From the Clinic to the Lab: Investigating Mechanisms of Response and Resistance to Immune Checkpoint Therapy

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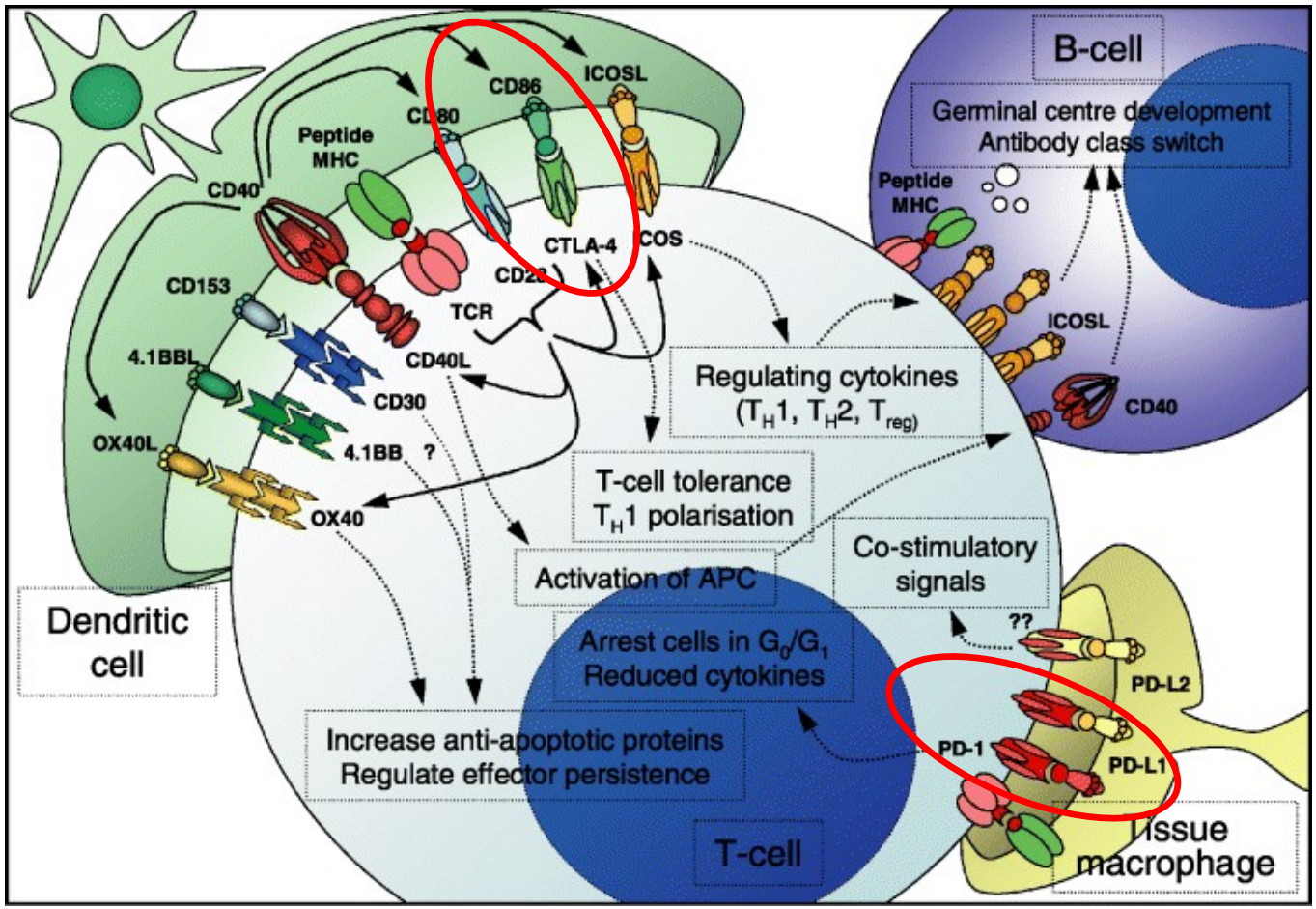
DISCLOSURES

- **Consulting Fees: Jounce, Oncolytics, BioAtla, Forty Seven**
- **Ownership Interest: Jounce, BioNTech, Constellation Pharmaceuticals, Oncolytics, BioAtla, Forty Seven**
- **Partner Consulting Fees: Jounce, Oncolytics, BioAtla, Forty Seven**
- **Partner Ownership Interest: Jounce, BioNTech, Constellation Pharmaceuticals, Oncolytics, BioAtla, Forty Seven**

Anti-CTLA-4 opened a new field called immune checkpoint therapy



FDA-Approvals

Melanoma
- **Ipilimumab (2011)**
- Nivolumab (2014)
- Ipilimumab + Nivolumab (2015)
- Pembrolizumab (2019)

Lung Carcinoma
- Nivolumab (2015)
- Pembrolizumab (2015)
- Atezolizumab (2016)
- Durvalumab (2018)
- Ipilimumab + Nivolumab (2020)

Renal Cell Carcinoma
- Nivolumab (2015)
- Ipilimumab + Nivolumab (2018)
- Avelumab (2019)

Colorectal Carcinoma (MSI-hi)
- Nivolumab (2017)
- Pembrolizumab (2017)
- Ipilimumab + Nivolumab (2018)

Head&Neck Sq Cell Carcinoma
- Nivolumab (2016)
- Pembrolizumab (2016)

Lymphoma
- Nivolumab (2016)
- Pembrolizumab (2017)

Hepatocellular Carcinoma
- Nivolumab (2017)
- Pembrolizumab (2018)

Merkel Cell Carcinoma
- Avelumab (2017)
- Pembrolizumab (2018)

Gastric/Gastroesophageal Adenocarcinoma
- Pembrolizumab (2017)

Cervical Carcinoma
- Pembrolizumab (2018)

Cutaneous Sq Cell Carcinoma
- Cemiplimab (2018)

Breast Carcinoma
- Atezolizumab (2019)

Esophageal Carcinoma
- Pembrolizumab (2019)

Uterine Carcinoma
- Pembrolizumab (2019)

Urothelial Carcinoma
- Atezolizumab (2016)
- Avelumab (2017)
- Durvalumab (2017)
- Nivolumab (2017)
- Pembrolizumab (2017)

Genomic Alterations
- Pembrolizumab for MSI-hi (2017)
- Pembrolizumab for TMB $\geq$10 mutations/megabase (2020)

Key Research Questions

Why do some patients respond and others do not?

Can we identify biomarkers that predict response? immune-related toxicities?

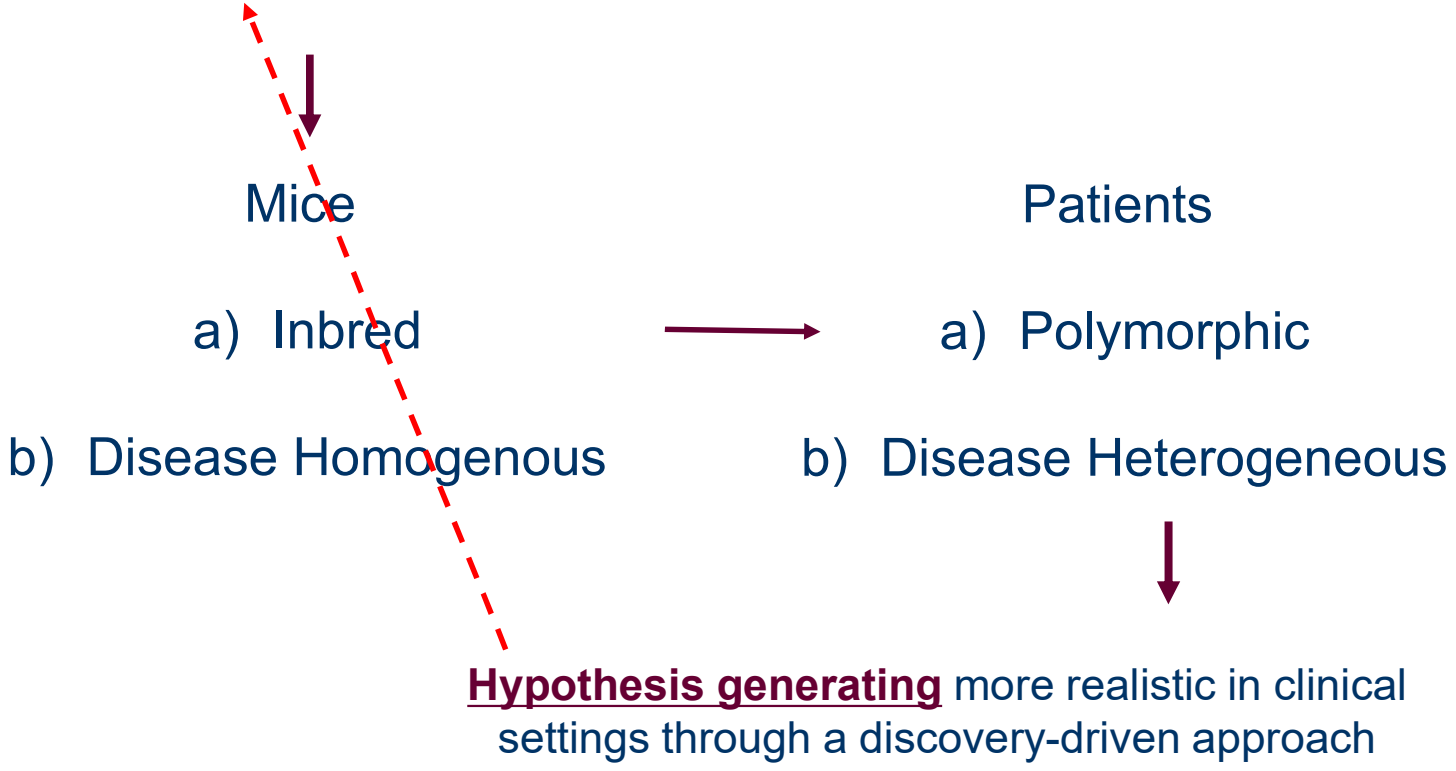
Are there biomarkers to enable patient selection for treatment with monotherapy vs combination?

Can we increase the number of patients who respond?

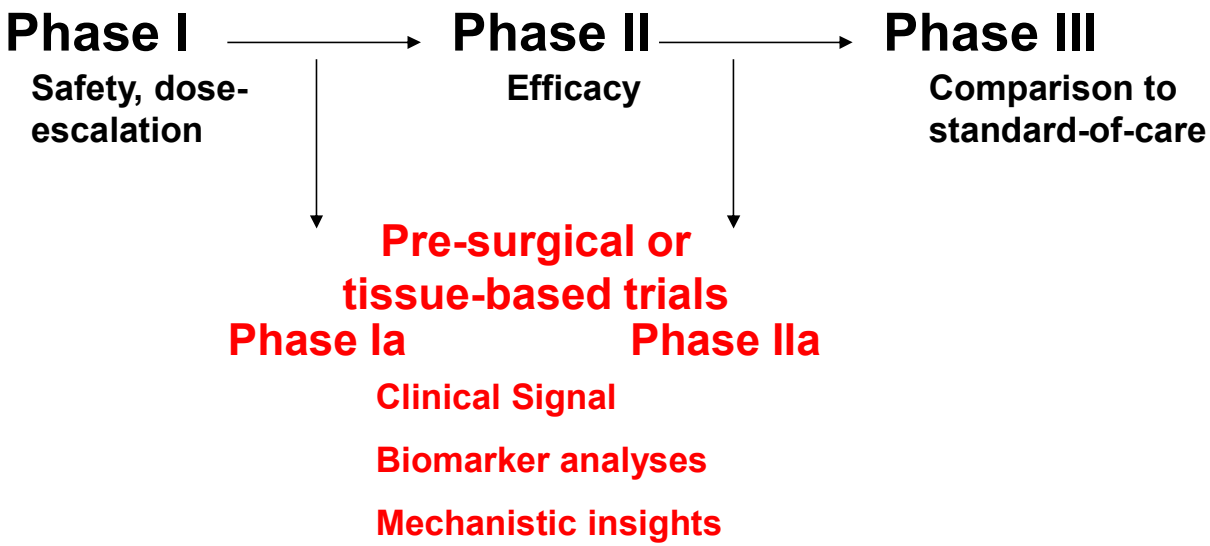
Are there other pathways that can be targeted to improve clinical outcomes?

>2000 Clinical Trials Ongoing Integrating Laboratory and Clinical Research: Reverse Translation

Hypothesis testing most efficient when one variable at the time is analyzed



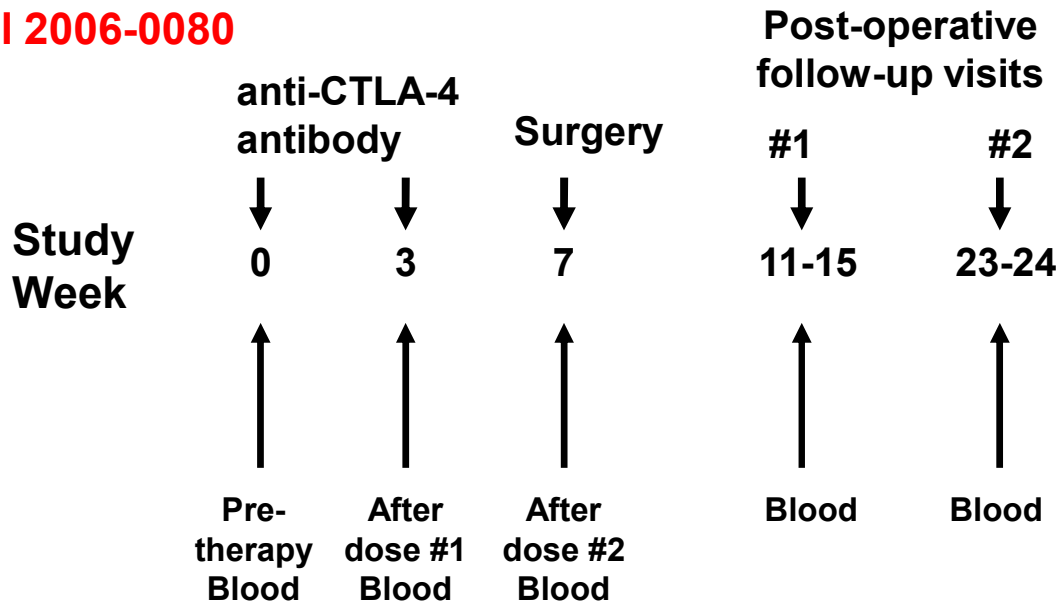
Re-thinking clinical trial design to obtain appropriate samples for laboratory studies



Initiated 2004.
First trial 2006 (protocol 2006-0080).
This occurred prior to any FDA approvals.

