The following protocol information is provided solely to describe how the authors conducted the research underlying the published report associated with the following article:

Randomized trial of hepatic artery embolization for hepatocellular carcinoma using doxorubicin-eluting microspheres compared to embolization with microspheres alone

DOI: 10.1200/JCO.2015.64.0821

[redacted]

[redacted]

IRB Protocol

IRB#: 07-099A(12)

A Randomized Single Blind Controlled Trial of Beads and Doxorubicin Eluting Beads for Arterial Embolization of Hepatocellular Carcinoma (HCC)

[redacted] THERAPEUTIC/DIAGNOSTIC PROTOCOL

Principal Investigator/Department:	[redacted]	Interventional Radiology
Co-Principal Investigator(s)/Department:	[redacted]	Medicine
Investigator(s)/Department:	[redacted]	Surgery Epidemiology and Biostatistics Interventional Radiology Interventional Radiology Interventional Radiology Pharmacy Radiology Radiology Radiology Radiology Radiology
Consenting Professional(s)/Department:	[redacted]	Interventional Radiology Interventional Radiology Interventional Radiology Interventional Radiology

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

Consultants: [redacted]

[redacted]

[redacted]

IRB Protocol

IRB#: 07-099A(12)

TABLE OF CONTENTS

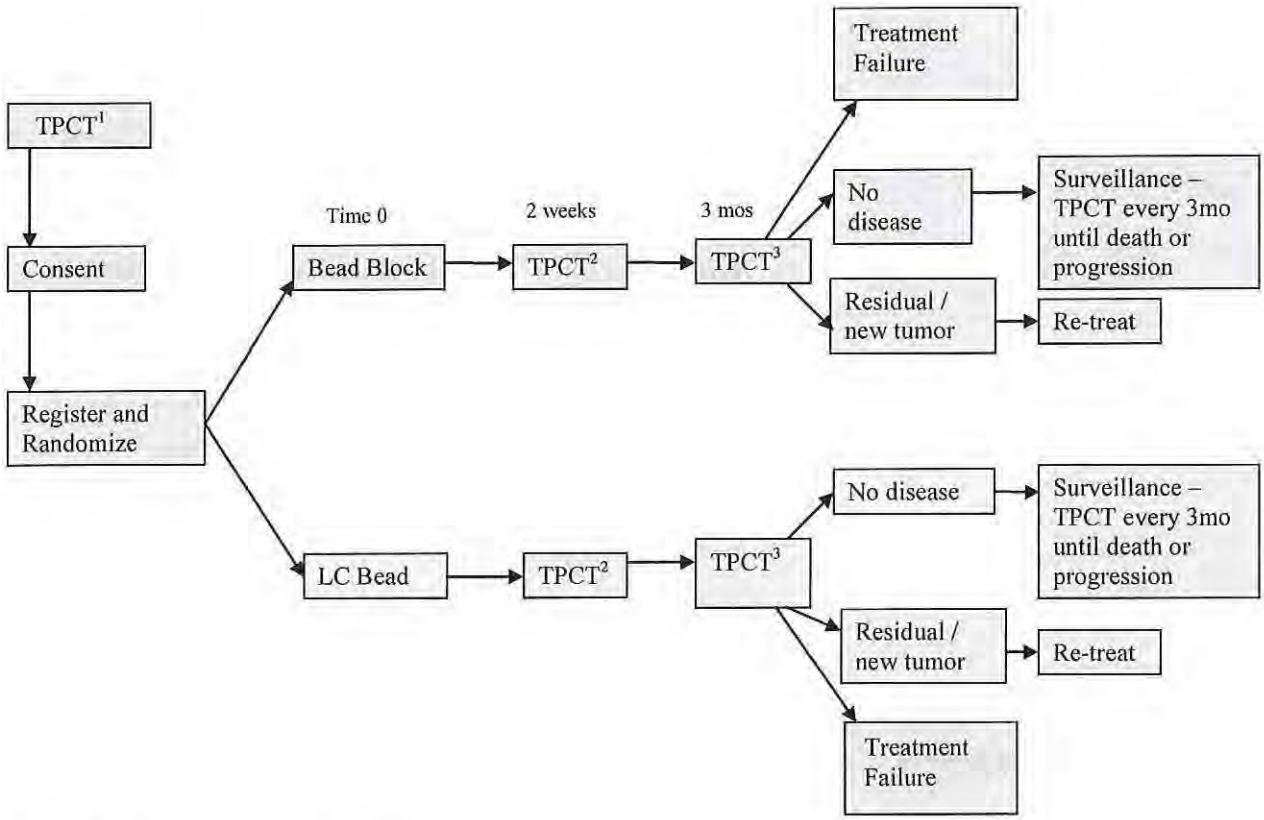
1.0 PROTOCOL SUMMARY AND/OR SCHEMA

Purpose:	The objective of this study is to evaluate hepatic arterial embolization for hepatocellular cancer using microscopic beads and the same microscopic beads loaded with doxorubicin.
Study design:	Single center, single blind, prospective, randomized phase II controlled, to be conducted without crossover with patients recruited over a 2 year period
Study agents:	Biocompatibles LC Bead **(Beads + doxorubicin)**, 100-300 microns, 150 mg doxorubicin per procedure with additional 100-300 micron and larger size beads (Bead Block) as necessary to achieve stasis Bead Block **(Beads)** microsphere, 100-300 micron with additional larger size beads as necessary to achieve stasis
Limitations of Study	LC Bead and Bead Block are both tinted blue. Because doxorubicin is red, the addition of doxorubicin to the embolization microspheres will change the color of the spheres to some degree, and thus it may be possible for the interventional radiologist performing the embolization to differentiate between the two embolics. All other participants performing image and data analysis will be blinded.
Enrollment:	100 patients will be enrolled, 50 in each study arm
Clinical site:	[redacted] NY, NY
Primary endpoint:	Response to treatment by RECIST criteria
Secondary endpoints:	• Tumor necrosis • Toxicity • Time to progression • Survival
Safety endpoint:	The safety endpoint will be treatment related Serious Adverse Event (SAE) rate, defined by SAE occurring within 30 days of a procedure. SAE is defined as a grade 3 or 4 adverse event using the Common Terminology Criteria for Adverse Events (CTCAE) 3.0.
Primary analysis:	Intent-to-treat analysis

Secondary Analysis: Per protocol analysis and treated population analysis

Principle Investigator: [redacted]
Member
Department of Radiology
[redacted]

Protocol Schema



TCPT[1] = Pre-treatment triple-phase CT
TPCT[2] = CT to be used to determine response to treatment (first study endpoint)
TPCT[3] - If there is evidence of persistent or progressive tumor on this follow-up CT (but not meeting criteria for treatment failure), patients are re-treated within their treatment arm, and then re-enter F/U pathway. Treatment failure = < 5% necrosis following the initial treatment, or development of >50% increase in uni-dimensional measurement (RECIST) following subsequent treatments.

2.0 OBJECTIVES AND SCIENTIFIC AIMS

Primary Objective

In this study one group of patients with hepatocellular cancer (HCC) will undergo hepatic arterial embolization using microscopic beads + doxorubicin and another group with the microscopic beads alone. Response to treatment will be determined in each group by conventional RECIST criteria. Since there is no historical data on imaging response to treatment for either group, the study will be randomized. The primary objective will be to estimate the response rate in the two arms.

Secondary Objectives

1. Quantify the amount of tumor necrosis before and after initial treatment in both groups by CT volumetry, and correlate with response to treatment by RECIST criteria.
2. Evaluate toxicity, time to progression (TTP) and survival in each treatment arm. Determine if initial imaging response to treatment, as manifested by either RECIST criteria or volume of necrosis correlates with either TTP (by RECIST or volumetric method) or survival.

3.0 BACKGROUND AND RATIONALE

Background:

Hepatocellular Carcinoma

Over 1 million new cases of hepatocellular cancer are diagnosed in the world each year. Primary liver cancer is not common in the United States; however the incidence is rising primarily because of the spread of hepatitis C. There are over 15,000 deaths in the U.S. each year from primary cancers of the liver and biliary tree. Hepatocellular carcinoma is one of the few cancers with well-defined major risk factors [Bosch et al 1999, Colombo 2003]. It develops within cirrhotic livers in 80% of cases and this pre-neoplastic condition is the strongest predisposing factor [Colombo 2003]. The cirrhosis is most often associated with either hepatitis B or C, or excessive alcohol use. Patients with hepatitis B or C who also consume alcohol increase their risk of developing HCC [NCI web site]. Because there was no test for hepatitis C before 1992, many Americans acquired the hepatitis C virus through blood transfusions received before that time. As awareness of hepatitis C virus transmission becomes more widespread, there is increased screening for hepatitis C, and a concomitant increase in the number of patients diagnosed with hepatocellular cancer. Currently 10-15,000 new cases of HCC are diagnosed in the US each year and it is estimated this number may double given the spread of hepatitis C [Wong JB 2000].

Surgical/Medical Therapy

Hepatocellular cancer is potentially curable by surgical resection, but most patients are not resectable at the time of presentation either because of the extent of the tumor or the severity of their underlying liver disease. Over 60% of patients who are resected will develop disease elsewhere in the liver within 5 years of resection as a result of their underlying liver disease [Sugioka A, 1993, Cha C, 2003]. Systemic chemotherapy has never been shown to be effective.

The ubiquitous nature of this disease, and the lack of effective traditional therapies, have led investigators to attempt many novel methods of treatments, including percutaneous ablation with alcohol, radiofrequency ablation, cryoablation and various methods of arterial infusion and embolotherapy. Hepatic arterial chemoembolization has become the accepted standard of care for patients who are not surgical candidates although, to date, no study has demonstrated a survival advantage to chemoembolization compared to particle embolization without chemotherapy [Brown DB, 2006].

