THOMAS JEFFERSON UNIVERSITY
Sidney Kimmel Cancer Center

Contrast-Enhanced Ultrasound for Diagnosis and Therapy of Cholangiocarcinoma

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Signature Page

The signature below constitutes the approval of this protocol and the attachments and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Principal Investigators:

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Name:	John Eisenbrey, PhD		
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Statement of Compliance

This study will be conducted in accordance with the International Conference on Harmonisation guidelines for Good Clinical Practice (ICH E6), the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), and Thomas Jefferson University research policies

List of Abbreviations

AE	Adverse Event/Adverse Experience
CEUS	Contrast-enhanced Ultrasound
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
CONSORT	Consolidated Standards of Reporting Trials
CR	Complete Response
CRF	Case Report Form
CRO	Clinical Research Organization
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DSMC	Data and Safety Monitoring Committee
DSMP	Data and Safety Monitoring Plan
ECOG	Eastern Cooperation Oncology Group
EMR	Electronic Medical Records
FDA	Food and Drug Administration
FWA	Federalwide Assurance
GCP	Good Clinical Practice
GWAS	Genome-Wide Association Studies
HCC	Hepatocellular Carcinoma
HI	Harmonic Imaging
HIPAA	Health Insurance Portability and Accountability Act
HVPG	Hepatic Venous Pressure Gradient
IB	Investigator’s Brochure
ICC	Intrahepatic Cholangiocarcinoma
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISPTA	Spatial-peak Temporal-average Intensity
LFT	Liver Function Test
MedDRA	Medical Dictionary for Regulatory Activities
MI	Mechanical Index
MOP	Manual of Procedures
MRI	Magnetic Resonance Imaging

mRECIST	Modified Response Evaluation Criteria in Solid Tumors
N	Number (typically refers to participants)
NCI	National Cancer Institute
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
OLS	Ordinary Least Squares
PHI	Protected Health Information
PR	Partial Response
PI	Principal Investigator
PRC	Protocol Review Committee
QA	Quality Assurance
QC	Quality Control
ROI	Region of Interest
SAE	Serious Adverse Event/Serious Adverse Experience
SDS	Safety Data Sheet (formerly MSDS; Material Safety Data Sheet)
SHAPE	Subharmonic Aided Pressure Estimation
SKCC	Sidney Kimmel Cancer Center
SoC	Standard of Care
SOP	Standard Operating Procedure
SPECT	Single Photon Emission Computed Tomography
TARE	Transarterial Radioembolization
TACE	Transarterial Chemoembolization
TJU	Thomas Jefferson University
UAP	Unanticipated Problem
UCA	Ultrasound Contrast Agent
UTMD	Ultrasound-triggered microbubble destruction
Y-90	Yttrium-90

Study Summary

Title: Contrast-Enhanced Ultrasound for Diagnosis and Therapy of Cholangiocarcinoma

Précis: 20 patients with biopsy-proven intrahepatic cholangiocarcinoma (ICC) or other non-HCC tumors in the liver will receive their standard of care (SoC) sub-lobar transarterial radioembolization (TARE), in addition to four sessions of ultrasound contrast infusion and ultrasound scanning. One session will be within 1 month prior to treatment and the other three will be at different time points after treatment. The treatment outcomes will be compared to a historical group of similar patients receiving TARE alone. The main hypotheses are that contrast-enhanced ultrasound (CEUS) can be used to predict non-HCC response to TARE prior to therapy, improve the sensitivity of these tumors to radiotherapy, and assess treatment response earlier than the current clinical standard.

Objectives: To evaluate the utility of CEUS-derived quantitative measures in predicting tumor response before and in the early phases after TARE treatment, and to evaluate the safety and efficacy of microbubble cavitation in improving TARE treatment.

Primary:

- To determine the ability of quantitative volumetric CEUS to predict tumoral response to TARE prior to therapy.

Secondary:

- To characterize the safety and preliminary efficacy of using localized UCA inertial cavitation to improve non-HCC tumor response to radioembolization.
- To determine if CEUS estimated tumor perfusion and residual vascularity can predict non-HCC tumor response to radioembolization 7-14 days post treatment.
- To evaluate tumoral response using the patient's 1 month MRI (obtained clinically) and determine the accuracy of MR or CT tumor evaluation at this earlier time point.

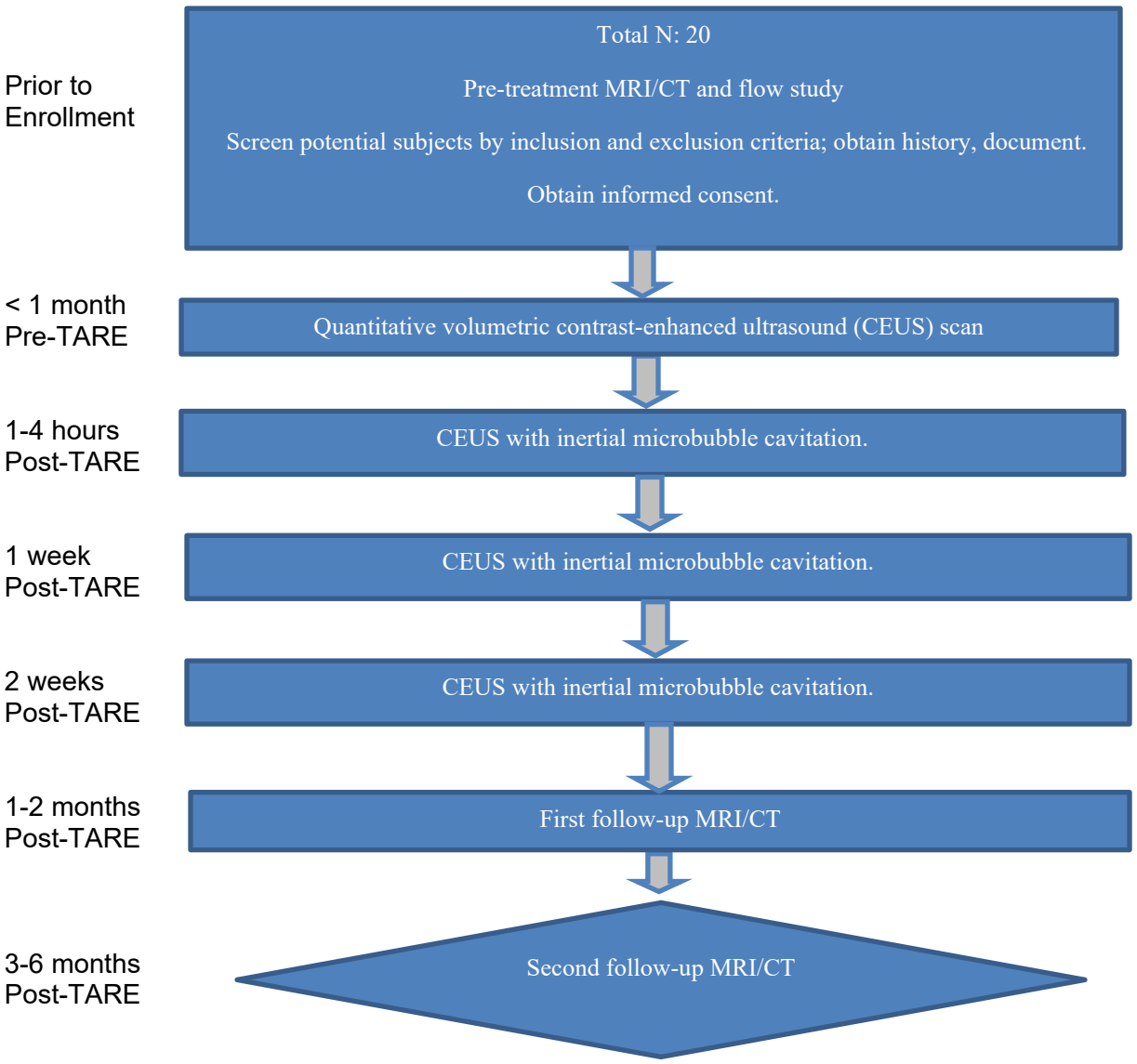
Exploratory:

- To examine the utility of SHAPE to noninvasively monitor tumoral interstitial fluid pressure (IFP) and provide an early biomarker of radiotherapy response.

