# What's Next for Cancer Immunotherapy: Intratumoral Therapy, Cancer Vaccines, and Agonistic Antibodies to Costimulatory Molecules

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**27 April 2021**

# Disclosures

**Consulting Fees:** CureVac, Polynoma

**IP Rights:** University of Virginia Licensing and Ventures Group

**Contracted Research:** Merck, Celldex, GlaxoSmithKline, Theraclion

**Other:** American Type Culture Collection (Licensing for cell lines)

# New directions in immune therapies

1. Intratumoral therapies for modulation of the tumor microenvironment to support immune rejection.
2. Enhanced activation of T cell responses to cancer, with cancer vaccines, including in situ vaccination.
3. Agonistic antibodies to costimulatory molecules, to enhance effects of cancer vaccines and to reduce regulatory T cells.

# Intratumoral therapies

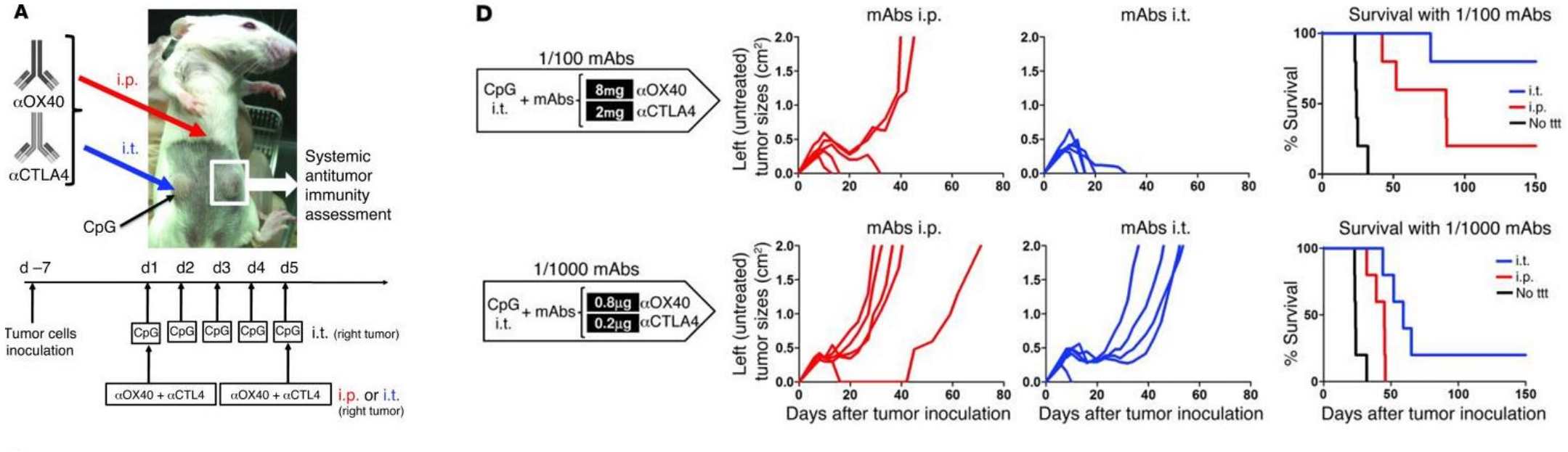
- Injection of an immune activating bacterium (BCG) has long been used for intravesical treatment of superficial bladder cancer.

- Intratumoral oncolytic viruses have activity in a range of cancers
  - T-vec induces clinical responses and is approved for advanced melanoma.
  - Overall response rates are still low.

- Intratumoral immune stimulation:
  - can enhance immune rejection of the treated tumors, and
  - may turn a cancer into a cancer vaccine (in situ vaccination),
  - which may induce regression of untreated tumors (abscopal effect).

# Preclinical experience with intratumoral CTLA-4 blockade

- Intratumoral CTLA-4 Ab + costimulatory Ab to Ox-40 + immune activator TLR9 agonist (Marabelle 2013):



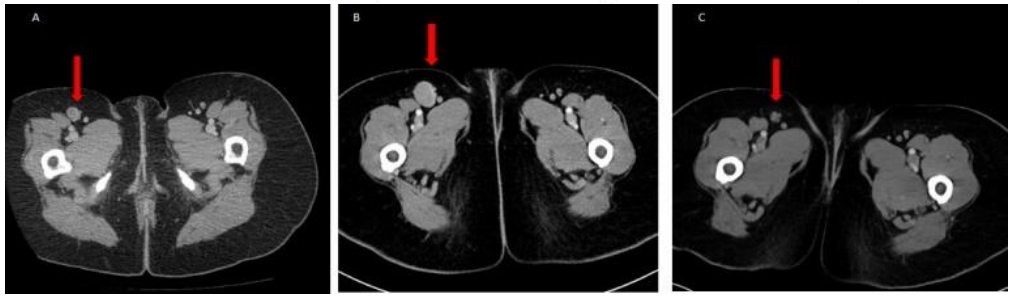

Marabelle A, JCI 2013 (Ron Levy)

# Human experience with intratumoral IL-2 and CTLA-4 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 after intratumoral IL-2 + CTLA-4 Ab:
  - No DLTs. One grade 3 injection site rxn.
  - Injected lesion:
    - 7 CR, 1 PR = 8/12 (67%) RR
  - Abscopal effects (9 with >1 lesion):
    - Regression at distant sites in 8/9 pts (89%)
  - Overall tumor regression (irRC): 3PR (25%) plus 1 with PD found to have path CR at surgery.
    - Included all 3 at highest dose of ipilimumab
  - Data suggest a vaccine effect.

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




Ray A, et al. Oncotarget 2016

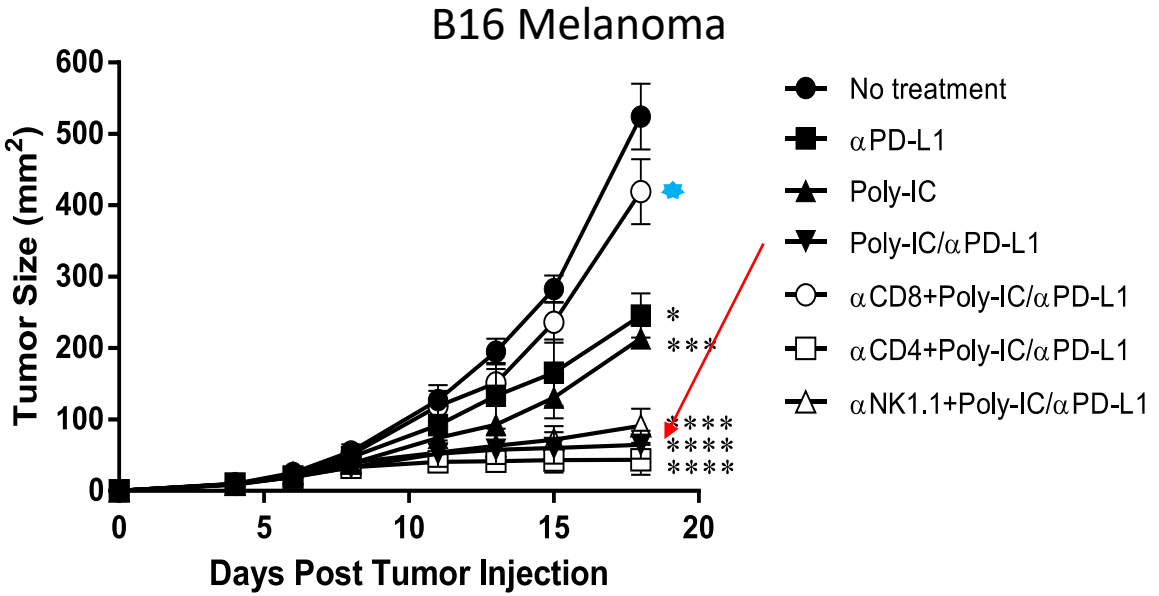
# Poly-ICLC (Hiltonol®) – Immune and therapeutic effects alone and in combination

