PROTOCOL

TITLE: ULTRASOUND MICROBUBBLE DESTRUCTION AND PERFUSION QUANTIFICATION FOR IMPROVING RADIOEMBOLIZATION THERAPY OF HEPATOCELLULAR CARCINOMA

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STUDY DURATION: 6 Years

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SYNOPSIS

Protocol Title: Ultrasound Microbubble Destruction for Improving Radioembolization Therapy of Hepatocellular Carcinoma

Trial Objectives: The primary objective of this trial is to:

- Characterize the ability of localized ultrasound contrast agent destruction to improve hepatocellular carcinoma (HCC) response to Y90 radioembolization.

The secondary aim of this trial is to:

- determine if contrast-enhanced ultrasound estimated tumor perfusion can reliably predict HCC response to radioembolization 1-14 days post treatment.

Trial Design: This is an open-label, randomized trial that will be conducted at one clinical site to determine if ultrasound-triggered microbubble destruction (UTMD) can be used to improve radioembolization of HCC. The subject population will be patients scheduled for radioembolization of a previously untreated HCC mass at Thomas Jefferson University. Patients will be randomly assigned to either the experimental (UTMD + radioembolization) or control group (radioembolization alone) by the research coordinator using a random assignment generator. For patients assigned to the experimental group, the study will consist of up to three contrast-enhanced ultrasound (CEUS) exams 1-6 hours post radioembolization, and approximately 7 and 14 days post treatment. Treatment response will be evaluated using modified response evaluation criteria in solid tumors (mRECIST) at 1 and 3-4 months post treatment based on the patient's clinically scheduled contrast-enhanced MRI or CT. For patients assigned to the control group, only outcomes data will be collected (without intervention).

Ultrasound imaging will be performed using a state of the art commercial ultrasound scanner with a curvilinear transducer. As part of each CEUS exam, patients will first undergo baseline grayscale B-mode and power Doppler imaging (PDI) of the treated mass. Patients will then receive an infusion of the ultrasound contrast agent (UCA) Optison (GE Healthcare, Princeton, NJ), while being imaged with two-dimensional (2D) dual mode B-mode / coded harmonic imaging. Once the presence of contrast has been confirmed in the tumor, a series of flash-replenishment sequences will be generated to destroy UCA within a region of interest encompassing the tumor. This sequence will be repeated at imaging planes throughout the tumor for the duration of the infusion.

Trial Population: This trial will consist of up to 104 adults (52 per group) scheduled for radioembolization of a previously untreated HCC mass less than 6 cm in diameter at Thomas Jefferson University.

Trial Procedures: Subjects eligible for trial enrollment will be identified by Jefferson's patient population of subjects being seen for sub-lobar radioembolization of HCC. These patients will be accrued from the Hepatology department, as well as from the HCC Multidisciplinary Tumor Board of the Kimmel Cancer Center at Thomas Jefferson University. A research coordinator will explain the study to the patient. The patient will be given time to consider the risks and benefits of the study and ask questions about participation. The coordinator will review the consent form with the patient and then the patient will be given the form to review. The patient, coordinator, and a study investigator will all sign the consent form. The patient will be given a copy of the signed consent form for their records. A research coordinator will explain the study to the patient. The patient will be given time to consider the risks and benefits of the study and ask questions about participation before providing informed consent. If the subject is a woman of childbearing potential, she will have a urine pregnancy test (the results of which will be made available to her prior to study initiation) prior to each ultrasound exam. Patients will be randomly assigned to either the experimental (UTMD + radioembolization) or control group (radioembolization alone) by the research coordinator using a random assignment generator.

All patients will undergo single-photon emission computed tomography (SPECT) imaging as part of their standard of care to confirm effective localization of the Y90 beads. For research purposes, imaging of the chest will be performed when possible to assess extrahepatic distribution of Y90 using a planar imaging acquisition. For patients in the experimental group, a brief abdominal ultrasound (without contrast) will also be performed prior to radioembolization to ensure the target lesion is visible on ultrasound. Imaging will be performed an ultrasonographer with experience in liver imaging) using an S3000 or Sequoia scanner with a 6C1, 5C1 or DAX probe and flash-replenishment and a nonlinear imaging package (Siemens Medical Solutions, Mountain View, CA). Ultrasound exams will take place 1-6 hours post radioembolization in Thomas Jefferson University's short procedure recovery unit, and approximately 7, and 14 days post treatment in Thomas Jefferson University's Interventional Radiology Division. Both of these locations have full time nursing support and with patient physiological monitoring. All contrast injections will be supervised by a board certified physician with resuscitation equipment in immediate proximity during contrast-enhanced ultrasound (CEUS) exam. The entire ultrasound imaging protocol will require approximately 90 mins including a 30 min observation period. These time points are based on times when patients are still present or returning to the hospital for regular follow up and when the Y-90 spheres are

still active. Patient vital signs will be monitored and recorded throughout the visit.

CEUS studies will only be conducted with patients assigned to the experimental group (UTMD + radioembolization). Five milliliters of activated Optison will be suspended in 50 ml of saline and infused through an angiocatheter placed in a peripheral arm vein at a rate of 120 ml/hour. This total Optison administration falls within the product insert dosage guidelines (up to 5 ml within a 10 minute period and up to 8.7 ml in any one study). After confirmation of contrast-enhancement within the mass, a series of ultrasound-triggered microbubble destruction (UTMD)-replenishment sequences will be generated to cavitate UCA within the imaging plane. The patient will be asked to temporarily halt respiration while a 4 second UTMD pulse is initiated (approximate transmit parameters of MI = 1.13 at 1.5 MHz, transmitting 2.3 μs pulses at a pulse repetition frequency of 100 Hz), followed by nonlinear imaging of contrast replenishment at lower intensity using Cadence Pulse Sequencing (MI = 0.06) for 10 seconds. Following patient respiration, this sequence will be repeated at multiple imaging planes throughout the tumor and then repeated throughout the entire tumor volume for the duration of the infusion (UTMD expected in 10-20 planes, repeated 3-4 times). In the few patients with multiple Y-90 treated tumors, the largest tumor will be selected for UTMD therapy and outcomes compared to the non-UTMD treated tumors outside the acoustic field.

