PROTOCOL

TITLE: 2D AND 4D CONTRAST-ENHANCED ULTRASOUND EVALUATION OF HEPATOCELLULAR CARCINOMA CHEMOEMBOLIZATION

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STUDY DURATION: 5 year project (January 2016-December 2020)

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SYNOPSIS

Protocol Title: 2D and 4D Contrast-enhanced Ultrasound Evaluation of Hepatocellular Carcinoma Chemoembolization

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

- To evaluate the sensitivity and specificity of 2D and 4D contrast enhanced ultrasound for monitoring transarterial chemoembolization (TACE) response 1-2 weeks and 1 month post treatment as an alternative to contrast-enhanced magnetic resonance (MRI) or computed tomography (CT) imaging

The secondary aim of this trial is to:

- To develop quantitative biomarkers based on the ultrasound contrast agent kinetics for identifying patients requiring retreatment of residual disease.

Trial Design: This is an open-label, non-randomized trial that will be conducted at three clinical sites. The subject population will be patients undergoing transarterial chemoembolization for the treatment of hepatocellular carcinoma (HCC) at Thomas Jefferson University, The University of California, San Diego, and The Hospital of the University of Pennsylvania. Patients will receive a contrast-enhanced ultrasound (CEUS) exam the morning prior to embolization, approximately one week post-embolization, and at their one month MRI follow up (scheduled as part of their clinical standard of care).

Trial Population: This trial will consist of up to 210 adults (with a maximum of up to 100 at any institution) undergoing transarterial chemoembolization for the treatment of HCC split between Thomas Jefferson University, The University of California, San Diego, and The Hospital of the University of Pennsylvania.

Trial Procedures: Patients will be identified and consecutively approached from each institution's Hepatology / Transplant Surgery and Interventional Radiology practices (by each site's Hepatology or Interventional Radiology co-investigators). An investigator or 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 to ask questions about participation. A consent form will be reviewed with the patient. A full history and physical

examination will be obtained from the patient's referring physician. If the subject is a woman of childbearing potential, she will have a urine pregnancy test prior to each CEUS study (the results of which will be made available to the subject prior to study initiation). In the event a patient presents with a lesion that is expected to be difficult to view on ultrasound (for example, smaller lesions located high on the liver dome), grayscale ultrasound imaging will quickly be performed to ensure the lesion is visible on ultrasound and that the patient is suitable for study inclusion.

Patients will undergo a total of three separate CEUS exams. These exams will consist of the baseline study prior to TACE therapy, a study 1-2 weeks post treatment that will coincide with clinical post-procedure follow-up by the interventional radiologist, and a study approximately one month post treatment when patients return for clinically scheduled CE-CT/MRI follow-up. If the patient fails to show up for the 1-2 week CEUS exam, they will not be excluded from the final ultrasound exam. Procedures and equipment for this trial will be used in accordance with standard clinical protocols and good clinical practices already in place at our hospitals.

The first three cases at each institution (baseline and at least one follow-up) will be performed under the guidance of at least one of the study PIs to ensure standardization amongst all three sites. The ultrasound examinations will be performed by a qualified sonographer. Efforts will be made to have all CEUS scans performed by the same dedicated sonographer, enabling us to evaluate operator dependence. During the ultrasound examination, the patient will be asked to lie in the supine position and a 20-22 gauge cannula will be placed in a superficial vein (preferably an antecubital vein). Ultrasound imaging will be performed using a state of the art Logiq E9 scanner with C1-5-D broad-spectrum convex transducer and a RAB2-5-D broad-spectrum real-time 4D transducer (GE Healthcare, Wauwatosa, WI). As part of this study, we have budgeted for the purchase of 3 4D probes (not routinely available in clinical practice) and the installation of GE's ultrasound volumetric and contrast imaging packages that provide 2D and 4D CEUS capabilities. In the event the patient has multiple lesions scheduled for treatment, up to 2 lesions will be imaged independently on CEUS. Patients will first undergo 2D baseline imaging. B-mode measurements and sweeps of the lesion in the transverse and sagittal planes will be performed, followed by standard power Doppler imaging (PDI). Following baseline imaging, patients will receive a bolus IV injection of up to 0.6 ml of Definity, followed by a 10 cc saline flush. Since Definity is currently only approved for echocardiography, we will apply to the FDA for an investigational new drug application for CEUS evaluation of TACE (similar to our previous study [Shaw at el. 2014]).

All CEUS imaging will be performed using the dual B-mode (used to locate anatomical features) and

nonlinear contrast (to identify the ultrasound contrast agent) imaging mode. A low mechanical index (< 0.1) will be used to minimize microbubble destruction during imaging. The standard nonlinear imaging frequency pairings in the contrast imaging software will be used (transmitting at 2 MHz, receiving at the 4 MHz harmonic), and gain settings will be adjusted to minimize nonlinear signals prior to contrast injection. Additionally, the focal zone will be placed at the approximate depth of the lesion to maximize the generation of nonlinear signals during CEUS. During the first contrast injection, 2D CEUS will be performed using the coded harmonics nonlinear imaging package on the unit. The approximate tumor mid-line will be imaged until homogenous liver enhancement is achieved (approximately 45 seconds post injection), followed by imaging sweeps through the tumor. Sweeps will then be acquired in the sagittal plane, before returning to the original plane. Imaging will be continued until contrast washout is observed (approximately 3-4 minutes), after which data will be digitally stored for later review. A ten to fifteen minute wait period will be observed between injections to allow for complete ultrasound contrast agent clearance. A region of interest encompassing the entire tumor volume and margin will then be selected in 4D mode. Baseline imaging of the tumor will be repeated in 4D mode in grayscale B-mode. A second bolus injection of up to 0.6 ml Definity followed by 10 cc saline flush will again be administered during continuous tumor imaging in 4D, which uses the machine's coded harmonics package. Data will be obtained until contrast washout is observed and then digitally stored in digital imaging and communications in medicine (DICOM) format for later review.

The proposed agent for the current study, Definity is a sterile non-pyrogenic suspension of liposome-encapsulated perfluoropropane microbubbles [Goldberg et al 2001; Miller & Nanda 2004]. The contrast agent is composed of a blend of three phospholipids contained in a matrix of sodium chloride, propylene glycol, and glycerin in water. The contrast agent is supplied in a vial that contains the phospholipids and perfluouropropane gas. The microbubble agent is supplied in a standard-size 2 ml vial and is prepared by shaking the vial with the aid of a shaking device (Vialmix: ESPE, Seefeld, Germany). Definity will be stored in a secure cabinet, with only the study investigators and research personnel having access. Definity is currently only approved for use in echocardiography. The agent will be used as an off-label indication for this study. We intend to apply for an FDA investigator-instantiated IND for the off-label usage of Definity for the evaluation of chemoembolization using drug eluting beads.

