SONOPORATION OF PANCREATIC CANCER

Flemming Forsberg, PhD

Department of Radiology, Thomas Jefferson University, Philadelphia, PA 19107, USA

Acknowledgements

D Adam, PhD, J Brody, PhD, LJ Delaney, PhD, T Dhir, MD, G Dimcevski, MD, PhD, J R Eisenbeey, PhD, S Feinstein, MD, OH Gilja, MD, KF, PhD, C Huang, MD, SK Kotopoulus, PhD, JH Liu, MD, X Luo, MD, A Lyshchik, MD, PhD, P Machado, MD, S Niu, MD, CW Schultz, PhD, M Torkzaban, MD, KE Wallace, PhD, C Wessner, RDMS, B Zhang, MD, Y Zhen, MD

Supported in part by NIH R01 CA199646, by GE Healthcare, Princeton, NJ, USA as well as Oslo, Norway and by Lantheus Medical Imaging, N Billerica, MA, USA

Disclosures

- Equipment loan from Butterfly Network, Canon Medical Systems, GE Medical Systems and Siemens Healthineers
- Contrast agent from Bracco Diagnostics, GE Healthcare and Lantheus Medical Imaging
- Consultant for Exact Therapeutics and Samumed

Pancreatic Cancer

- Approximately 3% of new cancers in the USA with 60,430 new cases expected in 2021
- Fourth leading cause of cancer-related deaths (48,220 deaths expected this year)
- Five year survival rates around 5-7%
- 50% of patients present with metastases
- No significant improvement in survival over the past 30 years

CEUS of Pancreatic Cancer

Contrast-to-Tissue Ratios

\[ CTR = \frac{(R_T - T)^2}{\sigma_T^2 + \sigma_R^2} \]

<table>
<thead>
<tr>
<th>CTR</th>
<th>EUS</th>
<th>SH</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>mass</td>
<td>5.63 ± 1.88</td>
<td>1.71 ± 1.63</td>
<td>0.016</td>
</tr>
<tr>
<td>vessel</td>
<td>5.50 ± 1.84</td>
<td>5.01 ± 1.85</td>
<td>0.72</td>
</tr>
</tbody>
</table>
Phase 1 Results

- Patients tolerated an increased number of treatment cycles compared to historical controls (N = 63); 8.3 ± 6.0 cycles vs. 13.8 ± 5.6 cycles (p = 0.008)
- The median survival also increased from 8.9 months to 17.6 months (p = 0.011)
- In addition, five patients showed a primary tumor diameter decrease
- Two patients were offered surgery
Phase 2 Clinical Trial of Sonoporation

- Selecting the optimal UCA for sonoporation of pancreatic cancer in a pre-clinical model
- Evaluate two different acoustic power regimes for sonoporation
- Conduct a large, multi-center clinical study of sonoporation
- 120 patients with metastatic or locally advanced and surgically unresectable pancreatic ductal adenocarcinoma will be enrolled at Thomas Jefferson University or Haukeland Hospital

Purpose

To evaluate the disruption of different ultrasound contrast agents (UCAs) for augmenting chemotherapy treatment (i.e., sonoporation) in a pre-clinical murine model of pancreatic cancer

- Selecting the optimal UCA for sonoporation of pancreatic cancer
- Evaluate two different acoustic power regimes for sonoporation

Sonoporation in a Murine Model of Pancreatic Cancer

- 120 athymic, nude mice
  - Injected with MIA PaCa-2 cells in the right flank
  - Randomized when tumors reached ~400 mm³
- 2 control groups and 4 treatment groups (10 mice/group)
  - Treated with vehicle only
  - Treated with chemotherapy only (Paclitaxel and Gemcitabine)
  - ChemoTx & Definity (Lantheus Medical Imaging, N Billerica, MA)
  - ChemoTx & Lumason/SonoVue (Bracco, Milan, Italy)
  - ChemoTx & Optison (GE Healthcare, Princeton, NJ)
  - ChemoTx & Sonazoid (GE Healthcare, Oslo, Norway)
- All animal work was conducted in compliance with the IACUC and Animal Care Policies of Thomas Jefferson University

Treatment Schema

- Standard of care chemotherapy was administrated once per week for 3 weeks
  - Paclitaxel at a dose of 30 mg/kg intraperitoneal (IP) 2 hours prior to UCA infusion
  - Gemcitabine at a dose of 100 mg/kg IP 15 minutes prior to infusion
  - At the maximum proportion of chemotherapy in the blood the UCAs were infused over 10 minutes
- Dosages were adjusted to 1.2 - 3.0 x 10⁸ bubbles/mL for all 4 UCAs

Imaging Schema

- A Logiq E9 scanner (GE Healthcare, Waukesha, WI) with a C6 curvilinear probe was utilized
- The highest line density was used with 12 pulses (20 μs pulse length) transmitted at a frequency of 2.1 MHz
- Operating at high or low acoustic power (Pmax of 200 or 60 mW/cm²)
- Oxygenation was measured using 3D photoacoustic imaging on a Vevo 2100 LAZR scanner (Fujifilm Visualsonics, Toronto, Canada)
- Measurements were obtained weekly for four weeks in subgroups of 3 random mice from each group

Pathology and Data Analysis

- Three mice per group were infused with tomato lectin 30 minutes prior to sacrifice
- Frozen sections were stained using CD31 (Dianova, Hamburg, Germany)
- Slides were imaged utilizing a DM4 B microscope (Leica Microsystems, Wetzlar, Germany) and perfusion was assessed
- Tumor growth and mouse survival curves were generated based on the remaining mice
- Comparisons between groups were performed in Stata 15.1 (StataCorp, College Station, TX, USA) using two-way, repeated measures ANOVA
In the high acoustic power cohort all 4 UCA treated groups had smaller tumors (p < 0.006), while only mice receiving Definity showed a significant tumor volume reduction (p = 0.003) in the low acoustic power cohort.

Vevo 2100/LAZR System

Co-registered real-time ultrasound and photoacoustic imaging using a tuneable pulsed Nd:YAG laser (680 nm – 970 nm)

Oxygen Saturation Calculation

Total Hemoglobin = Oxygenated Hemoglobin + Deoxygenated Hemoglobin

Oxygen Saturation Calculation

Photoacoustic Imaging Results

Oxygenation values across tumor volumes were greater in the high than in the low acoustic power cohort (p < 0.001)

The UCA treatment groups only showed significant increases for Definity (p = 0.048) and Sonazoid (p = 0.003)

Pathology Results

Representative images of stained mouse livers
- CD31 showing vasculature
- DAPI showing nuclei
- Tomato lectin demonstrating perfusion

Summary of Results

<table>
<thead>
<tr>
<th>UCA</th>
<th>Acoustic power</th>
<th>Reduced tumor volume</th>
<th>Increased perfusion</th>
<th>Increased oxygenation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definity</td>
<td>Low</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Lumason</td>
<td>Low</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Optison</td>
<td>Low</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Sonazoid</td>
<td>Low</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Definity</td>
<td>High</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Lumason</td>
<td>High</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Optison</td>
<td>High</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Sonazoid</td>
<td>High</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>
Conclusions

Based on pre-clinical studies in a murine xenograft model of pancreatic cancer, Sonazoid microbubbles imaged at high acoustic power was selected for our upcoming human clinical trial.

Sonoporation of pancreatic cancer is now being pursued in a multi-center, Phase 2, clinical trial expected to start enrolling in April/May, 2021.

THANK YOU!

flemming.forsberg@jefferson.edu