9-1-2021

# Predicting Long-Term Hepatocellular Carcinoma Response to Transarterial Radioembolization Using Contrast-Enhanced Ultrasound: Initial Experiences.

Lauren J. Delaney
*Thomas Jefferson University*

M. Tantawi
*Thomas Jefferson University*

Corinne Wessner
*Thomas Jefferson University*

Priscilla Machado
*Thomas Jefferson University*

Flemming Forsberg
*Thomas Jefferson University*



*See next page for additional authors*

# Predicting Long-term Hepatocellular Carcinoma Response to Transarterial Radioembolization Using Contrast-enhanced Ultrasound: Initial Experiences

Lauren J. Delaney [a], Mohamed Tantawi [a], Corinne E. Wessner [a], Priscilla Machado [a], Flemming Forsberg [a], Andrej Lyshchik [a], Patrick O'Kane [a], Ji-Bin Liu [a], Jesse Civan [b], Allison Tan [a], Kevin Anton [a], Colette M. Shaw [a], John R. Eisenbrey [a]

[a] Department of Radiology, Thomas Jefferson University, 132 South 10th Street, Philadelphia, PA 19107, USA

[b] Division of Gastroenterology and Hepatology, Department of Medicine, Thomas Jefferson University, 132 S. 10th Street, Philadelphia, PA 19107, USA

Corresponding Author:

John R. Eisenbrey, PhD

Department of Radiology

Thomas Jefferson University

132 South 10th Street, Main 796

Philadelphia, PA 19107

United States of America

Phone: 1-215-503-5188

Email: john.eisenbrey@jefferson.edu

**Abstract**

Conventional cross-sectional imaging done shortly after radioembolization of hepatocellular carcinoma (HCC) does not reliably predict long-term response to treatment. This study evaluated whether quantitative contrast-enhanced ultrasound (CEUS) can predict long-term response of HCC to Yttrium-90 (Y-90) treatment. Fifteen patients underwent CEUS at 3 time points: immediately following treatment, and 1 and 2 weeks post-treatment. Response 3-6 months after treatment was categorized on contrast-enhanced MRI by two experienced radiologists using mRECIST criteria. CEUS data was analyzed by quantifying tumor perfusion and residual fractional vascularity using time intensity curves. Patients with stable disease on MR had significantly greater fractional vascularity 2 weeks post-treatment (65.15%) than those with partial or complete response (13.8±9.9%, p=0.007, and 14.9±15.4%, p=0.009, respectively). Complete responders had lower tumor vascularity at 2 weeks than at post-op examination (-38.3±15.4%, p=0.045). Thus, this pilot study suggests CEUS may provide an earlier indication of Y-90 treatment response than cross-sectional imaging.

**Keywords**: contrast-enhanced ultrasound, CEUS, transarterial radioembolization, TARE, Y-90, hepatocellular carcinoma, HCC, perfusion, fractional vascularity

## Introduction

Hepatocellular carcinoma (HCC) is the fourth most common cause of cancer mortality worldwide (Yang, et al. 2019), with a dismal five-year survival rate of 18% (Jemal, et al. 2017). The incidence of HCC has increased by 3% in recent years (Siegel, et al. 2020). The treatment choice for HCC depends on the liver function, tumor burden, and the functional status of the patient. Although surgical resection has a five-year survival rate at 60% (Fan, et al. 2011), it is feasible in only 30% of HCC patients (Llovet and Bruix 2003). Liver transplantation is associated with better long-term survival rates, but requires a contained disease state (1 lesion less than 5 cm or 3 lesions less than 3 cm) (Llovet, et al. 1999). Furthermore, because of the increasing incidence along with organ shortages, liver transplants are feasible for only 7% of HCC patients in the United States (Moon, et al. 2018). Options for patients with unresectable HCC include locoregional therapies such as ablation and embolization. Embolization can be performed using transarterial chemoembolization (TACE) or Yttrium-90 (Y-90) transarterial radioembolization (TARE).

Targeted injection of Y-90-embedded glass microspheres induces extensive tumor necrosis with relatively low occurrence of adverse events. At our institution, radioembolization is performed using TheraSpheres (BTG International, London, United Kingdom), which consist of 20-30 µm glass beads containing Y-90. The TheraSpheres are delivered via a catheter temporarily placed in the hepatic artery branches supplying the tumor, and, upon lodging in the tumor neovasculature, provides a localized and sustained release of radiation within the tumor. Recent studies have reported prolonged time to progression (Salem, et al. 2011), better tumor control (Lewandowski, et al. 2009,

Riaz, et al. 2009, Riaz, et al. 2010), and improved quality of life in patients receiving radioembolization compared with traditional chemoembolization (Salem, et al. 2013a). However, another prospective trial reported similar efficacy of both approaches (Kolligs, et al. 2015).

Modified response evaluation criteria in solid tumors (mRECIST) is used to describe HCC response to locoregional therapy (Lencioni and Llovet 2010). These criteria take into account tumor viability as evidenced by arterial enhancement, using the linear sum of diameters of the viable tumor observed on contrast-enhanced CT or MRI. HCC response rate to TARE reported using mRECIST was 25-60% (Andreana, et al. 2012). Alternatively, studies evaluating HCC response based on reduction in tumor size and vascularity have reported response rates of 47-89% (Carr 2004, Salem, et al. 2011). The Y-90 treatment response is typically assessed with contrast-enhanced MRI or CT performed 3-6 months after treatment. As TARE does not depend on direct vessel embolization, evaluation of treatment effect in the immediate post-operative period is challenging. Earlier assessment of the tumor response could potentially improve the outcomes of HCC patients by allowing faster retreatments in those with residual viable tumor. Recent reports have shown that quantitative characteristics other than tumor size, such as changes in tumor perfusion, can provide an early predictor of HCC treatment response and outcome (Kim, et al. 2019, Serres, et al. 2014, Zocco, et al. 2013).