The primary measure of tumor response will be evaluated using mRECIST criteria as determined by Drs. Lyshchik and O'Kane in consensus using the patients clinically scheduled 3-4 month contrast enhanced MRI or CT. As a secondary measure of tumor response, changes in alpha fetal protein (AFP; collected as blood samples at approximately one month post embolization as standard of care) will be recorded when clinically available. Tumoral perfusion for each imaging plane will also be calculated as the slope of contrast reperfusion based on change in signal intensity over time. Perfusion values for each plane will then be averaged over the entire mass and normalized to values immediately post treatment (day 1). Finally, each patient's progression free survival and overall survival will be monitored, although this is a tertiary outcome for this study and many patients are expected to surpass the timeline of this pilot study. Safety outcomes will be determined using changes in vital signs, observed and self-reported adverse events, and changes in liver function tests (LFTs) at approximately 1 month post-embolization when available (preformed as part of clinical standard of care). Liver function tests (LFTs) include blood tests for alanine aminotransferase, alkaline phosphatase, asparate aminotransferase, bilirubin, albumin, total protein, and prothrombin time. SPECT imaging will be evaluated for all available cases by two blinded radiologists to evaluate if the initial UTMD session skews Therasphere distribution in the liver.

The proposed agent for the current study, Optison™ is a sterile non-pyrogenic suspension of encapsulated perflutren microbubbles [Goldberg et al 2001; Miller & Nanda 2004]. The contrast agent is consists of a human serum albumin shell with perflutren core and contains 5.0 - 8.0 x 10^8 microspheres/ ml. The microbubble agent is supplied in a standard-size 3 ml vial and is prepared by manually shaking the vial. Optison will be stored in a secure refrigerator, with only the study investigators and research personnel having access. Optison is currently only approved for use in echocardiography. The agent will be used as an off-label indication for this study, but within approved dosage recommendations.

Statistical Methodology: This project is a pilot study to determine if microbubble destruction and reperfusion can used to improve treatment response of HCC to radioembolization and predict early treatment response.

Our statistical analysis will address two major questions:

1. Does the systematic destruction of UCA within HCC masses treated by radioembolization improve treatment response relative to masses treated by radioembolization alone?
2. Do CEUS-based tumoral perfusion measurements provide an earlier indicator of radioembolization treatment response in HCC liver masses?

All statistical analysis will be performed by Dr. Keith (SKCC Biostatistics Core and faculty member) using SAS version 9.4 (Cary, NC). Aim 1: The primary hypothesis that UTMD improves the effect of radioembolization on tumor response in HCC patients will be tested with a non-parametric Mann-Whitney U-test of the difference in response distributions between control (radioembolization alone) and experimental group (UTMD + radioembolization), measured at 3-4 months post treatment. To test if tumor perfusion measured by CEUS between UTMD pulses predicts HCC response to radioembolization, perfusion will be characterized in terms of contrast replenishment time intensity curves fit with a 2-parameter exponential recovery curve as described above. The relationship between the normalized profusion values from this image processing and the patients' subsequent mRECIST scores in the UTMD + radioembolization group will be evaluated with Spearman's rank order correlation.

1. INTRODUCTION

Hepatocellular carcinoma is the third leading cause of cancer mortality worldwide and becoming increasingly common [Alterkruse et al. 2009]. Survival one year after diagnosis is less than 50%, and is worse in patients with advanced disease [Alterkruse et al. 2009]. Because systemic therapies have been proven largely ineffective, embolization represents the current clinical standard for treatment of non-resectable hepatocellular carcinomas [Llovet and Bruix 2003]. Embolization of HCC can be performed via

transarterial chemoembolization or radioembolization. Radioembolization for HCC is performed using TheraSpheres (BTG International). TheraSpheres consist of 20-30 μm glass beads containing yttrium-90 isotopes and the product has been authorized by the FDA with a Humanitarian Device Exemption for the treatment of unresectable hepatocellular carcinoma and our institutional review board has approved these agents for the treatment of HCC. These spheres are locally delivered via a catheter temporarily placed in the hepatic artery that supplies blood flow to the tumor thereby providing a localized and sustained release of radiation. Dosages range from 110-150 Gy**.** Yttrium-90 undergoes pure beta emission as it decays to stable zirconium-90 with a half-life of 64 hours and average energy emission of 0.94 MeV. The local beta emissions from this technique have been shown to provide therapeutic effect within the tumor [Herba et al. 1988; Houle et al. 1989]. Treatment response is then monitored using contrast-enhanced MRI or CT at 1 month and 3-4 months post treatment response.

Ultrasound contrast agents are gas filled microbubbles, encapsulated by a lipid shell for stability. These agents are small enough (1-8 μm in diameter), to pass through the pulmonary capillaries, but are still restricted to the vascular system [Goldberg et al. 2001]. These microbubbles have been approved for use in echocardiography in the United States and also for the characterization of liver lesions throughout Europe and Asia [Bouakaz and de Jong, 2007]. Our group has demonstrated the exceptional safety and accuracy of CEUS for monitoring chemoembolization of HCC [Shaw et al. 2015], and have also observed that UCA perfuse into HCC post radioembolization in these patients. These UCA can also be destroyed in the liver using commercially available flash-replenishment packages which enable visualization and quantification of contrast perfusion [Wakui et al. 2011; Shiraishi et al. 2008; Lefort T, et al. 2012]. These flash-replenishment sequences are performed using commercially available packages that generate a higher intensity (but still well within FDA MI limits) pulse within a selected region of interest to generate UCA cavitation, followed by lower MI nonlinear imaging to visualize contrast reperfusion. Microbubble cavitation has been demonstrated to temporarily increase both cellular and vascular permeability, making it a potential tool for improving localized chemotherapy [Kotopoulis et al. 2013]. Importantly, localized microbubble cavitation has also recently been shown to sensitize tissue to radiotherapy by inducing vascular endothelial cell apoptosis [Kim et al. 2014; Al-Mahrouki et al. 2014; Tran et al. 2012; Czarnota et. al 2012]. Thus, localized destruction of UCA after radioembolization of HCC may potentially improve tumor response by selectively sensitizing malignant tissue to radiotherapy. In this study, we propose a pilot clinical trial using localized UCA cavitation/reperfusion imaging to both improve patient outcomes and predict treatment response earlier than the current clinical standards.