## 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, Depends on CD8 T cells



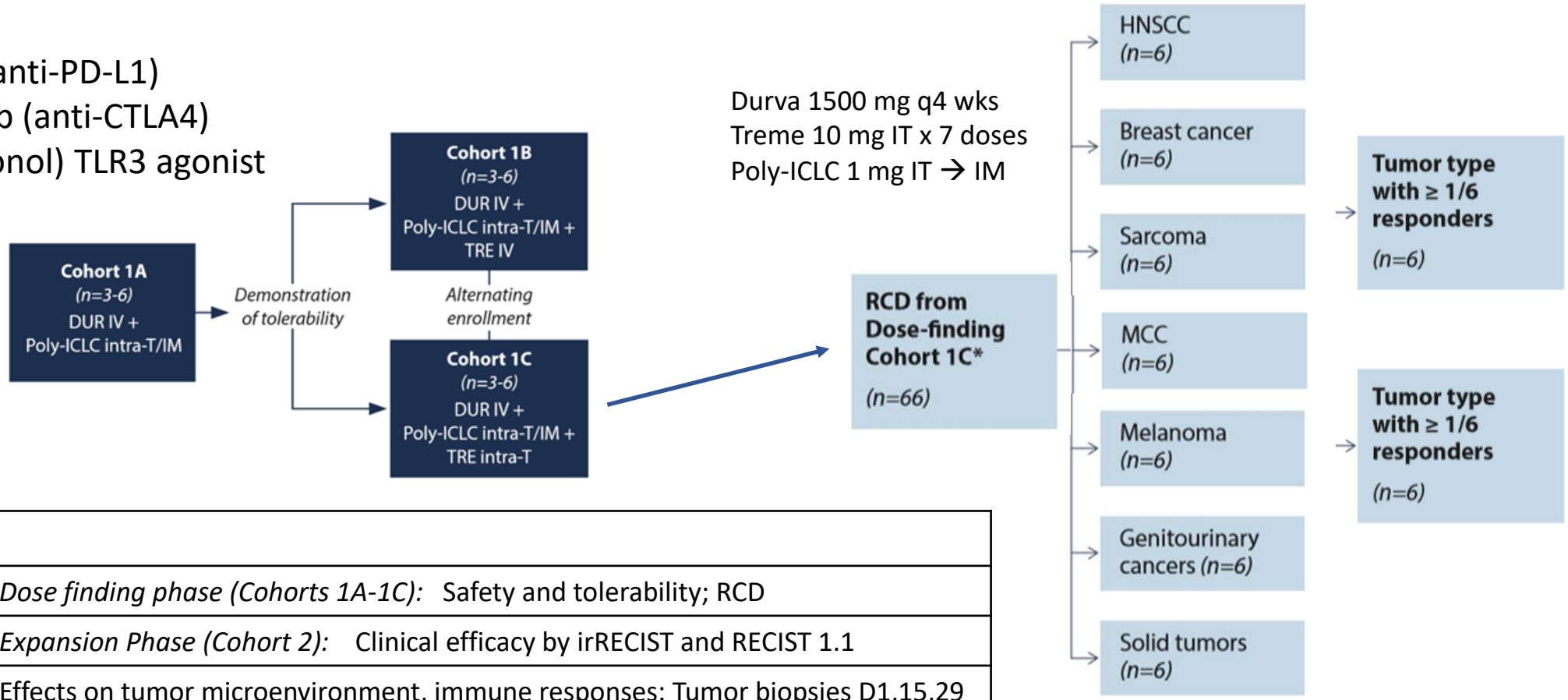

Poly-ICLC 50 mcg sc d7, 12, 17;
aPDL-1 200 mcg ip d 8, 10 13,15, 18, 20

-- 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

**Agents**:

- Durvalumab (anti-PD-L1)
- Tremelimumab (anti-CTLA4)
- polyICLC (Hiltonol) TLR3 agonist




| 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 |
| Exploratory | Effects on tumor microenvironment, immune responses: Tumor biopsies D1,15,29 |

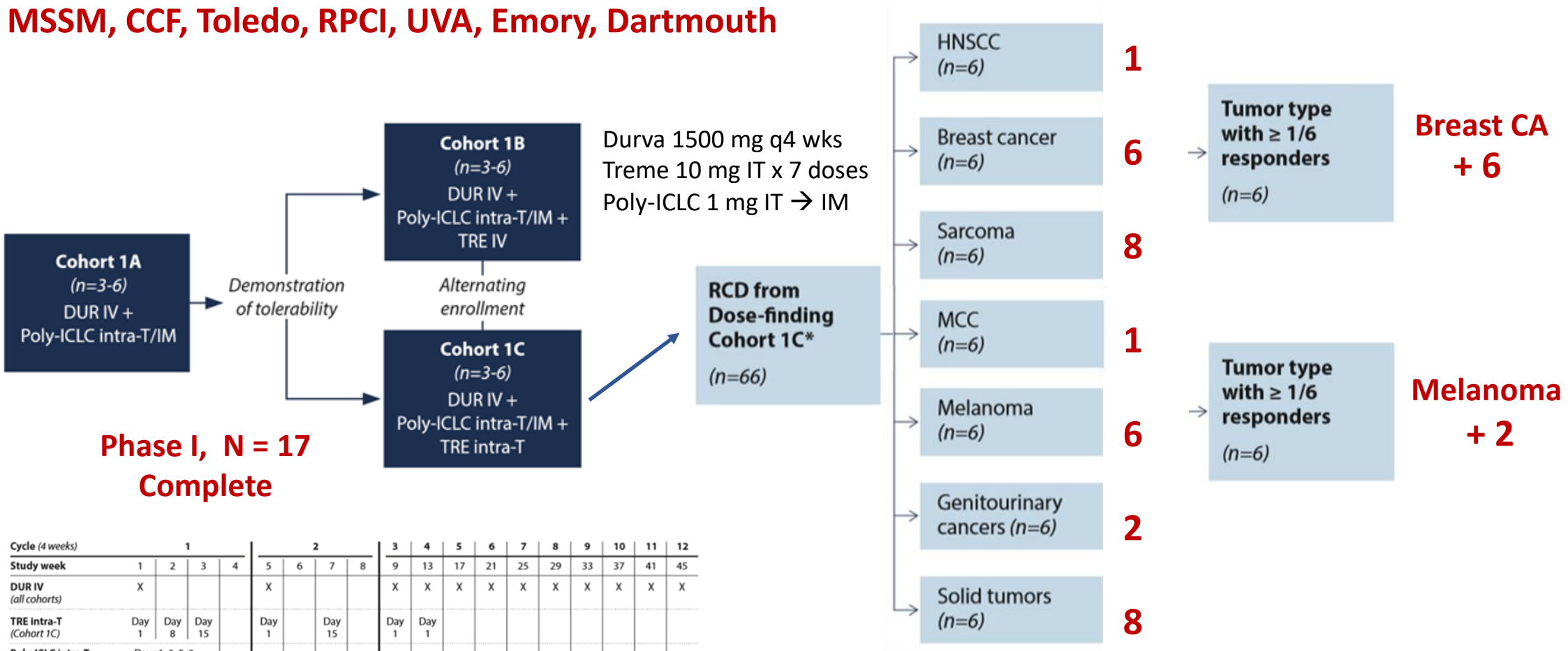
**This study is sponsored by Ludwig Institute for Cancer Research, with support from the Cancer Research Institute and MedImmune/Astrazeneca**

Study chairs: N Bhardwaj, C Slingluff

# Status of enrollment April 2021

**Participating centers:**
**MSSM, CCF, Toledo, RPCI, UVA, Emory, Dartmouth**

No unexpected higher-grade toxicities




**Phase I, N = 17**
**Complete**

| 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

**Phase II, N = 40 (32 + 8)**

**Current total: 57**
**Revised target: 60**

# CORRELATIVE/TRANSLATIONAL STUDIES

**UVA**

- Changes in TME with multispectral immunofluorescence histology (Ileana Mauldin, Sam Young):
  - Myeloid panel: CD11b, CD1a, CD68, CD83, CD40, Gal9
  - Immune suppression: IDO, Arginase, CD39, CD73
  - T helper: CD4, CD8, Tbet, GATA3, RORgt, FoxP3
  - Checkpoint blockade #1: CD8, CD56, SOX10, PDL1, IFNg
  - Checkpoint blockade #2: CD8, LAG3, TIM3, GAL9, CD155, MHCII
  - T cell activation panel: CD8, CD4, CD45RO, Ki67, Granzyme B, ICOS
  - TLS panel: CD20, CD8, PNAd, CD83, Ki67, FoxP3
- Induction of T cell responses to shared and mutated tumor antigens
- Flow cytometry of circulating T cells for activation

**MOUNT SINAI**

- Nanostring for changes immune gene expression in tumors
- Nanostring for changes in PBMC
- TCR/BCR profiling in tumors

