A Phase II Study of Individualized Stereotactic Body Radiation Therapy (SBRT) for Intrahepatic Cancer

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A. ABSTRACT

Approximately 25,000 patients die each year of unresectable intrahepatic cancer. Radiofrequency ablation (RFA) is an established treatment for small, unresectable HCC and for cancer metastatic to the liver. However, RFA is limited by tumor size (typically ≤4 cm), number of lesions, and the geometry of tumor(s) within the liver. Furthermore, patients with HCC who have undergone previous resections or ablations may have significant, but variable, underlying liver dysfunction, which makes it difficult to design a safe and effective therapy for all patients without a full understanding of the individual patient's tolerance to treatment. Likewise, although trans-arterial chemoembolization (TACE) has been shown to be superior over best supportive care for patients with HCC and good performance status (Child A) and tumors < 6 cm, essentially all patients ultimately recur. TACE has limited efficacy for metastatic disease to the liver (other than the relatively rare neuroendocrine tumor). Thus, improved treatment approaches are needed for patients with intrahepatic cancer who cannot undergo resection or transplant.

Recent advances in radiation treatment delivery have allowed for high-precision, high-dose stereotactic radiation techniques once reserved for intracranial malignancies to be applied to extracranial sites. Preliminary results suggest excellent local control rates (above 90%) of intrahepatic malignancies treated with stereotactic body radiation therapy (SBRT)(1-3) using 60 Gy in three fractions of 20 Gy each. These studies have been carried out on previously untreated patients with metastatic tumors less than 6 cm in size.

We hypothesize that it will be possible to extend SBRT to "non-standard" patients, i.e., those who have had previous liver treatment (such as resection, TACE, RFA, or SBRT) or who have primary HCC. These patients are at risk for toxicity from the established approach of 60 Gy in three fractions. Although we have more experience with liver radiation than any institution outside of China, we still have only a limited ability to predict who will develop toxicity based solely on pretreatment characteristics, particularly for patients with HCC and cirrhosis. Therefore, we propose a new approach to SBRT in which therapy is divided into two parts/sections, with a four week assessment period between parts. **This will permit us to increase the safety and efficacy of therapy by assessing the effect of the first part of therapy for the individual patient before administering the final portion of treatment.** Our primary aim is to characterize the safety of the dose we deliver using this approach and, secondarily, to estimate the response rate using this approach. Our goal is to achieve the same or superior control rates with acceptable toxicity in this group of patients that has been achieved for previously untreated patients using RFA, TACE, or primary SBRT.

B. SPECIFIC AIMS

Hypothesis: SBRT can be delivered safely in "non-standard" patients (previous liver treatment and/or primary HCC) with acceptable toxicity by dividing therapy into two parts so that the individual's response to treatment can be assessed.

1.0 Primary aim:

Characterize, in patients who have had previous liver treatment or who have primary HCC, the safety and efficacy of *individualized SBRT*, which consists of three fractions of SBRT, a one month break, an assessment of liver function (using Indocyanine Green (IC-Green)), and two additional fractions adjusted to account for the patient's tolerance to the first 3 fractions.

2.0 Secondary aims:

2.1 Collect data on plasma biomarkers of treatment efficacy, toxicity, and liver function to plan further enhancements to individualized SBRT.

2.2 Collect data on changes in the clinical measures of severity of liver dysfunction, including the Model for End-Stage Liver Disease (MELD) and Child-Turcotte-Pugh (CTP)

C. BACKGROUND AND SIGNIFICANCE
1.0 Epidemiology and scope
Intrahepatic malignancies, primary or metastatic, are an increasingly common problem in clinical practice. The American Cancer Society estimates that 148,810 people were diagnosed with colorectal cancer (CRC) in the US in 2008(4). Approximately half of these patients develop liver metastases at some point in the course of their disease. Liver metastases from primary tumors throughout the remainder of the GI tract and other sites (breast, lung) are also a significant cause of cancer related mortality.

Worldwide, primary liver cancer is a major health problem with more than 500,000 new cases diagnosed yearly. It is the fifth most common neoplasm, and the third most common cause of cancer-related death.[5] In some areas of Asia, HCC is the most common cause of death due to cancer. The American Cancer Society estimates that 30,890 people were diagnosed with HCC, gallbladder cancer or cholangiocarcinoma in the US in 2008.(4) The incidence has been increasing in Europe(6) and in the US(4, 7) and it is estimated that its incidence in the US will equal that currently reported in Japan within two decades.[8] An increase in the incidence of intrahepatic cholangiocarcinoma has also been reported.[9] Primary liver cancer is one of 4 cancer sites that has increased death rates between 1990 and 2004, and is the only cancer that has shown more than 10% increase (41% and 28% rate increases for males and females, respectively).[4] This disease is clearly a growing problem.

2.0 Current Therapies for unresectable intrahepatic cancer

Complete resection is the most effective therapy for HCC and bile-duct cancer. More recently, a similar role has been established for surgery in the management of CRC metastases. Surgical series have reported 25-40% 5-year survival following hepatic resection of solitary or a limited number of multiple metastases in these patients.(10-12) Unfortunately, curative surgery cannot be extended to most patients. Many patients are inoperable due to co-morbidity, and others present with disease extent that requires resections that would not leave sufficient residual functional liver parenchyma.   For instance, Abdalla et al. reported the M.D. Anderson experience with hepatic resection and radiofrequency ablation (RFA).(13) Of 418 patients who were deemed resectable and were explored, only 190 patients (45%) underwent complete resection. 38% of patients underwent either RFA alone (or RFA as part of a resection): 44%, recurred in the liver. With respect to metastases to the liver, a recent Cochrane review summarized its recommendations for the treatment by stating, "There is currently insufficient evidence to support a single approach, either surgical or non-surgical, for the management of colorectal liver metastases".(14)

Three major therapies have been used for these unresectable patients.

2.1 Radiofrequency Ablation (RFA)
In a prospective trial by Lencioni and colleagues(15), 10% of 206 patients initially evaluated for RFA had contraindications based on tumor location. After these patients were excluded, 20% had an inadequate response to RFA (viable tumor remaining after 1 month). Of the patients who had an inadequate response to RFA, only approximately half of the patients were controlled by a second RFA. Finally, 10% of patients who were thought to be controlled recurred locally within 3 years. In a systematic review of the use of RFA for HCC, local failures over a 3 year period tend to average from 10 - 29%, and failure elsewhere in the liver approximately 50%(16)**.** In the

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case of metastases from colorectal cancer, recurrences after RFA range from 2-39%, with other liver failures from 14-58%[16]. In all series, local failure increases with tumor size > 3-4 cm. Our experience at the University of Michigan suggests a similar or somewhat higher local failure rate (unpublished data). In this proposal, we anticipate focusing on patients who have recurred after RFA or have tumors that are not amenable to RFA, such as those abutting vasculature, lung, or bile duct.

2.2 Transcatheter Arterial ChemoEmbolization (TACE)

Although some trials and an initial randomized trial showed no difference between chemoembolization and best supportive care(17, 18), more recent studies have begun to suggest that chemoembolization can improve survival of a subset of patients with hepatocellular carcinoma(19, 20). A systematic review of all modern randomized trials suggests that TACE modestly prolongs survival (from approximately 16 months to 20 months), compared to best supportive case, for patients with tumors < 6 cm who have excellent performance status and do not have portal vein thrombosis(21, 22). However, TACE is not a curative therapy and tumors typically recur even after multiple TACE administrations, suggesting that additional therapies are required. Historical series demonstrate that chemoembolization is of minimal efficacy for colorectal cancer metastatic to the liver[23].

