Intratumoral Checkpoint Blockade Inhibition and New Opportunities for Intratumoral Therapy with Focused Ultrasound

Craig L. Slingluff, Jr., M.D.
Professor of Surgery
University of Virginia Cancer Center

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Disclosures

Scientific advisory board
Immatics (cancer vaccines)
Polynoma (PI for MAVIS cancer vaccine trial)

Research support to UVA:
Glaxo-Smith Kline: Cancer vaccine
Merck: Cancer vaccine + PD1 antibody; immunotherapy
Celldex: Cancer vaccine + CD27 antibody, CD40 antibody
Theraclion: focused ultrasound and immunotherapy
3M: drug provided for clinical trial
Agenus: pending collaboration

Patent holder for peptides used in cancer vaccines,
via UVA Licensing and Ventures Group

Off-label or experimental use of:
Focused ultrasound, polyICLC, cancer vaccines, intratumoral tremelimumab; durvalumab, imiquimod

Rationale for intratumoral checkpoint blockade

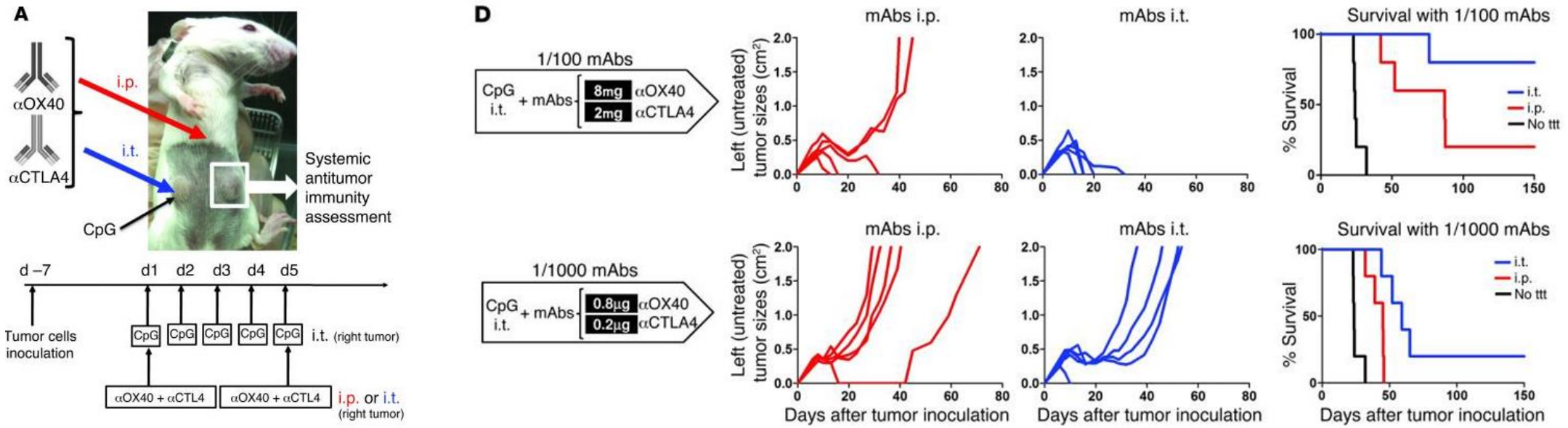
- Combination systemic checkpoint blockade (eg CTLA4 + PD1/PD-L1)
  - Enhances clinical response and survival
  - Increases toxicity over single agent PD1/PD-L1 blockade

- Intratumoral administration of CTLA4 antibody offers chance for oncologic benefit of combination therapy with low dose and thus lower toxicity.

- Intratumoral checkpoint blockade combined with other intratumoral therapy can create an in situ vaccination effect with systemic benefit

Preclinical experience with intratumoral CTLA-4 blockade (1)

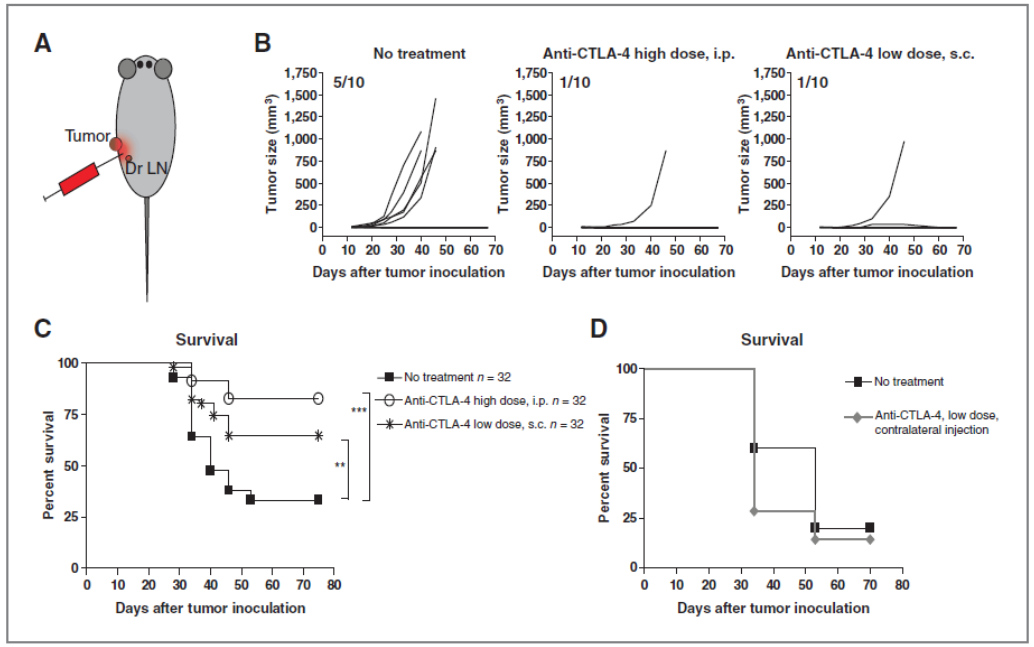
- TC-1 tumor cells (epithelial lung cancer) engineered to express CTLA-4 Ab combined with T-reg depletion → tumor control and avoids autoimmune toxicity of systemic CTLA-4 antibody (Tuve, Ca Res 2007)
- Intratumoral CTLA-4 Ab + Ox-40 Ab + TLR9 agonist



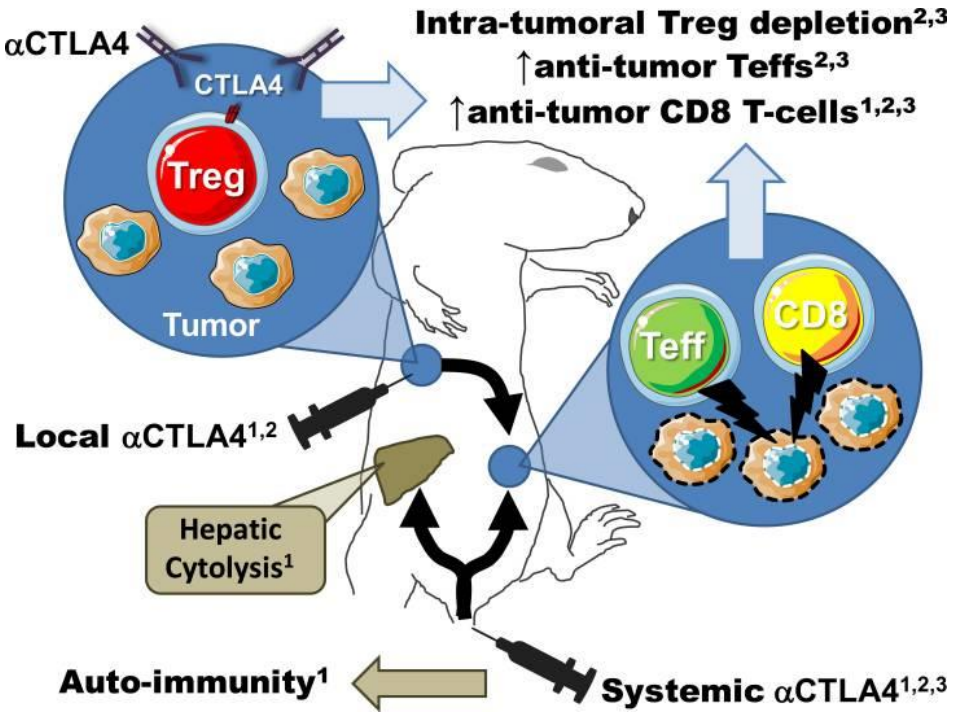
Marabelle A, JCI 2013

Preclinical experience with intratumoral CTLA-4 blockade (2)

