to comply with study requirements.
7. Determination by the investigator that it is no longer safe for the subject to continue therapy.

Patients removed for reasons 1, 2, 5 and 7 will count as events. All other patients will be replaced.

14.0 BIOSTATISTICS

Cohorts: This protocol will enroll patients with intrahepatic cholangiocarcinoma treated with HAI FUDR/Dex plus IV gemcitabine and oxaliplatin, or HAI FUDR/Dex plus IV gemcitabine alone, and will consist of three cohorts. Cohort 1 will be comprised of patients who either have not receive chemotherapy before for their liver disease, responded to or had stable disease on combination therapy with gemcitabine/oxaliplatin or gemcitabine/cis-platin, or progressed on single agent gemcitabine therapy alone. Cohort 2 will include patients who have failed systemic gemcitabine combined with either oxaliplatin or cis-platin. Cohort 3 will include patients who have had prior oxaliplatin and have existing neuropathy or an allergic reaction to Oxaliplatin, and therefore receiving HAI FUDR/Dex plus IV gemcitabine alone on this protocol. Primary objectives will be evaluated separately for the three cohorts.

Primary Objective for Cohort 1: The primary objective in Cohort 1 is to assess the 6-month progression free survival. Median progression-free survival for patients in Cohort 1 is estimated to be 6-8 months using various available regimens[3] [6] [7]. Our design for Cohort 1 is built

around a null rate of 60% progression-free survival at 6 months and requires 36 patients to detect an absolute difference of 20% for a target rate of 80% PFS at 6 months. If at least 25 patients are alive and progression-free six months after the initiation of therapy we will recommend this regimen for further study. This design has 10% Type I error (one-sided) and 90% power, typical of Phase II trials in oncology.

We will analyze and report survival using Kaplan-Meier estimates and tolerability using the proportion of patients with dose limiting toxicity, proportion of patients completing planned therapy and percentage of planned dose delivered.

Primary Objective for Cohort 2: The primary objective in Cohort 2 is to assess the 3-month progression free survival. Median progression-free survival for patients in Cohort 2 is estimated to be 2-5 months using various available regimens[3] [6] [7]. Our design for Cohort 2 is built around a null rate of 50% progression-free survival at 3 months and requires 39 patients to detect an absolute difference of 20% for a target rate of 70% PFS at 3 months. If at least 23 patients are alive and progression-free three months after the initiation of therapy we will recommend this regimen for further study. This design has 10% Type I error (one-sided) and 90% power.

We will analyze and report survival using Kaplan-Meier estimates and tolerability using the proportion of patients with dose limiting toxicity, proportion of patients completing planned therapy and percentage of planned dose delivered.

Primary Objective for Cohort 3: The primary objective in Cohort 3 is to assess the response rate. Since we anticipate this cohort to be smaller there is no formal hypothesis to test. If we enroll 10 patients in this cohort we will be able to estimate the response rate to at most within +/- 32%. We will analyze and report time to progression and survival keeping in mind that patients in this cohort may be heterogenous in their prognosis. Similar to the other cohorts, we will analyze tolerability using the proportion of patients with dose limiting toxicity, proportion of patients completing planned therapy and percentage of planned dose delivered.

Secondary Objectives: Correlative objectives include an assessment of the clinical relevance of dynamic contrast enhanced (DCE)-MRI and diffusion weighted imaging (DWI) of intrahepatic cholangiocarcinoma before treatment and early during the course of treatment. This study will investigate DCE-MRI and DWI as potential imaging biomakers and will measure tumor perfusion parameters and diffusion coefficients before initiating treatment and on follow-up MRI scans during treatment. Perfusion parameters (K^{trans} and IAUGC) and diffusion coefficients (ADC and ADC^{high}) at baseline, and early following treatment will be evaluated as prognostic factors for response and PFS. These will be accomplished using log-ranks tests for PFS and OS; and chi-square tests for response. We are planning to analyze the secondary objectives combining data from both cohorts since we think exposure to chemotherapy for liver disease (within the limits of inclusion/exclusion criteria) does not alter the prognostic ability of DCE-MRI and DWI-MRI .

Other correlative aims are to analyze tumors for expression of CEACAM1 and CEACAM6, a potential biomarker of outcome and resistance to chemotherapy and to analyze the genomic profiles of intrahepatic cholangiocarcinoma and to correlate the findings with clinical and

histopathological variables. This will be accomplished using chi-square and log-rank tests. We are planning to analyze these secondary objectives separately for the two cohorts since exposure to chemotherapy has the potential to alter gene expression and alterations.

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Inclusion/Exclusion Criteria. Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures. During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist. The individual signing the Eligibility Checklist is confirming whether or not the participant is eligible to enroll in the study. Study staff are responsible for ensuring that all institutional requirements necessary to enroll a participant to the study have been completed. See related Clinical Research Policy and Procedure #401 (Protocol Participant Registration).

15.2 Randomization

This is not a randomized study.

