Antigen specific anti-SPAS-1 CD8 T cell responses to in situ vaccination using irreversible electroporation (IRE) of prostate tumors

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Prostate Cancer

- Radical prostatectomy, external-beam radiotherapy, and brachytherapy are all standard local treatments.

- Best data for the efficacy and safety of local treatment are from two randomized trials that compared radical prostatectomy against watchful waiting.
  - Surgery was associated with greater urinary incontinence and erectile dysfunction.

- Disease in many patients recurs after local therapy.
  - Optimum timing to start systemic treatment initiation is not clear.
  - The best treatment of the prostatic primary tumor in patients with metastatic disease at diagnosis is also unclear.

- Novel treatment modalities:
  - Immunotherapy
  - Irreversible electroporation

Immunotherapy

Columbia University Medical Center

http://newsroom.cumc.columbia.edu/cancer-immunotherapy/
Tumor lysis and antigen release by irreversible electroporation (IRE)

SPAS-1 release from TRAMP C2 tumor cells by irreversible electroporation (IRE)

- First T cell-defined transgenic adenocarcinoma of mouse prostate (TRAMP) tumor antigen.
- Spas-1 ortholog SH3GLB2 is overexpressed in prostate cancer metastases.
- SH3 DOMAIN, GRB2-LIKE protein.
- SPAS-1 epitope SNC9-H8 arose from a point mutation in one allele of the gene in TRAMP tumor cells.
- Immunization with dendritic cells pulsed with SNC9-H8 peptide resulted in protection against TRAMP-C2 tumor challenge.
- MHC-I tetramers are available.
Hypothesis:

IRE combined with anti-CTLA4 immunotherapy will produce more SPAS-1 specific T cells and SPAS-1 specific T memory cells in vivo versus conventional resection with anti-CTLA4 antibody treatment.
Inject 10^6 Tramp C2 Tumor Cells

Allow tumor growth for specified number of days

CD8 T Cell Response to 1° Tumor and Spas-1 Tetramer

- Total # of H2-D^b [SPAS-1] T cells

- Graph showing increasing levels of T cells over time:
  - Naive
  - Day 7
  - Day 14
  - Day 28

Mice with tumors indicate progression of tumor growth.
Inject 10^6 Tramp C2 Tumor Cells

Allow tumor growth for 14 days

Treat with resection or IRE with or without anti-CTLA4 antibody

Treat with or without anti-CTLA4 antibody via IP injection

Sacrifice mouse and harvest spleen and lymph nodes

Use antibody tetramers to enrich for lymphocytes and SPAS1 T Cells
Gating Scheme for SPAS-1 Specific T Cells

- FSC-A
- SSC-A
- SSC-W
- Live/Dead
- Thy 1.2
- CD8 Beta
- CD8 alpha
- SPAS-1 Tetramer
Figure 1. Total SPAS-1 specific T CD8 T cells 11 days after resection or IRE with or without immunotherapy.
Figure 1. Total SPAS-1 specific CD8 T cells 11 days after resection or IRE with or without immunotherapy.
Figure 2. Total SPAS-1 specific T CD8 T cells 31 days after resection or IRE with or without immunotherapy.
Conclusions

• IRE combined with anti-CTLA4 immunotherapy results in greater total numbers of SPAS-1 specific CD8 T cells versus resection with anti-CTLA4 antibody.

• IRE may provide an alternative means to resection of prostatic disease due to preservation of local tissue, potentially decreasing the risk of fistula, urinary incontinence, and erectile dysfunction.

• IRE has potential for host vaccination to treat metastatic disease and recurrence.
Future Directions

• Anti-CTLA4 titration studies to eliminate potential immunopathologies.
• Treat with anti-CTLA4 antibody sooner (day 0, 3, 6, and 9 instead of day 1, 4, 7, 10)
• Enroll mice with tumor volumes ideal for resection by IRE.
• Enroll more mice
• Apply IRE to different mouse model
• Treat mice with only anti-CTLA4 antibody
• Assess T cell function with IFNγ and TNFα
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Figure 3. Percentage of mice tumor free following treatment and secondary tumor challenge.
Cryoablation with anti-CTLA-4 therapy
Cryoablation with anti-CTLA-4 therapy

Spas-1 Specific T Cell Function 18 Days Post 2° Tumor Challenge

IgG

Anti-CTLA4

Spleen

Tumor

% TNFα +

% IFNγ +
In vitro DC-mediated Trp2 T cell activation by B16 lysates: IRE > Cryo > Heat

**A**

- Trp2 T cells + DCs
  - No Antigen
- + Trp2 Peptide
  - 10ng/ml
- + B16 Cryocycle (total lysis)
  - 1.2 x 10^5 cell equiv
- + B16 IRE lysate
  - 1.2 x 10^5 cell equiv

**B**

- Total Lysis
- IRE
- Cryo (~80°C)
- Heat (50°C)

**C**

- Controls
- 122,996 ce
- 15,688 ce

Buck O’Flanagan BO047; BO054 ff First Batch, Ind. Expts