Neoadjuvant (pre-surgical) clinical trial with anti-CTLA-4 in patients with localized bladder cancer

Clinical Trial Protocol 2006-0080
N=12

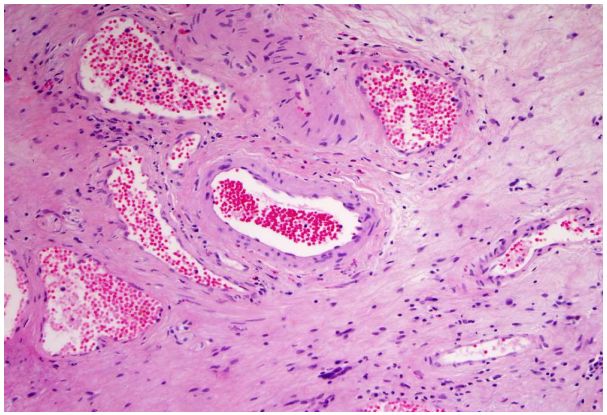


Clinical Signal for Safety: Immune checkpoint therapy can be given prior to surgery (neoadjuvant setting)

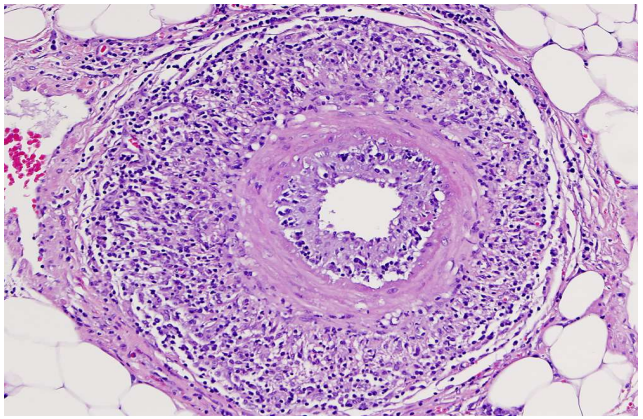
Clinical Signal for Efficacy: 3 patients developed pCR indicating that bladder cancer can be responsive to immune checkpoint therapy

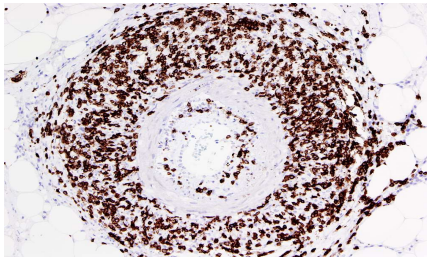
Pre-surgical trial: sufficient tissues at time of surgery for immune monitoring studies to identify biomarkers of response and resistance

Pre-therapy

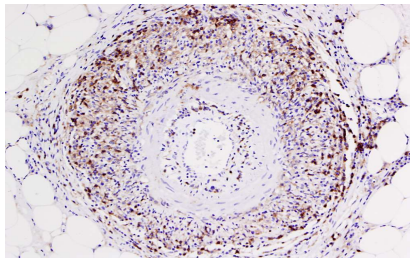


Post-therapy

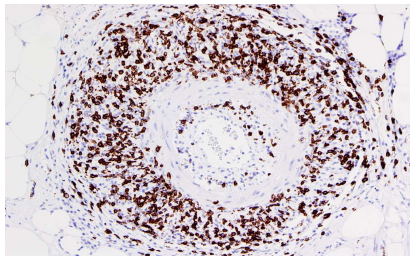




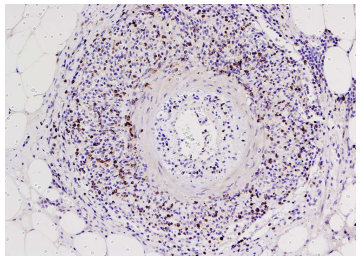
CD3



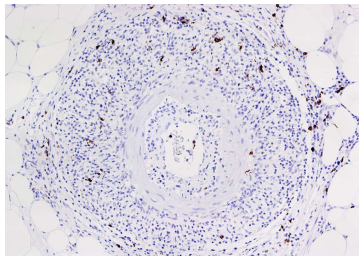
CD4



CD8



Granzyme



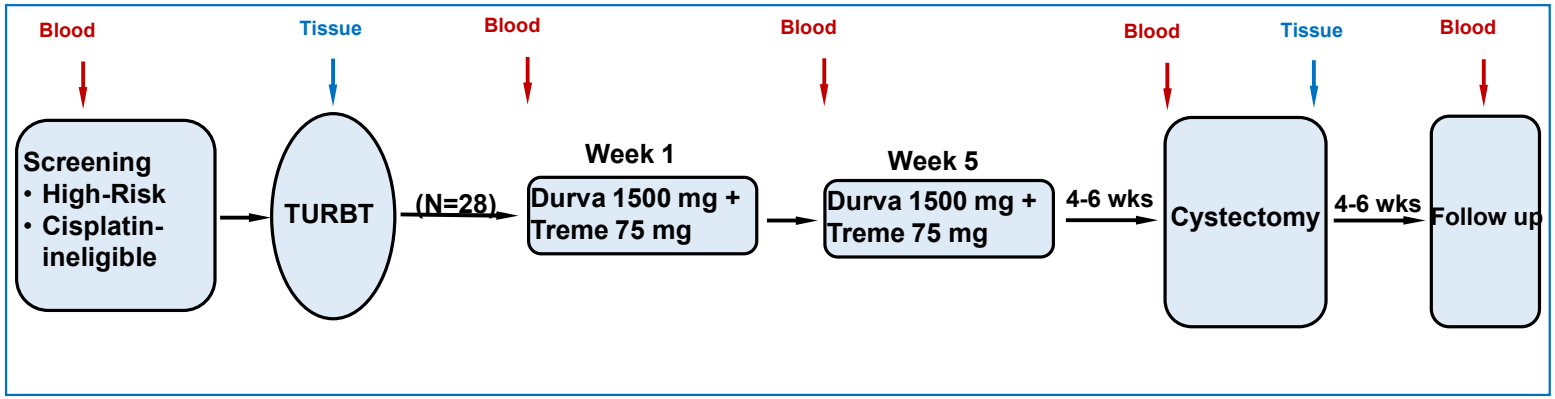
CD20

Liakou et al., *Proc Natl Acad Sci*, 2008

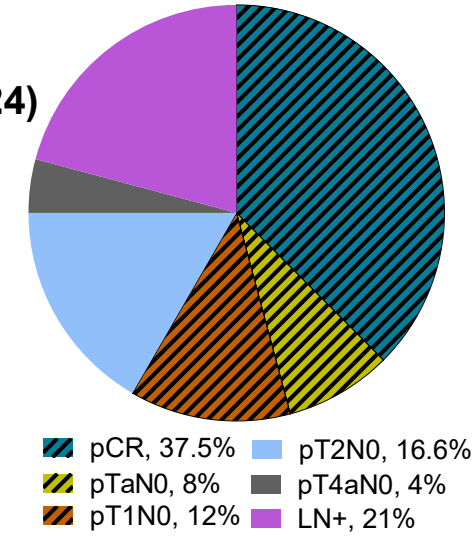
Comparison of pre- and post-treatment tumor samples for differentially expressed genes

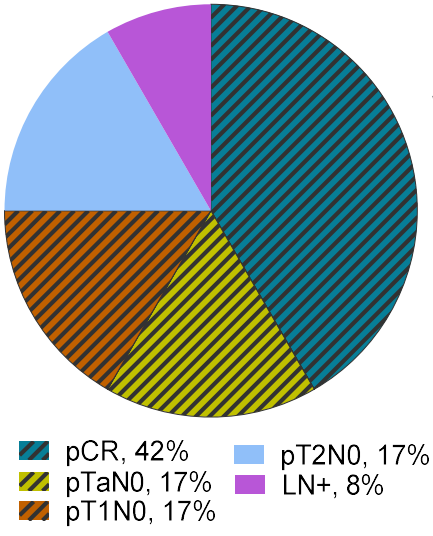
1. ICOS Pathway	$p = 9.8 \times 10^{-13}$
2. CD28	$p = 3.5 \times 10^{-10}$
3. TCR	$p = 4.7 \times 10^{-10}$
4. CTLA-4	$p = 1.2 \times 10^{-9}$
5. Leukocyte extravasation	$p = 2.2 \times 10^{-7}$
6. IL-12	$p = 1.8 \times 10^{-4}$
7. PI3/AKT	$p = 1.4 \times 10^{-3}$
8. JAK/STAT	$p = 8.7 \times 10^{-3}$
9. NF-kB	$p = 1.1 \times 10^{-2}$
10. ERK/MAP	$p = 2.3 \times 10^{-2}$

Neoadjuvant (pre-surgical) clinical trial with anti-CTLA-4 (Treme) plus anti-PD-L1 (Durva) in patients with localized bladder cancer



All patients who completed surgery (N=24)

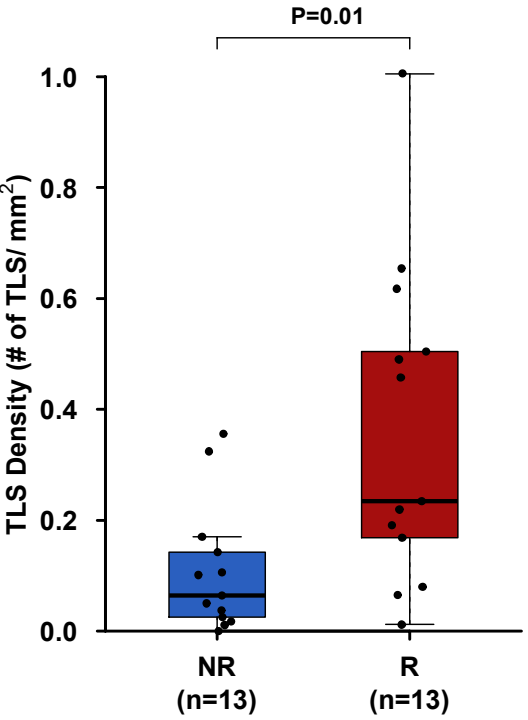


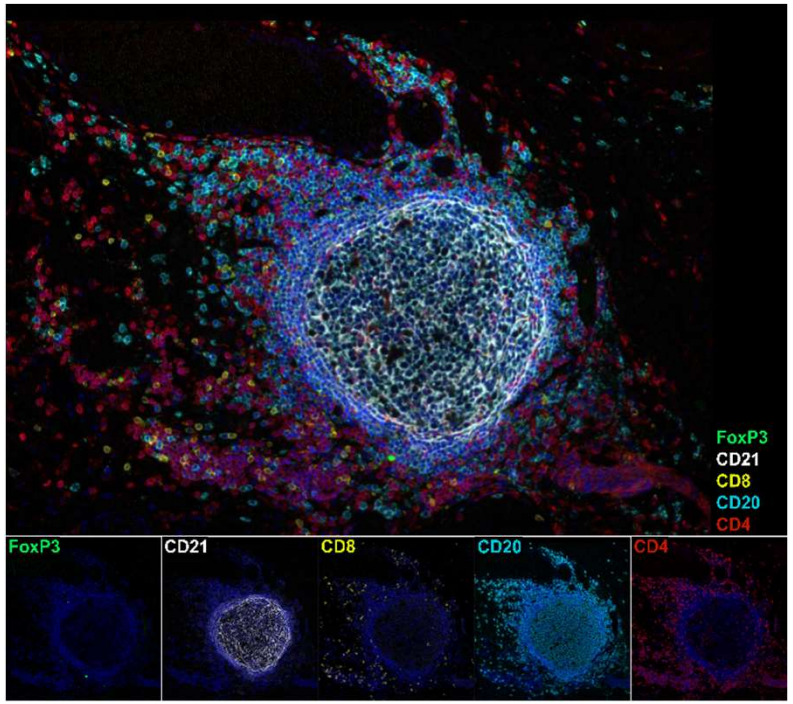


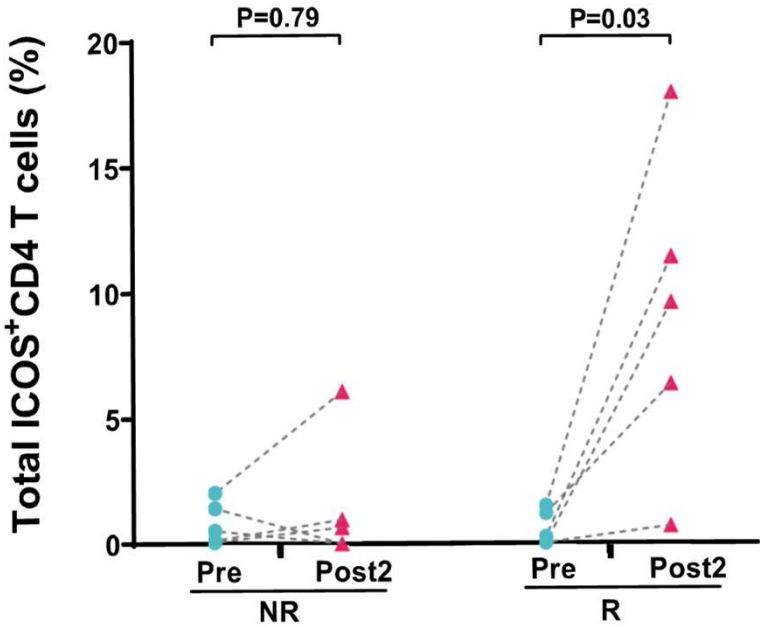
Patients with 3-D mass/cT4a who completed surgery (N=12)