- Peritumoral low-dose CTLA-4 Ab for murine colon cancer (MC38)
  - 200 mcg IP d0 + 3 vs. 50 mcg peritumoral d0 emulsified in Montanide ISA-51 (1/8th dose)
  - Induces systemic immune response protecting against tumor growth
  - Therapeutic effect depends on CD8 T cells.
  - Reduced serum CTLA-4 Ab; no autoimmune hepatitis



Fransen, CCR 2013

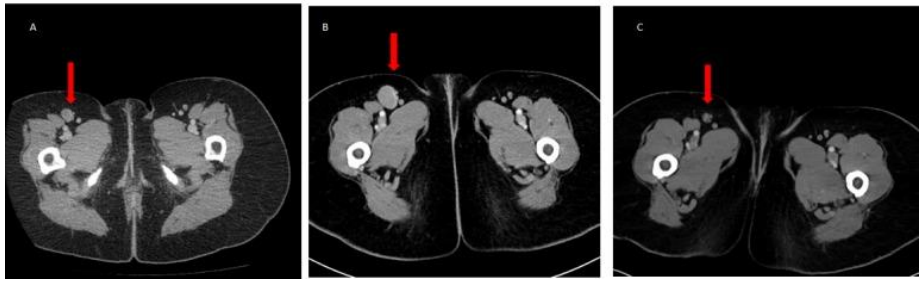


Marabelle A, CCR 2013

Human experience with intratumoral CTLA4 blockade

- In prior work, intratumoral IL-2 → regression of injected lesions in most patients but has no abscopal effect.
- Clinical trial in 12 patients with advanced melanoma were evaluated IT IL-2 + IT ipilimumab in dose escalation:
  - 0.5 → 1 → 2 mg Ipi: weekly x 8 (n = 3, 3, 6)
  - IL-2 at 3mIU: 3x/wk x 2, 2x /wk x 6
- Safety: No DLTs. One grade 3 injection site rxn.
- Injected lesion: 7 CR, 1 PR = 67% RR
- Abscopal effects:
  - Some regression at distant sites in 8/9 pts with >1 lesion (89%)
  - Overall irRC: 3PR (25%) plus 1 with PD found to have path CR at surgery.
    - Included all 3 at highest dose of ipilimumab

CT scan of abdomen and pelvis of non-injected lesion for patient 11



Ray A, et al. Oncotarget 2016

Poly-ICLC (Hiltonol®) – Immune and therapeutic effects alone and in combination

Poly-ICLC alone

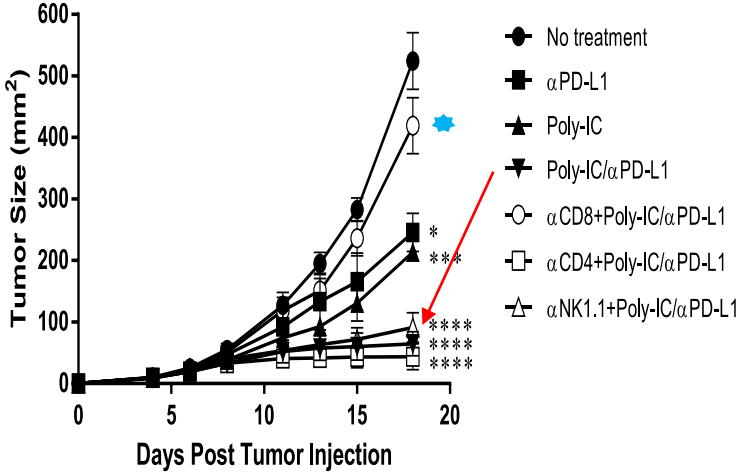
TLR 3 agonist

- Promotes DC maturation
- Potent induction of Type I IFNs
- Induces TNF, IP-10; low IL-10
- $CD8^+$ differentiation dependent upon IL-12
- Expands CD8 T cells reactive to melanoma antigens
- Repeated intratumoral injection in humans can enhance immune infiltrates and cause tumor regression.

-- Bogunovic et al., Cancer Res. 2011
-- Gallois et al., Frontiers in Immunology 2014
-- Salazar et al. Cancer Immunology Research 2014

Poly-ICLC plus αPD-L1 Therapeutic Effect in Murine Solid Cancers

B16 Melanoma



Poly-ICLC 50 mcg sc d7, 12, 17;
aPDL-1 200 mcg ip d 8, 10 13,15, 18, 20

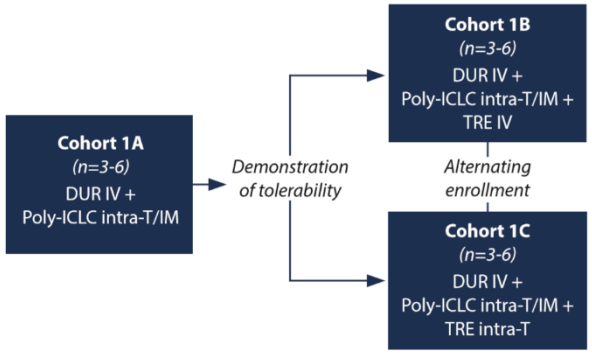
-- Nagato, Lee, Harabuchi & Celis, CCR 1/14

Phase 1/2 study of in situ vaccination with tremelimumab + intravenous durvalumab + poly-ICLC in patients with select relapsed, advanced cancers with measurable, biopsy-accessible tumors

Ongoing Phase 1/2, open-label, multicenter study (NCT02643303) designed to evaluate the safety, preliminary efficacy, and immune activity of combination therapies with poly-ICLC, tremelimumab (TRE) and durvalumab (DUR) in patients with advanced, measurable, biopsy-accessible cancers.

Agents:

- Durvalumab
  - human IgG1
  - anti-PD-L1
- Tremelimumab
  - human IgG2
  - anti-CTLA4
- polyICLC (Hiltonol) TLR3 agonist
  - preferentially activates mDC, → Th1/CTL
  - Activates NK cells, → cytotoxic potential.
  - Safely used IV to 300 mcg/kg
  - Has been given IT.



Study Objectives	
Primary objective (Endpoints)	*Dose finding phase (Cohorts 1A-1C):* Safety and tolerability; RCD
	Expansion Phase (Cohort 2): Clinical efficacy by irRECIST and RECIST 1.1 • Objective response rate (ORR), • Progression-free survival (PFS) • Overall Survival (OS)
Exploratory Objectives	Biologic activity [Effects on tumor micro-environment, immune responses] Tumor biopsies D1, D15, D29

This study is sponsored by Ludwig Institute for Cancer Research, with support from the Cancer Research Institute and MedImmune