Population: 20 patients with intrahepatic cholangiocarcinoma (ICC) or other non-HCC tumors receiving sub-lobar transarterial radioembolization

	(TARE). Patients at least 18 years of age, medically stable, and have a previously untreated tumor greater than 1 cm but small enough to be visualized in the ultrasound 3D volume will be included. These patients will be accrued from the Hepatology department, Interventional Radiology, and the Multidisciplinary Liver Tumor Board of the Sidney Kimmel Cancer Center at Thomas Jefferson University. All subjects must have read, signed, and dated the consent form(s) approved by the institutional review board of Thomas Jefferson University.
Phase:	Pilot
Number of Sites:	Thomas Jefferson University, Philadelphia, PA, USA
Description of Intervention:	Five ml of activated Optison will be suspended in 50 ml of saline and infused through an 18- to 22-gauge angiocatheter placed in an antecubital vein at a rate of 120 ml/hour (dosages based on prior approved Optison ultrasound-triggered microbubble destruction (UTMD) protocol and product insert) at the time points described above. All imaging will be performed using a modified Logiq E10 system with RAB6-D probe (GE Healthcare). For the second aim, a destructive (high mechanical index (MI)) pulse will be generated across the selected volume following peak enhancement (center frequency 2.1 MHz, pulse length 20 μs, line density 4). Acoustic pressure amplitudes will be adjusted as necessary to ensure inertial microbubble cavitation.
Study Duration:	24 months
Participant Participation Duration:	3-6 weeks
Estimated Time to Complete Enrollment:	36 months

Schematic of Study Design:



1 Introduction

1.1 Background Information

Intrahepatic non-HCC tumors

A variety of both primary and metastatic cancers present in the liver. Cholangiocarcinoma is an aggressive epithelial tumor arising from the lining of the biliary tract with high morbidity and extremely poor prognosis. There are three tumor subtypes defined by the anatomical region from which they arise – intrahepatic, peri-hilar and distal (extrahepatic) bile ducts. Approximately 10% of cholangiocarcinomas are intrahepatic. Intrahepatic cholangiocarcinoma (ICC) is the second most common primary liver malignancy after hepatocellular carcinoma (HCC), accounting for 10-20% of primary liver tumors[1]. Sadly, ICC incidence and mortality have been increasing in Western countries over the past two decades, highlighting the need to improve therapeutic options[2]. Additionally, many non-liver cancers will first metastasis to the liver. These include (but are not limited to) uveal melanoma, neuroendocrine tumors, pancreatic cancers, breast cancer, and colorectal cancers. Management options for non-HCC tumors in the liver are limited and the disease generally portends a poor prognosis. Liver transplant is currently not an option and while curative surgical resection is the first line of therapy in patients with ICC without extension beyond the regional lymph nodes, it is only possible in less than 30% of cases with median disease free survival of 26 months[1,3]. The majority of patients present with locally advanced or metastatic disease and are treated with systemic chemotherapy or loco-regional therapies. Liver-directed therapies including thermal ablation, transarterial chemoembolization (TACE), and transarterial radioembolization (TARE) are indicated for localized tumors[4,5].

Transarterial radioembolization

A meta-analysis of 12 studies in which 298 patients suffering ICC were treated with Yttrium-90 (Y-90) radioembolization reported overall median survival of 15.5 months[6]. TARE can be offered to patients with adequate performance status according to Eastern Cooperation Oncology Group (ECOG) performance score, blood cell count, liver function, and kidney function[4]. Additionally, TARE is being increasingly used for the treatment of other non-HCC solid tumors in the liver including colorectal cancer metastasis, uveal melanoma metastasis, and neuroendocrine metastasis. Using this approach, radioactive microspheres are delivered via a catheter placed in the hepatic artery branch supplying the tumor, thereby providing a localized and sustained release of radiation. The microspheres used are 20-30 μm diameter glass beads containing Y-90. Y-90 is a pure beta emitter. It decays to stable Zirconium-90 with a half-life of 64 hours and has an average energy emission of 0.94 MeV. The maximum tissue penetration in the liver is 10 mm [7,8,9]. Lobar doses range from 110-150 Gy, but radiation delivered to malignant tissue is dependent on distance from the yttrium source. Consequently, treatment response after radioembolization is between 25-60% when based on modified response criteria in solid tumors (mRECIST)[9,6,10].

Ultrasound contrast agents

Ultrasound contrast agents (UCA) are gas-filled microbubbles, encapsulated by a lipid or protein 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[11]. These microbubbles have been approved for use in echocardiography and also for the characterization of liver lesions in the United States, Europe and Asia[12]. UCA perfuse into the vasculature of liver tumors, and their wash-in/wash-out kinetics can be used to characterize liver masses (Figure 1). Both 2D and 4D CEUS can be acquired in hepatic tumors and used to quantify a variety of vascular parameters including blood flow dynamics and morphological features of the vasculature[13,14]. Our group has demonstrated the exceptional safety and accuracy of contrast-enhanced ultrasound (CEUS) for monitoring response to chemoembolization of HCC[15]. Importantly, we have shown that the vascular networks of HCC depicted by CEUS can be used to predict the likelihood of a response to TACE[14]. We have also shown that UCA perfuse into liver tumors post TARE, due to the fact that the larger Y-90 beads (20-30μm diameter) do not completely restrict blood flow. UCA can also be noninvasively destroyed in tissue using flash-replenishment sequences to generate inertial microbubble cavitation. Stable and inertial microbubble cavitation has demonstrated a variety of bioeffects useful for therapeutic applications[16]. Both stable and inertial cavitation have been shown to alter vascular and cellular permeability[17].

Ultrasound-triggered microbubble destruction (UTMD) has also been shown to sensitize tumors to radiotherapy by inducing vascular endothelial cell apoptosis[18,19,20]. This endothelial cell death has been attributed to increased ceramide production after interactions with inertially cavitating microbubbles[21]. Microbubble cavitation has also been shown to result in decreased tumor vascularity[22]. UTMD leads to increased DNA damage within the targeted tumor after radiotherapy, despite decreases in oxygenation[21]. Detailed parameter studies have been conducted in tumor xenografts, and synergistic combinations of UTMD and radiotherapy at mechanical indexes (MIs) > 0.8, radiation dosages of 1.8-8 Gy, and microbubble concentrations of 1-3% (v/v) have been explored[18,19]. Importantly, and yet somewhat paradoxically, UTMD in normal tissue increases vascular perfusion, due to cavitation-related increases in shear forces and activation of endothelial nitric oxide synthase[23]. Hence, UTMD combined with radiotherapy potentially offers a tumor-specific method of tissue sensitization.

Optison

The proposed agent for the current study, Optison (GE Healthcare, Princeton NJ), is a sterile non-pyrogenic suspension of encapsulated perflutren microbubbles[11,24]. 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, but within the approved dosage recommendations.

Optison Clinical Safety

Optison is well tolerated and has been used extensively in echocardiography applications[11]. 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. As a tool, contrast-enhanced ultrasound is well documented for the characterization of indeterminate liver lesions including HCC and ICC, as well as the identification of a variety of solid tumor metastasis within the liver including breast, neuroendocrine, pancreatic, and colorectal cancers[12]. Importantly, our group has also demonstrated the preliminary safety of using Optison with UTMD in patients undergoing Y-90 TARE of hepatocellular carcinoma[26].

Study goal

Our group has demonstrated the benefits of UTMD for sensitizing human HCC to radiotherapy in an orthotopic rat model[25]. More recently, we have translated this work to a first-in-humans randomized controlled clinical trial using localized UCA cavitation with 2D ultrasound within HCC vasculature to sensitize liver tumors to radiation following radioembolization (NCT# 03199274). An example imaging series from this trial is shown in Figure 1. Our recent interim analysis of this trial[26] has demonstrated this approach is safe and results in promising radiosensitization. We intend to leverage our prior data in interventional oncology-based CEUS to improve treatment of ICC and other solid tumors within the liver. Consequently, the scientific premise of this work is that volumetric CEUS can be used to predict non-HCC tumoral response to TARE prior to therapy (thereby guiding selection of treatment option), improve the sensitivity of non-HCC tumors to radiotherapy (thereby improving tumor response), and assess treatment response earlier than the current clinical standard (thereby enabling earlier tumor retreatment when necessary).