Gao et al., *Nature Medicine*, 2020

Biomarkers of response to ICT





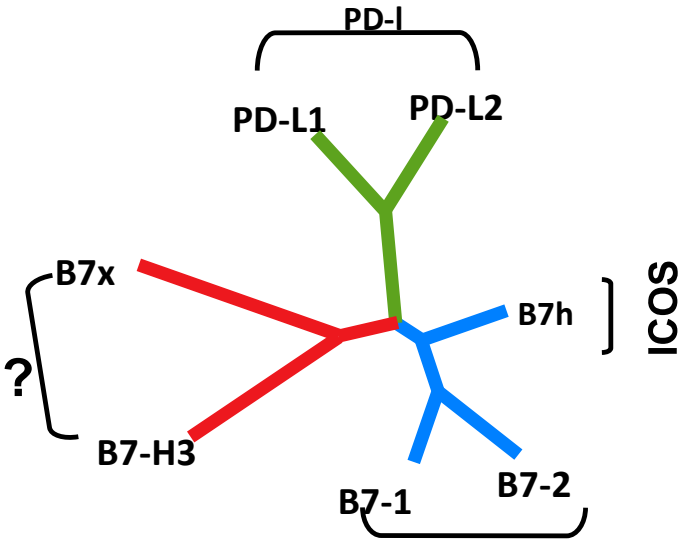


Gao et al., *Nature Medicine*, 2020

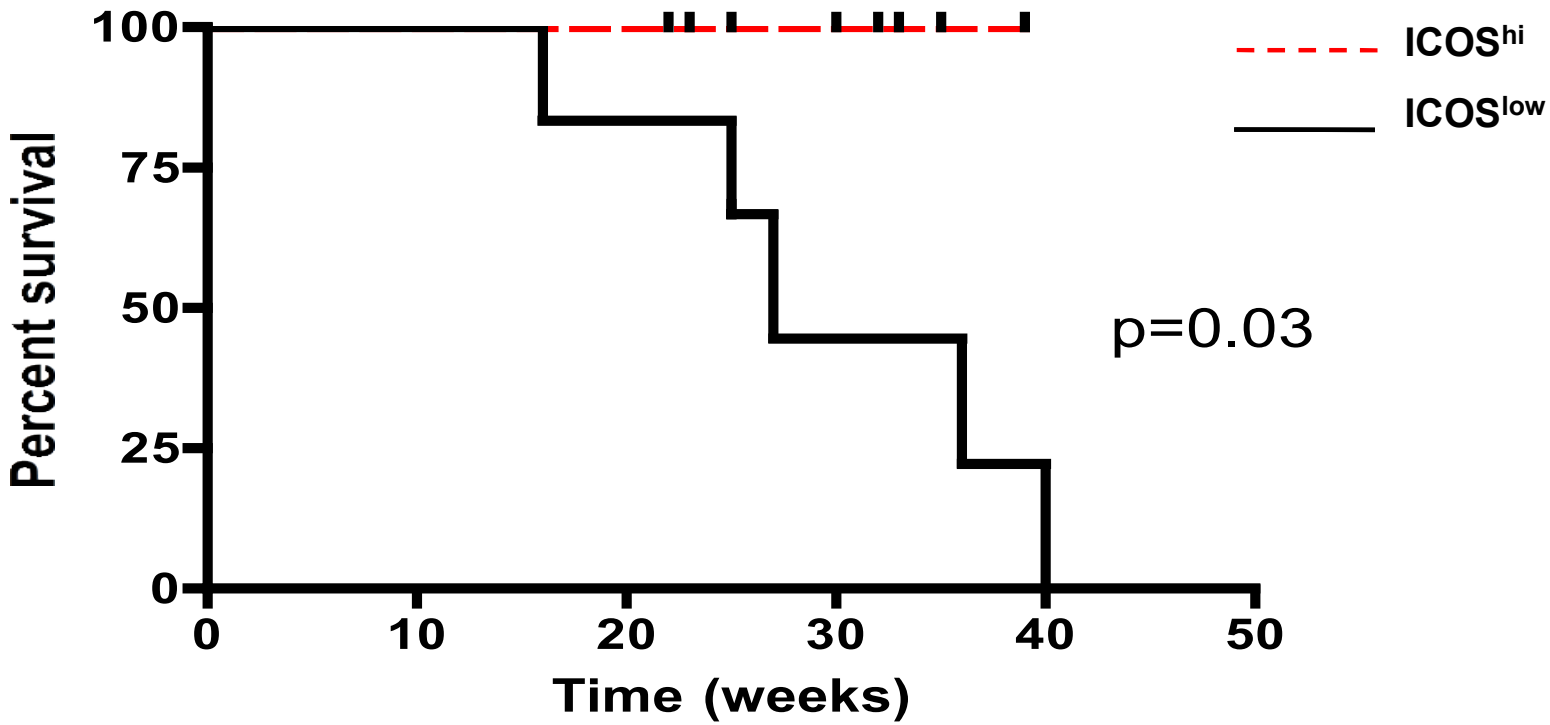
ICOS

- Inducible costimulator (ICOS)
  - -belongs to CD28/CTLA-4 family
  - -expression increased on activated T cells
  - -diverse role reported
  - -most associated with Tfh: ICOS+, PD-1+, CXCR5+, BCL6+, IL-10 production

Role in anti-tumor responses not established



Metastatic Melanoma: Sustained elevation of $CD4^{+}ICOS^{hi}$ T cells correlated with survival

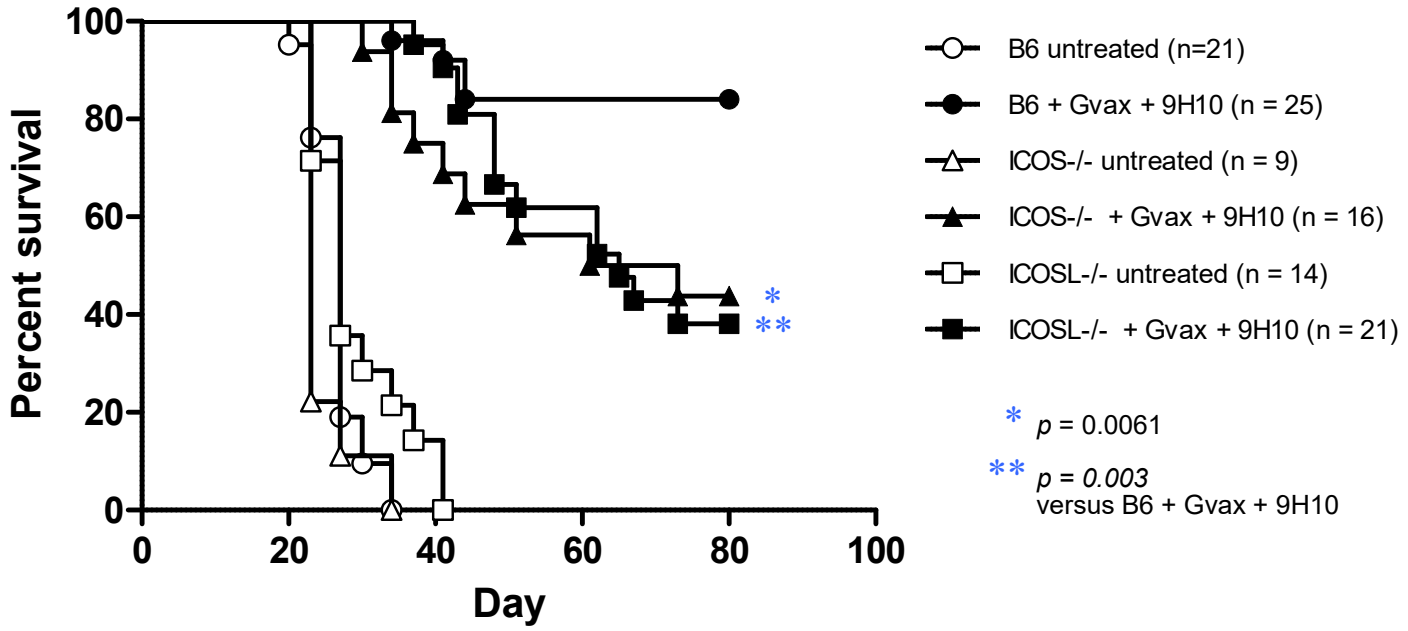


Carthon et al., *Clinical Cancer Research,* 2010

Hypothesis #1

The ICOS/ICOSL pathway is necessary for effective anti-tumor immune responses in the setting of anti-CTLA-4 therapy

ICOS/ICOSL pathway is necessary for optimal anti-tumor responses in the setting of CTLA-4 blockade



Fu et al., *Cancer Research, 2011*

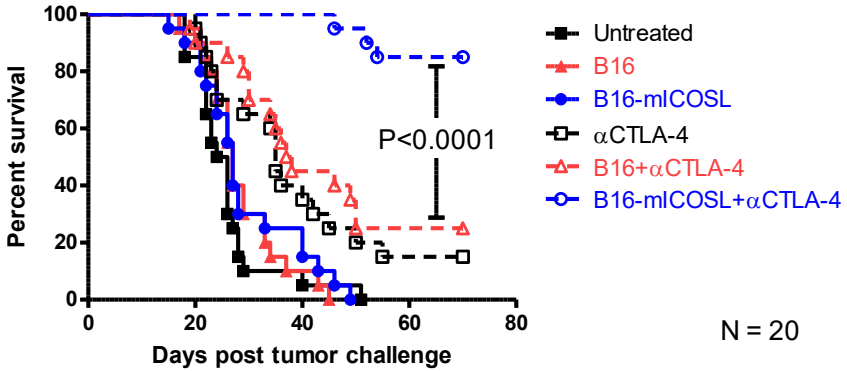
Hypothesis #2

The ICOS/ICOSL pathway can be targeted and developed as a combination therapy strategy with anti-CTLA-4 or other immunotherapies to improve anti-tumor responses

1) Agonistic anti-ICOS ab
2) ICOSL-Ig fusion protein
3) Tumor cell vaccine expressing ICOSL

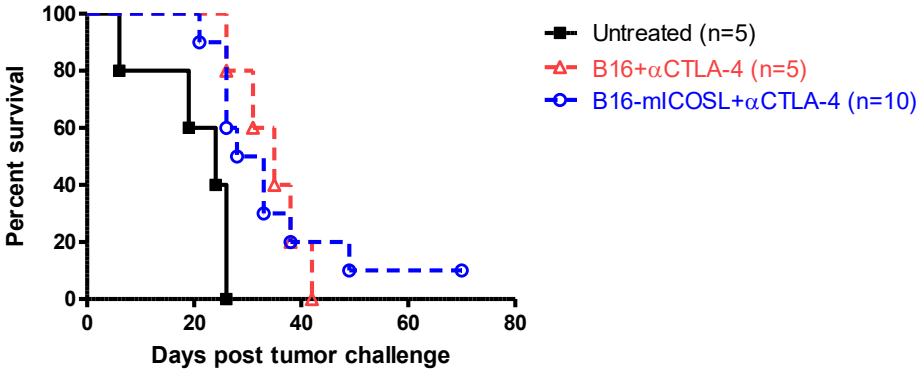
Targeting ICOS plus anti-CTLA-4 improves anti-tumor responses

