# What Information Provided by Models Inform Immune Drug Development and Use?

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*Executive Director, Exploratory Immuno-Oncology*

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# Presenter Disclosure Information

Philip Gotwals
*Executive Director, Exploratory Immuno-Oncology*
*Novartis Institutes for Biomedical Research*

I am an employee and shareholder of Novartis.

This presentation is not certified for CME/CE credit.

# What Information Provided by Models Inform Immune Drug Development and Use?

- Data from pre-clinical models, in conjunction with translational information, are used to specifically address the following:
  - Are we treating the correct patient...
    - Do we understand the underlying biology of disease and target?
  - With an optimal therapeutic...
    - Have we engineered appropriate potency and selectivity?
  - At the appropriate dose?
    - Do we understand dose response relationships?

# Therapeutic Approaches to cancer immunotherapy

*Selected Examples from the Novartis Immuno-Oncology Portfolio*

**Immune Priming**

- **IL-15**
- **STING**

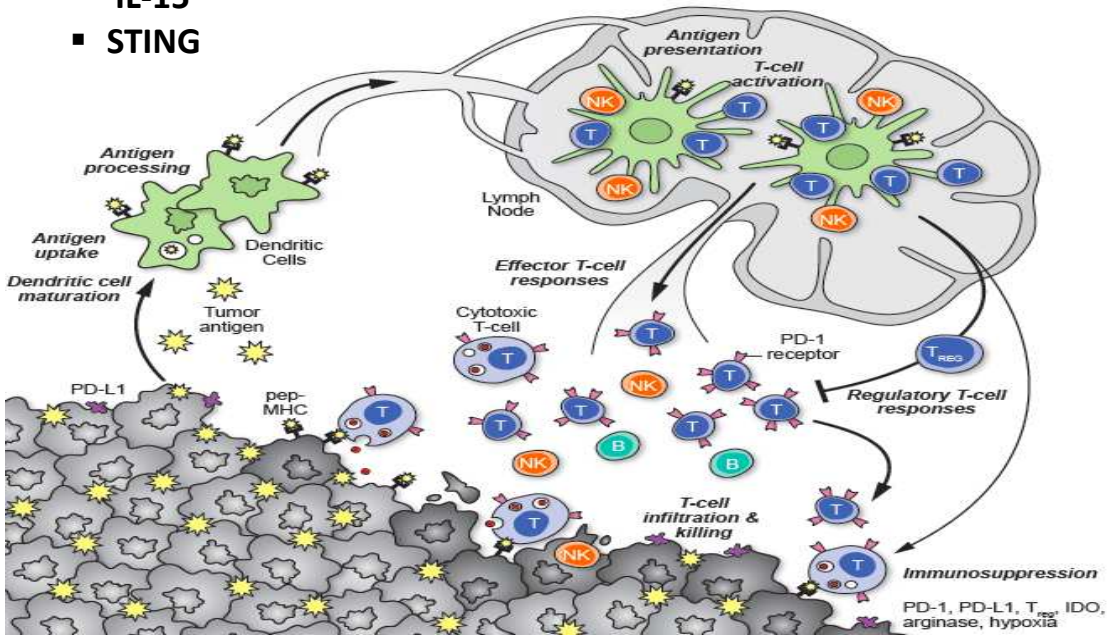



Image adapted from Mellman I, et al. *Nature*. 2011;480:480-489.

**T-cell Modulation**

- **PD-1**
- **PD-L1**
- **LAG-3**
- **TIM-3**
- **GITR**

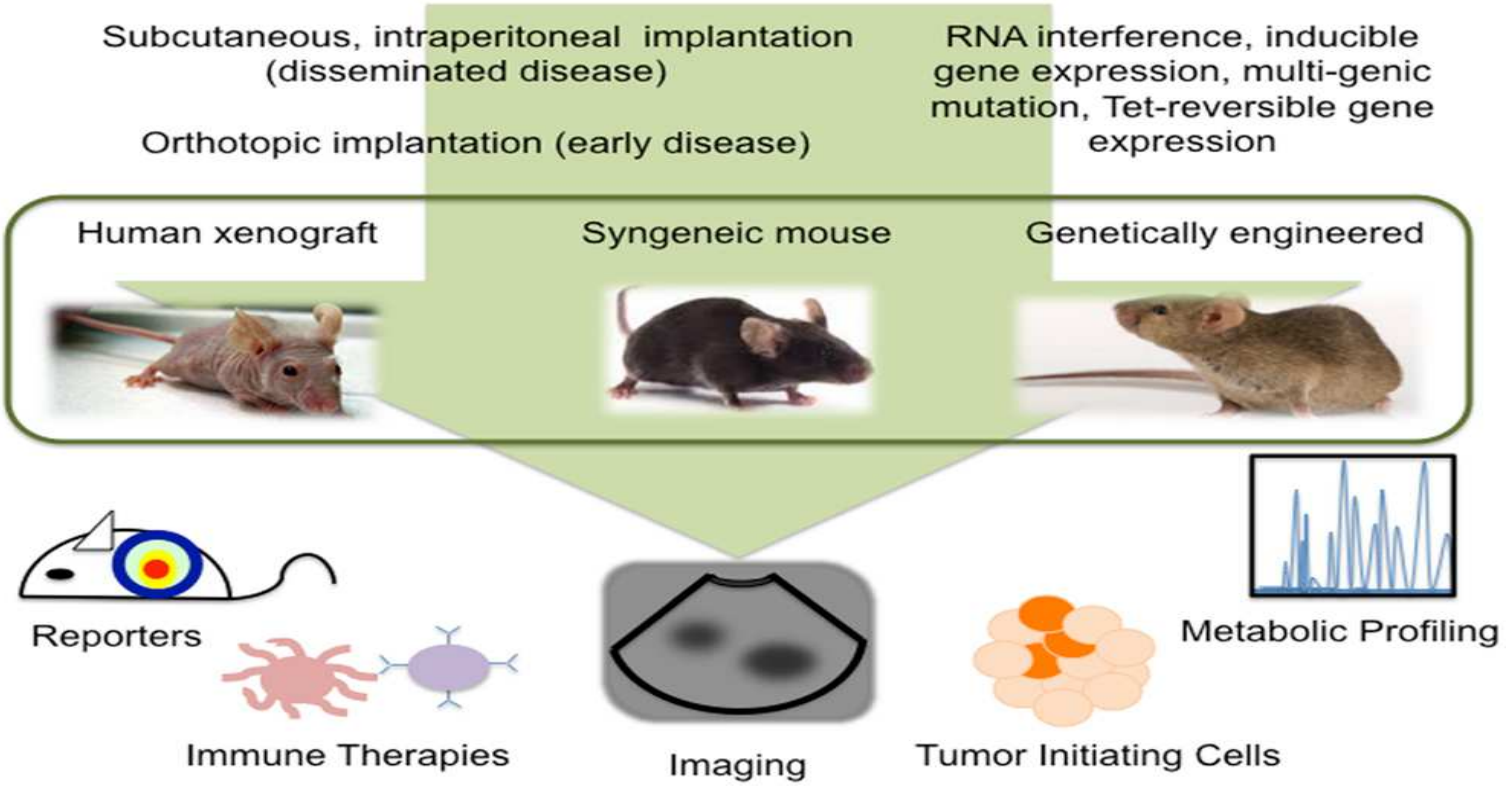
- **Engineered CAR-T**

**Tumor Environment**

- **TGF-β**
- **CSF-1**
- **$A_{2A}$ Adenosine Receptor**

# Approaches to preclinical modeling for oncology

House et al. Front Onc 2014



# Acknowledgments

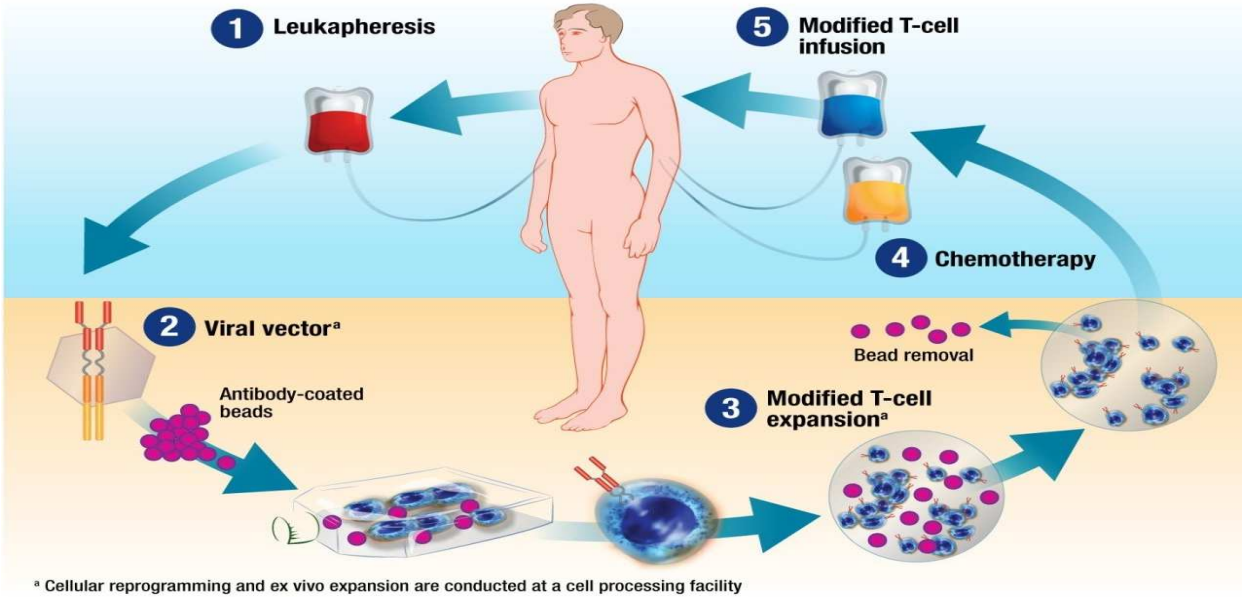

- CAR-T
  - Research Collaboration with the University of Pennsylvania, led by Carl June