16.0 DATA MANAGEMENT ISSUES

A MSKCC Clinical Research Coordinator (CRC) will be assigned to the study. The responsibilities of the CRC include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordination of the activities of the protocol study team.

The data collected for this study will be entered into CRDB. Source documentation will be available to support the computerized patient record. Grade 3 and 4 toxicities will be captured.

16.1 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 extent 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.

16.1.2 Response Review

If therapeutic efficacy is a stated primary objective, all participants' responses are subject to review by MSK's Therapeutic Response Review Committee (TRRC). Radiology, additional lab reports and possibly bone marrow biopsies and/or aspirates will need to be obtained for MSK TRRC review and confirmation of response assessment.

16.2 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 DSM Plans can be found on the MSKCC Intranet at:

http://inside2/clinresearch/Documents/MSKCC%20Data%20and%20Safety%20Monitoring%20Plans.pdf.

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 (eg. 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 (eg. 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.

16.3 Regulatory Documentation

Prior to implementing this protocol at MSKCC, the protocol, informed consent form, HIPAA authorization and any other information pertaining to participants must be approved by the MSKCC Institutional Review Board/Privacy Board (IRB/PB).

17.0 PROTECTION OF HUMAN SUBJECTS

Participation in this trial is voluntary. All patients will be required to sign a statement of informed consent, which must conform to IRB guidelines. The study will protect the rights of all human subjects and an informed consent will clearly define the risks, benefits, toxicities and side effects of treatment. The patients will also be informed of the alternative options for treatment.

Inclusion of Women and Minorities: Memorial Sloan-Kettering Cancer Center has filed forms HHS 441 (civil rights), HHS (handicapped individual), 639-A (sex discrimination), and 680 (age discrimination); we also take due notice of the NIH policy concerning inclusion of women and minorities in clinical research populations. Patients of all races, both male and female, will be accepted into the protocol. The proposed study population is as described in section 7.0.

Exclusion of Lactating or Pregnant Women: Children have been excluded from this study. Cholangiocarcinoma is an adult cancer. Thus, the relevance of this drug to the pediatric population has not been established. Lactating and pregnant women are also excluded because of potential antiproliferative effects of chemotherapy that may be harmful to the developing fetus or nursing infant.

Benefits: It is possible that this treatment will result in prevention of recurrence of cholangiocarcinoma. It is not known, of course, whether these or any other favorable events will occur. It is not known whether this treatment will affect the overall survival of the patients.

Incentives: No incentives will be offered to patients/subjects for participation in the study.

Alternatives: For patients with cholangiocarcinoma, alternative treatments may include other chemotherapy regimens as well as proceeding to surgery only, or receiving adjuvant chemotherapy and radiation. At present, no specific adjuvant chemotherapy regimen represents standard treatment for the disease. Patients may be eligible for other investigational studies.

Confidentiality: Every effort will be made to maintain patient confidentiality. Research and hospital records are confidential. Patient's name or any other personally identifying information will not be used in reports or publications resulting from this study. The Food and Drug Administration or other authorized agencies (eg, qualified monitors from MSKCC or the NCI, etc.), may review patients records and pathology slides, as required.

17.1 Privacy

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 (IRB/PB).

The consent indicates that individualized de identified information collected for the purposes of this study may be shared with other qualified researchers. Only researchers who have received approval from MSK will be allowed to access this information which will not include protected health information, such as the participant's name, except for dates. It is also stated in the Research Authorization that their research data may be shared with other qualified researchers.

17.2 Serious Adverse Event (SAE) Reporting

An adverse event is considered serious if it results in ANY of the following outcomes:

- Death
- A life-threatening adverse event
- An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.

SAE reporting is required as soon as the participant starts investigational treatment/intervention. SAE reporting is required for 30-days after the participant's last investigational treatment/intervention. Any event that occur after the 30-day period that is unexpected and at least possibly related to protocol treatment must be reported.

Please note: Any SAE that occurs prior to the start of investigational treatment/intervention and is related to a screening test or procedure (i.e., a screening biopsy) must be reported.

All SAEs must be submitted in PIMS. If an SAE requires submission to the HRPP office per IRB SOP RR-408 'Reporting of Serious Adverse Events', the SAE report must be submitted within 5 calendar days of the event. All other SAEs must be submitted within 30 calendar days of the event.

The report should contain the following information:

- The date the adverse event occurred
- The adverse event
- The grade of the event
- Relationship of the adverse event to the treatment(s)
- If the AE was expected
- Detailed text that includes the following
    - o An explanation of how the AE was handled
    - o A description of the participant's condition
    - o Indication if the participant remains on the study
- If an amendment will need to be made to the protocol and/or consent form
- If the SAE is an Unanticipated Problem

The SAE report should be completed as per above instructions. If appropriate, the report will be forwarded to the FDA by the IND Office

18.0 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.

5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

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20.0 APPENDICES

Appendix A: MSKCC Lab Requisition Form