Immune Priming with Ultrasound

Chandan Guha, MBBS, PhD
Professor and Vice Chair, Radiation Oncology
Professor, Urology and Pathology
Director, Einstein Institute of Onco-Physics
Montefiore Medical Center
Albert Einstein College of Medicine, Bronx, NY
cguhamd@gmail.com

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- NIH (R01 EB009040)
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- Project Energy with Johnson & Johnson

Tumor Evolution

Focal Oncology Clinical Adaptive Learning (FOCAL)
Cancer Clinic Network

1. Ablative Therapies for local control induces anti-tumoral immunity, which in turn helps local control.

2. Ablative Therapies for systemic immunity:
   Immune Priming Ablation (IPA) for In Situ Tumor Vaccines
   a. UPR ➔ ER stress ➔ Antigen Processing / Presentation
   b. "Eat Me" and DAMP signals
   c. Reversal of tolerance
   d. Antigen Presentation (neo-antigens & cryptic antigens)

   "Focal Therapy for Systemic Cure"
**Project ENERGY.01: Proposed Study Design**

**Immuno-Priming Ablation (IPA) Therapies**

- Study 1: IPA + DC Act./Check
  - Go/No-Go for IPA
  - Go/No-Go for local ablation
  - Thorsten Melcher

- Study 2: IPA + DC Act./Check
  - Immuno-Priming (IP)
  - LOFU1-2 HT RP IRE1-2 RFP1-2 Digoxin...
  - Electroportation

- Study 3: IPA + DC Act./Check
  - Biomarker, Tumor control, and survival endpoints

**Autologous in situ tumor vaccines**

**Treatment Protocol**

- Treatment Protocol
  - No Treatment
  - RT only
  - RT + IPA
  - RT + IPA + PD-1

**Advances in Brief**

**Flt3-Ligand Administration after Radiation Therapy Prolongs Survival in a Murine Model of Metastatic Lung Cancer**


8/1/2017
**FLT3 Ligand Immunotherapy and Stereotactic Radiotherapy for Advanced Non-small Cell Lung Cancer**

- SBRT will be administered during the first week of study therapy.
- A single pulmonary lesion that measures at least 1 cm in greatest dimension will be treated.
- Daily subcutaneous injections of CDX-301 (75 μg/kg) will be administered for 5 days, beginning on the first day of SBRT.

**Sample size:** 29 patients
Sample size: 29 patients

Ablative SBRT dose fractionation
- 34 Gy x 1 Fx
- 18 Gy x 3 Fx
- 10 Gy x 5 Fx

Study Subjects

<table>
<thead>
<tr>
<th>#</th>
<th>Demographics</th>
<th>Disease Burden</th>
<th>Prior Treatment(s)</th>
<th>SBRT</th>
<th>Clinical Course</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>73 year-old Korean male</td>
<td>Right lung squamous cell carcinoma with multiple lung masses and bilateral mediastinal adenopathy</td>
<td>Carboplatin + gemcitabine</td>
<td>50 Gy in 5 fractions to RLL mass</td>
<td>Progression at 6 weeks, death at 4 months</td>
</tr>
<tr>
<td>2</td>
<td>55 year-old Hispanic female</td>
<td>Right lung adenocarcinoma, with bilateral lung nodules</td>
<td>Chemoradiation therapy for localized disease, nivolumab for metastatic disease</td>
<td>34 Gy in 1 fraction to LUL nodule</td>
<td>Partial response at 2 months, stable at 4 months</td>
</tr>
<tr>
<td>3</td>
<td>80 year-old Caucasian female</td>
<td>Right lung squamous cell carcinoma with spine and pelvic metastases</td>
<td>Chemoradiation therapy for localized disease, carboplatin + gemcitabine for metastatic disease, pemetrexed</td>
<td>50 Gy in 5 fractions to right lung mass</td>
<td>Partial response at 2 months</td>
</tr>
<tr>
<td>4</td>
<td>73 year-old Caucasian female</td>
<td>Right lung adenocarcinoma with mesenodinal adenopathy and liver metastases</td>
<td>Carboplatin + pemetrexed, maintenance pemetrexed</td>
<td>50 Gy in 5 fractions to RLL mass</td>
<td>PET/CT on 4/27</td>
</tr>
</tbody>
</table>

Patient 2

Right lung mass reduced from 5.0 cm (maximum SUV 10.7) to 2.1 cm (maximum SUV 6.5) on first post-treatment PET/CT
Target Lesion Total Glycolytic Activity (TGA): 1.0 cc → 0.3 cc
Other lesions’ TGA: 44.7 cc → 4.5 cc
Patient 3