- STING
  - Collaborative effort with Aduro, Thomas Dubensky Jr., CSO

- NIBR Exploratory Immuno-Oncology
  - Glenn Dranoff
  - Jennifer Brogdon
  - Catherine Sabatos-Peyton and Pushpa Jayaraman

# Overview of CAR-T Therapy in the Clinic

*CTL019 treatment for CD19+ B cell leukemia*



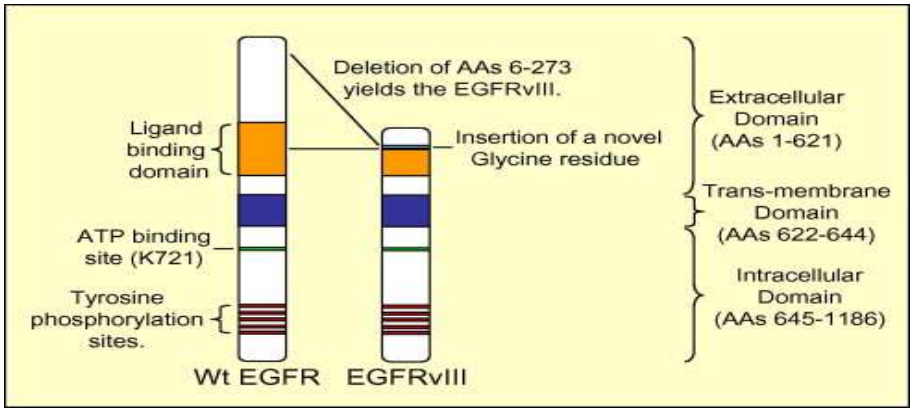

1. **Leukapheresis:** patient's T cells are harvested
2. **T cells are genetically transduced *ex vivo*** with a construct encoding the anti-CD19 chimeric antigen receptor
3. **CTL019 cells undergo *ex vivo* expansion** on magnetic antibody-coated beads
4. **Chemotherapy:** patient receives a preparative lymphodepleting regimen before T cell infusion
5. **CTL019 cells are re-infused** into the patient

# Expanding the use of CAR-T to solid tumors

*Development of an EGFRvIII CAR-T for CTL019 treatment for glioblastoma*

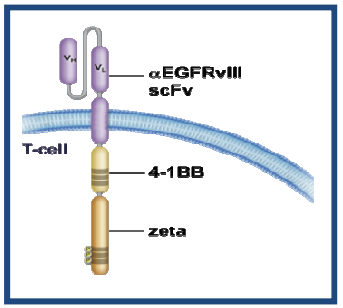
EGFRvIII:

- Tumor-specific, isoform variant of EGFR
  - In-frame deletion of exons 2-7 and generation of novel glycine residue at the junction of exons 1 and 8
  - Not expressed in normal tissues
- Expressed in 24-67% of GBM cases
- Activating EGFR mutation in GBM – signals constitutively, independent of ligand
- Frequent co-occurrence with amplified EGFR



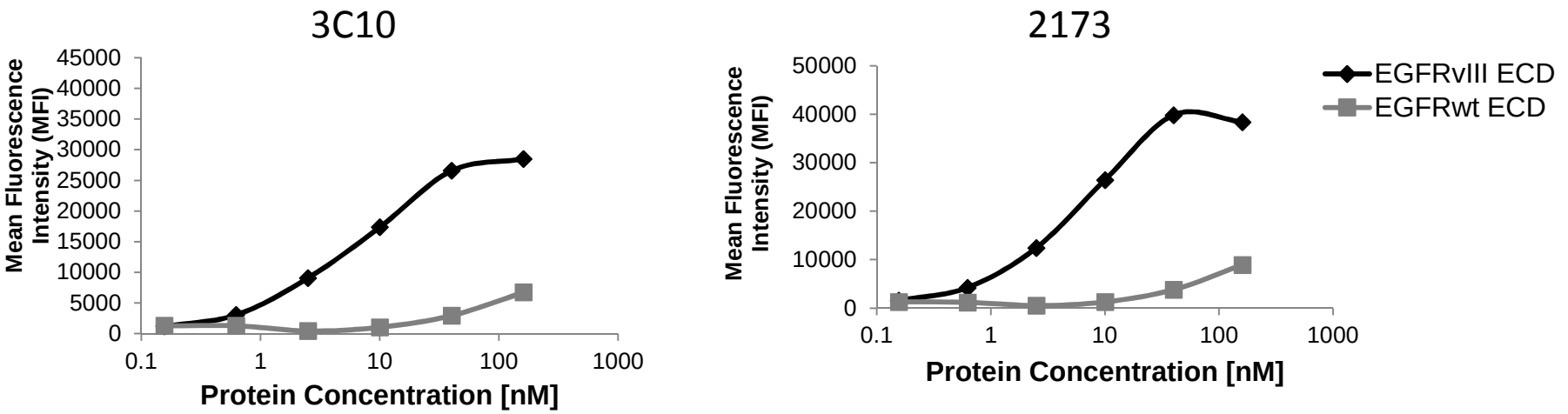

Rational development and characterization of humanized anti-EGFR variant III chimeric antigen receptor T cells For glioblastoma (2015) *Scl Transl Med 275: 275ra22*

Johnson LA, Scholler J, Ohkuri T, Kosaka A, Patel P, McGettin SE, Nace AK, Dentchev T, Thekkat P, Loew A, Boestaeanu AC, Cogdill AP, Chen T, Fraietta JA, Kloss CC, Posey AD, Engels B, Singh R, Ezell T, Idamakanti N, Ramones MH, Li N, Zhou L, Plesa G, Seykora JT, Okada H, June CH, Brogdon JL, and Maus MV



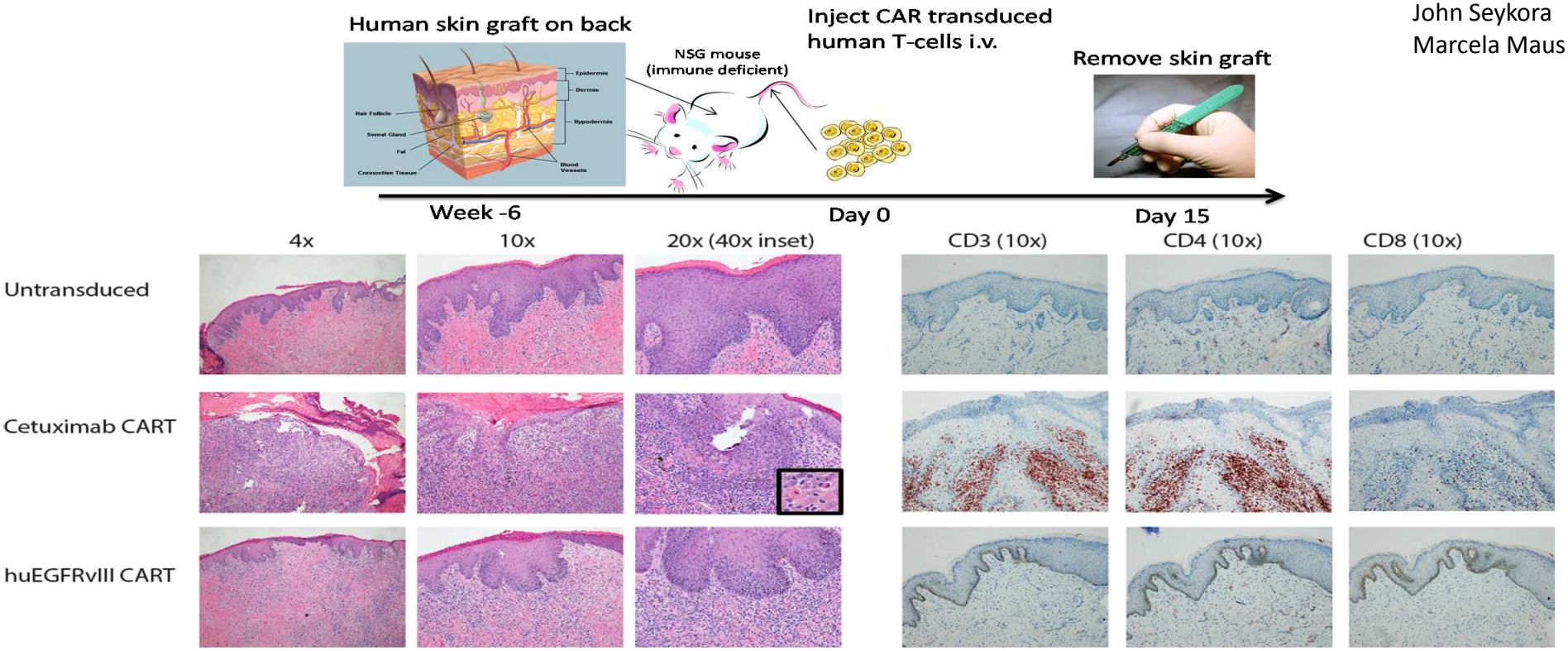
# A key EGFRvIII CAR-T design criteria is to limit cross-reactivity to wild type EGFR

Pramod Thekkat



# Use of NSG mice to demonstrate in vivo selectivity of the EGRvIII CAR-T

John Seykora
Marcela Maus

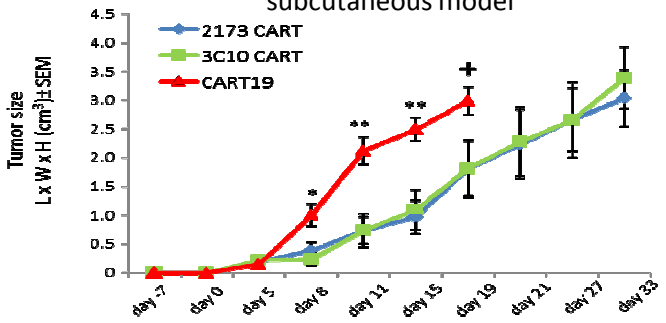


# Anti-tumor activity of EGFRvIII CAR-T in NSG mice injected with U87-EGFRvIII tumors

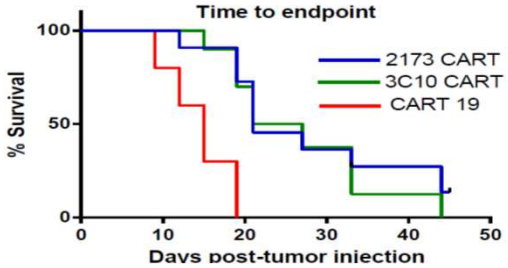
Tucker Ezell, Reshma Singh
Alina Boesteanu , Laura Johnson

A.