Study chairs: N Bhardwaj, C Slingluff

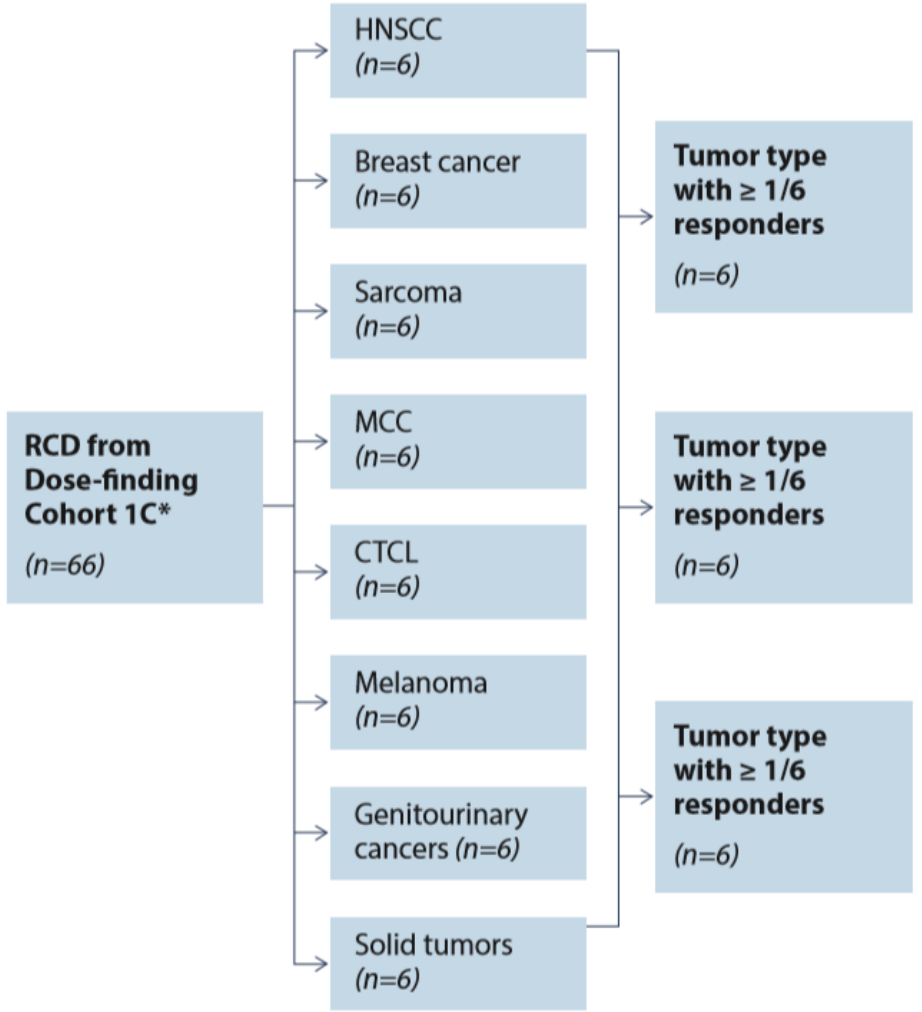
Phase 1/2 study of in situ vaccination with tremelimumab + intravenous durvalumab + poly-ICLC in patients with select relapsed, advanced cancers with measurable, biopsy-accessible tumors

KEY INCLUSION CRITERIA

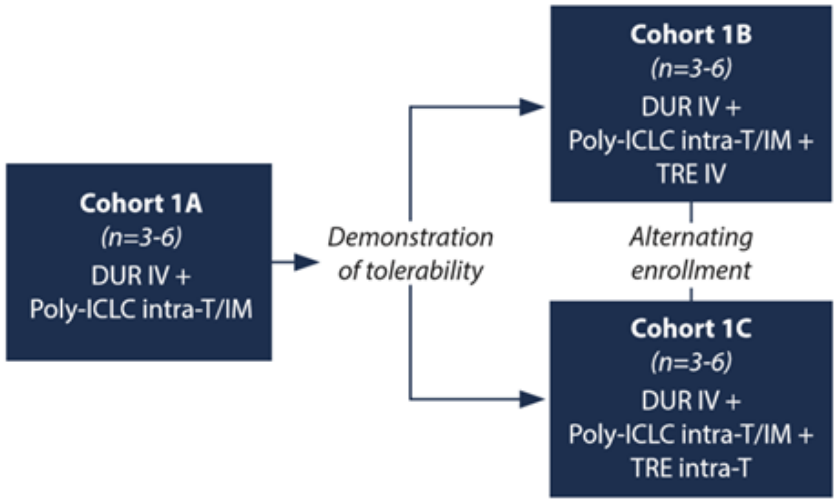
- Histologic confirmation of advanced, biopsy-accessible, measurable cancers
- ≥ 1 lesion that can be accurately measured in ≥ 1 dimension, and ≥ 2 lesions that can be biopsied or 1 lesion that can be biopsied at least twice
- ECOG performance status 0-1
- Age ≥ 18 years

KEY EXCLUSION CRITERIA

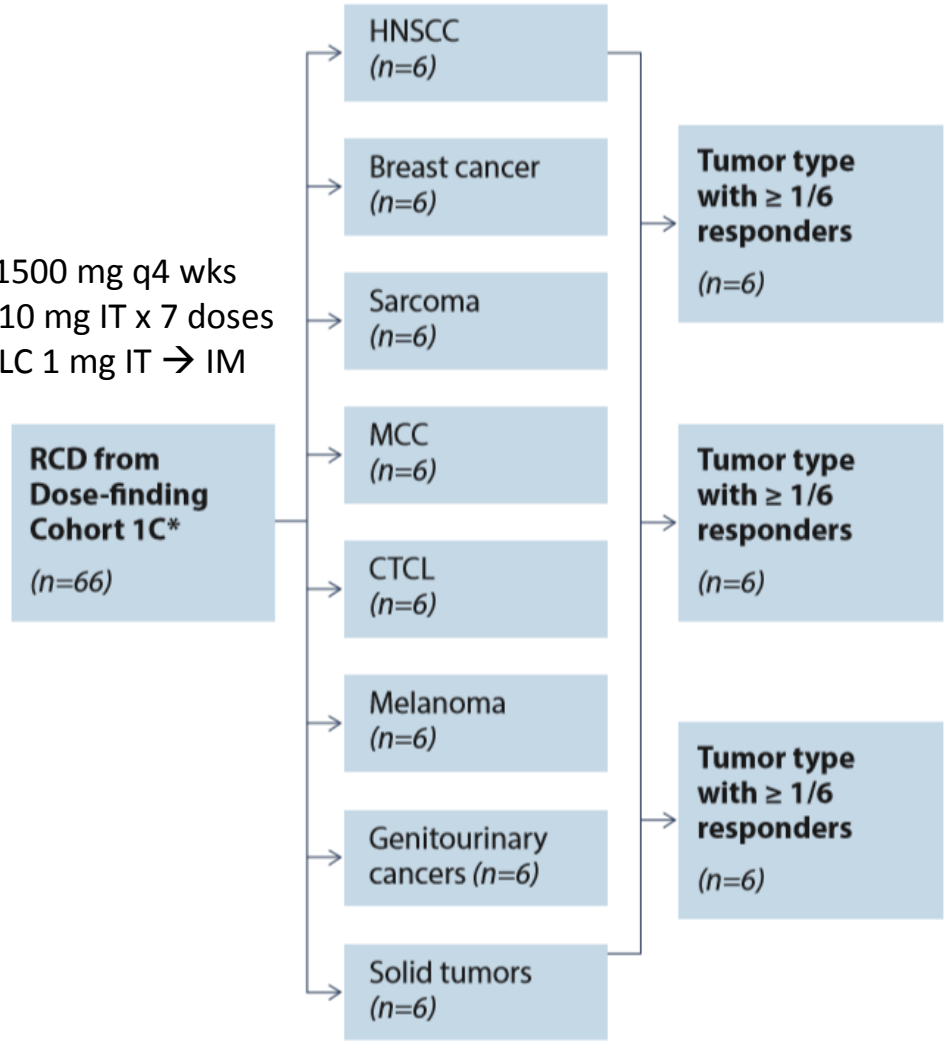
- Prior treatment with intra-T poly-ICLC or with combination blockade of CTLA-4 + PD-1/PD-L1 (now allowed for melanoma)
- History or evidence of CNS disease including brain metastases
- Underlying autoimmune disease or immunodeficiency
- Clinically significant cardiovascular disease
- Prior clinically significant or unresolved irAEs
- Symptoms or signs of GI obstruction or other serious illness



Phase 1/2 study of in situ vaccination with tremelimumab + intravenous durvalumab + poly-ICLC in patients with select relapsed, advanced cancers with measurable, biopsy-accessible tumors



Durva 1500 mg q4 wks
Treme 10 mg IT x 7 doses
Poly-ICLC 1 mg IT → IM



Cycle *(4 weeks)*	1				2				3	4	5	6	7	8	9	10	11	12
Study week	1	2	3	4	5	6	7	8	9	13	17	21	25	29	33	37	41	45
DUR IV *(all cohorts)*	X				X				X	X	X	X	X	X	X	X	X	X
TRE intra-T *(Cohort 1C)*	Day 1	Day 8	Day 15		Day 1		Day 15		Day 1	Day 1								
Poly-ICLC intra-T *(all cohorts)*	Days 1, 3, 5, 8, 10, 15																	
Poly-ICLC IM *(all cohorts)*			Days 17, 22, 24		Days 1, 3, 8, 10, 15 17, 22, 24				Days 1, 4									
Disease assessment										X			X		X		X	