Local/Regional Therapy

Studies performed in the early 1950s established that the primary blood supply to liver tumors was from the hepatic artery [Breedis C, 1954]. Before the development of sophisticated methods for liver resection, hepatic ligation was sometimes performed in unresectable cases, however it was not found to be effective owing to the large number of collateral routes to the hepatic circulation, as demonstrated by Michels in 1953 [Michels NA, 1953]. These collateral pathways result in rapid reperfusion of the hepatic vasculature distal to the ligature, not unlike that which is seen following proximal arterial embolization. Parallel with the development of angiography, catheter directed techniques of treating liver tumors began to be explored in the late 1970s and early 1980s. These therapies were particularly desirable for treating patients with hepatocellular cancer (HCC), for which there was no effective non-surgical treatment, and very few patients who were candidates for resection. Hepatic arterial embolization capitalizes on the dual blood supply to the liver. 75 to 80% of the trophic blood supply to the normal liver parenchyma arises from the portal vein, whereas the primary vascular supply to hepatocellular cancer, and most liver metastases, is from the hepatic artery. This dual blood supply, coupled with the relative ease of catheterizing the hepatic artery, allowed for the delivery of various agents intra-arterially that are intended to kill the tumor cells while having little or no effect on the normal hepatic parenchyma, and ideally no systemic effect.

Supporting Preliminary Data

Despite the fact that no systemic chemotherapy had ever been shown to be effective against hepatocellular cancer, chemotherapy has been administered by arterial infusion in an effort to deliver a higher concentration of chemotherapy directly to the tumor in the hope of enhancing the effect of the drug while limiting the systemic effect. It had been noticed that lipiodol, when administered intra-arterially, was taken up by most hepatic malignancies, and particularly HCC. Initially it was thought that intra-arterial lipiodol alone might have a therapeutic effect on HCC. This was soon discredited [Takayasu K, 1987], however the idea of using lipiodol as a carrier came into vogue. Pharmacokinetic studies failed to demonstrate high concentrations or prolonged retention of chemotherapeutic agents in liver tumors, or any difference in clinical outcome when these agents were administered alone, or with lipiodol [Carr BI, 1993, Johnson PJ, 1991, Madden MV, 1993]. A pharmacokinetic advantage to the use of chemotherapy and lipiodol could only be convincingly shown when unconventional methods were used to dissolve the chemotherapeutic agents in lipiodol [Konno T, 1990], or a lipophilic chemotherapeutic agent was mixed with the lipiodol [Egawa H, 1990], methods that are not used in clinical practice in the United States.

Based on data available in the literature in the early 1990s, it seemed as if the primary benefit from transarterial chemoembolization (TACE) for HCC might in fact be derived from ischemia related tumor necrosis, and not the chemotherapeutic agents or the lipiodol [Nakao N, 1991, Ngan H 1996]. Eliminating the chemotherapeutic agents would eliminate chemotherapy related side effects associated with TACE, decrease the cost of treatment, and reduce the technical difficulties of working with chemotherapy. Intra-arterial chemotherapy is conventionally dosed based on body surface area and not based on tumor volume or vascularity. A fixed amount of chemotherapeutic agent or agents are mixed with a fixed amount of lipiodol, and emulsified. This emulsion is then administered intra-arterially to the target area of the liver. Delivering an intra-arterial emulsion of chemotherapy is not straightforward. In some cases it is impossible to deliver the entire dose to smaller or less vascular tumors. In the case of large vascular tumors the entire dose of chemotherapy and lipiodol might be used early in the procedure while rapid flow to the tumor persists. The dose of chemotherapeutic agent is not proportional to tumor volume. At many centers TACE is performed non-selectively, exposing the uninvolved liver to the chemotherapy/lipiodol/particle cocktail. This may adversely affect the "normal" liver and result in deterioration of liver function.

To date, no study has demonstrated a difference in survival between patients treated with "bland" embolization (TAE) and those treated with chemoembolization (TACE). A metaanalysis of randomized controlled trials between 1980 and 2000 included 2466 patients, 178 of whom received "non-active" treatment [Camma C, 2002]. This study concluded that TACE significantly reduces overall 2 year mortality, but that TACE was not more effective than TAE. The most recent, and frequently cited, randomized controlled study comparing TACE, TAE, and supportive care [Llovett JM, 2002] again demonstrated a survival advantage to TACE over supportive care, but was stopped before any statement could be made about the utility of TAE. Despite this, chemoembolization with lipiodol has become accepted worldwide as an important treatment option for patients with unresectable HCC.

Preliminary Data

For the past 14 years the protocol used for hepatic arterial embolization at [redacted] employs only an embolic particle targeted to cause terminal vessel blockade and, as a result, ischemic tumor cell death. Knowing that occlusion of the more proximal hepatic vessels results in the development of intrahepatic collateral flow and continued perfusion of the more distal vascular bed, the smallest particles available are used to cause blockade of the terminal hepatic vasculature in an effort to enhance ischemia. Initially, polyvinyl alcohol (PVA) particles were in widespread use and the smallest particles available were 100μ, later 50μ particles became available. We published our early results using these particles to treat 46 patients in1998 [redacted]; obtaining results that were not substantially different than those reported contemporaneously by others using various methods of chemoembolization. Since then we have begun using spherical embolic particles, hydrophilic, nonresorbable microspheres produced from an acrylic polymer. Using these particles, and treating over 300 patients with similar characteristics to those reported on by Lo [Lo CM, 2002] and Llovett [Llovett JM, 2002] we have achieved 1, 2, and 3 year survival of 85%, 68%, and 42% [redacted] These results are better than those reported by Lo (56%, 31%, and 26% at 1, 2, and 3 years), and similar to the 82% 1year and 63% 2year survival reported by Llovett, but in a much larger group of patients.

A microscopic bead has been developed that can be used as an embolic agent alone, or loaded with doxorubicin to deliver high concentration of the drug to a target tumor within the liver while limiting systemic exposure. Before this agent that is much more expensive than conventional embolic material comes into widespread use, it would be valuable to assess its effectiveness. Since the agent is itself an embolic and can be used without loading doxorubicin, the opportunity exists to determine to what extent the addition of chemotherapy enhances the effect of embolization alone. Although survival is clearly the most important endpoint in evaluating treatments for HCC, survival studies require several years of follow-up in these patients, whereas preliminary data regarding response to treatment can be obtained using imaging. Traditionally, Response Evaluation Criteria In Solid Tumors (RECIST) have been used to evaluate response to treatment, and will be used as a primary endpoint in this study. These criteria are based on the product of bi-dimensional orthogonal measurements of a treated lesion, and do not take into account the degree of tumor necrosis resulting from treatment. The Barcelona-2000 EASL conference [Bruix J, et al 2001] concluded that tumor necrosis is an important indicator of response to treatment for HCC, and recommended that changes in viable tumor area, rather than overall tumor area, be used to assess response. RECIST criteria have been found to be insensitive when used to evaluate other tumors that develop significant necrosis in response to treatment, such as the response of GIST tumors to Imatinib [Benjamin RS, et al 2006]. In this study, % tumor necrosis will serve as a secondary endpoint. Tumor necrosis will be quantified on contrast enhanced CT scans using an automated volumetric method, and the relationship of tumor necrosis to response as measured by RECIST criteria will be determined. These 2 measures of response to treatment will be correlated with TTP and survival in an attempt to establish the utility of one or the other as a surrogate outcome marker.

Bead Block is a compressible hydrogel polyvinyl alcohol (PVA) microsphere available in sizes ranging from 100 μm to 900 μm. Manufactured by the same company, LC Bead is the same microsphere available in the same sizes. This agent is called DC Bead in Europe and Asia. It is intended to be loaded with doxorubicin in order to deliver a local, sustained release dose of doxorubicin to a tumor when used intra-arterially. A pharmacokinetic study [Geschwind 2004] has demonstrated sustained drug concentration within VX-2 tumor implanted in the liver of New Zealand white rabbits at 14 days, with low plasma concentration of doxorubicin at all times. The peak concentration of doxorubicin in the tumor is observed at 72 hours following treatment - demonstrating that these drug eluting beads do result in slow, local, sustained delivery of doxorubicin to the liver tumor. The amount of doxorubicin in the tumor is approximately 100 times greater when delivered from the drug eluting bead compared to direct intra-arterial injection. In addition, there is an 80% reduction in systemic availability of doxorubicin when using drug eluting bead. At 14 days there was also a significant increase in the fraction of non-viable tumor cells in animals treated with the drug eluting bead. Phase I/II clinical trials have been conducted in Asia and Europe using these doxorubicin eluting beads with the first cohort in each trial for dose escalation, and the second for efficacy [Attachment, DC Bead Product monograph]. A maximum dose of 150 mg of doxorubicin was given in a single procedure, and there was no dose limiting toxicity in either of the trials. In the Barcelona dose escalation trial, the peak level of doxorubicin was 2 log orders lower than that seen with TACE or intraarterial doxorubicin (Figure 1). The peak plasma concentration was reached immediately (5 minutes) in

each group, averaged 79 ± 38 ng/ml, with no significant differences between the groups, and serum level of doxorubicin was undetectable by 7 days. The AUC of the patients receiving the doxorubicin eluting bead was also significantly lower than patients treated with TACE or intra-arterial doxorubicin. The second cohort received 150 mg of doxorubicin per treatment with no systemic effects of doxorubicin. There was an objective response of 78% by EASL criteria at 6 months, with 30% CR.