Contrast-enhanced ultrasound (CEUS) utilizes ultrasound contrast agents (UCAs), which are gas-filled microbubbles (<10 μm) with a lipid, protein, or polymer shell. These

bubbles are roughly the same size as red blood cells, so they can pass through the pulmonary capillaries and are confined to the blood vessels (Lyshchik 2019). Consequently, they provide effective visualization of the vasculature in different tissues. CEUS imaging also provides several diagnostic advantages compared to MRI/CT. CEUS provides real-time imaging (on average 15 frames/second), whereas MRI/CT provide on average 1 frame every 30 seconds. Additionally, CEUS provides real blood pooling imaging as the UCAs are large enough to remain within the blood pool, compared to MR/CT contrast agents that escape the vasculature and pool in the interstitium (Chong, et al. 2018). UCAs perfuse into the vasculature of HCC tumors, and their wash-in/wash-out kinetics can be used to characterize liver masses (Shaw, et al. 2015). The safety and accuracy of CEUS for monitoring HCC response to TACE were demonstrated in a prospective study (Shaw, et al. 2015). UCAs are able to perfuse into HCC post radioembolization, due to the fact that the large Y-90 beads (20-30 μm in diameter) do not completely restrict blood flow to the tumor (Salem, et al. 2013b). Commercially available flash-replenishment imaging modes can be used to visualize and quantify contrast perfusion (Lefort, et al. 2012, Wakui, et al. 2011). These flash-replenishment sequences generate relatively high intensity pulses within a selected sector of interest to induce UCA cavitation and destruction, followed by lower intensity imaging to visualize contrast reperfusion. In this pilot study, we evaluated the ability of quantitative CEUS performed 1 and 2 weeks post-treatment to predict long term response of HCC to Y-90 radioembolization. The goal of this interim analysis is to determine the feasibility of this method. We hypothesize that UCA reperfusion following flash-replenishment ultrasound pulses will reflect changes in tumor perfusion and fractional vascularity and provide an

earlier predictor of Y-90 radioembolization treatment response than standard of care CT or MR imaging.

## Materials and Methods

Patient recruitment and clinical standard care

As part of an ongoing IRB-approved, prospective trial (NCT# 03199274, FDA IND #126,768) at Thomas Jefferson University, 15 participants scheduled for sub-lobar transarterial radioembolization (TARE) therapy of a previously untreated HCC tumor (< 6 cm) from July 2017 through February 2020 provided informed consent to be included in this study (Eisenbrey, et al. 2020), where participants are followed for up to 6 months post-TARE and the follow up imaging schedule is dictated by the patient's standard of care. As part of the standard of care at our institution, TARE with glass microspheres is used to downstage HCC tumors to within Milan criteria (a single tumor < 5 cm or up to 3 tumors where each is ≤ 3 cm, no vascular invasion, and no extrahepatic involvement). Radiotherapy was performed using sub-lobar delivery of Y-90 TheraSpheres (BTG International, London, UK) at doses ranging from 117-152 Gy. Exclusion criteria included known sensitivities to blood, blood products, albumin, and perflutren, as well as elevated (> 2 mg/dL) bilirubin levels.

Two board-certified radiologists (A.L. and P.O.) with over 15 years of experience in body imaging evaluated contrast-enhanced magnetic resonance imaging (MRI) pre-TARE and at 3-6 months after TARE for each patient and provided a consensus treatment response assessment using mRECIST (Lencioni and Llovet 2010). For the two

participants who received further tumor intervention or died prior to the 3-6 month imaging window, a 1- to 3-month follow up exam was used for the treatment response evaluation. Time to next treatment (TTNT), as decided by a multi-disciplinary tumor board as part of the clinical standard of care, overall survival, and transplant status were also monitored and recorded for each participant.

CEUS examination and analysis

CEUS exams were performed at three time points: 1-4 hours following radioembolization and at approximately 1 and 2 weeks post-treatment. All imaging was performed using a commercially available Siemens S3000 Helx Evolution scanner (Siemens Healthineers, Mountain View, CA, USA) with a 6C1 transducer in dual 2D B-mode/contrast mode by a sonographer (C.W.) with over 5 years of clinical and research experience. Participants received an infusion of 5 mL of the UCA Optison (GE Healthcare, Princeton, NJ, USA) suspended in 50 mL of sterile saline at a rate of 120 mL/hr, based on data from a larger therapeutic trial (Eisenbrey, et al. 2020), which chose Optison based on preclinical data (Daecher, et al. 2017). Microbubble infusion was preferred over bolus injection because the process is more reproducible and allows for more prolonged, consistent imaging enhancement and better assessment of intra-patient variability (Albrecht, et al. 1998, Correas, et al. 2000, Tang, et al. 2011).

During each CEUS exam, flash-replenishment sequences were performed at the tumor midline for UCA destruction/replenishment imaging. Participants were asked to halt respiration while a 4-second flash/replenishment sequence was transmitted (MI = 1.13 at

1.5 MHz, transmitting 2.3 μs pulses at 100 Hz), followed by nonlinear imaging of contrast replenishment at lower intensity using Cadence Pulse Sequencing (CPS, MI = 0.06) for 10 seconds. Three to five of these flash/replenishment sequences were performed at the tumor midline to evaluate and quantify tumor vascularity changes over time. These cine clips of the flash/replenishment sequences were collected within the first 3 to 4 minutes of the CEUS exam, each lasting from 5 to 15 seconds long.