Statistical Methodology: Data analysis from this multi-center clinical trial will be performed to characterize 2D and 4D CEUS as a follow-up imaging tool for the evaluation of residual tumoral blood flow in patients with HCC treated with TACE. Our statistical analysis will address three major questions:

1. Does 2D and 4D CEUS 1-2 weeks post TACE yield sensitivity and specificity greater than 90% for identifying residual tumor blood flow, thereby providing reliable earlier identification (relative to the current clinical standard) of patients in need of retreatment?
2. Does 2D and 4D CEUS 1 month post TACE yield sensitivity and specificity greater than 90% for identifying residual tumor blood flow ,thereby providing an accurate, safe, and cost-effective imaging alternative for identifying TACE patients in need of retreatment?
3. Are quantifiable biomarkers generated by parametric imaging of 2D and 4D CEUS exams able to differentiate between fully treated and incompletely treated masses by measuring blood flow kinetics?

The first three cases at each institution will be used as reader training cases and will not be included in the final analysis. In the event of all cases showing identical response (i.e. all incompletely or completely treated), the first case with an alternative outcome will be substituted for the 9th case. Following reader training, each reader (all of whom have previous experience with CEUS and volumetric ultrasound) will be asked to evaluate all 201 remaining cases. 2D and 4D CEUS exams will be read separately and only after randomization. Readers will first be asked to review both the patient's baseline CE-MRI/CT and CEUS exams to identify the size and location of the treated mass. Following baseline review, each reader will review the 1-2 week and 1 month CEUS exam (in random order). Intratumoral blood flow will be assessed as present or not present. Readers will then be asked to interpret the patient's 1 month follow-up CE-MRI/CT images and again evaluate the need to retreat based on residual blood flow. Finally, when available, readers will review subsequent CE-MRI/CT imaging studies (up to six months post TACE). MRI/CT evaluations will be based on mRECIST criteria. All imaging studies will be read twice by each reader. Studies will be presented in random order. The second round reads will be performed at least one month after the first. Outcomes will be defined as incompletely treated (i.e. requiring retreatment) based on (in order of preference of reference standard): a) pathological examination of explanted livers demonstrating live tumors; b) tumor enhancement seen with CT or MR and confirmed via angiography during retreatment; c) tumor growth on 6 month follow-up CE-CT/MRI; or d) asymmetrical or nodular tumor enhancement on CE-MRI/CT on 6 month follow-up. Incomplete treatment on CEUS (2D or 4D) will be evaluated by each reader and evidenced by the tumor showing either residual blood flow after treatment or nodular peripheral blood flow.

For statistical questions 1-2, sensitivity (the ability to detect patients in need of retreatment) and specificity (the ability to identify patients who do not require retreatment) will first be calculated on a reader by reader basis. Four 2 x 2 tables will be constructed comparing the need for re-treatment based on the clinical gold standard and CEUS, tabulating true positives, false positives, false negatives, and true negatives for CEUS

at 1-2 weeks and 1 month for both 2D and 4D CEUS and used to calculate sensitivity, specificity, accuracy, positive predictive value, and negative predictive value. Statistical significance between the reference standard and each of the 4 CEUS groups will be determined using a McNemar test. These values will also be calculated based on the majority decision (i.e. 4 or more readers in agreement). In the event of discordant readings during this analysis, tumor outcome will be decided by consensus by the two readers at TJU. Differences between techniques (2D vs. 4D) and time points (1-2 weeks vs. 1 month post treatment) will be determined by comparing areas under the receiver operating characteristic curves for each modality/time point based on necessity of tumor retreatment. Intra-rater and inter-rater variability will be calculated using a Shrout and Fleiss Interclass Correlation [Shrout and Fleiss 1979]. Finally, a logistical regression and analysis of variance will be performed to determine if significant variations exist between individual sites (to determine operator dependence), lesion size (tumor size dependency) or between patients treated with cTACE vs. DEB-TACE (treatment dependency).

For statistical question 3, all quantitative parameters will be compared between fully treated and incompletely treated masses using a Student's t-test. Additionally, lesions that demonstrate lack of residual enhancement at 1 month, but show recurrence at 6 month follow-up, will be compared to completely treated masses to determine if quantitative CEUS parameters can detect this lower level of residual disease. Statistical analysis will be performed with assistance from the biostatistician co-investigator using Stata 12.0 (Stata Corp, College Station, TX) with p values < 0.05 considered statistically significant after a Bonferroni-type adjustment for multiple tests. These statistical tests will answer our three major questions regarding the sensitivity and specificity of 2D and 4D CEUS as an imaging tool for the follow-up of TACE at 1-2 weeks and 1 month, as well as the influence of operator, reader, tumor size, and embolization material.

1. INTRODUCTION

Hepatocellular carcinoma (HCC) is the third leading cause of cancer mortality worldwide and the incidence is increasing. While surgical resection and transplantation offer the possibility of long-term cure, the majority of patients are not eligible for these treatments. Transarterial chemoembolization (TACE) is standard of care for patients with Barcelona Clinic Liver Cancer stage B disease but may also be used as bridging therapy in patients who are potential transplant candidates. Current guidelines recommend follow-up imaging 4-6 weeks after all tumor-bearing areas have been treated. Incomplete treatment is defined as persistence of enhancing areas inside the treated lesions seen at the first imaging study after locoregional treatment and is observed in up to two-thirds of patients post TACE. Contrast-enhanced magnetic resonance imaging (CE-MRI) has been established as the clinical standard imaging modality for evaluating treatment

response and contrast-enhanced computed tomography (CE-CT) is used when CE-MRI is contraindicated. However, there are multiple reasons patients may not undergo cross-sectional imaging follow-up - metallic implants, renal impairment, allergies to contrast medium, and anxieties related to confined space or radiation exposure. Additionally, the cost and availability of these techniques are disadvantageous compared to ultrasound imaging.

Contrast-enhanced ultrasound (CEUS) is a well-established technique for enhancing echocardiograms in the United States and is approved for a wide range of vascular and oncologic applications worldwide. These contrast agents function as blood pooling agents and unlike the contrast agents used in MRI/CT, are not nephrotoxic and have no renal contraindications, making them an exceptionally safe imaging agent. Additionally, CEUS imaging is not influenced by residual Ethiodol or post-surgical inflammation in the liver, potentially offering an earlier option for monitoring treatment response of TACE compared to the current clinical standard. In this study, we propose a multi-center clinical trial to determine the sensitivity and specificity of two and real time three-dimensional (2D and 4D, respectively) CEUS for evaluating TACE. In addition, quantitative biomarkers based on parametric imaging will be defined for identifying patients in need of retreatment. These techniques may provide a safer, more accurate imaging alternative for evaluating TACE effectiveness, as well as earlier identification of residual disease requiring retreatment. The multi-center nature of this proposed study allows sufficient sample sizes to define the sensitivity and specificity of CEUS within an adequate confidence interval, as well as investigate its operator and reader dependencies. Once properly validated, this technique is expected to improve patient outcomes by offering a viable imaging alternative in cases where CE-MRI/CT is contraindicated and enabling earlier identification of residual disease in need of retreatment.

1.1 Background

Hepatocellular carcinoma (HCC) is the fifth most prevalent cancer worldwide, with over half a million new cases reported per year [Alterkruse et al. 2009]. The rising incidence of HCC over the last 20 years is primarily attributed to the rise in hepatitis C virus infections [El-Serag and Mason 1999]. Surgery offers the best chance for long term cure; however, 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].Transplantation is another treatment option for HCC, with the 5 year survival rates for liver transplantation at 68%, [Watt et al. 2010] yetis severely limited by tumor burden and the availability of donors [Kim et al. 2013; Llovet et al. 1999].