Tumor volume
subcutaneous model

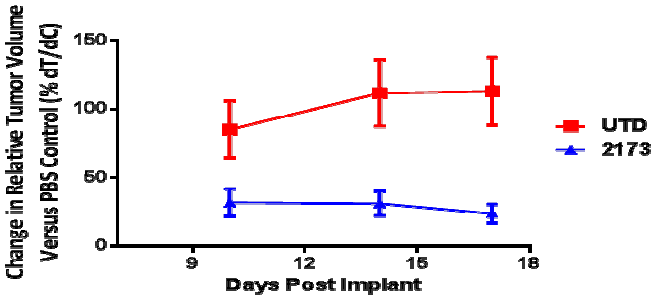



Overall survival
subcutaneous model

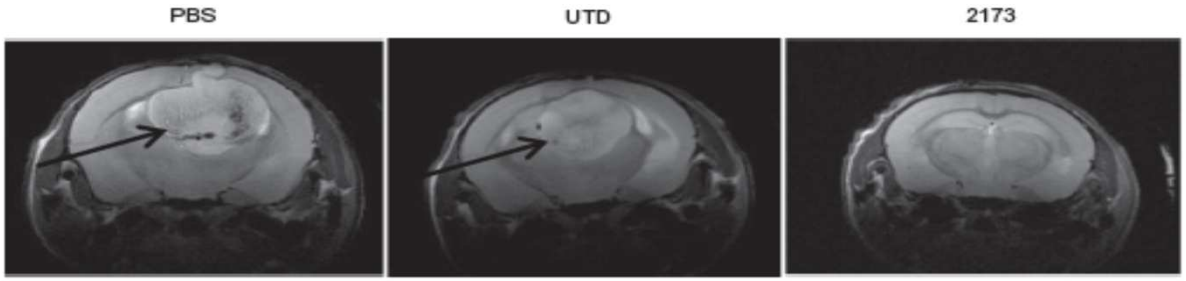



B.

Tumor vol relative to control
intracranial model



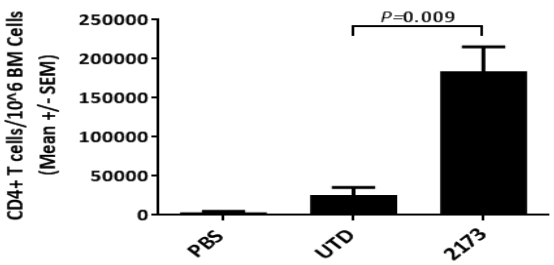

C.

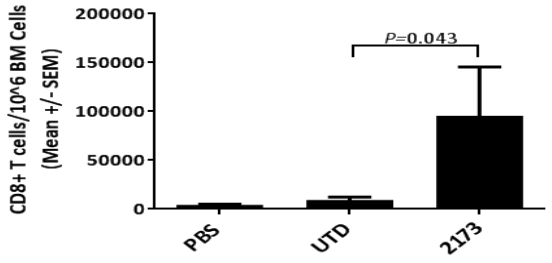


# Expansion and Trafficking of CART-EGFRvIII cells

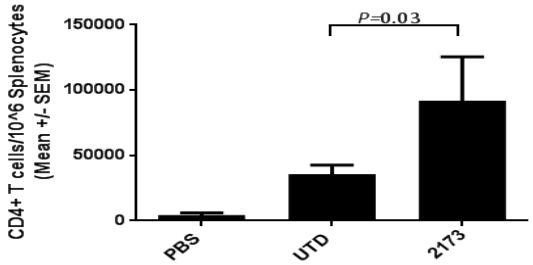
Tucker Ezell, Reshma Singh

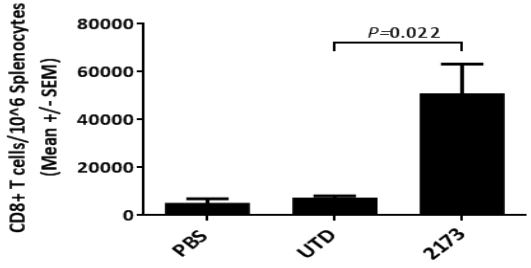
Bone marrow



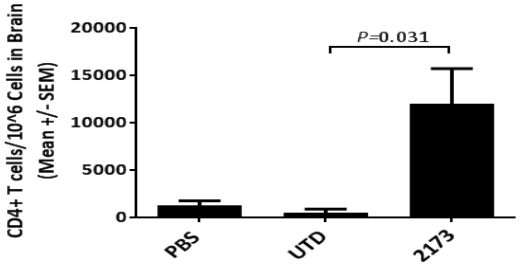



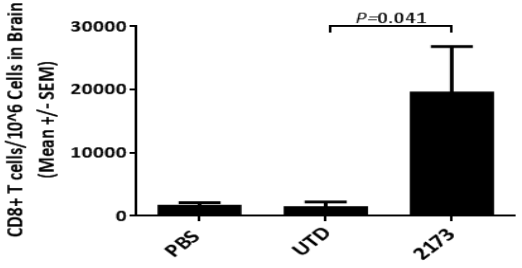

Spleen




Brain



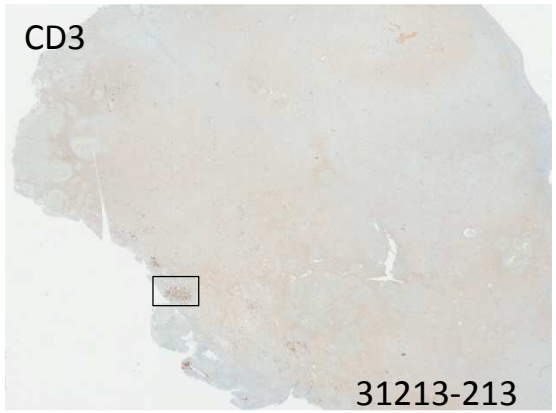
# Summary of EGFRvIII CAR therapy

- Infusion of CART-EGFRvIII is safe
- There is no EGFR-directed toxicity in 9 of 9 patients
- CART-EGFRvIII expand in blood
- Multifocal EGFRvIII CAR positive infiltrates were identified in biopsies obtained 6-7 days following infusion
  - T cell infiltrates contained IFN-γ, CD25 and CD134 positive cells, and cells which demonstrated a polarized granzyme B pattern, consistent with CAR cell activation observed in preclinical studies
- EGFRvIII CAR positive cells were not observed in patients biopsied at 35, 64 or 120 days following infusion

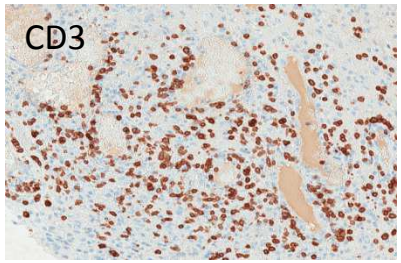
# Post EGFRvIII-CAR treatment biopsies of glioblastoma multiforme (6 days)

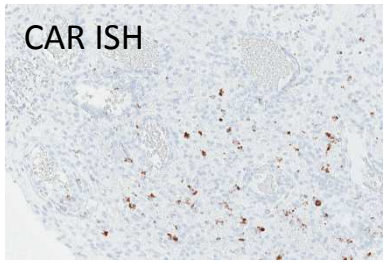
*Patient 31213-213, T cell infiltrated region*

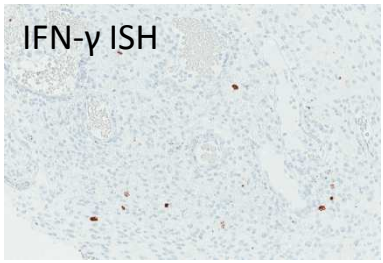
Keith Mansfield

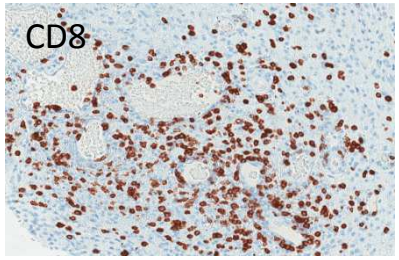


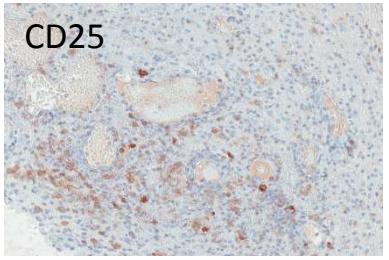

*Multifocal CAR cell infiltrates associated with increased expression of IFNG, CD25 and CD134 and tumor cell EGFRvIII expression*