2.3 Radiation Therapy

In our previous series of clinical trials of focal liver radiation and concurrent HA-floxuridine as described below in Preliminary Studies, patients were treated with 3-D conformal radiotherapy (3-D CRT). In our preliminary analysis of this trial(24, 25), we found improved survival based on our predetermined phase II endpoint. More importantly, dose delivered was the most important predictive factor for survival. **Indeed, approximately 20% of patients receiving >75 Gy are alive 4 years after treatment; historically that number would be very close to 0%.** These data are proof of principle that focused radiation can control intrahepatic cancer, and that we are experienced in these techniques.(26)

In the current study, we propose to use extracranial stereotactic body radiation therapy (SBRT). SBRT has been shown by multiple groups to be safe and effective in the treatment of colorectal metastases within the liver(1-3, 27-30). One study by Herfarth et al. demonstrated 81% local control rates of treated liver tumors at 18 months after single fraction SBRT(2). Another phase I/II trial of SBRT for liver metastases has demonstrated the safety of 60 Gy in 3 fractions, with a 93% local control rate at 18 months(1, 3). A phase II trial of SBRT for colorectal liver metastases demonstrated 86% local tumor control at 2 years(28). Additional studies confirm these positive results both for colorectal cancer metastatic to the liver and for primary liver tumors(1, 31, 32). A small study has shown that for patients with Childs A and Childs B liver status, 16 Gy x 3 fractions and 8 Gy x 5 fractions, respectively, can be safely delivered for tumors less than 6 cm in size(33).

3.0 Radiation Induced Liver Disease (RILD)

Attempts to deliver high dose radiotherapy to the liver have been limited by the development of RILD(34, 35). This toxicity, initially referred to as radiation hepatitis, was first reported in the mid 1960's(36). Symptoms generally occur 1-2 months following completion of radiotherapy and include tender hepatomegaly and weight gain secondary to ascites. Laboratory findings include elevated blood levels of alkaline phosphatase, transaminases and bilirubin. The clinical outcome ranges from mild reversible damage to rare fatality.(37-39) We were the first group to develop a quantitative model to estimate the risk of RILD based on dose to the liver, and we recently updated our parameter estimates by analyzing the results of over 200 patients treated at the University of Michigan with focal radiation(24). This refined model will be used to quantify the

risk of RILD and will guide the radiation dose selection for individuals participating in this trial. A specific toxicity criteria scale for RILD has been published following a consensus conference on late effects in normal tissue following radiation therapy and will be utilized in this study.(39)

Although we developed the most accurate population-based model for predicting RILD for patients receiving fractionated radiation therapy, we would like to develop an approach that permits us to individualize therapy for patients undergoing SBRT. A major impediment to this goal is that RILD takes time to develop. Patients typically do not show an evidence of toxicity until a minimum of 2 weeks after the completion of treatment, and, more typically, at 4-6 weeks after treatment is complete. Obviously, it is too late to adjust therapy a month after therapy is completed. **We hypothesize that the insertion of a 4 week period after 3 of the 5 planned fractions, and the use of clinical laboratory and imaging criteria to judge toxicity in the individual patient when it is still minor and reversible, could represent a major advance in increasing the safety of SBRT, particularly for patients with compromised liver function.**

We have made substantial progress toward developing three novel techniques to individualize the functional assessment of the liver during therapy:

3.1 <u>Extraction of indocyanine green</u>: Following intravenous injection, indocyanine green is rapidly bound to plasma protein, of which albumin is the principle carrier (95%). Indocyanine green is taken up from the plasma almost exclusively by the hepatic parenchymal cells and is secreted entirely into the bile. It undergoes no significant extrahepatic or enterohepatic circulation. Simultaneous arterial and venous blood estimations have shown negligible renal, peripheral, lung or cerebrospinal fluid uptake of the dye. Therefore, the serum clearance rate (determined from serial serum concentration measurements at various times after intravenous injection) can serve as a useful index of liver function, and has been used in thousands of patients to plan the extent of safe surgical resection. **Our preliminary studies of patients undergoing fractionated radiation show that IC-Green is an accurate measure of radiation-induced liver dysfunction and, as a continuous measure, could be used to adjust radiation dose.**
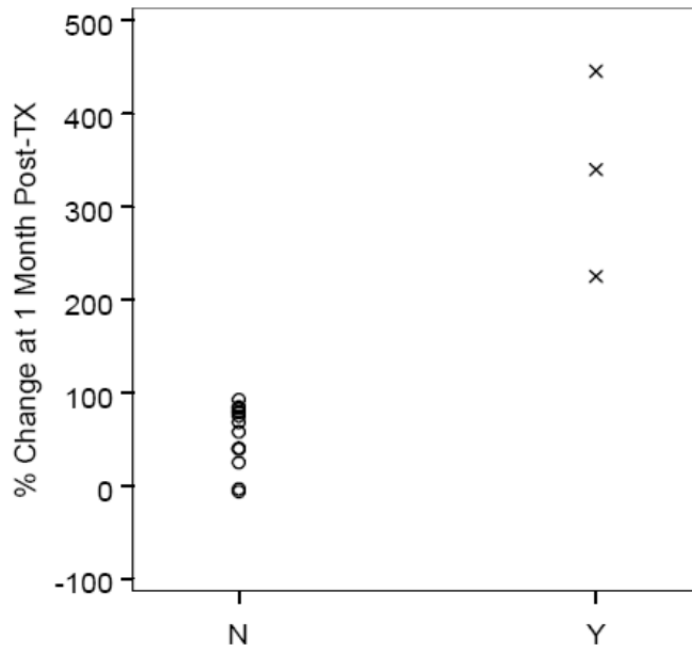
Figure 1. IC-Green uptake (measured in minutes of half-life and, here, expressed as percent change from baseline) for patients without evident of RILD 21 months after treatment (N) vs. those with RILD (Y). There is a significant increase in the time to take up IC-Green in patients with RILD (Pan et al, unpublished data).

3.2 Assessment of plasma cytokines: RILD is caused by veno-occlusive disease, which is likely related to endothelial cell apoptosis as an initiating lesion. Radiation also induces various proinflammatory cytokines, including tumor necrosis factor-a (TNF-a) and transforming growth factor-beta 1(40), and hepatic microvascular pathogenesis that lead to apoptosis in the liver(41). Elevation of TGF-beta has been associated with RILD in woman undergoing bone marrow transplantation for breast cancer(42). In addition, an increased TNF-a production has been associated with the progression of hepatic veno-occlusive diseases in stem cell transplant patients(43), suggesting the potential role of cytokines in radiation-induced liver apoptosis. Our preclinical studies demonstrate the TNF alpha may play a key role in radiation injury of the liver(44). We have experience measuring cytokines in patient plasma(45). **We propose to measure these cytokines in plasma, and retrospectively assess their potential contribution to individualize our assessment of liver injury that could be used to adjust liver dose**.