## 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

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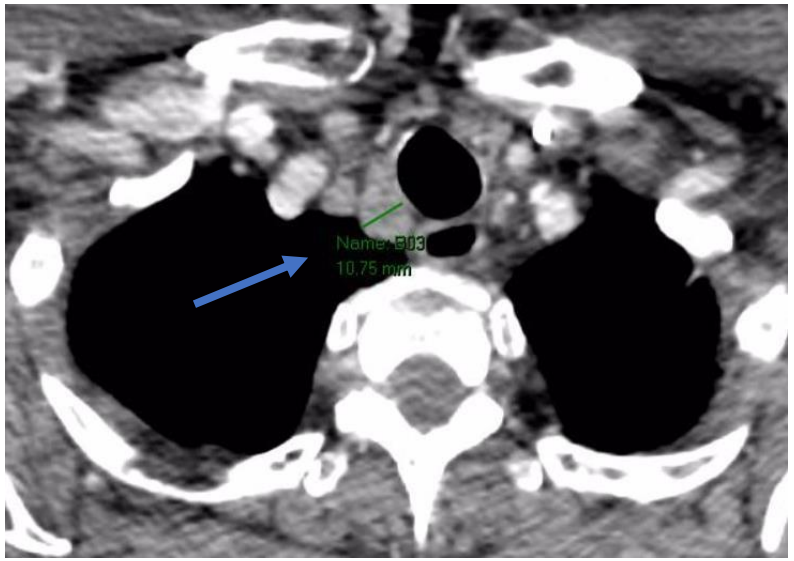
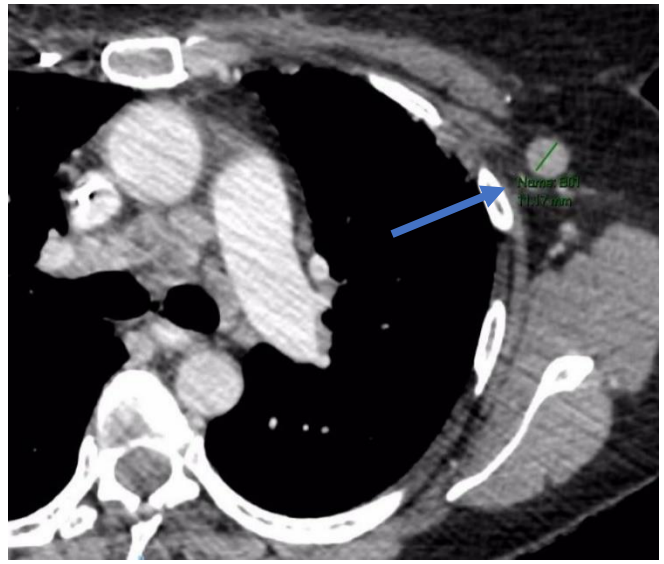
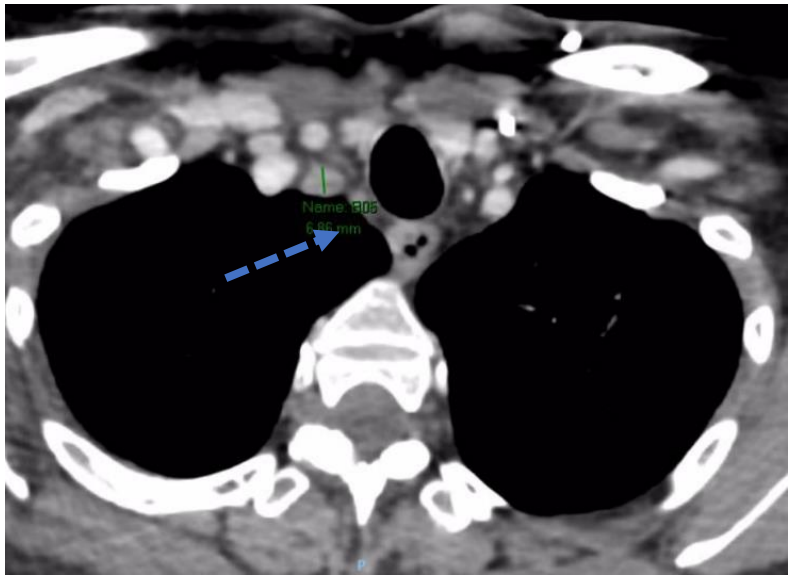
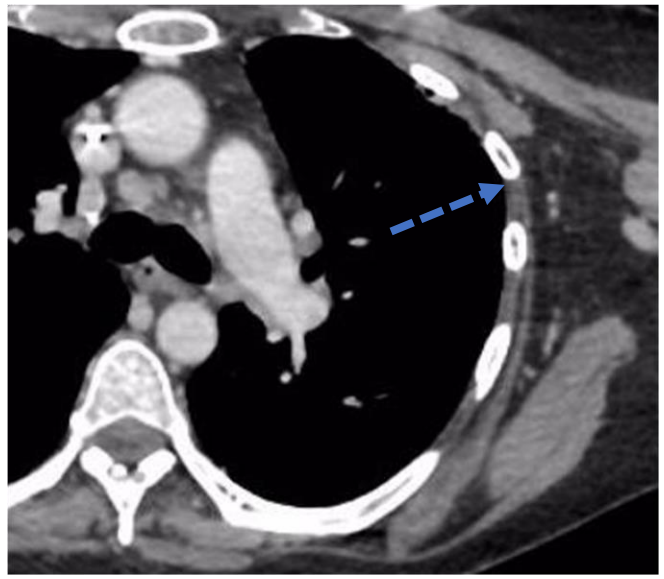
Breast cancer patients enrolled in expansion cohort, with other clinical activity.
Manuscript in preparation.



Pre

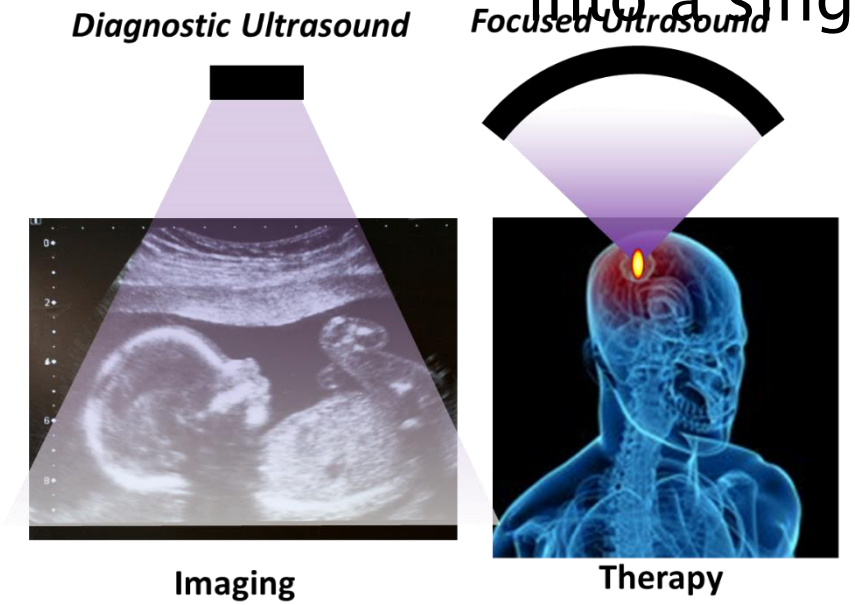


3 mos

# 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



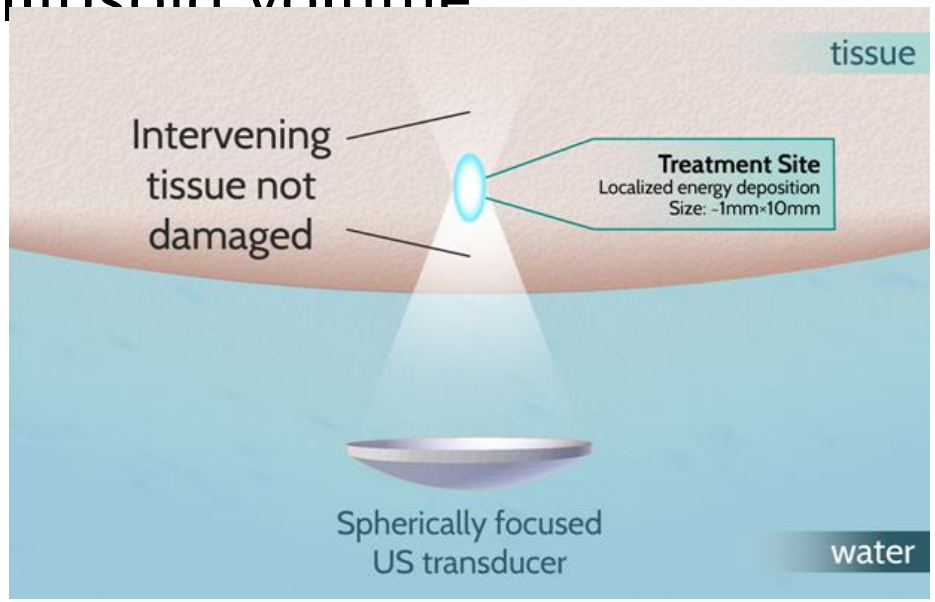



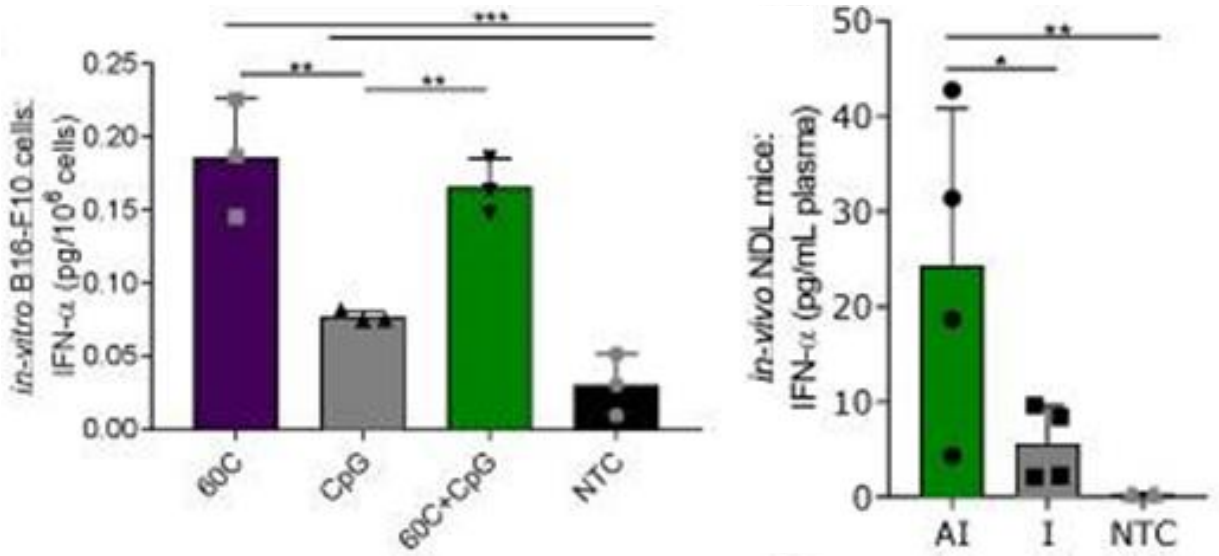

Image courtesy of FUS Instruments

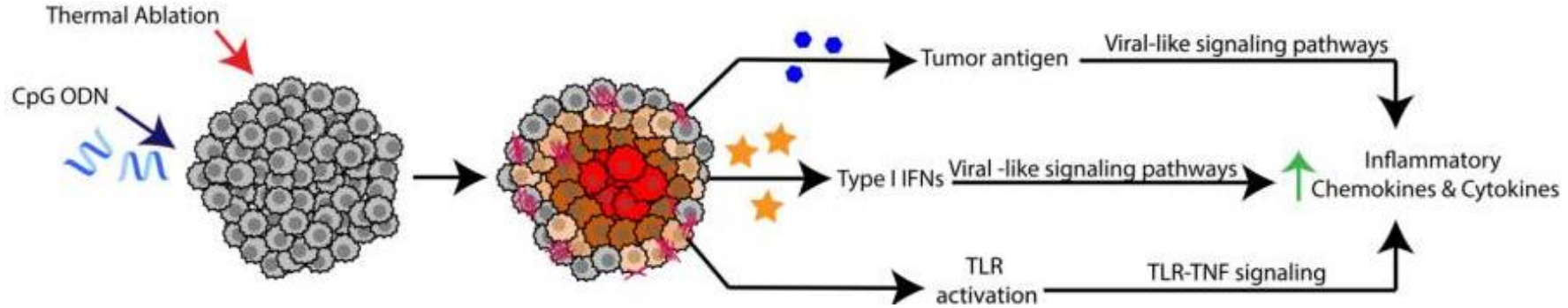
FUS is non-invasive (extracorporeal), non-ionizing, safe, repeatable, and localized.

