QUANTITATIVE CONTRAST-ENHANCED ULTRASOUND IMAGING FROM PRE-ClinICAL MODELS TO HUMAN TRIALS

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Disclosures

- Equipment loan from Canon Medical Systems
- Equipment loan from GE Medical Systems
- Equipment loan from Siemens Healthineers
- Contrast agent from GE Healthcare
- Contrast agent from Lantheus Medical Imaging
- Contrast agent from Bracco

Ultrasound Contrast Agents

- Gas filled 1 to 10 μm bubbles
- Injected intravenously and transpulmonary
- Air or higher molecular weight gases
- Bubbles are encapsulated for longevity
  - Albumin or polymer hard shell
  - Lipid or surfactant coated
- Up to 30 dB increase in SNR
- Signals mainly from vessels 20 - 60 μm

Microbubble Oscillations


Nonlinear Contrast Spectrum

[www.asci.org]
FDA Approved Contrast Agents

<table>
<thead>
<tr>
<th>Name &amp; manufacturer</th>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optison</td>
<td>LVO &amp; EBD</td>
</tr>
<tr>
<td>GE Healthcare, Princeton, NJ</td>
<td></td>
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<tr>
<td>Definity</td>
<td>LVO &amp; EBD</td>
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<tr>
<td>Lantheus Medical, N Billerica, MA</td>
<td></td>
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<tr>
<td>Lumason (SonoVue)</td>
<td>LVO &amp; EBD, focal liver lesions, vesicoureteral reflux</td>
</tr>
<tr>
<td>Bracco, Milan, Italy</td>
<td></td>
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</table>

Liver Lesion Characterization

Diagnosis: HCC

22 Seconds 1 Minute
2 Minutes 3 Minutes 4 Minutes

Subdermal Applications of Ultrasound Contrast

Supported in part by NIH R01 CA100370, R01 CA172336 and R21 CA137733 as well as by GE Healthcare

Animal Tumor Model

- 63 Sinclair swine with naturally occurring melanoma
- Weights 3.0 to 17.0 kg
- Melanomas are similar to human superficial spreading melanoma
- 70% incidence of SLN metastases
- 105 primary melanomas (average tumor diameter: 17 mm)

SLNs of the Female Breast

- Four subcutaneous injections (0.25 mL) of an ultrasound contrast agent
- Sonazoid (GE Healthcare, Oslo, Norway)
- Elegra ultrasound scanner with 7.5 MHz linear array (Siemens Healthineers, Mountain View, CA)
- Baseline: grayscale and color flow imaging
- Post contrast: grayscale phase inversion harmonic imaging (PIHI)
- Mechanical index (MI) from 0.2 to 0.4

Lymphosonography
Lymphoscintigraphy

- 50 µCi filtered Tc-99m sulfur colloid was injected in 4 locations around melanomas
  - Area massaged for 5 minutes
  - Starcam 300 Gamma camera (GE Healthcare, Milwaukee, WI)

Gold Standard: Blue Dye Injection

- Peritumoral injections of blue dye and surgical dissection after euthanasia
  - 0.5 mL Patent Blue V Sodium (Guerbet, Cedex, France) was injected at the same sites as Sonazoid and Tc-99m
  - Areas massaged to enhance up-take of dye

Accuracy of sonography and scintigraphy for SLN detection was compared to the gold standard using McNemar's test.