WT



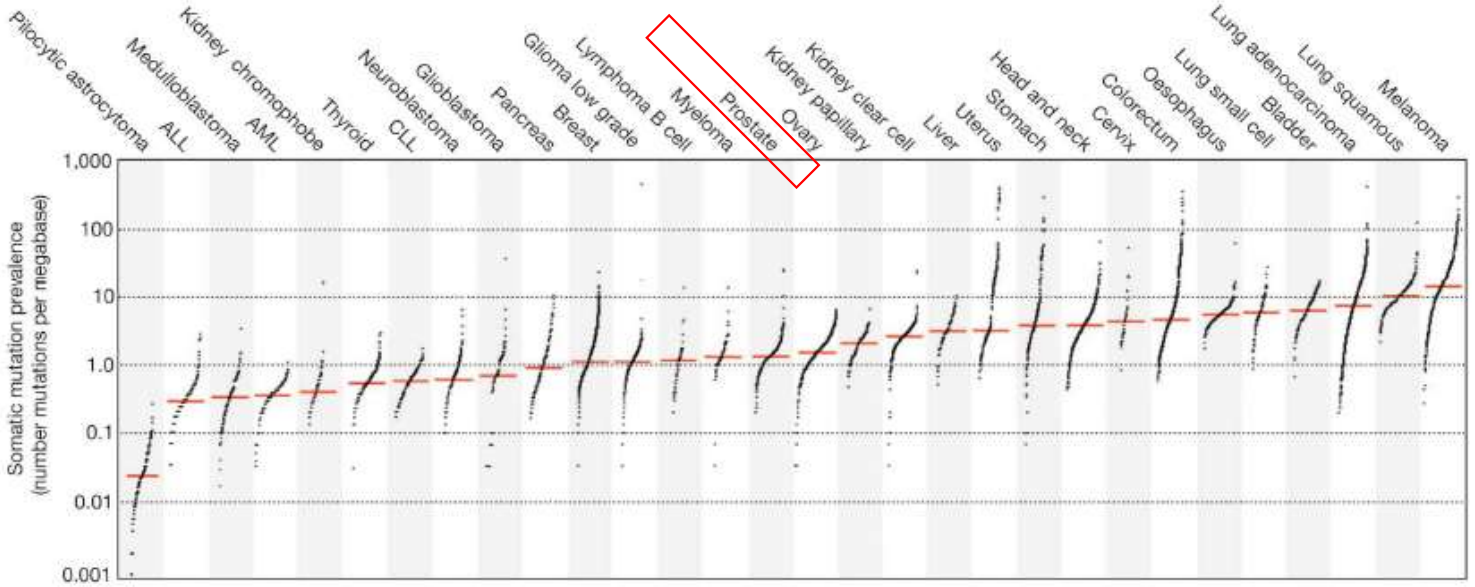
N = 20

ICOS KO



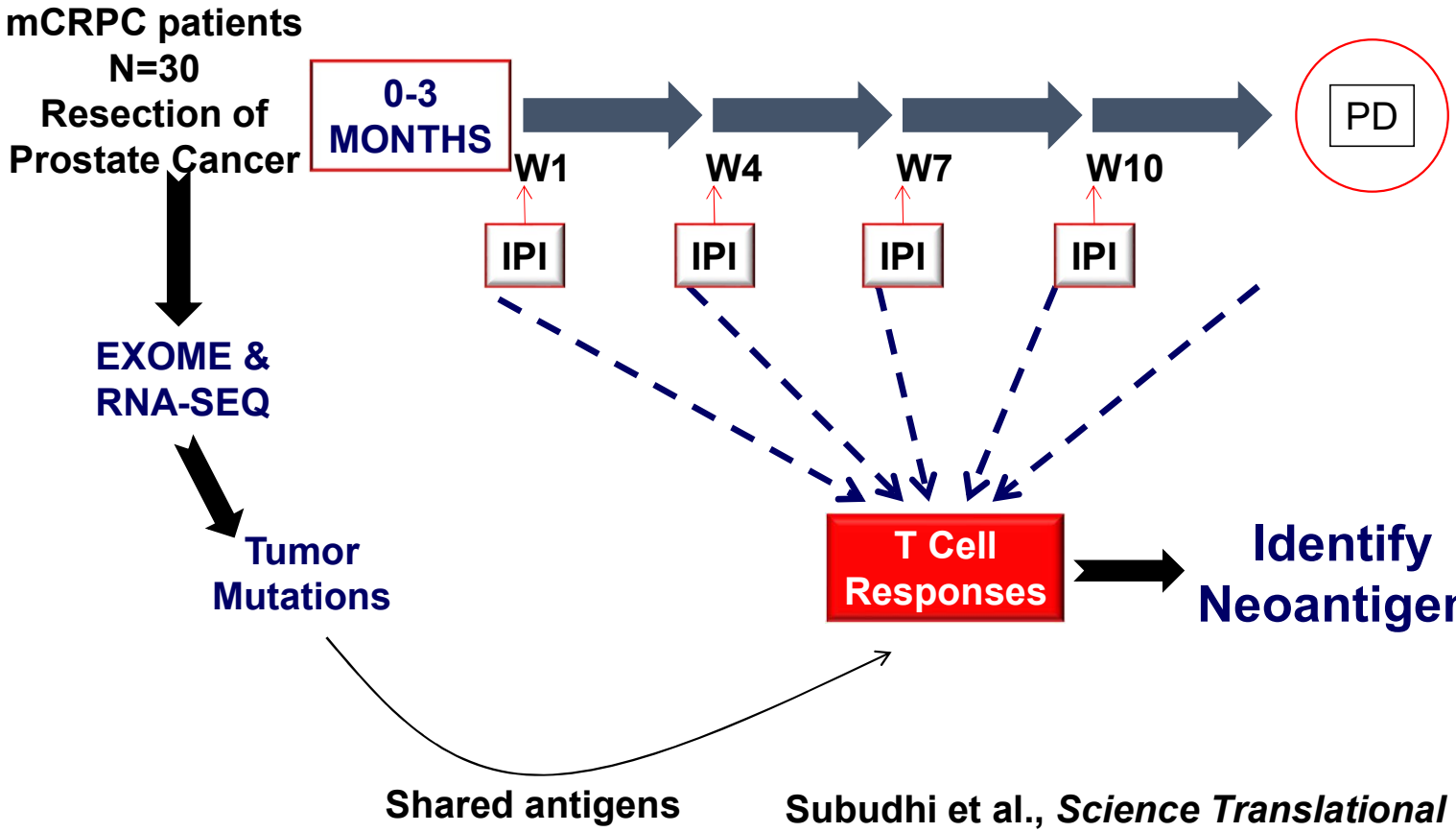
Fan et al., *JEM*, 2014

What about other tumors types?
Hot vs Cold?



Signatures of mutational processes in human cancer Alexandrov et al.
Nature Volume: 500,Pages:415–421Date published:(22 August 2013)DOI:doi:10.1038/nature12477

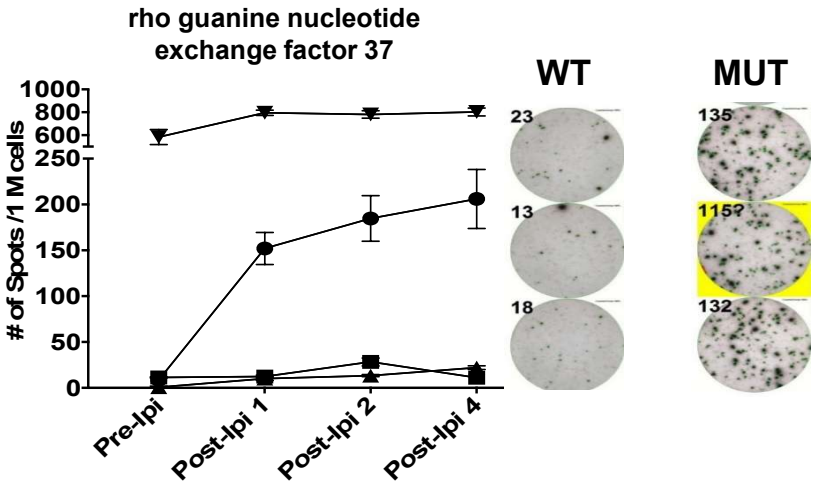
Can ipilimumab elicit T cell responses to PCa conventional and neoantigens?

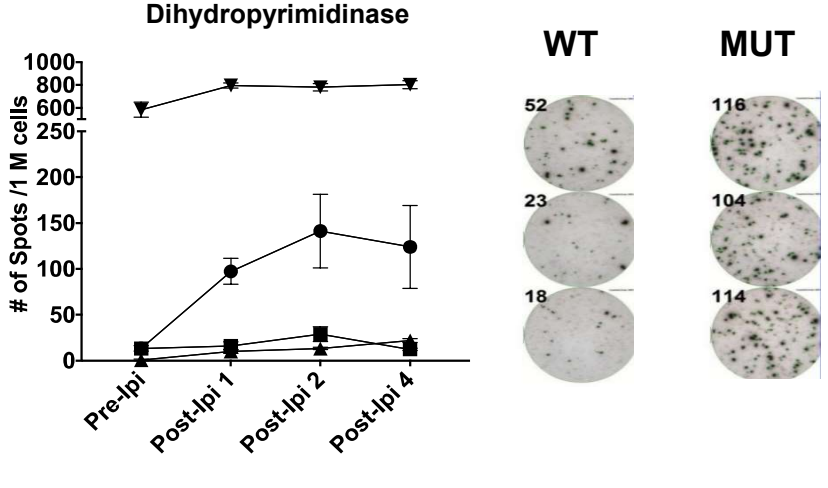


Subudhi et al., *Science Translational Medicine*, 2020

T cell responses to neoantigens in patient #7

Patient 7





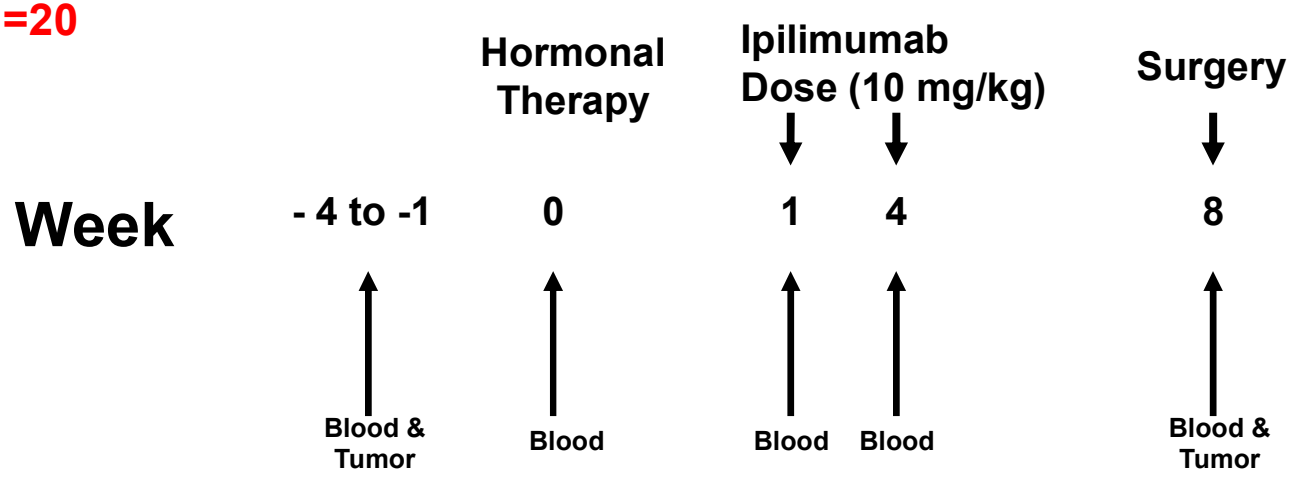
Peptide Name	Sequence
rho guanine nucleotide exchange factor 37 (WT)	H-GYVPSGFLARARSPVLWGWSLPS-OH
rho guanine nucleotide exchange factor 37 (MUT)	H-GYVPSGFLARAWSPVLWGWSLPS-OH

Peptide Name	Sequence
Dihydropyrimidinase (WT)	H-EDRMSVIWEKGVHSGKMDENRFV-OH
Dihydropyrimidinase (MUT)	H-EDRMSVIWEKGMHSGKMDENRFV-OH

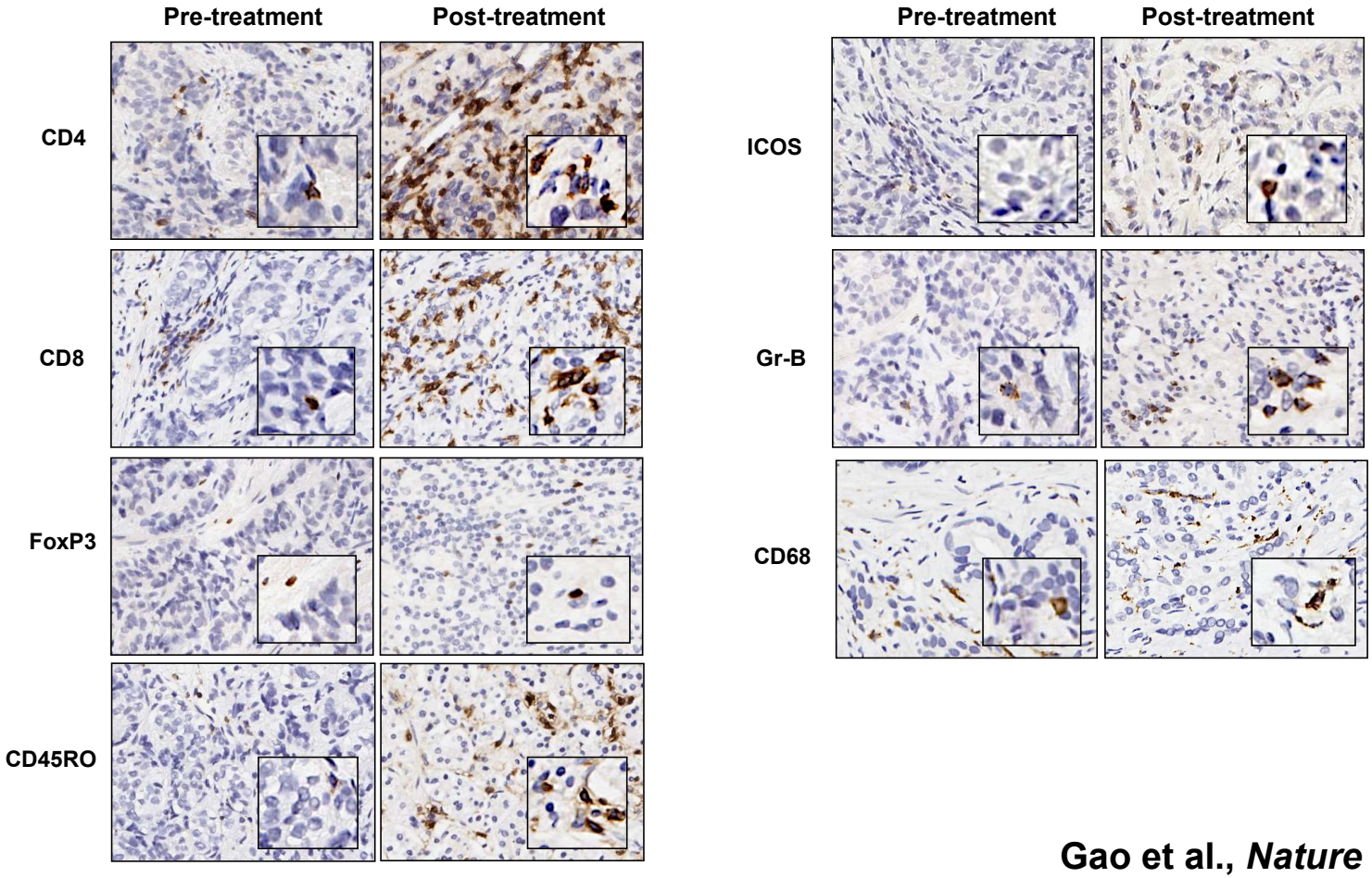
Mutations in prostate cancer can be recognized by T cells but are T cells infiltrating into prostate tumors?