3.3 Assessment of clinical measures of severity of liver disease: The Model for End-Stage Liver Disease(46) (MELD) and the Child-Turcotte-Pugh (CTP) classification are models used for the clinical assessment of patients with liver dysfunction (Appendix B). Patients with CTP classification Grade A versus Grade B appear to have increased sensitivity to radiation. Additionally, in a study of patients treated with SBRT for HCC or intrahepatic cholangiocarcinoma, 17% experienced progression from CTP Grade A to Grade B within 3 months after RT(31, 32), suggesting that CTP may be a useful assessment of worsening liver function. MELD may perform even better than CTP at evaluating liver function(47). **We propose to record these clinical measures (MELD and CTP), and assess their potential contribution to individualize our assessment of liver injury that could be used to adjust liver dose**.

In summary, we feel we are in a unique position to determine a patient's liver function approximately one month after the administration of three of five planned SBRT treatments. If the patient's liver function remains relatively stable at this point, it may be possible in future trials to administer substantially more radiation safely. If there has been some compromise of liver function, especially if this reflects a change in liver function, a more modest dose of radiation may still be safely given. If there has been a significant decrease, treatment can be stopped, and the risk of major toxicity avoided. **We feel this is a unique and powerful paradigm to individualize radiation therapy now in patients undergoing SBRT using IC-Green, and that a result of this study will be to develop the assessment of TNF-alpha and TGF beta, and clinical assessment to further improve our ability to individualize therapy.**

D. RESEARCH DESIGN AND METHODS

1.0 Eligibility

1.1 Inclusion Criteria
1.1.1   Patients with hepatocellular carcinoma or hepatic metastases from metastatic carcinomas are eligible for this trial. Hepatocellular carcinoma is defined as having at least one of the following:

a. Biopsy proven hepatocellular carcinoma (HCC); or

b. A discrete hepatic tumor(s) as defined by the Barcelona[48] criteria – for cirrhotic patients, >1cm with arterial hypervascularity and venous or delayed phase washout on CT or MRI

- 1.1.2 For patients with hepatic metastasis, patients with disease outside the liver are eligible for this study if the extrahepatic cancer is small volume (<5cm in diameter) or stable.
- 1.1.3 Patient is <u>not</u> eligible for curative liver resection.
- 1.1.4 Patients must have recovered from the acute effects of any prior surgery or radiation therapy.
- 1.1.5 Patients must have recovered from the acute effects of RFA or TACE, and a minimum of 6 weeks have passed since the last procedure.
- 1.1.6 Patients must have a Zubrod performance status of ≤2.
- 1.1.7 Patients must have a life expectancy of at least 12 weeks.
- 1.1.8 Patients must be 18 years of age or older. Adult patients of all ages, both sexes and all races will be included in this study.
- 1.1.9 Female patients within reproductive years may not be, nor become, pregnant during participation in this study. Both male and female patients within reproductive years must agree to use an effective contraceptive method during treatment. Women of child-bearing age will be required to undergo a urine or serum pregnancy test to ensure they are not pregnant.
- 1.1.10 Patients must have adequate organ function within 6 weeks of enrollment.
  - Bone marrow: Platelets ≥30,000/mm^3
  - Renal: BUN ≤40 mg/dl; creatinine ≤2.0 mg/dl
  - Hepatic: INR ≤1.3 or correctable by Vitamin K, unless anticoagulated for another medical reason
  - Bilirubin < 3 mg/dl (in the absence of obstruction or pre-existing disease of the biliary tract, e.g. primary sclerosing cholangitis)
- 1.1.11 Patients must sign an informed consent form approved for this purpose by the Institutional Review Board (IRB) of the University of Michigan Medical Center indicating that they are aware of the investigational aspects of the treatment and the potential risks.

1.2 Exclusion Criteria

- 1.2.1 Patients in a "special category" designated the Public Health Service, including patients younger than 18, pregnant women, and prisoners.
- 1.2.2 Uncontrolled ascites clinically evident on physical exam
- 1.2.3 Patients with metastatic cancer with normal liver function who have not undergone previous liver directed therapy and a single tumor < 6 cm in size.
- 1.2.4 Known allergy to IC-Green.
- 1.2.5 Known allergy to intravenous iodinated contrast agents

2.0 PRETREATMENT EVALUATION

2.1 Patients will undergo evaluation, including a complete history and physical examination, baseline assessments of organ function and documentation of measurable disease (CT or MRI) parameters (if measurable), signs and symptoms, weight, and performance status.

2.2 Laboratory evaluation per Study Calendar

3. 0 TREATMENT PLAN

3.1   Patients will be registered with the University of Michigan Comprehensive Cancer Center Clinical Trials Office prior to starting treatment.

3.2   Placement of Fiducial Markers
    3.2.1   If deemed clinically necessary, fiducial markers may be placed percutaneously within close proximity of the target tumor. Placement of markers is considered standard of care. These will be used for target localization.

3.3   Stereotactic Body Radiotherapy
    3.3.1   Three dimensional treatment planning will be used for all patients, based on a simulation CT scan.
    3.3.2   Energy: Treatment will be delivered with 6 - 25 MV photons and/or high energy electrons.
    3.3.3   Localization, simulation, and immobilization: All patients must undergo CT simulation prior to treatment, with IV and oral contrast if clinically appropriate. Patients will be immobilized with a body cast. Liver motion will be minimized with the use of active breathing control (ABC) or an equivalent technology when possible. Prior to each SBRT treatment, the liver tumor will be imaged and localized using either an on-board imaging orthogonal pair, using implanted fiducial markers, or a cone-beam CT scan.
    3.3.4   Radiation target volumes:
        The GTV will be defined using the planning CT scan or MRI.
        The CTV will be defined as the GTV.
        The PTV around the GTV/CTV will be determined based upon the immobilization device(s) used. If ABC is not used, then an approximately 0.5 cm circumferential margin for setup uncertainty, in addition to a breathing margin, will make up the PTV margin. The breathing PTV margin will be approximately 0.3 cm in the superior direction, anterior and posterior directions. The inferior margin will be the magnitude of the excursion of the diaphragm visualized during breathing, using fluoroscopy. If ABC is used (with daily localization), then the total PTV margin will be approximately 0.5 cm in superior, inferior, anterior and posterior directions and lateral directions.

3.4   SBRT Planning Guidelines - Radiation Doses:
    3.4.1   PTV Target Doses
        3.4.1.1 Doses will be prescribed to a peripheral covering isodose covering the PTV. Prescription isodose surface covers 99.5% of PTV. Aim to cover the PTV with an approximately 80% isodose surface (75-85%). Assuming dose is then normalized to this isodose at 100%, the minimum PTV dose will be 90%. Any dose > 110% must be within the PTV (except for adjacent tumors, in which the maximum dose outside the PTV must be < 115%).
        3.4.1.2 Variations
            3.4.1.2.1   Minor variation is defined as minimum PTV dose falling between 85 and 90% (of the required 100% isodose prescription).
            3.4.1.2.2   Major variation (unacceptable) is defined as minimum PTV dose < 85 % (for the required 100% isodose prescription).
        3.4.1.3 Maximum doses are defined at 1 cc of volume.
        3.4.1.4 Minimum dose to the PTV is defined as minimum dose to 99.0% of the PTV.