DUR IV = durvalumab intravenous, 1500 mg every 4 weeks x 12 cycles (dose de-escalation = 750 mg); TRE IV = tremelimumab intravenous, 75 mg every 4 weeks x 4 cycles (dose de-escalation = 22.5 mg); TRE intra-T = tremelimumab intratumoral, 10 mg (dose de-escalation = 3 mg); Poly-ICLC intra-T = intratumoral,1 mg; Poly-ICLC IM = intramuscular,1 mg

LUD2014-011
Patient # 1

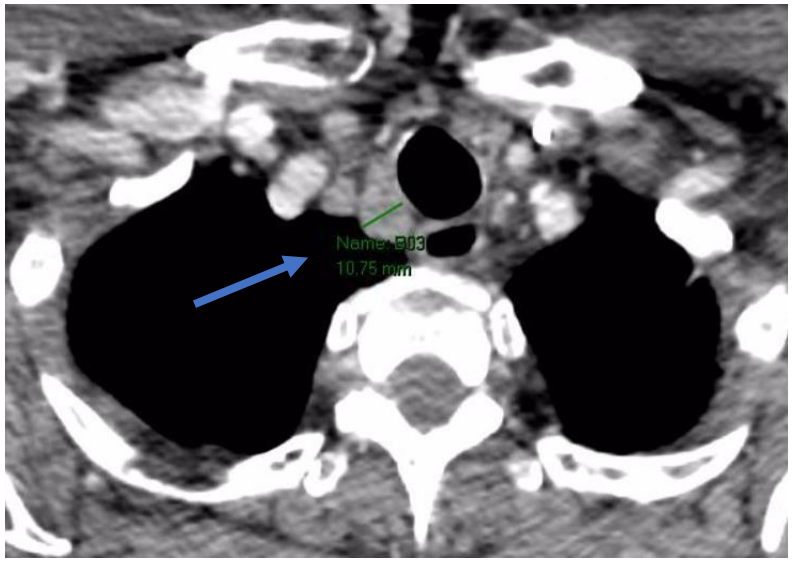
Locally/regionally advanced ER/PR negative, Her2 negative Breast cancer (TNBC)

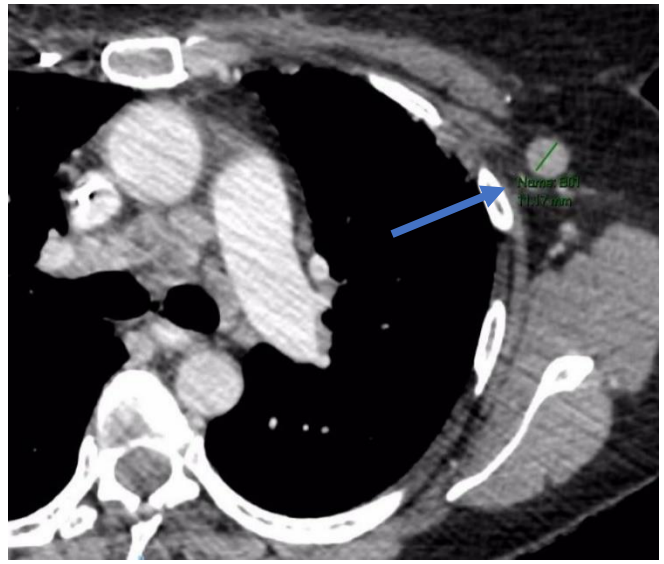
- Extensive skin metastases
- Large right SCL metastasis
- Paratracheal metastases
- Left axillary metastasis

Intratumoral polyICLC
Systemic Durvalumab

Complete response at week 12

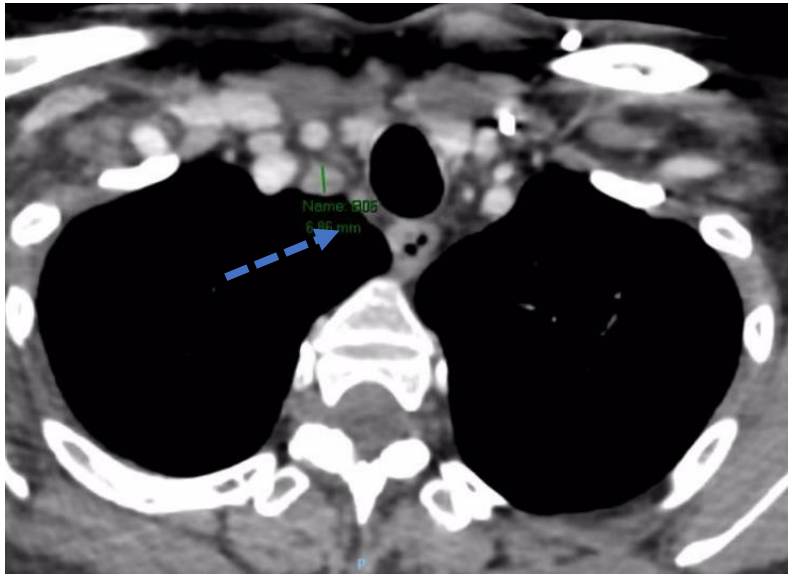
Breast cancer patients enrolling in expansion cohort.

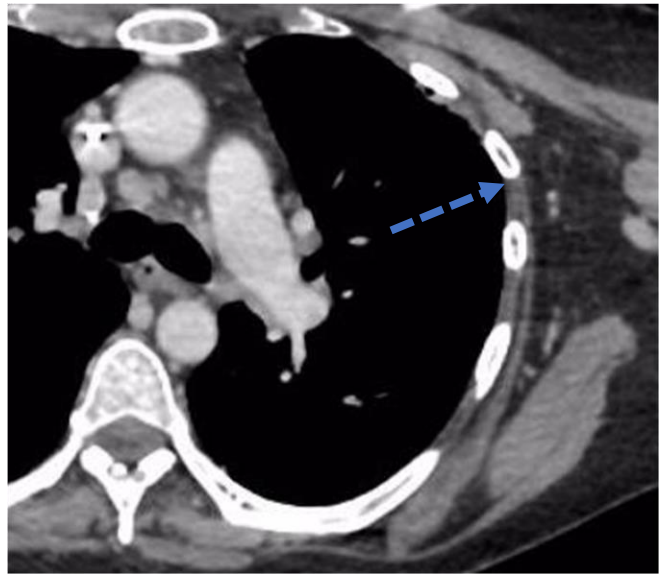




Pre

3 mos





Phase 1/2 study of in situ vaccination with tremelimumab + intravenous durvalumab + poly-ICLC in patients with select relapsed, advanced cancers with measurable, biopsy-accessible tumors

Sample collection & correlative laboratory studies planned

- **Tumor biopsy: non-injected, at screening, D15, D29, EOT*; and non-injected***
  - FFPE
  - Snap-frozen
  - RNA-later
- **PBMCs**
- **PAX RNA**

***Optional**

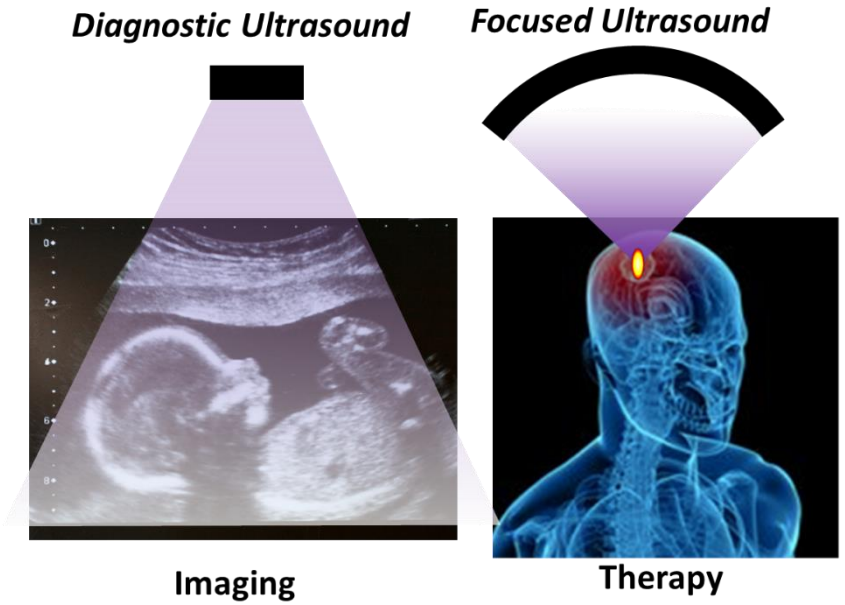
Challenges for Intratumoral Therapies with Checkpoint Antibodies and other Immune Modulators

- Cold tumors may not respond to checkpoint blockade without new antigen exposure
- Poor control of dispersion of therapeutic agent within the tumor
  - Failure to reach all of the tumor
  - Systemic diffusion from the tumor
- Poor accessibility of some tumors to percutaneous injection
  - Eg: periaortic nodes, retroperitoneal masses, brain metastases.