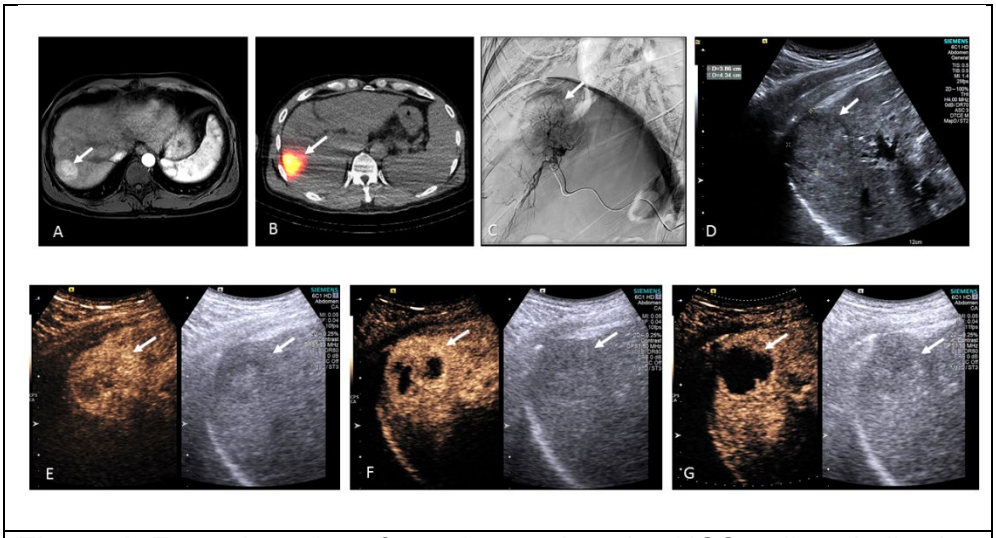


Figure 1. Example series of a patient undergoing HCC radioembolization with UTMD. The series shows baseline MRI (A), Tc-99m MAA (macroaggregated albumin) SPECT-CT during treatment planning (B), angiography during Y90 delivery (C), B-mode ultrasound immediately post radioembolization (D), and peak CEUS enhancement during UTMD 2 hours (E), 1 week (F), and 2 weeks (G) post radioembolization [Eisenbrey et al. 2021].

1.2 Rationale for the Proposed Study

Contrast-enhanced Ultrasound

The sensitivity and specificity of ultrasound imaging can be improved by intravenous (IV) injection of gas microbubbles as vascular contrast agents, which can enhance both Doppler and grayscale signals by up to 27 dB[27,11]. These contrast agents not only enhance the backscattered ultrasound signals, but at sufficient acoustic pressures they also act as nonlinear oscillators. These oscillations generate significant energy components in the received echo signals, which span the range of possible frequency emissions from subharmonics through ultraharmonics[11,28]. These nonlinear bubble echoes can be separated from tissue echoes and used to create contrast sensitive imaging modalities such as harmonic imaging (HI), which is commercially available on most state-of-the-art ultrasound scanners[27,11].

CEUS is one of the most important advances in ultrasonography over the last two decades. One example is the rapid success in detection and characterization of focal liver lesions, where CEUS in many studies have demonstrated similar value as Computer Tomography (CT) and Magnetic Resonance Imaging (MRI)[29,30,31].

Safety of ultrasound contrast agents

Ultrasound contrast agents have minimal side effects compared to CT or MRI contrast agents. They are not toxic, and do not influence the metabolism of the thyroid gland. The most serious adverse event is anaphylactic reaction. The frequency of anaphylaxis using registered ultrasound contrast agents is very low (0.001 % for ultrasound contrast agents versus 0.07 % for X-ray contrast agents)[32,33]. Contrast-enhanced ultrasound is well documented for the characterization of indeterminate liver lesions including HCC and ICC, as well as the identification of a variety of solid tumor metastasis within the liver including breast, neuroendocrine, pancreatic, and colorectal cancers[12].

Ultrasound-triggered microbubble destruction

Ultrasound-triggered microbubble destruction (UTMD) has also been shown to sensitize tumors to radiotherapy by inducing vascular endothelial cell apoptosis[18,19,20]. This endothelial cell death has been attributed to increased ceramide production after interactions with inertially cavitating microbubbles[21]. Microbubble cavitation has also been shown to result in decreased tumor vascularity[22]. UTMD leads to increased DNA damage within the targeted tumor after radiotherapy, despite decreases in oxygenation[21]. Detailed parameter studies have been conducted in tumor xenografts, and synergistic combinations of UTMD and radiotherapy at MIs > 0.8, radiation dosages of 1.8-8 Gy, and microbubble concentrations of 1-3% (v/v) have been explored [18,19.] Importantly, and yet somewhat paradoxically, UTMD in normal tissue increases vascular perfusion, due to cavitation-related increases in shear forces and activation of endothelial nitric oxide synthase[23]. Hence, UTMD combined with radiotherapy potentially offers a tumor-specific method of tissue sensitization.

Subharmonic-aided pressure estimation

Ultrasound contrast agents were proposed as pressure sensors for noninvasive pressure estimation in the cardiovascular system using a novel and innovative technique called subharmonic-aided pressure estimation (SHAPE)[34]. This method estimates internal pressure variations by transmitting at one frequency, receiving at its subharmonic frequency and then monitoring the subharmonic contrast bubble amplitude variations. We have demonstrated that the subharmonic signal component from contrast microbubbles is an excellent indicator of the hydrostatic pressure variation. High correlation ($r^2 > 0.95$) between the amplitude of the subharmonic component and hydrostatic pressure (from 0 to 186 mmHg) was obtained for several contrast agents in vitro [35].

We conducted a first-in-humans pilot study of this technique's ability to estimate portal pressures in 45 adult patients undergoing transjugular liver biopsy[36], which showed significantly higher SHAPE gradients between the portal and hepatic veins in subjects with clinically significant portal hypertension (HVPG > 10 mmHg) than in those with lower HVPGs (1.37 ± 0.59 dB vs. -1.68 ± 0.27 dB, $p<0.001$). Recently, we have expanded on the concept of using SHAPE for portal pressure estimation in a larger multi-center trial[37]. Results from 178 subjects across two sites

using modified GE Logic 9 systems (GE Healthcare) were very encouraging with an accuracy for diagnosing clinically significant portal hypertension of 95% (with a 95% confidence interval (CI): 89%-99%, p<0.001) and achieving a sensitivity of 91% (95% CI: 88%-93%) and a specificity of 82% (95% CI: 75%-85%). SHAPE offers the possibility of allowing pressure gradients in interstitial fluid pressure (IFP) in tumors to be obtained noninvasively[36,38]. The role of tumoral IFP in radiation response remains relatively understudied due to difficulties obtaining these measurements with traditional wick-in-needle approaches[39]. However, it has been proposed that tumor IFP drops following sufficient radiation exposure[39,40,41]. We hypothesize that tumoral IFP estimated via SHAPE can be used as a biomarker to predict tumor response to TARE therapy.

1.3 **Correlative Studies**

Abdominal MRI/CT 3 months to two weeks before study inclusion and approximately 1-2 months and 3-6 months after treatment will be collected (as part of SoC). Post-treatment single-photon emission computed tomography (SPECT) imaging will be evaluated for all patients to evaluate if the initial UTMD session skews microsphere distribution in the liver. After the study period, the patients' records will be monitored until progression or death is documented. Each patient's time to required next treatment and overall survival will be recorded. The clinical outcomes and MRI/CT results will used for evaluation of responses and local primary tumor progression.

1.4 **Potential Risks and Benefits**

Optison is well tolerated and has been used extensively in echocardiography applications[11]. Ultrasound contrast agents in general have a very favorable safety profile[42,43,44] with a serious adverse even risk below 0.03%. 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. Additionally, contrast-enhanced ultrasound is well documented for the characterization of indeterminate liver lesions including HCC and ICC, as well as the identification of a variety of solid tumor metastasis within the liver including breast, neuroendocrine, pancreatic, and colorectal cancers[12].

1.4.1 **Potential Risks**

Serious cardiopulmonary and allergic reactions including fatalities have occurred during or following administration of Optison, causing the food and drug administration (FDA) to place a black box warning on the agent. However these occurrences have been rare (less than 1 in 5,000 patients). As a result, patients with unstable cardiopulmonary conditions will be excluded. 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. Patients will also be monitored for 30 minutes after contrast administration for any adverse reactions.

Clinically significant adverse effects from the administration of Optison are unlikely. This approach is similar to our ongoing randomized control trial using 2D UTMD to sensitize HCC to TARE. An interim analysis from this study was recently published and demonstrated that using 2D UTMD in HCC patients is both safe and effective[26]. Safety results from 28 patients have demonstrated no significant changes in vital signs pre- and post-UTMD (or significant changes in LFTs between patients receiving TARE alone or UTMD combined with TARE ($p>0.25$). In addition, no radiopharmaceutical activity was detected outside the liver on SPECT, ruling out non-target embolization in patients who received UTMD.

