Immune Priming with Ultrasound

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- NIH (R01 EB009040)
- NCI SBIR grant with Celldex Therapeutics, Inc.
- Project Energy with Johnson & Johnson

Tumor Evolution

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

2. Ablative Therapies for systemic immunity:
   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
- Study 2: IPA
- Study 3: IPA

- Go/No-Go for RT/RFA
- Go/No-Go for IRE/RFA
- LoFU

**Immuno-Priming (IP) Therapies**

- RT (60 Gy)
- Surgery
- Autologous tumor vaccines

**Autologous in situ tumor vaccines**

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

**Flt3-Ligand Administration**

- Treatment Protocol

**Advances in Brief**

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

**Flt3-Ligand Administration**

- Treatment Protocol
Systemic Effects of Primary Tumor Irradiation

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 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 for localized disease, carboplatin + gemcitabine for metastatic disease, nivolumab</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 mediastinal adenopathy and liver metastasis</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

Patient 3

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

Energy activated in situ Tumor Vaccines

POC Studies

<table>
<thead>
<tr>
<th>Energy Activation</th>
<th>Tumor Models</th>
<th>End Points</th>
<th>Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Fraction SFRT 25-60 Gy</td>
<td>Lewis Lung 3LL in C57/Bl6; B16; Lewis palate (HCC) in B6/6</td>
<td>1. Primary Tumor Growth; 2. Metastases; 3. Survival; 4. Immune assays</td>
<td>Murine Ectopic Transplantation Models; RT Dose Fractionation; RT to Draining Lymph Node</td>
</tr>
<tr>
<td>20 Gy TL9 ag</td>
<td>Lewis Lung 3LL in C57/Bl6; Ba/F3</td>
<td>PTG, Mets, Survival</td>
<td>Break Tolerance to self antigens; Small Animal Treatment</td>
</tr>
<tr>
<td>10 Gy Listeria-PSA ADX33142</td>
<td>Lewis Lung 3LL in C57/Bl6; TRAMP</td>
<td>PTG and Immune Assays</td>
<td></td>
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<tr>
<td>20 Gy x 3 PDI-Fc</td>
<td>Lewis Lung 3LL in C57/Bl6; TRAMP</td>
<td>PTG, Mets, Survival</td>
<td></td>
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<tr>
<td>LOFU HIFU</td>
<td>TPSA320 Prostate</td>
<td>Immune Assays</td>
<td>Lack of Immune Surveillance and carcinogenic environment</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
• 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>Condition</th>
<th>Duty Cycle (%)</th>
<th>Power (W)</th>
<th>Time (ms)</th>
<th>Thermal Energy (J)</th>
<th>Peak Negative Pressure (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>1</td>
<td>32</td>
<td>1000</td>
<td>0.32</td>
<td>8.14</td>
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<tr>
<td>#2</td>
<td>25</td>
<td>16</td>
<td>625</td>
<td>2.3</td>
<td>6.08</td>
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<tr>
<td>#3</td>
<td>50</td>
<td>8</td>
<td>1250</td>
<td>5</td>
<td>4.58</td>
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<tr>
<td>#4</td>
<td>75</td>
<td>4</td>
<td>2500</td>
<td>7.5</td>
<td>3.34</td>
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<tr>
<td>#5</td>
<td>100</td>
<td>2</td>
<td>5000</td>
<td>10</td>
<td>2.46</td>
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</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>DNAJB1, HSPH1, HSPB1, HSPD1, HSPA4L, CRT, HSPA6, HSPA7, HSPG2A1, HSPG2A4, DNAJA4, FKBP4, LGSN, PGE2</td>
</tr>
<tr>
<td>2. Cell cycle regulation</td>
<td>IER5, JUN, CACYBP, GPR5CA, RRA2, 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>ATF3, ANKRD1, EPHA, KAT2A</td>
</tr>
<tr>
<td>7. Transporters</td>
<td>SLC22A2, SLC22A3, RHAG</td>
</tr>
<tr>
<td>8. Apoptosis regulation</td>
<td>NLR4A, ANGPT1A, BAG3</td>
</tr>
<tr>
<td>9. Peptidase</td>
<td>NAALADL1, MEP1A, PLD2</td>
</tr>
</tbody>
</table>
Gene Ontology

Figure 2 Gene ontology GoTerm network. After LOFU treatment, the gene response showed extensive upregulation of genes that are related to unfolded protein.

Gene Expression with qRT-PCR

Figure 4 qRT-PCR of select genes from the RNA sequencing. 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.

Increase in Hsp70 mRNA expression after LOFU treatment

Gene expression heatmap showing changes in Hsp70 mRNA levels after LOFU treatment.
Immunomodulation of tumor cells

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

Legend
- Tumor peptide
- CD1a
- Calreticulin
- BiP
- 100% CD86
- Sonication
- SRS
- Ch-Rt
- LOFU

Tumor Cell
- Induce cell death
- Calreticulin membrane translocation
- HSP activation
- HMGB1 release
- TLR binding
- DC activation
- Antigen uptake
- "eat me" signal
- NKG2D activation
- T cell activation
- Antigen presentation
- Antigen processing
- Induce Cell death
- Calreticulin membrane
- Translocation
- HSP activation
- HMGB1 release
- TLR binding
- DC Activation
- Antigen Uptake
- "eat me" signal
- NKG2D activation
- T cell activation
- Antigen presentation
- Antigen processing
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
- Re-stimulation
  - T cell Activation

- Anergic stimulus
  - CD4+ T cell
  - Hyporesponsive phenotype
- Re-stimulation
  - T cell Anergy

T cell Activation vs. T cell Anergy

- Full Activation
  - CD3
  - TCR
  - NFAT
  - MAP kinases
  - NFκB
  - AP-1
  - Activation-induced genes
- Anergic Stimulus
  - CD4+ T cell
  - TCR
  - NFAT
  - MAP kinases
  - NFκB
  - AP-1
  - 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^−/−CD4^−/− 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.

Low intensity focused ultrasound (LOFU) modulates unfolded protein response and sensitizes prostate cancer to 17AAG

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

Did the number of cells go down or expression?