3.4.2   Critical Normal Tissue Constraints

Normal Tissue Organ	Parameter	Limit (Gy/per fraction) for 5 fractions	Notes
Normal Liver		ALARA	NTCP to be calculated
Spinal Cord	Max	5.0	
Stomach		5.5	
Duodenum		6.0	
Lung		ALARA	
Esophagus		5.5	
Kidneys	<40% of both kidneys together, and <67% of one kidney	3.0	

3.4.3   Radiation Schedule: SBRT will be delivered in twice weekly fractions, with at a minimum of one day between any two treatments.

    3.4.3.1 Patient will receive 3 initial SBRT fractions, with the dose chosen as a function of NTCP.

    3.4.3.2 Liver function will be assessed approximately 4 weeks after completion of the initial 3 SBRT fractions

    3.4.3.3 An additional 2 SBRT fractions may be given after this assessment (refer to section 5.0).   Patients who have experienced Unacceptable Toxicity (as defined in section 8.2) at the reassessment point will receive no additional therapy at that time. Patients with normal liver function (estimated by IC-Green) will receive the full initial dose for the last 2 fractions. Those with intermediate liver function will receive an intermediate dose as defined by sections 5.0 and 10.0 of the protocol.

    3.4.3.4 If patients cannot receive 2 SBRT fractions because of issues stated in 3.4.3.3 (except for the patients that have UT from GI bleeding) they will be assessed one month later. If their liver status has improved, they can then undergo protocol therapy. If they have not improved they will be removed from protocol treatment.

3.4.4   Radiation Dose

Patients will have their initial dose prescribed according to our currently established NTCP model and SBRT practice.

    3.4.4.1 Three dimensional treatment planning will be used for all patients.

    3.4.4.2 Volumes of tumor and normal liver will be determined, and DVH based treatment planning will be carried out, targeted to the tumor only. We will bio-correct the normal liver dose distributions to 2 Gy equivalent fractions and then treat to an iso-NTCP level maximum of 15% computed with our models m=0.12; n=0.97 (a=1/n=1.03); $TD50_{metastases}$ = 40.7 Gy; $TD50_{primary}$ = 35.4 Gy). Typical radiation doses have been approximately 50 Gy in 5 fractions, 10 Gy/fraction; we expect that patients treated on this protocol will be treated with fractions sizes ranging from approximately 4.5 Gy/fraction to 15 Gy/fraction.

3.5   STUDY CALENDAR

	Pre-Rx Eval	Initial **3** SBRT	Evaluation Period Approximately 4-6 Weeks after [2]	Final **2** SBRT fractions[3]
		Active Treatment – SBRT		
History, Physical Exam, Performance Status	X	PE once		PE once
Weight	X	Once	X	Once
CBC/Platelets	X		X	
AST, ALT, Alk Phos, Bilirubin	X		X[4,5]	
BUN/Creatinine	X		X	
INR	X		X	
AFP (for HCC)	X		X	
CEA (for MCRC)	X		X	
Toxicity Notation	X	Once	X	Once
MELD/CTP assessment	X		X	
IC-Green	X[1,6]		X	
Simulation	X			
Abdominal imaging with either MRI or CT [7]	X			
Chest Imaging with either chest x-ray or CT[8]	X			
Plasma and serum for TGF-beta and TNF-alpha	X		X	
Treatment: SBRT		Twice Weekly		Twice Weekly

[1] Within 4 weeks prior to initiation of SBRT

[2] If patient has adequate liver function (ICG R15<0.44), proceed with final 2 SBRT fractions. If patient has poor liver status (per section 5), do not proceed with treatment. For those patients, repeat IC-Green in approximately 4 weeks. If liver function has improved, repeat evaluation procedures listed and proceed with protocol calendar. If liver status is still poor, they will not receive fraction 4 & 5, but will continue to be seen per follow up calendar.

[3] If the treatment is deemed complete after the initial 3 SBRT treatments (per section 10.1), then these items are not required.

[4] Repeat grade 4 LFTs within 5-10 days following 1st abnormal lab value.

[5] Patients with hepatic toxicity (LFTs $\geq$ 5-20x Baseline value) will be evaluated with radiologic imaging.

[6] If the patient was previously enrolled on any trial utilizing IC-Green (including this one), then an evaluation of IC-Green retention performed for the other trial may be used as the pre-treatment assessment for this enrollment, as long as it is within 4 weeks prior to initiation of this new course of SBRT.

[7] Abdominal imaging to be done within 2 months of enrollment. [8] Chest imaging to be done within 1 year of enrollment.

	Post-Treatment Evaluation Period Approximately 4-6 Weeks[1]	Follow-up After Treatment Concluded		
		3 months (+ 2 weeks)[2,4]	6 months (+/- 2 weeks)[2,]	Q 6 months to 24 months (+/- 4 weeks)[2,"]
Interval History, PE, Weight	X	X	X	X
Performance Status	X	X	X	X
CBC/Platelets	X	X	X	X
AST, ALT, Alk Phos, Bilirubin	X	X	X	X
BUN/Creatinine	X	X	X	X
INR	X	X	X	X
AFP (for HCC)	X	X	X	X
CEA (for MCRC)	X	X	X	X
Toxicity Notation	X	X	X	X
MELD/CTP assessment	X	X	X	X
IC-Green	X	X		
CT or MRI of the abdomen		X[3]	X	X

[1]Approximately 4-6 weeks after 5th SBRT fraction (or after 3rd fraction if 4th & 5th fractions will be not given).

[2] Patients determined to have any progression will be followed for survival only, without required followup visits, imaging, or laboratory assessments.

[3]If ascites develops at any time within 3 months following treatment, an abdominal CT and paracentesis with pathologic evaluation of the ascetic fluid is recommended.

4.0 [4]If the patient enrolls on a subsequent radiation treatment protocol, only the followup calendar for the subsequent study will be followed in order to avoid redundancy. DRUG INFORMATION: Indocyanine Green (IC-GREEN™)

4.1 Indocyanine Green has FDA approval for determining hepatic function and liver blood flow. Please refer to the Package Insert for completed details

4.2 Although there are currently no routine methods of estimating local function within the liver, there are established methods of measuring whole liver function. Probably the best-studied method of measuring liver function is through assessing the extraction of indocyanine green (IC-Green)(49-52). After i.v. administration, IC-Green dye is taken up from the plasma almost exclusively by the hepatic parenchymal cells and is secreted into the bile. It undergoes no significant extrahepatic or enterohepatic circulation. Simultaneous arterial and venous blood estimations have shown negligible renal, peripheral, lung, or cerebrospinal uptake of the dye. Therefore, the serum clearance rate of IC-Green serves as a reliable index of liver function. The clinical value of the IC-Green test has been demonstrated in the evaluation of donor organs for transplantation and the outcome of liver resections. Also, the IC-Green test was reported to be an

early indicator of hepatic dysfunction after injury, whereby prolonged IC-Green clearance preceded an increase in serum bilirubin levels(49).