SLN Identification

Subcutaneous administration of an RES-specific ultrasound contrast agent results in the ability to detect lymphatic channels and SLNs

Overall Detection of SLNs

<table>
<thead>
<tr>
<th></th>
<th>SLNs</th>
<th>FP</th>
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<tbody>
<tr>
<td>Lymphosonography</td>
<td>318</td>
<td>11</td>
</tr>
<tr>
<td>Lymphoscintigraphy</td>
<td>240</td>
<td>20</td>
</tr>
<tr>
<td>Blue dye / surgery</td>
<td>358</td>
<td></td>
</tr>
</tbody>
</table>

Accuracy:
- Lymphosonography: 82% (318/387)
- Lymphoscintigraphy: 63% (240/380)
- Difference was statistically significant ($p < 0.0001$)

Human Study

Subharmonic Pressure Estimation

*In Vitro and In Vivo*

Supported in part by the U.S. Army Medical Research Material Command under W81XWH-08-1-0503, by AHA grant no 065544U as well as by NIH R21 HL081902, R21 DK087365 and R01 DK095826 as well as by GE Healthcare, Oslo, Norway.
Subharmonic Imaging (SHI)

3 Stages of subharmonic signal generation:
• Occurrence
• Growth
• Saturation

Subharmonic Aided Pressure Estimation (SHAPE)

In Vivo Setup

In Vivo Pressure Measurements; Proof of Concept

In Vivo Cardiac Setup

Closed-chest dog model
Cardiac Pressure Waveforms

RV Pressures

Individual calibration factor

RV Pressure Waveform in a Patient

In Vivo Techniques and Analysis

Pilot Study of SHAPE in Portal Hypertension

- 45 patients (27 M, 18 F) scheduled for a transjugular liver biopsy
- HVNC measurements as reference
- IIB and FDA approved (IND: 100,083)
- Subjects provided written consent
- 12 subjects post-liver transplantation
- Subjects were 19 to 71 years old
- BMIs ranged from 17.2 to 57.2
- Sonazoid 0.72 μL microbubbles/kg/hour

[Dave et al., Ultrasound Med Biol, 2017]

[Barlow et al., Ultrasound Med Biol, 2017]
Acoustic Power Optimization

[Dave et al., Ultrasonics, 2013]

Subharmonic Signal versus HVPG

[Graph showing Subharmonic Signal versus HVPG]

Predicting Portal Hypertension

1.93 ± 0.61 vs. -1.47 ± 0.29 dB; p < 0.0001

Clinical Study of SHAPE for Diagnosing Portal Hypertension

- 178 patients scheduled for a transjugular liver biopsy at TJU or HUP
  - HVPG measurements as reference
- IRB and FDA approved (IND: 124,465)
- Subjects provided written consent
- Modified Logiq 9 scanner with 4C probe (Tx/Rx: 2.5/1.25 MHz)
- Output power optimized individually
- Gaussian windowed binomial filtered square wave
- Sonazoid 1.44 μL microbubbles/kg/hour

Subharmonic Signal versus HVPG

[Graph showing Subharmonic Signal versus HVPG]

Pilot Study and Clinical trial

- 2 mmHg
- 10 mmHg

HVPG (mmHg)
Diagnosing Portal Hypertension with SHAPE

\[ A_0 = 0.953 \text{ for HVPG > 10 mmHg} \]
\[ A_0 = 0.949 \text{ for HVPG > 12 mmHg} \]

N = 125

Contrast-Enhanced Ultrasound for Augmenting Therapy

Supported in part by NIH R01 CA194307, R01 CA199646, R21 CA190926, and an SIECC Breast Cancer Pilot Award, by GE Healthcare, Princeton, NJ, USA, Siemens Healthineers, Mountain View, CA as well as Lantheus Medical Imaging, Billerica, MA, USA

Tumor Hypoxia

- The tumor microenvironment is chronically hypoxic limiting the efficacy of radiation therapy
- A relatively small increase in oxygen partial pressure (pO2) in hypoxic cells can result in significant sensitization to radiation therapy
- Approaches using systemic delivery of O2 immediately prior or during radiotherapy have not translated to clinical usage, primarily due to the body's natural tendency to regulate O2
- Current research is now focused on localized delivery of O2 for improving radio-sensitivity

Ultrasound-sensitive oxygen-filled microbubbles may be a local, noninvasive, and effective method for overcoming hypoxia-associated radio-resistance