# Impact of and focused ultrasound (FUS) ablation on tumor tissues

- FUSA heats tumor rapidly to 45-90°C to induce controlled apoptotic cell death.

- FUS thermal ablation → enhanced expression of Type I IFNs in tumor.
- FUS thermal ablation + CpG (AI-T) → upregulation of Type I IFNs and tumor antigen release compared to CpG alone (I-T)

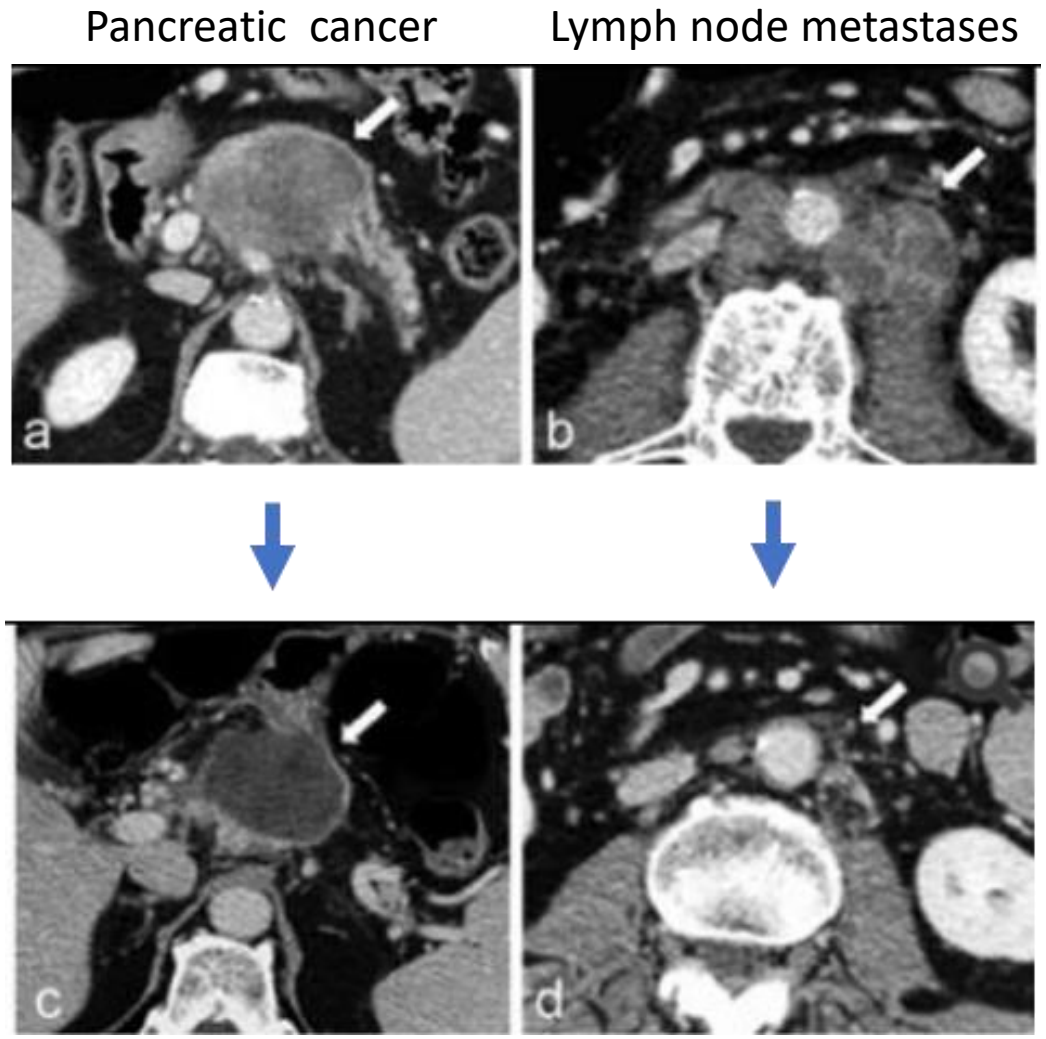



Chaves M, … Ferrara KW, Theranostics, 2018

# Abscopal effect in pancreatic cancer patient using HIFU for palliation

- HIFU ablation of pancreatic cancer primary (a) for pain, in a patient with metastases in distant lymph nodes (b: white arrows).
- F/u CT at 1 yr: large avascular area at treated primary tumor (c) & complete disappearance of the pathologic nodes (d: white arrow).
- 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

# UVA-AM-003 (NCT04116320)


Dengel

# PI: Lynn Dengel, MD

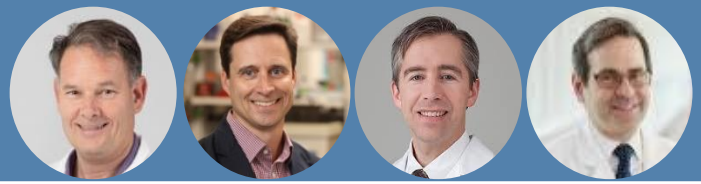
## PILOT EVALUATION OF FOCUSED ULTRASOUND ABLATION (FUSA) WITH OR WITHOUT PD-1 ANTIBODY BLOCKADE IN ADVANCED SOLID TUMORS

Eligible patients:

- *Cohort 1*: Advanced cancers treated with PD-1/PD-L1 blockade with SD after 12 weeks
- *Cohort 2:* Advanced cancers ineligible for PD-1/PD-L1 blockade.

Objectives:

- To assess the safety and toxicity of FUSA administered alone or combined with PD-1 antibody blockade.
- To evaluate the impact of FUSA treatment on immunologic features of the tumor microenvironment, including increases in $CD8^+$ T cell infiltration.
  - Biopsies pre-FUS, d1, d35

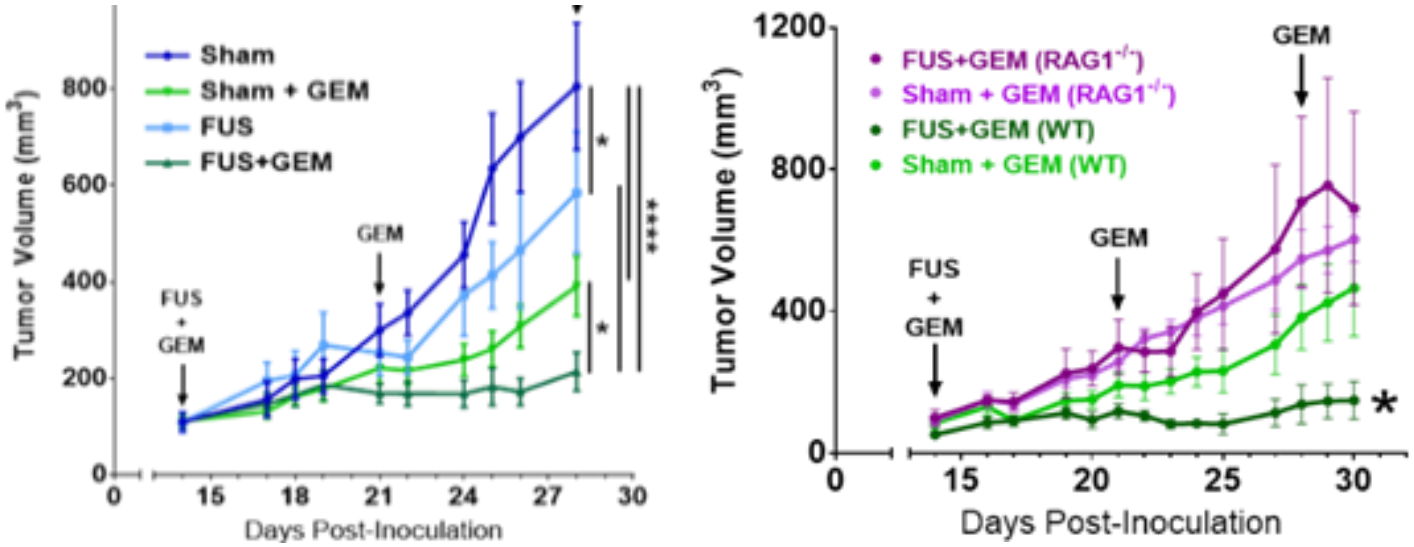


T. Bullock Price Dillon Brenin

UNIVERSITY of VIRGINIA HEALTH SYSTEM | UVA Cancer Center
An NCI-Designated Cancer Center