Improvement or resolution of most of the osseous foci

Target Lesion Total Glycolytic Activity (TGA): 49.1 cc → 6.2 cc

Other lesions' TGA: 130.2 cc → 45.4 cc

Energy activated \textit{in situ} Tumor Vaccines

POC Studies

<table>
<thead>
<tr>
<th>Energy Immunotherapy</th>
<th>Tumor Models</th>
<th>End Points</th>
<th>Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 Gy TLR9 agonist</td>
<td>Lewis Lung 3LL in C57/B6</td>
<td>PTG, Mets, Survival</td>
<td>RT Dose Fractionation RT to Draining Lymph Node</td>
</tr>
<tr>
<td>10 Gy Listeria-PSA Adx331 142</td>
<td>TRAMPC1 TP53A23 Prostate Cancer</td>
<td>PTG and Immune Assays</td>
<td>Break Tolerance to self antigens</td>
</tr>
<tr>
<td>20Gy x 3 PDI-Fc</td>
<td>Lewis Lung 3LL in C57/B6</td>
<td>PTG, Mets, Survival</td>
<td>Small Animal Treatment</td>
</tr>
<tr>
<td>LOFU HIFU</td>
<td>TP53A23 Prostate</td>
<td>PTG, Immune Assays</td>
<td>Lack of Immune Surveillance and carcinogenic environment</td>
</tr>
<tr>
<td>LOFU SBRT (10 Gy x 3)</td>
<td>B16 Melanoma</td>
<td>PTG, Mets, Survival Immune Assays</td>
<td></td>
</tr>
</tbody>
</table>
Acoustic priming

ULTRASOUND -- ADVANTAGES

- US Can do both Imaging and Treatment
- Quick Tissue Destruction
  - Bloodless
- Precise and Accurate
- Non-sterile environment

HIFU directed harmlessly across skin and rectum toward the tumor
**Therapeutic Ultrasound as an autologous in situ tumor vaccine**

- HIFU = High Intensity Focused Ultrasound
- MRgFUS = MR-guided Focused Ultrasound
- TULSA = Transurethral ultrasound ablation
- LOFU = Low intensity (energy) focused ultrasound (LOFU coined by Guha group)
- SST = Sonic Stress Therapy
- APT – Acoustic Priming Therapy

### LOFU parameters

<table>
<thead>
<tr>
<th>Duty Cycle (%)</th>
<th>Condition #1</th>
<th>Condition #2</th>
<th>Condition #3</th>
<th>Condition #4</th>
<th>Condition #5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power (W)</td>
<td>32</td>
<td>16</td>
<td>8</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Time (ms)</td>
<td>1000</td>
<td>625</td>
<td>1250</td>
<td>2500</td>
<td>5000</td>
</tr>
<tr>
<td>Thermal Energy (J)</td>
<td>0.32</td>
<td>1.5</td>
<td>5</td>
<td>7.5</td>
<td>10</td>
</tr>
<tr>
<td>Peak Negative Pressure (MPa)</td>
<td>8.14</td>
<td>6.08</td>
<td>4.58</td>
<td>3.34</td>
<td>2.46</td>
</tr>
</tbody>
</table>

The “sonic stress” of LOFU

<table>
<thead>
<tr>
<th>Gene function</th>
<th>Genes affected by LOFU treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Protein Folding</td>
<td>DNAB1, HSPH1, HSPB1, HSPD1, HSPA4L, CRYAB, HSPA6, HSPA7, HSPGAA1, HSPGAA1P, DNAJA4, FKBP4, LGSN, PTGES3</td>
</tr>
<tr>
<td>2. Cell cycle regulation</td>
<td>IERS, JUN, CACYBP, GPRCSA, RRA0, WEE2</td>
</tr>
<tr>
<td>3. Cytokines</td>
<td>IL8</td>
</tr>
<tr>
<td>4. Receptors</td>
<td>CSF2RB, IL7R, NPR1, RXFP2, FLT4, ITGA2</td>
</tr>
<tr>
<td>5. Cytoskeleton integrity</td>
<td>FAM101B, TCP1</td>
</tr>
<tr>
<td>6. Transcriptions</td>
<td>ATP3, ANKRD3, EWA, KAT2A</td>
</tr>
<tr>
<td>7. Transporters</td>
<td>SLC22A2, SLC22A16, RHAG</td>
</tr>
<tr>
<td>8. Apoptosis regulation</td>
<td>NLR4A, ANGPTL4, BAG3</td>
</tr>
<tr>
<td>9. Peptidase</td>
<td>NAALADL1, MEP1A, PLCD2</td>
</tr>
</tbody>
</table>
After LOFU treatment, the gene response showed extensive upregulation of genes that are related to unfolded protein.