1.1 Background

Hepatocellular carcinoma is the fifth most prevalent cancer worldwide with over half a million new cases reported per year [Alterkruse et al. 2009]. Liver transplant is the only cure for hepatocellular carcinoma, with 70% 5 year survival rates [Mazzaferro et al. 1996]. However, transplant requires a relatively contained disease state (1 lesion 3< 5 cm or up to 3 nodules < 3 cm) and the technique is limited by availability of donors [Llovet et al. 1999]. Only 30% of referred patients are eligible for surgical resection and this group is still plagued by 5 year recurrence rates higher than 70% [Bruix and Llovet 2002]. Because systemic therapies have been proven largely ineffective, embolization represents the current clinical standard for treatment of non-resectable hepatocellular carcinomas [Llovet and Bruix 2003]. Embolization of HCC can be performed via transarterial chemoembolization or radioembolization. Radioembolization is performed using TheraSpheres (BTG International). TheraSpheres consist of 20-30 μm glass beads containing yttrium-90 isotopes and are FDA approved under a Humanitarian Device Exemption for the treatment of HCC. These spheres are locally delivered via catheter through the hepatic artery to the tumor blood supply and provide a localized and sustained release of radiation. Dosages range from 110-150 Gy and radiation exposure to the surrounding liver is limited by the low penetration of the beta radiation**.** Yttrium-90 undergoes pure beta emission as it decays to stable zirconium-90 with a half-life of 64 hours and average energy emission of 0.94 MeV. The local beta emissions from this technique have been shown to provide therapeutic effect within the tumor [Herba et al. 1988; Houle et al. 1989]. Post-treatment, localized radioactivity is confirmed via SPECT imaging and patients are monitored for 2-6 hours before being discharged.

Treatment response of HCC to radioembolization is monitored using contrast-enhanced MRI or CT at 1 month and 3-6 months post treatment response. An earlier predictor of treatment response would be clinically beneficial as HCC patients are still eligible for chemoembolization or ablation post radioembolization. Changes in tumor perfusion have been identified as early indicators of HCC treatment response [Zocco et al 2013; Murakami et al. 2011]. Tumoral inflammation in the microenvironment may also be an early indicator of effective treatment [Serres et al. 2014]. Accurate quantification of tumoral perfusion is difficult using MRI or CT techniques, however, due to their relatively low temporal resolution.

CEUS, which has relatively high temporal resolution, has been able to identify inflammation-related changes in perfusion in other diseases, [Saevik et al. 2014] and thus may be useful in identifying inflammation following radioembolization. Ultrasound contrast agents are well tolerated and administered intravenously, making them an ideal noninvasive screening tool. Commercially available contrast imaging packages are now available on most ultrasound scanners. When insonated at sufficient acoustic pressures (MIs > 0.5), UCA undergo inertial cavitation and localized collapse [Goldberg et al. 2001]. This behavior

forms the basis of flash-replenishment imaging, in which reperfusion within an imaging plane can be repeatedly imaged using cycles of destructive pulses followed by lower MI imaging to monitor reperfusion. These flash-replenishment sequences can performed using commercially available packages that generate a higher intensity (but still well within FDA MI limits) pulse within a selected region of interest. Our group and others have also shown that blood perfusion can be quantified by measuring the rate of contrast perfusion after either a bolus injection or during flash-replenishment imaging using nonlinear CEUS [Sridharan et al. 2013; Wei et al. 1999; Gauthier 2011]. These techniques have been utilized clinically in the liver using commercially available flash-replenishment packages to quantify liver and liver tumor perfusion [Wakui et al. 2011; Shiraishi et al. 2008; Lefort, et al. 2012].

Microbubble cavitation has been demonstrated to temporarily increase both cellular and vascular permeability for improving chemotherapy delivery, and this approach has recently been used in a pilot clinical trial of patients with pancreatic cancer [Kotopoulis et al. 2013]. Importantly, localized microbubble cavitation has also been shown to sensitize tissue to radiotherapy by inducing vascular endothelial cell apoptosis [Kim et al. 2014; Al-Mahrouki et al. 2014; Tran et al. 2012; Czarnota et. al 2012]. Thus, we expect that flash-replenishment sequences that are useful for noninvasively characterizing tumor perfusion may also sensitize tumors to radiation.

The proposed agent for the current study, Optison, is a sterile non-pyrogenic suspension of encapsulated perflutren microbubbles [Goldberg et al 2001; Miller & Nanda 2004]. The contrast agent is consists of a human serum albumin shell with perflutren core and contains 5.0 - 8.0 x 10^8 microspheres/ ml. The microbubble agent is supplied in a standard-size 3 ml vial and is prepared by manually shaking the vial. Optison is currently only approved for use in echocardiography. The agent will be used as an off-label indication for this study.

Optison Clinical Safety

Optison is well tolerated and has been used extensively in echocardiography applications [Goldberg et al 2001]. In pre-market clinical trials, Optison was administered to 279 patients. In these patients 47 (16.8%) reported at least one adverse event. Of these events, 1 was classified as serious and required antihistamines for hypersensitivity manifestations of dizziness, nausea, flushing, and temperature elevation. No deaths were reported. Of the reported adverse reactions following the use of Optison the most frequently reported were headache (5.4%), nausea and/or vomiting (4.3%), warm sensation or flushing (3.6%), and dizziness (2.5%). Additional risks associated with the contrast material are described in the attached Optison Product

insert (Appendix B). All of the non-serious reported side effects have been transient, usually lasting only a few minutes.

Table 1. Selected Adverse Events Reported in ≥ 0.5% of the Subjects who Received Optison in Controlled Clinical Studies (From Optison Product Insert).

SELECTED ADVERSE EVENTS REPORTED IN ≥ 0.5% OF THE SUBJECTS WHO RECEIVED OPTISON™ IN CONTROLLED CLINICAL STUDIES	
No. of Patients Exposed to OPTISON™	279
No. of Patients Reporting on Adverse Event	47 (16.8%)
Body as a Whole	38 (13.6%)
Headache	15 (5.4%)
Warm Sensation/Flushing	10 (3.6%)
Chills/fever	4 (1.4%)
Flu-like Symptoms	3 (1.1%)
Malaise/Weakness/Fatigue	3 (1.1%)
Cardiovascular System	12 (4.3%)
Dizziness	7 (2.5%)
Chest Pain	3 (1.1%)
Digestive System	12 (4.3%)
Nausea and/or Vomiting	12 (4.3%)
Nervous System	3 (1.1%)
Respiratory System	5 (1.8%)
Dyspnea	3 (1.1%)
Skin & Appendages	11 (3.9%)
Injection Site Discomfort	3 (1.1%)
Erythema	2 (0.7%)
Special Senses	9 (3.2%)
Altered Taste	5 (1.8%)
(1) Patients are counted separately within each body system. (2) The body system is reported if the aggregate is ≥ 0.5%. Details are not shown if the subsystem is not ≥ 0.5%.	

Additional information concerning pre-clinical and clinical experience with Optison, including the dosing levels and reported subject complaints, can be found in the Optison Package Insert that is included as Appendix B.