# Focused Ultrasound (FUS) + myeloid cell depletion controls breast cancer growth

Preclinical data lead to a new clinical trial integrating gemcitabine and FUS




**4T1 breast cancer (TNBC)**
**GEM = gemcitabine; RAG1$^{-/-}$ = T cell deficient**

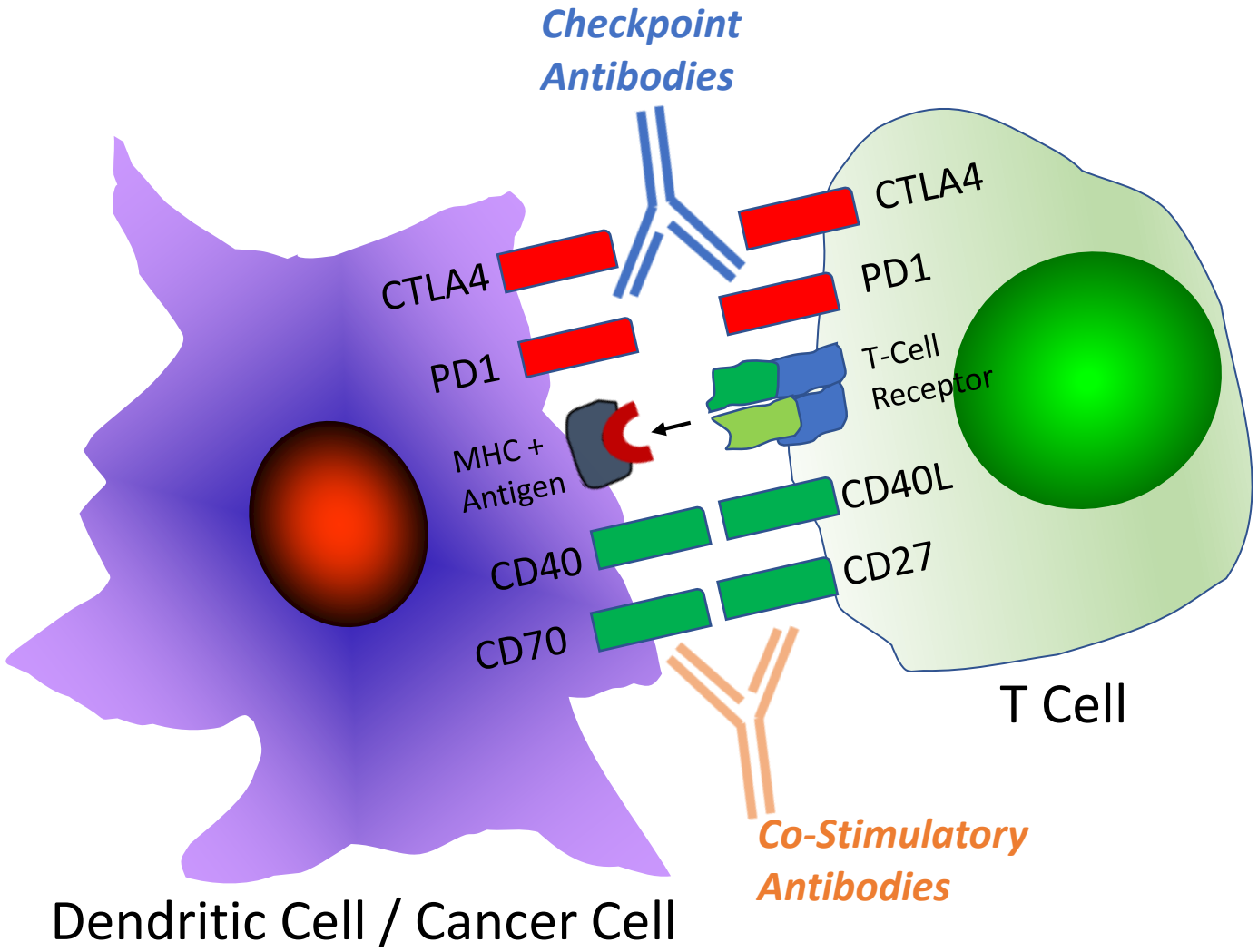
<u>Breast54 trial</u>
(NCT04796220)

*FUS + gemcitabine for advanced breast cancer.*

Opening
Spring 2021

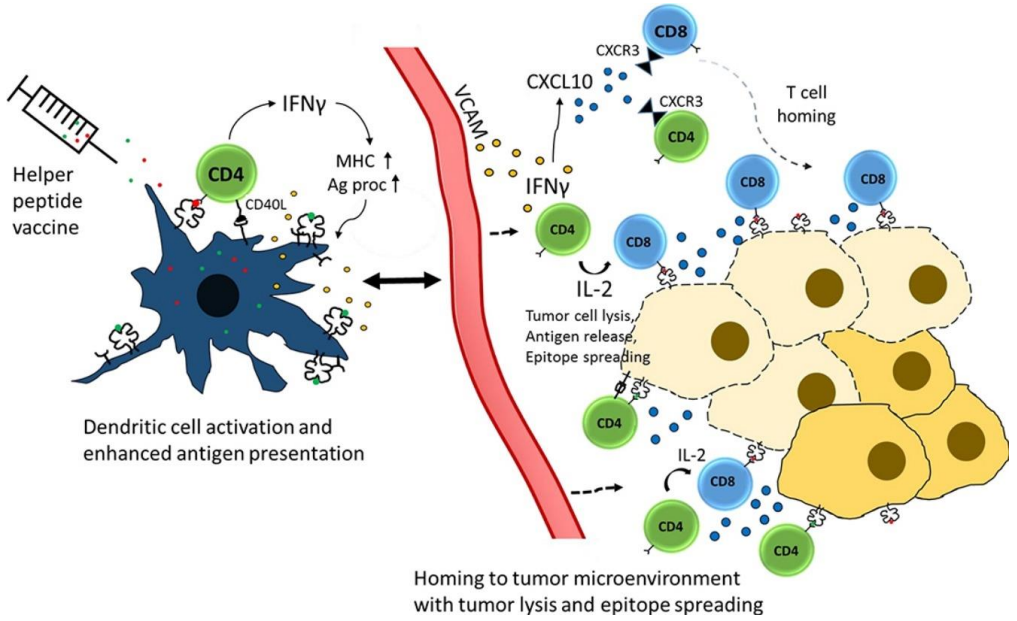
***Sheybani ND, et al. J Immunother Cancer. 2020***

# Cancer Vaccines, Checkpoints, and Costimulation:
## New ways to enhance immune responses to cancer



# The promise of “helper” peptide vaccines

- Activation of CD4 T cells may promote antitumor immunity by increasing antigen presentation, T cell homing and activation, effector function, enhancing T cell memory, and supporting CD8 responses, [1].



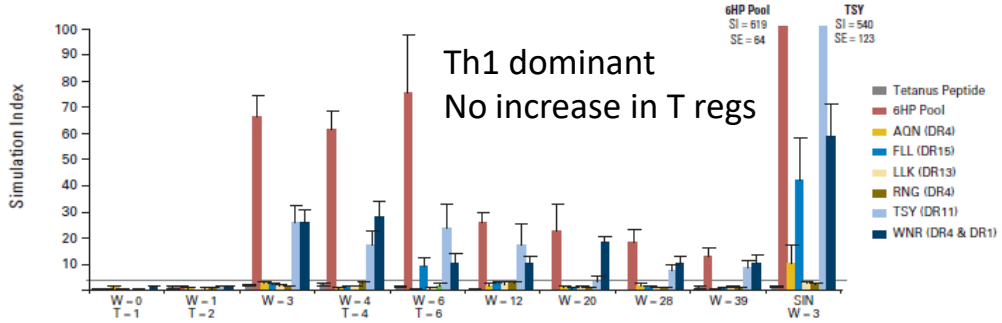

- A helper peptide vaccine for Her2 in breast cancer has induced complete regressions of DCIS in 19–29%; decreased Her2 expression [2,3].

[1] Melssen M. Curr Opin Immunol (2017); [2] Lowenfeld L. Clin Cancer Res (2016); [3] Staff C. Int J Oncol, (2014)

# Targeting CD4 T cells with peptides restricted by HLA-DR alleles

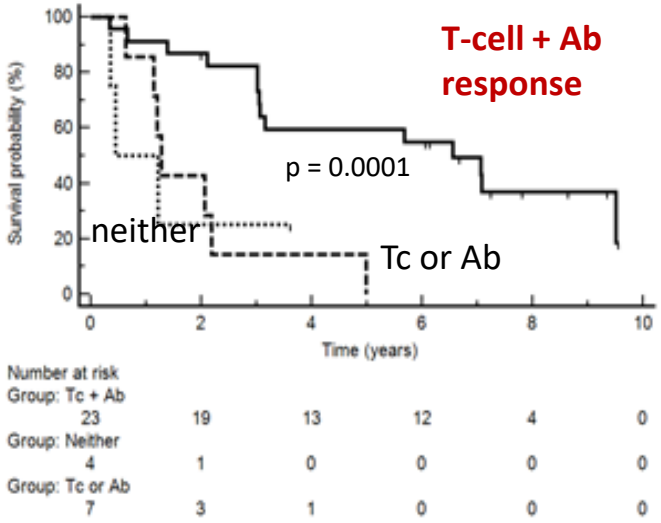
## 6 Class II-MHC Restricted Melanoma Peptides (6MHP)

| Protein (residues) | Allele | Peptide Sequence |
|---|---|---|
| Tyrosinase $_{56-70}$ | DR4 | (A)QNILLSNAPLGPQFP (Topalian) |
| Tyrosinase $_{388-406}$ | DR15 | FLLHHAFVDSIFEQWLQRHRP (Kobayashi) |
| MelanA $_{51-73}$ | DR4 | RNGYRALMDKSLHVGTQCALTRR (Zarour) |
| MAGE-3 $_{281-295}$ | DR11 | TSYVKVLHHMVKISG (Manici) |
| MAGE-1-3, 6 $_{121-134}$ | DR13 | LLKYRAREPVTKAE (Chaux) |
| gp100 $_{44-59}$ | DR1, DR4 | WNRQLYPEWTEAQRLD (Halder/Li) |