In patients with unresectable disease, locoregional treatment options include thermal ablation,

radioembolization and transarterial chemoembolization (TACE). Since hepatocellular cancers are almost exclusively supplied by the hepatic arterial system and the liver has a dual blood supply (approximately 75% from the portal vein and 25% from the hepatic artery), trans-arterial treatment of tumors can be performed while limiting toxicity to the surrounding uninvolved parenchyma [Chapman et al. 2008; Gonsalves and Brown, 2009; Bruix and Sherman 2010]. While this technique is primarily used for the treatment of HCC, it has also been successful for a variety of hepatic malignancies [Burger et al. 2005; Herber et al. 2007; Giroux et al. 2004; Brown et al. 2012; Fiorentini et al. 2007; Patel et al. 2005; Sato et al. 2005; Madoff et al 2006].

TACE involves the direct infusion of chemoembolic agents via a catheter placed in the tumor feeding artery [Yamada et al. 1995]. Conventional TACE (cTACE) uses chemotherapeutic agents mixed with Lipiodol, followed by an embolic material. Drug-eluting bead TACE (DEB-TACE) uses chemotherapeutic agents adsorbed onto poly-vinyl alcohol beads. The bead size is standardized and chosen based on tumor size and sub selective vascular access. Regardless of which embolic agents are used, the goal remains the same, to render the tumors ischemic while depositing high doses of chemotherapy in the tumor [Gonsalves and Brown 2009]. Tumor ischemia causes the disruption of intracellular glycoprotein pumps, which inhibits tumor cells from expelling chemotherapeutic agents, resulting in a six-fold increase in drug retention [Sasaki et al. 1987].

During cTACE, iodinated, ester derived poppy-seed oils serve as drug carriers, as temporary embolization material, and as imaging agents under fluoroscopy [Gonsalves and Brown, 2009]. The chemotherapeutic agents used may vary, but most frequently consist of a cisplatin, adriamycin, and mitomyocin (CAM) mixture [Van Ha 2009]. Following delivery, more permanent embolization is achieved with materials such as poly-vinyl alcohol or gelfoam [Gonsalves and Brown, 2009]. While cTACE is an effective therapy for slowing disease progression, improved patient responses with less toxicity have been observed with the use of DEB-TACE [Vogl et al. 2011; Lammer et al. 2010; Gaba 2012]. Embolization with DEB-TACE results in occlusion of the tumor vasculature, combined with a sustained, intratumoral release of the chemotherapeutic [Lewis et al. 2006]. Compared with cTACE, DEB-TACE is associated with greater treatment tolerance and significantly less liver toxicity and doxorubicin-related side effects [Vogl et al. 2011, Lammer et al 2010].

Both TACE procedures are performed under fluoroscopic guidance for catheter placement and infusion monitoring, with infusions continuing until near stasis in the tumor-feeding artery is observed [Van Ha 2009]. Access to the tumor vasculature is achieved via a catheter placed in the hepatic artery. Technical

success, defined as correct catheter placement and drug administration, is achieved in approximately 98% of patients [Brown et al. 2012]. Residual tumoral viability at imaging post-embolization is the primary criterion used in determining the need to re-treat. TACE is frequently iterative – up to 65-75% of tumors will demonstrate residual blood flow and require repeat TACE or alternative treatments. The lack of efficacy on the initial treatment may be due to reaching premature stasis because of slow flow conditions, vasospasm limiting delivery, or redistribution of flow to the lesion from an alternative hepatic branch after primary branch occlusion.

Contrast-enhanced magnetic resonance imaging (CE-MRI) is the standard imaging modality used to assess response to TACE by evaluating residual blood flow within the tumor [Brown et al. 2012; Hammerstingl et al. 2008; Akai et al. 2011]. The Society of Interventional Radiology guidelines recommend imaging follow-up four weeks post treatment [Brown et al. 2012]. A lack of arterial phase enhancement is used to indicate the lack of residual tumoral blood flow due to complete embolization and tumor necrosis. This one month time point has been adopted based on experience with the use of Lipiodol and on follow-up with contrast-enhanced computed tomography (CECT) scans; the non-tumor bearing liver requires 3-4 weeks to eliminate the Lipiodol by Kupffer cell phagocytosis [Brown et al. 2012]. In CE-MRI, the use of low molecular weight and water soluble contrast agents renders it difficult to differentiate granulation tissue and residual tumor perfusion and to differentiate peripheral viable tumors from inflammatory peritumoral infiltration if scanned within four weeks of treatment [Yan et al. 2002]. Differentiation between viable tumor and inflammation can be made in some cases by evaluating enhancement washout kinetics. Nevertheless, the cost of imaging and difficulty of inflammation in many cases has resulted in follow-up imaging recommendation to remain at 1 month [Brown et al. 2012]. CECT is used in patients in whom MRI is contraindicated. Confluent, dense Lipiodol uptake within treated tumors correlates with complete tumor necrosis, but may mask some residual tumor perfusion on CECT [Imaeda et al. 1993; Ito et al. 1995]. Following imaging, TACE or alternative treatment is repeated until a complete lack of arterial enhancement is achieved, after which tumoral response (evaluated by modified response evaluation criteria in solid tumors (mRECIST)) is assessed at 3-6 month intervals.

While follow-up imaging standards are well established, several limitations exist. CE-MRI may not be feasible in patients with metallic implants, renal contraindications (glomerular filtration rates <30 mL/min/ 1.73 m^2 [Shellock and Spinazzi 2008]), the inability to breath hold or claustrophobia. CECT may be contraindicated in patients with severe allergy to iodinated contrast and renal impairment. The radiation exposure associated with repeated CT imaging (particularly in patients who undergo multiple TACE treatments) also renders CT a suboptimal imaging modality. Additionally, the costs and limited availability

of CE-MRI or CT may place a significant, unnecessary burden on the health system. A final limitation of existing imaging techniques is the required delay between treatment and imaging to evaluate response due to the inherent properties of the MRI/CT contrast media and the presence of Lipiodol in the background liver. Consequently, time to retreatment is prolonged and in patients where TACE is used to downstage disease, a decision regarding transplantation is delayed. Phase II efficacy and pharmacokinetic studies show liver function generally returns to normal within 7 days after DEB-TACE [Varela et al. 2007]; thus, retreatment before the standard 5-7 weeks is feasible. In addition, retreatment by ablation may also be performed earlier with advanced screening of effective embolization. An imaging test that provides earlier identification of tumoral blood flow after TACE would shorten the time between treatments and potentially improve patient outcomes. Once tumor avascularity is confirmed, patients could resume standardized CE-MRI/CT long term follow-up protocols.