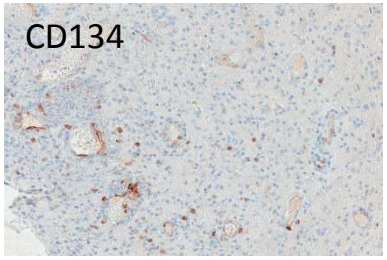


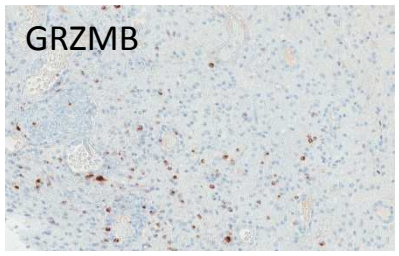



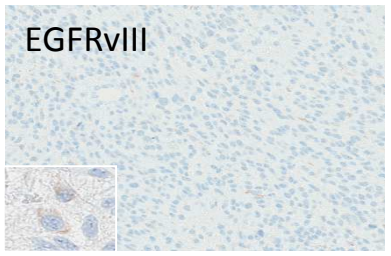



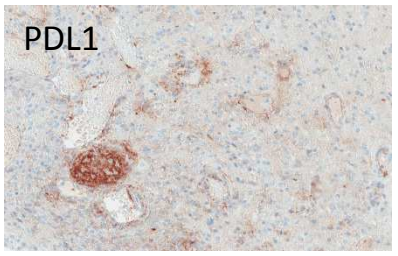



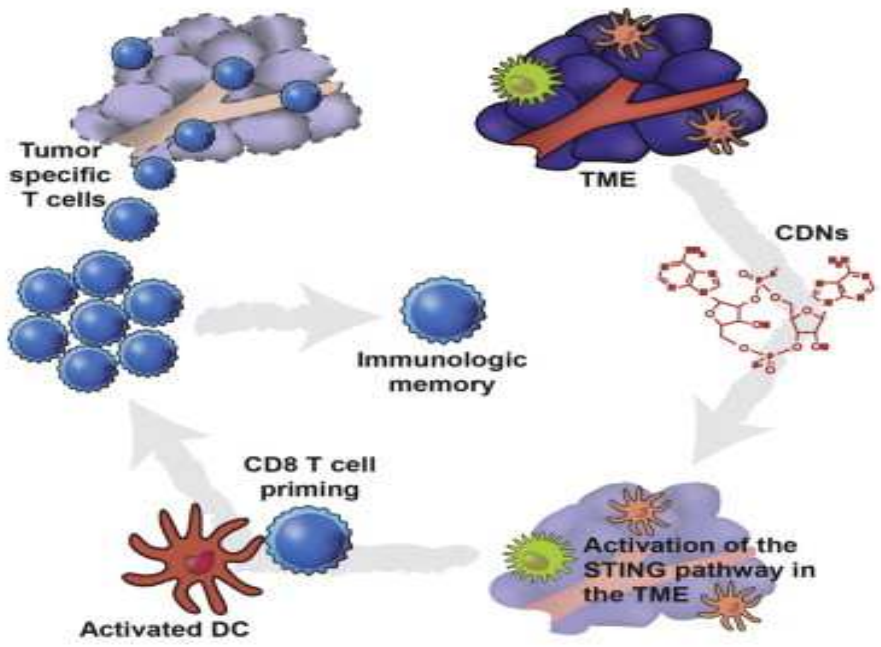



# STING (STimulator of INterferon Genes) agonists activate dendritic cells




Corrales L, et al. *Cell Rep*. 2015;11(7):1018-30.

- STING is a central sensor of cytosolic double-stranded nucleic acids from infectious pathogens, and is essential for efficient induction of a type I interferon immune response[1,2]
- STING senses and binds to bacterial signaling molecules known as cyclic dinucleotides (CDNs),[3] which leads to the expression of T-cell recruitment factors, including pro-inflammatory cytokines and interferon[1]
- CDNs may be useful as vaccine adjuvants, given the strong immune response triggered by foreign double-stranded DNA[1,4]
- In mouse tumor models, intratumoral injections of synthetic CDNs resulted in a substantial regression of injected and distant tumors, and provided immunologic memory[5]

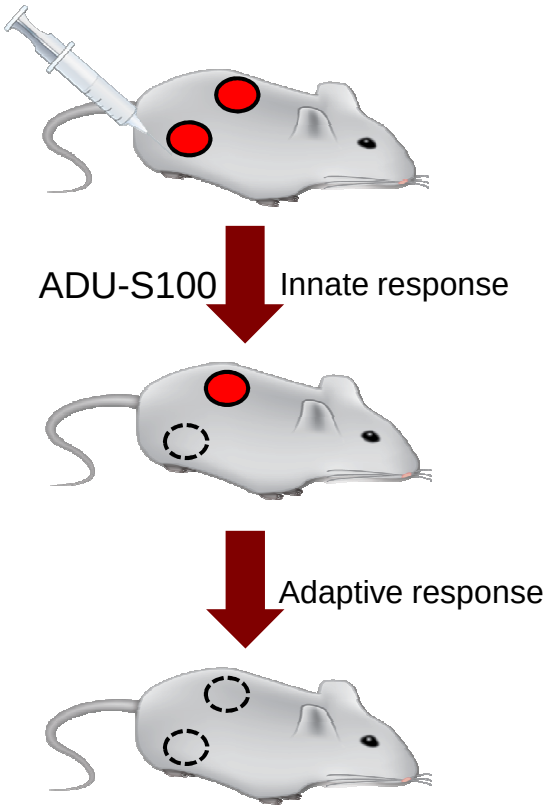
DC, dendritic cell; TME, tumor microenvironment.

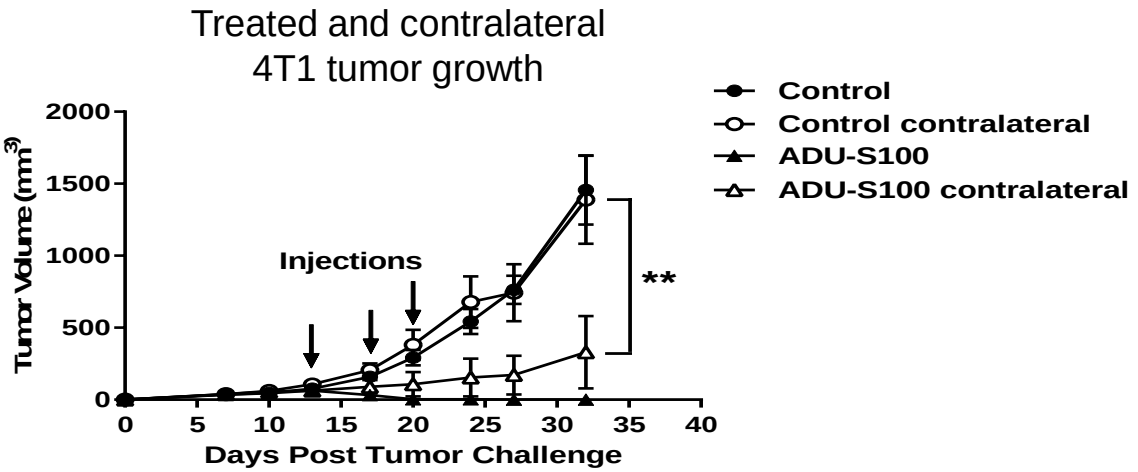
1. Dubensky TW Jr, et al. *Ther Adv Vaccines*. 2013;1(4):131-43. 2. Diner EJ, et al. *Cell Rep*. 2013;3(5):1355-61.
3. Burdette DL , Vance RE. *Nat Immunol*. 2013;14(1):19-26. 4. Chen W, et al. *Vaccine*. 2010;28(18):3080-5.
5. Corrales L, et al. Cell Rep. 2015;11(7):1018-30.

# ADU-S100: a potent cyclic dinucleotide STING agonist

*Abscopal Effect in Dual Flank Model*

Corrales and Glickman et al., Cell Reports (2015)

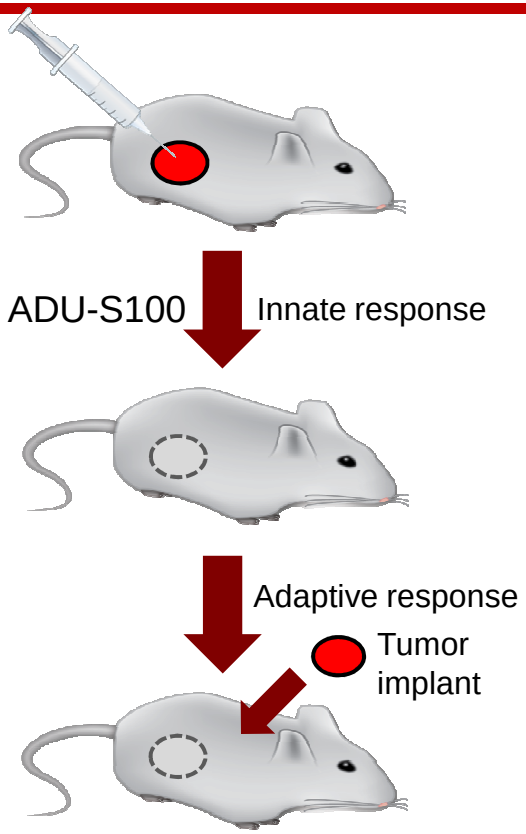


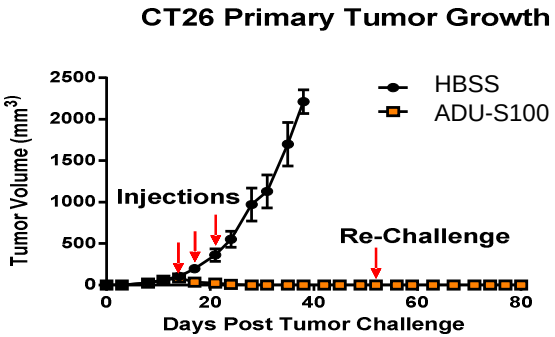

Laura Hix Glickman, Aduro Biotech
Poster #399
Friday, November 11
12:15 – 1:30 and 6:15 – 7:30