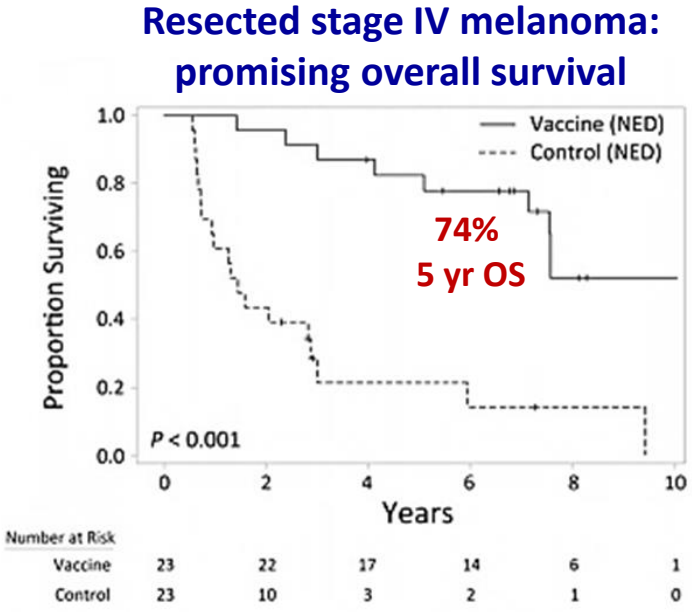


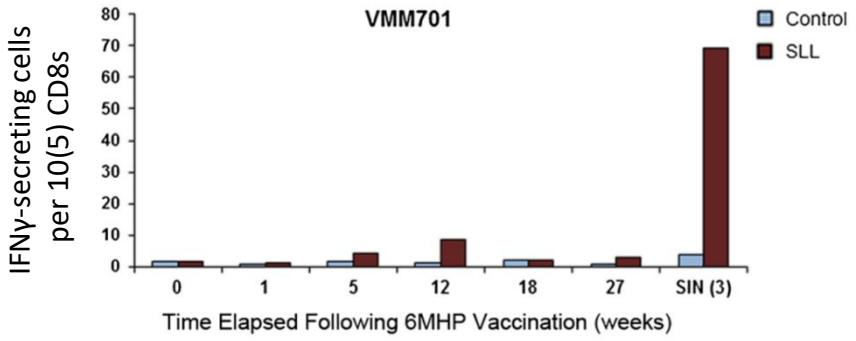

Immune response rate: 57% PBMC, 81% Node

Clinical activity of vaccine alone for advanced melanoma:
2PR, 2SD of 17 patients, durable 1-7 years




Strong correlation between immune response and survival



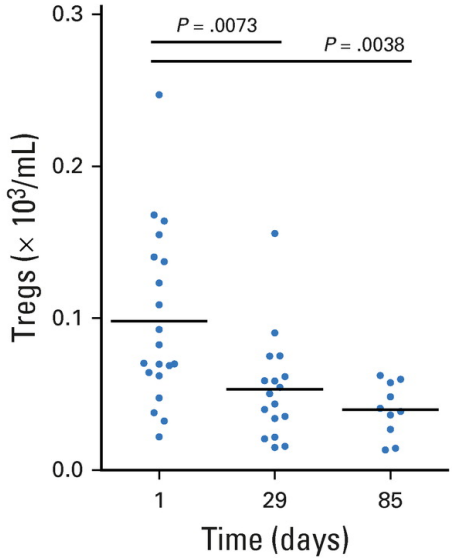



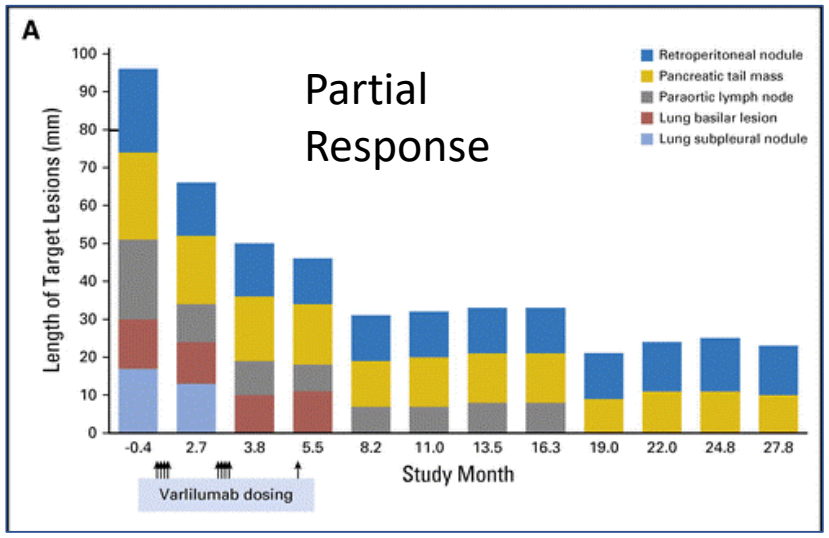

Epitope spreading to CD8 T cells, recognizing gp100, NYESO1, and MAGE-A3 peptides , in 5/11 patients tested

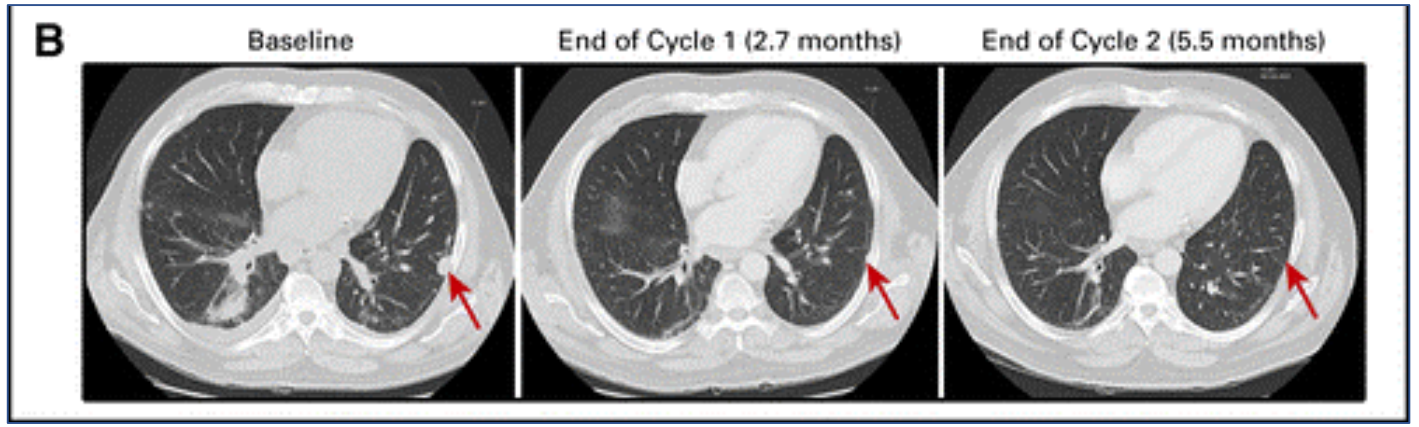
Reed, CCR 2015; Hu, Ann Surg 2015; Dillon, JITC 2014; Slingluff, CCR 2013; Slingluff, JCO 2008

# Rationale: CDX-1127 (varlilumab): Anti-CD27

- CD27 enhances activation and survival of T cells.
- CD27 stimulation lowers T cell expression of inhibitory molecules.
- CDX-1127
  - fully human agonistic CD27 antibody with proven safety.
  - depletes circulating regulatory T-cells.
  - has clinical activity.



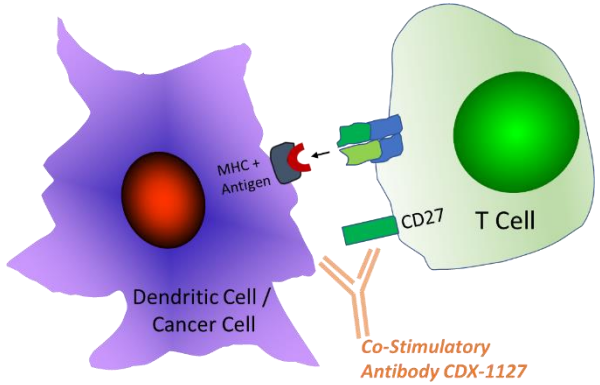




Durable clinical response, RCC: 78% regression > 2.3 years
Eight others w SD > 3 mos.

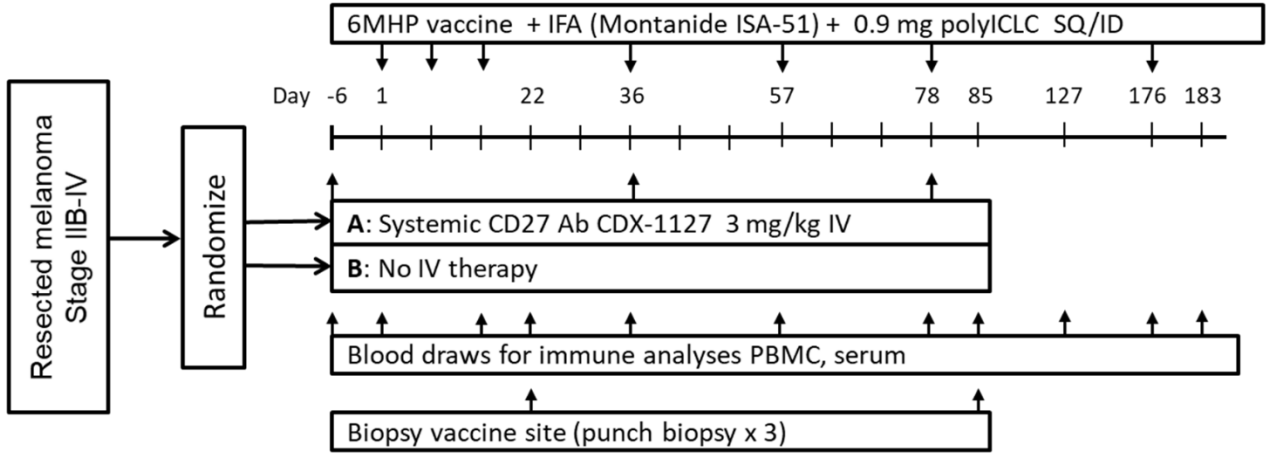
Burris (Bullock) JCI 2017

# MEL65 (NCT03617328) EVALUATION OF SAFETY AND DURABLE IMMUNOGENICITY OF MELANOMA VACCINATION, WITH OR WITHOUT SYSTEMIC CDX-1127, IN PATIENTS WITH STAGE II-IV MELANOMA (University of Virginia & Virginia Commonwealth University)




