Immune Gene Expression as a Discovery Tool for Actionable Immunotherapy Resistance Mechanisms

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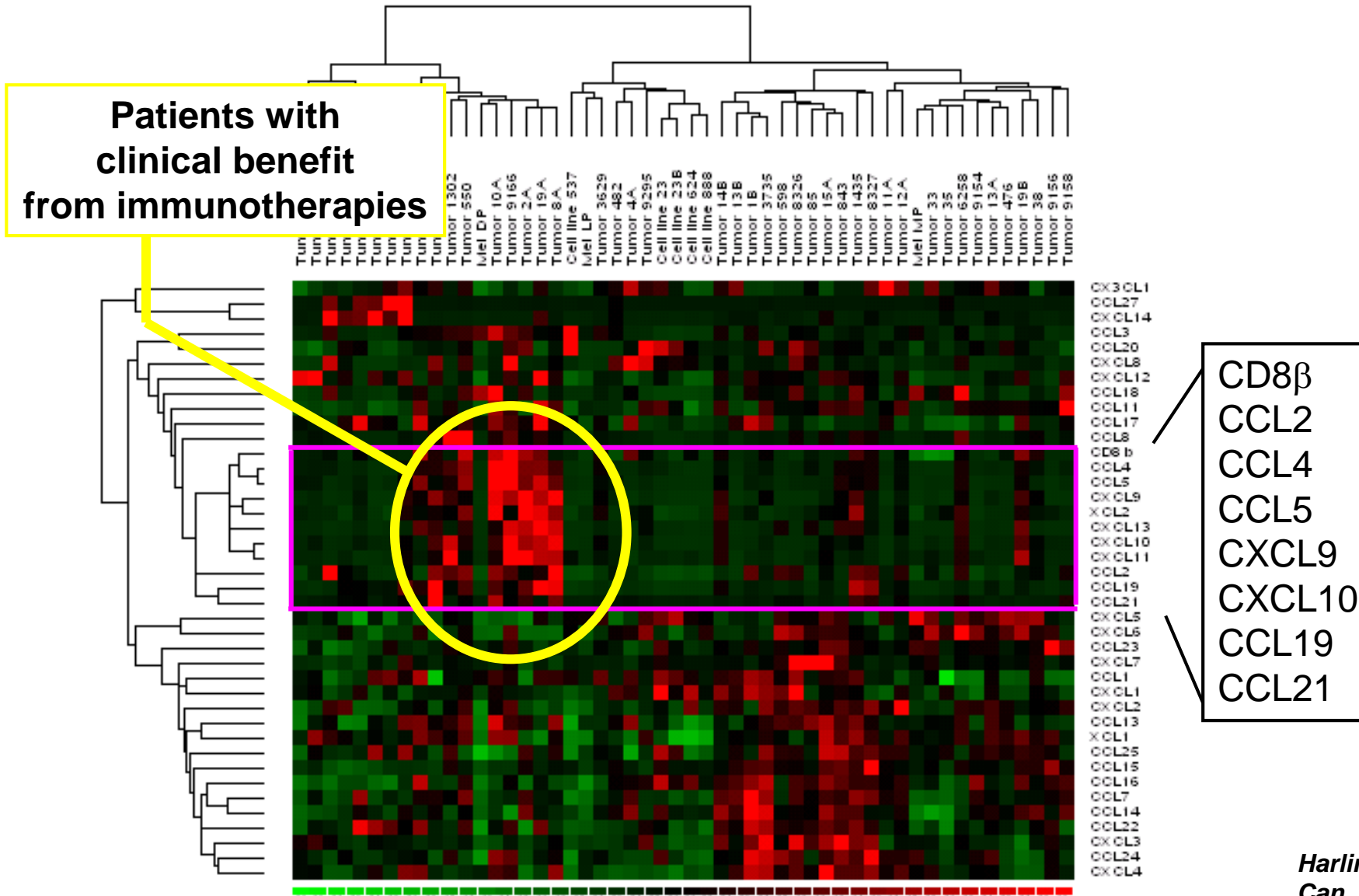
@RandySweisMD

Disclosures

- **Consulting:** AstraZeneca, BMS, Eisai, Exelixis, Puma, Mirati, Merck

- **Honoraria/speaking**: Medscape, AstraZeneca, BMS, Exelixis

- **Grant/Research support (to institution):** Bayer, BMS, Eisai, Eli Lilly, EpiVax Oncology, Evelo, Genentech, CytomX, Merck

- I will not discuss off-label use of any product

Objectives

- Review the T cell-inflamed tumor microenvironment as a paradigm for current investigation

- Highlight uses of this tool to discover actionable mechanisms of resistance

- Explore emerging data on the characterization of cancer immune phenotypes

Expression of a subset of chemokine genes is associated with presence of CD8⁺ T cells in melanoma metastases


Patients with clinical benefit from immunotherapies

CD8β
CCL2
CCL4
CCL5
CXCL9
CXCL10
CCL19
CCL21

Harlin et al.
Can. Res. 2009

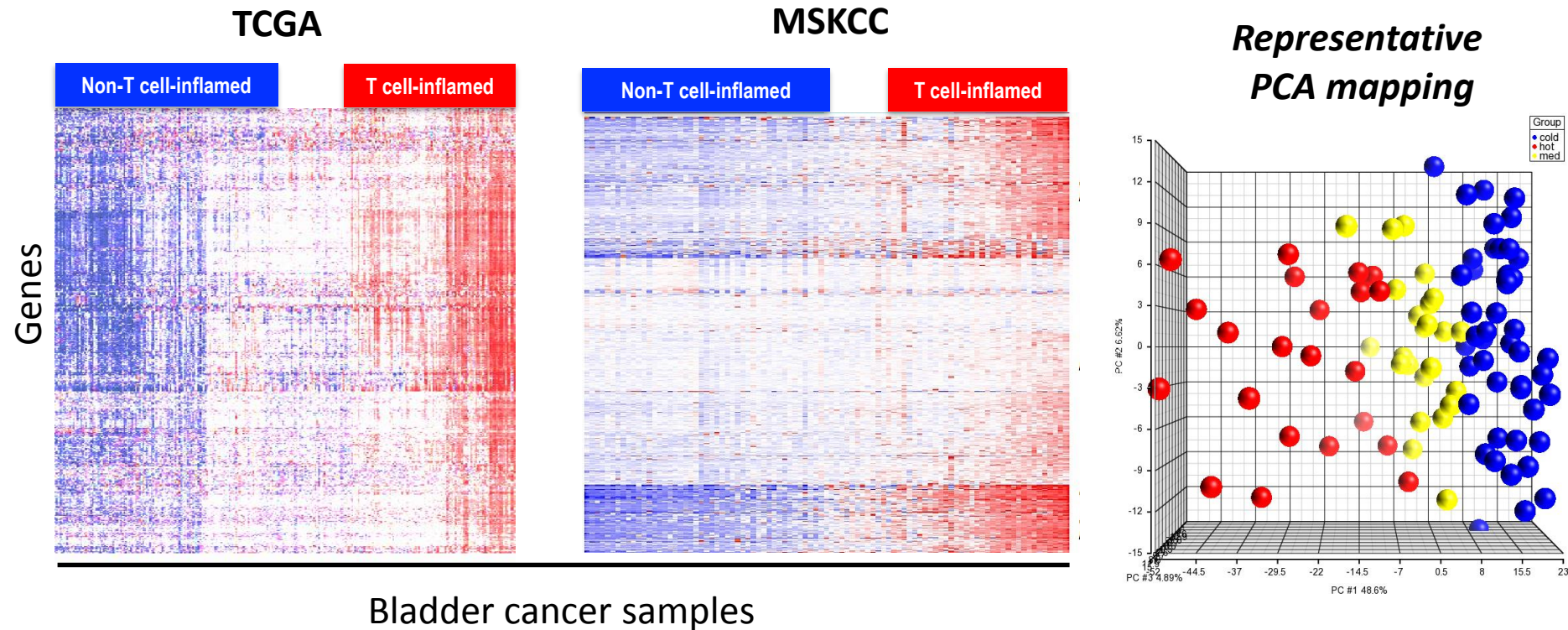# Immune phenotypes can be identified by gene expression profiling using RNA seq

Immune Gene Signature

CD8A, CCL2, CCL3, CCL4, CXCL9, CXCL10, ICOS, GZMK HLA-DMA, HLA-DMB, HLA-DOA & HLA-DOB

- The Cancer Genome Atlas (TCGA): Over 30 tumor types
  - Over 10,000 samples with publically available data
  - Bladder cancer cohort: >400 samples including multi-omics data