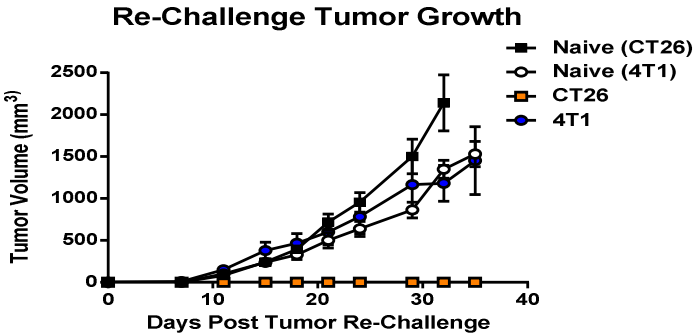
# Intratumoral ADU-S100 in the CT26 Model

*Injected tumor response and rejection upon re-challenge*



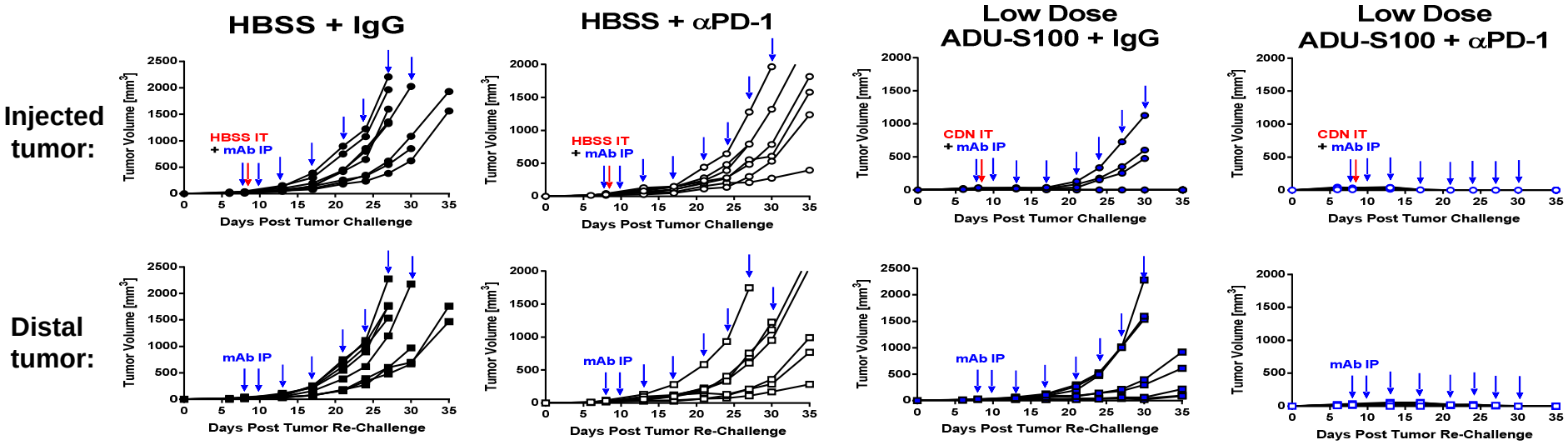

Corrales and Glickman et al., Cell Reports (2015)



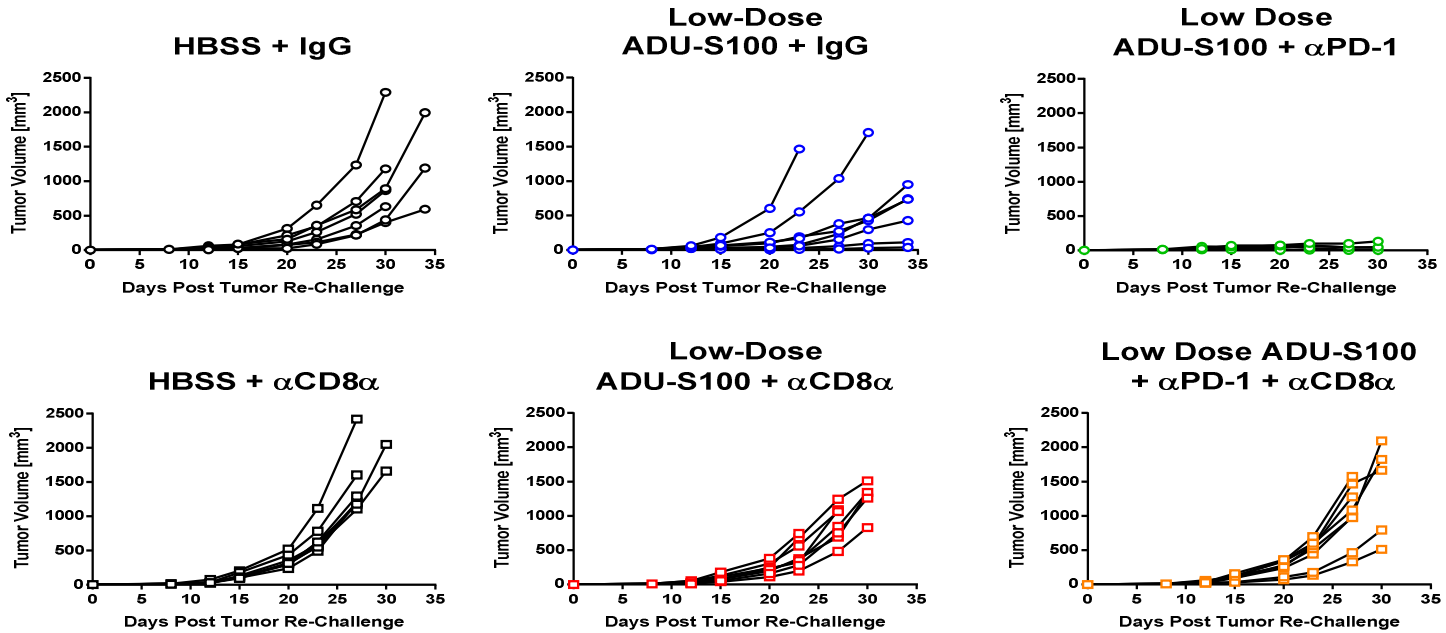
# Increased anti-tumor efficacy of ADU-S100 with immune checkpoint inhibition

*Dual Flank 4T1 Model*



# Abscopal efficacy of ADU-S100 combined with immune checkpoint inhibition is CD8 T cell dependent

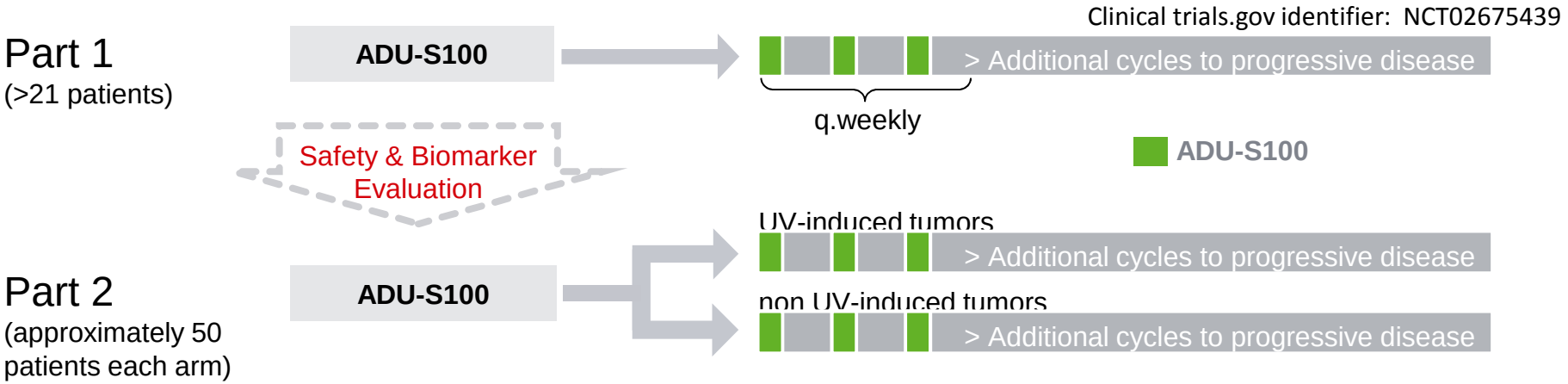
*Distal Tumor Responses in Dual Flank 4T1 Model*




***Laura Hix Glickman, Aduro Biotech***

# ADU-S100 Phase I Clinical Trial Design

*FIH STING Agonist Clinical Trial*




**Primary Objective**

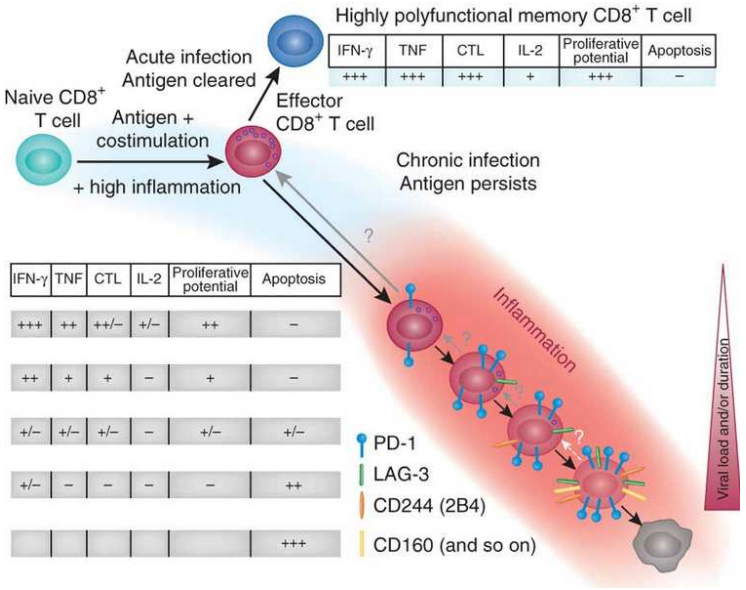
- Characterize safety and tolerability of ADU-S100 and identify a recommended dose and schedule for future studies