Open-label, multi-center randomized phase I/II study

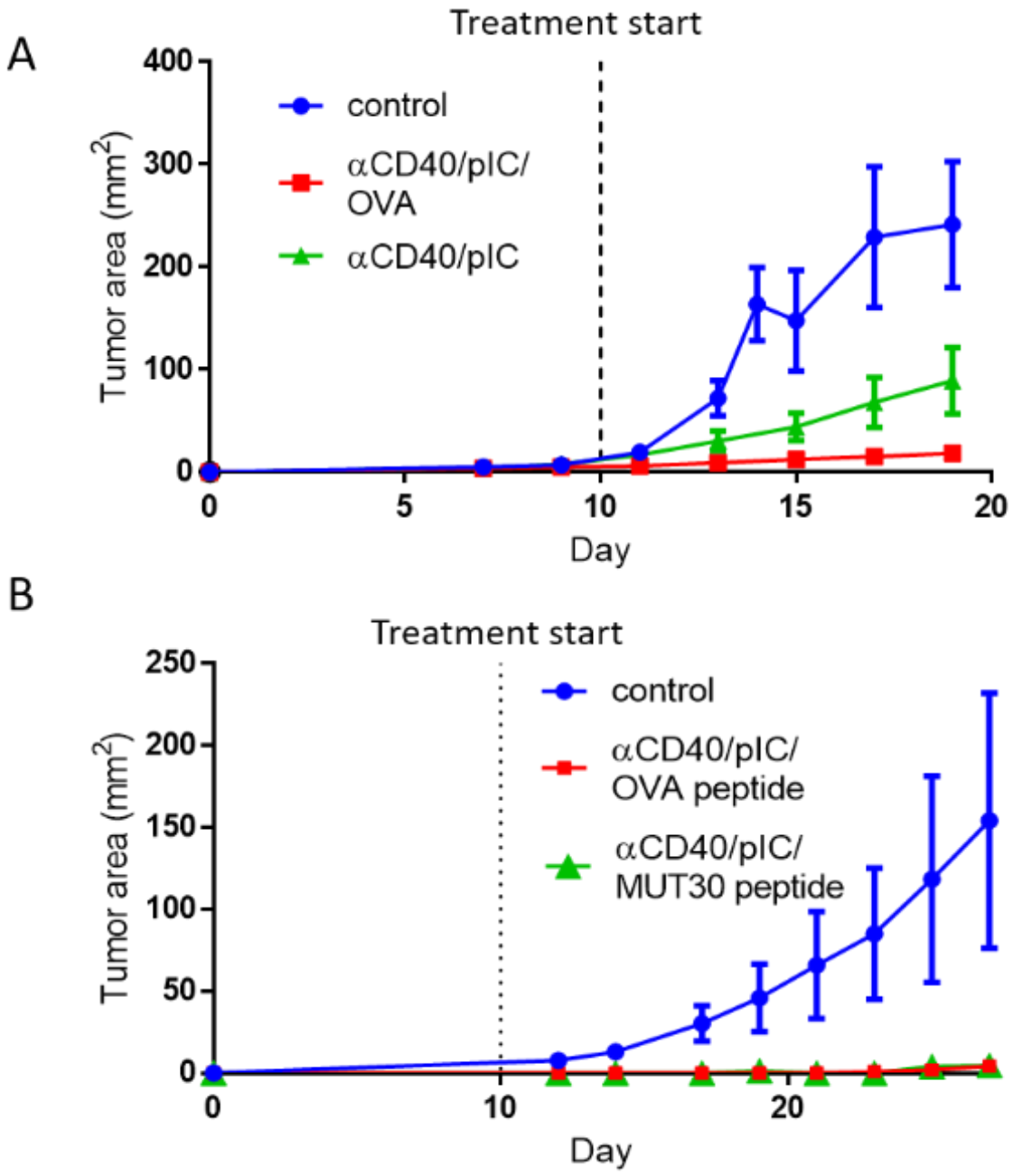
- to assess combination of CDX-1127 plus helper peptide vaccine: safety, persistence of T cell response, reduction of T-regs
- Target accrual = 30 eligible patients.




Note: A prior lot of Hiltonol (Lot PJ215-1-10-01) was used in other studies (Mel60) and the amount of dry weight polyICLC was 2.0 mg/ml for that formulation. Current lots are packaged at 1.8 mg/mL dry weight polyICLC. Thus, 0.5 mL of polyICLC for Mel65 will be equivalent to 0.9 mg of polyICLC.

**Preclinical data for tumor control with CD40 Ab + polyIC + antigen.**
αCD40-based therapeutic vaccine regimens using xenoantigen (OVA) or mutant (MUT30) neoantigen control melanoma outgrowth. In both experiments, mice were implanted with 4 x $10^5$ B16cOVA cells. 10d after implantation when tumors were palpable, mice were injected intraperitoneally with a mix of 100μg αCD40, 75μg polyIC with or without 200 μg of the indicated protein (A) or peptide (B). Tumor growth was measured by caliper.

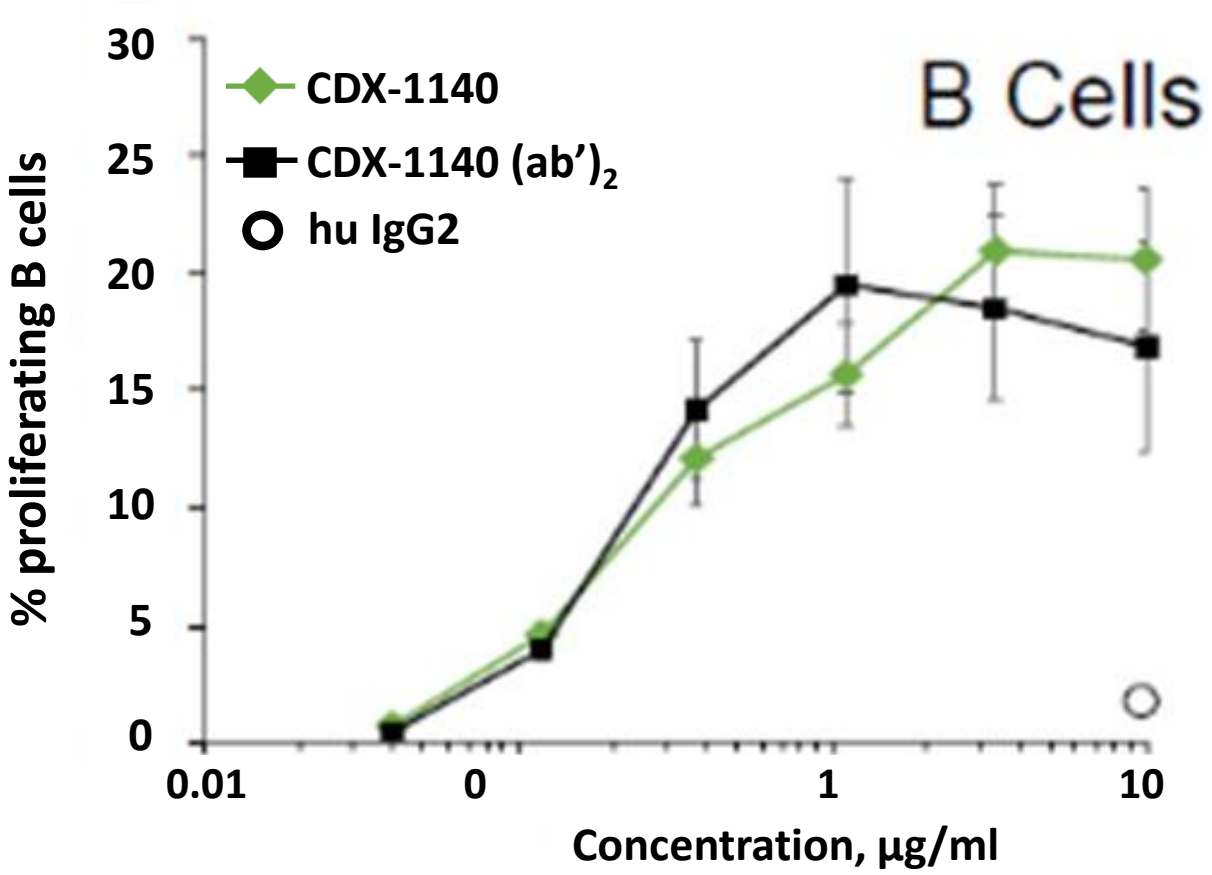
--Tim Bullock, UVA

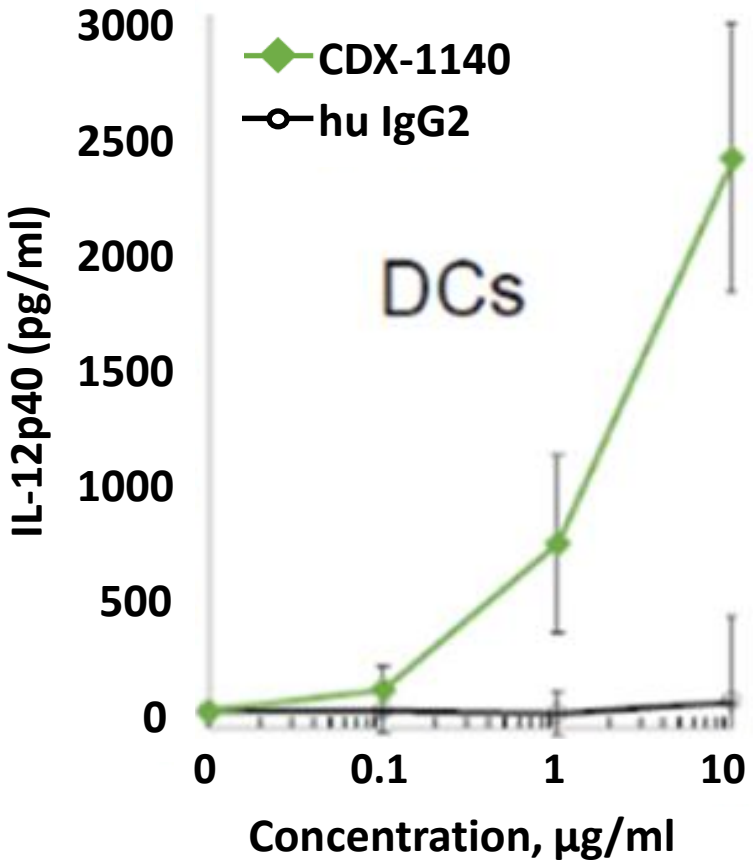


# Agonistic CD40 antibody: CDX-1140

Vitale, AACR, 2016.