- Immune gene expression signature derived from prior studies used to characterize T cell-inflamed phenotype
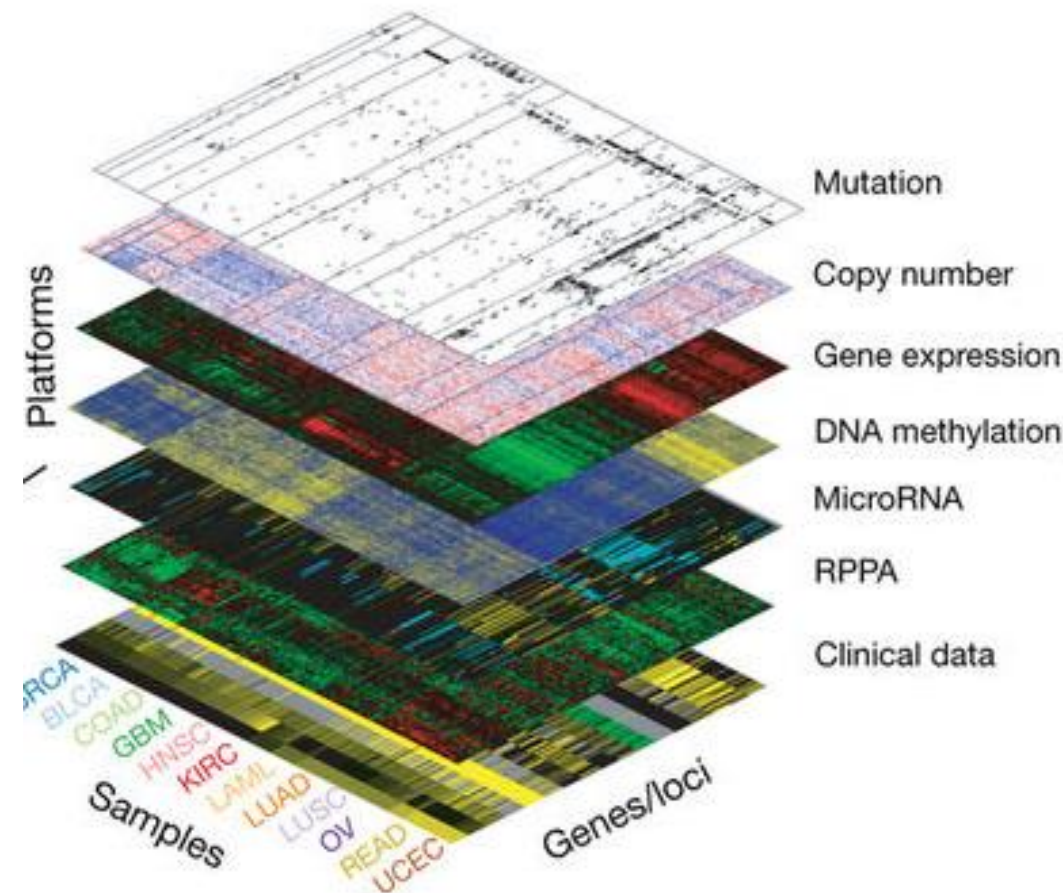
Immunophenotypes can be identified by gene expression profiling across multiple datasets

TCGA

Non-T cell-inflamed   T cell-inflamed

MSKCC

Non-T cell-inflamed   T cell-inflamed

Representative PCA mapping

Genes

Bladder cancer samples

Immunophenotypes can be identified by gene expression profiling across multiple cancers