We propose the use of contrast-enhanced ultrasound (CEUS) as a potential solution. Ultrasound contrast agents are composed of a gas microbubble, encapsulated by an outer protein or lipid shell [Goldberg et al. 2001]. Ultrasound imaging is portable, provides real time imaging, uses non-ionizing radiation, is less expensive than CT/MRI, and is more readily available than MRI/CT imaging [Lewin 2004]. While CEUS is currently only approved by the FDA for echocardiography, the technique represents the primary imaging modality for numerous hepatic and vascular applications worldwide [Claudon et al. 2012; Bouakaz and de Jong, 2007]. CEUS also offers significant cost savings relative to CE-MRI/CT in the characterization of liver lesions [Westwood et al. 2013]. After intravenous injection, ultrasound contrast agents permeate throughout the blood supply, including the neovascular tumor supply, thereby providing enhancement of the tumor vasculature [Eisenbrey and Forsberg 2010]. The diameter of the contrast microbubbles (1-8 μm) enables them to pass through the pulmonary capillaries, but still restricts them to the vasculature, making them excellent intravascular blood pool agents [Correas et al. 2001]. Unlike the contrast agents used in MRI/CT, ultrasound contrast agents are not nephrotoxic and have no renal contraindications, making them an exceptionally safe imaging test. The presence of Lipiodol in the lesion does not interfere with ultrasound imaging. Unlike CT or MRI contrast agents, ultrasound contrast agents are completely confined to the vascular space and thus enable clear visualization of the tumor vasculature, despite any presence of inflammation. Finally, the higher temporal resolution of ultrasound avoids motion artifacts in patients with poor breath holding capability. Thus, CEUS is an attractive imaging alternative for identifying residual vascularity post-TACE.

Recent technological advances in the field of medical ultrasound are expected to aid in these goals. Contrast-specific imaging techniques can isolate nonlinear microbubble signals from the surrounding tissue

to enhance visualization of vascularity [Philips 2004; Eisenbrey 2011a]. Additionally, the recent implementation of real-time 3D (i.e. 4D) transducers enables volumetric imaging at acceptable acquisition rates (2-6 volumes/second for 4D ultrasound vs. 20+ frames/ second for 2D ultrasound imaging) and image quality [Eisenbrey et al. 2012]. Finally, blood flow kinetics in tissue can be quantified using dynamic-CEUS (D-CEUS) or parametric imaging [Eisenbrey et al. 2011b] and these techniques have also been applied using 4D CEUS to enhance quantification of blood flow within a tissue volume [Sridharan et al. 2012].

Pilot studies by our groups suggest that 2D CEUS provides accurate and earlier identification of residual tumoral blood flow (relative to current imaging guidelines [Brown et al. 2012]) in TACE patients treated with Lipiodol [Kono et al. 2007] and drug-eluting beads [Shaw et al. 2014]. Importantly, results from our CEUS study with c-TACE also indicate that CEUS may be more accurate than CE-MRI/CT at 1 month for evaluating residual blood flow [Kono et al. 2007]. Other studies evaluating the use of 2D CEUS as a follow-up imaging tool for TACE therapy reported that the lack of tumor enhancement correlates well with an avascular treatment response [Vallone et al. 2003; Salvaggio et al. 2010; Moschouris et al. 2010; Takizawa et al. 2013]. Xu et al. [2010] used static volumetric (3D) CEUS to monitor HCC response to local therapy and found the addition of volumetric ultrasound provided improved diagnostic confidence relative to 2D CEUS. Nevertheless, in this study, static 3D CEUS was used (potentially missing important dynamic volumetric data), only 12 patients underwent TACE therapy (and the majority of these TACE cases were treated in combination with ablation), effects of follow-up times were not investigated, and follow-up time ranged from 10 minutes to 28 months.

Sufficient data relating to a contrast agent approved for use in the USA, the use of 4D volumetric CEUS, the influence of reader or operator dependence, or the effect of follow-up time of CEUS for evaluating TACE therapy are not available. Additionally, D-CEUS remains a relatively unexplored option for characterizing treatment response to TACE therapy and may offer further improvements in diagnostic accuracy. Such findings could provide a safe, cost-effective and readily available follow-up imaging alternative, while also potentially improving patient outcomes by providing earlier and more accurate identification of patients requiring re-treatment. We propose a multi-center clinical trial to determine the sensitivity and specificity of 2D and 4D CEUS for evaluating TACE 1-2 weeks and 1 month post-treatment.

The proposed agent for the current study, Definity is a sterile non-pyrogenic suspension of liposome-encapsulated perfluoropropane microbubbles [Goldberg et al 2001; Miller & Nanda 2004]. The contrast agent is composed of a blend of three phospholipids contained in a matrix of sodium chloride, propylene glycol, and glycerin in water. The contrast agent is supplied in a vial that contains the phospholipids and

perfluouropropane gas. The microbubble agent is supplied in a standard-size 2 ml vial and is prepared by shaking the vial with the aid of a shaking device (Vialmix: ESPE, Seefeld, Germany).

Definity is currently only approved for use in echocardiography. The agent will be used as an off-label indication for this study. We intend to apply for an FDA investigator-instantiated IND for the off-label usage of Definity for the evaluation of chemoembolization.

Definity Clinical Safety

Definity is well tolerated and has been used extensively in echocardiography applications [Goldberg et al 2001]. In pre-market clinical trials, Definity was administered to 1716 patients. In these patients 269 (8.4%) reported at least one adverse event. Of these events, 26 were classified as serious including 19 (1.1%) patients experiencing serious cardiopulmonary symptoms including eight deaths. The deaths occurred several days after activated Definity administration and appear to be related to the course of underlying disease. Of the 11 other serious adverse events, which appeared within days of the drug administration (2-15 days), all appeared to be a progression of underlying cardiac and non-cardiac disease. However, a role for Definity in the initiation or course of these adverse events can not be ruled out. Of the reported adverse reactions following the use of Definity the most frequently reported were headache (2.3%), back and renal pain (2.1%), flushing (1.1%), and nausea (1.0%). Additional risks associated with the contrast material are described in the attached Definity 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 Definity in Controlled Clinical Studies

No. of Patients Exposed to Definity	1716	
No. of Patients Reporting an Adverse Event	269	(8.8%)
Central and peripheral nervous system	54	(3.2%)
Headache	40	(2.3%)
Dizziness	11	(0.6%)
Body as a Whole	41	(2.4%)
Back/Renal Pain	20	(1.2%)
Chest Pain	13	(0.8%)
Digestive System	31	(1.8%)
Nausea	17	(1.0%)
Vascular (extracardiac) disorders	19	(1.1%)
Flushing	19	(1.1%)
Application Site Disorders	11	(0.6%)
Injection Site Reactions	11	(0.6%)

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

1.2 Rationale

Successful TACE of a tumor using drug eluting beads is expected to completely obstruct the tumor blood supply. Thus, the fundamental theory behind this study is that CEUS, which uses a blood pooling agent for contrast, will provide a good indicator of the degree of occlusion. If CEUS at one month correlates with the current clinical standard of a contrast-enhanced MRI, this alternative will lead to substantial cost savings. If CEUS at one to two weeks correlates with the current clinical standard of a contrast-enhanced MRI at one month, this will reduce time to retreatment, potentially improving patient outcomes.

We propose a clinical trial to determine the accuracy of using CEUS for the evaluation of TACE of HCC. The purpose of this study is to compare ultrasound derived vascularity and blood flow measurements at varying time intervals to both the clinical evaluation standard and patient outcomes.