→ **Focused ultrasound therapy** offers opportunities to address these needs for intratumoral immune therapies.

Focused ultrasound (FUS) offers opportunities to modulate tumor microenvironments and to deliver therapeutic agents

FUS is the propagation and concentration of sound waves into a single ellipsoid volume



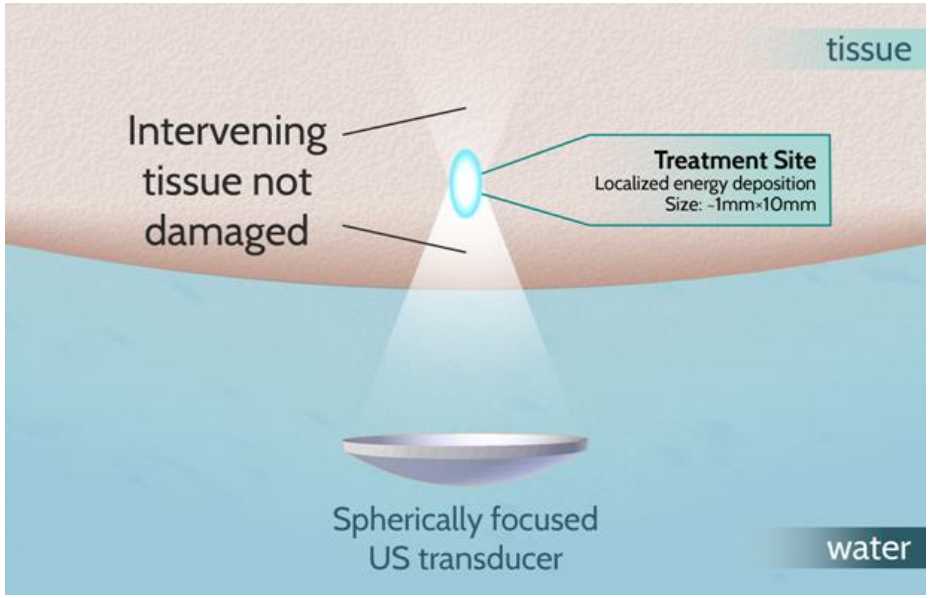
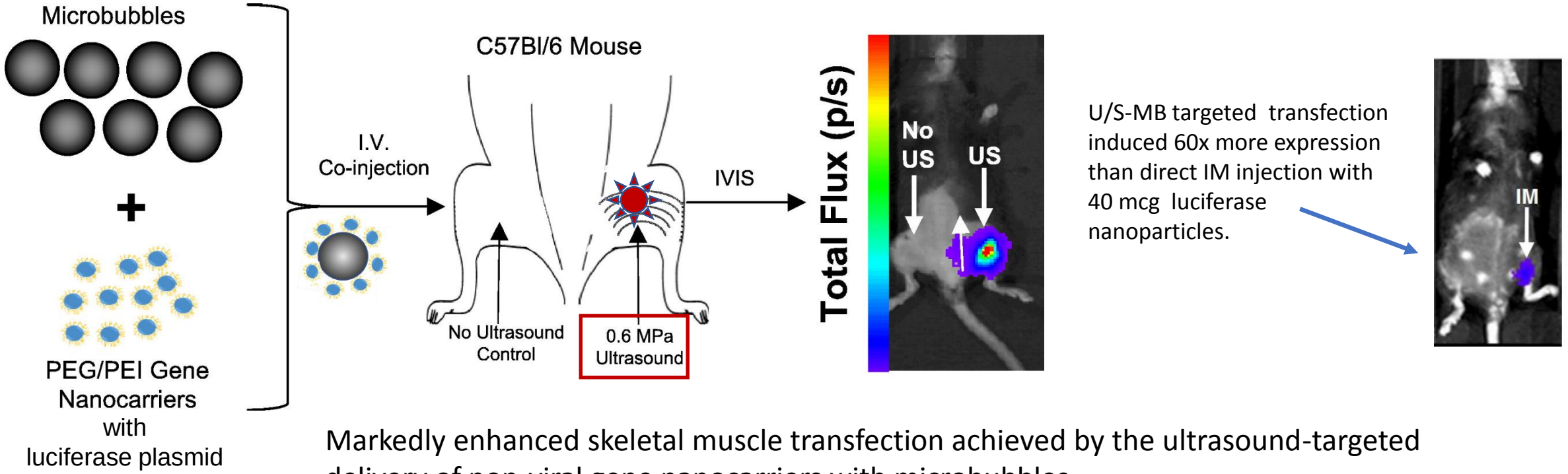


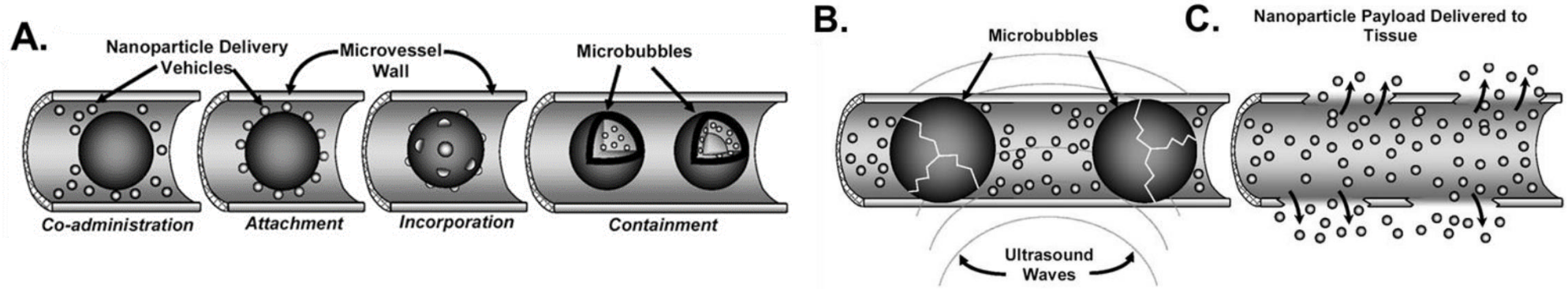
Image courtesy of FUS Instruments

FUS is non-invasive (extracorporeal), non-ionizing, safe, repeatable, and localized.

Focused delivery of gene payload with ultrasound and nanoparticle-loaded microbubbles.



Markedly enhanced skeletal muscle transfection achieved by the ultrasound-targeted delivery of non-viral gene nanocarriers with microbubbles



- Burke CW . J Control Release 2012
- Gorick et al. Int. J. Mol. Sci. 2019

Brain-Penetrating Nanoparticle (BPN) Delivery to Gliomas in Mice with MR Image-Guided Focused Ultrasound and Microbubbles (6h)