The use of an intravenous needle and the fluids given through the needle may cause minor discomfort, bleeding under the skin (bruise), and possible infection at the site of needle insertion. Based on the available non-clinical and clinical safety data and the dosage levels of Optison that will be used in this study, safety concerns are minimal. The potential side effects related to Optison administration are described in Table 1 above.

Optison destruction within the nontumor liver may sensitize this tissue to Y-90 beta emissions. However, destruction will be primarily located within the target area due to selective placement of the flash/replenishment ROI. Additionally, liver parenchyma outside the treatment area will have limited radiation exposure due to the selective delivery of Y-90 as well as the limited penetration of its beta emissions.

To minimize and/or eliminate risks a nurse will be present during the entire procedure. Adverse events will be monitored during the entire procedure and for 30 minutes post injection.

1.4.2 **Benefits**

Patients may potentially benefit from being in this study by experiencing improved tumoral outcomes to radiotherapy as a result of CEUS. Based on our recent animal data[25] and preliminary human data[26] shown in figure 1, we expect a 40-60% improvement in tumor response when radioembolization is combined with microbubble cavitation. CEUS-based data will also be collected as a means for predicting tumor response, but no treatment decisions will be based on this information.

This study aims to define the efficacy and safety of using volumetric microbubble cavitation for sensitizing malignant tissue to radiotherapy and predicting treatment outcomes using CEUS-based perfusion estimation. Once properly validated, this technique is ultimately expected to improve patient outcomes by locally and selectively sensitizing malignant tissue to Y90 radiotherapy, identifying which patients would most benefit from TARE, and by enabling alternative forms of treatment through earlier identification of patients not responding to initial/first-

line therapy. In addition, findings are expected to be applicable to other solid tumor applications in radiation oncology and microbubble cavitation research.

2 Study Objectives

2.1 Objectives

To evaluate the utility of CEUS in prediction of tumoral response to TARE prior to therapy, improving the sensitivity of non-HCC tumors to radiotherapy, and assessment of treatment response earlier than the current clinical standard.

2.1.1 Primary

To determine the ability of quantitative volumetric CEUS to predict non-HCC tumor response to TARE prior to therapy. Existing volumetric parametric imaging algorithms will also be employed to quantify vascular heterogeneity, volumetric perfusion, and area under the curve will be quantified. These variables will then be compared to modified response evaluation criteria in solid tumors (mRECIST) findings 3-6 months post treatment to identify CEUS-derived hallmarks of tumor vascularity that predict treatment response.

2.1.2 Secondary

- To characterize the safety and preliminary efficacy of using localized UCA inertial cavitation to improve tumoral response to radioembolization.
- To determine if CEUS estimated tumor perfusion and residual vascularity 7-14 days post treatment can predict tumoral response to radioembolization.
- To evaluate tumoral response using the patient's 1 month MRI (obtained clinically) and determine the accuracy of MR or CT tumor evaluation at this earlier time point.

2.1.3 Exploratory

To examine the utility of SHAPE to noninvasively monitor tumoral interstitial fluid pressure (IFP) and provide an early biomarker of radiotherapy response.

2.2 Endpoints/Outcome Measures

2.2.1 **Primary**

Tumor complete response (CR) and partial response (PR) on mRECIST reads of contrast-enhanced CT or MRI obtained 3-6 months after treatment determined by two independent readers

2.2.1 **Secondary**

Tumor complete response (CR) and partial response (PR) on mRECIST reads of contrast-enhanced CT or MRI obtained 3-6 months after treatment determined by two independent readers

Changes in Liver Function Tests (LFTs)

Observed and self-reported adverse events

Time required to next treatment

Overall survival

2.2.2 **Exploratory**

Tumor complete response (CR) and partial response (PR) on mRECIST reads of contrast-enhanced CT or MRI obtained 3-6 months after treatment determined by two independent readers

3 **Study Design**

3.1 **Characteristics**

Patients at least 18 years of age, medically stable with a previously untreated non-HCC tumor in the liver greater than 1 cm but small enough to be visualized in the ultrasound 3D volume, will be included. These patients will be recruited from the Hepatology department, Interventional Radiology, and the Multidisciplinary Liver Tumor Board of the Sidney Kimmel Cancer Center at Thomas Jefferson University.

3.2 **Number of Participants**

20 patients

3.3 **Duration of Therapy**

Patients will receive a quantitative volumetric CEUS scan < 1 month before TARE therapy. Consequently, they will receive UTMD sessions 1-4 hours, approximately 1 week, and approximately 2 weeks after TARE therapy. The entire ultrasound imaging protocol will require approximately 1.5 hours including a 30 min observation period.

3.4 Duration of Follow Up

After the subjects have completed treatment, they will be followed through the electronic medical record (EMR) system for up to 1 year to document long term outcomes.

3.5 Treatment Assignment Procedures

3.5.1 Randomization Procedures (if applicable)

This is a non-randomized trial; therefore, no randomization procedure required.

3.6 Study Timeline

3.6.1 Primary Completion

Primary completion is anticipated in June of 2025.

3.6.2 Study Completion

Study completion and publication of results is anticipated in July of 2025

4 Study Enrollment and Withdrawal

Patients at least 18 years of age, medically stable who have a previously untreated intrahepatic non-HCC tumor greater than 1 cm but small enough to be visualized in the ultrasound 3D volume will be included. These patients will be recruited from the Hepatology department, Interventional Radiology, and the Multidisciplinary Liver Tumor Board of the Kimmel Cancer Center at Thomas Jefferson University.

A sufficient number of patients will be screened in order to enroll 20 patients with non-HCC tumors receiving TARE over the duration of the study.

Patients who are discontinued from the study prior to the completion of all planned procedures and assessments will not be replaced.

Each subject is identified in the study by a unique subject number that is assigned when subject enrolls after signing the Informed Consent Form. Once assigned the subject number cannot be reused for any other subject. The same primary identifier will be used throughout the study.

4.1 Eligibility Criteria

4.1.1 Inclusion Criteria

The patients enrolled in this project will be adults over the age of 18 capable of providing written informed consent. Based on the study protocol and Optison contraindications, inclusion criteria for this trial are as follows:

- Be scheduled for sub-lobar radioembolization therapy of a previously untreated non-HCC tumor greater than 1 cm but small enough to be fully visualized in the ultrasound 3D volume (approximately 6 cm maximum diameter, but depth dependent)
- 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.

4.1.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

Exclusion criteria for this study will consist of:

- Females who are pregnant or nursing.
- Patients with recent cerebral hemorrhage.
- Patients with known sensitivities to albumin, blood, or blood products
- Patients with known hypersensitivity to perflutren
- Patients with known congenital heart defects.
- Patients with severe emphysema, pulmonary vasculitis, or a history of pulmonary emboli.
- Patients with bilirubin levels > 2 mg/dL

4.2 Gender/Minority/Pediatric Inclusion for Research

The subject population for this study will be made up of approximately 30% women based upon a retrospective review of the demographics of the population presenting with non-HCC tumors at the Oncology section of Thomas Jefferson University.

No patients will be excluded on the basis of race. The patient population of this project will reflect the population demographics found at major American urban academic health centers who present with cancer of the intrahepatic bile duct. The overall hospital demographics for TJU include 58-66 % Caucasian, 28-30 % African American, and 2-4 % Asian, with 10-12 % representing Hispanic patients.

No children will be included. The patients enrolled in the human studies portion of this project will be adults over the age of 18 diagnosed with non-HCC tumors, who are scheduled to receive sub-lobar TARE.

4.3 Strategies for Recruitment and Retention

Suitable subjects referred to the Division of Interventional Radiology within the Department of Radiology at Thomas Jefferson University for radioembolization of non-HCC tumors. There will be no further recruitment of subjects. The research coordinators for the study will invite the subjects to participate in the study. The study will be explained to potential subjects and, if they would like to participate, they will be given an informed consent to read. The consent will be reviewed with the patient, providing the patients time to consider participation in the study and ask questions about the research study. The subject will sign the consent in the presence of a co-investigator and will receive a copy of the signed consent form.

4.4 Participant Withdrawal

4.4.1 Reasons for Withdrawal

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may terminate a study participant's participation in the study if:

- Any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.
- The participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.

4.4.2 Handling of Participant Withdrawals and Participant Discontinuation of Study Intervention

Patients who withdraw or are withdrawn from the study, will stop further UTMD procedures, as described by the study protocol. The reason for discontinuation shall be recorded. The investigator will follow up any significant adverse events related to the novel agent with appropriate clinical care.

