

New frontiers in therapeutic ultrasound: transfection and immune modulation

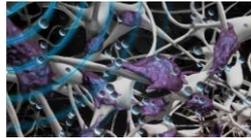
New frontiers in therapeutic ultrasound: transfection and immune modulation

Kathy Ferrara



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

T-cell invigoration to tumour burden ratio associated with anti-PD-1 response

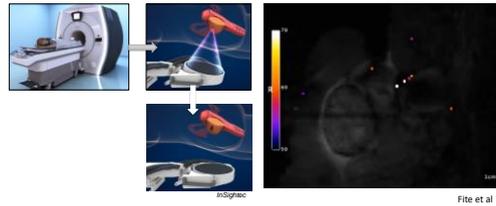
Sharma SC, Allison JP, Brahmer CR, et al. (2015) T-cell invigoration to tumour burden ratio associated with anti-PD-1 response. *Nature Reviews Clinical Oncology* 11:1315-1334. doi:10.1038/nrco.2015.11

Therapeutic ultrasound protocols explored with immunotherapy include:

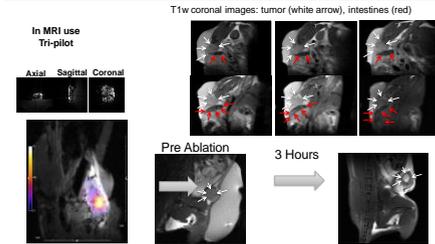
- Thermal ablation
- Hyperthermia
- Microbubble-based membrane and vascular destabilization
- Mechanical disruption

We will focus on thermal ablation.

MRgFUS ablation

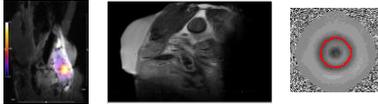


Motivation- MRI guidance



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



Fite et al.
Liu et al

Experimental Setup (3 MHz for thermal effect)

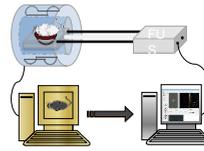
- Tumors ablated in 2mm circular pattern
 - CW for 30s, 5W acoustic, PNP = -3.1 Mpa

FUS system

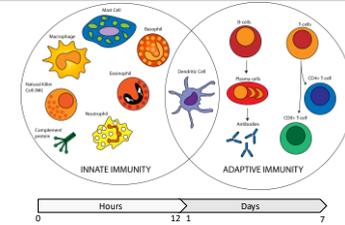
- 16-element annular array
- 3 MHz
- 300 kHz bandwidth
- 120 W peak acoustic power
- 120 W peak acoustic power
- 35 mm radius of curvature
- 0.5 × 0.5 × 2.5 mm³ focal spot

MR scanner

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

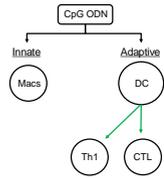


Innate and adaptive immunity

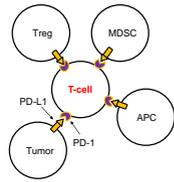


Immune Agonists and Checkpoint Inhibition

Agonists: Press on the gas



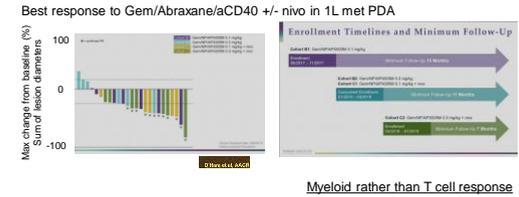
Checkpoint: Release the brakes



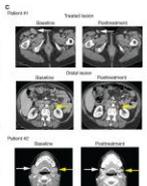
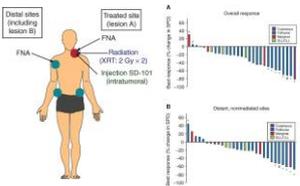
Motivation

- 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



In situ vaccination: Lymphoma (Levy Lab)



Frank MJ et al Cancer Discov. 2018 Oct;8(10):1258-1269.