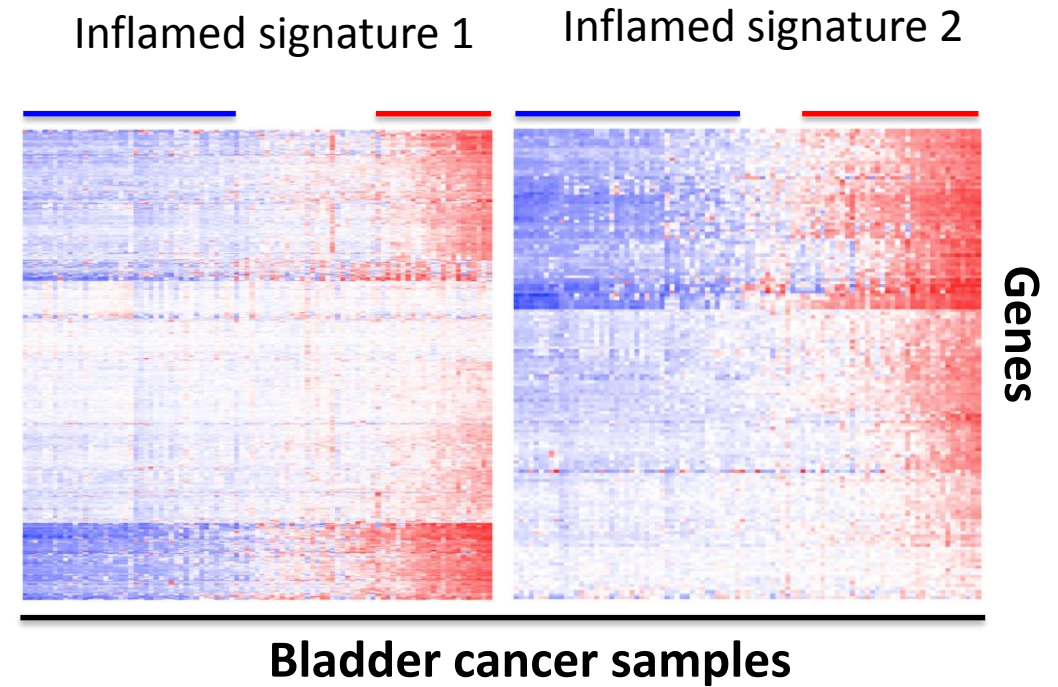*Luke, Bao, Sweis, et al Clin Cancer Res. 2019*
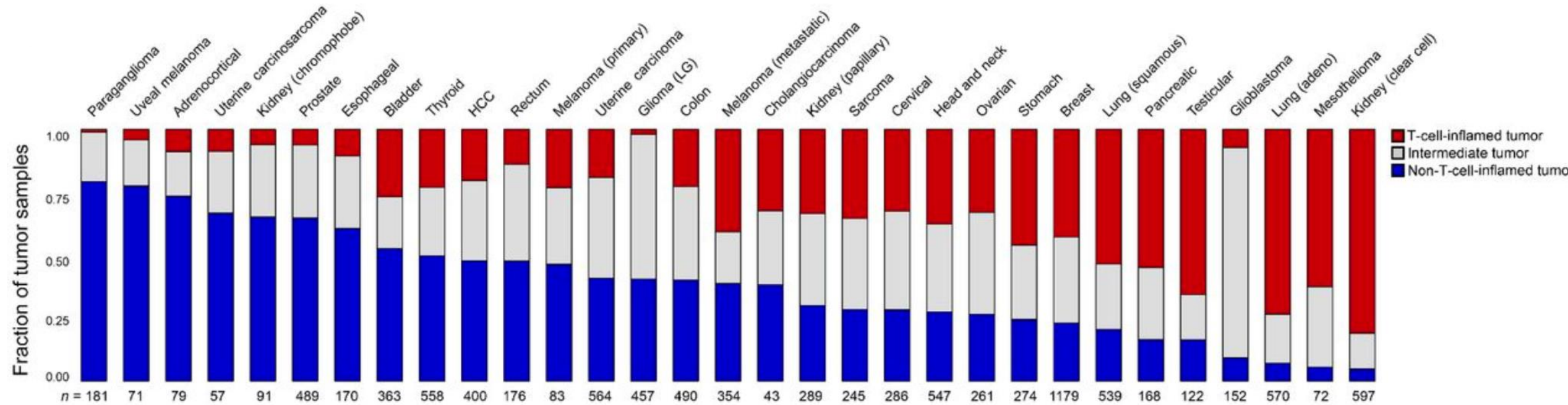
T cell-inflamed tumors have higher expression of immune inhibitory markers
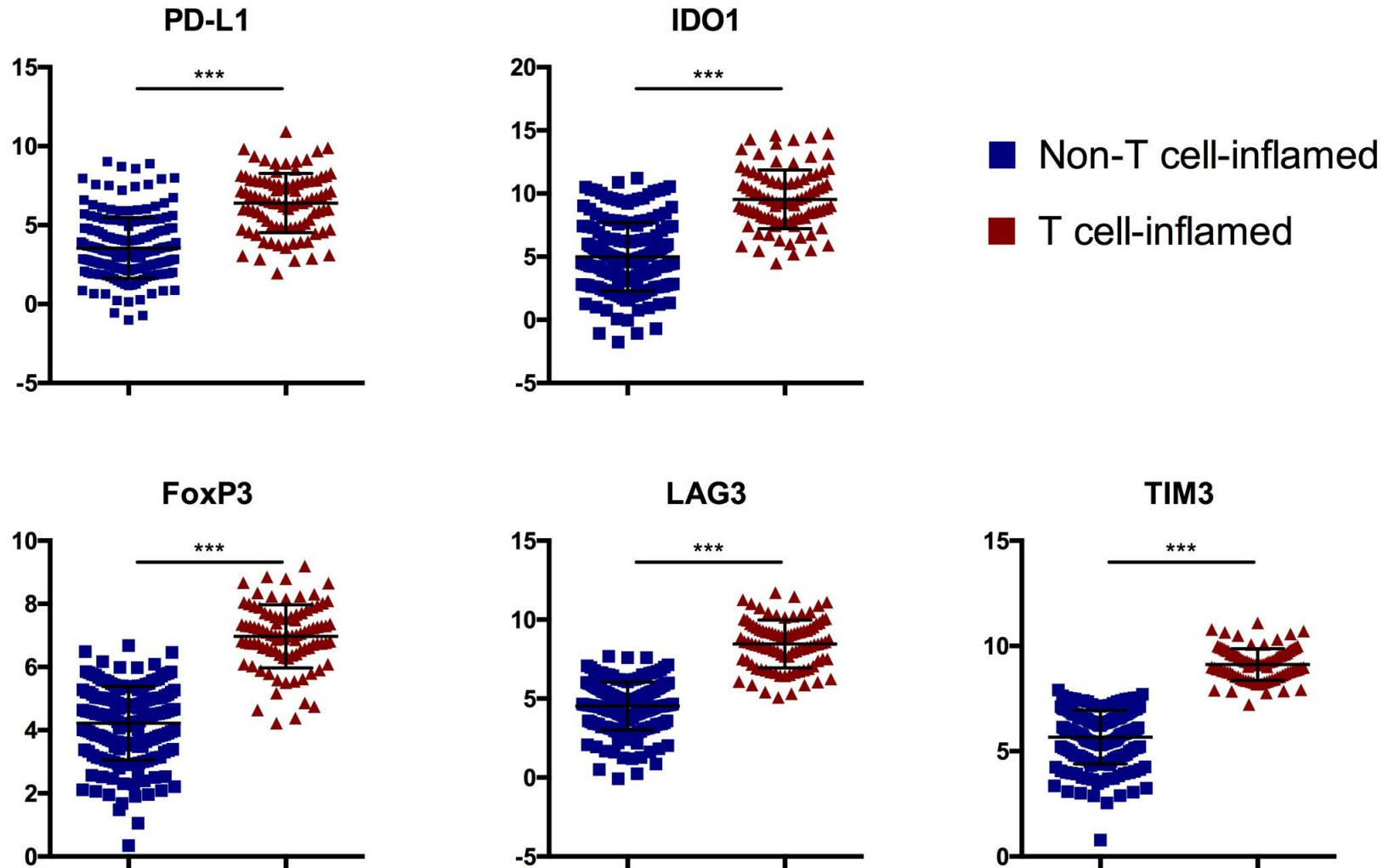
Immune checkpoints show concordant expression and varying T cell-inflamed gene signatures yield similar phenotype calls.
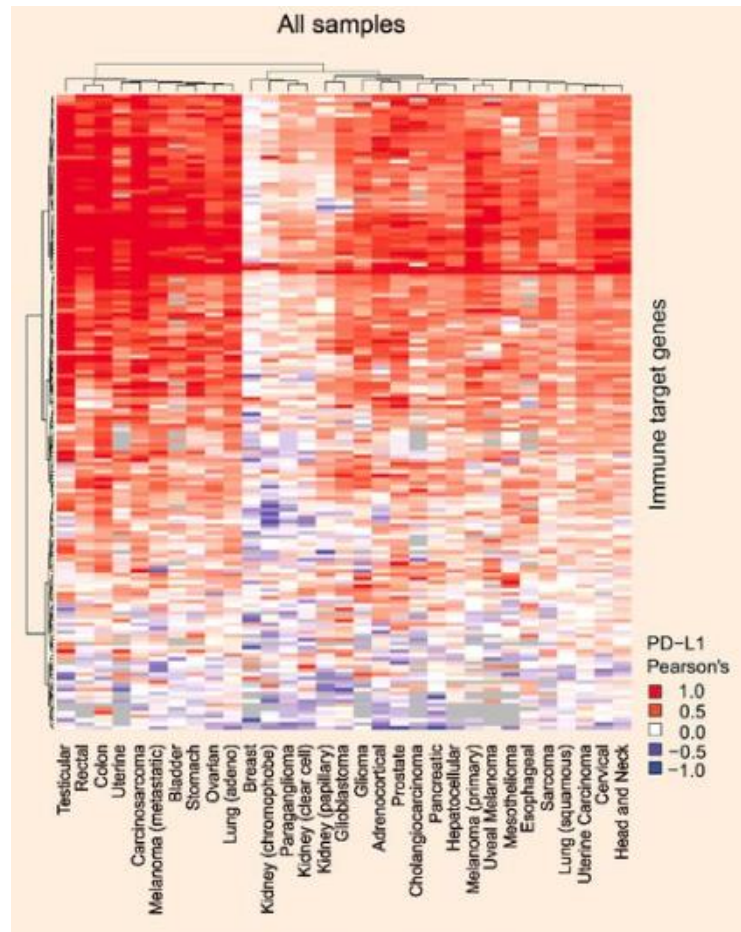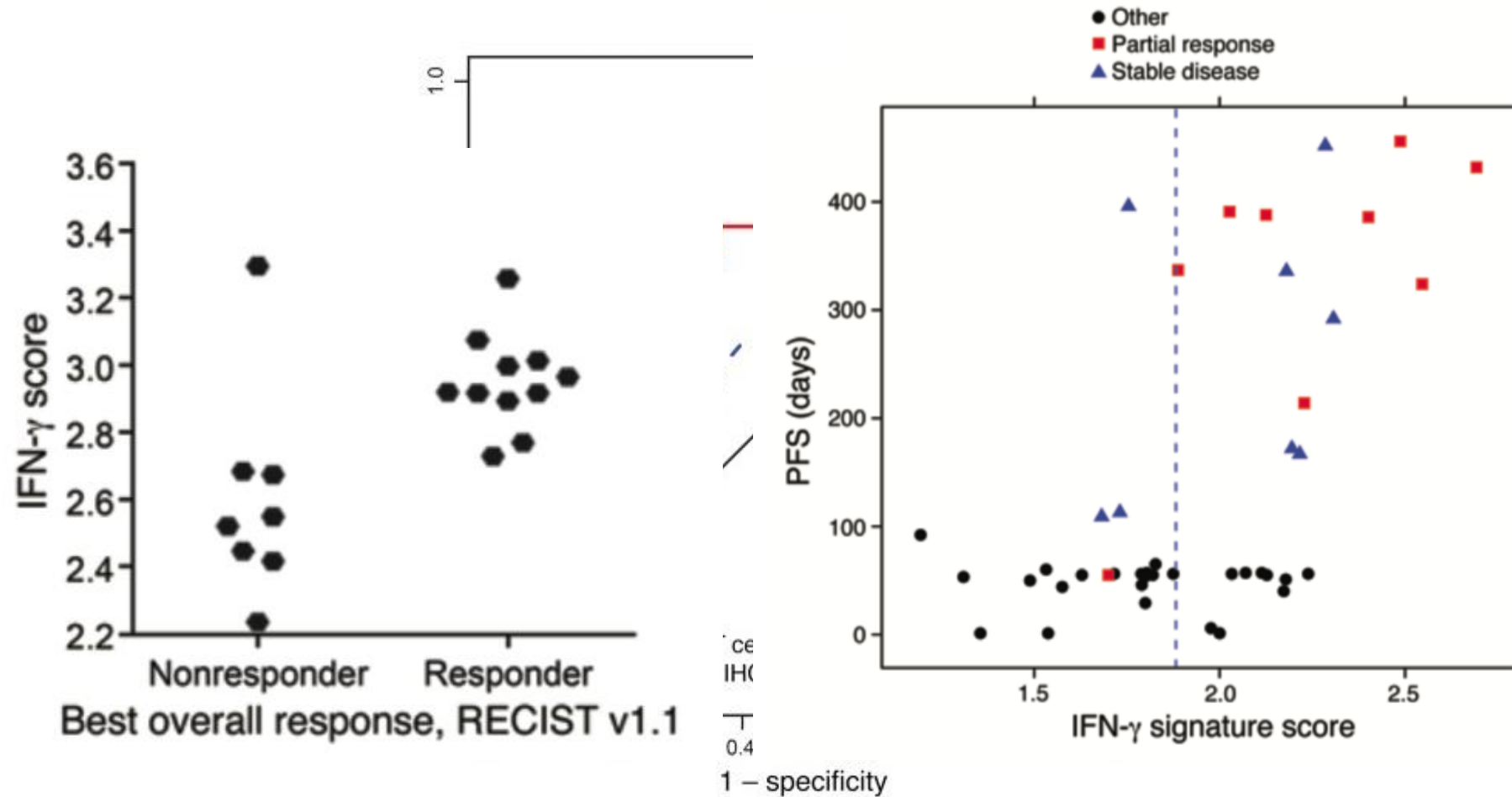

Trujillo, Sweis, Bao, Luke CIR 2018

Inflamed signature 1   Inflamed signature 2

Genes

Bladder cancer samples

89% concordance calling non-T cell-inflamed and T cell inflamed using three T cell signatures

Sweis, unpublished data

T cell-inflamed gene expression signature associates with favorable response to immunotherapy