4.5 Premature Termination or Suspension of Study

This study may be suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to all regulatory authorities. If the study is prematurely terminated or suspended, the principal investigator will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants.
- Insufficient adherence to protocol requirements.
- Data that is not sufficiently complete and/or evaluable.
- Determination of futility.

5 Study Intervention

5.1 Study Product

One ultrasound contrast agent will be used (Optison). The agent will be used as an off-label indication for this study, but within the approved dosage recommendations. Optison is a sterile non-pyrogenic suspension of encapsulated perflutren microbubbles. The contrast agent consists of a human serum albumin shell with perflutren core and contains 5.0 - 8.0 x 108 microspheres/ ml.

5.1.1 Acquisition

The ultrasound contrast agent will be purchased from GE Healthcare, Princeton, NJ.

5.1.2 Formulation, Packaging, and Labeling

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.

5.1.3 Product Storage and Stability

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.

5.2 Dosage, Preparation, and Administration

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 18- to 22-gauge angiocatheter placed in a peripheral arm vein, preferably an antecubital vein. Five milliliters of Optison will be activated 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 UCA.

5.3 Dose Modifications and Dosing Delays

The standard dose of Optison is 5 mL that will be activated and suspended in a 50 ml bag of saline. The Optison mixture will then be infused through the IV at a rate of 120 ml/hour. The reported adverse event (AE) rates at the dosages employed in this study are very low, but have not been fully evaluated in conjunction with radioembolization. No reasons for dose modifications for Optison are considered.

5.4 Study Product Accountability

The study coordinators at each site will keep an electronic log accounting for the use of each kit of Optison and individual vials based on the sign-out sheets.

5.5 Assessing Participant Compliance with Study Product Administration

Compliance will be recorded through attendance for the scheduled visits.

5.6 Concomitant Medications/Treatments

No restrictions related to concomitant medications or treatments are considered.

5.7 Dietary Restrictions

No special dietary or “life-style” requirements are needed during the infusion time.

5.8 Study Procedural Intervention(s) Description

All imaging will be performed using a modified Logiq E10 system with RAB6-D probe (GE Healthcare). A brief abdominal ultrasound (without contrast) will be performed prior to

radioembolization to ensure the target lesion is visible on ultrasound. Volumes of the mass on B-mode and power Doppler will be acquired for later processing.

For the pretreatment exam, 5 ml of activated Optison will be suspended in 50 ml of saline and infused through an 18- to 22-gauge angiocatheter placed in an antecubital vein at a rate of 120 ml/hour (dosages based on prior approved Optison UTMD protocol and product insert). Following confirmation of UCA arrival, 4D CEUS data will be acquired. Imaging parameters (gain, MI, frequency preset) will be optimized on a patient-by-patient basis, while still acquiring adequate signal at the required depth. The volumetric region of interest will be placed to encompass the entire lesion and surrounding normal liver tissue while still being minimized enough to ensure an adequate volume acquisition rate[45] (0.5-2 volumes/second in our experience). Every 60 seconds, microbubbles within the volume will be destroyed using a 4 second flash pulse (MI>1.1), followed by lower MI (<0.15) nondestructive imaging to monitor tumor reperfusion. Data from > 5 flash-replenishment cycles will be obtained, allowing us to define intra-patient variability for each quantitative parameter. For the last 2 minutes of the infusion, a SHAPE acoustic output optimization algorithm will be initiated on the E10 system[46]. Following identification of the optimal acoustic output, 10 seconds of 4D SHAPE data will then be collected from both the tumor and surrounding liver tissue in triplicate. After complete washout of the UCA (around 10 minutes) baseline 4D SHAPE data at the optimal acoustic output without bubbles present will be acquired. This acquisition involves standard ultrasound only and can be obtained during the 30 minutes post-contrast observation period.

Post treatment ultrasound exams will take place 2-4 hours post radioembolization in Interventional Radiology's recovery unit prior to single photon emission computed tomography (SPECT) imaging, and again 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. Specific imaging time points are based on times when patients are present or returning to the hospital for regular follow up, experiences with our study enrollment in ongoing HCC trials, and at a time when the Y90 spheres are still active. All contrast injections will be supervised by a board certified physician (Drs. Anton, Civan, O'Kane, or Lyshchik) with resuscitation equipment in immediate proximity during CEUS exam. Patient vital signs will be monitored and recorded throughout the visit. Tumor characteristics (size and location) will also be recorded for subset analysis to study how these factors influence treatment.

Similar to the pre-treatment exam, B-mode and Doppler volumes will be acquired for each exam post-TARE. Following these acquisitions, 5 milliliters of activated Optison will be suspended in 50 ml of saline and infused through an 18- to 22-gauge 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). Following peak enhancement, a destructive (high MI) pulse will be generated across the selected volume. This will be performed using a modified software platform (created as part of R01 CA199646) that can be customized for transmission of UTMD flash replenishment sequences across a volume. The system will be set a center frequency of 2.1 MHz, with pulse lengths of 20 μs and a line density of 4. Acoustic pressure amplitudes will be adjusted as necessary to ensure inertial microbubble cavitation. Importantly, this research software system provides range gated

detection of stable and inertial cavitation through analysis of received radiofrequency data and real time display of spatial-peak temporal-average intensity (ISPTA). For each new patient, an ISPTA of 300 mW/cm^2 will be used as a starting point and increased in 50 mW/cm2 increments until inertial cavitation is detected. However, the system is also limited to not exceed any acoustic output limits set by the FDA and the MI will be kept well below 1.7. This acoustic output setting will then be used for all further UTMD sequences. UTMD sequences followed by 10 second low MI microbubble replenishment sequences will then be repeated across the tumor volume for the remainder of the infusion (approximately 20-30 sequences). Finally, for the last 30 seconds of the infusion, 4D SHAPE data will be acquired using the optimal acoustic output determined at baseline. The patient will be monitored for 30 minutes per our institutional CEUS protocol before being discharged (during which time the baseline 4D SHAPE data will be obtained).

5.9 Administration of Procedural Intervention

Imaging will be performed an ultrasonographer with over 10 years of experience in liver imaging. All contrast injections will be performed by a research nurse with CEUS experience under with resuscitation equipment in immediate proximity. All imaging will be performed using a modified Logiq E10 system with RAB6-D probe (GE Healthcare).

5.10 Procedures for Training of Clinicians on Procedural Intervention

The personnel performing the UTMD procedures are all from the Ultrasound Research lab and have extensive experience with CEUS and ultrasound imaging procedures. These individuals will all receive training by the PI in this specific protocol.

5.11 Assessment of Clinician and/or Participant Compliance with Study Procedural Intervention

Compliance of the subjects will be recorded through attendance for the scheduled visits. There are no clinicians outside of the Ultrasound Research lab involved in administrating the UTMD procedure.

6 Study Schedule

6.1 Pretreatment Period/Screening

Subjects eligible for trial enrollment will be identified by Drs. Anton, Gonsalves, and Civan from their patient population (approximately 50-60 eligible patients per year recruited across 4 Jefferson Health campuses and treated in our main Center City Hospital in Philadelphia). Patients will be accrued from the Hepatology department, Interventional Radiology, and the

Multidisciplinary Liver Tumor Board of the Sidney Kimmel Cancer Center at Thomas Jefferson University.

6.2 Enrollment/Baseline

Study Entry visit (Day 0)

Screening assessments will be performed when the patients are on campus for their pre-treatment evaluation. 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 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. Pre-treatment volumetric CEUS exam will be performed within 1 month prior to radioembolization treatment.

6.3 Treatment Period

Transarterial Radioembolization (TARE) treatment visit (Day 1-31)

- A brief abdominal ultrasound (without contrast) will also be performed prior to radioembolization to ensure the target lesion is visible on ultrasound.
- Post treatment CEUS exams with microbubble cavitation 1-4 hours after TARE

Post-TARE visit 1 (Day 8-38)

- Post treatment CEUS exams with microbubble cavitation approximately 1 week after TARE

Post-TARE visit 2 (Day 15-45)

- Post treatment CEUS exams with microbubble cavitation approximately 2 weeks after TARE

6.4 Long Term/Survival Follow-up

After the subjects have completed treatment, they will be followed through the EMR system to for up to 1 year document long term outcomes.

6.5 Withdrawal Visit/Discontinuation of Therapy

Patients who withdraw or are withdrawn from the study, will stop further CEUS procedures, as described by the study protocol. The reason for discontinuation shall be recorded. The investigators will follow any patient who develops significant adverse events.