From R. Levy

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

pDC Inducers				mDC Maturation Inducers			
Adenoviral Ad.R	GM-CSF	IL-3a	TLR2 agonists	Biotinylated/Fluorescent Antigen/antigen	IL-1 beta/IL-1F2	TLR8 agonists	
Adenoviral Ad3.R	GRF78/SPAS	IFN-beta	TLR4 agonists	CD40 Ligand/TNFSP3	IL-5	TLR7 Agonists	
Adenoviral Ad3.R	Histones	P2X7.R Agonists	TLR7 agonists	CD40 Agonists/ Antibodies	IL-18.R alpha Blocking	Toll-like Receptor Agonists	
Adenoviral Ad3.R	ISPN9	P2X7.R Agonists	TLR9	DC-SIGN/CD203	IL-18.R beta Blocking	TNF-alpha	
Non-enveloped Adenovirus	HSP70/SPAI	Phosphatidylinositol	TLR9	Dendritic-SIGN/LECTA	IL-12	VEGF-R1 Blocking	
Adenovirus 5	Delta	RAGE	Receptor Agonists	GM-CSF	Neurostatin B	VEGF-R2 Blocking	
CD40	HMGB1/HD-1	Synuclein-alpha		IL-18	Receptor Antibodies	Antibodies	
Liposomes/TNFSP3	CD40 Agonists/ Antibodies	IL-3	TIM-3 Agonists/ Antibodies	IFN-alpha	CD137		
				IFN-gamma	TLR3 Agonists		

https://www.rndsystems.com/products/cancer-immunotherapy-inducing-dendritic-cell-maturation

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

SD-101 and BMS-986178 in Treating Patients with Advanced or Metastatic Solid Malignancies
[NCT03831295](https://clinicaltrials.gov/ct2/show/study/NCT03831295)

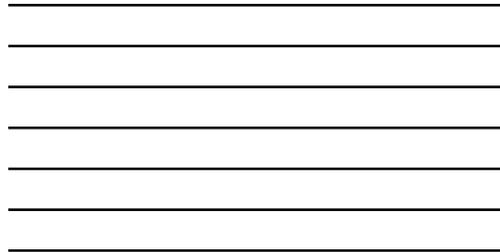
TLR9 Agonist SD-101, Ibrutinib, and Radiation Therapy in Treating Patients with Relapsed or Refractory Grade 1-3A Follicular Lymphoma
[NCT02927264](https://clinicaltrials.gov/ct2/show/study/NCT02927264)

Androgen Deprivation Therapy, Pembrolizumab, and Stereotactic Body Radiation Therapy with or without TLR9 Agonist SD-101 in Treating Patients with Metastatic Prostate Cancer
[NCT03097732](https://clinicaltrials.gov/ct2/show/study/NCT03097732)

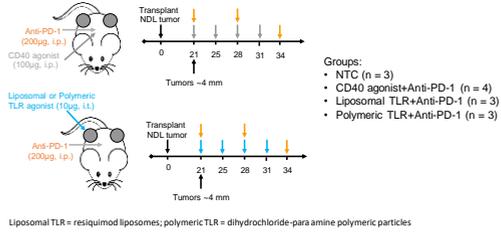
Epacadostat, Toll-Like Receptor 9 Agonist SD-101, and Radiation Therapy in Treating Participants with Advanced, Metastatic, or Refractory Solid Tumors or Lymphoma
[NCT03322384](https://clinicaltrials.gov/ct2/show/study/NCT03322384)

I-SPY 2 TRIAL: Neoadjuvant and Personalized Adaptive Novel Agents to Treat Breast Cancer
[NCT01042373](https://clinicaltrials.gov/ct2/show/study/NCT01042373) (includes SD-101 + Pembrolizumab)

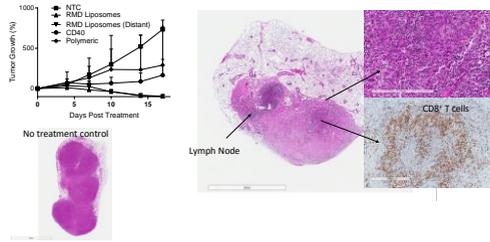
Safety and Efficacy of APX005M With Gemcitabine and Nab-Paclitaxel With or Without Nivolumab in Patients With Previously Untreated Metastatic Pancreatic Adenocarcinoma
[NCT03214250](https://clinicaltrials.gov/ct2/show/study/NCT03214250)