Prostate cancer neoadjuvant (pre-surgical) study with anti-CTLA-4 (ipilimumab)

Clinical Trial Protocol 2009-0135
N=20

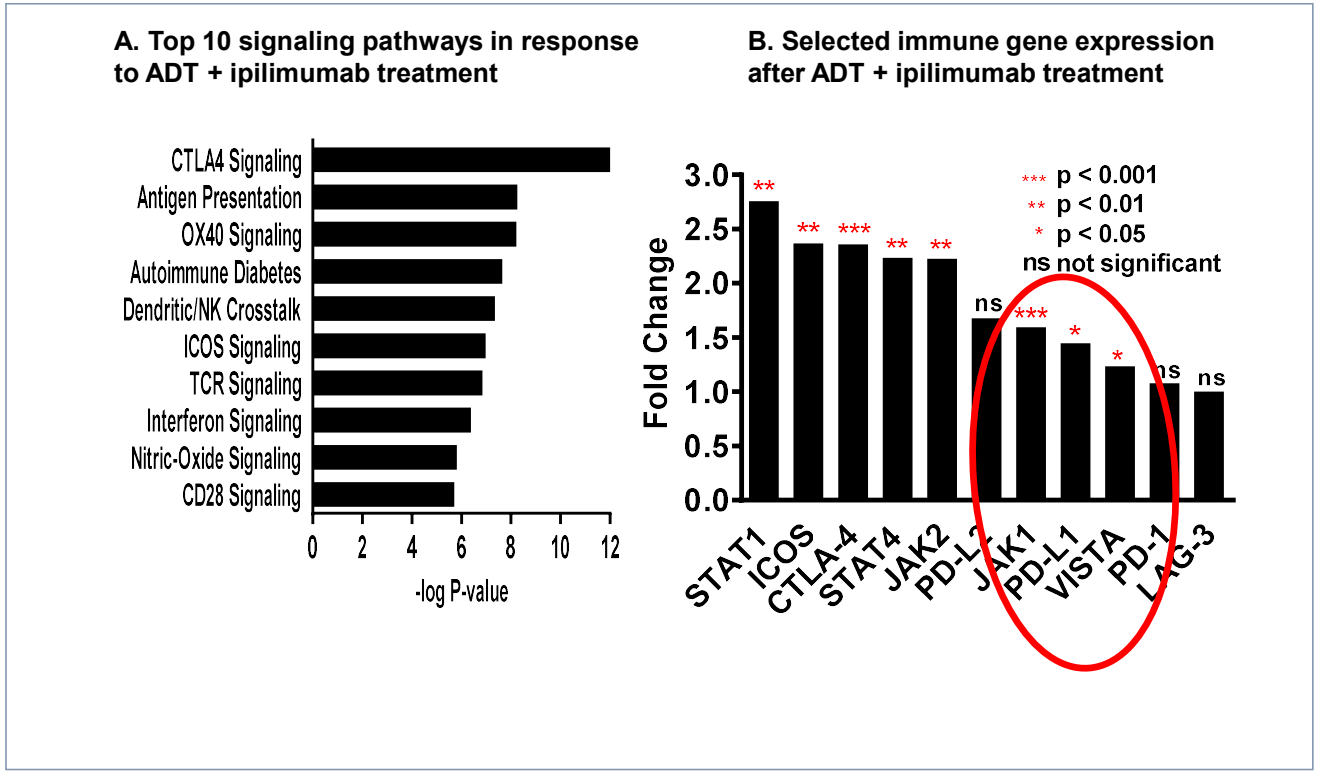


Converting a “cold” prostate tumor microenvironment to “hot”



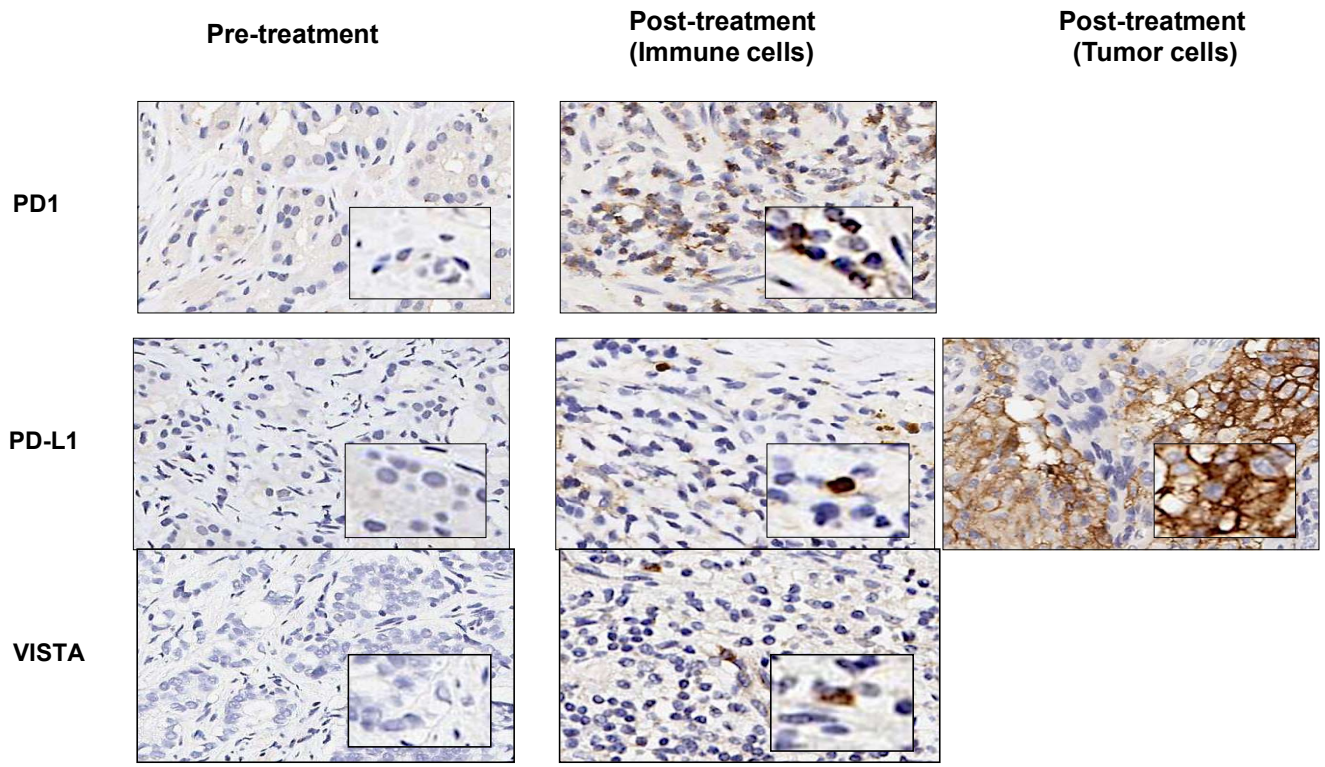
Gao et al., *Nature Medicine*, 2017

Immune response is dynamic and constantly evolving: Compensatory immune inhibitory pathways after treatment with immune checkpoint therapy



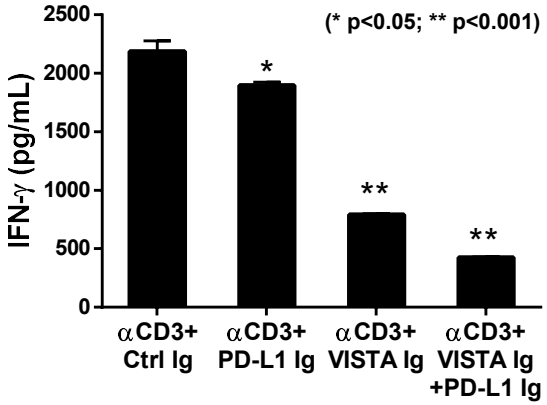
Gao et al., *Nature Medicine*, 2017

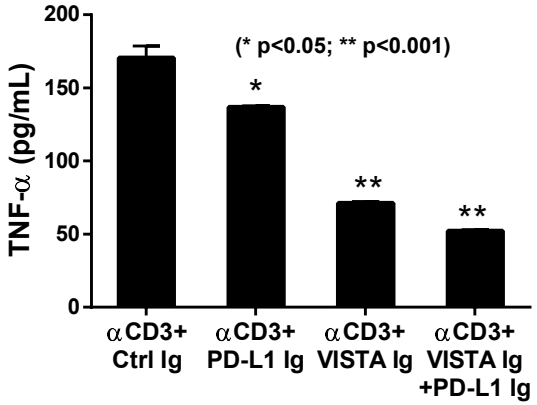
Compensatory inhibitory pathways in prostate tumor microenvironment



Gao et al., *Nature Medicine*, 2017

VISTA and PD-L1 are potent inhibitors of human T cell responses

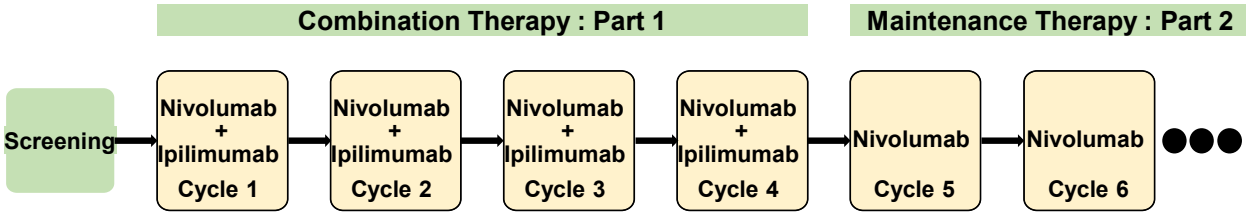




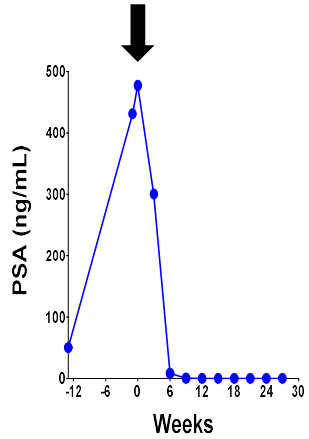
Gao et al., *Nature Medicine*, 2017

Anti-CTLA-4 (Ipi) + Anti-PD-1(Nivo) in patients with mCRPC

N=90



Expanding study to larger Phase II with 4 treatment arms

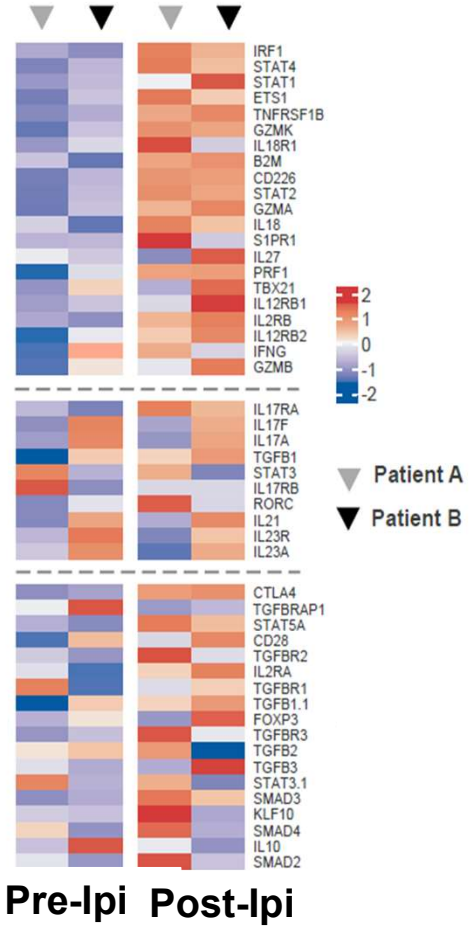


Sharma et al., *Cancer Cell*, 2020

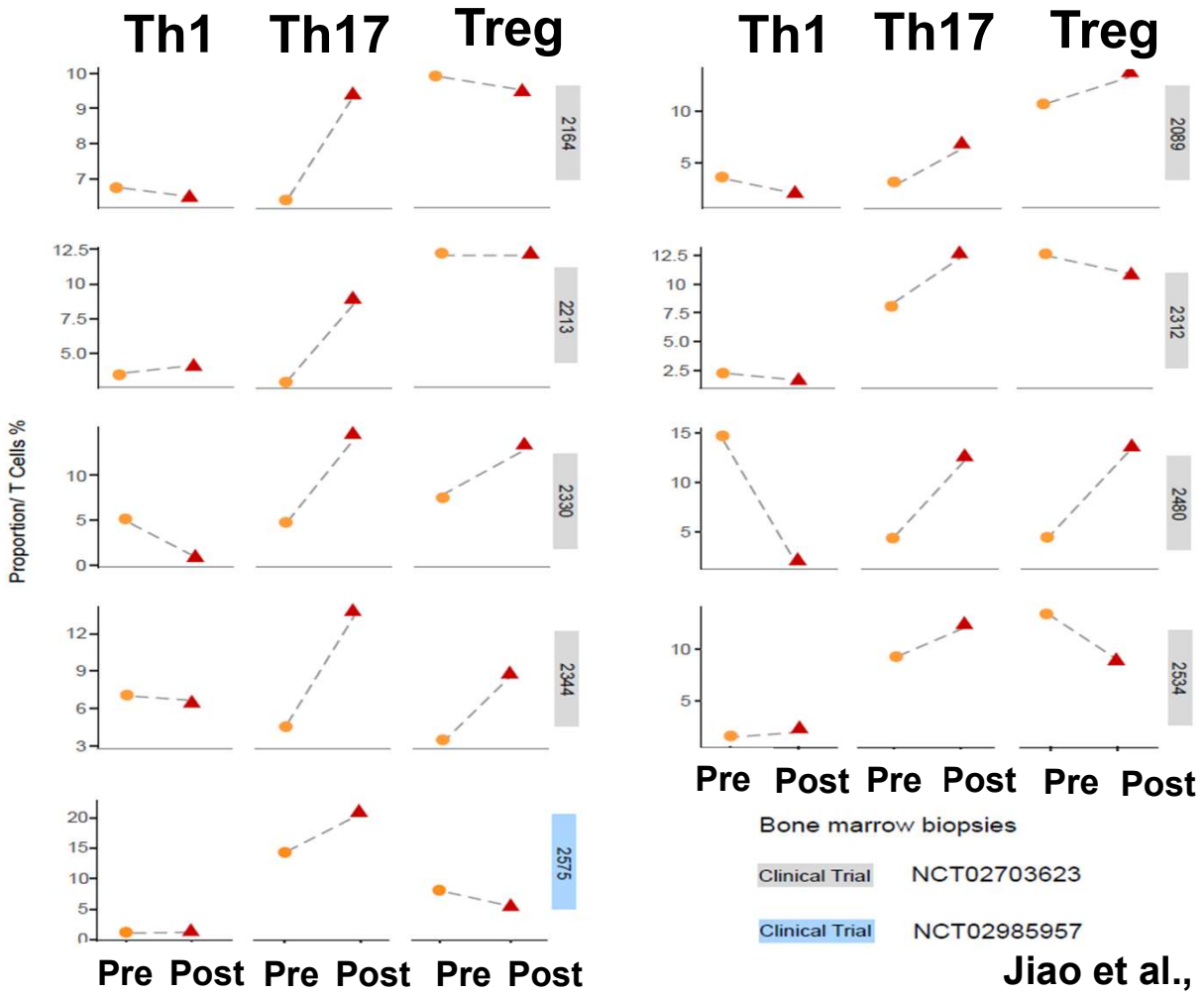
Why do clinical responses occur less frequently in patients with bone metastases?