1.2 Rationale

The fundamental theory behind this study is that localized UCA cavitation within the tumor vasculature will sensitize this tissue to Y-90 radiation and improve patient outcomes. Additionally, we hypothesize that quantification of UCA reperfusion following flash destructive ultrasound pulses will reflect inflammation changes, and provide an earlier predicator of radioembolization treatment response. If UCA

cavitation leads to effective sensitization of HCC to radioembolization, such an approach would improve treatment efficacy and prolong survival. If CEUS-based perfusion measurements were found to accurately predict treatment response, non-responding patients could be identified earlier and scheduled for alternative therapies such as chemoembolization or ablation (which in turn would also prolong survival).

We propose a pilot clinical trial to determine the potential of using CEUS flash-replenishment imaging for improving radioembolization therapy and predicting tumor response. The purpose of this study is to compare tumoral response in 26 patients treated with this approach to 26 patients treated via standard of care (radioembolization alone) and also to determine if quantitative CEUS data acquired from the experimental arm can predict future RECIST outcomes.

2. TRIAL OBJECTIVES

Trial Objectives: The primary objective of this trial is to:

- Characterize the ability of localized ultrasound contrast agent destruction to improve hepatocellular carcinoma (HCC) response to Y90 radioembolization.

The secondary aim of this trial is to:

- Determine if contrast-enhanced ultrasound estimated tumor perfusion can reliably predict HCC response to radioembolization 1-14 days post treatment.

3. TRIAL DESIGN

This is an open-label, randomized trial that will be conducted at one clinical site to determine if ultrasound-triggered microbubble destruction (UTMD) can be used to improve radioembolization of HCC. The subject population will be patients scheduled for radioembolization of a previously untreated HCC mass at Thomas Jefferson University. All patients will undergo single-photon emission computed tomography (SPECT) imaging as part of their standard of care to confirm effective localization of the Y90 beads. For research purposes, imaging of the chest will be performed when possible to assess extrahepatic distribution of Y90 using a planar imaging acquisition. Patients will be randomly assigned to either the experimental (UTMD + radioembolization) or control group (radioembolization alone) by the research coordinator using a random assignment generator. For patients assigned to the experimental group, the study will consist of up to three contrast-enhanced ultrasound (CEUS) exams: 1-6 hours post radioembolization, and approximately 7 and 14 days post treatment. Treatment response will be evaluated using modified response evaluation criteria in solid tumors (mRECIST) at 1 and 4 months post treatment based on the patient's clinically scheduled

contrast-enhanced MRI or CT. For patients assigned to the control group, only outcomes data will be collected (without intervention).

Study participation for those assigned to the experimental group will consist of up to three contrast-enhanced ultrasound exams. The primary measure of tumor response will be evaluated using mRECIST criteria as determined by Drs. Lyshchik and O'Kane in consensus using the patients clinically scheduled 3-4 month contrast enhanced MRI or CT. As a secondary measure of tumor response, when available, changes in alpha fetal protein (AFP; collected as blood samples at 1 month as standard of care) will be recorded. Finally, each patient's progression free survival and overall survival will be monitored, although this is a tertiary outcome for this study and many patients are expected to surpass the timeline of this pilot study. Safety outcomes will be determined using changes in vital signs, observed and self-reported adverse events, changes in liver function tests (LFTs) at approximately 1 month post-embolization (preformed as part of clinical standard of care), and distribution of radioactivity on SPECT imaging. Liver function tests (LFTs) include blood tests for alanine aminotransferase, alkaline phosphatase, asparate aminotransferase, bilirubin, albumin, total protein, and prothrombin time.

3.1 Trial Duration

Individual participation for patients within the experimental arm will be limited to 3 ultrasound exams. If patients are unable to make one of the 7, or 14 day visits they will still be eligible to participate. The first CEUS exam will take place 1-6 hours post radioembolization during the patient's clinically scheduled observation period. Exams at approximately 7 and 14 days post treatment will take place during separate visits to Thomas Jefferson University. The entire ultrasound imaging protocol will require approximately 90 mins including a 30 min observation period. For patients assigned to the control arm, no additional participation will be required. Patients who receive ultrasound contrast agent will be monitored for at least 30 minutes before being discharged. The research coordinator will obtain laboratory values, imaging data, and outcomes for comparison to the experimental group (all obtained as part of the patient's clinical standard of care). Subject recruitment is expected to last 83 months (March 2017 – December 2023). Analysis and publication of results are expected to take an additional 3 months (January 2024– March 2024). Volunteer Registry Database forms will be submitted to the National Institute of Health's Office of Regulatory Compliance and Quality at the completion of the research study.

4. TRIAL POPULATION

Trial Population: This trial will consist of up to 104 adults (18 years of age or older) undergoing radioembolization of a previously untreated HCC mass less than 6 cm in diameter.

Subjects eligible for trial enrollment will be identified by the co-investigators from their patient population of subjects being seen for radioembolization of HCC. These patients will be accrued from the Hepatology department, as well as from the HCC Multidisciplinary Tumor Board of the Kimmel Cancer Center at Thomas Jefferson University. A research coordinator will explain the study to the patient. The patient will be given time to consider the risks and benefits of the study and ask questions about participation. The coordinator will review the consent form with the patient and then the patient will be given the form to review. The patient, coordinator, and a study investigator will all sign the consent form. The patient will be given a copy of the signed consent form for their records.

4.1 Inclusion Criteria

All subjects accepted for this trial must:

- Be scheduled for sub-lobar radioembolization therapy of a previously untreated HCC mass < 6 cm visible on grayscale ultrasound.
- Be at least 18 years of age.
- Be medically stable.
- If a female of child-bearing age, have a negative pregnancy test prior to each ultrasound exam.
- Have signed Informed Consent to participate in the study.

4.2 Exclusion Criteria

Subjects with any of the following conditions or who have had the following procedures will be excluded from this trial:

- Females who are pregnant or nursing.
- Patients who are medically unstable, patients who are seriously or terminally ill, and patients whose clinical course is unpredictable. For example:
  - Patients on life support or in a critical care unit.
  - Patients with unstable occlusive disease (eg, crescendo angina)
  - Patients with clinically unstable cardiac arrhythmias, such as recurrent ventricular tachycardia.
  - Patients with uncontrolled congestive heart failure (NYHA Class IV)
- Patients with recent cerebral hemorrhage.
- Patients with known sensitivities to albumin, blood, or blood products
- Patients with known hypersensitivity to perflutren
- Patients with known cardiac shunts.
- Patients with known congenital heart defects.
- Patients with severe emphysema, pulmonary vasculitis, or a history of pulmonary emboli.
- Patients with respiratory distress syndrome.
- Patients with a history of bleeding disorders
- Patients with bilirubin levels > 2 mg/dL

Subject identification will be maintained with a study specific alphanumeric code including the subject number (01-104).