Ayers, et al, J clin Invest. 2017

Cancers develop divergent immune phenotypes

Non-T cell-inflamed

- Absence of pre-existing intratumoral T cells
- Worse prognosis
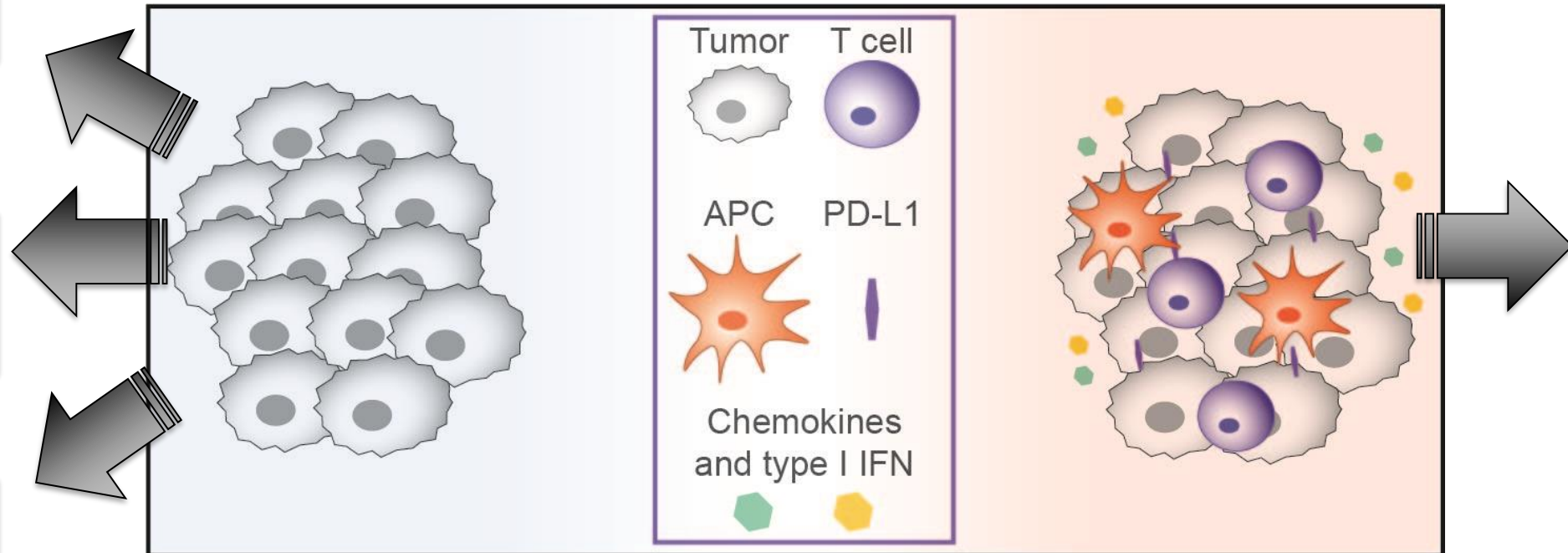- Associated with resistance to immunotherapy

T cell-inflamed

- Characterized by T cells, chemokines, and an IFN signature
- Favorable prognosis
- Predictive of immunotherapy response

Lack of priming

Restrictive vasculature/ biophysical

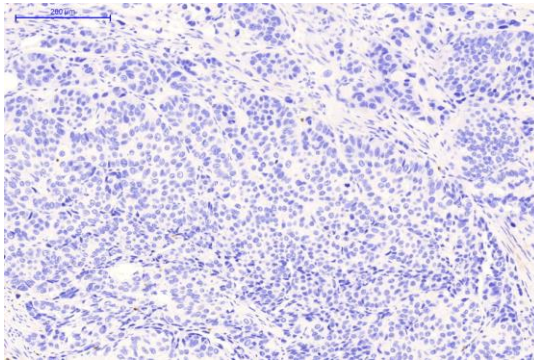Impaired trafficking of T cells

Tumor   T cell

APC   PD-L1

Chemokines and type I IFN

How is this phenotype defined?

What components are necessary and /or sufficient?

Bladder cancer

Non-T cell-inflamed

T cell-inflamed

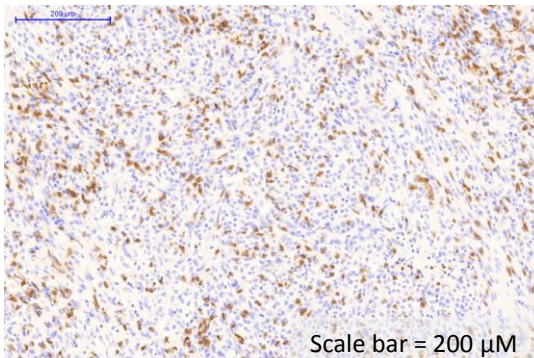Can we better define this immune phenotype histologically?
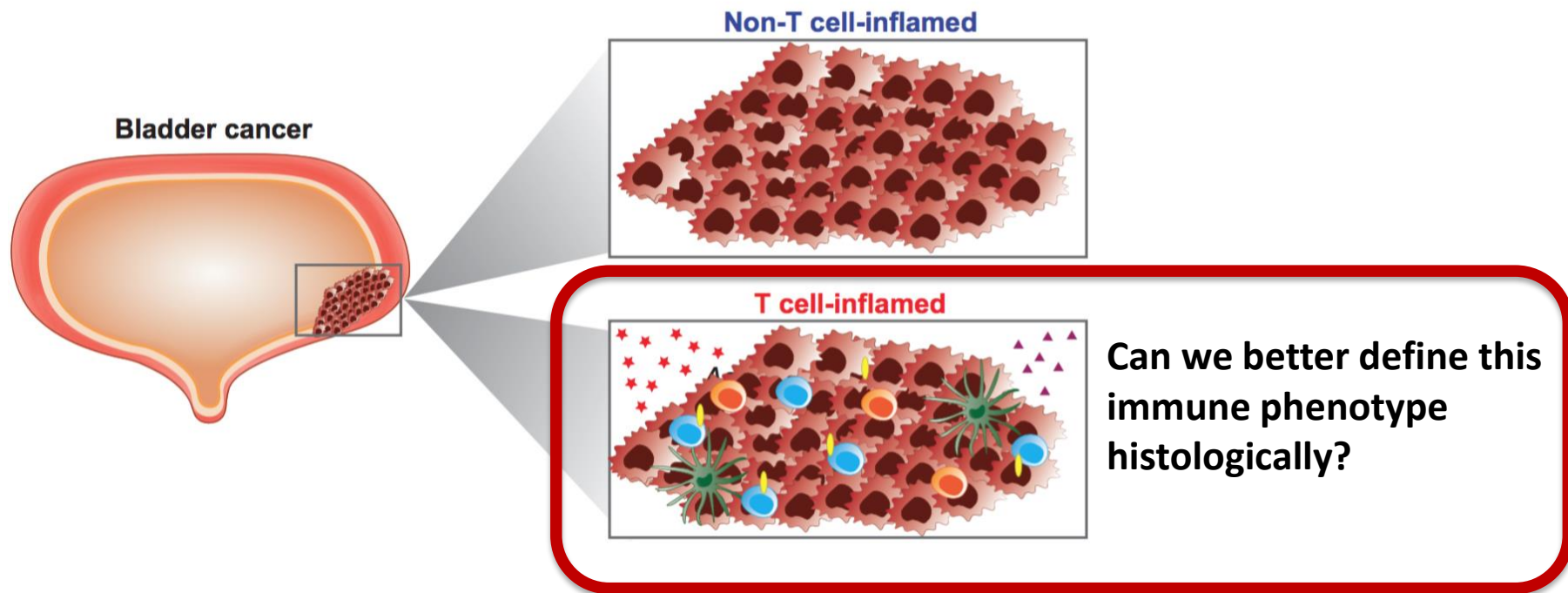
Immune gene signature is associated with CD8⁺ T cell infiltration by IHC, but not mutational density
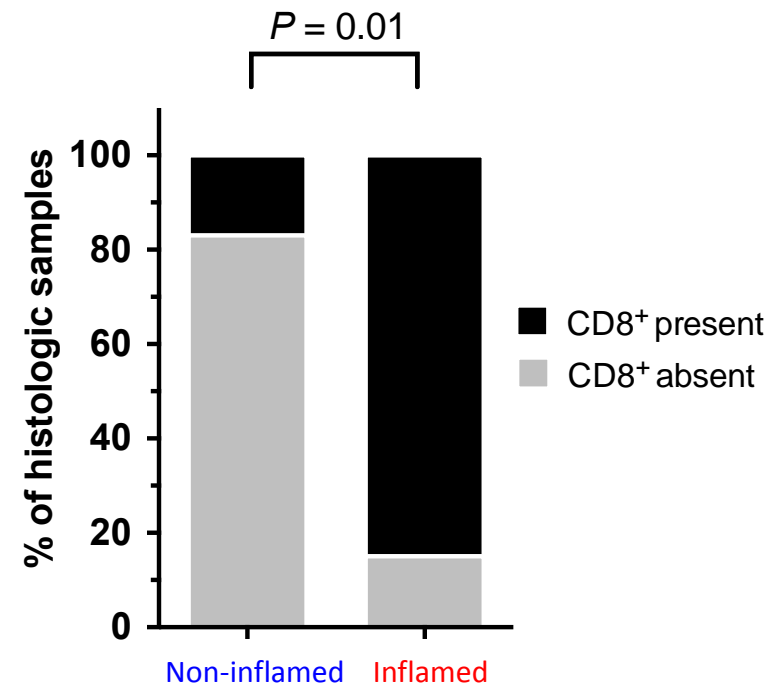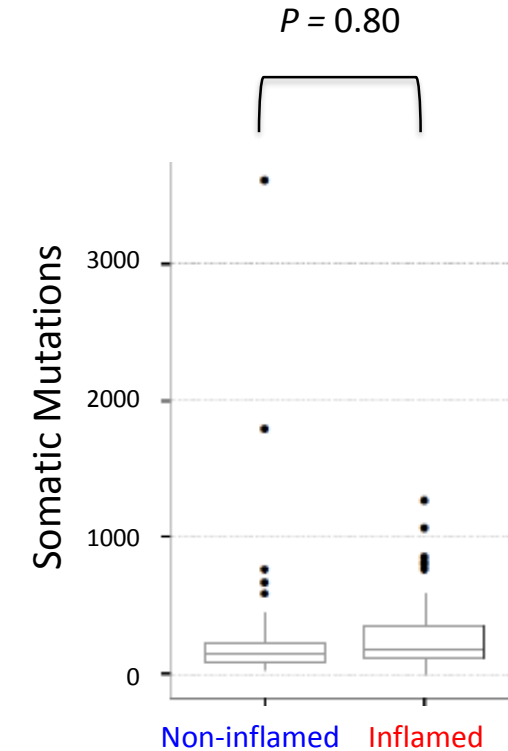

