New frontiers in therapeutic ultrasound:
transfection and immune modulation

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Outline: New frontiers in therapeutic ultrasound

- Synergy between ultrasound and immunotherapy
- Image-guided transfection

Why combine focal and immunotherapies?

- Great progress in treating some disseminated cancers with immunotherapy
- Still many do not respond: particularly solid tumors
- Goal: create a T cell response through combination of agonists and focal therapy
Therapeutic ultrasound protocols explored with immunotherapy include:

- Thermal ablation
- Hyperthermia
- Microbubble-based membrane and vascular destabilization
- Mechanical disruption

We will focus on thermal ablation.

**MRgFUS ablation**

**Motivation - MRI guidance**
Magnetic resonance guided ablation facilitates:

- 3D view of the anatomy within the region of interest
- Quantification of the change in temperature at the ablated site
- Estimation of the ultrasound pressure in the region of interest through radiation force estimation
- Estimation of changes in the stiffness of the treated region

**Experimental Setup (3 MHz for thermal effect)**

- Tumors ablated in 2mm circular pattern
  - CW for 30s, 5W acoustic, PNP = -3.1 Mpa

**FUSS system**

- 16-element annular array
- 3 MHz
- 300 kHz bandwidth
- 120 W peak acoustic power
- 120 W peak acoustic power
- 35 mm radius of curvature
- 0.5 x 0.5 x 2.5 mm³ focal spot

**MR scanner**

7T MR (Biospec 70/30 USR, Bruker Biospin, Germany)

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**Innate and adaptive immunity**

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Immune Agonists and Checkpoint Inhibition

**Agonists: Press on the gas**
- CpG ODN
- DC
- Treg
- MDSC
- APC
- Tumor

**Checkpoint: Release the brakes**
- T
- T-cell
- PD-1
- PD-L1
- Checkpoint: Release the brakes
- CpG (toll like receptor agonist)
- CD40
- Checkpoint inhibitors include anti-PD-1 and anti-CTLA4

Motivation
- Agonist immunotherapy has recently been shown to combine with focal or chemotherapy (creating immunogenic cell death) and checkpoint blockade (improving T cell functionality) to reduce tumor growth even in challenging cancers
  - Agonists include CpG (toll like receptor agonist) and CD40
  - Checkpoint inhibitors include anti-PD-1 and anti-CTLA4
- What is the role for focal therapy?

From AACR 2019: NCT03214250 (Vonderheide lab-UPenn)
Agonist immunotherapy promising even in PDA

Best response to Gem/Abraxane/aCD40 +/- nivo in 1L met PDA

Max change from baseline (%)
Sum of lesion diameters

Myeloid rather than T cell response
**In situ vaccination: Lymphoma (Levy Lab)**


**Agonists- many act on dendritic cells to enhance antigen presentation and activate T cells**

- **pDC Inducers**
  - GM-CSF, IL-10
  - TLR2 agonists
- **pDC Maturation Inducers**
  - Adenosine A2a R agonists
  - GRP78/HSPA5, MFG-E8
- **TLR4 agonists**
  - CD24
- **Adenosine A2b R Agonists**
  - Histones
  - P2X R Agonists
  - TLR7 agonists
- **Adenosine A3 R Agonists**
  - HSP60
  - P2Y R Agonists
  - TLR9
- **Non-selective Adenosine R Agonists**
  - HSP70/HSPA1A
  - Phosphatidylserine
  - Toll-like Receptor Agonists
- **CD24 Agonistic Antibodies**
  - IL-3
- **TIM-3 Agonistic Antibodies**
  - CD40 Agonistic Antibodies
- **SD-101 and BMS-986178 in Treating Patients with Advanced or Metastatic Solid Malignancies**
- **TLR9 Agonist SD-101, Ibrutinib, and Radiation Therapy in Treating Patients with Relapsed or Refractory Grade 1-3A Follicular Lymphoma**
- **Epacadostat, Toll-like Receptor 9 Agonist SD-101, and Radiation Therapy in Treating Participants with Advanced, Metastatic, or Refractory Solid Tumors or Lymphoma**
- **I-SPY 2 TRIAL: Neoadjuvant and Personalized Adaptive Novel Agents to Treat Breast Cancer**
- **Safety and Efficacy of APX005M With Gemcitabine and Nab-Paclitaxel With or Without Nivolumab in Patients With Previously Untreated Metastatic Pancreatic Adenocarcinoma**


**Agonist therapies/checkpoint/RT therapy combinations are moving forward!**

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Toll-like receptor 7/8 agonists loaded liposomes

In vivo, without ablation

- Anti CD40 (100μg, i.p.)
- Anti-PD-1 (200μg, i.p.)

Groups:
- NTC (n = 3)
- CD40 agonist+Anti-PD-1 (n = 4)
- Liposomal TLR+Anti-PD-1 (n = 3)
- Polymeric TLR+Anti-PD-1 (n = 3)

Tumors ~4 mm

Liposomal TLR = resiquimod liposomes; polymeric TLR = dihydrochloride-para-amine polymeric particles

Dense T-cell mediated response in treated and distant tumors

A good marriage: ablation and agonists
Intervene locally and achieve a controlled systemic effect

Expression of 10,000 genes altered by combining ablation and immunotherapy

- Anti-tumor gene expression is enhanced.
- Anti-tumor efficacy is enhanced.