Microbubble Fragmentation

(1 cycle, 2.4 MHz at 1.1 MPa)

O2 Delivery from Microbubbles In Vivo

- In all tumors treated with O2-microbubbles and ultrasound, pO2 levels increased by on average 22.9 ± 6.4 mmHg
- Increases in pO2 levels occurred within 10 seconds and lasted at least 2 minutes with maximum oxygenation achieved 75 ± 28.9 seconds post injection

O2 Microbubble Tumor Radiotherapy Sensitization

50 mice with MDA-MB-231 breast tumor xenografts received O2- or N2-microbubbles and 75 seconds of ultrasound with or without 5 Gy external beam radiation

<table>
<thead>
<tr>
<th>Group</th>
<th>Radiation Therapy</th>
<th>Microbubble</th>
<th>Ultrasound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5 Gy</td>
<td>Oxygen</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>5 Gy</td>
<td>Oxygen</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>0 Gy</td>
<td>Oxygen</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>5 Gy</td>
<td>Nitrogen</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>5 Gy</td>
<td>None</td>
<td>Yes</td>
</tr>
</tbody>
</table>
O₂ Microbubble Tumor Radiotherapy Sensitization

N = 50

[Eisenbrey et al., Int J Radiation Oncol Biol Phys., 2018]

Inertial Cavitation of Microbubbles Sensitizes Tumors to Radiation

Ultrasound Targeted Microbubble Destruction (UTMD)

Prostate cancer xenografts

HCC xenografts

[Czarnota et al., Proc Natl Acad Sci USA, 2012] [Daecher et al., Cancer Lett, 2017]

Phase 1/2 Clinical Trial of UTMD Augmented HCC Radioembolization

- Target is 52 patients with HCC scheduled for radioembolization with yttrium-90 (Y90) beads
- IRB and FDA approved (IND no. 126,768)
- Subjects randomized to a control group (Y90 alone) or to a UTMD + Y90 group
- Optison infusion (5 mL in 50 mL saline) over 10 min
- Siemens S3000 with 6C1 probe & flash-replenishment
- UTMD 2-4 hours, 1 week and 2 weeks post treatment
  - 3 safety outcomes (liver function test, physiologic & AE monitoring)
  - 3 efficacy outcomes (mRECIST, time to next treatment & survival)


Example Case

Baseline MRI showing HCV male with 5.3 cm HCC in segment 6

Angiography showing segment 6 hepatic vein access and tumor localization prior to Y90 delivery

Nuclear medicine SPECT showing effective localized delivery to HCC via catheter

Example Case

Baseline MRI showing HCV male with 5.3 cm HCC in segment 6

Angiography showing segment 6 hepatic vein access and tumor localization prior to Y90 delivery

Nuclear medicine SPECT showing effective localized delivery to HCC via catheter

Example Case

UTMD session 3 hours post radioembolization

Example Case

UTMD session 7 days post radioembolization
Results to Date

- 19 patients enrolled to date (12 in UTMD + Y90 group and 7 in Y90 alone)
- 2 AE in control group (fatigue, MI), 1 AE in UTMD group (fatigue)
- 3 deaths in Y90 alone group, 1 death in UTMD + Y90 (unrelated)
- No differences in physiological parameters pre to post UTMD (p ≥ 0.10)
  - Heart rate, temperature, systolic and diastolic pressures
- No difference in blood work after 1 month (p ≥ 0.17)
  - Bilirubin, AST, ALT, creatinine, WBC, albumin
- Follow up imaging available on 15 patients to date

Preliminary Efficacy

Conclusions

An introduction to IV as well as subdermal contrast enhanced ultrasound imaging was given

SHI and SHAPE are new techniques for contrast specific imaging and non-invasive pressure estimation based on subharmonic signals from contrast microbubbles

Augmenting Y90 radiation treatment of HCC using contrast microbubbles was described

THANK YOU!

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