Patients may be discontinued from study treatment and assessments at any time. Specific reasons for discontinuing a patient for this study are:

- Voluntary discontinuation by the patient who is at any time free to discontinue his/her participation in the study, without prejudice to further SoC treatment.
- Patient lost to follow-up.
- Safety reason as judged by the Principal Investigators.
- Severe non-compliance to protocol as judged by the Principal Investigators.
- Incorrect enrolment i.e., the patient does not meet the required inclusion/exclusion criteria for the study.
- Disease progression and/or rapidly deteriorating ECOG performance status.
- Deterioration in the patient's condition which in the opinion of the Principal Investigator warrants UTMD discontinuation (to be recorded as an AE or under Investigator Discretion)
    - Patient requires dialysis at any visit
    - Deteriorating liver function
- Related Serious Adverse Event
- A female patient becoming pregnant
- Any reason why, in the opinion of the investigator, the patient should not participate

The whole trial may be discontinued at the discretion of the PI or the sponsor in the event of any of the following:

- Occurrence of AEs unknown to date in respect of their nature, severity and duration
- Medical or ethical reasons affecting the continued performance of the trial

The sponsor and principal investigator will promptly inform all investigators and the relevant regulatory authorities of the termination of the trial along with the reasons for such action

7 Study Procedures and Evaluations

7.1 Study Procedures/Evaluations

- Medical history will be obtained from the electronic medical records (EMR) system and during the first study visit < 1 month before the TARE.

- Volumetric CEUS exam will be obtained approximately < 1 month before the treatment as described in section 5.8.

- Three CEUS exam with microbubble cavitation will be performed 2-4 hours, 1 week, and 2 weeks after treatment as described in section 5.8.

8 Evaluation of Safety

8.1 Specification of Safety Parameters

8.1.1 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.

8.1.2 Adverse Events

An AE 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. . All AEs, including observed or volunteered problems, complaints, signs or symptoms, and diagnoses, occurring from the study treatment will be recorded on a serious or non-serious AE data form. Whenever possible, the AE will be evaluated and reported as a diagnosis rather than individual signs and symptoms. If a definitive diagnosis is not possible, the individual signs and symptoms will be recorded. The investigator will evaluate and note the duration, intensity, and relationship to (association with) the treatment or imaging procedures, the action taken, and the determination of seriousness for each AE. The intensity of the AE will be characterized as mild, moderate, or severe.

Optison is well tolerated and has been used extensively in echocardiography applications. 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. All of the non-serious reported side effects have been transient, usually lasting only a few minutes. Please see table 1 and the attached Optison Product insert (Appendix B).

8.1.3 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.

8.2 **Safety Assessment and Follow-Up**

The PI will follow adverse events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. 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.

8.3 **Recording Adverse Events**

The following subsections detail what information must be documented for each AE occurring during the time period specified in Section 8.2 Safety Assessment and Follow-Up.

If the patient has experienced AE(s), the investigator will record the following information in the AE log:

- The nature of the event(s) will be described by the investigator in precise standard medical terminology (i.e. not necessarily the exact words used by the patient).
- The duration of the event will be described in terms of event onset date and event ended data.
- The intensity of the AE will be described according to Common Terminology Criteria for Adverse Events version 5.0 (CTCAE), for details see National Cancer Institute home page http:\evs.nci.nih.gov\ftp1\CTCAE\About.html of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:
    - Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
    - Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living.
    - Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care activities of daily living.
    - Grade 4: Life-threatening consequences; urgent intervention indicated.
    - Grade 5: Death related to AE.
- The Causal relationship of the event to the study medication will be assessed as one of the following:
    - Unrelated: There is not a temporal relationship to investigational product administration (too early, or late, or investigational product not taken), or there is

a reasonable causal relationship between noninvestigational product, concurrent disease, or circumstance and the AE.
    - Unlikely: There is a temporal relationship to investigational product administration, but there is not a reasonable causal relationship between the investigational product and the AE.
    - Possible: There is reasonable causal relationship between the investigational product and the AE. Dechallenge information is lacking or unclear.
    - Probable: There is a reasonable causal relationship between the investigational product and the AE. The event responds to dechallenge. Rechallenge is not required.
    - Definite: There is a reasonable causal relationship between the investigational product and the AE.
- The Expectedness of the AE
- Action taken
- The outcome of the AE – whether the event is resolved or still ongoing.

8.3.1 **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:

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.

8.3.2 **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.

8.3.3 **Severity of Event**

Adverse events will be graded for severity according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

8.3.4 **Intervention**

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

8.4 **Safety Reporting**

All adverse events and serious adverse events that should be reported as defined in section 8.1.1 will be recorded in the patient's CRF.

All events will be graded by the Investigator according to the CTCAE version 5.0. A grading (severity) scale is provided for each AE term. Every SAE must be documented by the investigator on the SAE pages (to be found as part of the CRF). The SAE Report Form must be completed and signed. SAEs and possible SAEs will be reported to the regulatory authorities as described below. The initial report shall promptly be followed by detailed, written reports if necessary. The initial and follow-up reports shall identify the trial subjects by unique code numbers assigned to the latter.

The sponsor keeps detailed records of all SAEs reported by the investigators and performs an evaluation with respect to causality and expectedness. Based on, among other, SAE reports the sponsor will evaluate whether the risk/benefit ratio associated with study is changed.
We will in all cases follow the ICH Harmonised Guideline Integraded Addendum to ICH E6(R1): Guideline for Good Clinical Practise ICH E6(R2) ICH Consensus Guideline. Link: https://ichgcp.net/411-safety-reporting/

8.4.1 **Reporting to IRB**

8.4.1.1 ***Unanticipated Problems***

All incidents or events that meet criteria for unanticipated problems (UAPs) as defined in Section 8.1.1 Unanticipated Problems 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 10 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.

8.4.1.2 *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.

8.4.1.3 *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.

8.4.2 **Reporting to SKCC DSMC**

All AEs and SAEs, safety and toxicity data, and any corrective actions will be submitted to the DSMC per the frequency described in the SKCC DSMP. The report to the SKCC 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 table below:

DSMC AE/SAE Reporting Requirements

	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)

8.4.3 Reporting to Funding Sponsor

Once a year throughout the clinical trial, the sponsor will provide the Competent Authority with a listing of all suspected serious adverse reactions which have occurred over this period and a report of the subjects' safety. Annual reports are submitted in accordance with ICH guideline E2F - Note for guidance on development safety update reports.

8.4.4 Reporting to FDA

The PI and the SKCC regulatory team will be responsible for submitting annual reports to the FDA on the progress of the study. For event reporting, any event that is both serious and unexpected must be reported to the Food and Drug Administration (FDA) as soon as possible and no later than 7 days (for a death or life-threatening event) or 15 days (for all other SAEs) after the investigator's or institution's initial receipt of the information. Thomas Jefferson University will submit SAE reports to the FDA. The TJU Study Site Contact is responsible for submitting SAEs to the funder that occur at the affiliate site.

SAEs should be reported on MedWatch Form 3500A, which can be accessed at: http://www.accessdata.fda.gov/scripts/medwatch/

MedWatch SAE forms should be sent to the FDA at:

MEDWATCH
5600 Fishers Lane
Rockville, MD 20852-9787
Fax: 1-800-FDA-0178 (1-800-332-0178)
http://www.accessdata.fda.gov/scripts/medwatch/

- An SAE report should be completed for any event where doubt exists regarding its seriousness.
- For studies with long-term follow-up periods in which safety data are being reported, include the timing of SAE collection in the protocol.
- If the investigator believes that an SAE is not related to study drug, but is potentially related to the conditions of the study (such as withdrawal of previous therapy or a complication of a study procedure), the relationship should be specified in the narrative section of the SAE Report Form.
- If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported.)

8.4.5 Reporting of Pregnancy

Pregnancy is an exclusion criteria and women of a child-bearing potential must use adequate protection measures. If a subject becomes pregnant during the study they will be discontinued from the study.

8.5 Halting Rules

The whole trial may be discontinued at the discretion of the PI or the sponsor in the event of any of the following:

- Occurrence of AEs unknown to date in respect of their nature, severity and duration
- Medical or ethical reasons affecting the continued performance of the trial

9 Study Oversight

In addition to the PI's responsibility for oversight, study oversight will be under the direction of the SKCC's Data and Safety Monitoring Committee (DSMC). The SKCC DSMC operates in compliance with a Data and Safety Monitoring Plan (DSMP) that is approved by the NCI.