Non-inflamed tumor lacking CD8+ cells
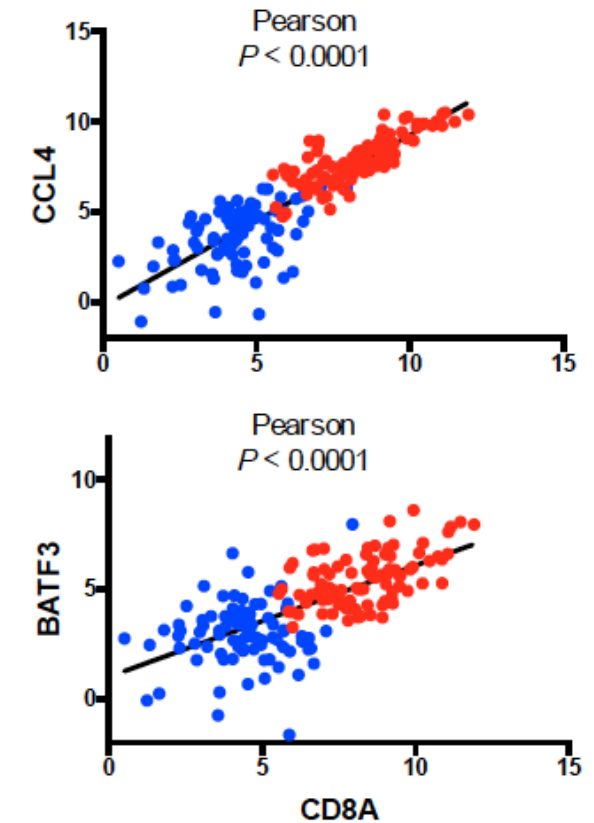
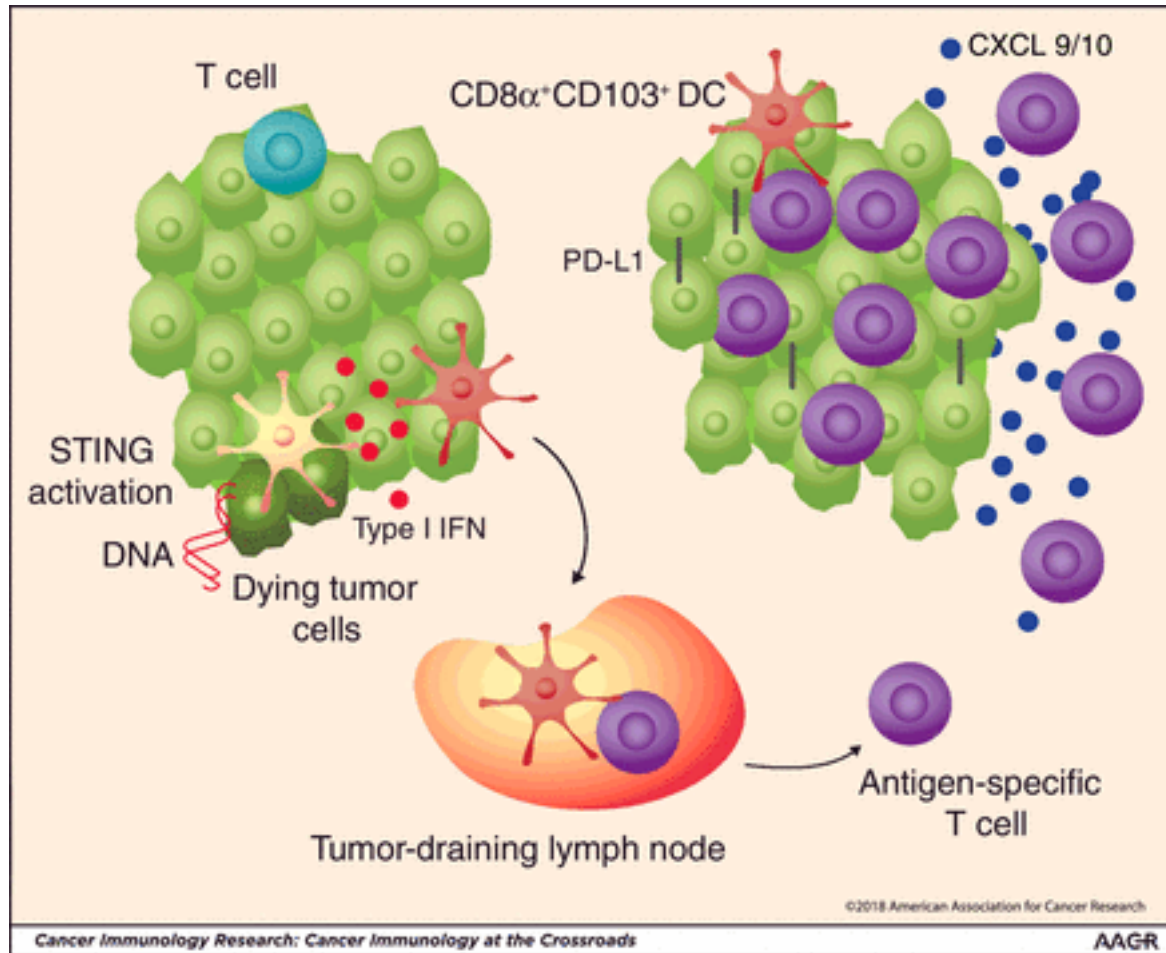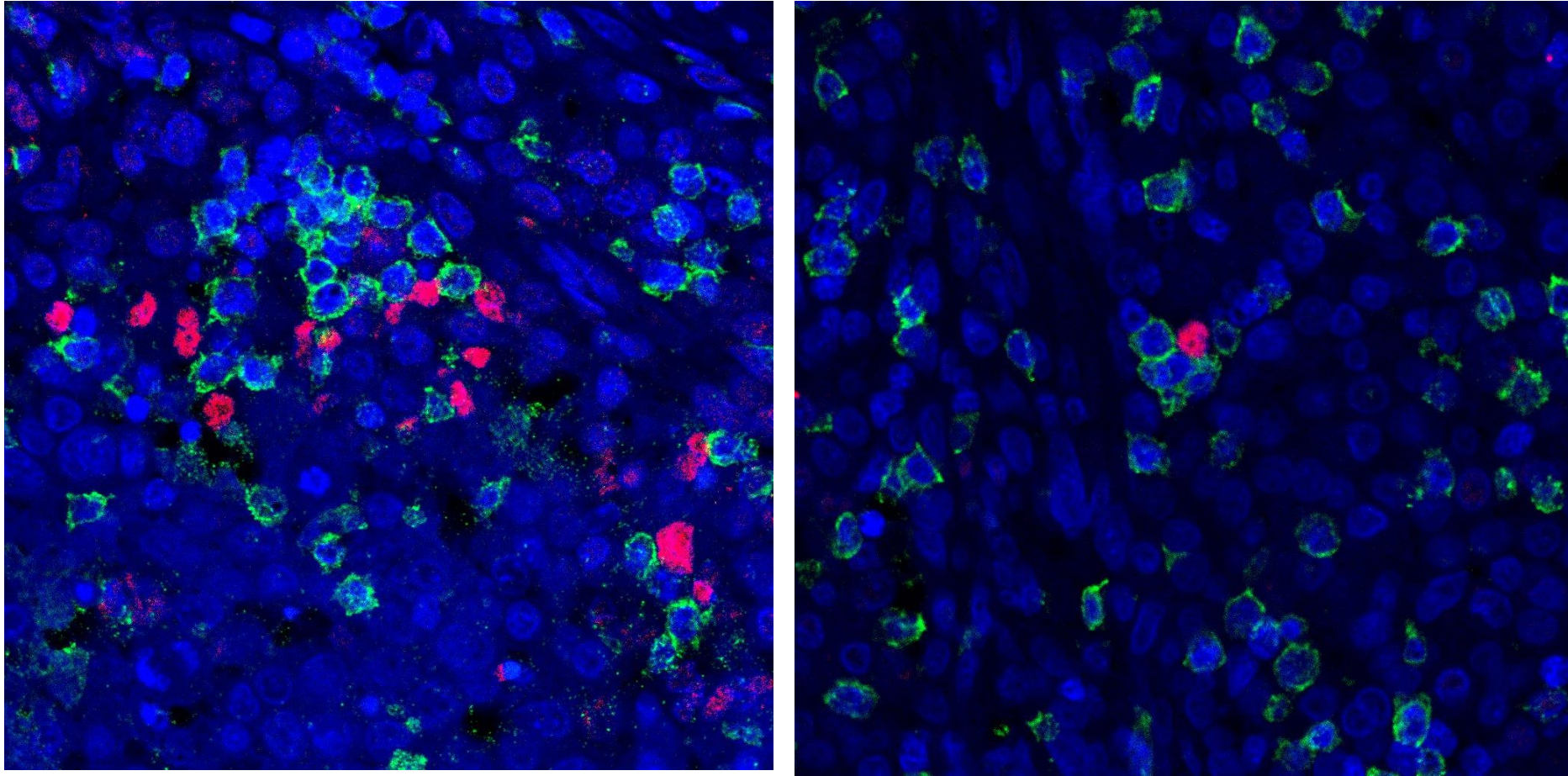Inflamed tumor with numerous CD8+ cells