5. MEDICATIONS

Optison will be purchased from GE Healthcare, Princeton, NJ.

Optison is a sterile non-pyrogenic suspension of encapsulated perflutren microbubbles [Goldberg et al 2001; Miller & Nanda 2004]. The contrast agent consists of a human serum albumin shell with perflutren core and contains 5.0 - 8.0 x 10^8 microspheres/ ml. The microbubble agent is supplied in a standard-size 3 ml vial and is prepared by manually shaking the vial. Detailed instructions are provided in the Optison Product Insert, found in Appendix B.

Optison will be stored in a secure refrigerator, with only the study investigators and research personnel having access. Unused drug and empty vials will be properly disposed of after reconciling the log of study drug kept at Thomas Jefferson University. Study drug will be transported from the secure refrigerator to the patient bedside by the research coordinator or principal investigators.

5.1 Administration

Contrast agent will be prepared and administered by the research nurse or a physician co-investigator. All contrast injections will be supervised by a board certified physician. Resuscitation equipment and trained personnel will be immediate proximity to the patient during each contrast-enhanced ultrasound exam. Optison will be administered by IV infusion through an angiocatheter placed in a peripheral arm vein, preferably an antecubital vein. Five milliliters of Optison will be activated by hand mixing for 30 seconds and suspended in a 50 ml bag of saline. The saline/UCA mixture will then be infused through the IV at a rate of 120 ml/hour. Subjects will be instructed not to move their arm during the administration of the contrast agent.

5.2 Contraindications

Optison should not be administered to patients with known or suspected hypersensitivity to perflutren, blood, or blood products. The safety of Optison in patients with 1) right-to-left, bi-directional or transient right-to-left cardiac shunts; 2) severe emphysema, pulmonary vasculitis or a history of pulmonary emboli; 3) respiratory distress syndrome has not been studied. Therefore, patients with any of these conditions will be excluded from participation.

5.3 Randomization

Patients will be randomly assigned to either the experimental (UTMD + radioembolization) or control group (radioembolization alone) by the research coordinator using a random assignment generator.

5.4 Blinding and Unblinding Methods

No blinding will be performed as this is a pilot study and all data analysis will be performed immediately following ultrasound intervention.

5.5 Storage

Optison vials will be stored in a secure refrigerator, with only the study investigators and research personnel having access. The study research coordinator will be responsible for drug suspension and inventory control.

6. TRIAL PROCEDURES

6.1 Patient Enrollment

Subjects eligible for trial enrollment will be identified by Drs. Shaw, Posey, Civan, and Maley from their patient population (approximately 52 patients annually being treated by yttrium-90 for hepatocellular carcinoma). These patients will be accrued from the Hepatology department, as well as from the HCC Multidisciplinary Tumor Board of the Kimmel Cancer Center at Thomas Jefferson University. A research coordinator will explain the study to the patient. The patient will be given time to consider the risks and benefits of the study and ask questions about participation. The coordinator will review the consent form with the patient and then the patient will be given the form to review. The patient, coordinator, and a study investigator will all sign the consent form. The patient will be given a copy of the signed consent form for their records. A research coordinator will explain the study to the patient. The patient will be given time to consider the risks and benefits of the study and ask questions about participation before providing informed consent. If the subject is a woman of childbearing potential, she will have a urine pregnancy test (the results of which will be made available to her prior to study initiation) prior to each ultrasound exam. Patients will be randomly assigned to either the experimental (UTMD + radioembolization) or control group (radioembolization alone) by the research coordinator using a random assignment generator. For patients in the experimental group, a brief abdominal ultrasound (without contrast) will also be performed prior to radioembolization to ensure the target lesion is visible on ultrasound.

6.2 Screening Assessments

Screening assessments will be performed prior to CEUS imaging. Trial participants will have the presence of inclusion criteria and absence of exclusion criteria verified by providing a medical history. A full demographic profile, known drug allergies or intolerances, and a review of the subject's medical/surgical history will be recorded. If the subject is a woman of childbearing age, she will have a urine pregnancy test (the results of which will be made available to the subject prior to study initiation) prior to each ultrasound exam. A brief abdominal ultrasound (without contrast) will also be performed prior to radioembolization to ensure the target lesion is visible on ultrasound.

6.3 Ultrasound Imaging

All contrast injections will be supervised by a board certified physician. Resuscitation equipment and trained personnel will be in immediate proximity to the patient during each CEUS exam. The ultrasound examinations will be performed by a qualified sonographer. Ultrasound exams will take place 1-6 hours post radioembolization in Thomas Jefferson University's short procedure recovery unit, and approximately 7, and 14 days post treatment in Thomas Jefferson University's Ultrasound Division.

Procedures and equipment for this trial will be used in accordance with typical clinical procedures. All trial procedures will be conducted in accordance with Good Clinical Practice. For the ultrasound examination, the patient will be asked to lie in a supine or decubitous position and a catheter will be placed in a superficial vein (preferably an antecubital vein). Acoustic coupling gel will be applied to the area of interest. A state of the art, commercially available ultrasound scanner with curvilinear probe will be used. All acoustic parameters will be well within FDA limits. Imaging will be performed by an ultrasonographer with experience in liver imaging using an S3000 or Sequoia scanner with a 6C1, 5C1 or DAX probe and flash-replenishment and a nonlinear imaging package (Siemens Medical Solutions, Mountain View, CA). Ultrasound exams will take place 1-6 hours post radioembolization in Thomas Jefferson University's short procedure recovery unit, and 7, and 14 days post treatment in Thomas Jefferson University's Interventional Radiology or Ultrasound Division. Both of these locations have full time nursing support and with patient physiological monitoring. All contrast injections will be supervised by a board certified physician with resuscitation equipment in immediate proximity during contrast-enhanced ultrasound (CEUS) exam. The entire ultrasound imaging protocol will require approximately 90 mins including a 30 min observation period. These time points are based on times when patients are still present or returning to the hospital for regular follow up and when the Y-90 spheres are still active. Patient vital signs will be monitored and recorded throughout the visit.