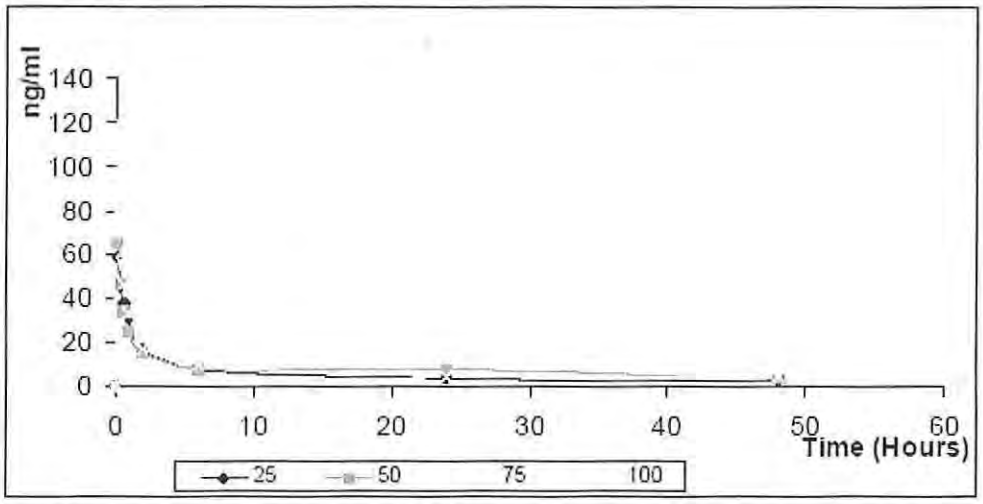


Figure 1 Combined plasma doxorubicin concentration-time curves according to dose groups (mg of doxorubicin)

Although trans-arterial chemoembolization (TACE) has become the standard treatment for patients with unresectable HCC, there has never been convincing evidence that the chemotherapy used for TACE remains in prolonged contact with the tumor in high concentration, or that there is any difference in survival when chemotherapy is added to the embolization agents. With the recent availability of drug eluting beads that have been shown to result in high local concentrations of chemotherapy (LC Bead) when used for embolization of HCC, it is possible to perform a study that evaluates the specific role of a chemotherapeutic agent (doxorubicin) delivered intra-arterially for the treatment of HCC. The results of this study are intended primarily to estimate response to treatment within the study and control groups. They may also help to clarify the role of chemotherapy when added to the ischemic effect of arterial embolization. A substantial difference in response to treatment would serve as a signal that the doxorubicin eluting bead may improve outcome in patients with HCC, and warrants further study. Coupled with and increase in TTP or survival, this might serve as the basis for an appropriately powered phase III trial. In addition, this study may validate a new imaging technique for evaluating response to treatment, and indicate if this imaging response to treatment may be used as a surrogate biomarker for outcome.

The expected outcomes of this study are to determine the response to treatment following arterial embolization of HCC using microspheres alone or microspheres + doxorubicin by RECIST criteria, and to determine the % tumor necrosis that results following treatment in each group. The response to treatment by RECIST criteria will be correlated with % tumor necrosis in both groups, and the relationship of both of these response parameters to TTP and survival will be assessed. The study may serve to validate an automated volumetric method of assessing response to treatment, and determine if this imaging response to treatment can be used as a surrogate biomarker for outcome. The findings will increase our understanding of how regional hepatic arterial therapies work, and may improve upon current imaging methods of assessing response to treatment. This study will offer a preliminary assessment of whether the additional expense, complexity, and potential side effects of adding chemotherapy to arterial embolization are worthy of further investigation. The results of this study are important not only for patients with HCC, but also for patients with other hypervascular liver tumors that are commonly treated with hepatic embolization.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

Study Design
This is a single center, single blind, prospective, randomized, phase II controlled trial to be conducted without crossover.

Trial Arms
Patients will be randomized to one of two arms designated **Bead Arm** and **Bead + Dox Arm**, where the planned interventions are:

- **Bead Arm:** Hepatic arterial embolization with Bead Block microspheres, beginning with 100 – 300 micron beads, and using larger particles if necessary until stasis is evident.
- **Bead + Dox Arm:** Hepatic arterial embolization with 100-300 micron drug eluting microspheres (LC Bead) loaded with 150 mg Doxorubicin, followed by embolization with Bead Block microspheres (100-300 micron and larger size beads as necessary) until stasis is evident.

Limitations of Study Design
It is not possible to perform the embolization procedure in a completely double blinded fashion. Doxorubicin is red, and when added to the embolization beads results in red colored spheres, thus the interventional radiologist performing the embolization will know which embolic agent is being used. Subsequently, CT readers assessing response to treatment, and TTP will be blinded to the treatment method.

Tumor Response
For the purposes of the primary objective, the triple phase CT obtained within 2-3 weeks of initial treatment will be used to assess response to treatment. Tumor response will be catalogued according to RECIST criteria as well as by % necrosis determined using the study CT volumetric method.

RECIST Response Criteria
RECIST criteria responses are defined as follows:

Complete Response (CR):	disappearance of all target lesions (up to 10 target lesions)
Partial Response (PR):	≥ 30% decrease from baseline
Stable Disease (SD):	All other cases
Progressive Disease (PD):	≥ 20% increase from the smallest sum of measurements since the start of treatment
Objective Response:	CR and PR

Measurement of Treatment Response
Contrast enhanced CT and MRI are the best currently available methods of evaluating response to treatment for HCC, and the most reproducible means of measuring target lesions and assessing necrosis. Triple phase contrast enhanced CT scans will be performed 2-3 weeks after the first complete treatment. Patients will be grouped according to RECIST criteria; however the volumetric assessment will use a continuous scale of percent tumor necrosis.

Verification of Tumor Response
Tumor response assessments will be recorded in CRDB. Triple phase CT scans will be read from PACS workstation by independent readers without knowledge of the treatment group or investigator opinion. Tumor response will be classified by RECIST criteria, as well as by volume of enhancing tumor.

Local Tumor Response
Local tumor response will be measured following each complete treatment. Local tumor progression (LTP) will be recorded when the patient develops > 20% increase in sum of uni-dimensional measurement (RECIST), or > 75% increase in volume of enhancing tumor since beginning treatment.

Time to Symptomatic Progression
Time to symptomatic progression will be measured as a 2-point increase in ECOG score, or the presence of constitutional symptoms, or the occurrence of pain in previously asymptomatic patient compared to baseline. ECOG performance status (Oken et al 1982) is used widely in cancer clinical trials to assess the progression of disease and how this affects patient's daily life (Appendix 1). ECOG performance status will be assessed at baseline and at each of the follow-up visits until study completion, with the exception of the 2-3 week post treatment visit when patients might still be experiencing post-embolization syndrome.

Time to Progression
Time to progression (TTP) will include and be categorized as time to: local tumor progression (LTP), distant hepatic progression (DHP), and extra-hepatic progression (EHP) Local tumor progression (LTP) will be measured as the time from randomization until the first measurement of progression as determined by the central reading group using RECIST criteria as well as volumetric criteria for the target tumor. Local tumor progression (LTP) will be defined

specifically as ≥20% increase from the smallest sum of measurements since the start of treatment (RECIST criteria, see above), or ≥ 75% increase in enhancing tumor volume. Distant hepatic progression (DHP) will be defined as the development of any new discontinuous disease within the liver, and the development of any extra-hepatic disease will constitute extra-hepatic progression (EHP).

Duration of Objective Response
Duration of treatment response will be measured as the time from the date of first objective response until the first measurement of progression as determined by the central readers using the RECIST criteria.

Time to Treatment Failure
Time to treatment failure (TTF) will be measured as the time from randomization until treatment discontinuation for any reason including, development of ≤ 5% necrosis, by volume, following the initial treatment or >50% increase in sum of uni-dimensional measurements following later treatments, treatment toxicity, patient preference, or death.

Change in Alpha-fetoprotein
Alpha-fetoprotein (AFP) will be measured at baseline, and then at each follow-up visit.

Time to Hospital Discharge
Time to discharge will be measured as the time from the date of embolization until discharge for each embolization, and be reported as per embolization, not per patient.

Other Procedures or Interventions Required Secondary to Embolization
Any additional procedures or interventions will be recorded in CRDB

Study Population
Subjects for study will be recruited from [redacted] patient population, and will include patients with a diagnosis of HCC seen primarily by Hepatobiliary Surgery, GI Oncology, or GI Medicine. These patients may have had previous surgery, but will not have had previous radiation therapy, embolization or local-regional treatment of the current target tumor volume. Every effort will be made to recruit a diverse group of patients, including men and women of different ethnic backgrounds from the varied Memorial Sloan-Kettering referral population. The disease is more common in men and patients of Asian ethnicity. We plan to enter 100 patients into the study over a two-year period.

4.2 Intervention

The intervention being studied is hepatic arterial embolization for the treatment of hepatocellular carcinoma using either a plain microscopic bead or the same bead loaded with doxorubicin. Baseline angiography including celiac and superior mesenteric angiography will be performed to delineate arterial anatomy, and blood supply to the tumor followed by catheter embolization.

Solitary HCC

In the case of a solitary tumor, selective embolization will be performed. In the **Bead + Dox Arm** an attempt will be made to administer the entire 2-3 vial dose of 100-300 micron LC Bead containing a total of 150 mg of doxorubicin, followed by administration of unloaded microspheres (Bead Block) until stasis occurs. Whether the 150 mg of doxorubicin will be loaded in 2 or 3 vials (37.5 mg/ml or 25 mg/ml) will depend on the volume of tumor being treated. Patients in the **Bead arm** will be embolized beginning with 100-300 micron Bead Block and using larger size beads as necessary until stasis is evident.