**Secondary Objectives**

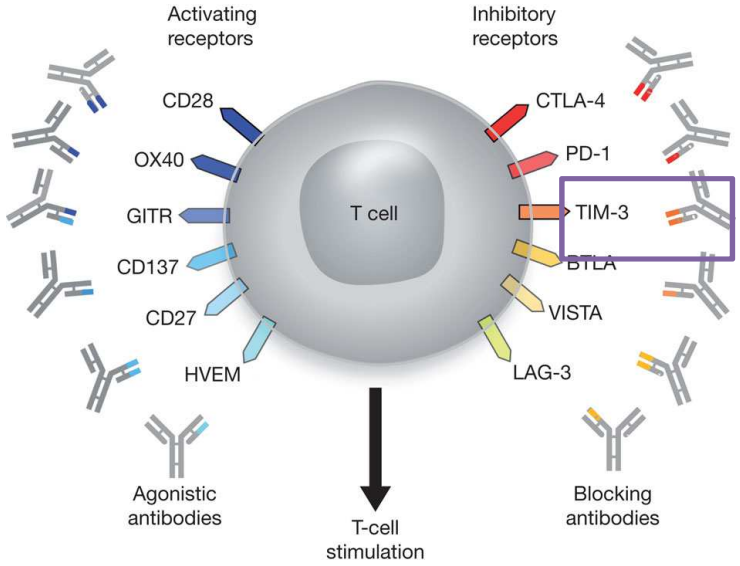
- Evaluate the preliminary anti-tumor activity of ADU-S100
- Characterize the pharmacokinetics (PK) of ADU-S100
- Assess pharmacodynamic (PD) effects in injected and distal lesions

# Accumulation of inhibitory receptors on dysfunctional T cells provide multiple targets

Accumulation of inhibitory receptors is associated with increasing T cell dysfunction during chronic antigenic stimulation



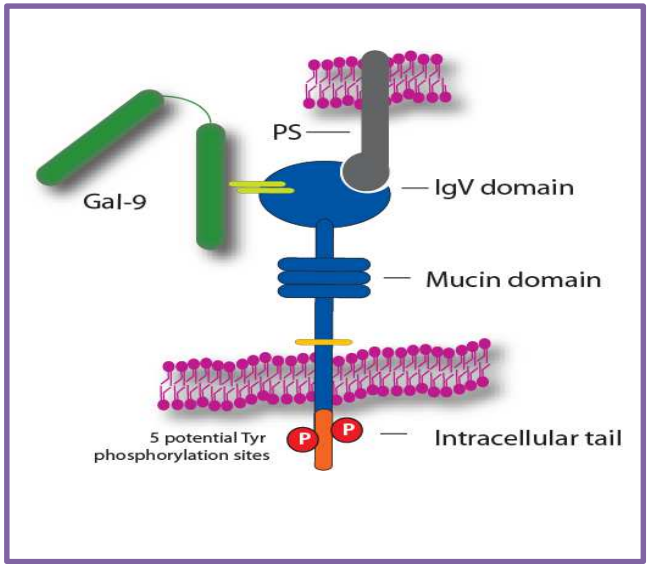

*Wherry (2011) Nat. Immunol. 12:492*




*Mellman et al (2011) Nature 480:480*

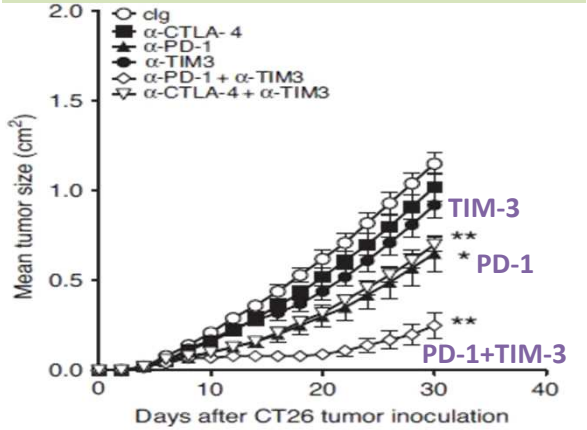
# TIM-3: critical role on T cells and myeloid cells

*Initially described as a T cell marker, emerging biology supports broader role*




***Preclinical rationale***

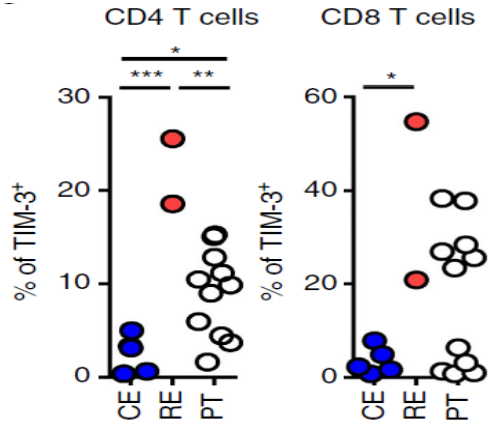
**PD-1/TIM-3 co-blockade is efficacious**




*Ngiow et al (2011) Cancer Res 71:3540*

***Clinical PD-1 resistance***

**PD-1 resistance: TIM-3 upregulated**

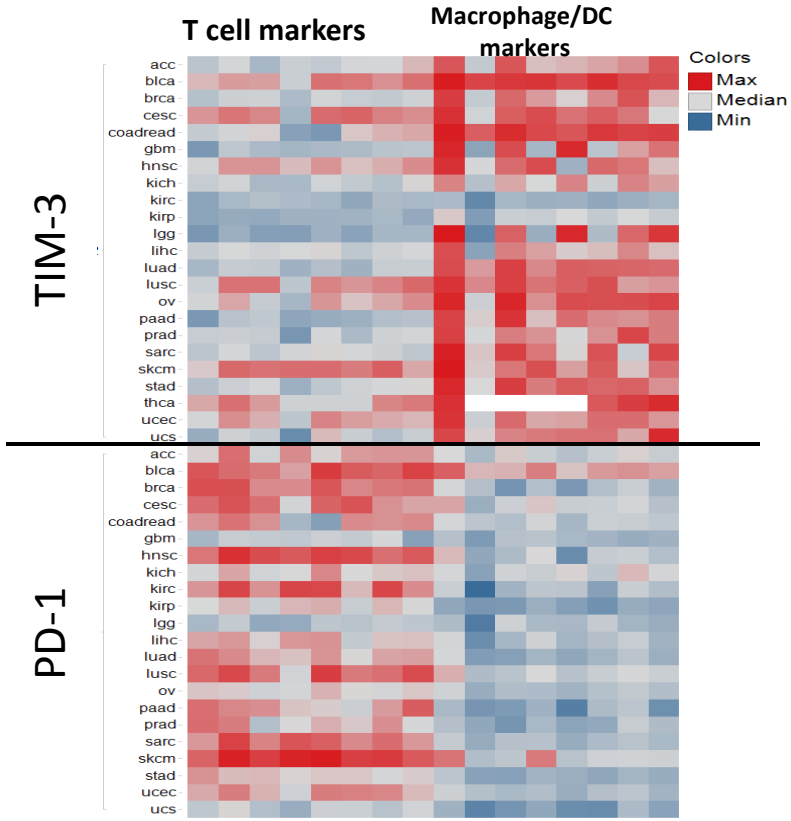


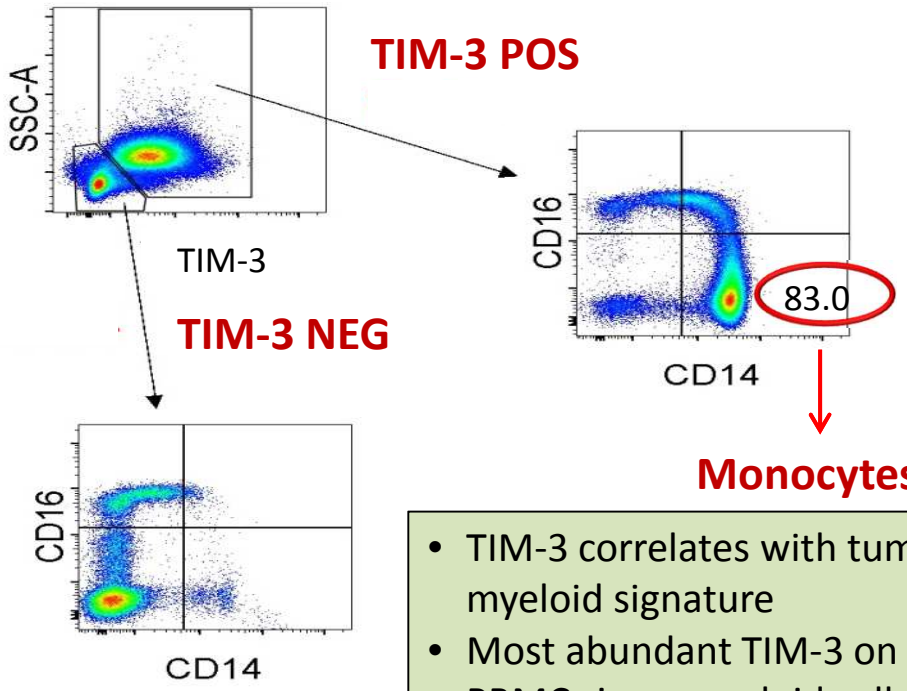

*Koyama et al (2016) Nat Comm 7:10501*

- Induced on activated and regulatory T cells; constitutively expressed on multiple innate immune populations, including myeloid cells
- Four reported ligands: Phosphatidylserine; (Galectin-9, CEACAM1, HMGB1)
- Anti-TIM-3 blockade restores Teff activity, diminishes T reg suppressor activity, enhances myeloid inflammatory cytokine secretion, and enhances anti-PD-1/PD-L1 antitumor and antiviral activity