CEUS studies will only be conducted with patients assigned to the experimental group (UTMD + radioembolization). Five milliliters of activated Optison will be suspended in 50 ml of saline and infused through an angiocatheter placed in a peripheral arm vein at a rate of 120 ml/hour. This total Optison administration falls within the product insert dosage guidelines (up to 5 ml within a 10 minute period and up to 8.7 ml in any one study). After confirmation of contrast-enhancement within the mass, a series of ultrasound-triggered microbubble destruction (UTMD)-replenishment sequences will be generated to cavitate UCA within the imaging plane. The patient will be asked to temporarily halt respiration while a 4 second UTMD pulse is initiated (approximate transmit parameters of MI = 1.13 at 1.5 MHz, transmitting 2.3 μs pulses at a pulse repetition frequency of 100 Hz), followed by nonlinear imaging of contrast replenishment at lower intensity using Cadence Pulse Sequencing (MI = 0.06) for 10 seconds. Following patient respiration, this sequence will be repeated at multiple imaging planes throughout the tumor and then repeated throughout the entire tumor volume for the duration of the infusion (UTMD expected in 10-20 planes, repeated 3-4 times). In the few patients with multiple Y-90 treated tumors, the largest tumor will be selected for UTMD therapy and outcomes compared to the non-UTMD treated tumors outside the acoustic field. This sequence will be repeated at imaging planes throughout the tumor for the duration of the infusion. Following completion of the infusion, patients receiving ultrasound contrast agent will be monitored for adverse events for 30 minutes to ensure stable response.

6.4 Safety Monitoring

Adverse events in patients receiving ultrasound contrast agents will be monitored during the entire procedure and for 30 minutes from the completion of Optison infusion. Specifically, the patient will be monitored with non-leading questions to monitor the patient for the transient side effects that are described below. Changes in liver function tests (preformed clinically prior to radioembolization and 1 month) will be recorded and compared to the control group to evaluate safety and liver toxicity.

6.5 Efficacy Assessments

The primary measure of tumor response will be evaluated using mRECIST criteria as determined by Drs. Lyshchik and O'Kane in consensus using the patients clinically scheduled 3-4 month contrast enhanced MRI or CT. As a secondary measure of tumor response, changes in alpha fetal protein (AFP; collected as blood samples at 1 month as standard of care) will be recorded. Finally, each patient's progression free survival and overall survival will be monitored, although this is a tertiary outcome for this study and many patients are expected to surpass the timeline of this pilot study. Safety outcomes will be determined using changes in vital signs, observed and self-reported adverse events, and changes in liver function tests (LFTs) at 1 and month post-embolization (preformed as part of clinical standard of care). Liver function tests

(LFTs) include blood tests for alanine aminotransferase, alkaline phosphatase, asparate aminotransferase, bilirubin, albumin, total protein, and prothrombin time. SPECT imaging will be evaluated for all available cases by two blinded radiologists to evaluate if the initial UTMD session skews Therasphere distribution in the liver.

To answer the secondary hypothesis of whether contrast reperfusion can predict treatment outcomes earlier than conventional MRI/CT imaging at 3-4 months, changes in imaging data from the experimental group will be quantified. The contrast replenishment time intensity curve will be fit with a 2-parameter exponential recovery curve: $VI = \alpha(1 - e^{-\beta t})$, in which VI represents video intensity; α (decibels) represents the asymptotic plateau correlative of the microvessel cross-sectional area, and β (in mm/s) represents the blood velocity. The product $\alpha \times \beta$ is an estimate of perfusion or blood flow per tissue unit (approximately ml/(s mg)) [Krix et al. 2003]. Parametric images from multiple slices in the tumor will also be compiled into a parametric volume to enable visualize of perfusion throughout the mass. Regions of interest will then be selected by Drs. Lyshchik and O'Kane to determine average perfusion of the mass, peripheral perfusion, and vascular heterogeneity. These values will be quantified for each time point and normalized to D-CEUS at day 1. Normalized values will be grouped based on tumoral response as complete response, partial response, stable disease, and progressive disease (based on mRECIST by 3-4 month contrast-enhanced MRI/CT) to determine if D-CEUS provides an earlier indication of treatment response. Finally, changes in perfusion during the same infusion will be quantified to determine if tumor vascularity is altered during the process of UTMD.

6.6 Safety Assessments

6.6.1 Unanticipated Problems

Unanticipated problems (UAPs) include, in general, any incident, experience, or outcome that meets the following criteria:

- unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;

UAPs are considered to pose risk to participants or others when they suggest that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

6.6.2 Adverse Events

An adverse event is any untoward or unfavorable medical occurrence in a human participant, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the participant's participation in the research, whether or not considered related to the participant's participation in the research.

6.6.3 Serious Adverse Events

A serious adverse event (SAE) is one that meets one or more of the following criteria:

- Results in death
- Is life-threatening (places the participant at immediate risk of death from the event as it occurred)
- Is disabling or incapacitating
- Results in inpatient hospitalization or prolongation of existing hospitalization
- Results in a persistent or significant disability or incapacity
- Results in a congenital anomaly or birth defect
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the participant or may require intervention to prevent one of the outcomes listed in this definition.

6.6.4 Safety Assessment and Follow-Up

At each study visit, the investigator (or designee) will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

The subjects who receive ultrasound contrast agent will be monitored for AEs during the entire procedure. All AEs, including observed or volunteered problems, complaints, signs or symptoms, and diagnoses, occurring from the initiation of Optison dosing until 30 minutes from the completion of the Optison administration will be recorded on a serious or non-serious AE data form, whether or not associated with the use of the trial medication.

6.6.5 Recording Adverse Events

The following subsections detail what information must be documented for each adverse event occurring during the time period specified in Appendix C: SKCC DSMP's AE/SAE Reporting Requirements.

Relationship to Study Intervention

The relationship to study intervention or study participation must be assessed and documented for all adverse events. Evaluation of relatedness must consider etiologies such as natural history of the

underlying disease, concurrent illness, concomitant therapy, study-related procedures, accidents, and other external factors.

The following guidelines are used to assess relationship of an event to study intervention:

Relationship

1. Related (Possible, Probable, Definite)
    a. The event is known to occur with the study intervention.
    b. There is a temporal relationship between the intervention and event onset.
    c. The event abates when the intervention is discontinued.
    d. The event reappears upon a re-challenge with the intervention.
2. Not Related (Unlikely, Not Related)
    a. There is no temporal relationship between the intervention and event onset.
    b. An alternate etiology has been established.

Expectedness

The PI is responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the intervention. Risk information to assess expectedness can be obtained from preclinical studies, the investigator's brochure, published medical literature, the protocol, or the informed consent document.