Scale bar = 200 μM

P = 0.01

P = 0.80

% of histologic samples

CD8⁺ present
CD8⁺ absent

Non-inflamed   Inflamed

Somatic Mutations

Non-inflamed   Inflamed

Gene Expression Subtype

Sweis, et al., Cancer Immunol Res. 2016

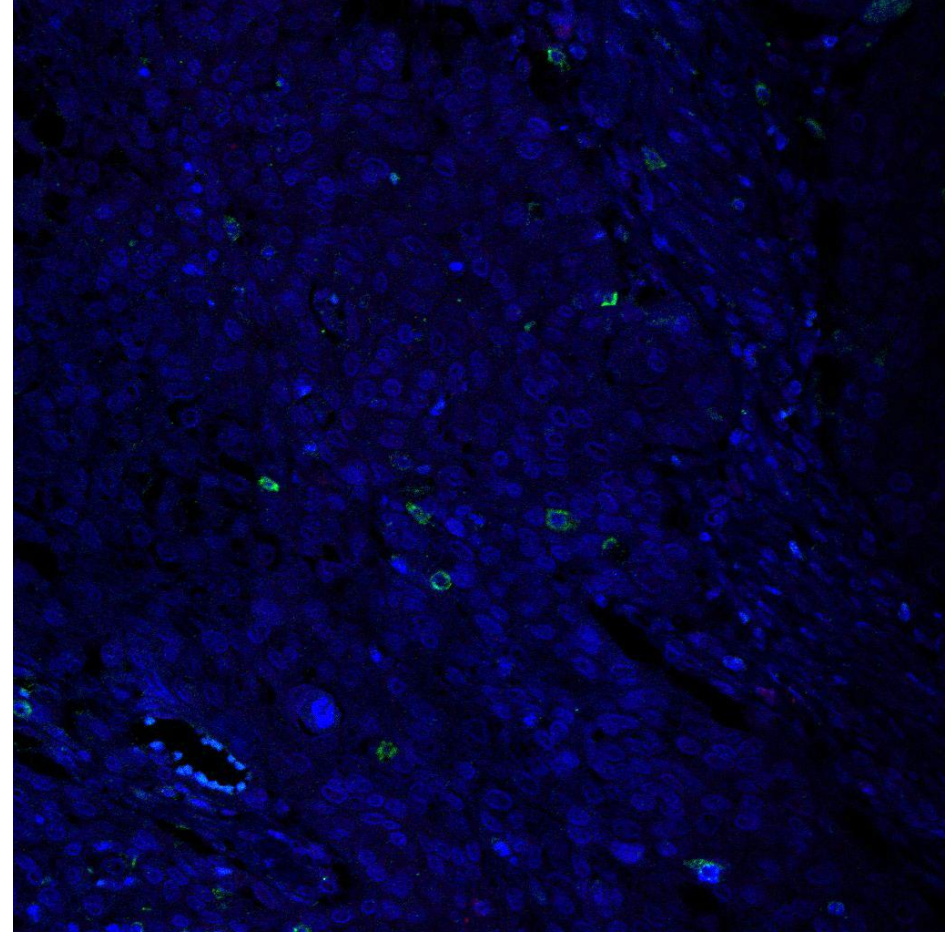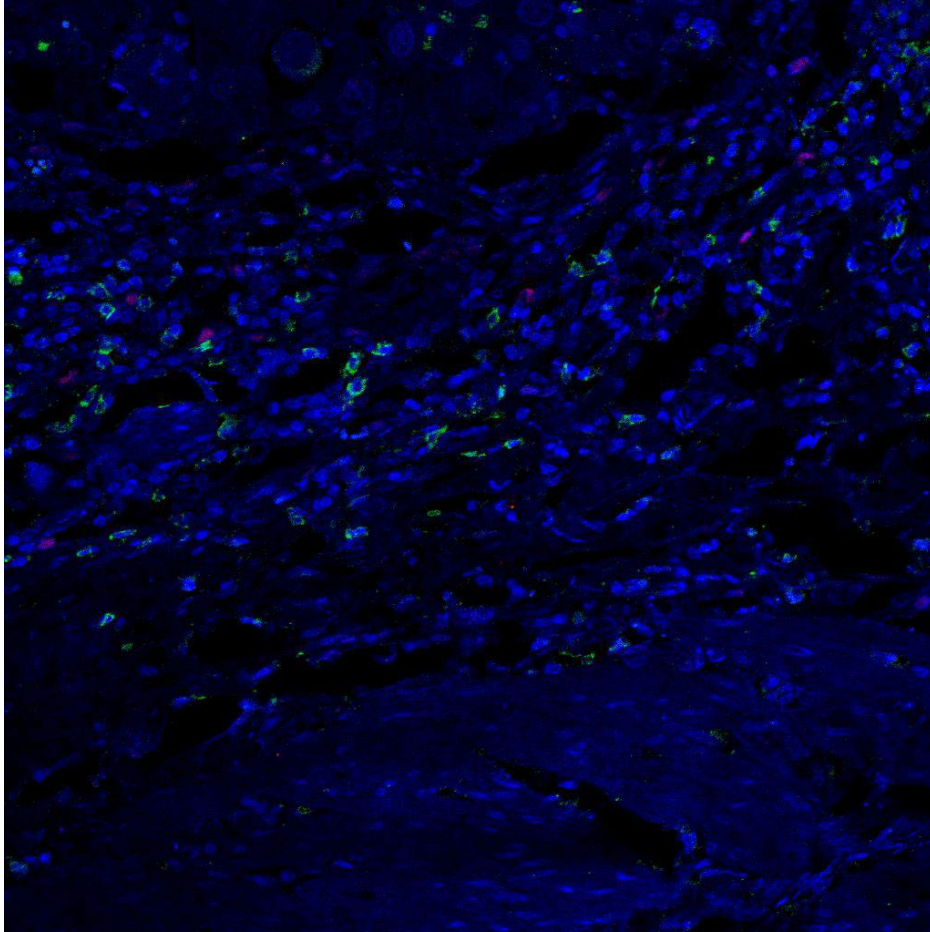# Beyond T cells and immune checkpoints: BATF3-DCs

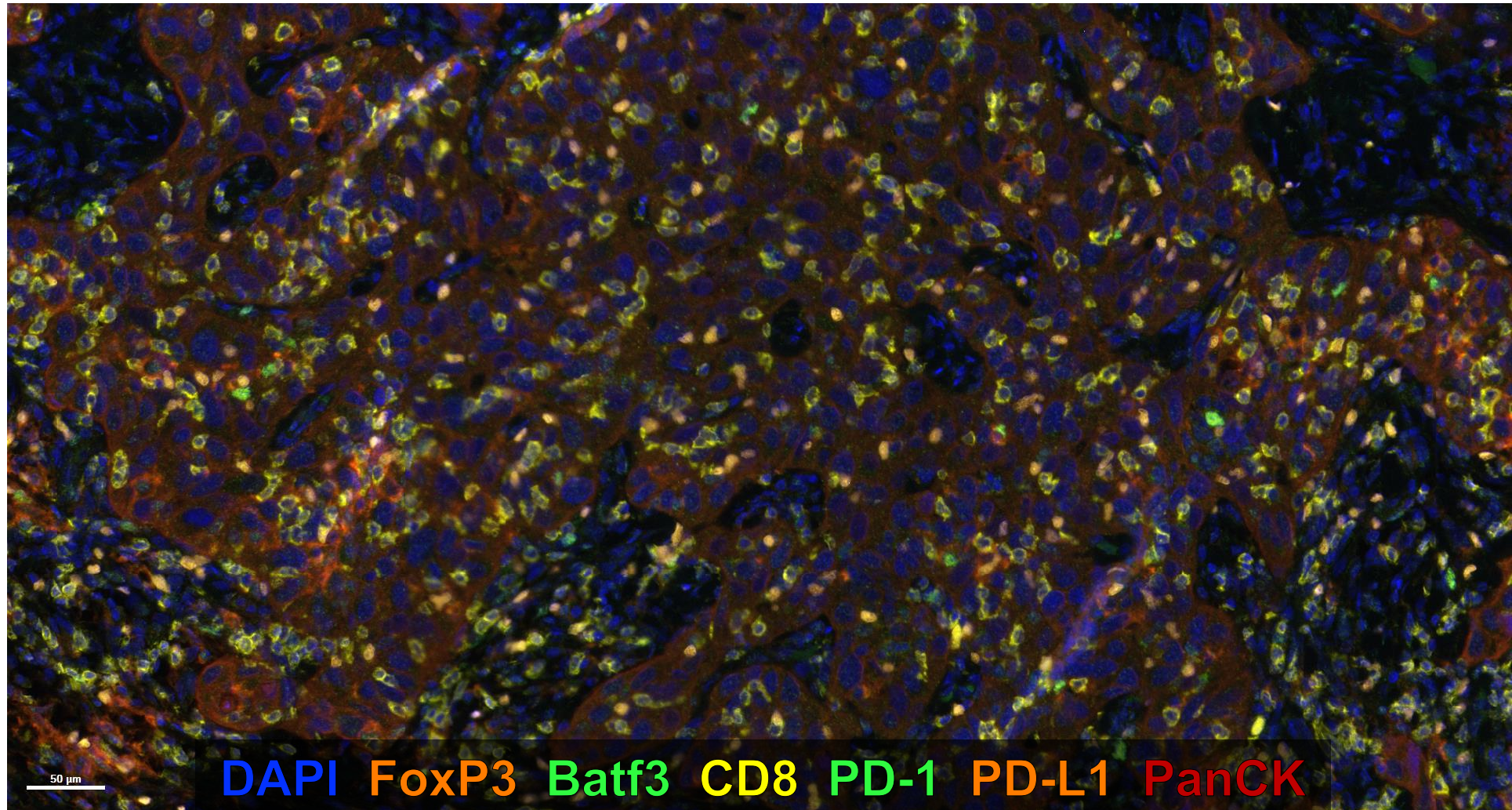# Batf3$^+$ DCs observed in T cell-inflamed tumors