4.3 Formulation & Supply

    4.3.1   IC-GREEN™ is a sterile, lyophilized green powder containing 25 mg of indocyanine green with no more than 5% sodium iodide. It is packaged with Aqueous Solvent consisting of Sterile Water for Injection used to dissolve the indocyanine green. IC‑GREEN™ is to be administered intravenously. Indocyanine green is a water soluble, tricarbocyanine dye with a peak spectral absorption at 800 nm. The chemical name for Indocyanine Green is 1 H-Benz[e]indolium,2-[7-[1,3-dihydro-1,1-dimethyl-3-(4-sulfobutyl)-2H-benz[e]indol-2-ylidene]-1,3,5-heptatrienyl]-1,1-dimethyl-3-(4-sulfobutyl)-hydroxide, inner salt, sodium salt.

$$C_{43}H_{47}N_2NaO_6S_2 \qquad \text{Molecular Weight 774.96}$$
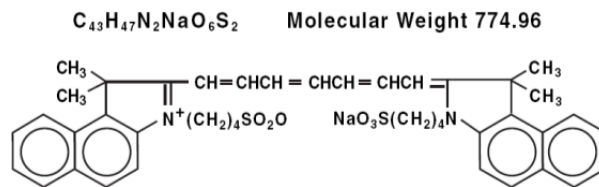
    4.3.2   IC-GREEN™ has a pH of approximately 6.5 when reconstituted. Each vial of IC-GREEN™ contains 25 mg of indocyanine green as a sterile lyophilized powder.

4.4 Preparation and Administration

    4.4.1   Under sterile conditions, the IC-GREEN™ powder should be dissolved with the Aqueous Solvent provided for this product, and the solution used within 6 hours after it is prepared. If a precipitate is present, discard the solution. The amount of solvent to be used can be calculated from the dosage form which follows. It is recommended that the syringe used for injection of the dye be rinsed with this diluent. Isotonic saline should be used to flush the residual dye from the cardiac catheter into the circulation so as to avoid hemolysis. With the exception of the rinsing of the dye injection syringe, saline is used in all other parts of the catheterization procedure.

    4.4.2   The IC-GREEN test will be administered on the following schedule:

        4.4.2.1 Within four weeks prior to start of radiotherapy, although if a patient undergoes an IC-GREEN test unrelated to current enrollment in this study, it may be used as the pre-treatment test up to 4 weeks prior to start of radiotherapy, and a new IC-GREEN test is not required.

        4.4.2.2 At approximately 4-6 weeks after completion of the first 3 SBRT treatments.

        4.4.2.3 Approximately one month after radiation therapy is completed. If treatment is deemed complete after 3 SBRT treatments per section 10.1, then this IC-GREEN test is not required.

        4.4.2.4 Approximately two months after radiation therapy is completed.

    4.4.3   For patients that are enrolled in this study multiple times, we will use the 2-month IC green results as the baseline draws for the next course of treatment, whenever possible.

    4.4.4   The patient will be studied in a fasting state. Patients should not eat 4 hours prior to the test. Coffee and/or water are allowed up to approximately two hours before the test. Patients should take all of their medications as usual. The patient will be weighed and the dosage calculated on the basis of a recommended 0.5 mg/kg of body weight. The dose given must be within 20% of the total recommended dose. There will be approximately a 6 cc blood draw followed by rapid IV push of the IC-GREEN at time 0. Following I.V. administration via catheter, serum samples will be collected at approximately 5, 10, 15 and 20 minutes after injecting the dye. Each blood draw will be approximately 6 cc. The patient will have two different catheters

one will be used for the IC-GREEN infusion, and the other catheter will be used to draw the samples. The catheter will be flushed with saline following each blood draw.

4.4.5 The IC-GREEN test will be administered by trained personnel in the Michigan Clinical Research Unit "MCRU". The dye is commercially available and will be ordered from UMHS pharmacy (formulary). Radiographic contrast agents are administered routinely in the department and it is fully equipped to treat an anaphylactic reaction, should one occur.

4.5 Contraindications
4.5.1 IC-GREEN™ contains sodium iodide and should be used with caution in patients who have a history of allergy to iodides.

4.6 Warnings
4.6.1 Anaphylactic deaths have been reported following IC-GREEN™ administration during cardiac catheterization.

4.7 Drug Interactions
4.7.1 Heparin preparations containing sodium bisulfite reduce the absorption peak of IC-GREEN™ in blood and, therefore, should not be used as an anticoagulant for the collection of samples for analysis.

4.8 Adverse Effects
4.8.1 Anaphylactic or urticarial reactions have been reported in patients with or without history of allergy to iodides. If such reactions occur, treatment with the appropriate agents, e.g., epinephrine, antihistamines, and corticosteroids should be administered.

5.0 TREATMENT MODIFICATIONS

5.1 <u>Hepatic Toxicity:</u> Patients will be evaluated for symptoms and signs of RILD or other toxicity.

5.1.1 IC-Green: Expressed as a prolongation of the clearance time compared to pretreatment. Refer to Section 10.1 for details on its use for treatment modifications.

5.1.2 Plasma cytokines will be measured, but will not affect dosing.

5.1.3 It is expected that a proportion of patients will have transient elevation of liver enzymes during treatment. Repeat of all Grade 4 LFTs is required within 5-10 days following the first abnormal lab value to determine if the Grade 4 levels are transient (defined here as <10 days) or persistent. Patients exhibiting hepatic toxicity $\geq$ 5-20x baseline LFT's will be evaluated with radiological imaging procedures to assess whether change in LFTs are due to tumor progression or treatment toxicity. Patients whose progressive liver function abnormalities while under treatment are deemed due to tumor progression will stop all protocol treatment and will be managed and followed as described in Section 9.0 (Study Monitoring). Patients with treatment induced hepatic toxicity of greater than 20x baseline elevation will not receive further protocol treatment unless and until liver function tests have returned to less than 5x patients baseline value. Patients will be evaluated for symptoms and signs of RILD or other toxicity.

5.2 <u>Other Toxicity</u>: The occurrence of treatment-related Grade 4 adverse events in any organ system will prompt discontinuance of protocol therapy while appropriate physical examination, laboratory, and imaging assessments are undertaken. Protocol treatment will not be resumed in the absence of recovery from adverse events of this magnitude. Once recovery to grade 2 has occurred, treatment may continue at the discretion of the treating physician.

5.3 Exceptions that will not be reported to IRB or require discontinuation of therapy: Grade 3 or 4 asymptomatic albumin levels or lymphopenia. Transient (< 48 hours) asymptomatic grade 3 fasting glucose levels in type II diabetics.

6.0 SBRT DOSE ADJUSTMENT

Each patient will be treated in two parts. In the first part, the dose will be determined using the normal tissue complication probability (NTCP) model described in Section D.3.4.2., Radiation Dose. The patient's baseline and interstage liver function assessments will be used to individualize their second part of treatment to ensure that the patient's ICG retention proportion at 15 minutes does not exceed 0.44(53). Accumulated data from the trial will be used to update the individualization model, as described in Section D. 9.0, Statistical Considerations. The steps for dose adjustment for each patient are:

6.1 Assess the patient's baseline ICG 15-minute retention proportion prior to SBRT, and plan five equal-sized fractions of SBRT as described in Section D.3.4.2. to a fixed probability of RILD.

6.2 Administer three fractions of SBRT at the dose described in Section 3.4.

6.3 4-6 weeks after SBRT Part 1, again assess the patient's ICG 15-minute retention proportion.

6.4 Adjust the fractional dose for SBRT Part 2 to ensure, based on the patient's ICG response to SBRT Part 1 and ICG data accrued from the previous patients in the trial, that the probability is at least 0.90 that the patient's ICG 15 minute retention proportion will not increase above -0.44. This may result in a decrease in dose per fraction in Part 2 compared to Part 1, but will never result in an increase. Details of this calculation are presented in Section 10 Statistical Considerations.