CDX-1140 has Dose-dependent and Fc-independent Agonist Activity.




B Cells: CFSE labeled B cells were incubated 6 days with whole Ab or F(ab')2. The % of proliferating cells was measured by flow cytometry.

DCs: Monocyte-derived DCs were incubated with Ab 48hrs. Supernatant was analyzed for IL-12p40 by ELISA.[74]

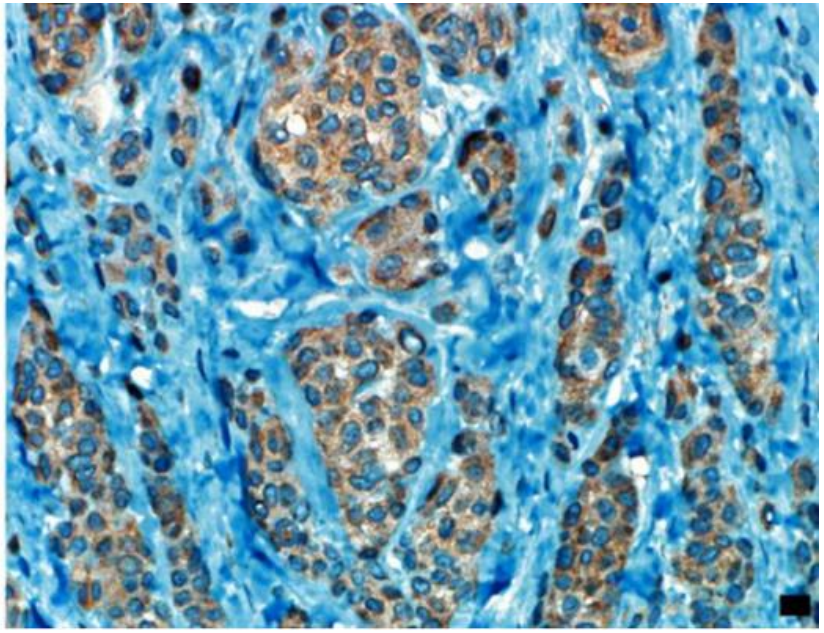
# Peptide antigens for Mel66 trial include: shared antigens and a mutated neoantigen

| Table 1: Peptides used in the Melanoma Helper Peptide Vaccine | | | |
|---|---|---|---|
| | **Sequence** | **Epitope** | **Ref** |
| 6MHP | AQNILLSNAPLGPQFP | Tyrosinase $_{56-70}$ * | 1 |
| | FLLHHAFVDSIFEQWLQRHRP | Tyrosinase $_{386-406}$ | 2 |
| | RNGYRALMDKSLHVGTQCALTRR | Melan-A/MART-1$_{51-73}$ | 3 |
| | TSYVKVLHHMVKISG | MAGE-3 $_{281-295}$ | 4 |
| | LLKYRAREPVTKAE | MAGE-1,2,3,6 $_{121-134}$ | 5 |
| | WNRQLYPEWTEAQRLD | gp100 $_{44-59}$ | 6,7 |
| NeoAg-mBRAF | HEDLTVKIGDFGLATEKSRWSGSHQFEQLS | BRAF $_{585-614\ (V600E)}$ ** | 8 |
| * alanine added at the N terminus; ** histidine added at the N terminus. | | | |

**BRAF V600E mutation in most melanocytic nevi**

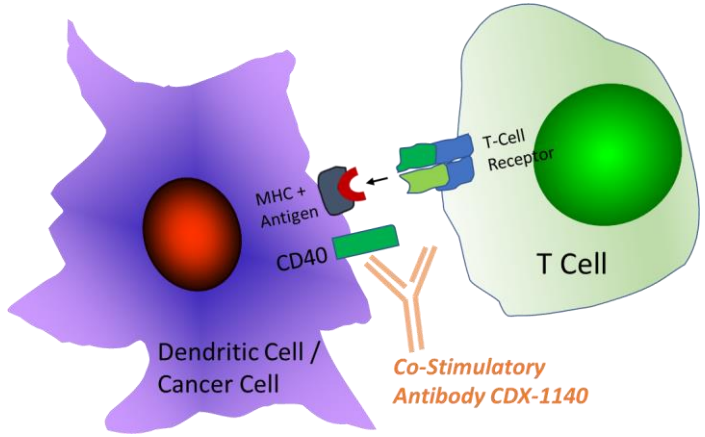
373/478 (78%) of acquired melanocytic nevi express BRAF V600E [11 studies; Roh, Pigment Cell and Melanoma Research, 2015].

High-power view with IHC with VE1 antibody, showing BRAF V600E mutation in a nevus.



Yeh I, von Deimling A, Bastian BC. J Natl Cancer Inst. 2013.

# ENHANCED MELANOMA VACCINE AGAINST NEOANTIGEN AND SHARED ANTIGENS BY CD40 ACTIVATION AND TLR AGONISTS IN PATIENTS WITH STAGE II-IV MELANOMA: UVA MEL 66 (NCT04364230)

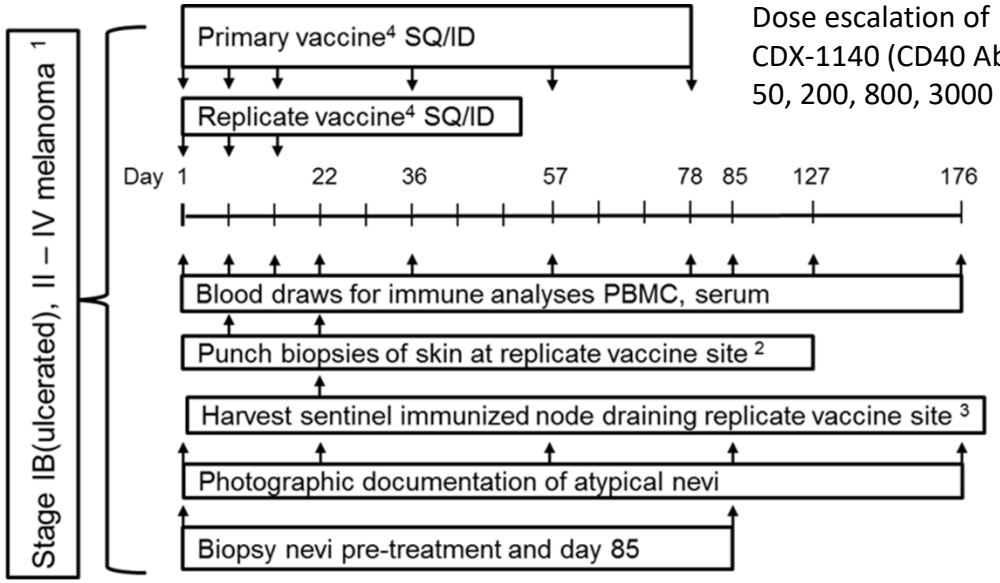



University of Virginia and Cleveland Clinic

Funded by Department of Defense

Open-label, multi-center phase I/II study:

- To test the safety and immunogenicity of vaccination with helper peptides + CD40 Ab CDX-1140 + TLR3 agonist polyICLC.
- To test for DC activation and maturation.
- To evaluate whether vaccination against a mutant BRAF peptide induces T cells that recognize BRAF-mutant melanomas and infiltrate nevi.




Dose escalation of CDX-1140 (CD40 Ab): 50, 200, 800, 3000 mcg

[1] Enroll patients clinically free of disease. An individual with small radiologic or clinical findings of an indeterminate nature may still be eligible. See Inclusion/Exclusion criteria for details.
[2] Vaccine site biopsies will be done only at the replicate vaccine site.
[3] Biopsy of the sentinel immunized node will only be done for the replicate vaccine site.
[4]Participants will be allocated to Cohort A or Cohort B. Each participant will receive vaccines at two sites. Six vaccines will be given at the primary vaccine site. Three vaccines will be given at the replicate vaccine site.

# 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:
  - Favorably modulate the tumor microenvironment to support immune therapies
  - May benefit from combination with checkpoint blockade or chemotherapy
- Cancer vaccines can have clinical activity targeting shared tumor antigens
  - Cancer testis antigens; Differentiation antigens; Mutated neoantigens
- Co-stimulatory antibodies (to CD27 and CD40) may:
  - Enhance activation of dendritic cells
  - Reduce regulatory T cells
  - Enhance T cell activation and survival
- Ongoing clinical trials will illuminate the impact of these therapies in tumor, blood, and vaccine sites in humans.

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