Severity of Event

Adverse events will be graded for severity according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0

Intervention

Any intervention implemented to treat the adverse event must be documented for all adverse events.

6.6.6 Safety Reporting

Reporting to the IRB

Unanticipated Problems

All incidents or events that meet criteria for unanticipated problems (UAPs) as defined in Appendix C: SKCC DSMP's AE/SAE Reporting Requirements require the creation and completion of an unanticipated problem report form (OHR-20).

UAPs that pose risk to participants or others, and that are not AEs, will be submitted to the IRB on an OHR-20 form via the eazUP system within 5 working days of the investigator becoming aware of the event.

UAPs that do not pose risk to participants or others will be submitted to the IRB at the next continuing review.

Adverse Events

Grade 1 AEs will be reported to the IRB at continuing review.

Grade 2 AEs will be reported to the IRB at the time of continuing review.

Serious Adverse Events

SAEs will be reported to the IRB on OHR-10 forms via the electronic reporting system (eSAEy) according to the required time frames described below.

Grade 3-4 AEs that are unexpected and deemed to be at least possibly related to the study will be reported to the IRB within 2 working days of knowledge of the event.

Grade 3-4 AEs that are deemed unrelated to the study will be reported to the IRB within 5 working days.

Grade 5 AEs will be reported to the IRB within one working day of knowledge of the event.

All SAEs will be submitted to the IRB at continuing review, including those that were reported previously.

Reporting to the Sidney Kimmel Cancer Center DSMB

On a quarterly basis, all AEs and SAEs, safety and toxicity data, and any corrective actions will be submitted to the DSMC per the DSMP. The quarterly report to DSMC will also include any unanticipated problems that in the opinion of the PI should be reported to the DSMC.

For expedited reporting requirements, see DSMC AE/SAE Reporting Requirements.

FDA Reporting

The investigator is required to submit all unexpected and serious adverse events to the FDA within 48 hours. Fatal adverse events related to treatment which are unexpected must be reported within 24 hours. Fatalities not related to the study drug/device must be reported within 5 days.

6.7 End-of-Treatment and End-of-Trial Evaluations

Subjects will be free to discontinue trial participation at any time. The investigator will also discontinue any subject from the trial if, in the investigator's opinion, it is not safe for the subject to continue. The date the subject is withdrawn from a treatment and/or from the trial and the reason for discontinuation will be recorded on the CRF.

Trial participation will be considered completed if the subject has met the following trial requirements:

- Has received four CEUS exams during infusion of Optison combined with flash/replenishment imaging of the radioembolized HCC mass if enrolled in the experimental group.

- Has provided informed consent to share liver function, imaging, and outcomes data if enrolled in the control group.

If a subject's participation in the trial is interrupted for any reason (e.g., because of an AE) and the subject has met the criteria described above for completing the trial, the subject's trial participation will be considered completed. If a subject's trial participation is interrupted for any reason by the subject's or investigator's choice and the subject has not met all of the criteria listed above, then the subject will be considered a discontinued subject.

Findings from this study will be submitted in abstract form to the annual meetings of the American Institute of Ultrasound in Medicine, the Radiological Society of North American, and the Society of Interventional Ultrasound. A manuscript detailing study findings will also be submitted to either a radiology or cancer journal.

7. DATA MANAGEMENT AND STATISTICAL ANALYSES

7.1 Data Management

Data forms will be completed for all subjects enrolled in the trial. The subject study files will be stored in a secure file cabinet and maintained by the research study coordinator. Subject study files will be kept for 7 years after the completion of the study. The final data will be entered into a database. The investigator will be responsible for management of the database. The database will be maintained within an organized and secure directory system.

Findings from this study will be submitted in abstract form to the annual meetings of the American Institute of Ultrasound in Medicine, the Radiological Society of North American, and the Society of Interventional Ultrasound. A manuscript detailing study findings will also be submitted to either a radiology or cancer journal.

7.2 Statistical Analyses

Qualitative findings regarding treatment response will be generated by two radiologists based on mRECIST criteria at 1 and 3-4 months post treatment (using the patient’s clinically scheduled contrast-enhanced MRI or CT exams). In the event of conflicting outcome reports, outcomes will be evaluated in consensus.

All statistical analysis will be performed by Dr. Keith (SKCC Biostatistics Core and faculty member) using SAS version 9.4 (Cary, NC). **Aim 1:** The primary hypothesis that UTMD improves the effect of

radioembolization on tumor response in HCC patients will be tested with a non-parametric Mann-Whitney U-test of the difference in response distributions between control (radioembolization alone) and experimental group (UTMD + radioembolization), measured at 3-4 months post treatment. The outcome variable in this analysis, the mRECIST score, is treated as an ordinal variable in this analysis. **Aim 2:** To test if tumor perfusion measured by CEUS between UTMD pulses predicts HCC response to radioembolization, perfusion will be characterized in terms of contrast replenishment time intensity curves fit with a 2-parameter exponential recovery curve as described above. The relationship between the normalized profusion values from this image processing and the patients' subsequent mRECIST scores in the UTMD + radioembolization group will be evaluated with Spearman's rank order correlation.

7.2.1 Sample Size Calculation

Aim 1: Little is known about the effect of UTMD on radioembolization in humans. When comparing similar groups in our animal studies, we observed relative effect sizes of 0.4 and 0.9 for tumor growth and survival, respectively. These animal study results may not apply directly to humans, but they demonstrate that large effect sizes are plausible for this intervention in subjects with HCC tumors. Under these assumptions, we have determined that a sample size of 52 patients randomized to each group would be needed in order for a Mann-Whitney U-test to have 85% power to detect a similarly moderately large relative effect of UTMD on the mRECIST score (i.e., 0.6) with a 5% type I error rate. A similar analysis is planned to compare mRECIST evaluations between 1 month and 3-4 month MRI to determine if earlier cross-sectional imaging can be used to predict long-term treatment response. Relative effect sizes for LFT, AFP, pathology, blinded reads from SPECT, planar imaging, and time to next treatment are expected to be similarly large and will also be evaluated using appropriate non-parametric or exact tests, and are each subject to the same power considerations as for the primary endpoint. Confounding of the results will be investigated and potential confounders will be included in ordinary least squares (OLS) regression models (or quantile regression models, depending on the adequacy of the data meeting OLS modeling assumptions) of these endpoints. **Aim 2**: A sample size of 52 would provide a two-sided test having 5% type I error rate at least 80% power to detect a Spearman's correlation of 0.4 or greater in the population. Confounding will also be evaluated for this aim and regression analysis will be considered similarly as described for Aim 1.