2. TRIAL OBJECTIVES

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

- To evaluate the sensitivity and specificity of 2D and 4D contrast enhanced ultrasound for monitoring transarterial chemoembolization (TACE) response 1-2 weeks and 1 month post treatment as an alternative to contrast-enhanced magnetic resonance (MRI) or computed tomography (CT) imaging

The secondary aim of this trial is to:

- To develop quantitative biomarkers based on the ultrasound contrast agent kinetics for identifying patients requiring retreatment of residual disease.

3. TRIAL DESIGN

This is an open-label, non-randomized trial that will be conducted at three clinical sites. The subject population will be patients undergoing transarterial chemoembolization for the treatment of hepatocellular carcinoma (HCC) at Thomas Jefferson University, The University of California, San Diego, and The Hospital of the University of Pennsylvania. Patients will receive a contrast-enhanced ultrasound (CEUS) exam the morning prior to embolization, approximately one week post-embolization, and at their one month

MRI follow up (scheduled as part of their clinical standard of care).

3.1 Trial Duration

Individual participation in this trial will be limited to three ultrasound imaging studies. Exams will take place the morning prior to treatment, one to two weeks post embolization, and prior to the patient's one month follow-up (scheduled as part of clinical care). If the patient fails to show up for the one week, CEUS exam, they will not be excluded from the final ultrasound exam. The entire ultrasound imaging protocol will require approximately one hour including a 30 min observation period. Patients will be monitored for at least 30 minutes before being escorted to procedure or clinically scheduled imaging.

Subject recruitment is expected to last 5 years (January 2016 – December 2020). Analysis and publication of results are expected to take an additional 2 months (December 2020– January 2021). 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 210 adults (with a maximum of up to 100 at any institution) undergoing transarterial chemoembolization for the treatment of HCC split between Thomas Jefferson University, The University of California, San Diego, and The Hospital of the University of Pennsylvania. The patients enrolled in this project will be adults over the age of 21 capable of providing informed consent.

4.1 Inclusion Criteria

All subjects accepted for this trial must:

- Patients $\geq$ 21 years of age
- Patient capable of making informed decisions regarding his/her treatment
- Scheduled for TACE treatment of a HCC mass (lesions reported as Liver Imaging Reporting and Data Systems 4B or 5 or Organ Procurement and Transplantation Network 5a or 5b)
- Negative pregnancy test in a female of child-bearing age (regardless of sexual orientation, having undergone a tubal ligation, or remaining celibate by choice) who meets the following criteria
  - Has not undergone a hysterectomy or bilateral oophorectomy; OR
  - Has not been naturally postmenapasual for at least 12 consecutive months (i.e., has had menses at any time in the preceding 12 consecutive months)
- Have an HCC mass viewable on grayscale B-mode ultrasound.

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 not eligible or scheduled for TACE of a HCC mass.
- Patients who have received an investigational drug in the 30 days before study drug administration, or will receive one within 72 h after their final CEUS exam.
- Patients who have received prior radioembolization (Y90) of the lesion of interest.
- Patients with known or suspected hypersensitivity to perflutren
- Patients with pulmonary hypertension or unstable cardiopulmonary conditions.
- Patients who are medically unstable, terminally ill, or whose clinical course is unpredictable.

Subject identification will be maintained with a study specific alphanumeric code including the study site (TJU, HUP, UCSD) and study number (01-100).

Subsite Enrollment Procedures

When a potential patient is identified at the sub-site, the Thomas Jefferson University (TJU) Study Site Contact should be contacted within 1 week via email or phone. Please see the Site Contact List for email and phone number for the TJU Study Site Contact. The sub-site will send the following to the TJU Study Site Contact

1. Notify them of the patient registration
2. Confirm the method of sending registration documents (i.e. fax, email, etc.)
3. Communicate the desired timeline of study completion.

A master study enrollment log will be maintained by the study team at Thomas Jefferson University. The sub-site site will also be asked to maintain an enrollment/screening log on-site, and email this information to the TJU Study Site Contact at least once a month.

5. MEDICATIONS

Definity will be provided by Lantheus Medical Imaging, Billerica, MA. An FDA Sponsor-Investigator IND will be obtained prior to beginning the trial.

Definity is a sterile, non-pyrogenic suspension of liposome-encapsulated perfluoropropane microbubbles [Goldberg et al 2001; Miller & Nanda 2004]. The contrast agent is composed of a blend of three phospholipids contained in a matrix of sodium chloride, propylene glycol, and glycerin in water. The contrast agent is supplied in a vial that contains the phospholipids and perfluouropropane gas. The microbubble agent is supplied in a standard-size 2 ml vial and is prepared by shaking the vial with the aid

of a shaking device (Vialmix: ESPE, Seefeld, Germany). Detailed resuspension instructions are provided in the Definity Product Insert, found in Appendix B.

Definity will be stored in a secure cabinet, with only the study investigators and research personnel having access. Unused drug and empty vials will be properly disposed of after reconciling in the log of study drug.

5.1 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. Definity will be administered by bolus IV injection through an 18- to 20-gauge angiocatheter placed in a peripheral arm vein, preferably an antecubital vein. Subjects will be instructed not to move their arm during the administration of the contrast agent. Subjects will receive a bolus injection of up to 0.6 ml. All injections will be performed at a steady rate not to exceed 1 ml/sec. If contrast is not detected within the tumor, the injection may be repeated with the total cumulative dose not to exceed 1.5 ml. Each bolus injection of Definity will be followed with a very slow flush of 10 ml of normal saline.

5.2 Contraindications

Definity should not be administered to patients with known or suspected hypersensitivity to perflutren. The safety of Definity in patients with 1) severe emphysema, pulmonary vasculitis or a history of pulmonary emboli; 2) confirmed or suspected severe liver lesions; and 3) respirator distress syndrome has not been studied. Therefore, patients with any of these conditions will be excluded from participation.

5.3 Randomization

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

5.4 Blinding and Unblinding Methods

This is an open-label trial; therefore, no blinding or unblinding procedures for the trial drug are required.

5.5 Storage

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

6. TRIAL PROCEDURES

6.1 Patient Enrollment

Patients will be identified and consecutively approached from each institution's Hepatology / Transplant Surgery and Interventional Radiology practices (by each site's Hepatology or Interventional Radiology co-investigators). An investigator or 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 to ask questions about participation. A consent form will be reviewed with the patient

6.2 Screening Assessments

Trial participants will have the presence of inclusion criteria and absence of exclusion criteria verified by providing a medical history. A full history and physical examination will be obtained from the patient's referring physician. If the subject is a woman of childbearing age as defined in the inclusion criteria (section 4.1), she will have a urine pregnancy test prior to each CEUS study (the results of which will be made available to the subject prior to study initiation). In the event a patient presents with a lesion that is expected to be difficult to view on ultrasound (for example, smaller lesions located high on the liver dome), grayscale ultrasound imaging will quickly be performed to ensure the lesion is visible on ultrasound and that the patient is suitable for study inclusion. Based on preliminary studies, approximately 10-30% of TACE patients will present with lesions that are not detectable on baseline ultrasound (generally a smaller, nonvisible percentage compared to traditional liver mass ultrasound characterization studies). Particular issues tend to arise in cirrhotic patients with smaller lesions at the dome of the liver [Kono et al. 2007]. Patients with masses not visible on B-mode ultrasound will be treated as failed cases and reported, but not followed with CEUS or counted towards our 210 enrollment limit for the work proposed in our specific aims.