6.5 Complete SBRT Part 2 (if additional radiation allowed per Section 10.1), and, 4-6 weeks after the final two fractions, measure the patient's ICG 15-minute retention proportion for use in adjusting the dose of subsequent patients. If only 3 treatments are allowed per section 10.1, then this ICG-15 is not required.

7.0 RESPONSE CRITERIA

7.1 Local intrahepatic tumor: The status of each treated tumor will be assessed by MRI or CT scan and classified as progression if there is tumor growth (excluding growth due to biloma or abscess formation), residual or new enhancement of ablated tumor (excluding benign peri-ablational enhancement), or contiguous viable tumor. Each treated intrahepatic lesion will be evaluated as

7.2.1 Complete Response: disappearance of the lesion

7.2.2 Progression: $\geq 20\%$ growth of lesion from smallest lesion measurement;

7.2.3 Partial Response: $\geq 30\%$ decrease in lesion from baseline measurement or

7.2.4 Stable: not meeting progression or response

7.2 Remote intrahepatic tumor: The presence of new tumor remote from previously treated tumor sites will be interpreted based on the standard CT criteria for the diagnosis of metastatic disease or HCC and recorded.

7.3 Extrahepatic tumor: All of the CT scans will be evaluated for the presence of extrahepatic tumor.

7.4 Relapse: The appearance of new lesions, or the reappearance of old lesions in patients who previously had obtained a complete response. In the presence of a partial response, an increase of

20% increase in a lesion compared to its measurement taken at the time of maximum tumor regression.

7.5   Local control: For this study, local control is defined as the lack of progressive local disease following CR or PR, or lack of progressive local disease in patients with non-evaluable disease, who have no progressive elevation in serum tumor markers. Progression or development of new tumors within the liver but outside of the radiation field would not constitute a local control failure.

7.6   Disease-Specific Mortality: For this study, disease-specific mortality will be defined as death due to the patient's disease, or death due to treatment for the patient's disease. Time zero will be defined the day of the last treatment fraction.

8.0 TOXICITY CONSIDERATIONS

8.1   The criteria used for the grading of toxicities in this study are based upon and modified from those listed in the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0, May 2009

8.2   Unacceptable Toxicities
Unacceptable toxicities are the following:
- Grade ≥4 hepatic toxicity for changes in AST, ALT, alkaline phosphatase, or platelet counts not attributable to disease progression. Transient grade 4 (less than 10 days) hepatic toxicities are acceptable, as detailed in Section 5.
- Grade ≥4 Upper GI bleeding (attributed to treatment, and not attributable to disease progression)

Items that will not be considered unacceptable toxicities:
- Any complications or death due to disease progression
- Any complications (i.e. infection) resulting from a PTC tube
- Grade 4 elevation in bilirubin during the course of therapy

8.3   Expected Toxicities
RT: The administration of RT may cause or aggravate nausea or vomiting. It can cause gastric or duodenal ulceration. Radiation can also cause skin irritation, fatigue, and decreased blood counts. Radiation-induced liver disease (veno-occlusive disease), including the possibility of damage severe enough to result in liver failure which could lead to death. In some patients it is not possible to avoid kidney irradiation which could produce a decrease in renal function. Careful RT planning can minimize these effects.

8.4   Liver Toxicity
Patients will be evaluated during therapy, and at 4-6 weeks, 2-month, and 4-month follow-up visits after last RT for symptoms and signs of RILD. RILD is a clinical syndrome of anicteric ascites, hepatomegaly and elevation of alkaline phosphatase (ALP) relative to other transaminases that may occur 2 weeks to 4 months following radiation to the liver. ALP must be at least 2-fold increased above the baseline ALP. If ascites develops at any time within 3 months following treatment, an abdominal CT and paracentesis with pathological evaluation of the ascitic fluid is recommended. If the ascitic fluid does not reveal malignancy and there is no evidence of disease progression in the liver or abdomen, it will be assumed that RILD has occurred. If disease progression in the liver or abdomen has occurred, no diagnosis of RILD can be made. In patients who have elevation of liver enzymes near Grade 4 levels and/or in patients with early non-specific signs or symptoms of liver injury, close follow-up is recommended with repeat blood work. If no tumor progression is documented in these patients, liver injury will be presumed to be treatment related.

8.5   GI Toxicity

The dose constraints required for the normal stomach and small intestine should limit the GI toxicity observed and it is not expected that GI toxicity will be dose limiting. However, if a portion of the stomach or small intestine is treated (> 30 Gy), H2 blockers or proton pump inhibitors will be recommended to attempt to decrease the chance of late GI bleeding. Patients will be followed for GI toxicity at each follow up visit. Toxicity of all grades ≥2 measuring only the following conditions will be collected in the database: Gastritis, hepatic pain, vomiting and fatigue.

8.6   Management of Toxicity
    8.6.1   RT:
        Will be administered only by professional personnel trained, certified, and experienced in the administration of the respective treatment modalities. Patients will undergo frequent examination and laboratory assessment, as outlined in the protocol, to detect the early signs of toxicity so that treatment can be adjusted as required.
    8.6.2   Toxicity:
        Supportive care for toxicity will be determined by physicians based on each individual situation. This may include blood component transfusions, intravenous fluids, antihistamines and pressors (for acute anaphylaxis), anti-epileptic agents (for seizures), and antibiotics, allopurinol, etc. Decision as to the extent of cardiopulmonary support (i.e., resuscitation, mechanical ventilator support, etc.) will be made on a case-by-case basis.

9.0 STUDY MONITORING

9.1   Off-Treatment Conditions
    9.1.1   Unacceptable toxicity as defined in section 5.
    9.1.2   Therapy may be discontinued prematurely at any time by patient request without prejudice to subsequent care.
    9.1.3   Patients may be removed from the treatment at any time per investigator discretion.

9.2 Off Study Conditions
    9.2.1 Patients will be removed if they are unable to complete the pre-treatment and post-3 fraction IC-Green tests.
    9.2.2 Patients will be removed if they are unable to receive SBRT treatments.
    9.2.3   Patients may be removed from study at any time by patient request.
    9.2.4   Patients who enroll in subsequent radiation therapeutic trials are considered off study.
    9.2.5   Patients who go on to receive additional therapy (non-radiation therapy, on or off protocol) will be followed for survival only.

9.3   Adverse Event Reporting Guidelines
    9.3.1 Adverse Event definitions
        9.3.1.1 An Adverse Event is any untoward medical event that occurs in a patient who has received an investigational treatment, and does not necessarily have a causal relationship with the investigational treatment. An AE can therefore be any unfavorable and unintended sign (including abnormal laboratory finding), symptom,

or disease temporally associated with the use of an investigational treatment, whether or not related to the treatment.

9.3.1.2 Pre-existing diseases or symptoms or abnormal laboratory values present upon recruitment are not considered an AE even when observed during the further course of the study. However, every worsening of a pre-existing condition is considered as an adverse event.

9.3.1.3 All grade 3 and above AEs will be collected, in addition to any grade ≥2 gastritis, hepatic pain, vomiting, and fatigue. The NCI Common Terminology Criteria for Adverse Events 4.0 (CTCAE) will be utilized to grade AE's for AE reporting.

9.3.1.4 During the course of an adverse event, severity and/or causality and/or seriousness may change. For CRF documentation this adverse event represents one entity from onset to resolution and the worst of the observed categories shall be attributed.