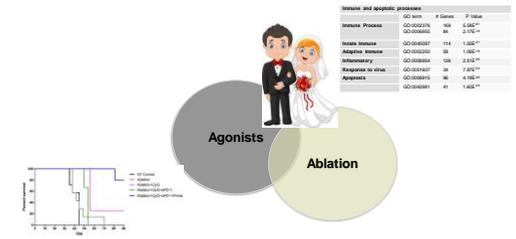
Toll-like receptor 7/8 agonists loaded liposomes
In vivo, without ablation



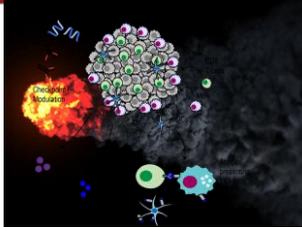
Dense T-cell mediated response in treated and distant tumors



A good marriage: ablation and agonists

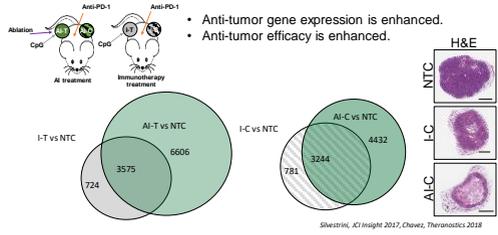


Intervene locally and achieve a controlled systemic effect

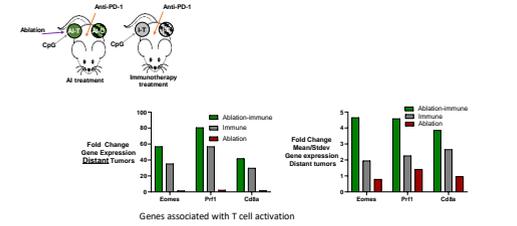


Silverstein, JCI insight 2017, Chavez, Theranostics 2018

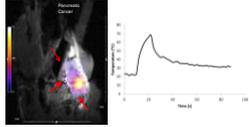
Expression of 10,000 genes altered by combining ablation and immunotherapy



Intra-tumoral injection with ablation: 80-fold changes in distant T cell response



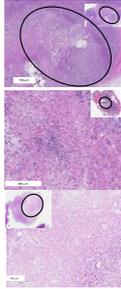
Debulk and create scar



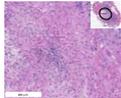
Clear on frozen sections with NADH Diaphorase (red circle)



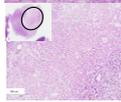
2 hours post ablation



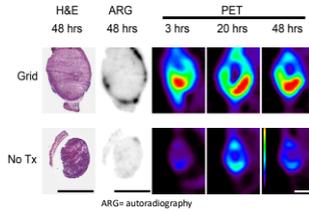
24 hours



4 days post ablation

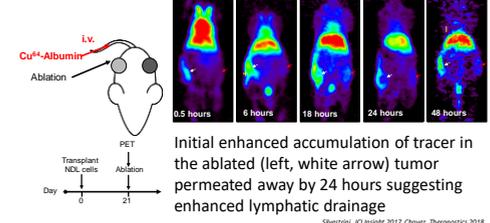


HIFU ablation enhances accumulation of liposomes surrounding the lesion

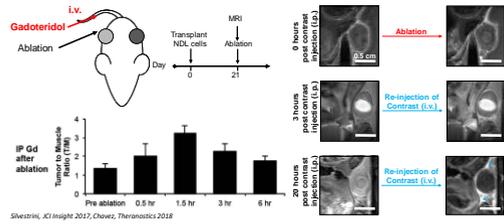


Wong et al, JCI

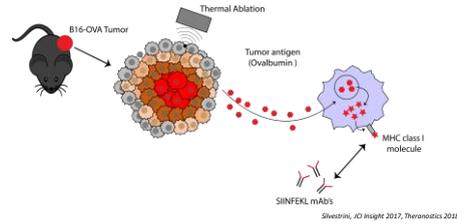
Ablation Alters Transport of Proteins



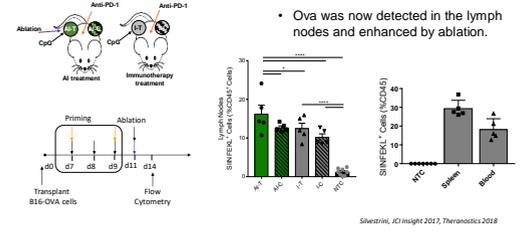
Ablation Alters Transport of Small Molecules