Multifocal HCC

In the case of multifocal bilobar disease or some large tumors with significant blood supply from both the right and left hepatic artery, either the right or left hepatic territory will be treated at the first session. This will be performed by placing the angiographic catheter selectively into either the right or left hepatic artery, and embolizing to stasis. In the **Bead + Dox Arm** 2-3 vials of 100-300 micron microspheres (LC Bead) will be prepared with 50 mg of doxorubicin/vial in an attempt to administer a total of 150 mg of doxorubicin. The goal will be to administer all 2 or 3 vials when possible and, if persistent antegrade flow is noted after the drug eluting beads have been used, then embolization will be continued using unloaded microspheres (Bead Block) until stasis occurs. Patients in the **Bead arm** will be embolized in a similar fashion using unloaded microspheres (Bead Block) only.

Patients requiring a second embolization to complete their treatment will undergo the second embolization within 2-6 weeks, depending on their recovery from the first session.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

- **Bead Arm:** Hepatic arterial embolization with Bead Block microspheres, beginning with 100-300 micron spheres, and using larger sizes as outlined above until stasis is evident.
- **Bead + Dox Arm:** Hepatic arterial embolization with 100-300 micron drug eluting microspheres (LC Bead) loaded with 150 mg Doxorubicin, followed by embolization with Bead Block microspheres until stasis.

5.1 Bead Block Product Description

Bead Block comprises a range of sizes of hydrophilic microspheres produced from polyvinyl alcohol (PVA) and supplied in sterile prefilled syringes containing 1 ml or 2 ml of microspheres in sterile saline. These are intended for single use, should be stored in a cool, dry and dark place, and used by the date indicated on the syringe label. They are FDA approved for use in the embolization of hypervascular tumors and arteriovenous malformations. These microspheres, like all agents used for arterial embolization, are mixed with radiographic contrast prior to administration in order to allow for fluoroscopic control of the embolization procedure.

5.2 LC Bead Product Description

Biocompatibles LC Bead (a.k.a. DC Bead in Asia & Europe) microspheres are preformed soft, deformable microspheres that may be loaded with doxorubicin and used to occlude blood flow to a cancerous tumor. LC Bead microspheres consist of a macromere derived from PVA. The fully polymerized microsphere is approximately 90% water and is compressible to approximately 30%

by diameter. The microspheres can be delivered through conventional catheters (4-5Fr) or micro-catheters in the 2-3Fr range. These microspheres, like all agents used for arterial embolization, are mixed with radiographic contrast prior to administration in order to allow for fluoroscopic control of the embolization procedure.

LC Bead microspheres are supplied in glass vials containing 2ml of microspheres in 6ml of saline that do not require special handling, and should be stored in a cool, dry, dark place in original vial. The product is sterilized by steam.

Loading Prior to Use: LC Bead may be loaded with Doxorubicin immediately prior to use or by storing the vial with loading solution in a fridge (2-8°C) overnight prior to the procedure.

The recommended loading dose is 25mg Doxorubicin per 1ml of LC Bead. The maximum loading dose is 37.5mg Doxorubicin per 1 ml of LC Bead. The recommended total dose of Doxorubicin combined with LC Bead per procedure is 150mg.

Contrast can be added to the LC Bead /Doxorubicin mix immediately prior to use without affecting the Doxorubicin loading or the stability of the loaded drug. After mixing with contrast, the LC Bead Doxorubicin solution is stable for up to 8 hours at ambient temperature. In order to obtain a homogenous suspension of Doxorubicin loaded LC Bead an equivalent volume of non-ionic contrast media should be added to the loaded LC Bead mixture.

Disposal of Unused DC Bead: Unused LC Bead should be disposed of as cytotoxic clinical waste.

5.3 LC Bead Doxorubicin Loading Instructions

LC Beads are suitable for loading doxorubicin-HCl ONLY. Liposomal formulations of doxorubicin are not suitable for loading into LC Bead.

150 mg (2 vials, 75 mg each, or 3 vials, 50 mg each) doxorubicin HCL will be obtained from the pharmacy. Our pharmacy will load the doxorubicin

To obtain a final loading of 50mg doxorubicin per 2ml vial of LC Bead

i. Reconstitute a vial containing 50mg of doxorubicin with 2ml of sterile water for injection. Mix well to obtain a clear solution (25mg/ml).
ii. Remove as much saline as possible from a vial of using a syringe with a small gauge needle.
iii. Using a syringe and needle add the 2ml of reconstituted doxorubicin solution directly to the vial of LC Bead.
iv. Agitate the LC Bead/doxorubicin solution gently to encourage mixing, then allow to stand for the required time. At this point the LC Bead will be red and loaded to the extent stated. The solution usually will still have a red coloration,. For static loading the following times must be adhered to:

Product Cap Color	Nominal Size Range	Concentration of Doxorubicin Loading Solution	Minimum Time to achieve >90% Loading	Minimum Time to achieve 98% Loading
	100μ-300μ	**25mg/ml**	**30 minutes**	**60 minutes**
	300μ -500μ	**25mg/ml**	**60 minutes**	**120 minutes**

Although the solution retains a pink color, the majority of the doxorubicin will be loaded.

v. Loading will take a minimum of 30 minutes for the smallest size LC Beads and up to 120 minutes for the largest size LC Bead.
vi. Repeat above procedure with 2nd 50 mg vial of doxorubicin
vii. Prior to use, transfer the LC Bead loaded with doxorubicin to a syringe and add an equal volume of non-ionic contrast media. Invert the syringe gently to obtain an even suspension of LC Bead.

Use the suspension of loaded LC Bead within 4 hours of addition of the contrast media. A pink coloration will be present in the suspension as approximately 0.3% (0.1mg) of the loaded drug elutes into the contrast media suspension.

vii. A dose of up to 37.5mg doxorubicin per ml LC Bead can be loaded.
viii. The maximum recommended total dose of doxorubicin per procedure is 150mg.

For 37.5mgs, the following loading times should be used:

Product Cap Color	Nominal Size Range	Concentration of Doxorubicin Loading Solution	Minimum Time to achieve >90% Loading	Minimum Time to achieve 98% Loading
	100μ-300μ	**37.5mg/ml**	**30 minutes**	**60 minutes**
	300μ -500μ	**37. 5mg/ml**	**45 minutes**	**90 minutes**

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

Patients will be eligible for the study if they fulfill the following criteria at entry and prior to each treatment

6.1 Subject Inclusion Criteria

1. Patient with a confirmed diagnosis of HCC according to EASL criteria for diagnosis, see Appendix 2 who is not surgical resection candidate, or refuses surgery.
2. Patient must be 18 years of age or older.
3. Patient must be Okuda stage I or II, see Appendix 3.
4. Patient must have an ECOG performance status of 0 or 1, see Appendix 1.
5. No prior chemotherapy or biotherapy within 4 weeks of scheduled embolization, with all toxicities, if any, resolved to grade ≤ 1
6. Patient must have the following laboratory values confirmed within 4 weeks of registration:
   a. Creatinine ≤ 2.0 the institution ULN
   b. Platelets $\geq 50{,}000/mm^3$

c. INR ≤ 2.0 for patients who are not on Coumadin
d. aPTT $\leq$ twice control
e. Bilirubin < 3 mg/dl
f. WBC > 3000 cells/mm^3
g. ANC >1500 cells/mm^3
h. Negative serum pregnancy test (Female of childbearing potential only)

A patient will **NOT** be eligible for inclusion in this study if any of the following apply:

6.2 Subject Exclusion Criteria

1. Patient has another primary tumor, with the exception of conventional basal cell CA, superficial bladder cancer, melanoma in situ, or treated prostate cancer currently without biochemical or radiographic evidence of active disease.
2. Women who are pregnant or lactating
3. Patient previously treated with doxorubicin
4. Contraindication to angiography/embolization including:
    a. Patients who cannot receive contrast
        i. Severe allergic reaction to contrast despite pre-medication
        ii. Poor renal function
    b. Lack of arterial access (e.g. femoral artery occlusion)
    c. other, based on judgment of the investigator
5. Patient has already undergone hepatic arterial embolization for the hepatocellular cancer for which they are currently being evaluated.
6. Patient has received prior radiotherapy for the hepatocellular cancer for which they are currently being evaluated.
7. Patient has had previous local-regional treatment of the current target tumor volume.
8. Patient who cannot have CT scan
9. Patient at very high risk for post-embolization hepatic failure
    a. Child's C cirrhosis
    b. >75% liver replaced by tumor
10. Cardiac exclusion for:
    a. Myocardial infarction within 90 days of study
    b. Uncontrolled arrhythmia
    c. LVEF $<$ 50%, for patients randomized to receive LC Bead
11. Patients with tumors exhibiting characteristics considered contra-indications to particle embolization, including:
    a. Collateral vessel pathways potentially endangering normal territories during embolization
    b. Arteries supplying tumor not large enough to accept LC Bead or Bead Block
    c. Presence of arterial to systemic venous shunts
    d. Presence of arterial to pulmonary vascular shunts

7.0 RECRUITMENT PLAN

Potential research subjects will be identified by a member of the patient's treatment team (GI oncologist, or Surgical Oncologist), the protocol investigator, or research team at [redacted] ([redacted] If the investigator is a member of the treatment team, s/he will screen their patient's medical records for suitable research study participants and discuss the study and their potential for enrolling in the research study. Potential subjects contacted by their treating physician will be referred to the investigator/research staff of the study.

8.0 PRETREATMENT EVALUATION

Day – 6 weeks
Within 6 weeks of registration, patients will be seen by a hepatobiliary surgeon, GI oncologist, or interventional radiologist and, if general criteria for the study are met, he/she will have a triple phase CT. They will have vital signs (weight, blood pressure, temperature, heart rate) checked. After review of the CT, informed consent will be obtained by the interventional radiologist PI or Co-I, and the patient will be randomized. This will allow for stratification into patients whose treatment is expected to be complete after one embolization procedure, and those who will need two embolization procedures to complete their first treatment. Patients randomized to the LC Bead (Bead + Dox) group will also have a cardiac echo or a MUGA scan to determine baseline ejection fraction (EF) if they have not had an evaluation of their EF within 3 months.