Ultrasound contrast time-intensity curves were generated offline using Matlab software (MathWorks, Natick, MA, USA) to quantify residual fractional vascularity and perfusion post-treatment over the entire cross-sectional area of the tumor midline using a segmentation algorithm. Contrast replenishment time intensity curves were fitted to a 2-parameter exponential recovery curve: $VI = \alpha(1 - e^{\beta t})$, where VI represents video intensity; α (in dB) represents the asymptotic plateau correlative of the microvessel cross-sectional area; and β (in mm/s) represents the blood velocity (Krix, et al. 2003a, Krix, et al. 2003b).

Statistical analysis

Statistical analysis was performed with GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA), with p-values below 0.05 indicating statistical significance. Consensus mRECIST outcomes were used as the reference standard in all cases. Comparisons between treatment groups were performed using a one-way ANOVA with Bonferroni correction for multiple comparisons. Comparisons between exam time points were

160 performed using paired t-tests, and comparisons between treatment groups at each time
161 point were performed using unpaired t-tests. Error bars represent standard deviation.
162
163 **Results**
164 Participant Characteristics
165 Participant demographics are summarized as a whole and by treatment group in Table
166 1. There were no significant differences in participant age (range 45 – 87 years) or tumor
167 size (range 1.3 – 5.1 cm) between treatment groups ($p > 0.08$). The participant who did
168 not respond to TARE, and was classified as stable disease, had a significantly greater
169 BMI (41.6 kg/m$^2$) than the average BMI for the partial response group (25.8 ± 3.6 kg/m$^2$,
170 $p = 0.009$) and the complete response group (28.1 ± 4.5 kg/m$^2$, $p = 0.026$). There was no
171 difference in BMI (range 19.3 – 33.9 kg/m$^2$) between partial response and complete
172 response ($p = 0.89$). All participants received similar radiation doses to the targeted lesion
173 (range 125.1 – 155.2 Gy, $p = 0.95$ between treatment groups). All participants were
174 ambulatory and capable of self-care, as evidenced by a clinical Eastern Cooperative
175 Oncology Group (ECOG) score of 2 or less (Oken, et al. 1982).
176
177 Clinical Outcomes
178 According to the mRECIST scoring at 3-6 months post-TARE, only 1 participant had
179 stable disease (6.7%), 8 participants had partial response (53.3%), and 6 participants had
180 complete response (40.0%).
181

Clinical outcomes, including the reported LI-RADS treatment response at the 1-3 month MRI and 6-month MRI, are given for each participant in Table 2. The participant with stable disease required re-treatment with TACE 2.5 months post-TARE, and ultimately received a liver transplant 12 months following re-treatment. As for the 8 participants with partial response, 3 received transplants (37.5%), 2 are on the transplant waiting list (25.0%), and 3 were not eligible for transplant due to age or other factors (37.5%). Of those 3 patients, 2 presented with a small focus of disease at follow up, and 1 died of causes unrelated to disease progression. For the 6 participants with complete response, 4 received transplants (66.7%), and 2 were not eligible for transplant due to age, but presented with no viable tumor as of publication (33.3%).

CEUS Examination

Administration of Optison and subsequent CEUS examinations were well-tolerated in all patients, with no serious adverse effects. Ultrasound contrast enhancement and UCA destruction were observed in all cases. An example sequence of CEUS destruction/reperfusion images is shown in Figure 1.

*Perfusion*

Tumor perfusion outcomes are summarized in Table 3. There were no significant differences in tumor perfusion between response groups at any of the 3 time points ($p > 0.44$). Within response groups, there were no significant differences in perfusion at any time point for partial responders ($p > 0.27$) or complete responders ($p > 0.99$). However, for the participant with stable disease, increased perfusion was observed at 1 week (1.14

205 x $10^{-1}$ mL/s*mg, $p < 0.0001$) compared to both post-operatively (6.42 x $10^{-2}$ mL/s*mg) and
206 2 weeks (6.17 x $10^{-2}$ mL/s*mg). There was no difference in tumor perfusion for the
207 participant with stable disease between 2 weeks post-TARE and 1-4 hours post-TARE ($p > 0.99$).
208 Figure 2 shows examples of the temporal changes in the time intensity curves modeling
209 perfusion for the participant with stable disease (Figure 2A), a participant with partial
210 response (Figure 2B), and a participant with complete response (Figure 2C).
211
212 Since the baseline tumor perfusion estimate is not normalized, but unique to each
213 participant's clinical presentation, we also evaluated the degree of change in tumor
214 perfusion for each participant in an attempt to normalize the data. We found no significant
215 differences in the degree of change between 1 week and the 1-4 hours post-operative
216 exam ($p > 0.99$), nor in the degree of change between 2 weeks and the 1-4 hours post-
217 operative exam ($p > 0.06$); however, the difference between the degree of change for
218 partial responders (-1.90 x $10^{-2}$ ± 9.04 x $10^{-3}$) and the participant with stable disease (-
219 2.48 x $10^{-3}$) between the 2 weeks and 1-4 hours post-operative examinations suggested
220 a trend ($p = 0.06$).
221
222 *Fractional Vascularity*
223 Fractional vascularity provided additional insight into the differences between
224 treatment response groups, and may suggest the likelihood of patient response to Y-90
225 therapy. Representative CEUS images of the temporal changes in fractional vascularity
226 for each treatment response group are shown in Figure 3. Fractional vascularity outcomes

are summarized in Table 4 and shown in Figure 4 and are also provided for each participant in Table 2 for comparison with clinical outcomes.