Different T cell subsets in soft tissue vs bone mets after ICT

Prostate Ca: Soft Tissue Mets

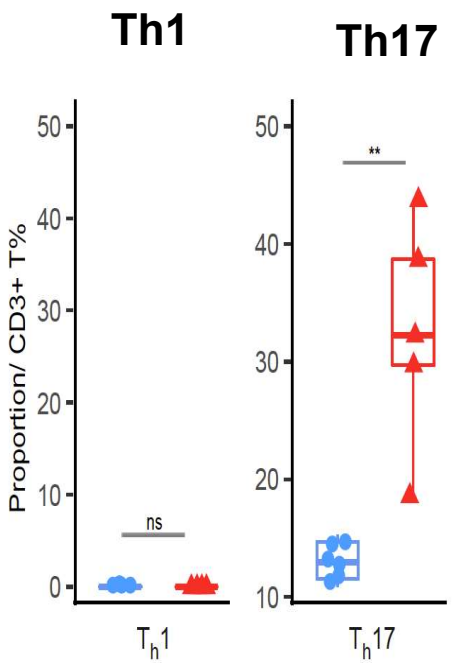


Prostate Ca: Bone Metastases

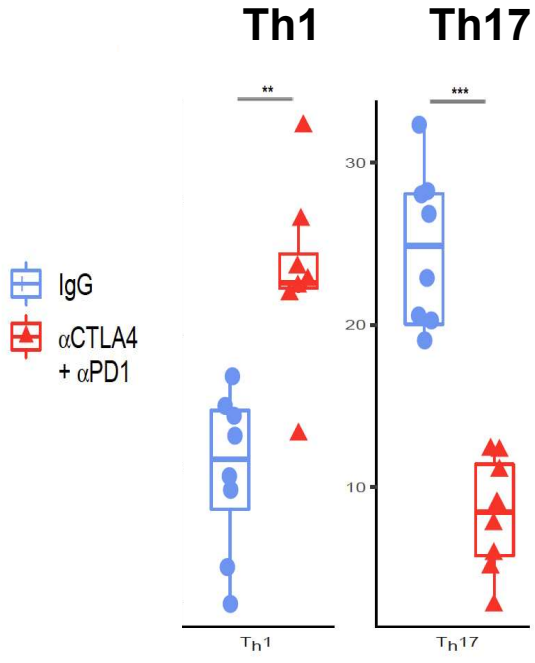


Jiao et al., *Cell*, 2019

Immunologic subsets dictated by organ-specific microenvironment



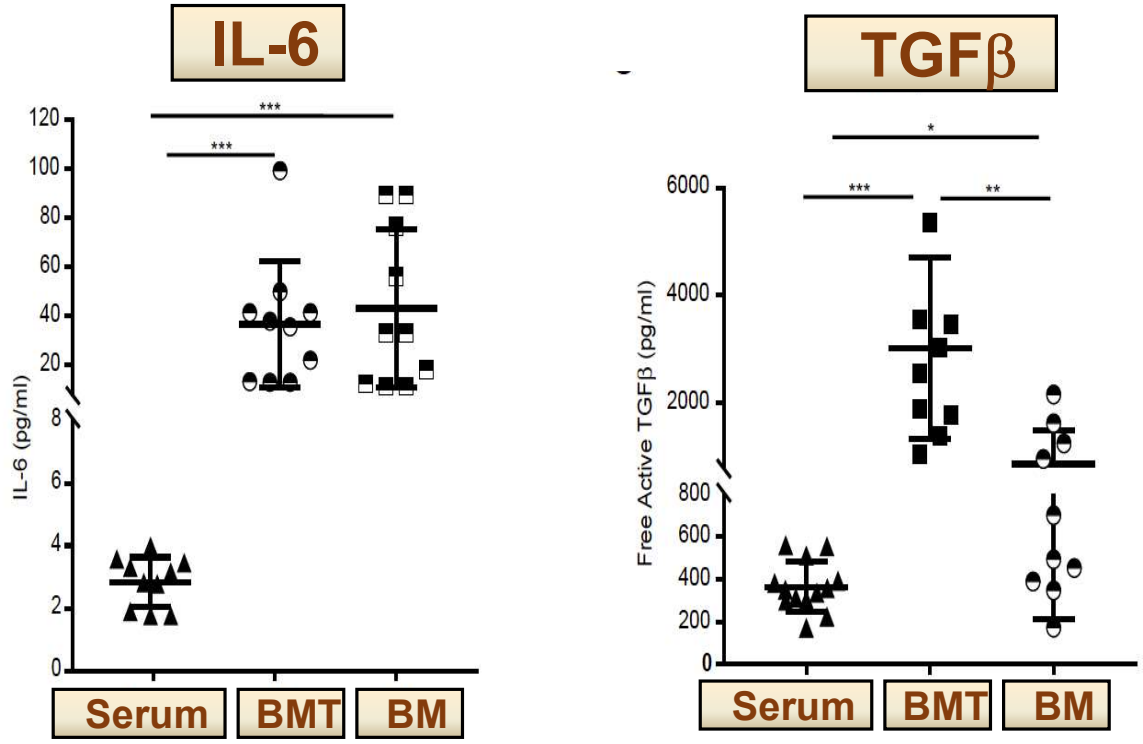
CD4 T cell subsets in bone-CRPC



CD4 T cell subsets in subcutaneous CRPC

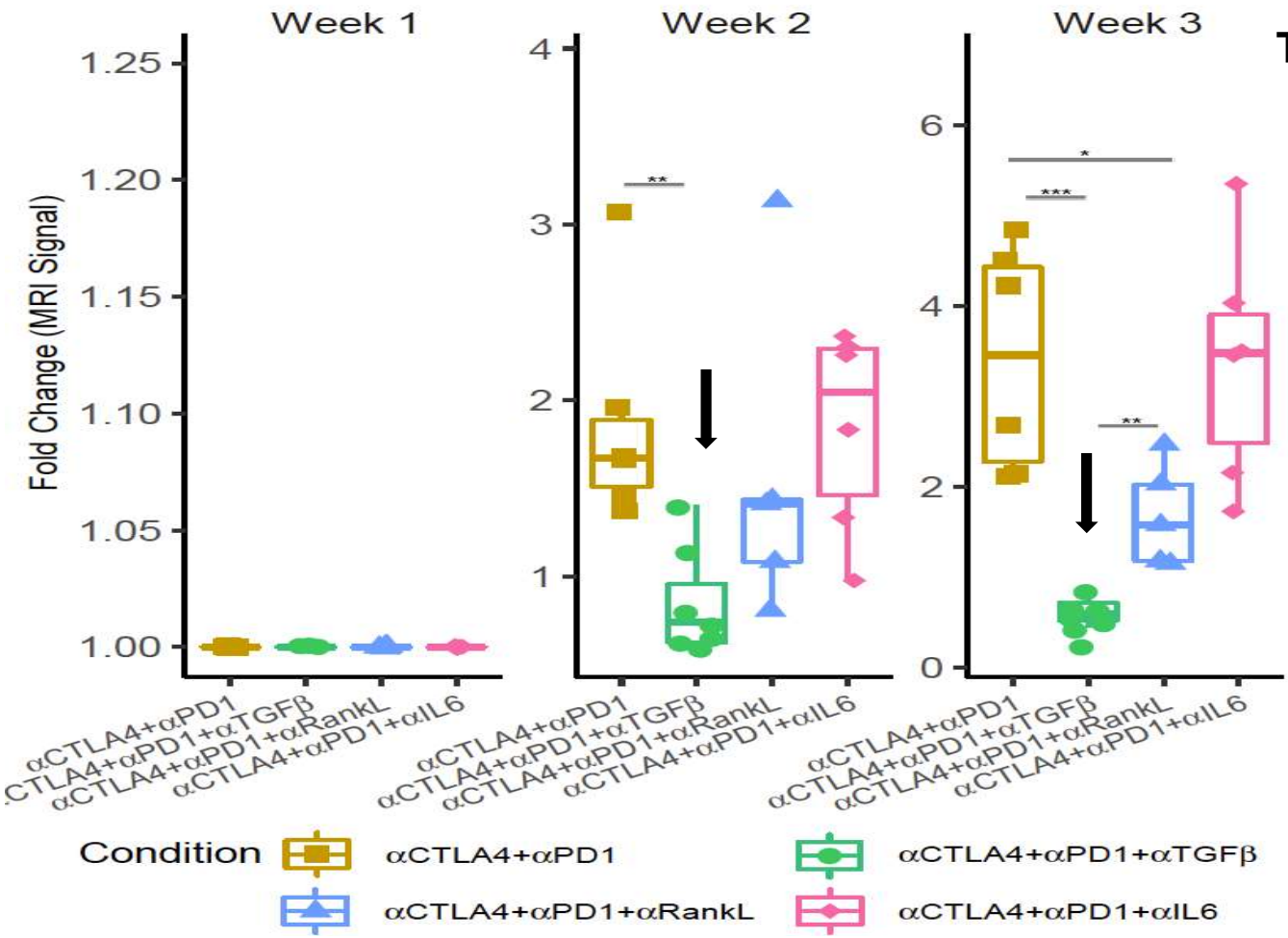
Jiao et al., *Cell*, 2019

Elevated levels of IL-6 and TGFβ are present in bone



Jiao et al., *Cell*, 2019

Combination of ICT + anti-TGFβ leads to tumor regression in bone

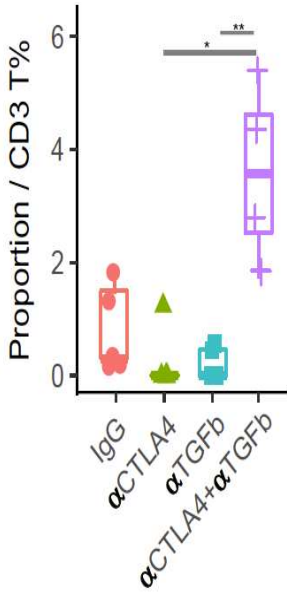


Tumor volume

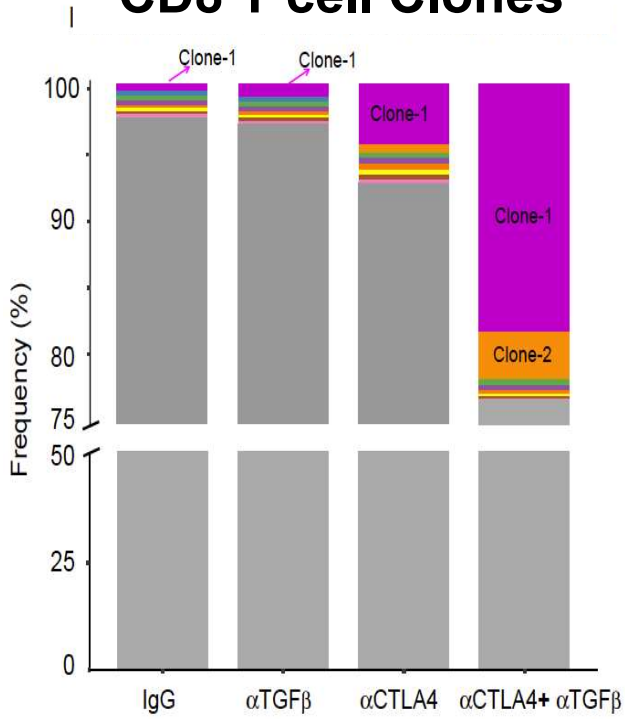
Jiao et al., *Cell*, 2019

Combination of ICT + anti-TGFβ leads to an increase in frequency of Th1 CD4 cells and clonal expansion of CD8 T cells