Day - 4 weeks
Within four weeks of the first embolization procedure patients will have a CBC, PT, INR, PTT, electrolytes, BUN/creatinine, alfa-fetoprotein, liver function tests including albumin, total protein, total bilirubin, LDH, alkaline phosphatase, ALT, and AST. The patients will be screened for hepatitis B and C, if this has not already been done. They will have an ECG.

9.0 TREATMENT/INTERVENTION PLAN

Hepatic Embolization Procedure
On the day of the procedure patients will take only clear liquids after midnight. The morning of the procedure an IV will be started, and they will receive an anti-emetic (palonosetron hydrochloride, 0.25 mg IV), and a pre-procedure antibiotic (cephazolin, 1 gram IV or, for those who have had a bilioenteric anastomosis, sphincterotomy or any other reason to lack an intact, functional sphincter of Oddi, Piperacillin/Tazobactam, 4.5 grams IV. Patients who are allergic to those medications will receive Clindamycin 900 mg and Gentamicin 1.5 mg/kg IV). Patients with a creatinine >1.5 will receive intravenous sodium bicarbonate. 150 mEq of sodium bicarbonate is mixed in 1 L of D5W and administered at a rate of 3 cc/kg/hr beginning 1 hour before the procedure, with a maximum rate of 300 cc/hr. After the initial hour, the rate is decreased to 1 cc/kg/hr during the procedure, and continued for 6 hours after the procedure. Those who are allergic to contrast will be pre-medicated with steroids. These patients receive 50 mg of prednisone by mouth every 6 hours for 3 doses, beginning 13 hours before the procedure,

thus administered at -13 hours, -6hours, and -1hr. In addition, 50 mg of diphenhydramine hydrochloride is given by mouth 1 hour before the procedure.

Baseline angiography including celiac and superior mesenteric angiography will be performed to delineate arterial anatomy and blood supply to the tumor.

Solitary HCC

In the case of a solitary tumor, selective embolization will be performed. For patients randomized to receive LC Bead, if a single vessel supplies the tumor then an attempt will be made to administer the entire 2-3 vial dose of 100-300 micron LC Bead containing 150 mg of doxorubicin into that vessel. If stasis occurs before the entire dose is delivered, the amount administered is recorded, and the remainder is discarded. If there is continued antegrade flow after the LC Bead has been used, embolization will be continued using100-300 micron Bead Block until **stasis*** occurs, or a total of 10 cc of 100-300 micron particles have been used (LC Bead + Bead Block). If there is persistent antegrade flow, then embolization will be continued with 300-500 micron Bead Block until 10 cc of that size microsphere has been used, at which point embolization may be continued using up to 10 cc of 500-700 micron particles continuing with 10 cc of larger sizes until stasis is evident. 100 micron PVA may be used at any time to stabilize the embolization endpoint assuming all of the tumor vessels have been embolized. If there are 2 or more vessels supplying the tumor, then the LC Bead will be divided proportionally and administered first in each branch. After the entire dose of 100-300 micron LC Bead has been used, the embolized vessels will be re-catheterized and embolized to stasis with Bead Block beginning with 100-300 micron spheres and using a maximum of 10 cc of any given size per vessel before moving to the next size microsphere, as outlined above. Patients will never be treated with sizes other than 100-300 micron LC Bead.

Patients to be treated with beads alone will have vessel(s) supplying the tumor catheterized as selectively as possible, and embolized with 100-300 micron Bead Block, using up to 10 cc of 100-300 micron Bead Block in a single vessel. If stasis is not evident, then 10 cc of the next size particle will be used, and so on, as outlined above, also allowing for the use of 100 micron PVA when indicated. This will be repeated in each vessel supplying tumor.

Multifocal HCC

In the case of multifocal bilobar disease, either the right or left hepatic territory will be treated at the first session. This will be performed by placing the angiographic catheter selectively into either the right or left hepatic artery, and embolizing to stasis. For patients receiving LC Bead, 2-3 vials of 100-300 micron LC Bead will be prepared. The goal will be to administer all vials when possible, and if persistent antegrade flow is noted after the drug eluting beads have been used, then embolization will be continued using100-300 micron Bead Block until **stasis*** occurs. When stasis is not evident after a total of 10 cc of microspheres (including the LC Bead) has been administered in this vessel, the next larger size of microspheres (300-500 micron) will be used. If stasis is not evident after 10 cc of 300-500 micron Bead Block has been given in that vessel, the next size (500-700 micron) particles will be used. In the event that sectoral or segmental branches of one side of the liver have to be catheterized selectively (for instance when there is an accessory vessel that arises separately, or when selective catheterization is necessary

to avoid non-target embolization), after evaluation of the initial angiogram the LC Bead will be divided proportionally, and administered proportionally in each branch. After the entire dose of 100-300 micron LC Bead has been used, the vessels will be re-catheterized and embolized to **stasis*** beginning with 100-300 micron Bead Block according to the method outlined above using up to 10 cc of each size microsphere in each vessel. Patients will never be treated with sizes other than 100-300 micron LC Bead.

Patients being treated with beads alone will have the vessels supplying one side of the liver catheterized as selectively as necessary, and embolized with 100-300 micron Bead Block, using up to 10 cc of 100-300 micron Bead Block in each vessel embolized. If **stasis*** is not then evident, 10 cc of the next size particle will be used, and so on, as outlined above. This will be repeated in each vessel supplying tumor.

****Stasis** is defined as the absence of antegrade flow within a vessel, such that even slow administration of contrast material results in reflux, or retrograde flow. This corresponds to an angiographic image of the target vessel and branches filled with contrast that persists, and does not washout, for 5 cardiac beats after the injection of 2 ml of contrast. Once this angiographic endpoint is achieved, the operator will wait 3 minutes to ensure that antegrade flow has not been re-established. If there is evidence of antegrade flow, embolization will be continued until the desired endpoint is reached again. After an additional 3 minutes, the endpoint will be reconfirmed as stable. This sequence of events will be repeated as necessary to achieve reproducible "stasis".*

What Constitutes a "Treatment"?

A single "treatment" is defined as the administration of Bead Block, or LC Bead and Bead Block to the entire tumor burden evident on pre-procedure imaging. In the case of multifocal bi-lobar disease this would, by definition, involve embolization of both right and left hepatic arteries. Solitary tumors may also be supplied by right and left hepatic arteries, particularly when they are large and arise in the anterior right liver, or in the medial segment of the left. Patients with HCC typically have underlying liver disease, and cirrhosis. As portal hypertension progresses, these patients become more reliant on hepatic arterial blood flow and hepatic failure following arterial embolization is a recognized risk of the procedure. For this reason, the entire hepatic arterial circulation is not embolized at once. Even patients without cirrhosis who are embolized for metastatic disease have a higher risk of hepatic failure when the entire liver is treated at one sitting [Brown KT, 1998, 1999]. Thus, a single session of embolization may not constitute a "treatment" as defined above.

For the purposes of this study when multifocal bilobar disease is present, a "treatment" is defined as treatment of both the right and left hepatic artery, and therefore will consist of two embolization sessions. This will be evident on the pre-procedure CT, and patients stratified accordingly. Patients with large solitary tumors may require embolization of more than one side of the liver as well. For example, patients with large tumors arising in segment IV may require embolization of the entire left hepatic artery as well as the anterior division of the right hepatic artery. Similarly, patients with large tumors in the anterior division of the right liver may require embolization of the entire right hepatic artery, plus segment IV. This is typically evident on the

pre-procedure CT, and the need for 1 or 2 embolizations to complete treatment will be stratified for. If, in the operator's judgment, it is not safe to perform any embolization in one session the treatment will be staged, even if this only becomes evident at the time of embolization. For the purposes of the study, two embolization sessions necessary to completely treat the initial tumor burden would constitute a single "treatment".

Patients with small tumors, or tumors localized to either the right or left liver, typically have the entire tumor burden treated in one session. Findings on the initial triple phase CT will typically identify which patients can be completely treated in one embolization session, and which patients will require a second session, thus randomization performed following the initial triple phase CT will be stratified to take this into account.

Patients Requiring 2nd Embolization for Completion of Treatment

Patients requiring a second embolization to complete one treatment will be scheduled for their second embolization within 2-6 weeks of their initial embolization. They will have a clinic visit within 2 weeks of their second treatment, at which time they will have vital signs (weight, blood pressure, temperature, heart rate), CBC, chemistry profile with liver function tests, coagulation studies, AFP level, and evaluation for any treatment toxicity.

At the time of the second embolization the patients will be admitted through the Pre-surgical Center and receive the same pre-procedure care as they did before their initial embolization. Hepatic angiography will be performed, and the vessels not treated at the time of the first embolization will be embolized using the same treatment algorithm outlined in section 9.0 Hepatic Embolization Procedure. Thus, these patients will have received a total of 300 mg of doxorubicin, 150 mg at each treatment.