Intra-tumoral injection with ablation: 80-fold changes in distant T cell response
Debulk and create scar

2 hours post ablation

24 hours

4 days post ablation

HIFU ablation enhances accumulation of liposomes surrounding the lesion

Ablation Alters Transport of Proteins

Initial enhanced accumulation of tracer in the ablated (left, white arrow) tumor permeated away by 24 hours suggesting enhanced lymphatic drainage

Wong et al. (1)

Clear on frozen sections with NADH Diaphorase (red circle)

Debulk and create scar

Clear on frozen sections with NADH Diaphorase (red circle)

Ablation Alters Transport of Proteins

Initial enhanced accumulation of tracer in the ablated (left, white arrow) tumor permeated away by 24 hours suggesting enhanced lymphatic drainage

Wong et al. (1)
Ablation Alters Transport of Small Molecules

Ablation releases tumor antigen

Adding immunotherapy before ablation, enhances lymph node (and blood and spleen) antigen

- Ova was now detected in the lymph nodes and enhanced by ablation.
**Summary: Innate immune locally, adaptive immune distantly**

- **< 6 hrs**
  - Systemic Type
  - IFN local and in blood

- **< 48 hours**
  - Activated T cells local site

- **> 7 days and onward**
  - Dense myeloid response at local site
  - Activated T cells at distant sites
  - T cell clonality similar in blood and tumor

**169cDb**

**Cu**

**Cl**

**2**

**Cu**

**- 169cDb**

**TCEP**

**- 169cDb**

**CD8**

**+ T cell imaging (collaboration with Anna Wu)**


**30% ID/cc**

**0% ID/cc**

**S**

**K**

**I**

**S**

**K**

**I**

**LN**

**24 h**

Agonists & in situ vaccination: conclusion

- Encouraging preclinical and clinical data
- Local intervention achieves systemic anti-cancer effect
- Clinical trials expanding to advanced solid tumors (need for interventional imaging)
- MRgFUS can play a role in debulking
- Much to learn about the signals between the treated and distant sites
- Imaging of T cells and macrophages now established

Outline: New frontiers in therapeutic ultrasound

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The unmet clinical need

- Severe bone loss often results from trauma, infection and tumor resection.
- Nonhealing fractures (nonunions): 5-10% of fractures.
- 2.1M bone grafts are implanted each year.
- Autografts – not always available, pain, infection.
- Allografts – fail to integrate, disease transmission.
- Bone regeneration is an unmet clinical need.
Fundamentals of microbubbles

- Expand and contract in response to ultrasound
  - Morgan et al. IEEE TUFFC 1998
- Nonlinear relationship to pulse frequency and phase
  - Morgan et al. IEEE TUFFC 1998
- Echoes are predictable
- Many applications in imaging:
  - Millions of exams/year
- Reimbursement in US for radiology applications in 2019

When driven at high pressure, membrane permeability altered

- In vitro
- In vivo

- In vitro
- Before US
- US

Microbubble collapse occurs at 100s of meters/second

- Microbubble collapse at 100s of meters/second
  - Chomas et al. Applied Physics Letters
Local Transfection

Shapiro et al. JCR, 2016

DNA
DNA + MB
DNA + US
DNA + MB + US

Hypothesis: Targeted BMP gene delivery to endogenous stem cells could lead to effective repair of nonunions in a large animal model.

Regenerative medicine solution for severe bone loss

Ultrasound-mediated Activation of Endogenous Stem Cells for Bone Regeneration

Endogenous mesenchymal stem cells migrate to a minipig segmental fracture

Collagen scaffold implantation enhanced endogenous mesenchymal stem cell migration to fracture site.

Day 7
Day 14
Day 21
0
20
40
60
% CD29+

N=3 per **

% CD90+

Bez et al. STM, 2017

p<0.01

p<0.001
Ultrasound-mediated, microbubble-enhanced gene delivery procedure

Philips Sonos 5500, S3 probe, Definity, 1.3 MHz, mechanical index of 0.6, (0.68 MPa) a depth of 4 cm for ~ 2 minutes. Insonify 1 cm defect with >200 kPa, <800 kPa


Reporter gene expression in mini-pigs' tibial fractures following ultrasound-mediated gene delivery

No US US
0
50
100%CD29+ of GFP+ *

Mean fluorescence intensity (arbitrary units) ****

No US US
50
100
150
200
250
Luciferase activity (RLU/mg protein) ****

No US US
0
50
100%CD90+ of GFP+ **

No US US
0
50
100%CD44+ of GFP+ **

Ultrasound-mediated gene delivery induced transient, localized, overexpression of BMP-6 at the fracture site

Bez et al STM, 2017

RQ: relative quantification. ** p<0.01, *** p<0.001

No significant changes U/S treated animals compared to control animals.
Ultrasound-mediated BMP-6 gene delivery enhanced bone regeneration

**Conclusions (transfection)**

- US+MB transfection in large joint space was feasible
- Ultrasound-based therapy resulted in well-localized transient transgene expression.
- Gene delivery was targeted to recruit endogenous stem cells.
- BMP-6 gene delivery to endogenous stem cells resulted in complete fracture repair.

**Conclusion (overall)**

- MRgFUS ablation progressing in clinical applications yielding minimally invasive and image-guided treatments
- *In situ* vaccination in human trials: enabled by combinations of agonists and T cell signaling modulators
- *In situ* transduction on the horizon
- Needs:
  - Training in combining imaging and therapy
  - Training spanning molecular assays and imaging
Thanks!

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