6.3 Ultrasound Imaging

Patients will first undergo 2D baseline imaging. B-mode measurements and sweeps of the lesion in the transverse and sagittal planes will be performed, followed by standard power Doppler imaging (PDI). Following baseline imaging, patients will receive a bolus IV injection of up to 0.6 ml of Definity, followed by a 10 cc saline flush. Since Definity is currently only approved for echocardiography, we will apply to the FDA for an investigational new drug application for CEUS evaluation of TACE (similar to our previous study [Shaw at el. 2014]).

All CEUS imaging will be performed using the dual B-mode (used to locate anatomical features) and nonlinear contrast (to identify the ultrasound contrast agent) imaging mode. A low mechanical index (< 0.1) will be used to minimize microbubble destruction during imaging. The standard nonlinear imaging

frequency pairings in the contrast imaging software will be used (transmitting at 2 MHz, receiving at the 4 MHz harmonic), and gain settings will be adjusted to minimize nonlinear signals prior to contrast injection. Additionally, the focal zone will be placed at the approximate depth of the lesion to maximize the generation of nonlinear signals during CEUS. During the first contrast injection, 2D CEUS will be performed using the coded harmonics nonlinear imaging package on the unit. The approximate tumor mid-line will be imaged until homogenous liver enhancement is achieved (approximately 45 seconds post injection), followed by imaging sweeps through the tumor. Sweeps will then be acquired in the sagittal plane, before returning to the original plane. Imaging will be continued until contrast washout is observed (approximately 3-4 minutes), after which data will be digitally stored for later review. A ten to fifteen minute wait period will be observed between injections to allow for complete ultrasound contrast agent clearance. A region of interest encompassing the entire tumor volume and margin will then be selected in 4D mode. Baseline imaging of the tumor will be repeated in 4D mode in grayscale B-mode. The line density (a measure of image quality) may also be adjusted to achieve adequate volume acquisition rates (>0.5 volumes/ second). A second bolus injection of up to 0.6 ml Definity followed by 10 cc saline flush will again be administered during continuous tumor imaging in 4D, which uses the machine's coded harmonics package. If sufficient contrast is not detected in either mode, the injection may be repeated with the total cumulative study dose not to exceed 1.5 ml. Data will be obtained until contrast washout is observed and then digitally stored in digital imaging and communications in medicine (DICOM) format for later review.

6.4 Safety Monitoring

Patients will be monitored for AEs during and 30 mins after contrast administration. All other procedures will be performed according to standard of care.

6.5 Efficacy Assessments

Following reader training, each reader (all of whom have previous experience with CEUS and volumetric ultrasound) will be asked to evaluate all 201 remaining cases. 2D and 4D CEUS exams will be read separately and only after randomization. Readers will first be asked to review both the patient's baseline CE-MRI/CT and CEUS exams to identify the size and location of the treated mass. Following baseline review, each reader will review the 1-2 week and 1 month CEUS exam (in random order). Intratumoral blood flow will be assessed as present or not present. Readers will then be asked to interpret the patient's 1 month follow-up CE-MRI/CT images and again evaluate the need to retreat based on residual blood flow. Finally, when available, readers will review subsequent CE-MRI/CT imaging studies (up to six months post TACE). MRI/CT evaluations will be based on mRECIST criteria. All imaging studies will be read twice by each reader. Studies will be presented in random order. The second round reads will be

performed at least one month after the first. Outcomes will be defined as incompletely treated (i.e. requiring retreatment) based on (in order of preference of reference standard): a) pathological examination of explanted livers demonstrating live tumors; b) tumor enhancement seen with CT or MR and confirmed via angiography during retreatment; c) tumor growth on 6 month follow-up CE-CT/MRI; or d) asymmetrical or nodular tumor enhancement on CE-MRI/CT on 6 month follow-up. Incomplete treatment on CEUS (2D or 4D) will be evaluated by each reader and evidenced by the tumor showing either residual blood flow after treatment or nodular peripheral blood flow.

Quantitative analysis of 2D and 4D CEUS datasets will be performed off-line using Matlab (Mathworks, Natick, MA). Motion compensation will first be performed using modified approaches of a either a kernel matching [Dave et al. 2009] or a dual mode registration [Bouhlel et al. 2014] motion compensation technique. Parametric images of both the mass and surrounding liver tissue will be generated using 2D [Eisenbrey et al. 2011b] and 4D [Sridharan et al. 2014] algorithms previously developed by our lab to quantify maximum intensity, perfusion, time to peak, and area under the time intensity curve. Based on images or volumes from the arterial wash-in phase, images will be cropped to include the tumor and margins of 20% of the mass diameter. Vascular skeletonization of the mass and surrounding margins will then be performed [Eisenbrey et al. 2011c] and fractal dimensionality of the skeletonized images calculated (another potential quantifiable indicator of malignancy [Taverna et al. 2009]). Differences in all quantified parameters between pre- and post- treatment will then be calculated and compared between groups to determine if significant biomarkers exist in identifying patients in need of re-treatment.

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

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.

The subjects 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 Definity dosing until 30 minutes from the completion of the Definity 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 Section 6.6.4.

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 Section 6.6.1 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.

Sub-Site Unanticipated Problems Reporting

Unanticipated problems (UAPs) occurring at the sub-site are to be reported to the sub-site IRB per institutional guidelines.

UAPs occurring at the sub-site must also be reported to Thomas Jefferson University using the Unanticipated Problems Form (see Appendix C). The TJU Study Site Contact will submit UAPs occurring at the sub-site to the TJU IRB.

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.

SAEs occurring at the sub-site are to be reported to the sub-site IRB per institutional guidelines.

Sub-Site SAE Reporting

All SAEs occurring at the sub-site must be reported to the TJU Study Site Contact within 24 hours of notification. This initial notification can take place via email or phone, followed by the submission of a formal report.

SAEs should be reported to TJU using the FDA Medwatch 3500A, and should comprise a full written summary, detailing relevant aspects of the adverse events in questions, including grading

and attribution to study drug. Where applicable, information from relevant hospital case records and autopsy reports should be included.

SAE Reports should be signed by the sub-site PI, and then emailed to the Thomas Jefferson University Study Site Contact within 24 hours.

The TJU coordinator will notify the TJU PI and obtain the TJU PI signature, and report these events to the TJU Medical Monitor/IRB appropriately (within 5 working days if it deems an amendment, or in a spreadsheet at the time of annual review if no amendment is necessary).

Additional follow-up SAE reports should be submitted when available.

All reportable Adverse Events (AEs) should be reported to the TJU Research Coordinator within 48 hours using the FDA MedWatch 3500 form.

A reportable AE is any adverse event NOT identified in the IB or consent form as a risk.

Any non-reportable AE must be kept by the sub-site on an ongoing tracking log to be reviewed by TJU quarterly.