Th1 CD4 cells

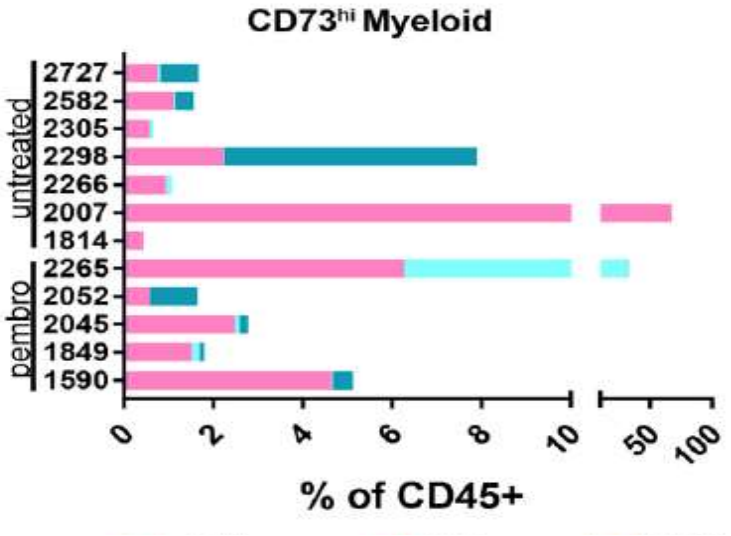


CD8 T cell Clones



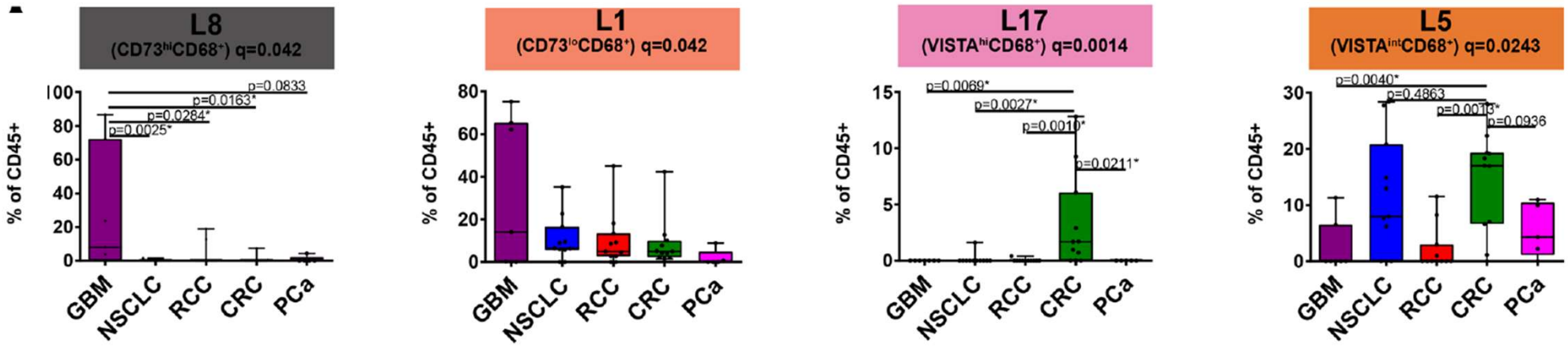
Jiao et al., *Cell*, 2019

Identifying unique subsets in other tumor niches: GBM



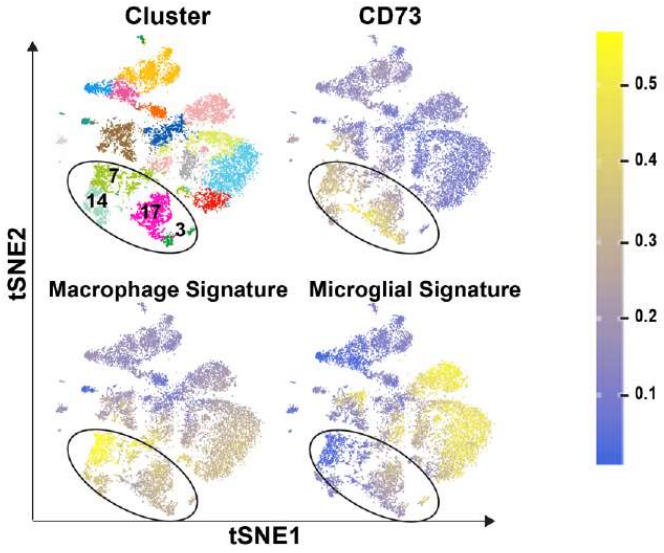
Untreated GBM Patients

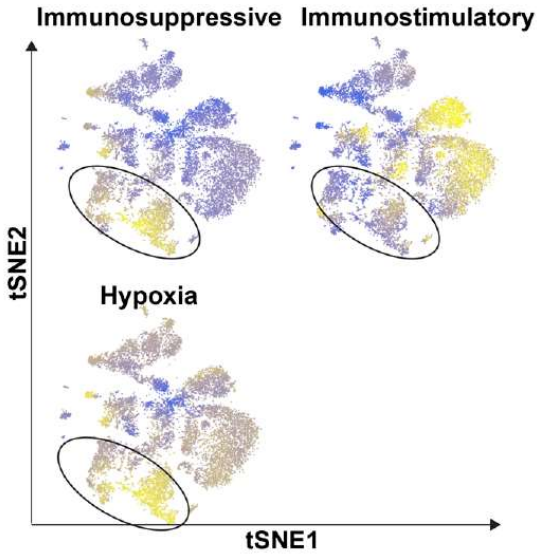
Anti-PD-1 Treated GBM Patients



Goswami et al., *Nature Medicine*, 2020

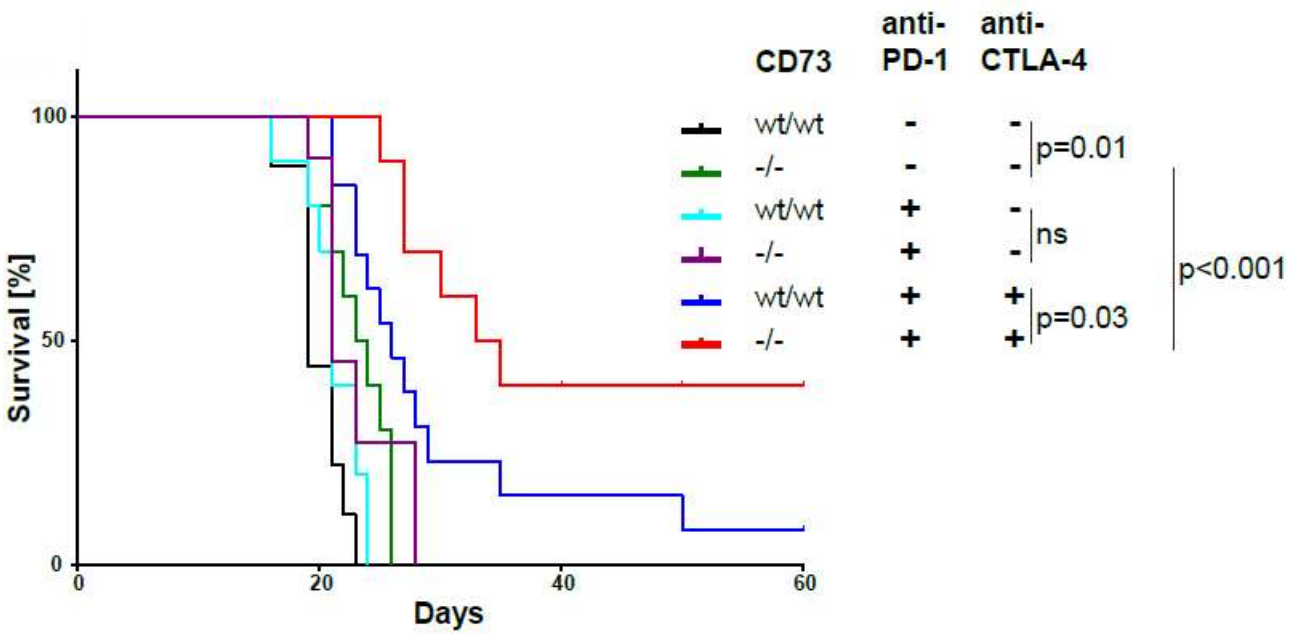
scRNASeq demonstrated immunosuppressive signature for CD73 subset





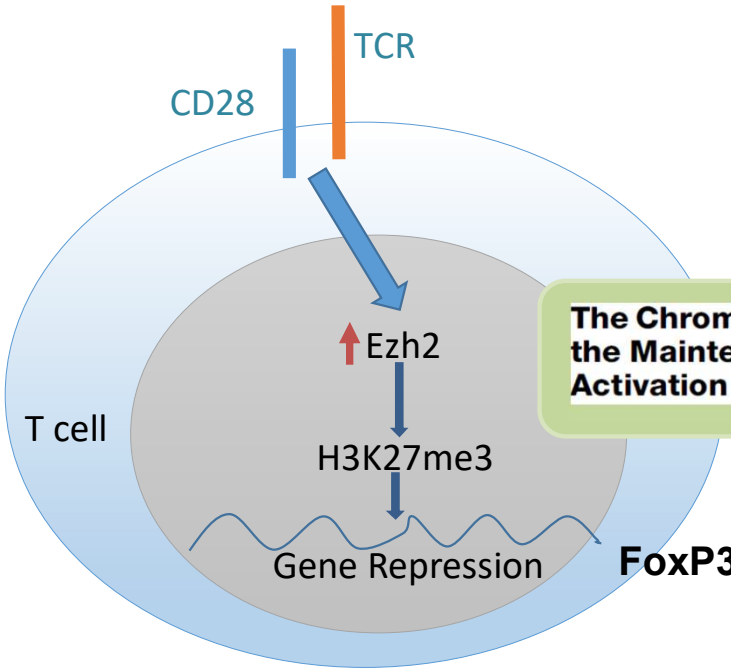
Goswami et al., *Nature Medicine*, 2020

Improved tumor rejection and survival with ICT in absence of CD73



Goswami et al., *Nature Medicine*, 2020

Other Targets: Epigenetic Pathways

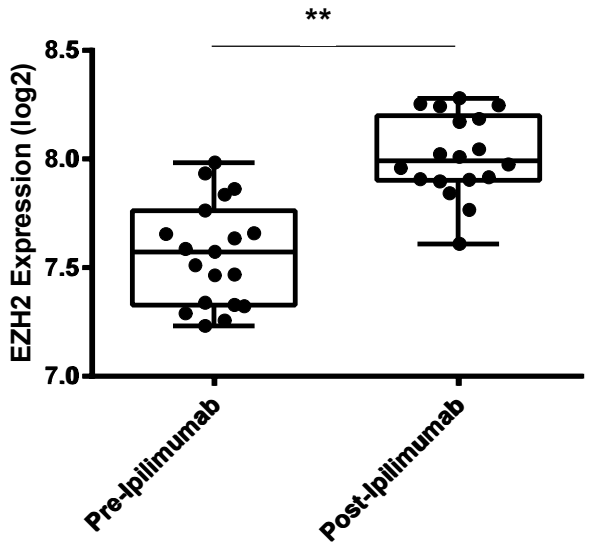


The Chromatin-Modifying Enzyme Ezh2 Is Critical for the Maintenance of Regulatory T Cell Identity after Activation

DuPage et al., 2015, Immunity *42*, 227–238

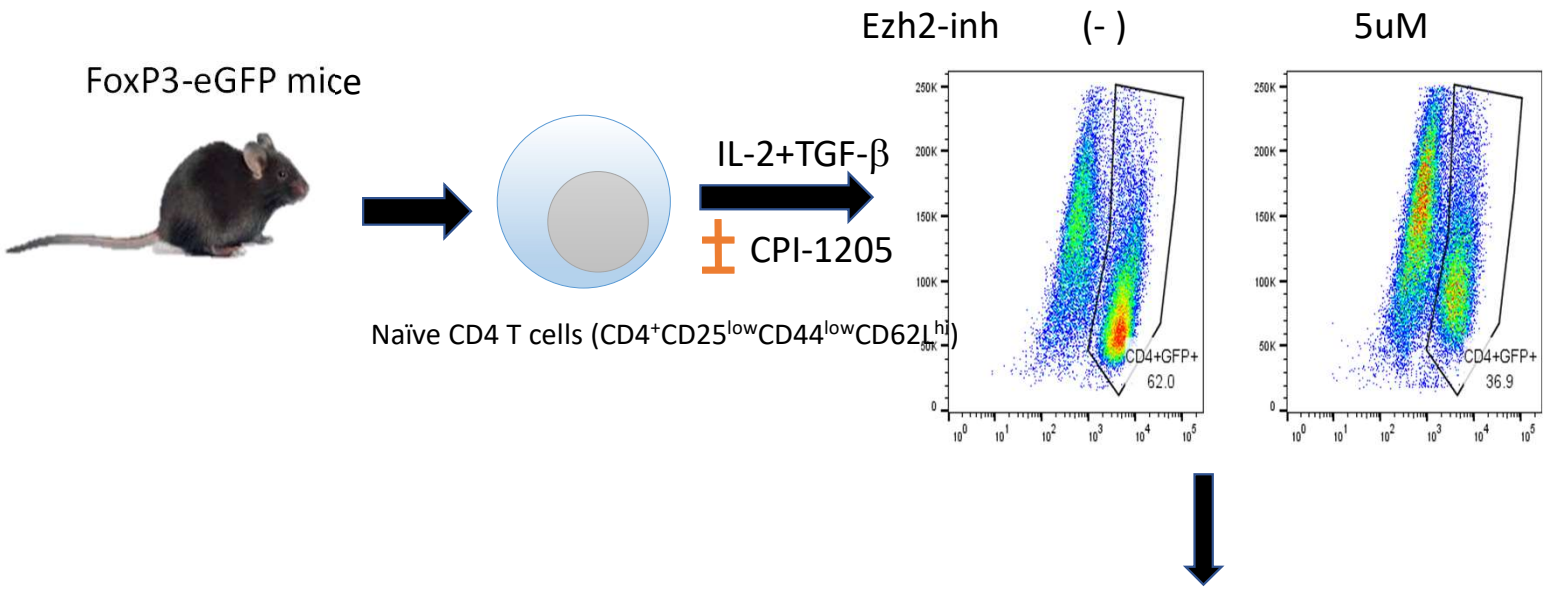
FoxP3 Stabilization

Anti-CTLA-4 therapy increases EZH2 expression in CD4 T cells from treated patients