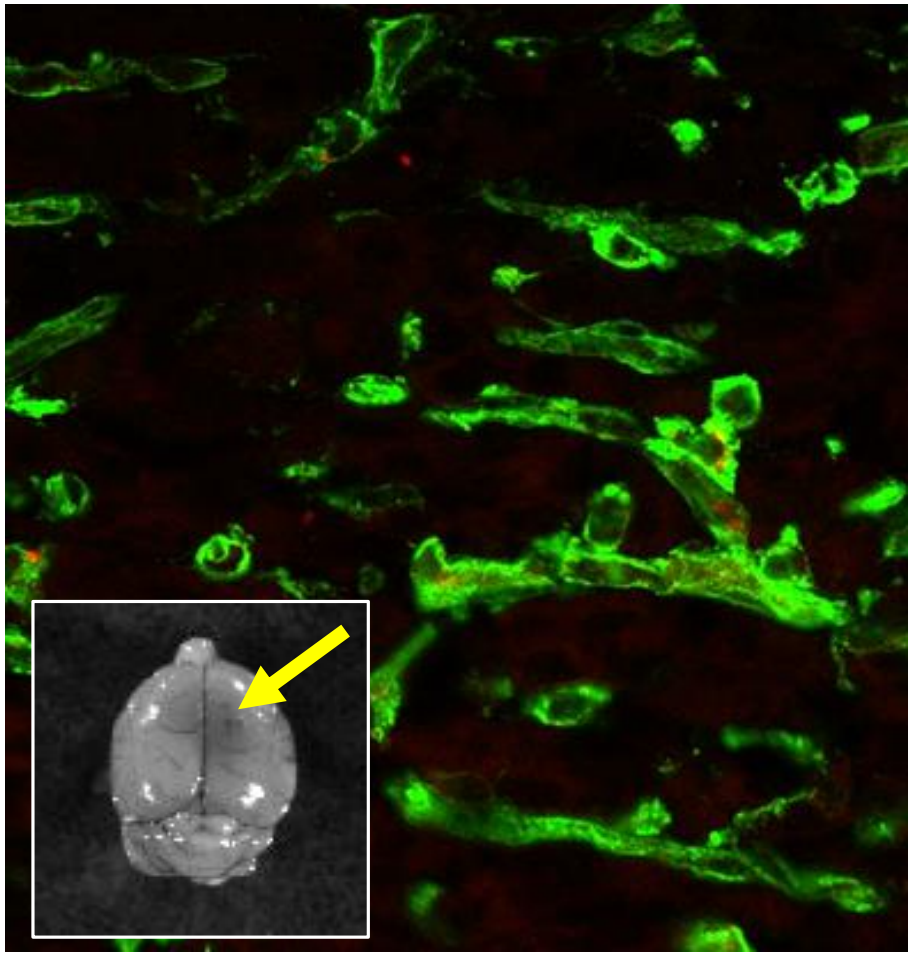
BPN is a non-viral gene-bearing nanoparticle with with a Cy5-labeled plasmid.

BS-I Lectin stains endothelium.

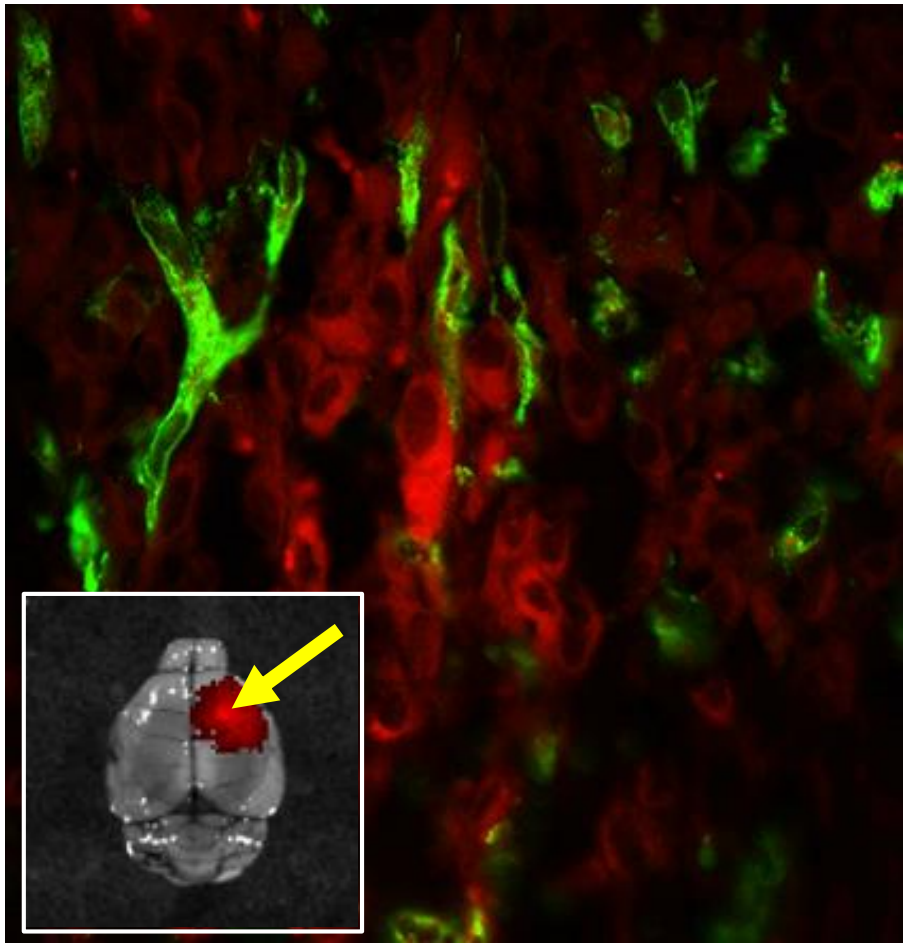
Cy5 staining of tumor cells demonstrates delivery through endothelium to tumor cells.