As we observed with modelled perfusion, there were no differences in fractional vascularity between treatment response groups at the 1-4 hours post-operative exam ($p > 0.99$) or at 1 week ($p > 0.32$). However, at 2 weeks post-TARE, the participant classified as having stable disease had significantly greater fractional vascularity (65.15%) than both the partial disease group (13.80 ± 9.92%, $p = 0.007$) and the complete response group (14.86 ± 15.35%, $p = 0.009$). There was no difference in the fractional vascularity at 2 weeks between partial responders and complete responders ($p > 0.99$).

The participant with stable disease showed greater fractional vascularity at 1 week (70.96%) than at the 1-4 hours post-op exam (60.92%, $p = 0.007$). This was not observed in partial responders (42.34 ± 31.02% post-op vs. 26.29 ± 20.95% at 1 week, $p = 0.50$) nor complete responders (53.13 ± 27.16% post-op vs. 33.39 ± 27.76% at 1 week, $p = 0.53$). There were no other temporal differences for the participant with stable disease ($p > 0.18$). Additionally, there were no temporal differences in fractional vascularity for partial responders ($p > 0.06$). For complete responders, there was significantly reduced fractional vascularity at 2 weeks (14.86 ± 15.35%) than at the 1-4 hours post-operative exam (53.13 ± 27.16%, $p = 0.045$). There were no other temporal differences for complete responders ($p > 0.53$).

As with perfusion, we also evaluated the degree of change in fractional vascularity for each participant in an attempt to normalize the data. We found no significant differences in the degree of change between 1 week and the 1-4 hours post-operative exam ($p > 0.82$). However, when evaluating the degree of change in fractional vascularity from 1-4 hours post-TARE to 2 weeks, the participant with stable disease showed increased fractional vascularity (+4.23%) while the partial response group (-28.54 ± 9.92%, $p = 0.009$) and complete response group (-38.27 ± 15.35%, $p = 0.025$) both showed reduced fractional vascularity. There was no difference between partial responders and complete responders ($p = 0.09$).

**Discussion**

This work represents, to our knowledge, the first human clinical trial using UCAs to predict tumor response to Y-90 TARE therapy in HCC patients at 2 weeks post-operatively. Results showed that participants who responded to Y-90 TARE, whether partial or complete response, exhibit decreased tumor vascularity (reduced by at least 28.54 ± 9.92%, $p < 0.025$) 2 weeks post-TARE, while the participant who did not respond (stable disease) exhibited unchanged or increasing tumor perfusion and vascularity as quantified with CEUS. The potential clinical impact of these findings is promising. The interventional radiologist would be able to intervene at 2 weeks post-TARE in patients with stable disease, instead of waiting for the 3-6 month MRI evaluation as part of the current clinical standard of care, potentially improving the overall patient outcome.

Multiple studies report better survival outcomes with TARE therapy compared with conventional TACE (Inchingolo, et al. 2019, Yang and Si 2018). However, the available evidence is insufficient to make a conclusion about the superiority of either approach (Abdel-Rahman and Elsayed 2020). The primary mechanism of radiotherapy, especially at higher doses, is believed to be destruction of the tumor microvasculature, causing secondary death of cancer cells (Garcia-Barros, et al. 2003). Changes in tumor perfusion and vascularity can provide an early indication of tumor response to such therapy (Serres, et al. 2014). Studies have described the potential of CEUS-derived parameters in predicting HCC response to anti-angiogenic therapy (Frampas, et al. 2013, Zocco, et al. 2013). In those studies, a decrease in perfusion parameters calculated from the time-intensity curve correlated with better HCC response. Previously, our group and others have shown that UCA perfuse into HCC tumors in cases where TACE treatment is incomplete, and that CEUS can be used to accurately quantify residual disease (Kono, et al. 2007, Nam, et al. 2018, Shaw, et al. 2015).

Since the Y-90 TheraSpheres (20-30 μm) do not fully occlude the blood supply to the tumor (Salem, et al. 2013b), CEUS is capable of quantifying the perfusion and fractional vascularity of the tumor post-TARE. In our current study, we have used a modified 2-parameter exponential recovery curve to estimate the tumor perfusion from the time-intensity curve (Krix, et al. 2003a, Krix, et al. 2003b). On the other hand, the fractional vascularity model utilizes a thresholding function on the image deemed as having the maximum contrast intensity, and is a function that can be easily adjusted to address the image standardization and breathing motion artifact issues confounding the perfusion

294 model. Therefore, the fractional vascularity determined by CEUS may provide a more
295 accurate predictive measure of HCC response to Y-90 TARE therapy.
296
297 Such an early evaluation of treatment response has great potential for clinical impact
298 compared to the current standards of care for evaluating HCC response to TARE.
299 Patients are generally assessed at 1-3 months post-TARE with MRI or CT, but these
300 exams are frequently read as equivocal mainly due to presence of patchy regions of
301 arterial phase enhancement, mimicking diffuse heterogeneous tumor, which typically
302 resolve over 1-5 months. Therefore, most guidelines state that imaging obtained within
303 the first 6 months after TARE should be interpreted with caution (Kielar, et al. 2018).
304 Select patients may be retreated based on this early post-TARE MRI/CT imaging if little
305 to no response is observed, but a second MRI/CT scan at 4-6 months post-TARE is much
306 more reliable for assessing viable tumor (Ibrahim, et al. 2009). Therefore, a quantitative
307 prediction of treatment response at 2 weeks post-TARE would greatly improve patient
308 outcomes by allowing for earlier intervention in cases where disease progression or lack
309 of response is determined.
310
311 This study does have limitations, which must be acknowledged. The perfusion
312 estimation model does not account for differences in gain between time points and
313 participants, imaging plane, movement or breathing, or speed of contrast infusion. Great
314 care was taken to standardize these parameters across all participants, but some factors
315 were beyond our experimental control. Future investigation should also include baseline
316 CEUS evaluation prior to TARE for additional analysis to evaluate tumor necrosis and