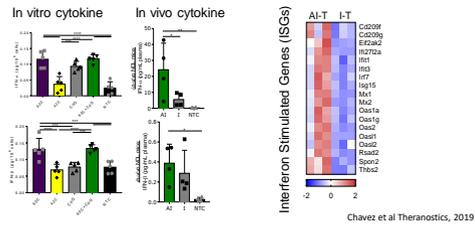
Ablation releases tumor antigen



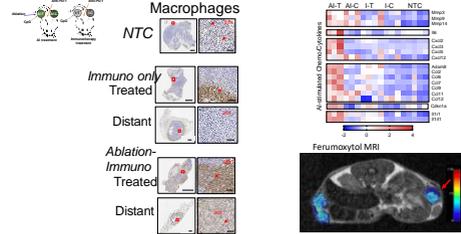
Adding immunotherapy before ablation, enhances lymph node (and blood and spleen) antigen



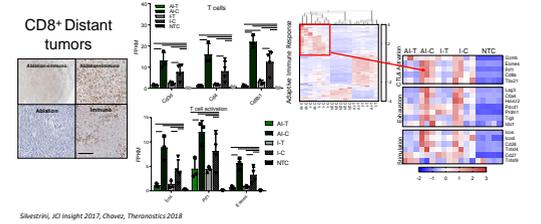
Ablation induces type I IFN (cytokine) release



Ablation-immunotherapy enhances LOCAL innate immune response

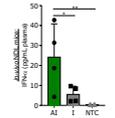


Ablation-immunotherapy increases DISTANT T-cell markers





< 6 hrs
Systemic Type 1 IFN local and in blood

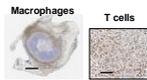


< 48 hours
Activated T cells local site

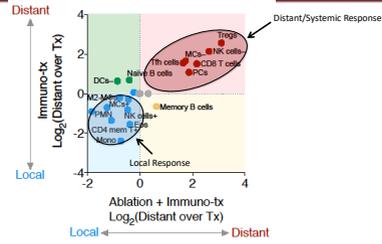


Alam IS et al J Clin Invest. 2018 Jun 1;128(6):2569-2580.

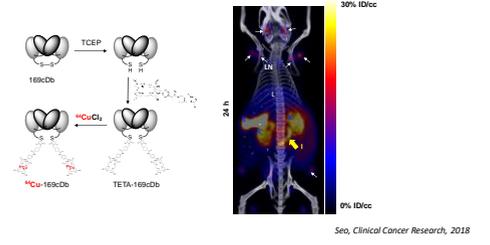
- > 7 days and onward**
- Dense myeloid response at local site
 - Activated T cells distant sites
 - T cell clonality similar blood and tumor



Summary: Innate immune locally, adaptive immune distantly



CD8+ T cell imaging (collaboration with Anna Wu)

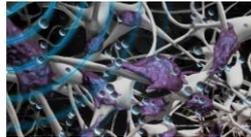


Agonists & *in situ* vaccination: conclusion

- 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

- Synergy between ultrasound and immunotherapy
- **Image-guided transfection**

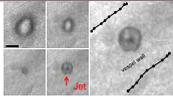


The unmet clinical need

- 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

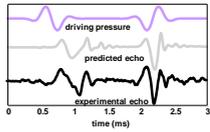


Expand and contract in response to ultrasound
 Dayton et al, IEEE, 1999
 Caskey et al, JASA, 2007

Many applications in imaging:

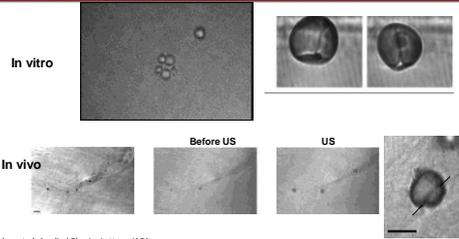
Millions of exams/year

Reimbursement in US for radiology applications in 2019



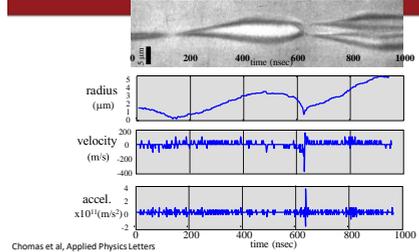
Echoes are predictable
 Nonlinear relationship to pulse frequency and phase
 Morgan et al IEEE TUFFC 1998