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APPENDIX A - INVESTIGATOR OBLIGATIONS

A. Institutional Review Board (IRB) and Human Subjects Research Review Board (HSRRB) Review/Approval

The protocol and informed consent for this study must be reviewed and approved by an appropriate IRB and HSRRB prior to enrollment of participants in the study. It is the responsibility of the investigator to assure that all aspects of the ethical review are conducted in accordance with FDA Regulations 21 CFR Part 56. A letter documenting the IRB and HSRRB approval which specifically identifies the study/protocol must be obtained by the investigator prior to initiation of the study. Amendments to the protocol will be subject to the same requirements as the original protocol. The HSRRB must review and approve each modification to the study prior to implementation.

A progress report with a request for re-evaluation and re-approval will be submitted by the investigator to the IRB and HSRRB at intervals required by the IRB, and not less than annually.

After completion or termination of the study, the investigator will submit a final report to the IRB. This report should include: deviations from the protocol, the number and types of participants evaluated, the number of participants who discontinued (with reasons), results of the study, if known, and all AEs, including deaths.

B. Informed Consent

Signed, written informed consent which conforms to FDA Regulation 21 CFR Part 50, must be obtained from each participant prior to entering the study. Each participant will be provided a written consent form and verbal information in an understandable manner which describes the nature and duration of the study. The research study coordinator or the investigator will conduct the informed consent interview in a private examination room. The potential subject will be allowed to discuss the study with the investigator, research study coordinator, or any persons who may have accompanied the potential subject. Additionally, the participant must be allowed adequate time to consider the potential risks and benefits associated with his participation in the study. The research study coordinator will sign the informed consent as the person conducting the consent interview.

C. Data Reporting and Data Forms

Data reflecting participant's experiences with the study will be recorded on CRFs by the investigator.

D. Records Retention

All records pertaining to the conduct of the clinical study, including CRFs, informed consent forms, source documents, and other study documentation must be retained for seven (7) years after the end of the study.

Other study documentation includes all protocols and amendments, drug supply receipt, dispensing and final disposition records, IRB correspondence and approvals, signed consent forms, a blank copy of study consent forms, Form 1572, curriculum vitae or biosketches of members of the research team including the medical monitor, HSRRB correspondence and approval, and Statement of Investigator forms.

Source documents include all original records of observations, results, and activities necessary to reconstruct and evaluate the study. Source documents include but are not limited to laboratory reports,

electrocardiogram tracings, X-ray films, ultrasound images, subject diaries, subject progress notes, hospital charts, appointment books, radiologic reports or pharmacy records, and any other records or reports of procedures performed during the study. Source documents also may include copies of the CRF or sponsor supplied worksheets when original information is recorded directly onto these forms.

Whenever possible, an original recording of an observation should be retained as the source document. However, a photocopy of a record is acceptable provided it is legible and is a verified copy of the original document.

E. Deviation from the Protocol

The investigator will not deviate from the protocol without prior written approval from the IRB and the HSRRB. In medical emergencies, the investigator will use medical judgment and remove the participant from immediate hazard. The HSRRB and the IRB will be notified regarding the type of emergency and course of action taken. Any other changes to or deviations from the protocol will be made as an amendment to the protocol. The amendment must be submitted for review and approval to the local IRB and the HSRRB for review and approval.

F. Roles and Responsibilities of Study Personnel

John Eisenbrey, PhD, Assistant Professor of Radiology, will serve as Principal Investigator on this project. He will be responsible for the scientific goals of the project. Dr. Eisenbrey will oversee patient recruitment, informed consent, ultrasound studies, and the data entry and statistical analyses. He will also supervise the data acquisition from patients.

Colette Shaw, MD, Assistant Professor of Radiology will be co-PI on this project and assist with the patient recruitment, interpret ultrasound images and advise on clinical issues. She will also help with final data analysis and publication of results.

Flemming Forsberg, PhD, Professor of Radiology and Director of Ultrasound Research will assist in data collection, data analysis, and publication of results.

James Posey III, MD, Director of the Gastrointestinal Program in Medical Oncology will assist with the patient recruitment, interpret tumoral response and liver function, and advise on clinical issues.

Jesse Civan, MD, Assistant Professor of Radiology will assist with the patient recruitment, interpret ultrasound images and advise on clinical issues.

Warren Maley, MD, Professor of Surgery will assist with the patient recruitment, interpret ultrasound images and advise on clinical issues.

Amanda Smolock, MD, Assistant Professor of Radiology will assist with the patient recruitment, interpret ultrasound images and advise on clinical issues.

Allison Tan, MD, Assistant Professor of Radiology will assist with the patient recruitment, interpret ultrasound images and advise on clinical issues.

Andrej Lyshchik, MD, Assistant Professor of Radiology will assist with interpretation of results and advise on clinical issues

Patrick O'Kane, MD, Assistant Professor of Radiology will assist with interpretation of results and advise on clinical issues

Robert Den, M.D., Associate Professor of Radiation Oncology, will act as the medical monitor for this project.

Scott Keith, PhD, Assistant Professor of Biostatistics, will perform final statistical analysis of all acquired data.

Corinne Wessner, MS, Research Sonographer in the Department of Radiology will be responsible for performing the ultrasound examinations and interpreting results.

Nancy Pedano, RTR, will be responsible for screening, recruiting, and scheduling patients and will explain the study to them. In addition, she will perform data entry.

Kristen Bradigan, RN will be the research nurse and assist in contrast administration and AE monitoring.

Signature of PI: _____
John Eisenbrey, PhD

APPENDIX B – OPTISON PRODUCT INSERT

APPENDIX C: SKCC DSMP's AE/SAE Reporting Requirements

This table is from the SKCC DSMP version 3.0 dated 3/20/2014, made effective 5/12/2014.

	Grade 1	Grade 2		Grade 3				Grades 4 and 5
				Unexpected		Expected		
	Unexpected and Expected	Unexpected	Expected	With Hospitalization	Without Hospitalization	With Hospitalization	Without Hospitalization	Unexpected and Expected
Unrelated Unlikely	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	5 Working Days	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	5 Working Days	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Phase I - 48 Hours (Death: 24 Hours) Phase II - 5 working days
Possible Probably Definite	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	48 Hours (Death: 24 Hours)	Phase I - 48 Hours Phase II - 5 working days	48 Hours (Death: 24 Hours)	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Phase I and Phase II - 48 Hours (Death: 24 Hours)