10 Clinical Site Monitoring and Auditing

Clinical site monitoring and auditing is conducted to ensure that the rights of human participants are protected, that the study is implemented in accordance with the protocol and/or other operating procedures, and that the quality and integrity of study data and data collection methods are maintained. Monitoring and auditing for this study will be performed in accordance with the SKCC's Data and Safety Monitoring Plan (DSMP) developed by the SKCC Data and Safety Monitoring Committee (DSMC). The DSMP specifies the frequency of monitoring, monitoring procedures, the level of clinical site monitoring activities (e.g., the percentage of participant data to be reviewed), and the distribution of monitoring reports. Some monitoring activities may be performed remotely, while others will take place at the study site(s). Appropriate staff will conduct monitoring activities and provide reports of the findings and associated action items in accordance with the details described in the SKCC DSMP.

11 Statistical Considerations

11.1 Study Hypotheses

H1: Contrast reperfusion time, degree of tumor enhancement relative to the surrounding parenchyma, tumoral IFP and 3D morphological features of the vasculature quantified via CEUS before therapy correlate with SoC mRECIST reads of non-HCC tumor patients treated with TARE.

H2: mRECIST reads for non-HCC tumor patients receiving TARE combined with microbubble inertial cavitation have more complete response (CR) and partial response (PR) reads compared to the historical group treated with TARE alone.

H3: Patient's time to required next treatment and overall survival are longer for non-HCC tumor patients receiving TARE combined with microbubble inertial cavitation compared to the historical group treated with TARE alone.

H4: Observed and self-reported adverse events, and changes in liver function tests (LFTs) one month post-TARE in non-HCC tumor patients receiving TARE combined with microbubble inertial cavitation are similar compared to the historical group treated with TARE alone.

H5: Values of tumor perfusion and residual vascularity quantified by CEUS 2-4 hours, 1 week, and 2 weeks after TARE therapy are lower in CR and PR mRECIST reads of non-HCC tumor patients treated with TARE.

H6: Reductions of tumor perfusion and residual vascularity values quantified by CEUS 2-4 hours, 1 week, and 2 weeks after TARE therapy are more frequent in CR and PR mRECIST reads of non-HCC tumor patients treated with TARE.

H7: Tumoral response in mRECIST reads of MRI or CT (obtained clinically from patients treated with TARE) at 1 month post treatment will agree with response in their mRECIST reads at 3-6 months.

H8: Values of IFP quantified by SHAPE 2-4 hours, 1 week, and 2 weeks after TARE therapy are lower in CR and PR mRECIST reads of patients treated with TARE.

H9: Reductions of IFP values quantified by SHAPE 2-4 hours, 1 week, and 2 weeks after TARE therapy are more frequent in CR and PR mRECIST reads of patients treated with TARE.

11.2 **Analysis Plans**

In general, categorical data will be summarized as counts and percentages (or proportions) and continuous data will be summarized with descriptive statistics such as mean, standard deviation (SD), median, and range. All statistical analysis will be performed by Dr. Keith using SAS version 9.4 (Cary, NC). All statistical tests will be conducted at a nominal α=0.05 level. For H1 (see Section 11.1 above), the respective relationships between the CEUS-derived contrast reperfusion time, degree of tumor enhancement relative to the surrounding parenchyma, tumoral IFP and 3D morphological features of the vasculature with the patients' subsequent mRECIST diagnosis (3 levels: stable disease, partial response, or complete response) will be evaluated by testing for mean difference in the CEUS measures between response levels by analysis of variance (ANOVA). Historical controls (n=20) will be identified from a pool of recently treated non-HCC patients at Jefferson that meet study eligibility criteria for the study and match our enrollees by propensity score based on a logistic regression model including patient age, tumor size, bilirubin and ECOG performance score and utilizing a greedy matching algorithm. Testing for the effect of radioembolization on tumor response in H2 will be conducted using non-parametric Mann-Whitney U-tests of the differences in mRECIST distributions between historical controls and enrolled patients. The difference in time-to-event endpoints (i.e., to next treatment or death, respectively) between the treated and historical control patients for H3 will be estimated and tested using Kaplan-Meier survival methods and log-rank tests. Differences in safety data will be evaluated for H4 with descriptive statistics, but not statistical hypothesis tests. A similar ANOVA approach taken for evaluating H1 will be taken for evaluating the means of CEUS measures at different times (2-4 hours, 1 week, and 2 week, respectively) of tumor perfusion and residual vascularity values between response levels to address H5 and SHAPE IFP values for addressing H8, as well as the means of changes from baseline in these measure for addressing H6 and H9. The agreement on response between mRECIST response diagnoses at 1 month and 3-6 months post-TARE will be tested using McNemar's test.

11.3 Interim Analyses and Stopping Rules

This is a pilot study, no interim analysis will be considered.

11.3.1 Safety Review

Safety outcomes will be compared between the treatment group and the historical group using observed and self-reported adverse events, and changes in LFTs one month post-embolization (performed as part of clinical standard of care). LFTs will include blood tests for alanine aminotransferase, alkaline phosphatase, asparate aminotransferase, bilirubin, albumin, total protein, and prothrombin time. SPECT imaging will be evaluated for all patients by two blinded radiologists to evaluate if the initial UTMD session skews microsphere distribution in the liver.

11.3.2 Efficacy Review

The primary measure of tumor response will be evaluated using mRECIST criteria as determined by Dr. Lyshchik and O'Kane in consensus using 3-6 month contrast enhanced MRI or CT (blinded to patient group). Following a wait period and randomization of the patient order, both readers will also evaluate tumoral response using the patient's 1 month MRI (obtained clinically) to determine the accuracy of MR or CT tumor evaluation at this earlier time point and its use as an alternative to CEUS. Finally, each patient's time to required next treatment and overall survival will be monitored, although this is a secondary outcome for this study and expected to surpass the timeline of this study.

11.4 Sample Size Considerations

Sample size for this pilot study is primarily driven by patient availability and larger, multi-center randomized trials will likely be needed to fully demonstrate efficacy. However, for the primary objective, a sample size of 20 patients (of which we expect 25% having stable disease, 50% partial responders, and 25% complete responders) will provide 80% power to detect a 0.76 standard deviation difference in CEUS measurement means (e.g., mean contrast perfusion time) between mRECIST response levels, by analysis of variance (ANOVA) with a nominal 5% type I error rate. Similarly, in analyses comparing the prospective treated patients to matched historical control patients, 20 patients per arm will provide 80% power to detect a relative effect size of 0.66 standard deviations by t-test with a nominal 5% type I error rate (i.e., a moderately large, but reasonable effect given our current data in HCC patients).

11.4.1 Replacement Policy

Subjects that withdraw from the study will not be replaced. If a sufficiently high number of subjects withdraw, the protocol will be amended to allow for greater enrollment.

11.4.2 Accrual Estimates

We estimate it will take 2 years to recruit 20 subjects for this trial.

11.5 **Exploratory Analysis**

Exploratory analysis is anticipated for the associations between IFP or reductions in IFP and mRECIST response. The plans for that analysis are included in the statistical analysis plans above.

11.6 **Evaluation of Safety**

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE or SAE. Each patient will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.

To determine the safety profile of the treatment the following safety assessments will be done:

- Clinical laboratory tests
- Physical examinations
- Sitting vital signs, body temperature and body weight
- Adverse events registration with attribution in accordance with section 8.3.1

The methods for collection safety data are described below.

Expected AE's for the SoC TARE and the ultrasound contrast agent (i.e., Optison) will be recorded.

12 **Source Documents and Access to Source Data/Documents**

Study staff will maintain appropriate medical and research records for this study, in compliance with ICH E6, and regulatory and institutional requirements for the protection of confidentiality of participant information. Study staff will permit authorized representatives of SKCC and regulatory agencies to examine (and when required by applicable law, to copy) research records for the purposes of quality assurance reviews, audits, and evaluation of the study safety, progress and data validity.

Source Data

The medical records for each patient should contain information which is important for the patient's safety and continued care and to fulfil the requirement that critical study data should be verifiable. To achieve this, the medical records of each patient should clearly describe at least:

- That the patient is participating in the study e.g., by including the enrollment number and the study code or other study identification;
- Date when Informed Consent was obtained from the patient for the following and statement that patient received a copy of the signed and dated Informed Consent;
- Results of all assessments confirming a patient's eligibility for the study;
- Diseases (past and current; both the disease studied and others, as relevant);
- Surgical history, as relevant;
- Treatments withdrawn/withheld due to participation in the study;
- Results of all assessments performed during the study;
- WHO performance status assessments conducted as part of the study;
- Treatments given, changes in treatments during the study and the time points for the changes;
- Visits to the clinic during the study, including those for study purposes only;
- AEs and SAEs (if any) including causality assessments;
- Date of, and reason for, discontinuation from study treatment;
- Date of, and reason for, withdrawal from study;
- Date of death and cause of death, if available;
- Additional information according to local regulations and practice.