9.3.1.5 When event reoccurs after it disappeared, it should be handled as a new AE. However, AEs that occur intermittently can be recorded as one AE.

9.3.1.6 A serious adverse event (SAE) shall be defined as an adverse advent which fulfills one or more of the following criteria:

- Results in death
- Is immediately life threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is an important medical event that may jeopardize the patient or may require medical intervention to prevent 1 of the outcomes listed above.

Any events or hospitalizations that are unequivocally due to progression of disease should not be reported as a SAE. The causality of SAEs (i.e., their relationship to study treatment) will be assessed by the investigators and will be labeled Definitely related to treatment, Probably related to treatment, Possibly related to treatment, Unlikely related to treatment or Not related to treatment.

9.3.2 Only adverse events events deemed serious and related will be reported to the IRB and the PI within 10 days of awareness of the event. All other events will be noted in the patients medical record.

9.3.3 Adverse events will no longer be reported if the patient has another liver-directed therapy or starts chemotherapy.

9.3.3 The following types of hospitalizations do not constitute SAEs:

9.3.3.1 Hospitalization or Emergency room visits secondary to expected cancer morbidity: Admission for palliative care or pain management

9.3.3.2 Planned hospitalizations for surgical procedures, either related or unrelated to the patient's cancer.

9.4 Data and Safety Monitoring

9.4.1 This trial will be monitored in accordance with the NCI approved University of Michigan Comprehensive Cancer Center Data and Safety Monitoring Plan.

The study specific Data and Safety Monitoring Committee (DSMC), consisting of the protocol investigators, data manager or designee and other members of the study team involved with the conduct of the trial, will meet quarterly or more frequently depending on the activity of the protocol. The discussion will include matters related to the safety of study participants (SAE/UaP reporting), validity and integrity of the data, enrollment rate relative to expectations, characteristics of participants, retention of participants, adherence to the protocol (potential or real protocol deviations) and data completeness.

At the regular DSMC meetings, the protocol specific Data and Safety Monitoring Report form will be completed. The report will be signed by the Principal Investigator or by one of the co-investigators.

Data and Safety Monitoring Reports will be submitted to the University of Michigan Comprehensive Cancer Center Data and Safety Monitoring Board (DSMB) on a quarterly basis for independent Review.

10.0 STATISTICAL CONSIDERATIONS

This is a Phase II trial to characterize the safety and efficacy of individualized SRBT for patients who have had previous liver treatment or who have primary HCC. The trial endpoints are toxicity, local control, time to progression and survival; plasma biomarkers will also be collected to explore their use as tools for treatment individualization in future trials. During the current trial, an indicator of liver function, indocyanine green (ICG), will be used to identify during treatment patients who are at excess risk for radiation-induced liver disease (RILD) so that their radiation dose may be reduced. The model used for individualization will be updated as trial data accrue, so this is an *adaptive* trial of an *individualized* therapy. The planned accrual is ninety (90) evaluable patients over three years. An evaluable patient is a patient that has received their complete prescribed treatment (including those whose IC-green measurements precluded the second phase of treatment). We will continue to enroll patients until we have a total of 90 evaluable patients, by replacing non-evaluable patients.

10.1 Adaptive Estimate of Dose-Response Model

Data from previously treated patients will be represented by estimated parameters in the individualization model, which will be updated continually throughout the trial. The primary indicator of liver function at any time is the proportion of indocyanine green that is retained 15 minutes after administration, which is collected prior to any treatment, one month after the first part of treatment (three fractions), and one month after the second part of treatment (two additional fractions). The basic concept is to use the change in liver function over the first set of fractions to predict the change over the second set of fractions, reduce the dose if there is evidence that patients are less tolerant of the second set of fractions, and adjust the second fraction size for a given patient if it appears that patient's liver function will be unacceptably decreased by the fraction size originally planned.

Write the three observed ICG 15-minute retention proportions of Patient h as k_{0h}, k_{1h} and k_{2h}, and the SBRT Part 1 and Part 2 fraction sizes as d_{1h} and d_{2h}. The sensitivity of liver function to SBRT Part 1 is $b_{1h}=(k_{1h}-k_{0h})/(3d_{1h})$; the 3 in the formula represents the three fractions in Part 10.1.1. Similarly, the sensitivity of the total Part 2 dose equals $b_{2h}=(k_{2h}-k_{1h})/(2d_{2h})$. The ratio of these sensitivities for patient h is denoted by $\gamma_h =b_{2h}/b_{1h}$. The γ_h are assumed to follow a Gaussian(μ,σ^2) distribution, with priors given by $\mu\sim$Gaussian(*1.5,0.25*) and $\sigma^2\sim$Gamma(*2.5,0.08*). The algorithm for selecting the Stage 2 fraction for Patient i, where the goal is to determine the dose d_{2i} such that $P\{k_{2i}<0.44\}>0.9$, is:

10.1.2. If $i=1$, set $\gamma=1.5$. Otherwise, for each patient who has completed treatment, $h=1,...,i-1$, calculate the values $b_{1h}=(k_{1h}-k_{0h})/3d_{1h}$ and $b_{2h}=(k_{2h}-k_{1h})/2d_{2h}$, and the ratio b_{2h}/b_{1h}.

10.1.3 . If $i>1$, estimate the predictive distribution for the next γ_i from the known ratios γ_h ; h=1, … i-1, on all previously treated patients. Using that distribution, find γ^* such that $P\{\gamma<\gamma^*\}=0.9$.

10.1.4 . *If $(k_{0i} > .44)$, then $d_{2i} = 0$*

    If $(k_{1i} < .44)$ AND $(k_{1i} < k_{0i})$, then $d_{2i} = d_{1i}$

    If $(k_{0i} < k_{1i} < .44)$, then $d_{2i} = min((0.44-k_{1i})/(2(\gamma^*)b_{1i}),d_{1i})$

(The posterior distribution in Step 2, above is estimated by means of Markov Chain Monte Carlo assuming that $\gamma\sim$Gaussian(μ,σ^2), $\mu\sim$Gaussian$(1.5,0.25)$ and $\sigma^2\sim$Gamma$(2.5,0.08)$. γ^* is determined from the median of $\mu+\Phi^{-1}(0.9)\sigma^2$, sampled from the posterior distribution). For example, if a patient begins the trial with $k_{0i}=0.23,$ and, after SBRT Part 1, has $k_{1i}=0.45,$ he will receive no Part 2 treatment (d_{2i}=0) because he has already exceeded the upper boundary for liver function. If she begins the trial with $k_{0i}=0.18,$ has $k_{1i}=0.22$ after SBRT Part 1, and the current estimate of γ^* is 1.3, she will receive full SBRT Part 2. If a patient has $k_{0i}=0.14$ and $k_{1i}=0.29,$ and the current estimate of γ^* is 2.5, his Part 2 fraction size will be 60% of his Part 1 fraction size, since two full fractions would be expected to result in estimated $k_{2h}= 0.294+(0.29-0.14)*2.5*2/3=0.54$.

10.2 Stopping Rules

The method for monitoring binary adverse criteria in Phase IIa trials proposed by Simon, Thall and Estey(54) will be employed to monitor patients for decreased treatment efficacy.