Unanticipated problems (UAPs) that pose risk to subjects or others, and that are not AEs/SAEs should be reported to TJU within 10 working days using form Unanticipated Problems Form (see Appendix C) and should be emailed to the TJU Study Site Contact within 5 business days.

Reporting to the Sidney Kimmel Cancer Center DSMB

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 SKCC DSMC will also include any unanticipated problems that in the opinion of the PI should be reported to the DSMC.
The sub-site is required to provide copies of any Unanticipated Problems, protocol deviations, and AE logs to the TJU Study Site Contact for submission 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)

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.

Thomas Jefferson University will submit SAE reports for all sites to the FDA.

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/

The TJU Study Site Contact is responsible for submitting all SAE reports occurring at TJU and the sub-site to the FDA.

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

6.7.1 Discontinuation of Subjects

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 all of the following trial requirements:

- Has received three contrast-enhanced ultrasound exams
- Has undergone the one month contrast-enhanced follow up MRI scheduled as part of their clinical care.

If a subject's participation in the trial is interrupted for any reason (e.g., because of an AE or if the subject is lost to follow-up) 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.

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.

7.1.1 Sub-Site Data Management

Data forms will be completed by sub-site research staff for all subjects enrolled at the sub-site. Completed data forms must be sent to Thomas Jefferson University for inputting into the database. Image data will be de-identified, transferred to Thomas Jefferson University, and placed on a secure server.

7.2 Statistical Analyses

Data analysis from this multi-center clinical trial will be performed to characterize 2D and 4D CEUS as a follow-up imaging tool for the evaluation of residual tumoral blood flow in patients with HCC treated with TACE.

Our statistical analysis will address three major questions:

1. Does 2D and 4D CEUS 1-2 weeks post TACE yield sensitivity and specificity greater than 90% for identifying residual tumor blood flow, thereby providing reliable earlier identification (relative to the current clinical standard) of patients in need of retreatment?
2. Does 2D and 4D CEUS 1 month post TACE yield sensitivity and specificity greater than 90% for identifying residual tumor blood flow ,thereby providing an accurate, safe, and cost-effective imaging alternative for identifying TACE patients in need of retreatment?
3. Are quantifiable biomarkers generated by parametric imaging of 2D and 4D CEUS exams able to differentiate between fully treated and incompletely treated masses by measuring blood flow kinetics?

The first three cases at each institution will be used as reader training cases and will not be included in the final analysis. In the event of all cases showing identical response (i.e. all incompletely or completely treated), the first case with an alternative outcome will be substituted for the 9^{th} case. Following reader training, each reader (all of whom have previous experience with CEUS and volumetric ultrasound) will be asked to evaluate all 201 remaining cases. 2D and 4D CEUS exams will be read separately and only after randomization. 2D cases will be viewed in a standard DICOM viewer, while 4D cases will be viewed in GE's 4D View software. Readers will first be asked to review both the patient's baseline CE-MRI/CT and CEUS exams to identify the size and location of the treated mass. Following baseline review, each reader will review the 1-2 week and 1 month CEUS exam (in random order). Intratumoral blood flow will be assessed as present or not present. Readers will then be asked to interpret the patient's 1 month follow-up CE-MRI/CT images and again evaluate the need to retreat based on residual blood flow. Finally, when available, readers will review subsequent CE-MRI/CT imaging studies (up to six months post TACE). MRI/CT evaluations will be based on mRECIST criteria. All imaging studies will be read twice by each

reader. Studies will be presented in random order. The second round reads will be performed at least one month after the first. Outcomes will be defined as incompletely treated (i.e. requiring retreatment) based on (in order of preference of reference standard): a) pathological examination of explanted livers demonstrating live tumors; b) tumor enhancement seen with CT or MR and confirmed via angiography during retreatment; c) tumor growth on 6 month follow-up CE-CT/MRI; or d) asymmetrical or nodular tumor enhancement on CE-MRI/CT on 6 month follow-up. Incomplete treatment on CEUS (2D or 4D) will be evaluated by each reader and evidenced by the tumor showing either residual blood flow after treatment or nodular peripheral blood flow.

For questions 1-2, sensitivity (the ability to detect patients in need of retreatment) and specificity (the ability to identify patients who do not require retreatment) will first be calculated on a reader by reader basis. Four 2 x 2 tables will be constructed comparing the need for re-treatment based on the clinical gold standard described above and CEUS, tabulating true positives, false positives, false negatives, and true negatives. A table will be created for CEUS at 1-2 weeks and 1 month for both 2D and 4D CEUS and used to calculate sensitivity, specificity, accuracy, positive predictive value, and negative predictive value. Statistical significance between the reference standard and each of the 4 CEUS groups will be determined using a McNemar test. These values will also be calculated based on the majority decision (i.e. 4 or more readers in agreement). In the event of discordant readings during this analysis, tumor outcome will be decided by consensus by the two readers at TJU. Differences between techniques (2D vs. 4D) and time points (1-2 weeks vs. 1 month post treatment) will be determined by comparing areas under the receiver operating characteristic curves for each modality/time point based on necessity of tumor retreatment. Intra-rater and inter-rater variability will be calculated using a Shrout and Fleiss Interclass Correlation [Shrout and Fleiss 1979]. Finally, a logistical regression and analysis of variance will be performed to determine if significant variations exist between individual sites (to determine operator dependence), lesion size (tumor size dependency) or between patients treated with cTACE vs. DEB-TACE (treatment dependency).

For statistical question 3, all quantitative parameters will be compared between fully treated and incompletely treated masses using a Student's t-test. Additionally, lesions that demonstrate lack of residual enhancement at 1 month, but show recurrence at 6 month follow-up, will be compared to completely treated masses to determine if quantitative CEUS parameters can detect this lower level of residual disease. Statistical analysis will be performed with assistance from the biostatistician co-investigator using Stata 12.0 (Stata Corp, College Station, TX) with p values < 0.05 considered statistically significant after a Bonferroni-type adjustment for multiple tests. These statistical tests will answer our three major questions regarding the sensitivity and specificity of 2D and 4D CEUS as an imaging tool for the follow-up of TACE

at 1-2 weeks and 1 month (specific aims 1-2), as well as the influence of operator, reader, tumor size, and embolization material.

7.2.1 Sample Size Justification

As the most clinically important aims, aims 1-2 will be used as the basis for the sample size analysis, where each patient represents an independent data point used to evaluate the technique's sensitivity and specificity at both 1-2 weeks and 1 month follow-up times. Based on our pilot studies using 2D CEUS to evaluate TACE performed using Lipiodol [Kono et al. 2007] or drug-eluting-beads [Shaw et al. 2014], we expect to encounter a patient population in which up to 67% of patients require retreatment. A sample size analysis was performed using a binomial test with One-Sample Sensitivity and Specificity Analysis in NCSS/PASS 2008 (NCSS, East Kaysville, UT) to estimate the number of cases required to produce a over 85% power for this study. This analysis assumes sensitivity and specificities of 98%, an alpha of 0.05, prevalence of disease of 66.7%, and null sensitivity and specificities of 90%. Results from this analysis are provided in tabular format below.