Source Data Verification

The investigator will be visited on a regular basis by the Study Monitor. Monitoring will include source data verification (SDV) and discuss the progress of the study. SDV is confirmed by comparing completed CRFs with matched source documentation in subject's research binder. The PI will perform spot checks to verify CRFs and database entries match source documents. Verified data is entered into a computer database by the study coordinator from the completed CRF's for statistical evaluation at Thomas Jefferson University.

The monitor and/or regulatory authorities will be allowed audits at the investigation site and source data verification in which case a review of those parts of the hospital records relevant to the study may be required.

13 Quality Control and Quality Assurance

Case report forms (CRF) will be provided for the recording of all data. Data will be recorded directly and legibly onto the record forms, in blue/black ink. The signature of the investigator will attest the accuracy of the data on each CRF. If any assessments are omitted, the reason for such omissions will be noted on the CRFs. Corrections, with the reason for the corrections, should be made legibly, dated and initialed. Correction fluid is not allowed. All original data collected with paper and pen will immediately be recorded electronically by the study coordinator within the database.

Data collection and accurate documentation are the responsibility of the study staff under the supervision of the investigator. All source documents and laboratory reports must be reviewed by the study team and data entry staff, who will ensure that they are accurate and complete. Unanticipated problems and adverse events must be reviewed by the investigator or designee. The monitor and/or regulatory authorities will be allowed audits at the investigation site and source data verification in which case a review of those parts of the hospital records relevant to the study may be required.

14 Ethics/Protection of Human Participants

14.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, as drafted by the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6.

The study will be conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice and applicable regulatory requirements.

14.2 Institutional Review Board

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented in the study.

14.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout study participation. Extensive discussion of risks and possible benefits of study participation will be provided to participants and their families, if applicable. A consent form describing in detail the study procedures and risks will be given to the participant. Consent forms will be IRB-approved, and the participant is required to read and review the

document or have the document read to him or her. The investigator or designee will explain the research study to the participant and answer any questions that may arise. The participant will sign the informed consent document prior to any study-related assessments or procedures. Participants will be given the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. They may withdraw consent at any time throughout the course of the study. A copy of the signed informed consent document will be given to participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their clinical care will not be adversely affected if they decline to participate in this study. The consent process will be documented in the clinical or research record.

The investigators are responsible for giving the patients full and adequate verbal and written information about the nature, purpose, possible risk and benefit of the study. They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician.

It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever she/he wants. This will not prejudice the patient's subsequent care. Documented informed consent must be obtained for all patients included in the study before they are registered in the study. This will be done in accordance with the national and local regulatory requirements of each site. The investigators are responsible for obtaining signed informed consent.

A copy of the patient information and consent will be given to the patients. The signed and dated patient consent forms will be filed in the Investigator File binder and also scanned to be part of the patient's electronic medical record at the hospital.

14.4 Exclusion of Women, Minorities, and Children (Special Populations)

No children will be included. The patients enrolled in this study will be adults over the age of 18 diagnosed with a non-HCC solid tumor, who are scheduled for TARE. Malignant intrahepatic bile duct tumors are rare in children and adolescents (0.7% of new cases are in patients younger than 20 years), and the median age of diagnosis is 65 years old [https://seer.cancer.gov/statfacts/html/livibd.html].

14.5 Participant Confidentiality

Subject identification, other than subject number, date of study and date of birth, will not appear in any eCRF pages or other documents given to the Sponsor. Only the PIs as well as the persons authorized to verify the quality and integrity of the study will have access to subject records where the subject can be identified. The PIs shall arrange for the secure retention of the patient identification and the code list.

15 Data Handling and Record Keeping

The investigators are responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents must be completed in a neat, legible manner to ensure accurate interpretation of data. The investigators will maintain adequate case histories of study participants, including accurate CRFs and source documentation. The monitor and/or

regulatory authorities will be allowed audits at the investigation site and source data verification in which case a review of those parts of the hospital records relevant to the study may be required.

15.1 **Data Management Responsibilities**

Data management will be performed by the ultrasound research laboratory using paper charts hosted at Thomas Jefferson University in accordance with the department's SOPs and ICH guidelines. The data management process will be described in the study specific data handling report after database closure.

15.2 **Data Capture Methods**

Data management will be performed by the ultrasound research laboratory using paper charts hosted at Thomas Jefferson University in accordance with the department's SOPs and ICH guidelines. The data management process will be described in the study specific data handling report after database closure.

Data entered into the database will be validated as defined in the data validation plan. Validation includes, but is not limited to, validity checks (e.g. range checks), consistency checks and customized checks (logical checks between variables to ensure that study data are accurately reported) for data captured in the database and external data (e.g. laboratory data).

Data management personnel will perform both manual CRF review and review of additional electronic edit checks to ensure that the data are complete, consistent and reasonable. The electronic edit checks will run continually throughout the course of the study and the issues will be reviewed manually online to determine what action needs to be taken.

Manual queries may be written by clinical data management or study monitor. Queries will be sent to the investigator for resolving. All updates to queried data will be made by clinical data management only and all modifications to the database will be recorded in an audit trail.

Adverse events and medical history will be coded from the verbatim description (Investigator term) using the Medical Dictionary for Regulatory Activities (MedDRA). Prior and concomitant medications and therapies will be coded according to MedDRA.

Once the database has been completed and locked, the PI will authorize database lock and all electronic data will be analyzed. Subsequent changes to the database will then be made only by written agreement.

The data will be stored in a dedicated and secured area at Thomas Jefferson University. Electronic data will be stored at the Thomas Jefferson University servers. Data will be stored in a de-identified manner, where each study participant is recognizable by his/her unique trial subject number. The data will be stored at least 15 years after study closure.

15.3 **Types of Data**

Imaging data (ultrasound, CT, and MRI), clinical and laboratory data, AEs

15.4 Study Records Retention

The data will be stored in a dedicated and secured area at Thomas Jefferson University. Electronic data will be stored at the Thomas Jefferson University servers. Data will be stored in a de-identified manner, where each study participant is recognizable by his/her unique trial subject number. The data will be stored at least 15 years after study closure.

15.5 Protocol Deviations

A protocol deviation is any noncompliance with the clinical study protocol, Good Clinical Practice, or Manual of Procedures requirements. The noncompliance may be on the part of the participant, the investigator, or study staff. As a result of deviations, corrective actions are to be developed by the study staff and implemented promptly.

All deviations from the protocol must be addressed in study participant source documents and promptly reported to the IRB and other regulatory bodies according to their requirements.

16 Study Finances

16.1 Funding Source

This study is financed through a grant from the United States National Institute of Health.

16.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All Jefferson University Investigators will follow the TJU Conflicts of Interest Policy for Employees (107.03).

16.3 Participant Stipends or Payments

Parking or travel reimbursement will be provided to patients as needed to cover transportation costs of participation. The total payment will not exceed $100 per patient.

17 Publication and Data Sharing Policy

Upon study completion and finalization of the study report the results of this study will either be submitted for publication and/or posted in a publicly assessable database of clinical study results. The results of this study will also be submitted to the Competent Authority and the Ethics Committee.

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SUPPLEMENTAL MATERIALS

These documents are relevant to the protocol, but they are not considered part of the protocol. They are stored and modified separately. As such, modifications to these documents do not require protocol amendments.

Appendices

The following documents are officially affiliated with the protocol and will be submitted to the IRB as a part of the protocol. As such, changes to these items require a protocol amendment.

Appendix A: Schedule of Events

Appendix B: Optison product insert

APPENDIX A: SCHEDULE OF EVENTS

Procedures		Screening & baseline (Day 1)	Study Visit 1 (Day 7 ± 14)	Study Visit 2 (Day 14 ± 21)	Study Visit 3 (Day 21 ± 28)
Signed Consent Form		X			
Assessment of Eligibility Criteria		X			
Review of Medical/Dental History		X			
Review of Concomitant Medications		X	X	X	X
Contrast-enhanced ultrasound (CEUS) exam		X	X	X	X
Ultrasound-triggered microbubble destruction (UTMD)			X	X	X
Physical Examination	Complete				
	Symptom-Directed	X	X	X	X
	Vital Signs	X	X	X	X
Assessment of Adverse Events		X	X	X	X
Clinical Laboratory	Pregnancy test	X	X	X	X
	Chemistry				