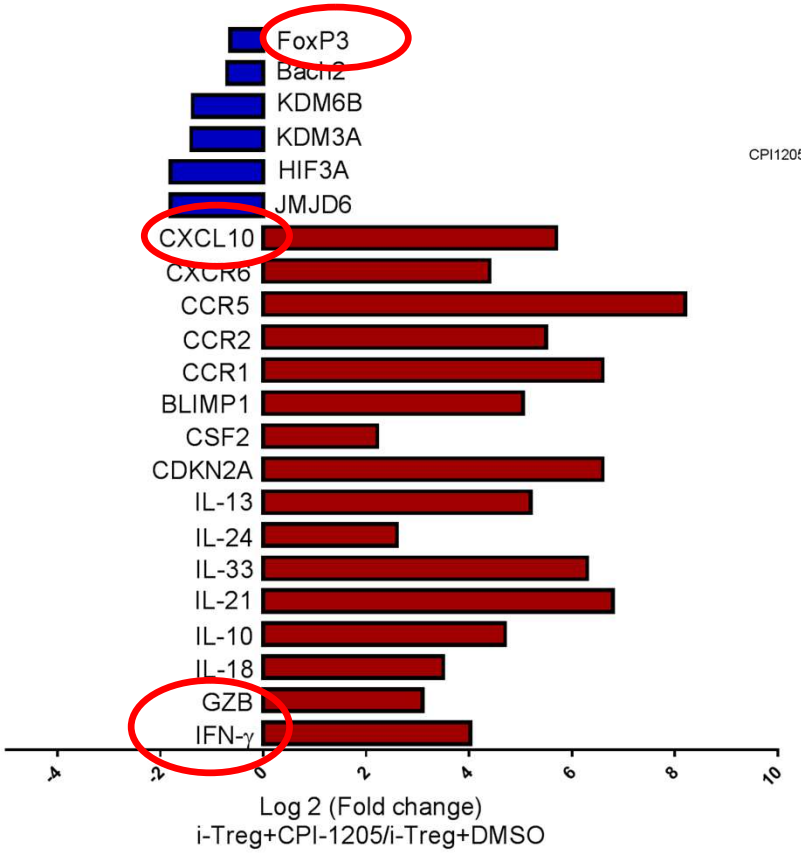
Goswami et al, *JCI*, 2018

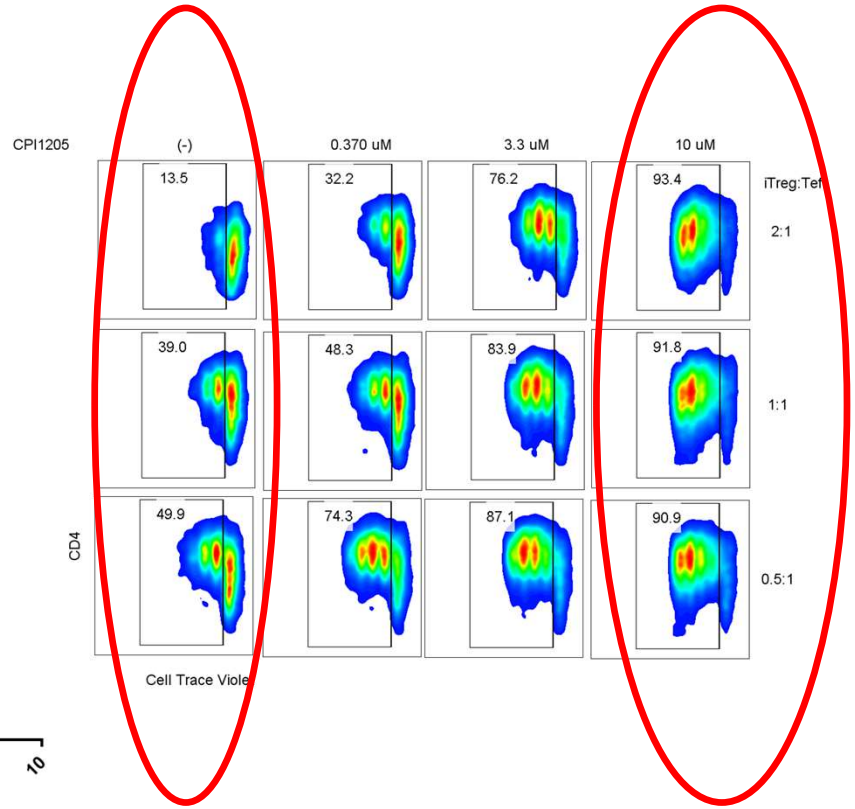
Transcriptional profiling of i-Treg following Ezh2 inhibition



Goswami et al, *JCI*, 2018

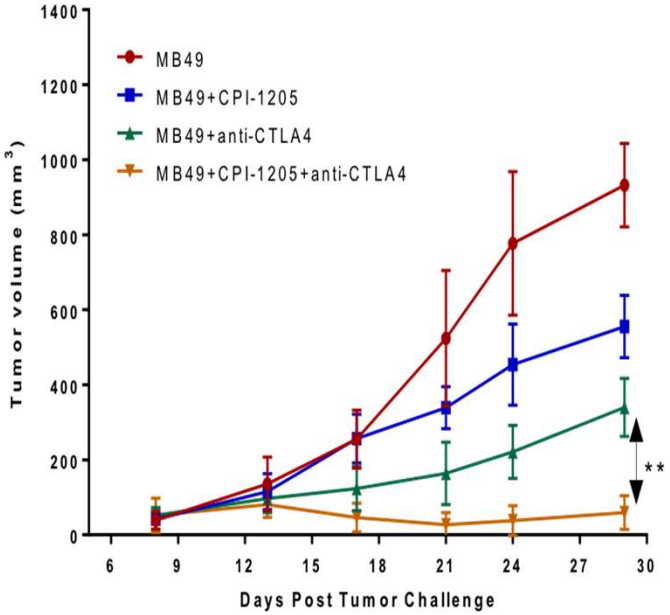
EZH2 inhibition impacts phenotype and function of Tregs

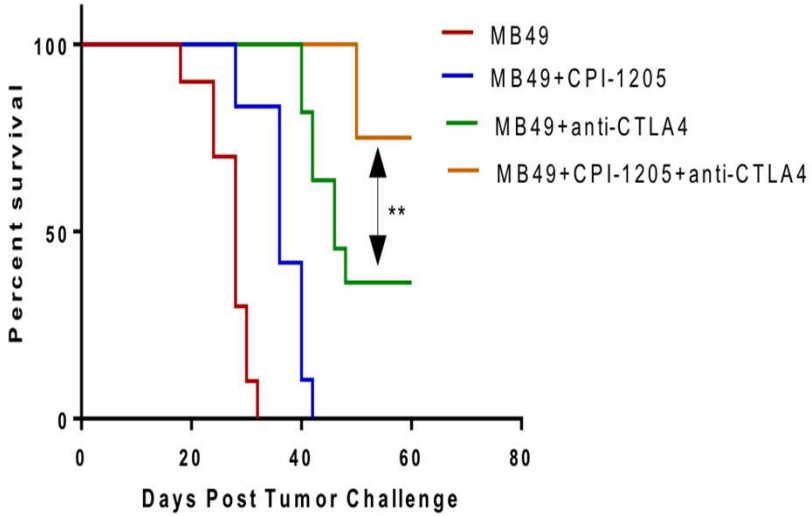




Goswami et al, *JCI*, 2018

Inhibiting EZH2 to improve anti-tumor responses

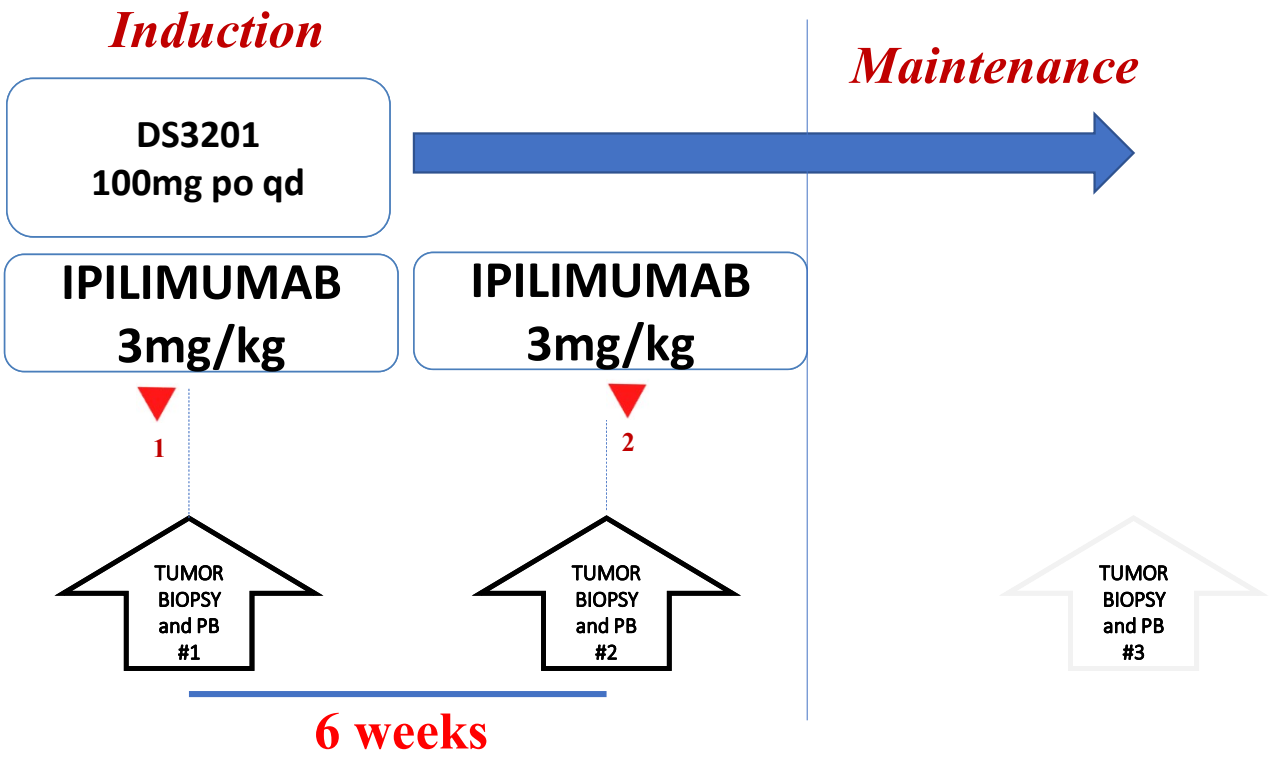




Goswami et al, *JCI*, 2018

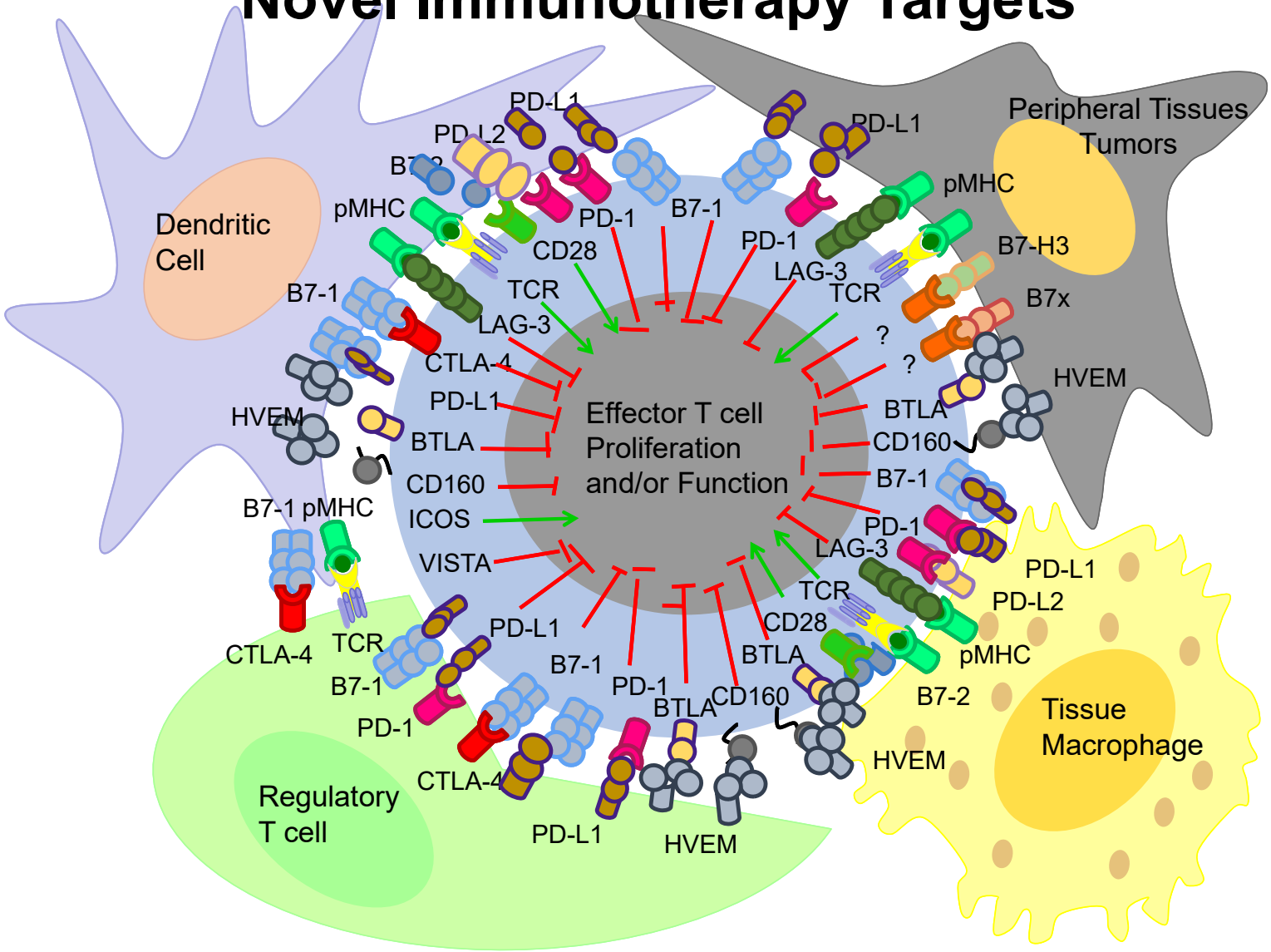
From the Clinic to the Lab and Back to the Clinic
IRB-approved protocol 2019-0967: EZH1/2i plus Ipi

Enrolling patients with metastatic prostate ca (N=15), bladder ca (N=15) and RCC (N=15). 3 patients treated to date.



DS3201-Ipilimumab Clinical Trial Schema.

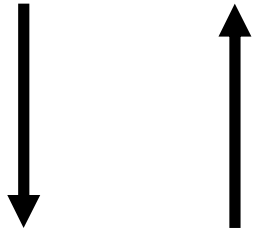
Novel Immunotherapy Targets



Immunotherapy Platform at M. D. Anderson

Cancer Immunotherapy Clinical Trials

PA13-0291: Umbrella lab protocol, which enables collection of samples from all patients at MDACC for immune monitoring studies



Laboratory Interrogation

As of September 2020:

1) participating in >100 ongoing clinical trials, across 18 MDACC departments

2) > 4000 patients have been enrolled to date

3) Longitudinal samples collected with >7000 blood samples (> 40,000 tubes of blood) and >4000 fresh solid tumor tissue samples and >1000 hematologic tumor samples have been collected and analyzed

4) >40,000 tumor tissue slides evaluated for immune infiltration

Conclusions

- **Immune checkpoint therapy has joined the ranks of surgery, radiation and chemotherapy as a pillar of cancer treatment: combination strategies are the future.**

- **Multiple immune checkpoints exist and are dynamic in their expression; therefore, they should be evaluated in *both* pre- and on-treatment human tumor samples to guide therapeutic decisions.**

- **The organ-specific microenvironment will need to be considered in order to understand immunologic subsets and subsequent immune responses against cancer cells in these organs.**

- **Pre-surgical and tissue-based clinical trials provide a feasible platform to study biologic effects in patients, which provide insights into mechanisms that can be targeted for rational combination therapies.**

- **Sharma Lab Team**
  - Derek Ng Tang
  - Liangwen Xiong
  - Salah Tahir
  - Sangeeta Goswami
  - Swetha Anandhan
  - Jielin Liu
  - JJ Gao
  - Sumit Subudhi

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MDACC Bladder SPORE
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- **Protocol and data managers and research nurses**
- And, the **PATIENTS!**