# TIM-3 correlation in the TCGA database with a myeloid signature; confirmed on normal PBMCs

Kenzie MacIsaac
Pushpa Jayaraman




**Monocytes**

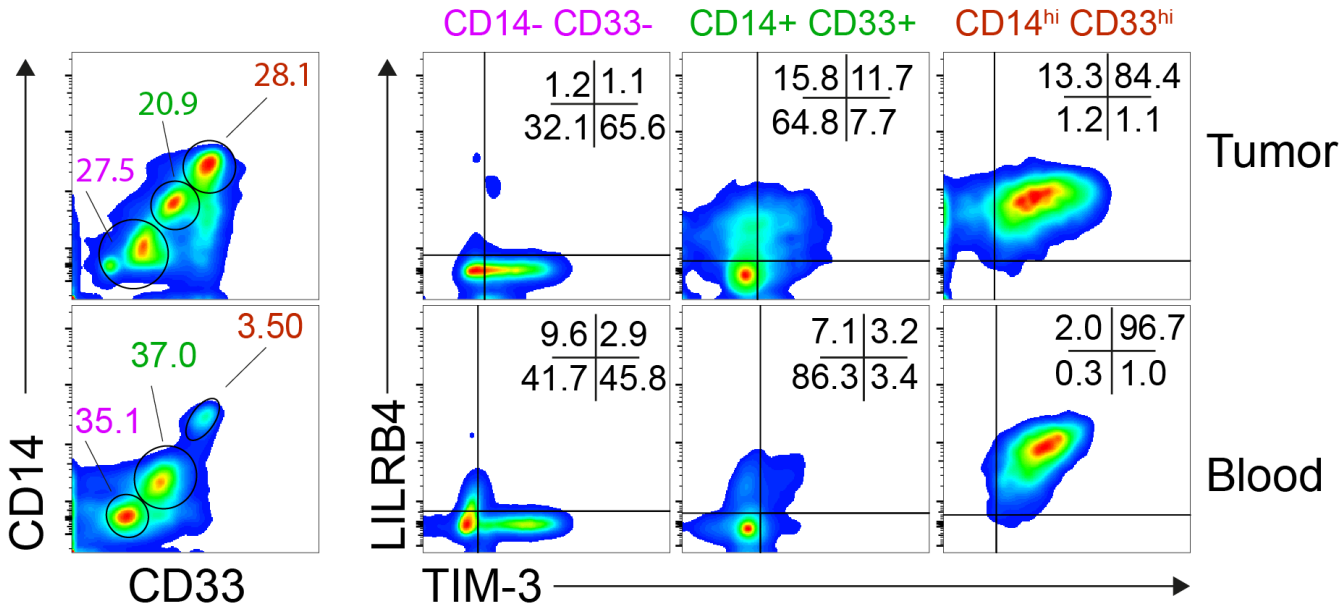
- TIM-3 correlates with tumor myeloid signature
- Most abundant TIM-3 on normal PBMCs is on myeloid cells

# TIM-3 correlation in the TCGA database with a myeloid signature; confirmed on human TAMs from RCC patient

Data representative of four independent donors

Radha Ramesh

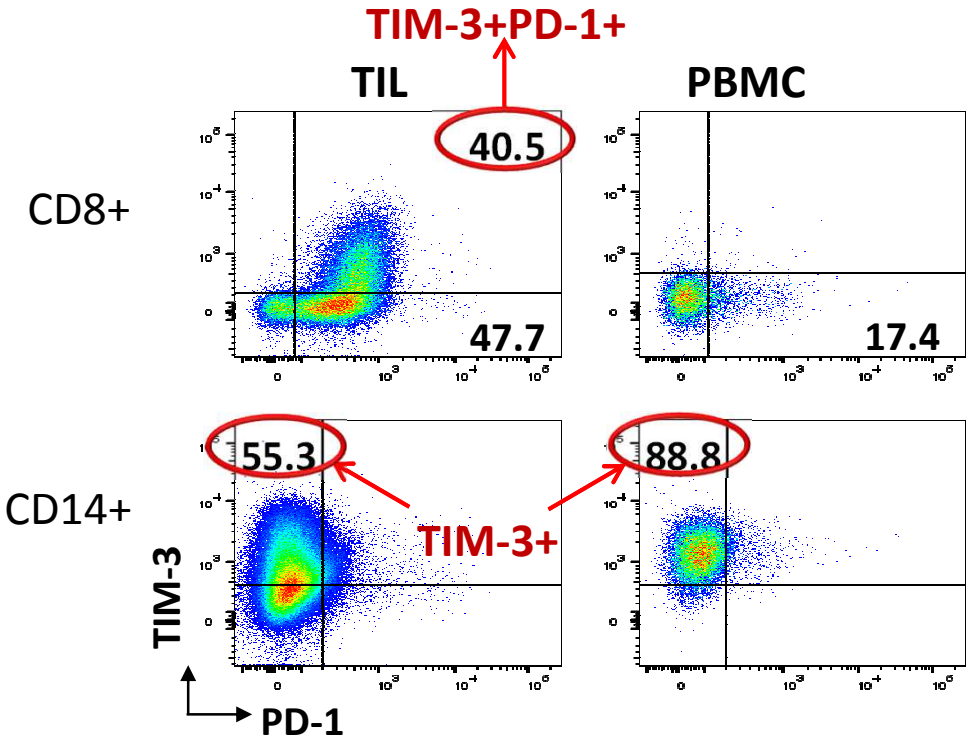
- RCC, treatment naive patient
- TIM-3 expression highest within CD14+ CD33+ myeloid cells
- Coexpression with LILRB4 confirms heatmap of RNA signature from TCGA database; also highest amongst CD14+ CD33+ MDSCs