echogenicity prior to therapy. Additional follow up CEUS exams at later time points, such as 1 to 2 months post-TARE, may improve the accuracy of this method while still being clinically impactful and will be considered in future investigations. Another limitation of this pilot study is the small sample size, with only 15 participants completing the study at a single medical center. Additionally, only 1 participant was later deemed to have stable disease and not responding to TARE. While that is advantageous for the participants in our study, it does limit the ability to statistically analyze that treatment group, as 1 participant does not necessarily constitute a "group". Therefore, we cannot definitively determine whether the observed differences in tumor perfusion and fractional vascularity, as measured with CEUS, can serve as an effective method of predicting patient response. However, we are encouraged that all the significant findings in our study support this conclusion.

Future work will include further modifications and improvements to the perfusion modeling, including motion compensation and enhanced image processing techniques. Further investigation with larger sample sizes, multiple follow up time points, and at multiple study centers is necessary to fully determine whether CEUS can be used to effectively provide an early prediction of tumor response to Y-90 TARE therapy in HCC patients. However, this pilot study provides a strong proof of concept demonstrating the feasibility of this method.

## Conclusion and Summary

339 While larger sample sizes are required to fully evaluate effectiveness, CEUS appears
340 to provide an earlier indicator of Y-90 TARE response at 2 weeks compared to the current
341 clinical standard of care mRECIST evaluation performed 3-6 months post-treatment. The
342 potential clinical impact of these findings is promising, in that quantitative CEUS
343 performed 2 weeks after treatment may be useful in predicting long-term response of
344 HCC tumors to Y-90 TARE therapy.
345

346 **Acknowledgements**

347 The authors would like to thank Kristen Bradigan for nursing support and Nancy
348 Pedano, Marsha Robinson, and Diana Zaccagnini for coordinator support. We also thank
349 Siemens Healthineers for equipment support. The work was supported by the National
350 Institutes of Health (R01 CA238241, R01 CA194307, and F32 AR072491).
351

**Figure Captions**

Figure 1: Example CEUS destruction/reperfusion images at 2 weeks post-TARE from a patient with partial response, arrows indicate location of treated tumor. A) CEUS image prior to destructive flash pulse, showing presence of UCA in liver and tumor tissue. B) CEUS image during destructive flash pulse. C) CEUS image immediately following (1 second later) destructive pulse, showing destruction of UCA from within liver and tumor tissue. D) CEUS image showing UCA reperfusion into liver and tumor tissue following (5 seconds later) flash pulse.

Figure 2: Example time intensity curves modeling tumor perfusion showing temporal changes in A) stable disease, B) partial response, and C) complete response. Blue = 2-4 hours post-TARE, orange = 1 week post-TARE, purple = 2 weeks post-TARE.

Figure 3: Representative CEUS images of temporal changes in fractional vascularity for each treatment response group, with the tumor outlined by the dotted white circle on each grayscale (right) image. The left image is the contrast-enhanced image, where UCA appear as orange image enhancement. A-C) Representative images from the participant with stable disease. D-F) Representative images from a participant with partial response. G-I) Representative images from a participant with complete response.

Figure 4: Summary of tumor vascularity at each study time point, stratified by treatment response group. SD = Stable Disease, left, n = 1, PR = Partial Response, center, n = 8,

CR = Complete Response, right, n = 6. CEUS exam time points are designated by shading pattern with post-op as solid white, 1 week post-TARE as horizontal striped, and 2 weeks post-TARE as crosshatched. Error bars = standard deviation. *p = 0.045, **p = 0.009, ***p = 0.007.

379 **Tables**

| Table 1: Participant demographics | | | | |
|---|---|---|---|---|
| | All Participants (n=15) | Stable Disease (n=1) | Partial Response (n=8) | Complete Response (n=6) |
| Age (years) | 68 ± 11 | 45 | 70 ± 9 | 69 ± 10 |
| Gender | 9 male (60%)<br>6 female (40%) | 0 male (0%)<br>1 female (100%) | 4 male (50%)<br>4 female (50%) | 5 male (83%)<br>1 female (17%) |
| BMI ($kg/m^2$) | 27.8 ± 5.5 | 41.6 | 25.8 ± 3.6 | 28.1 ± 4.5 |
| Tumor Size (cm) | 3.5 ± 1.3 | 3.5 | 3.4 ± 1.4 | 3.6 ± 1.4 |
| Radiation Dose (Gy) | 139.8 ± 9.5 | 145.5 | 139.4 ± 10.3 | 139.5 ± 9.7 |
| ECOG Score | 2 with ECOG 2<br>3 with ECOG 1<br>10 with ECOG 0 | 1 with ECOG 2 | 1 with ECOG 2<br>1 with ECOG 1<br>6 with ECOG 0 | 2 with ECOG 1<br>4 with ECOG 0 |
| Presence of Ascites (# positive) | 5 (33%) | 1 (100%) | 2 (25%) | 2 (33%) |