			--- Sensitivity ---		--- Specificity ---		---------- Alpha ----------			
Power	Prevalence	Sample Size	H0	H1		Sens.	Spec.	Actual		
Sens.	Spec.	N1 and N	Se0	Se1	Sp0	Sp1	Target	Actual	Actual	P
0.0000	0.0000	7								
10	0.9000	0.9800	0.9000	0.9800	0.0500	0.0000	0.0000	0.6670		
0.0000	0.0000	13								
20	0.9000	0.9800	0.9000	0.9800	0.0500	0.0000	0.0000	0.6670		
0.0000	0.0000	20								
30	0.9000	0.9800	0.9000	0.9800	0.0500	0.0000	0.0000	0.6670		
0.0000	0.0000	27								
40	0.9000	0.9800	0.9000	0.9800	0.0500	0.0000	0.0000	0.6670		
0.5134	0.0000	33								
50	0.9000	0.9800	0.9000	0.9800	0.0500	0.0309	0.0000	0.6670		
0.4457	0.0000	40								
60	0.9000	0.9800	0.9000	0.9800	0.0500	0.0148	0.0000	0.6670		
0.7581	0.0000	47								
70	0.9000	0.9800	0.9000	0.9800	0.0500	0.0440	0.0000	0.6670		
0.7135	0.0000	53								
80	0.9000	0.9800	0.9000	0.9800	0.0500	0.0259	0.0000	0.6670		
0.6619	0.5455	60								
90	0.9000	0.9800	0.9000	0.9800	0.0500	0.0138	0.0424	0.6670		
0.8494	0.5134	67								
100	0.9000	0.9800	0.9000	0.9800	0.0500	0.0307	0.0309	0.6670		
0.8202	0.4735	73								
110	0.9000	0.9800	0.9000	0.9800	0.0500	0.0190	0.0203	0.6670		
0.9231	0.4457	80								
120	0.9000	0.9800	0.9000	0.9800	0.0500	0.0353	0.0148	0.6670		
0.9027	0.4195	87								
130	0.9000	0.9800	0.9000	0.9800	0.0500	0.0211	0.0108	0.6670		
0.9608	0.7581	93								
140	0.9000	0.9800	0.9000	0.9800	0.0500	0.0382	0.0440	0.6670		

0.9492	0.7358	100						
150	0.9000	0.9800	0.9000	0.9800	0.0500	0.0237	0.0338	0.6670
0.9791	0.7135	107						
160	0.9000	0.9800	0.9000	0.9800	0.0500	0.0374	0.0259	0.6670
0.9735	0.6839	113						
170	0.9000	0.9800	0.9000	0.9800	0.0500	0.0255	0.0181	0.6670
0.9893	0.6619	120						
180	0.9000	0.9800	0.9000	0.9800	0.0500	0.0382	0.0138	0.6670
0.9857	0.8879	127						
190	0.9000	0.9800	0.9000	0.9800	0.0500	0.0250	0.0421	0.6670
0.9945	0.8494	133						
200	0.9000	0.9800	0.9000	0.9800	0.0500	0.0385	0.0307	0.6670

A total of 201 patients will be used in the final analysis for this study (the first 9 will be used as training cases and excluded), of which 135 (66.7%) will require retreatment. A total sample size of 201 (which includes 133 subjects with the disease) achieves 99% power to reject the null hypothesis for sensitivity, and 85% power to reject the null hypothesis for specificity.

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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, Ph.D., Assistant Professor of Radiology, TJU 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, TJU is co-PI on the grant and will be responsible for all scientific aims within the study. She will assist with the patient recruitment at Thomas Jefferson University, interpret ultrasound images and advise on clinical issues.

Flemming Forsberg, PhD Professor of Radiology, TJU will assist with the patient recruitment, interpret ultrasound images and advise on data processing issues.

Jesse Civan, MD, Assistant Professor of Medicine, Division of Gastroenterology and Hepatology, TJU, will assist with the patient recruitment at Thomas Jefferson University, interpret results and advise on clinical issues.

Andrej Lyshchik, MD, PhD, Assistant Professor of Radiology, TJU will assist with the patient recruitment at Thomas Jefferson University, provide input during site training and scanning, interpret ultrasound images and advise on clinical issues.

Patrick O'Kane, MD , Assistant Professor of Radiology, TJU will assist with the patient recruitment at Thomas Jefferson University, provide input during site training and scanning, interpret ultrasound images and advise on clinical issues.

Amanda Smolock, MD, Assistant Professor of Radiology, TJU will assist with the patient recruitment at Thomas Jefferson University, provide input during site training and scanning, interpret ultrasound images and advise on clinical issues.

Allison Tan, MD, Assistant Professor of Radiology, TJU will assist with the patient recruitment at Thomas Jefferson University, provide input during site training and scanning, interpret ultrasound images and advise on clinical issues

Yoko Kono, MD, PhD, Assistant Professor of Medicine and Radiology, UCSD will oversee all work within The University of California, San Diego. She will oversee patient recruitment, supervise ultrasound scanning, interpret ultrasound images and advise on clinical issues.

Robert Mattrey, MD, Professor of Radiology, UTSW, will provide input during site training and scanning, interpret ultrasound images and advise on clinical issues.

Steven Rose, MD, Professor of Radiology, UCSD, will provide input during site training and scanning, and advise on clinical issues.

Michael Soulen, MD, Professor of Radiology, HUP, will oversee all work within The Hospital of the University of Pennsylvania. He will oversee patient recruitment, supervise ultrasound scanning, advise on clinical issues, and interpret CEUS cases.

Arthur Fleischer, MD, Professor of Radiology, Vanderbilt, will interpret ultrasound images and advise on clinical issues.

Corrine Wessner, RDMS, is a research sonographer. She will be responsible for performing the ultrasound examinations under the supervision of the radiologists listed as co-investigators and the PI of the study.

Susan Schultz, RDMS, is a research sonographer at HUP. She will be responsible for performing the ultrasound examinations under the supervision of the radiologists listed as co-investigators and the PI of the study.

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

Cynthia Miller, RN will prepare and administer the contrast agent, record medications, and monitor the patients appropriately during and after the procedure.

Kirsten Bradigan, RN will prepare and administer the contrast agent, record medications, and monitor the patients appropriately during and after the procedure.

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

Signature of PI: _____
John Eisenbrey, PhD

APPENDIX B – DEFINITY PRODUCT INSERT

Appendix C

UNANTICIPATED PROBLEM REPORT FORM

For Sub-Site Reporting

Thomas Jefferson University Principal Investigator: _____
Sub-Site Principal Investigator: _____
TJU IRB Control Number/Sub-Site Identifier: _____

Protocol Title: _____
_____

Subject ID: _____ Approx. Date of Problem: _____ Date Aware: ____
Description of Problem: _____
_____

Is this Unanticipated Problem a Protocol Deviation? Yes ☐ No ☐

Did the Unanticipated Problem pose risk to subjects or others? Yes ☐ No ☐

If no, have PI or Co-I sign the form. If YES, describe the risk below:

Describe the Corrective Action Plan: _____
_____

Has the problem been resolved? Yes ☐ No ☐

Does the consent or protocol require modification? Yes ☐ No ☐

_____
Signature of person preparing report Date Email/Phone number

_____
Sub-site PI signature Date Email/Phone number