Post-Procedure Care

Following embolization patients are monitored in the Post Anesthesia Care Unit for 3-5 hours. They are then transferred to the floor where they receive 24 hours of IV hydration and antibiotics, as well as symptomatic treatment for postembolization syndrome (PES). Postembolization syndrome consists of pain, fever, nausea and vomiting, and can be thought of as a type of tumor lysis syndrome and therefore as a side effect of treatment rather than a complication. Patients may experience one or all of the symptoms of PES, and are treated with narcotics, antiemetics and antipyretics as needed to control their symptoms. When the patients have completed 24 hours of antibiotics, their temperature is $\leq 38.5^{\circ}$ C, they are taking adequate nutrition by mouth, and have their pain controlled by oral analgesics they are discharged home.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

Time line	Within 6 weeks	Within 4 weeks	Within 2 weeks[2]	Time 0	Day 1	10-14 Days	Within 2 -3 weeks	10-14 weeks	5-6 months	8-9 months	11-12 months
Clinic Visit[8]	X		X				X[9]	X	X	X	X
Laboratory tests											
CBC		X	X		X	X[4]	X	X	X	X	X
PT, INR, PTT		X	X				X	X	X	X	X
electrolytes		X	X				X	X	X	X	X
BUN/ creatinine		X	X				X	X	X	X	X
AFP		X	X				X	X	X	X	X
Albumin, total protein, bilirubin (total)		X	X				X	X	X	X	X
LDH, Alk. Phosphatase, ALT, AST		X	X				X	X	X	X	X
Hepatitis screening (B and C)		X[10]									
Radiologic tests											
Triple phase CT (TPCT)	X						X[5]	X[6]	X[7]	X[7]	X[7]
Treatment											
Hepatic Embolization procedure				X[3]							
Others											
ECG		X									
MUGA scan or Cardiac Echo		X[1]									
ECOG assessment								X	X	X	X
Vital Signs checked	X		X				X	X	X	X	X

[1]For patients randomized to the LC Bead (particle + doxorubicin) group. Patients will have repeat MUGA scan or cardiac echo after every 3 treatments, or 450 mg of doxorubicin, and prior to exiting protocol if feasible. If patient exits protocol unexpectedly (e.g. due to death), no MUGA scan or cardiac echo is necessary.

[2]Only for patients who require 2 embolizations to complete one treatment, within 2 weeks of the second embolization the patient will return for clinic, vitals and laboratory tests.

[3]Time 0 is the day of the patient's completed treatment, either single or two embolizations sessions, as anticipated by pre-treatment CT.

[4]CBC may be done locally.

[5]CT used to determine response to treatment.

[6]CT used to determine the need for re-treatment.

[7]If there is evidence of disease progression; a repeat treatment will be scheduled within 2 months ± 2 weeks. The patients will be re-treated with the same embolic agent used in the first treatment. Patients will not receive more than 450 mg of doxorubicin per annum. They will then re-enter the follow-up pathway.
[8] Clinic visit will include recording of vital signs, ECOG status and assessment of any SAE
[9] No ECOG assessment will be done at 2-3 weeks post embolization visit since patients might still be experiencing post-embolization syndrome
[10]If not already done.

Day 1
All patients will have a CBC drawn the day after embolization.

Time 0 + 10-14 days
Patients will have a CBC drawn 10-14 days after embolization. This may be done locally.

Time 0 + 2-3 weeks
Patients will be seen in clinic within 2-3 weeks of complete treatment (Time 0). They will have vital signs (weight, blood pressure, temperature, and heart rate), CBC, chemistry profile with liver function tests, coagulation studies, AFP level, and evaluation of any treatment toxicity. The patients will also have a triple phase CT scan. This is the CT scan that will be used to determine response to treatment

Time 0 + 10-14 weeks
Patients will be seen in clinic and have vital signs evaluation (weight, blood pressure, temperature, and heart rate), CBC, chemistry profile with liver function tests, coagulation studies, AFP level, and ECOG assessment. They will have a triple phase CT scan. This CT scan will be used to determine if the patients need re-treatment. Patients with evidence of persistent or progressive tumor but who do not meet the criteria for treatment failure, will be re-treated within their original treatment arm within 6 weeks of this follow-up scan. The patients will be re-treated with same embolic agent used for first treatment, with embolization protocol identical to that outlined above. They will then re-enter the follow-up pathway.

Time 0 +5- 6 months
Patients will be seen in clinic and have vital signs (weight, blood pressure, temperature, heart rate), CBC, chemistry profile with liver function tests, coagulation studies, and AFP level, and ECOG assessment. They will have a triple phase CT scan. Patients with stable disease compared to the previous scan will be followed up in 3 months. If there is evidence of recurrent or new tumor a repeat treatment will be scheduled within their original treatment arm within 6 weeks of the follow-up scan. The patients will be re-treated with same embolic agent used for first treatment, with embolization protocol identical to that outlined above. They will then re-enter the follow-up pathway.

Time 0 + 8-9 months
Patients will be seen in clinic and have vital signs (weight, blood pressure, temperature, heart rate), CBC, chemistry profile with liver function tests, coagulation studies, and AFP level, and ECOG assessment. They will have a triple phase CT scan. Patients with stable disease compared

to the previous scan will be followed up in 3 months. If there is evidence of recurrent or new tumor a repeat treatment will be scheduled within their original treatment arm within 6 weeks of the follow-up scan. The patients will be re-treated with same embolic agent used for first treatment, with embolization protocol identical to that outlined above. They will then re-enter the follow-up pathway.

Time 0 + 11-12 months
Patients will be seen in clinic and have vital signs (weight, blood pressure, temperature, heart rate), CBC, chemistry profile with liver function tests, coagulation studies, and AFP, and ECOG assessment. They will have a triple phase CT scan. Patients with stable disease compared to the previous scan will be followed up in 3 months. If there is evidence of recurrent or new tumor a repeat treatment will be scheduled within their original treatment arm within 6 weeks of the follow-up scan. The patients will be re-treated with same embolic agent used for first treatment, with embolization protocol identical to that outlined above. They will then re-enter the follow-up pathway. Within one year patients will not receive more than 3 embolizations with LC Bead in order to ensure that they are not exposed to >450 mg of doxorubicin/annum.

Patients who remain disease free (CR), or who have stable disease (SD) at this point, will have follow-up triple phase CT every 3-6 months until death or progression and will only be re-treated for progression or new tumor.

11.0 TOXICITIES/SIDE EFFECTS

Following embolization 80% of patients experience some degree of post-embolization syndrome consisting of pain, fever, nausea and/or vomiting for which they receive supportive care. Liver abscess is a recognized complication, but is typically seen only in patients who have had a bilio-enteric bypass, or for some other reason do not have an intact sphincter of Oddi. Liver abscesses are usually managed with antibiotics and catheter drainage rarely is surgical debridement necessary. Post embolization cholecystitis is occasionally seen, presumably from inadvertent embolization of the cystic artery, although this usually resolves without treatment pain and nausea/vomiting may prolong hospital stay. Very rarely more severe consequences of non-target embolization are seen, such as pancreatitis or gastric or duodenal ulceration. Transient deterioration in liver function or even frank liver failure may occur, more commonly following embolization of more than 75% of the liver volume, or in patients with marginal underlying hepatic function. Cutaneous manifestation of embolization may be seen when non-hepatic vessels such as the right phrenic or internal mammary arteries are embolized when they provide collateral flow to hepatic tumors. These cutaneous findings are usually asymptomatic and resolve without treatment. Very rarely death has occurred from pulmonary embolization when particles pass through arterial-systemic venous shunts into the pulmonary artery branches. There is no known treatment for this complication.

Cardiac toxicity is a risk when doxorubicin is administered systemically. Given the affinity of doxorubicin for LC Bead, results of previous clinical, and the favorable AUC curve for doxorubicin eluting LC Bead it is possible, but unlikely, that cardiac toxicity might occur.

Reproductive risks: Patients should not become pregnant or father a baby while on this study because the drugs and radiation used in this study can affect an unborn baby.

Toxicity will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) 3.0.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

All patients will undergo contrast enhanced triple phase CT within 6 weeks of registration and then again within 2-3 weeks of completing their first treatment. Prior to treatment index lesions according to RECIST will be identified and conventional uni-dimensional measurements obtained blinded to the patients treatment group assignment. Response to treatment will be assessed by conventional RECIST criteria (see **Section 4.1**). The electronic data will then be sent to an independent imaging laboratory where an imager not familiar with the same patient's initial data set will create a volumetric profile including volume of tumor and volume of necrosis at time 0. On the initial post-treatment scans the index tumor(s) will once again be identified and measured by an imager blinded to the patient's treatment group, and once again the electronic data will be sent to the imaging laboratory where it will be independently analyzed volumetrically, once again recording tumor volume, and volume of necrosis. These results will be recorded in the electronic database. These linear and volumetric assessments will be repeated each time the patient is imaged for the duration of the study. Any increase in standard manual measurements of >20%, or the development of > 75% increase in enhancing tumor volume will be considered evidence of progression of disease (PD) and so recorded. In addition, patients who develop ≤ 5% necrosis, by volume, following the initial treatment, or increase of >50% in sum of uni-dimensional measurements following subsequent treatments will be considered treatment failures.

Volumetric Imaging Method

We have developed a system with a practical user interface that allows operator to synchronically view baseline and corresponding follow-up images of the liver side-by-side, semi automatically delineate tumor contours, and segment necrosis with an optimal threshold determined by observing segmentation results while varying thresholds or setting a system wide threshold value. If a segmentation result is suboptimal, the system allows the operator to make manual correction (Fig 2).