DAPI CD8 Batf3
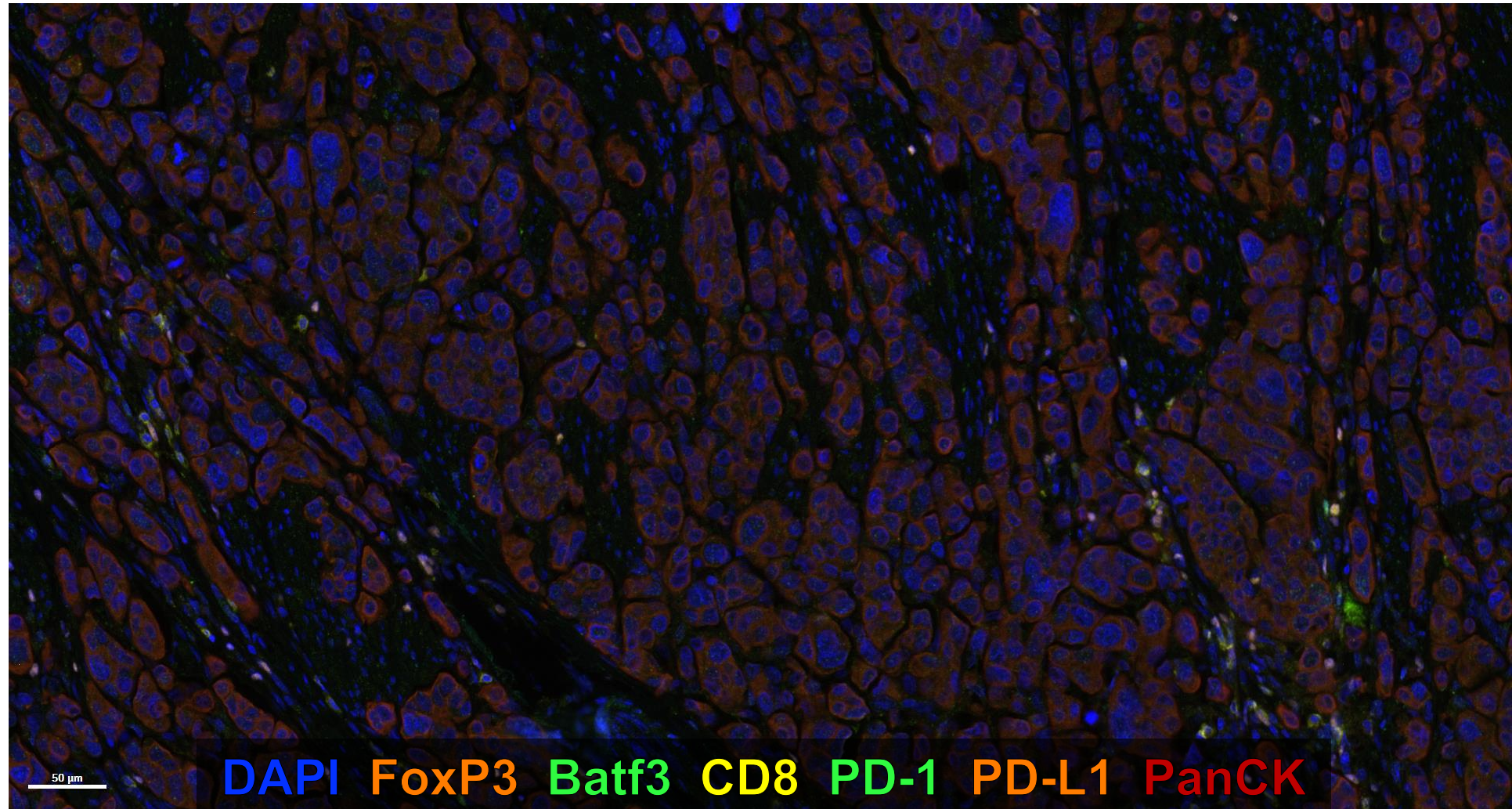
Batf3⁺ DCs absent in non-T cell-inflamed tumors

DAPI CD8 Batf3
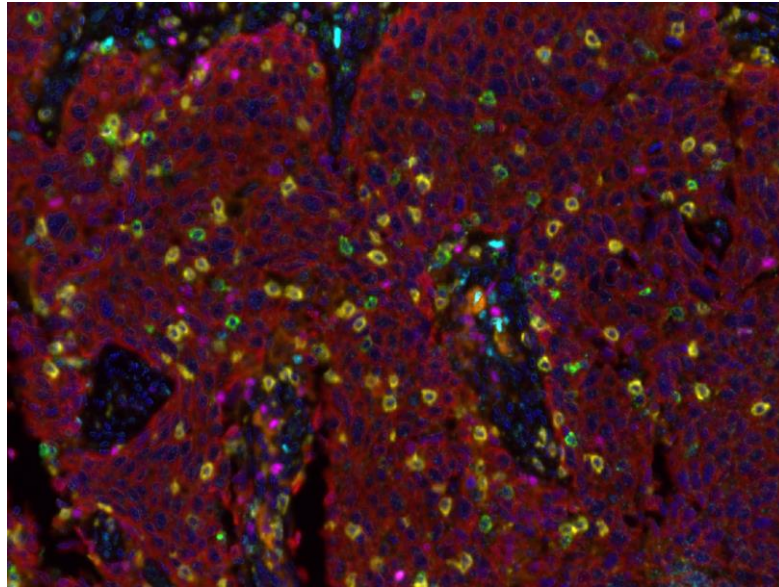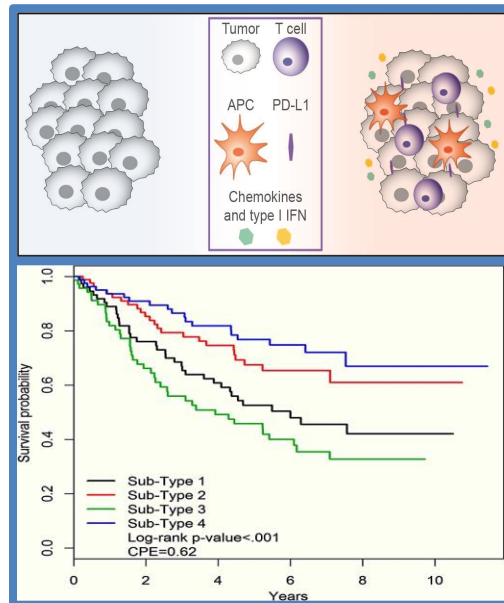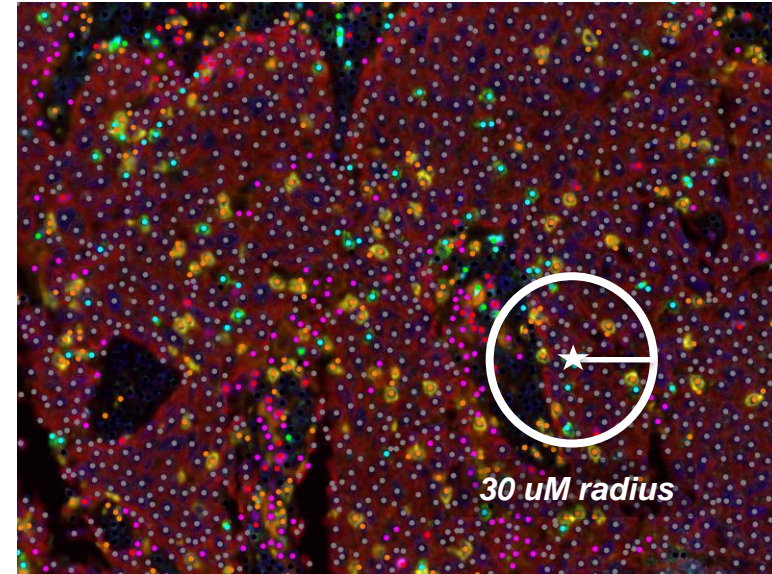
Hepta-color imaging of the tumor microenvironment