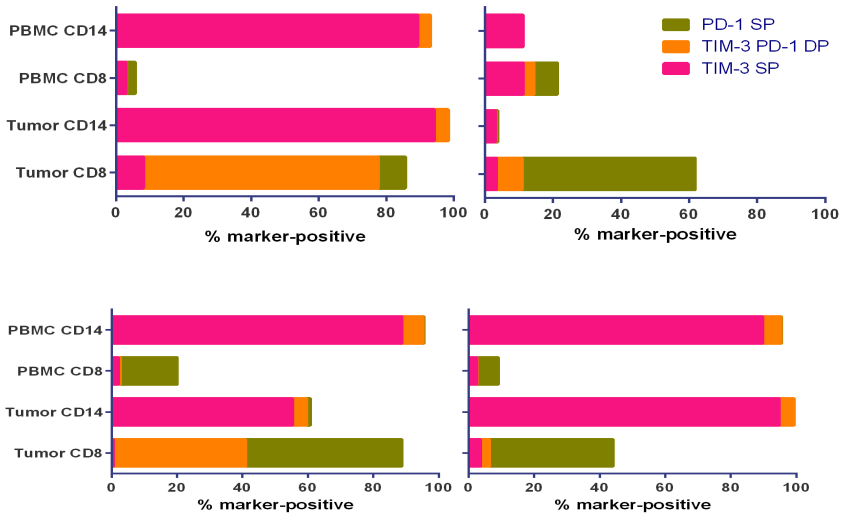
# TIM-3 and PD-1 expression on RCC

*Dominant TIM-3 SP myeloid cells; PD-1+TIM-3+ DP CD8+ in TILs*

Radha Ramesh

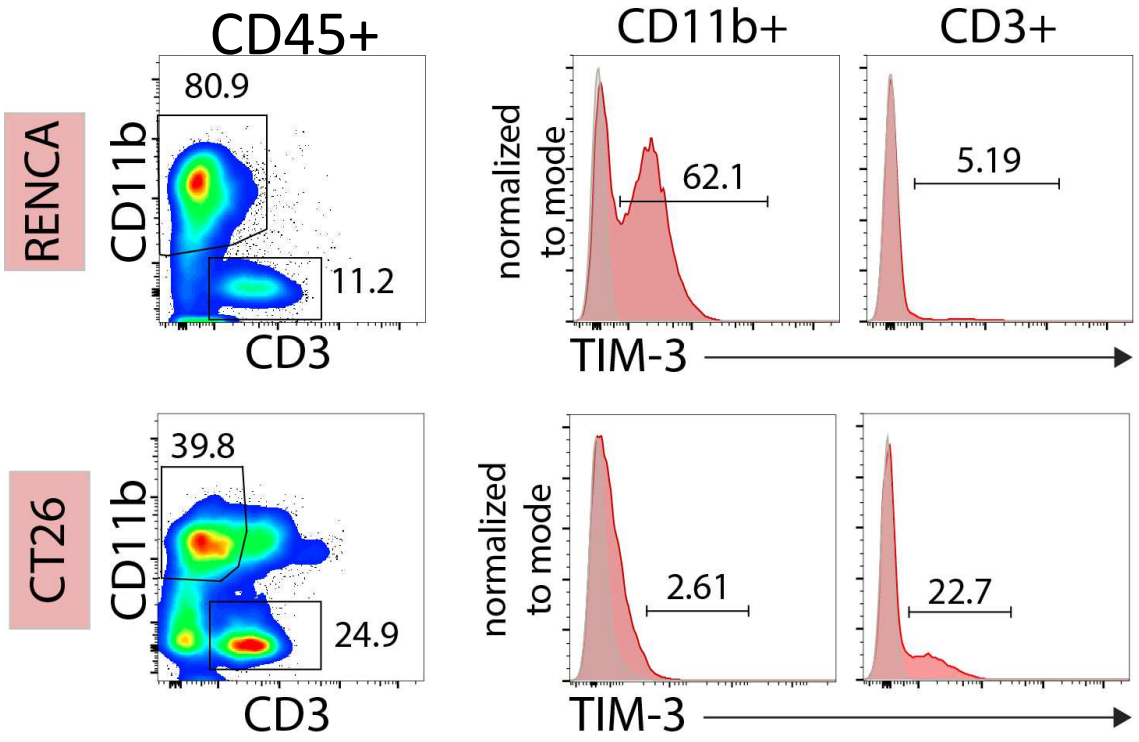



Dominant TIM-3+ CD14+ cells and
PD-1+ CD8+ T cells



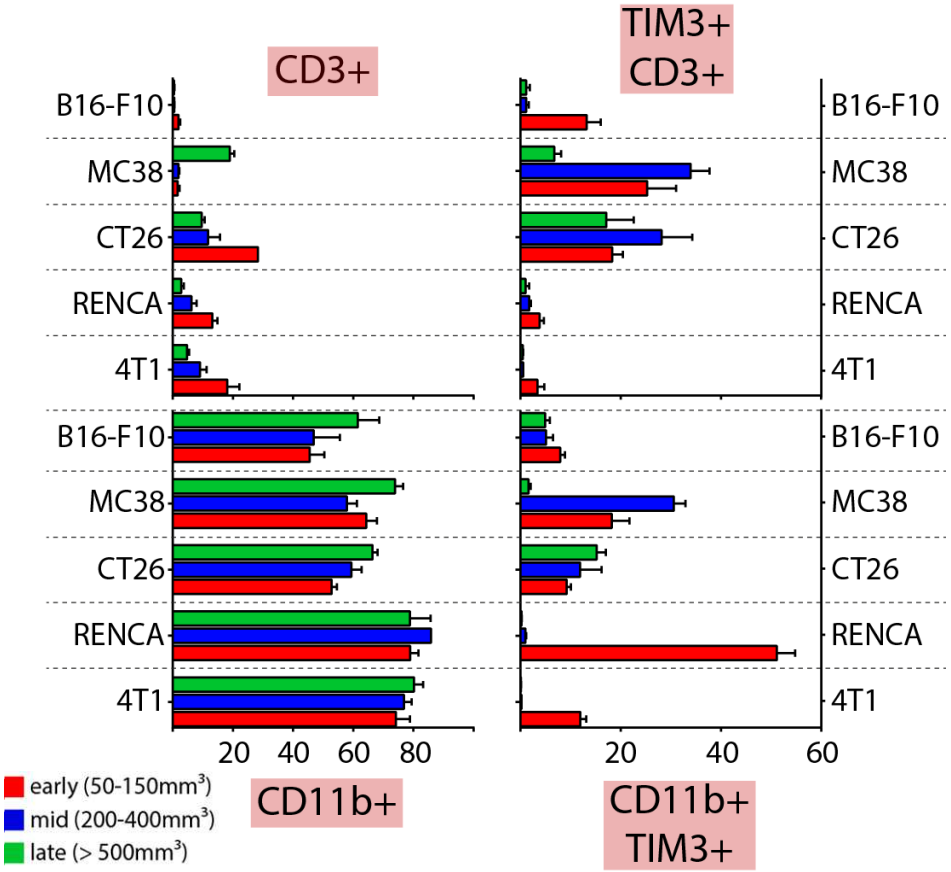
# TIM-3-positive myeloid cells seen in early tumors in murine syngeneic tumor models

Pushpa Jayaraman




- Similar to RCC, RENCA syngeneic model also shows increased TIM-3 expression on myeloid cells compared to T cells
- CT26 model exhibits opposite phenotype with increased TIM3+ T cells compared to myeloid cells
- Decreased TIM-3+ T cells in Renca not due to decreased frequency of CD3+ T cells

# Variability across syngeneic tumor models; higher TIM-3+ myeloid populations in 4T1, RENCA at early stages




Pushpa Jayaraman

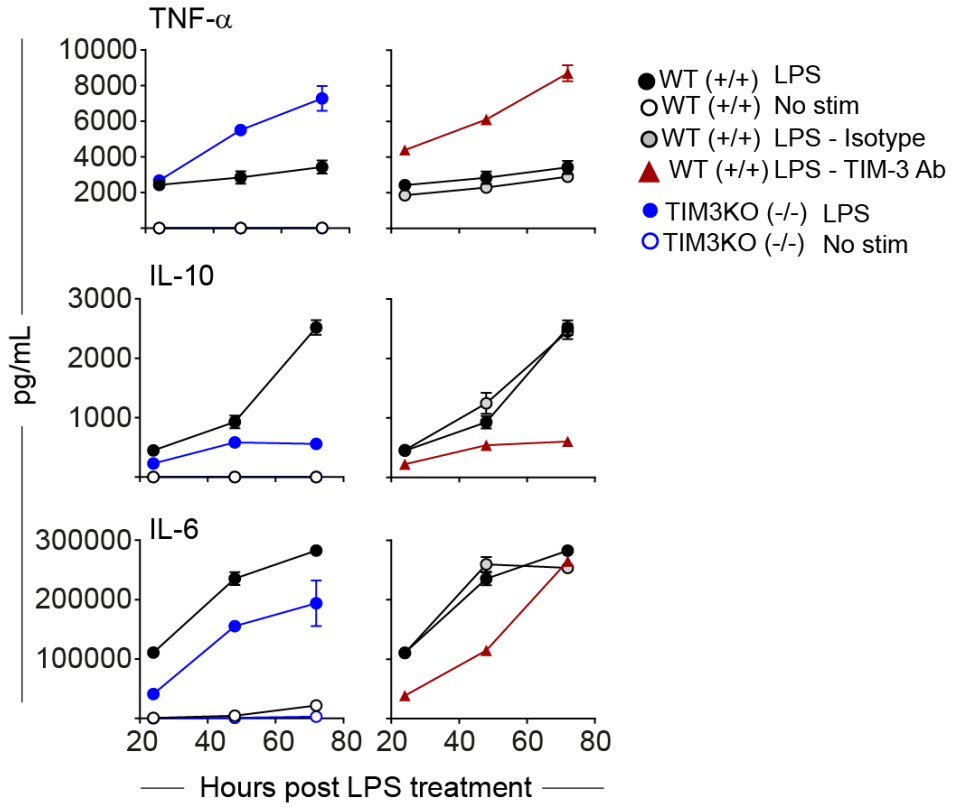
- 4T1 and Renca express TIM-3 on myeloid cells to a higher extent than T cells

- CT26 and MC38 colon carcinomas express TIM-3 on both myeloid and T cells to comparable levels

**Distinct syngeneic tumor models exhibit bias towards a dominant TIM-3 expressing myeloid or T cell infiltrate**

- *tractable models to test TIM-3 biology on distinct immune populations*

# TIM-3KO macrophages exhibit dysfunctional response to LPS with increased TNF, decreased IL-6

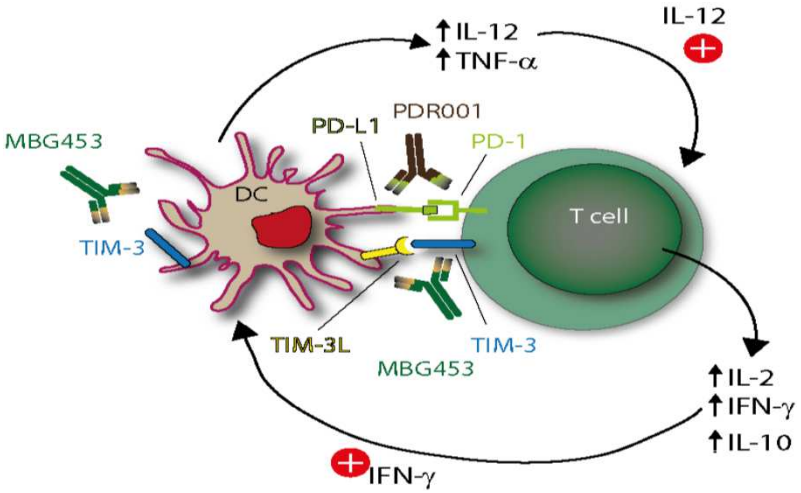


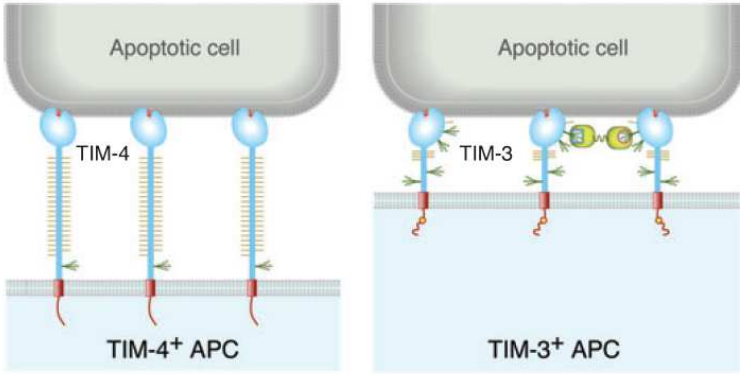

Nidhi Patel
Pushpa Jayaraman

- TIM-3KO macrophages exhibit dysfunctional response to LPS with increased TNF, decreased IL-6, IL-10
- No phenotype seen with IL-12
- TIM3 blockade in WT macrophages phenocopies effect seen in TIM-3KO macrophages

LPS, 500ng/mL
24, 24, 72 hour supe harvest
Resident peritoneal macrophages
Data representative of two independent experiments

# Model: TIM-3 blockade on myeloid cells impacts CD8 T cell IFN-γ secretion




*Freeman et al (2010) Imm Rev 235:172*

Apoptotic clearance: anti-inflammatory effects?

- MBG453 and PDR001 blockade synergize to increase inflammatory cytokine secretion in multiple *in vitro* assays (as in preclinical models with surrogate mAbs)
- MoA for TIM-3: On T cells, DCs or both?

# What Information Provided by Models Inform Immune Drug Development and Use?

- Data from pre-clinical models, in conjunction with translational information, are used to specifically address the following:
  - Are we treating the correct patient...
    - Do we understand the underlying biology of disease and target?
  - With an optimal therapeutic...
    - Have we engineered appropriate potency and selectivity?
  - At the appropriate dose?
    - Do we understand dose response relationships?