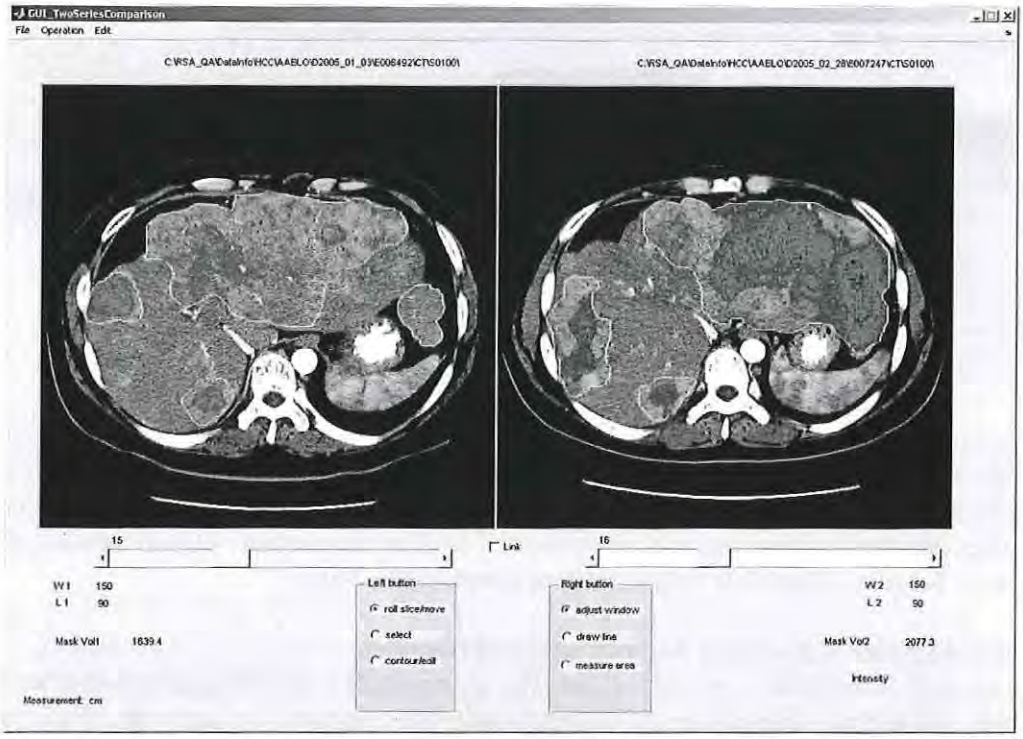


Figure 2 An image processing system allows manual segmentation of liver tumor (with blue border) and tumor necrosis (area in red). Segmented tumor contours and necrosis areas are superimposed on baseline (left) and follow-up (right) CT images. Tumor volume increased 29.5%, whereas ratio of tumor necrosis volume to tumor volume increased 388.8%.

Segmented tumor contours and necrosis areas are superimposed on baseline (left) and follow-up (right) CT images. Tumor volume increased 29.5%, whereas ratio of necrosis volume to tumor volume increased 388.8%.

In addition to the manual segmentation and semi-automated segmentation, we plan to develop a fully automated segmentation algorithm for separating tumor from normal hepatic parenchyma as well as necrosis from tumor. These are extremely difficult segmentation tasks, because 1) the intensity contrast between tumor and liver parenchyma can be very low, and 2) necrosis can be heterogeneous throughout the tumor and may be difficult to be distinguished from hypointense portions of the tumor.

Automated/Semi-Automated Segmentation of Tumor

Previously, we developed a computer algorithm for semi-automated delineation of liver metastases from colorectal cancer. The algorithm starts with a manual selection of a seed lesion region-of-interest (ROI). Based on intensity distributions of the seed ROI and the liver parenchyma, several features were computed and used to adaptively guide the region-growing. To prevent the region-growing from leaking into surrounding tissues of similar characteristics, specific shape constraints, including a local shape, a global shape and a gravity-shift index, were

developed to jointly control the iteration of the region-growing. [Zhao B 2006] Figure 3 shows two examples of the segmentation results obtained using the algorithm.

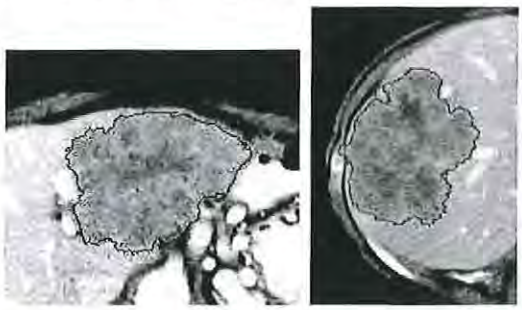


Figure 3 Semi-automated segmentation of liver metastasis using a shape-constrained region-growing algorithm.

In this study, due to similarity of the tumor appearance, we will adopt the same segmentation strategy. However, some portions of the algorithm may need to be modified and some of the intensity-based features may need to be redefined, especially their threshold values. For instance, the lower threshold of the intensity sub-range IV used in the previous algorithm may need to be increased because of possible bright parts of tumor in this study.

Automated/Semi-Automated Segmentation of Necrosis

Once a tumor is delineated, necrosis inside the tumor needs to be detected and segmented. As necrosis will not be enhanced by the contract material, it appears low attenuation and possesses a certain low range of intensity on contrast-enhanced CT images. We determine this intensity range through manually extracting necroses in a large number of liver tumors and calculating the mean and standard deviation (std) of the necroses' intensity. A fixed threshold can then be determined by the equation: $Thr = \text{mean} + a*\text{std}$, where a is a parameter that needs to be optimized in the study. Any voxel in the tumor having an intensity value lower than *Thr* will be considered as a necrosis voxel. This value may be modified on a scan or patient basis and will be visually validated by the Radiologist. Volumetric tumor size and necrosis can therefore be calculated simply for an entire tumor or series of tumors.

Tumor Response

Tumor response will be measured according to RECIST criteria, as well as using the new volumetric method of assessing non-viable tumor. In the case of differences between these criteria, the RECIST criteria will be used for clinical judgment and decisions.

RECIST criteria responses are defined as follows:

Complete Response (CR):	disappearance of all target lesions (up to 10 target lesions) confirmed at ≥ 4 weeks
Partial Response (PR):	≥ 30% decrease from baseline
Stable Disease (SD):	All other cases
Progressive Disease (PD):	≥ 20% increase from the smallest sum of measurements since the start of treatment
Objective Response:	CR and PR

Measurement of Treatment Response
Contrast enhanced CT and MRI are the best currently available methods of evaluating response to treatment for HCC, and the most reproducible means of measuring target lesions and assessing necrosis. Triple phase contrast enhanced scans will be performed in accordance with protocol detailed in "Follow-up after complete treatment" section.

Verification of Tumor Response
Tumor response assessments will be recorded in CRF by the investigator. Triple phase CT scans will be read from PACS workstation by independent readers without knowledge of the treatment group or investigator opinion.

Local Tumor Response
Local tumor response will be determined for the initial tumor volume treated following each complete treatment and be recorded as CR, PR, SD, or PD by RECIST and volumetric methods. New tumor developing in a distant previously untreated area of the liver will be considered new hepatic progression (NHP)

13.0 CRITERIA FOR REMOVAL FROM STUDY

If the patient develops < 5% necrosis by volume following the initial treatment, or increase of >50% in sum of uni-dimensional measurements following subsequent treatments he/she will be considered a treatment failure and will be taken off study and referred for alternative therapy.

If at any time the patient develops unacceptable toxicity he/she will be removed from study.

Known or suspected pregnancy will result in withdrawal of patient from study.

If at anytime the patient is found to be ineligible for the protocol as designated in the section on Criteria for Patient/Subject Eligibility (i.e., a change in diagnosis or condition), including an LVEF of <50%, the patient will be removed from the study.

Non-compliance, patient preference.

14.0 BIOSTATISTICS

This primary objective of this study is to estimate the response rates (CR + PR) to treatment following hepatic arterial embolization of hepatocellular cancer (HCC) using microscopic beads + Doxorubicin and beads alone by conventional RECIST criteria. With 50 patients in each arm response rates can be estimated to within +/- 14%. Patients will be randomly assigned to one the two treatment arms. A two-arm study was deemed necessary because there was not sufficient preliminary data on the control arm to design a single-arm study with historical controls. This is not a study where the goal is to test a hypothesis, rather the emphasis is on the estimation of response rates in the two arms. For this reason the sample size justification is provided in terms of the precision (half length of the asymptotic confidence interval) of these estimates instead of a power calculation. This is in the same spirit with most randomized Phase II trials of chemotherapeutic agents.

Randomization will be performed using the Clinical Research Database (CRDB), the central computing facility for clinical research at [redacted] This system will generate a treatment assignment using the method of permuted blocks, at the time the patient is registered. Due to the nature of the treatment, the interventional radiologist will not be blinded but the radiologists assessing response to treatment will be blinded to treatment assignment.

One of the secondary objectives is to quantify the amount of necrosis in the two treatment arms and correlate this with conventional RECIST response as well as time to progression. With 50 patients in each arm, percent necrosis can be estimated to within +/- 14% of its standard deviation. Using 50% necrosis as threshold we plan to evaluate the degree of agreement between RECIST and necrosis response using Cohen's kappa and McNemar's test.

Another secondary objective is to establish whether the use of the doxorubicin eluting beads might prolong time to progression (TTP), or survival, and if initial imaging response to treatment, as manifested by necrosis, correlates with either endpoint. This aim relates to analysis with censored outcomes (time-to-progression and survival time). We will use the Kaplan-Meier method to estimate the survival probabilities and we will use the log-rank test to compare groups. With 50 patients in each arm, we will have approximately 80% power to detect a hazard ratio of 3, assuming a median follow-up of 3 years (i.e., about half of the patients expiring) and controlling the Type I error rate at 5%. A hazard ratio of 3 corresponds to improving the median survival by three-fold or improving two-year survival by approximately 20%. We do not anticipate a survival difference of this magnitude so this aim will primarily serve to obtain preliminary data to plan a future definitive trial.

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

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

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.

All participants must be registered through the Protocol Participant Registration (PPR) Office at [redacted] PPR is available Monday through Friday from 8:30am – 5:30pm at [redacted]. The PPR fax numbers are [redacted]. Registrations can be phoned in or faxed. The completed signature page of the written consent/verbal script and a completed Eligibility Checklist must be faxed to PPR.