Genes were selected from pathways highlighted in the KEGG pathway analysis and validated using qRT-PCR. The data correlated well with the RNA sequencing results. HSPA6 and HSPA7 were highly regulated to 200+ and 25+ fold respectively.
## Immunomodulation of Tumor Cells

### Surface Calreticulin (CRT)

- **Pre-LOFU**: 0.50%
- **6h LOFU**: 1.00%
- **24h LOFU**: 2.00%
- **48h LOFU**: 3.00%

### Intracellular HSP70

- **Pre-LOFU**: 1.50%
- **6h LOFU**: 2.00%
- **24h LOFU**: 3.00%
- **48h LOFU**: 4.00%

## In vitro Effect of LOFU on Cell Surface Markers

**Summary of in vitro data under JJI-ENERGY**
- LOFU is non-ablative
- LOFU induces "sonic stress" on cancer cells
- Sonic stress signature is consistent across cell types and indicative of immune stimulation

### Table: In vitro Effect of LOFU on Cell Surface Markers

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>0h</th>
<th>5h</th>
<th>10h</th>
<th>20h</th>
<th>30h</th>
<th>6h</th>
<th>24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>3LL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>4T1</td>
<td></td>
<td></td>
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<tr>
<td>TPSA23</td>
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</tbody>
</table>

**Legend**
- LOFU
- Tumor Cell
- Cytotoxicity
- Induce Cell death
- Calreticulin membrane
- Translocation
- HSP activation
- HMGB1 release
- TIA binding
- Antigen uptake
- "eat me" signal

**Icons**
- LOFU
- TPSA23
- 3LL
- 4T1

**Graphs**
- LOFU-induced immune response
- T cell activation
- Antigen processing
- DC migration
- T cell proliferation
- "danger" signal
- "eat me" signal
- Calreticulin translocation
- HSP activation
- HMGB1 release
Treat the tumor draining lymph node (TDLN – immune privilege site)

- Reprogram tolerogenic DCs (IDO inhibitors)
- Inhibit regulatory T cells (Treg)
- Reverse T-cell anergy in TDLN – LOFU primary tumor

T cell activation vs. T cell anergy

- Full stimulation
  - CD3, CD28
  - Cytokine production
  - Cell Proliferation
  - T cell Activation
- Re-stimulation
  - CD4+ T cell
  - Hyporesponsive phenotype

Anergic stimulus

- Full activation
  - Ca
  - NFAT
  - p
  - p
  - p
  - MAP kinases
  - NF-kB
  - AP-1
  - NFAT p50, p65
  - Activation-induced genes
- Anergy
  - Ca
  - Ca
  - Co-partners
  - Anergy-inducing genes

T cell Activation vs. T cell Anergy

- Full Actiuation
  - CD3
  - TCR
- Anergic Stimulus
  - CD3
- Activation-induced genes
  - Anergy-inducing genes
B16 tumors induced CD4+ T cell anergy

LOFU prevents B16-induced CD4+ T cell anergy

T cell anergy: transcriptional program
LOFU prevents B16-induced CD4+ T cell anergy

LOFU can reverse established T cell anergy

LOFU as immune adjuvant for IGRT
LOFU as adjuvant for IGRT requires T cells

Nude mice
B16 melanoma

LOFU as adjuvant to potentiate effect of RT and improve control of distal metastases

Untreat
LOFU
IGRT
LOFU+IGRT

CD62L<sup>-</sup>/CD4<sup>+</sup> population in Tumor infiltrating lymphocytes (TILs)

A marked shift from naive to activated phenotype was observed in the combination treatment TILs.
CD62L⁺/CD8⁺ in Tumor infiltrating lymphocytes (TILs)

LOFU+17AAG treatment causes a significant increase in activated CD8⁺ T cells in TILs.

LOFU+17AAG reduces the expression of prostate cancer stem cell marker

SCA1   CD44   CD133

LOFU+17AAG  Control

Did the number of cells go down or expression?
Acoustic Priming

↑Immunosensitization
Sensitization of ablative therapies

HIFU
RFA
Cryotherapy

Chemotherapy

†Immunosensitization

Radiosensitization

Chemosensitization

Autologous in situ tumor vaccines

The FOCAL Team

Guha Laboratory
Students:
Lorena Agoni
Karin Skalina
Tatjana Savage
Justin Tang

Research Associates:
Huagang Zhang

Clinical Trials
Rafi Kabariti
Nitin Oferi
Mudhur Garg

Medical Physics
Assoc.: Patrik Brodin
Director: Wolfgang Tome

Immune Tolerance
Sanmay Bandyopadhyay
Fernando Macian

Immune Therapeutics
Steve Almo

Immune Tolerance
Sanmay Bandyopadhyay
Fernando Macian

Thank you!