BS-I Lectin/Cy5 BPN

Untreated Control



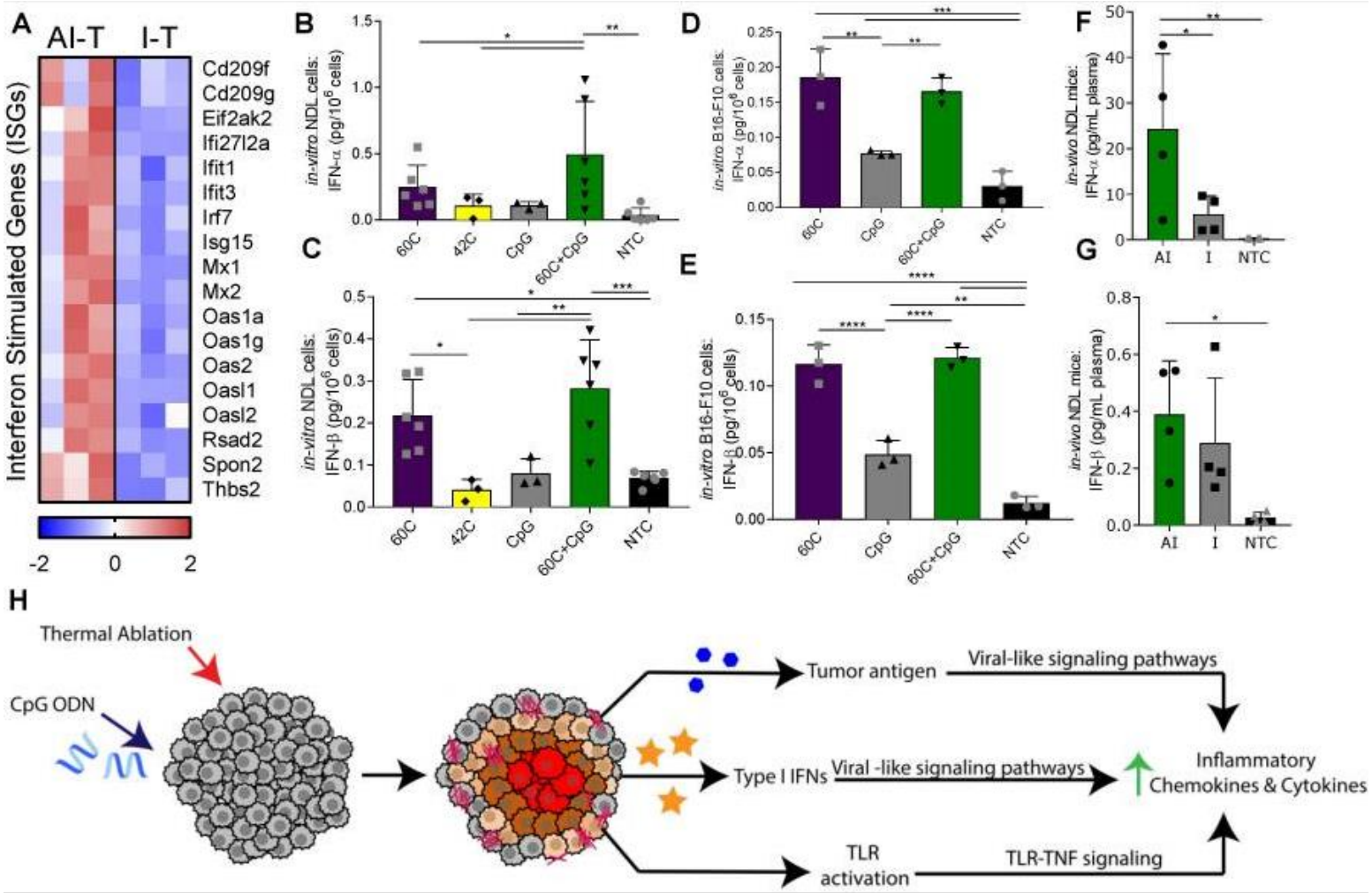
FUS + MB + Cy5 BPN



Curley et al. In Revision

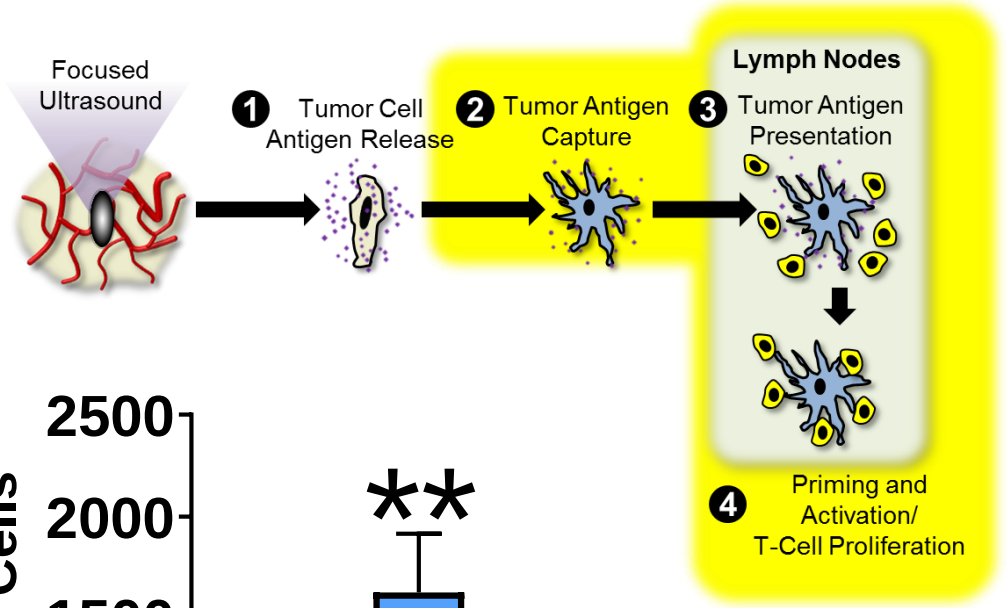
Combination therapy with intratumoral TLR agonist and focused ultrasound ablation

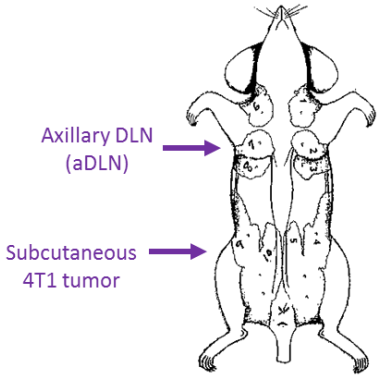
- FUS thermal ablation + CpG (AI-T) → upregulation of TLR/TNF signaling, Type I IFNs and tumor antigen release compared to CpG alone (I-T)



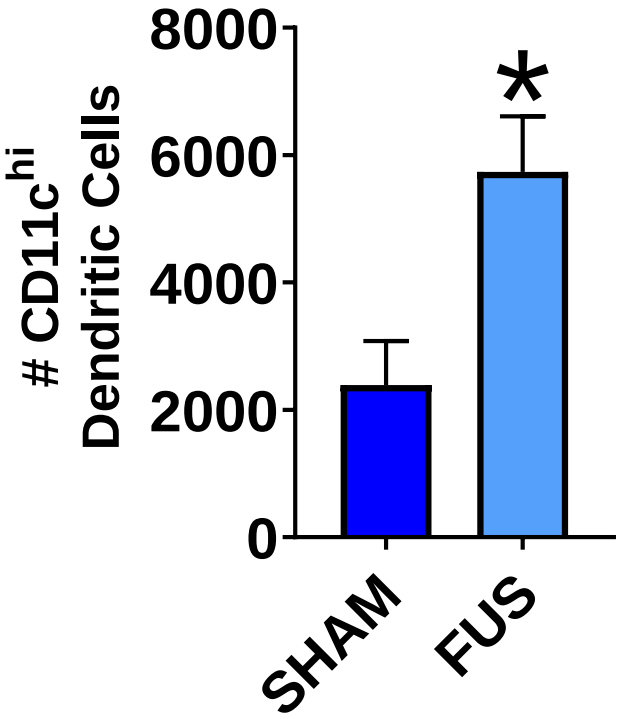
Chaves M, … Ferrara KW, Theranostics, 2018

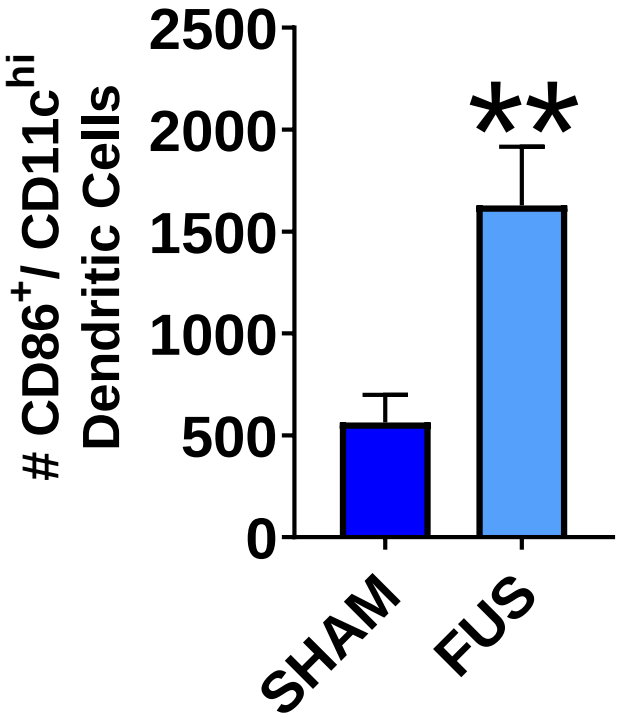
FUS_{PTA} Enhances Absolute Frequency & Maturity of Dendritic Cells in Draining Lymph Nodes (DLN)





FUS: 3 MHz, 30W, 4s sonication

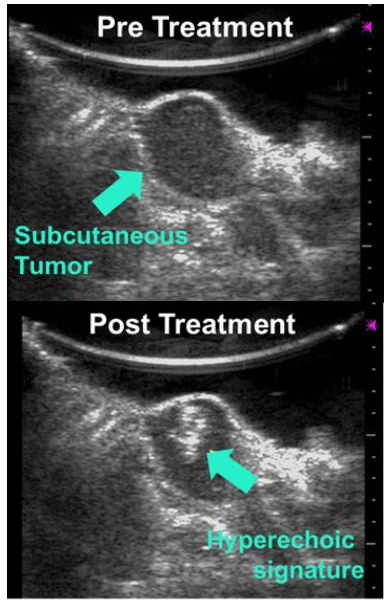




Focused Ultrasound & Gemcitabine for Murine Breast Cancer 4T1-HA

Hypothesis: Partial FUS ablation will enhance immune infiltrates in the TME, and tumor control, which will be enhanced by gemcitabine.