380

381

**Table 2: Participant clinical outcomes**

| mRECIST Response Classification | LI-RADS Treatment Response Classification at 1-3 months | LI-RADS Treatment Response Classification at 6 months | Clinical Outcome | Change in Fractional Vascularity at 2 week CEUS |
|---|---|---|---|---|
| SD | Viable | Viable | Retreated via TACE at 3 months, transplant after 14 months | + 4.23 ± 5.04% |
| PR | Nonviable | Equivocal | Lost to follow up | - 35.11 ± 1.74% |
| PR | Nonviable | Nonviable | Retreated via microwave ablation at 16 months | - 25.91 ± 10.36% |
| PR | Viable | N/A | Non-disease related death at 3 months | - 29.28 ± 10.92% |
| CR | Nonviable | Nonviable | Surveillance (not eligible for transplant due to age) | - 47.14 ± 1.60% |

| | | | | |
|---|---|---|---|---|
| CR | Nonviable | Nonviable | Surveillance (not eligible for transplant due to age) | - 53.06 ± 0.10% |
| CR | Equivocal | Nonviable | Transplant after 16 months | - 46.96 ± 2.97% |
| PR | Viable | N/A | Retreated via TACE at 2 months, then transplant at 5 months | - 23.97 ± 8.11% |
| PR | Viable | Viable | Retreated via TACE at 11 months, transplant after 18 months, explant showed no viable tumor | - 35.34 ± 3.28% |
| CR | Nonviable | Nonviable | Transplant after 17 months | - 41.15 ± 4.71% |
| PR | Equivocal | N/A | Retreated via TACE at 2 months, on transplant waitlist | - 40.87 ± 1.34% |

| | | | | |
|---|---|---|---|---|
| PR | Viable | N/A | Retreated via TACE at 4 months, transplant after 33 months | - 29.72 ± 3.26% |
| CR | Equivocal | Nonviable | Transplant after 8 months | - 11.26 ± 11.80% |
| CR | Equivocal | Equivocal | Retreated via TACE at 7 months, transplant after 10 months | - 30.05 ± 5.67% |
| PR | Viable | N/A | Retreated via TARE at 4 months, not eligible for transplant due to extrahepatic malignancy | - 8.12 ± 6.53% |

382

383

384

385

386

387

**Table 3: Summary of average tumor perfusion (mL/s*mg) by treatment response and observational time point.**

| | Stable Disease (n=1) | Partial Response (n=8) | Complete Response (n=6) |
|---|---|---|---|
| Post-Op | $6.42 \times 10^{-2}$ | $4.34 \times 10^{-2} \pm 2.95 \times 10^{-2}$ | $9.26 \times 10^{-2} \pm 8.42 \times 10^{-2}$ |
| 1 Week | $1.14 \times 10^{-1}$ | $5.94 \times 10^{-2} \pm 6.11 \times 10^{-2}$ | $7.55 \times 10^{-2} \pm 8.75 \times 10^{-2}$ |
| 2 Weeks | $6.17 \times 10^{-2}$ | $2.44 \times 10^{-2} \pm 9.04 \times 10^{-3}$ | $5.10 \times 10^{-2} \pm 5.18 \times 10^{-2}$ |
| | | | |
| 2 Weeks – Post-Op | $-2.48 \times 10^{-3}$ | $-1.90 \times 10^{-2} \pm 9.04 \times 10^{-3}$ | $-4.16 \times 10^{-2} \pm 5.18 \times 10^{-2}$ |
| 1 Week – Post-Op | $4.97 \times 10^{-2}$ | $1.61 \times 10^{-2} \pm 6.11 \times 10^{-2}$ | $-1.71 \times 10^{-2} \pm 8.75 \times 10^{-2}$ |

**Table 4: Summary of average fractional vascularity (% of tumor) by treatment response and observational time point.**

| | Stable Disease (n=1) | Partial Response (n=8) | Complete Response (n=6) |
|---|---|---|---|
| Post-Op | 60.92% | 42.34 ± 31.02% | 53.13 ± 27.16% |
| 1 Week | 70.96% | 26.29 ± 20.95% | 33.39 ± 27.76% |
| 2 Weeks | 65.15% | 13.80 ± 9.92% | 14.86 ± 15.35% |
| | | | |
| 2 Weeks – Post-Op | +4.23% | -28.54 ± 9.92% | -38.27 ± 15.35% |
| 1 Week – Post-Op | +10.04% | -16.05 ± 20.95% | -19.67 ± 27.76% |

## References

Abdel-Rahman O, Elsayed Z. Yttrium-90 microsphere radioembolisation for unresectable hepatocellular carcinoma. Cochrane Database of Systematic Reviews 2020.

Albrecht T, Urbank A, Mahler M, Bauer A, Doré CJ, Blomley MJ, Cosgrove DO, Schlief R. Prolongation and optimization of Doppler enhancement with a microbubble US contrast agent by using continuous infusion: preliminary experience. Radiology 1998; 207:339-47.

Andreana L, Isgrò G, Marelli L, Davies N, Yu D, Navalkissoor S, Burroughs AK. Treatment of hepatocellular carcinoma (HCC) by intra-arterial infusion of radio-emitter compounds: Trans-arterial radio-embolisation of HCC. Cancer Treatment Reviews 2012; 38:641-49.

Carr BI. Hepatic arterial 90Yttrium glass microspheres (Therasphere) for unresectable hepatocellular carcinoma: Interim safety and survival data on 65 patients. Liver Transplantation 2004; 10:S107-S10.

Chong WK, Papadopoulou V, Dayton PA. Imaging with ultrasound contrast agents: current status and future. Abdominal Radiology 2018; 43:762-72.

Correas JM, Burns PN, Lai X, Qi X. Infusion versus bolus of an ultrasound contrast agent: in vivo dose-response measurements of BR1. Invest Radiol 2000; 35:72-9.

Daecher A, Stanczak M, Liu J-B, Zhang J, Du S, Forsberg F, Leeper DB, Eisenbrey JR. Localized microbubble cavitation-based antivascular therapy for improving HCC treatment response to radiotherapy. Cancer Letters 2017; 411:100-05.