When driven at high pressure, membrane permeability altered



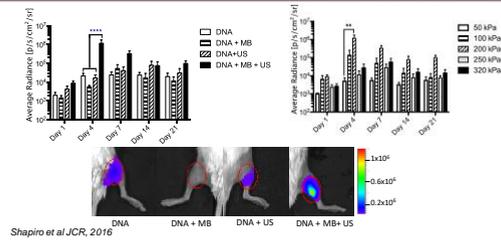
Caskey et al, Applied Physics Letters, JASA

Microbubble collapse occurs at 100s of meters/second



Chomas et al, Applied Physics Letters

Local Transfection

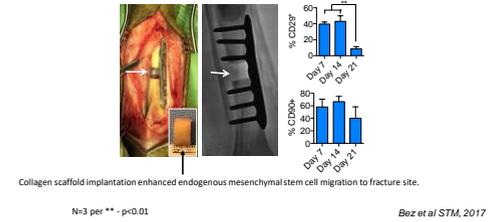


Regenerative medicine solution for severe bone loss

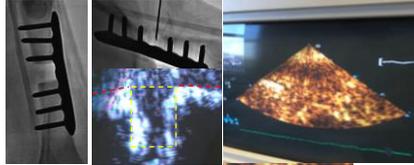


Hypothesis: Targeted BMP gene delivery to endogenous stem cells could lead to effective repair of nonunions in a large-animal model.

Endogenous mesenchymal stem cells migrate to a minipig segmental fracture

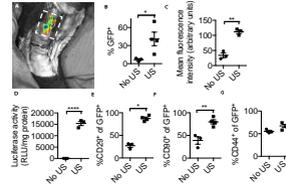


Ultrasound-mediated, microbubble-enhanced gene delivery procedure



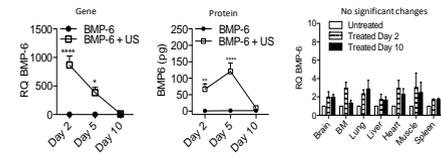
Philips Sonos 5500, S3 probe, Definity, 1.3 MHz, mechanical index of 0.6, (0.68 MPa) a depth of 4 cm for ~ 2 minutes. Insonify 1 cm defect with >200 kPa, <800 kPa
 Boz et al STM, 2017, Nature Protocols, 2019

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



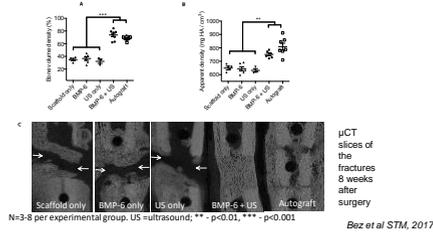
N=3-4 per experimental group. US =ultrasound, * - p<0.05, ** - p<0.01, **** - p<0.0001 Boz et al STM, 2017

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



N=3 per experimental group. US =ultrasound, * - p<0.05, ** - p<0.01, **** - p<0.0001
 RQ: relative quantification. 850- and 400- fold higher BMP-6 expression in ultrasound-treated animals compared to control animals Boz et al STM, 2017

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!

National Cancer Institute
Focused Ultrasound Foundation
Image-Guided Therapy (Erik Dumont)

Cedars Sinai: Gadi Palled, Dan Gazit

Duke University: Gregg Trahey

U Bergen: Frits Thorsen, Cecilie Brekke,

Rolf Reed

UCD: Sandy Borowsky, Bob Cardiff

USC: Robert Wodnicki, Qifa Zhou

Stanford: Sam Gambhir, Aaron Newman,

Ron Levy, Stanley Qi, Kim Butts Pauly,

Peji Ghanouni

Xiran Cai
Michael Chavez
Lisa Even
Brett Fite
Josquin Foiret
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Tali Ilovitsh
Elizabeth Ingham
Sarah Johnson Tam
Azadeh Kheirolomoom
Hamilton Kakwere
Chun-Yen Lai
Jai Woong Seo
Matt Silvestrini
Doug Stephens
Samantha Tucci
Spencer Tumbale
Hua Zhang