15.2 Randomization

Randomization will be performed using the Clinical Research Database (CRDB), the central computing facility for clinical research at [redacted] This system will generate a treatment assignment using the method of permuted blocks, at the time the patient is registered. Due to the nature of the treatment, the interventional radiologist may not be blinded but the radiologists assessing response to treatment will be blinded to treatment assignment.

16.0 DATA MANAGEMENT ISSUES

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

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

16.1 Quality Assurance

Quarterly 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.

16.2 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at [redacted] were approved by the National Cancer Institute in March 2007. 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 [redacted] were established and are monitored by the Office of Clinical Research. The [redacted] Data and Safety Monitoring Plans can be found on the [redacted] Intranet at: [redacted]

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

clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Center's Research Council and Institutional Review Board.

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

17.0 PROTECTION OF HUMAN SUBJECTS

All the patients will sign informed consents and will have all their questions fully addressed before enrolling the study. By definition patients will not be surgical candidates, however alternative options for treatment such as conventional embolization outside the study, will be discussed thoroughly, as well as the risks and benefits of embolization. Although only the usual risks of hepatic artery embolization are expected in both groups, study patients will be carefully monitored for unusual or unanticipated events. Cardiac function will be evaluated in the LC Bead group to look for doxorubicin related cardiotoxicity. All the data will be confidential, maintained in a password protected electronic database and will comply with all HIPAA guidelines.

17.1 Privacy

[redacted] 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.

17.2 Serious Adverse Event (SAE) Reporting

An SAE is defined as a grade 3 or 4 adverse event using the Common Terminology Criteria for Adverse Events (CTCAE) 3.0. For the purpose of this study, an unanticipated device effect (UADE) will mean any event that is rarely observed after hepatic arterial embolization, rarely seen in patients hospitalized with diagnosis of cancer or patients receiving doxorubin.

Any SAE must be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at [redacted] containing the following information:

Fields Populated from the CRDB:

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

Data Needing to be Entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention

- Detailed text that includes the following information:
  - A explanation of how the AE was handled
  - A description of the subject's condition
  - Indication if the subject remains on the study
  - If an amendment will need to be made to the protocol and/or consent form

The PI's signature and the date it was signed are required on the completed report.
For IND/IDE protocols:
Only UADEs and patient deaths will be reported to the FDA as they occur. For these reports, The CRDB AE report should be completed as above and the FDA assigned IND/IDE number written at the top of the report. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office. **All other SAEs will be recorded as part of the study's data and will be included in the study's progress reports to the FDA.**

This study will collect all SAEs that occur from date of consent until the patient is off study. Since elevation in liver function tests is expected in the immediate post treatment period, no elevation in liver function tests occurring within 2 weeks following treatment will be reported as an SAE, although all elevations will be recorded and captured among the data for the study. The following is a list of the liver function tests: Alkaline Phosphatase, ALT, AST, and Bilirubin All other elevations to Grade 3 or 4, along with any hospitalization will be reported as SAEs and only reported to the FDA as they occur if considered an UADE.

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.

19.0 REFERENCE(S)

Bibliography

- Bosch FX, Ribes J and Boras J. Epidemiology of primary liver cancer. Semin Liver Dis 1999;19(3):271-285
- Breedis C, Young G. Blood supply of neoplasms of the liver. Am J Pathol 1954; 30:969-985.
- Bronwicki JP, Vetter D, Dumas F, et al. Transcatheter oily chemoembolization for hepatocellular carcinoma. Cancer 1994;74:16-24.
- Brown DB, Cardella JF, Sacks D, Goldberg SH, Gervais DA, Rajan D, Vedantham S, Miller DL, Brountzos EN, Grassi CJ, and Towbin RB. Quality improvement guidelines for transhepatic arterial chemoembolization, embolization and chemotherapeutic infusion for hepatic malignancy. J Vasc Interv Radiol 2006;17:225-232.
- Brown DB, Geschwind JFH, Soulen MC, Millward SF, and Sacks D. Society of Interventional Radiology position statement on chemoembolization of hepatic malignancies. J Vasc Interv Radiol 2006;17:217-223.
- Brown DB, Geschwind JH, Soulen MC, et al. Society of Interventional Radiology position statement on chemoembolization of hepatic malignancies. J Vasc Interv Radiol 2006;17:217-223.
- [redacted]
- Bruix J, Sherman M, Llovett JM, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. Journal of Hepatology 2001;35:421-430.
- Camma C, Schepis F, Orlando A, et al. Transarterial chemoembolization for unresectable hepatocellular carcinoma: Meta-analysis of randomized controlled trials. Radiology 2002;224:47-54.
- Carr BI, Iwatsuki S, Baron R, et al. Intrahepatic arterial cis-platinum and doxorubicin with or without lipiodol for advanced hepatocellular carcinoma (HCC): a prospective randomized study (abstr). 2003 Proc Am Soc Clin Oncol.
- Cha C, Fong Y, Jarnagin WR, et al. Predictors and patterns of recurrence after resection of hepatocellular cancer. J Am Coll Surg 2003;197:753-758.
- Chung JW, Park JH, Han JK, et al. Hepatocellular carcinoma and portal vein invasion: results of treatment with transcatheter oily chemoembolization. AJR 1995;165:315-321.

- Colombo M. Risk groups and preventive strategies. In: Berr F, Bruix J, Hauss J, Wands J, Wittekind Ch and Wands J, eds. Malignant liver tumors: Basic concepts and clinical management. Kluwer Academic Publishers BV and Falk Foundation. Dondrecht 2003, 67-74.
- Egawa H, Maki A, Mori K, et al. Effects of intra-arterial chemotherapy with a new lipophilic anticancer agent, Estradiol-Chlorambucil (KM2210), dissolved in lipiodol on experimental liver tumors in rats. J Surg Oncol 1990; 44:109-114
- Hong X, Choi H, Loyer EM, et al. Gastrointentinal stromal tumor: Role of CT in diagnosis & in response evaluation and surveillance after treatment with Imatinib. Radiographics 2006;26:481-495.
- Johnson PJ, Kalayci C,Dobbs, Raby N, Metivier EM, Summers L, Harper P, and Williams R. Pharmacokinetics and toxicity of intraarterial Adriamycin for hepatocellular carcinoma: Effect of coadministration of lipiodol. J of Hepatology 1991;13:120-127.
- Kim W, Clark TWI, Baum RA, et al. Risk factors for liver abscess formation after chemoembolization. J Vasc Interv Radiol 2001;12:965-968.
- Konno T. Targeting cancer chemotherapeutic agents by use of lipiodol contrast medium. Cancer 1990; 66:1897-1903.
- Llovet JM, Real MI, Montana X, et al. Arterial embolization or chemoembolization versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomized controlled trial. Lancet 2002; 359:1734-1739.
- Lo CM, Ngan H, Tso WK, et al. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. Hepatology 2002;35(5):1164-1171.
- Madden MV, Krige JEJ, Bailey S, et al. Randomised trial of targeted chemotherapy with lipiodol and 5-epidoxorubicin compared with symptomatic treatment for hepatoma. Gut 1993; 34:1598-1600
- Michels NA. Collateral arterial pathways to the liver after ligation of the hepatic artery and removal of the coeliac axis. Cancer 1953;6:708
- Nakao N, Kamino K, Miura K, et al. Recurrent hepatocellular carcinoma after partial hepatectomy: Value of treatment with transcatheter arterial chemoembolization. AJR 1991;156:1177-1179.
- NCI Cancer topics website, http://www.cancer.gov/cancertopics/pdq/treatment/adult-primary-liver/healthprofessional
- Ngan H, Lai C, Fan S, et al. Transcatheter arterial chemoembolization in inoperable hepatocellular carcinoma: Four-year follow up. JVIR 1996;7:419-425.
- Siskin GP, Dowling K, Virmani R, et al. Pathological evaluation of a spherical polyvinyl alcohol agent in a porcine renal model. J Vasc Interv Radiol 2003;14:89-98.
- Sugioka A, Tsuzuki T, and Kanai T. Postresection prognosis of patients with hepatocellular carcinoma. Surgery1993; 113(6):612-618.
- Takayasu K, Yasuo S, Muramatsu Y, et al. Hepatocellular carcinoma: Treatment with intraarterial iodized oil with and without chemotherapeutic agents. Radiology 1987; 162:345-351.
- Varela M, Real MI, Brunet M, et al. Chemoembolization of hepatocellular carcinoma with drug eluting beads reduces the systemic availability of doxorubicin. A

pharmacokinetic assessment. Presented at the American Association for the Study of Liver Diseases, San Francisco, November, 2005.

- [redacted]

20.0 APPENDICES

Appendix 1. ECOG Performance Status Criteria.

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

Appendix 2. Diagnostic Criteria for HCC
EASL Diagnostic Criteria for HCC
Cytology-histological criteria.

- Non-invasive criteria (restricted to cirrhotic patients)

1. Radiological criteria: Two coincident imaging techniques*
   - Focal lesion > 2cm with arterial hyper vascularization
2. Combined criteria: One imaging technique associated with AFP
   - Focal lesion > 2 cm with arterial hyper vascularization
   - AFP levels > 400 ng/mL

*Four techniques considered: US, Spiral CT, MRI and angiography

Appendix 3. Okuda Staging

Tumor size >50%	"+"
Ascites clinically detectable	"+"
Albumin <3 g/dl	"+"
Bilirubin > 3	"+"
Stage I	no positive indicators
Stage II	1-2 positive indicators
Stage III	3-4 positive indicators

7.0 RECRUITMENT PLAN