Eisenbrey JR, Forsberg F, Wessner CE, Delaney LJ, Bradigan K, Gummadi S, Tantawi M, Lyshchik A, O'Kane P, Liu J-B, Intenzo C, Civan J, Maley W, Keith SW, Anton

428 K, Tan A, Smolock A, Shamimi-Noori S, Shaw CM. US-triggered Microbubble
429 Destruction for Augmenting Hepatocellular Carcinoma Response to Transarterial
430 Radioembolization: A Randomized Pilot Clinical Trial. Radiology 2020; 298:450-
431 57.
432 Fan ST, Mau Lo C, Poon RTP, Yeung C, Leung Liu C, Yuen WK, Ming Lam C, Ng KKC,
433 Ching Chan S. Continuous Improvement of Survival Outcomes of Resection of
434 Hepatocellular Carcinoma: A 20-Year Experience. Annals of Surgery 2011;
435 253:745-58.
436 Frampas E, Lassau N, Zappa M, Vullierme M-P, Koscielny S, Vilgrain V. Advanced
437 Hepatocellular Carcinoma: Early evaluation of response to targeted therapy and
438 prognostic value of Perfusion CT and Dynamic Contrast Enhanced-Ultrasound.
439 Preliminary results. European Journal of Radiology 2013; 82:e205-e11.
440 Garcia-Barros M, Paris F, Cordon-Cardo C, Lyden D, Rafii S, Haimovitz-Friedman A,
441 Fuks Z, Kolesnick R. Tumor Response to Radiotherapy Regulated by Endothelial
442 Cell Apoptosis. Science 2003; 300:1155-59.
443 Ibrahim SM, Nikolaidis P, Miller FH, Lewandowski RJ, Ryu RK, Sato KT, Senthilnathan
444 S, Riaz A, Kulik L, Mulcahy MF, Omary RA, Salem R. Radiologic findings following
445 Y90 radioembolization for primary liver malignancies. Abdom Imaging 2009;
446 34:566-81.
447 Inchingolo R, Posa A, Mariappan M, Spiliopoulos S. Locoregional treatments for
448 hepatocellular carcinoma: Current evidence and future directions. World J
449 Gastroenterol 2019; 25:4614-28.

450 Jemal A, Ward EM, Johnson CJ, Cronin KA, Ma J, Ryerson B, Mariotto A, Lake AJ, Wilson
451 R, Sherman RL, Anderson RN, Henley SJ, Kohler BA, Penberthy L, Feuer EJ, Weir
452 HK. Annual Report to the Nation on the Status of Cancer, 1975-2014, Featuring
453 Survival. J Natl Cancer Inst 2017; 109:djx030.
454 Kielar A, Fowler KJ, Lewis S, Yaghmai V, Miller FH, Yarmohammadi H, Kim C, Chernyak
455 V, Yokoo T, Meyer J, Newton I, Do RK. Locoregional therapies for hepatocellular
456 carcinoma and the new LI-RADS treatment response algorithm. Abdom Radiol
457 (NY) 2018; 43:218-30.
458 Kim S, Kim DY, An C, Han K, Won JY, Kim GM, Kim MJ, Choi JY. Evaluation of Early
459 Response to Treatment of Hepatocellular Carcinoma with Yttrium-90
460 Radioembolization Using Quantitative Computed Tomography Analysis. Korean J
461 Radiol 2019; 20:449-58.
462 Kolligs FT, Bilbao JI, Jakobs T, Iñarrairaegui M, Nagel JM, Rodriguez M, Haug A, D'Avola
463 D, op den Winkel M, Martinez-Cuesta A, Trumm C, Benito A, Tatsch K, Zech CJ,
464 Hoffmann R-T, Sangro B. Pilot randomized trial of selective internal radiation
465 therapy vs. chemoembolization in unresectable hepatocellular carcinoma. Liver
466 International 2015; 35:1715-21.
467 Kono Y, Lucidarme O, Choi S-H, Rose SC, Hassanein TI, Alpert E, Mattrey RF. Contrast-
468 enhanced Ultrasound as a Predictor of Treatment Efficacy within 2 Weeks after
469 Transarterial Chemoembolization of Hepatocellular Carcinoma. Journal of
470 Vascular and Interventional Radiology 2007; 18:57-65.

Krix M, Kiessling F, Farhan N, Schmidt K, Hoffend J, Delorme S. A multivessel model describing replenishment kinetics of ultrasound contrast agent for quantification of tissue perfusion. Ultrasound in Medicine & Biology 2003a; 29:1421-30.

Krix M, Kiessling F, Vosseler S, Kiessling I, Le-Huu M, Fusenig NE, Delorme S. Comparison of intermittent-bolus contrast imaging with conventional power Doppler sonography: quantification of tumour perfusion in small animals. Ultrasound in Medicine & Biology 2003b; 29:1093-103.

Lefort T, Pilleul F, Mulé S, Bridal SL, Frouin F, Lombard-Bohas C, Walter T, Lucidarme O, Guibal A. Correlation and Agreement Between Contrast-Enhanced Ultrasonography and Perfusion Computed Tomography for Assessment of Liver Metastases from Endocrine Tumors: Normalization Enhances Correlation. Ultrasound in Medicine & Biology 2012; 38:953-61.

Lencioni R, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. Seminars in Liver Disease 2010; 30:52-60.

Lewandowski RJ, Kulik LM, Riaz A, Senthilnathan S, Mulcahy MF, Ryu RK, Ibrahim SM, Sato KT, Baker T, Miller FH, Omary R, Abecassis M, Salem R. A Comparative Analysis of Transarterial Downstaging for Hepatocellular Carcinoma: Chemoembolization Versus Radioembolization. American Journal of Transplantation 2009; 9:1920-28.