The rule is designed to stop the trial if either the probability that the proportion of patients experiencing local progression (as defined in Section 7.6) within six months of treatment is greater than 0.3 or the probability that the proportion of patients experiencing treatment-related toxicity (as defined in Section 8.2) is greater than 0.1 exceeds 0.8. The trial will be stopped if the number of patients experiencing disease-related progression or treatment-related toxicity is greater than or equal to the values in columns 2 or three in Table 1.

# Evaluable	# Local Progression Within Six Months	# Toxicity
10	6	3
20	10	5
30	14	6
40	18	8
50	22	10
60	27	11

Table 1 Stopping rules – The trial is halted if the number of patients with local progression within six months of treatment or treatment-related toxicity is greater than or equal to the numbers in the second or third columns, respectively.

For example, if six or more out of the first ten evaluable patients experience local progression (as defined in Section 7.6) within six months after treatment, the trial must be stopped. Similarly, if five or more of the first twenty patients experience unacceptable toxicity (as defined in Section

8.2), the trial must be halted. The operating characteristics of this rule, determined by Monte Carlo simulation, are displayed in Table 2.

			Percentiles of Sample Size				
True P(Progression)	True P(Toxicity)	P(Trial Stops Early)	10	25	50	75	90
0.3	0.1	0.23	10	70	70	70	70
0.3	0.2	0.80	10	10	30	60	70
0.5	0.1	0.93	10	10	20	30	50

Table 2 Operating characteristics of the stopping rule for progression and toxicity.

For instance, if the true probability of toxicity is 0.2 and the probability of local progression is 0.3, the probability the trial will stop early is 0.8, and the median number of patients treated at trial termination will equal 30.

10.3   Analysis Plan
10.3.1   Primary Objective
Characterize, in patients who have had previous liver treatment or who have primary HCC, the safety and efficacy of individualized SBRT, which consists of three fractions of SBRT, a one month break, an assessment of liver function (using Indocyanine Green (IC-Green)), and two additional fractions adjusted to account for the patient's tolerance to the first 3 fractions.

10.3.1.1 Lesion control (absence of progression) at 1 year will be assessed as a dichotomous variable (see Section 7.6) in patients who complete therapy, and, in a secondary analysis, in all patients who undergo any per-protocol therapy. The proportion of patients achieving lesion control will be estimated with 90% exact binomial confidence intervals. An exact binomial test of the null hypothesis H_O: $\pi<0.65$ (where π is the proportion of treatment successes) will be performed at a 1-sided 5% significance level on patients who complete treatment and patients who undergo any therapy. Secondary analyses will include evaluation of potential predictors, such as demographic and clinical factors, in logistic regression models.

10.3.1.2 Overall survival of patients who complete therapy and patients who receive any therapy will be described by product-limit (Kaplan-Meier) estimates of the survival function, along with 90% confidence limits. Secondary analyses will include evaluation of potential predictors, such as demographic and clinical factors, in proportional hazards regression (Cox) models.

10.3.1.3 Toxicities will be summarized with frequency tables and will be tabulated by CTCAE grade and relatedness to treatment (as assessed by the investigator or PI). The proportion of patients who receive any therapy that experience unacceptable toxicity (as defined in section 8.2) will be estimated. The distribution of indocyanine green 15 minute retention proportion at each time point, and its change over time, will be graphed; an appropriate probability function will be determined using the graph, and parameters estimated so that the expected probability of SBRT patients with and without individualization having unacceptable liver function can be determined. Baseline clinical and demographic variables will be added to determine potential patient subsets who may

be more or less tolerant of the Part 1 treatment, or for whom indocyanine green assessment may be a better or worse predictor of toxicity; the form of these models will depend on the choice of the probability model.

10.3.2 Secondary Objective

Collect data on plasma biomarkers of treatment efficacy and toxicity and liver function to plan further enhancements to individualized SBRT.

> 10.3.2.1 The utility of plasma cytokines for individualizing SBRT will be explored in the same fashion as liver perfusion, above. The goal of both of these secondary analyses is to generate hypotheses and provide statistics for use in the design of subsequent experiments.

Collect data on changes in the clinical measures of severity of liver dysfunction, including the Model for End-Stage Liver Disease (MELD) and Child-Turcotte-Pugh (CTP)

> 10.3.2.2 MELD and CTP will be assessed and recorded at baseline, four weeks after Part 1 of SBRT, and four weeks after Part 2 of SBRT. Logistic regression will be used to determine if MELD and/or CTP, or changes in these clinical assessments, will be useful as predictors of subsequent RILD. Mixed effects models will be used to relate them to IC-Green. Because the number of patients experiencing RILD is expected to be small, these analyses will be considered exploratory.

10.4 Justification of Design
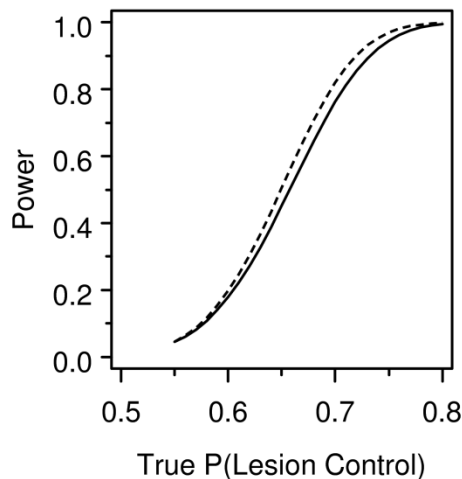
The sample size is justified in terms of the hypothesis test of lesion control in the primary objective. We expect a local control rate at 1 year of 80% and would like high power to rule out values lower than 65%. The required number of subjects to yield 80% power is 70. The power calculations are based on a 1-sided exact test at a 0.05 level.

We expect the proportions of metastatic, Child A and Child B patients to be roughly equal, so exploratory models of clinical subsets will be feasible. Other simulations (not shown) show that the estimate of the individualization parameter should be adequate after 10-20 patients. An additional 20 patients (for a total of 90) will also provide excellent pilot data for exploratory analyses of cytokines as predictors of toxicity, for use in designing the next clinical trial incorporating these into the adaptive process.

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Appendix A

Model for End-Stage Liver Disease (MELD)

MELD score is calculated using a relatively simple formula that relies on three readily available objective variables:

Serum creatinine (Scr; mg/dL)
Total bilirubin (Tbil; mg/dL)
INR (international normalized ratio)

MELD Score, UNOS modified
=[9.57 Ln(Serum Creatinine (mg/dl)) + 3.78 Ln(Total bilirubin (mg/dl)) + 11.2 Ln(INR) + 6.43]

The following rules must be observed when using this formula:
- 1 is the minimum acceptable value for any of the three variables.
- The maximum acceptable value for serum creatinine is 4, to avoid higher MELD scores in patients with concomitant intrinsic renal disease
- The maximum value for the MELD score is 40.

Child-Turcotte-Pugh (CTP)

	Points		
	1	**2**	**3**
Encephalopathy	None	Grade 1-2 (or precipitant-induced)	Grade 3-4 (or chronic)
Ascites	None	Mild/Moderate (diuretic-responsive)	Severe (diuretic-refractory)
Bilirubin (mg/dL)	<2	2-3	>3
Albumin (g/dL)	>3.5	2.8-3.5	<2.8
PT (sec prolonged) or	<4	4-6	>6
INR	<1.7	1.7-2.3	>2.3

CTP score is obtained by adding the score for each of the 5 parameters.

CTP class:
A = 5-6 points
B = 7-9 points
C = 10-15 points