A



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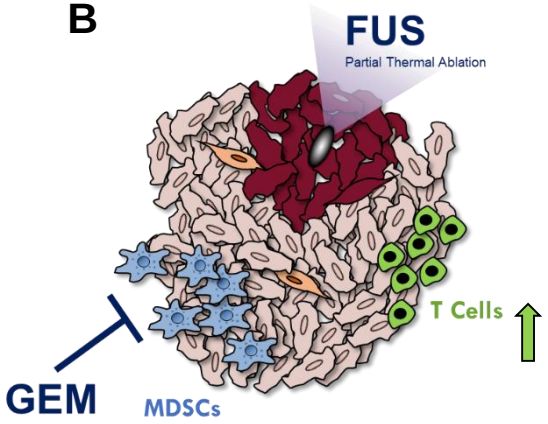

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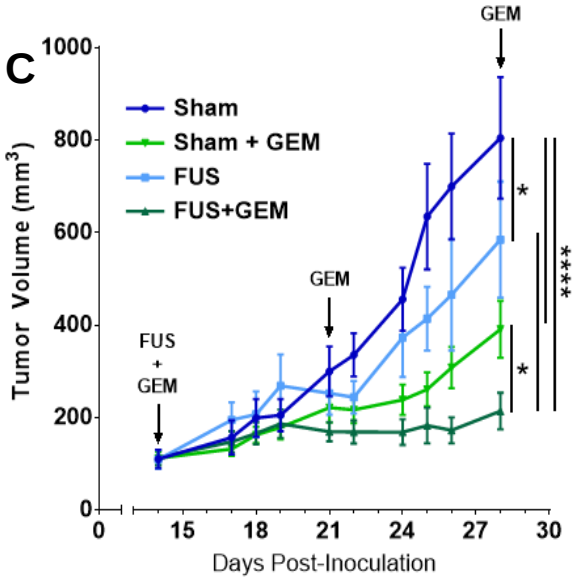

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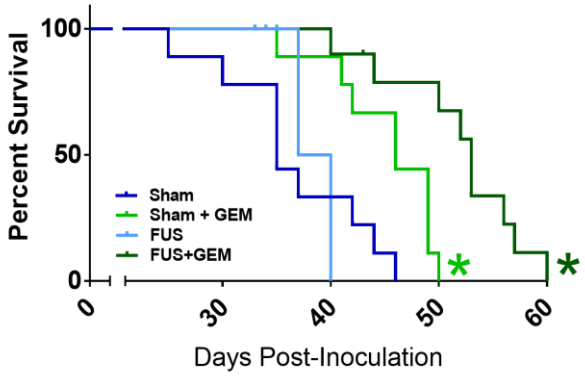

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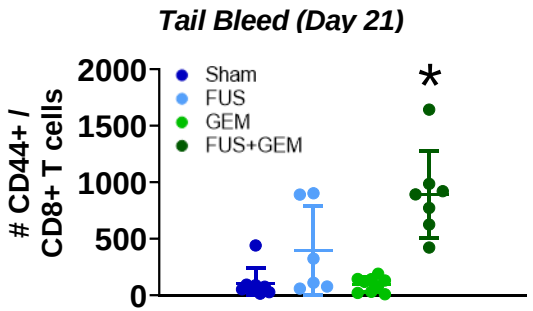

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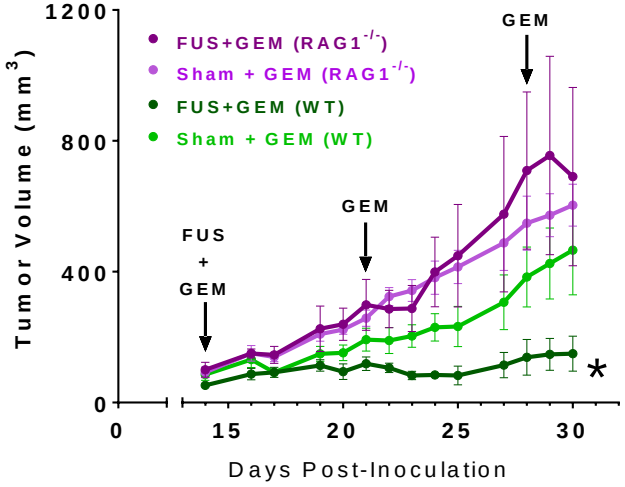
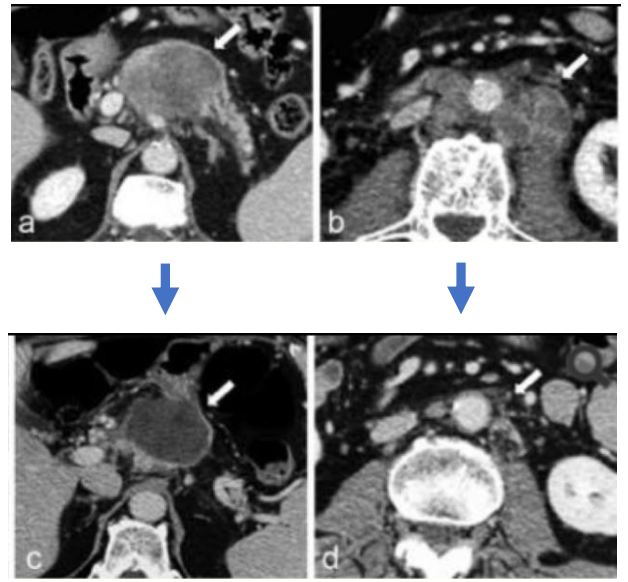


(A) Partial focused ultrasound ablation
(B) combined with systemic gemcitabine (GEM)
(C) reduces tumor growth significantly vs either rx alone
(D) and enhances survival
(E) FUS + GEM significantly increases activated (CD44+) CD8+ cells in circulation.
(F) Improved tumor control is confirmed in a repeat experiment for FUS + GM vs GEM alone. Yet in RAG1-KO mice lacking mature T and B cells (purple lines), the benefit of adding FUS to GEM is lost.

Natasha Sheybani, Tim Bullock, Rich Price

Abscopal effects have been observed in 5/80 pancreatic cancer patients using HIFU for palliation

- HIFU ablation of pancreatic primary with abscopal response of distant lymph node metastases.



- F/u CT at 1 yr large avascular area at treated tumor & complete disappearance of the pathologic nodes.
- 4 other patients with similar outcomes.

Ungaro, Orsi, et al, Ecancermedicalscience 2016
https://www.fusfoundation.org/component/content/article?id=1932:physicians-share-latest-research-with-full-house-at-awareness-event

Preclinical data support continued investigation of FUSA combined with other immune therapies for human cancer therapy

- Clinical trial of partial FUSA + Pembrolizumab in patients with advanced breast cancer
  - PI Dillon. NCT03237572.
  - Open to enrollment
  - Biopsies pre- and post-treatment

- Clinical trial of partial FUSA ± PD-1/PD-L1 blockade ± TLR7 agonism (imiquimod) for advanced solid tumors for which PD-1/PD-L1 antibody is approved.
  - PI Dengel. NCT04116320
  - IDE approved. Expect to open 4Q2019
  - Biopsies pre- and post-treatment

Summary

- Intratumoral therapy with checkpoint blockade antibodies offers promise for enhanced tumor control and reduced toxicity
  - Especially for CTLA4 blockade
  - Promising in combinations with TLR agonists and with PD1/PD-L1 blockade
- Focused ultrasound technologies can:
  - Enable drug delivery selectively to the tumor microenvironment after systemic delivery of nanoparticle-loaded microbubbles
  - Favorably modulate the tumor microenvironment to support immune therapies
- Ongoing clinical trials will illuminate the impact of these therapies, with pre/during/post tumor biopsies

Collaborators and Research Team

Research Fellows and Students

Kevin Lynch
Katie Leick
Marit Melssen
Sofia Shea
Max Meneveau
Min Kwak
Karlyn Pollack
Joel Pinczewski

Research Faculty

Lynn Dengel
Ileana Mauldin
Walter Olson

Protocol Development

Kim Bullock
Meagan Darling
Sarah Lewis
Cara

Immunologic Analyses

Kelly Smith
Cheryl Murphy
Donna Deacon
Andrea Czarkowski

Statisticians: Public Health Sciences

Gina Petroni
Mark Smolkin
Nolan Wages

All of our patients

Collaborating Laboratories at UVA

Tim Bullock: Aly Witter
Rich Price: Natasha Sheybani

Clinical Melanoma Team - UVA

Elizabeth Gaughan
Varinder Kaur
Alejandro Gru
William W. Grosh
Kathleen Haden
Emily Allred

Collaborators in Multicenter trials

Nina Bhardwaj, Mt Sinai
Mike Lowe, Emory
Keisuke Shirai, Dartmouth
Megan Kruse, Cleveland Clinic
John Nemunaitis, Toledo

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