Llovet JM, Brú C, Bruix J. Prognosis of Hepatocellular Carcinoma: The BCLC Staging Classification. Seminars in Liver Disease 1999; 19:329-38.

Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. Hepatology 2003; 37:429-42.

494 Lyshchik A. Specialty Imaging: Fundamentals of CEUS. Philadelphia, PA: Elsevier Health
495 Sciences, 2019.
496 Moon AM, Weiss NS, Beste LA, Su F, Ho SB, Jin G-Y, Lowy E, Berry K, Ioannou GN. No
497 Association Between Screening for Hepatocellular Carcinoma and Reduced
498 Cancer-Related Mortality in Patients With Cirrhosis. Gastroenterology 2018;
499 155:1128-39.e6.
500 Nam K, Stanczak M, Lyshchik A, Machado P, Kono Y, Forsberg F, Shaw CM, Eisenbrey
501 JR. Evaluation of hepatocellular carcinoma transarterial chemoembolization using
502 quantitative analysis of 2D and 3D real-time contrast enhanced ultrasound.
503 Biomedical physics & engineering express 2018; 4:035039.
504 Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, Carbone PP.
505 Toxicity and response criteria of the Eastern Cooperative Oncology Group.
506 American Journal of Clinical Oncology 1982; 5:649-55.
507 Riaz A, Kulik L, Lewandowski RJ, Ryu RK, Giakoumis Spear G, Mulcahy MF, Abecassis
508 M, Baker T, Gates V, Nayar R, Miller FH, Sato KT, Omary RA, Salem R.
509 Radiologic–pathologic correlation of hepatocellular carcinoma treated with internal
510 radiation using yttrium-90 microspheres. Hepatology 2009; 49:1185-93.
511 Riaz A, Lewandowski RJ, Kulik L, Ryu RK, Mulcahy MF, Baker T, Gates V, Nayar R,
512 Wang E, Miller FH, Sato KT, Omary RA, Abecassis M, Salem R. Radiologic-
513 pathologic correlation of hepatocellular carcinoma treated with
514 chemoembolization. Cardiovasc Intervent Radiol 2010; 33:1143-52.
515 Salem R, Gilbertsen M, Butt Z, Memon K, Vouche M, Hickey R, Baker T, Abecassis MM,
516 Atassi R, Riaz A, Cella D, Burns JL, Ganger D, Benson AB, Mulcahy MF, Kulik L,

Lewandowski R. Increased Quality of Life Among Hepatocellular Carcinoma Patients Treated With Radioembolization, Compared With Chemoembolization. Clinical Gastroenterology and Hepatology 2013a; 11:1358-65.e1.

Salem R, Lewandowski RJ, Kulik L, Wang E, Riaz A, Ryu RK, Sato KT, Gupta R, Nikolaidis P, Miller FH, Yaghmai V, Ibrahim SM, Senthilnathan S, Baker T, Gates VL, Atassi B, Newman S, Memon K, Chen R, Vogelzang RL, Nemcek AA, Resnick SA, Chrisman HB, Carr J, Omary RA, Abecassis M, Benson AB, 3rd, Mulcahy MF. Radioembolization results in longer time-to-progression and reduced toxicity compared with chemoembolization in patients with hepatocellular carcinoma. Gastroenterology 2011; 140:497-507.e2.

Salem R, Mazzaferro V, Sangro B. Yttrium 90 radioembolization for the treatment of hepatocellular carcinoma: Biological lessons, current challenges, and clinical perspectives. Hepatology 2013b; 58:2188-97.

Serres S, O'Brien ER, Sibson NR. Imaging Angiogenesis, Inflammation, and Metastasis in the Tumor Microenvironment with Magnetic Resonance Imaging. Tumor Microenvironment and Cellular Stress 2014; 772:263-83.

Shaw CM, Eisenbrey JR, Lyshchik A, O'Kane PL, Merton DA, Machado P, Pino L, Brown DB, Forsberg F. Contrast-Enhanced Ultrasound Evaluation of Residual Blood Flow to Hepatocellular Carcinoma After Treatment With Transarterial Chemoembolization Using Drug-Eluting Beads. Journal of Ultrasound in Medicine 2015; 34:859-67.

Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA: A Cancer Journal for Clinicians 2020; 70:7-30.

Tang MX, Mulvana H, Gauthier T, Lim AK, Cosgrove DO, Eckersley RJ, Stride E. Quantitative contrast-enhanced ultrasound imaging: a review of sources of variability. Interface Focus 2011; 1:520-39.

Wakui N, Takayama R, Kamiyama N, Takahashi M, Shiozawa K, Nagai H, Watanabe M, Ishii K, Iida K, Igarashi Y. Diagnosis of hepatic hemangioma by parametric imaging using sonazoid-enhanced US. Hepatogastroenterology 2011; 58:1431-35.

Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR. A global view of hepatocellular carcinoma: trends, risk, prevention and management. Nat Rev Gastroenterol Hepatol 2019; 16:589-604.

Yang Y, Si T. Yttrium-90 transarterial radioembolization versus conventional transarterial chemoembolization for patients with hepatocellular carcinoma: a systematic review and meta-analysis. Cancer Biol Med 2018; 15:299-310.

Zocco MA, Garcovich M, Lupascu A, Di Stasio E, Roccarina D, Annicchiarico BE, Riccardi L, Ainora ME, Ponziani F, Caracciolo G, Rapaccini GL, Landolfi R, Siciliano M, Pompili M, Gasbarrini A. Early prediction of response to sorafenib in patients with advanced hepatocellular carcinoma: The role of dynamic contrast enhanced ultrasound. Journal of Hepatology 2013; 59:1014-21.