DAPI FoxP3 Batf3 CD8 PD-1 PD-L1 PanCK

50 μm

Ken Hatogai

Hepta-color imaging of the tumor microenvironment

DAPI FoxP3 Batf3 CD8 PD-1 PD-L1 PanCK

50 µm

Ken Hatogai

Deconvolution and analysis of large imaging data

Cellular phenotyping

30 uM radius

Image deconvolution

Tumor   T cell

APC   PD-L1

Chemokines
and type I IFN

Machine Learning

Statistical analysis

Survival probability

Sub-Type 1
Sub-Type 2
Sub-Type 3
Sub-Type 4
Log-rank p-value<.001
CPE=0.62

Years

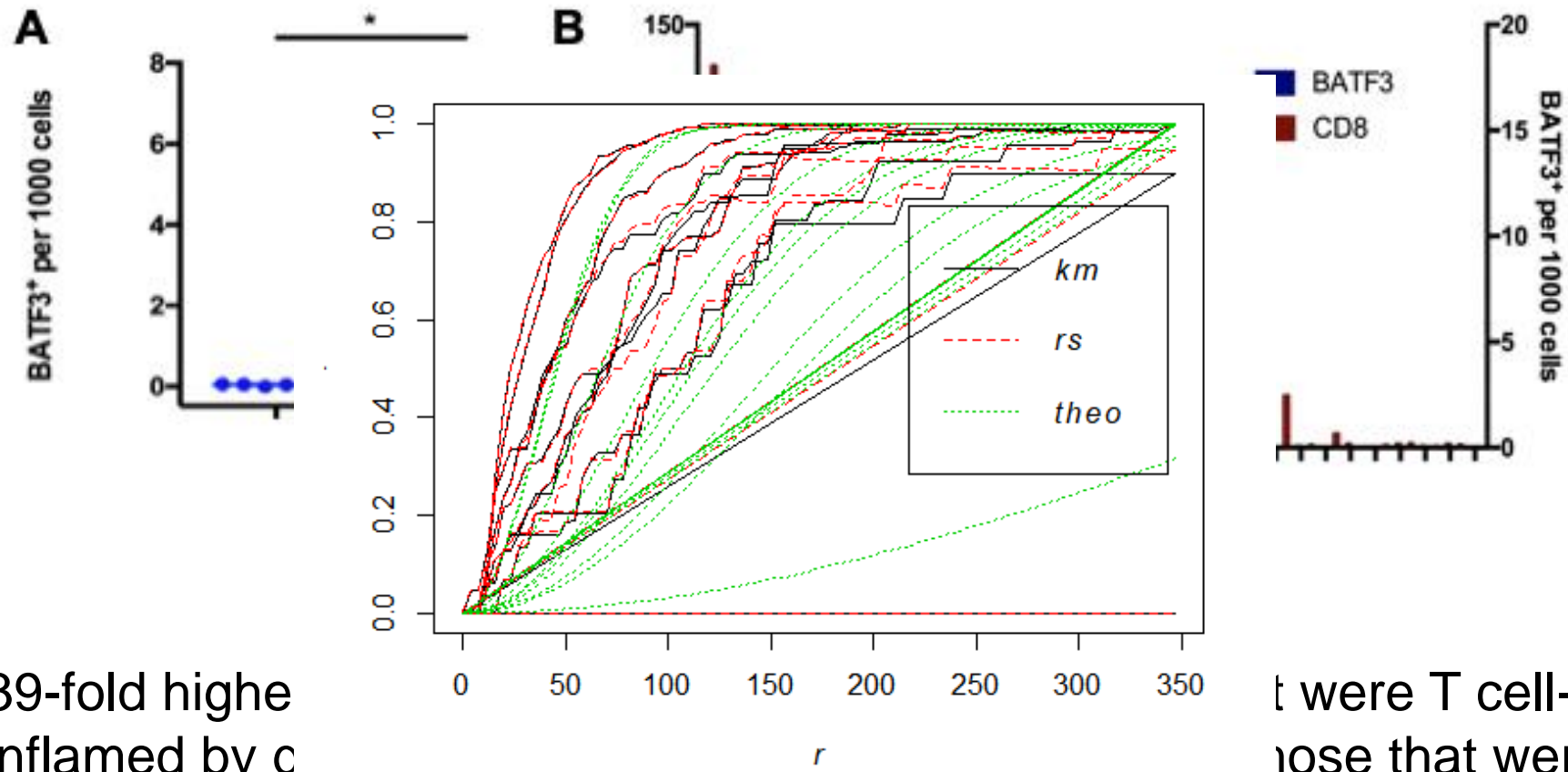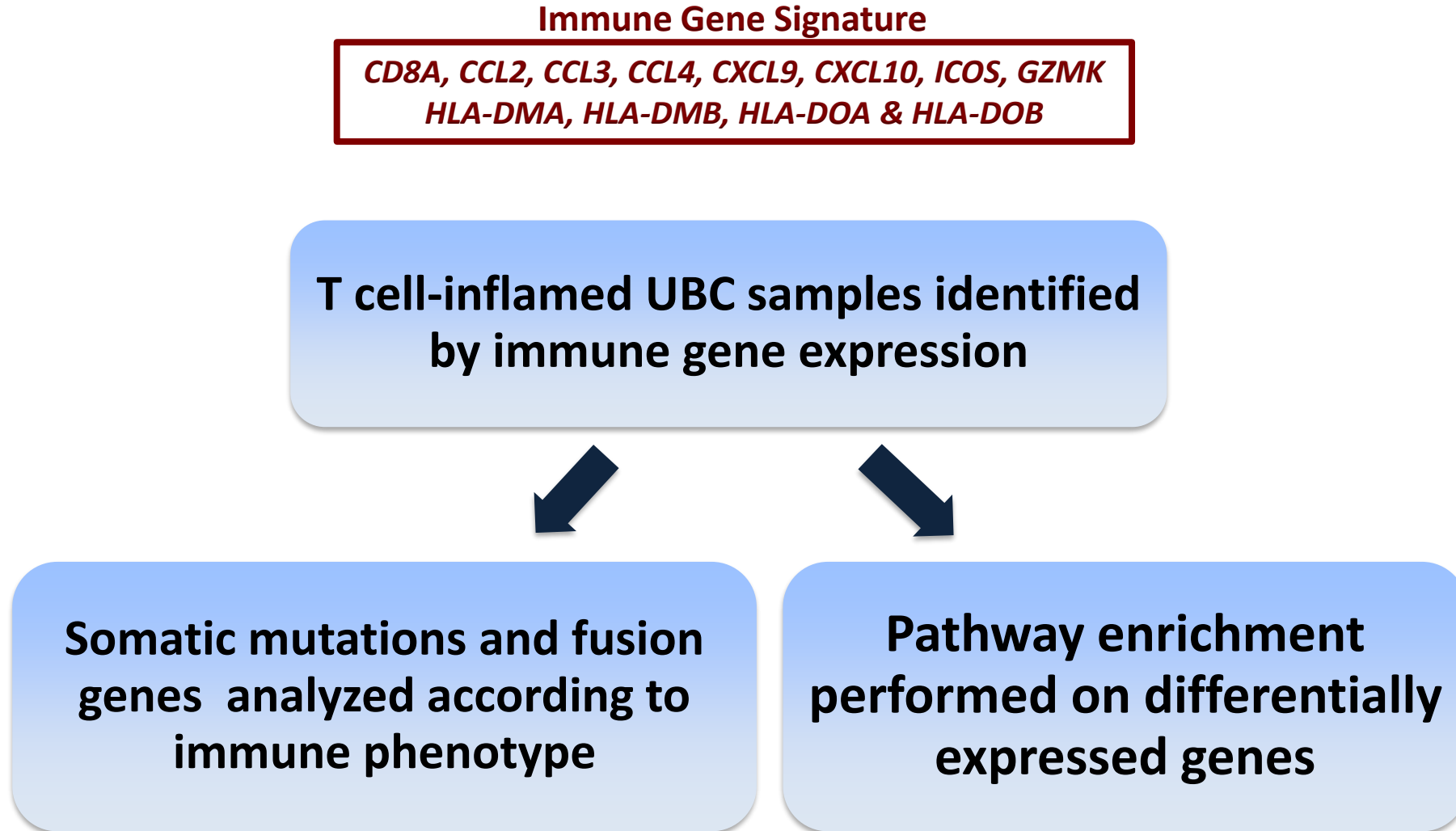*Collaboration with Alex Pearson Lab*

BATF-3 DCs cluster with CD8+ T cells and are strongly linked with T cell inflamed gene expression

- 39-fold highe   inflamed by g   non-T cell-inflamed   t were T cell-   iose that were

Methods to identify immune phenotypes and determine genomic correlates

Immune Gene Signature

CD8A, CCL2, CCL3, CCL4, CXCL9, CXCL10, ICOS, GZMK
HLA-DMA, HLA-DMB, HLA-DOA & HLA-DOB

T cell-inflamed UBC samples identified by immune gene expression

Somatic mutations and fusion genes analyzed according to immune phenotype

Pathway enrichment performed on differentially expressed genes

- DEG analysis: ANOVA – FDR q <0.01, fold change >2.0 – 3,112 genes
- Genes upregulated in non-T cell-inflamed tumors analyzed for pathway enrichment

Activation of β-catenin and PPAR-gamma is strongly linked with non-T cell-inflamed tumors

- ANOVA – FDR q < 0.01, fold change >2.0 – 3,112 genes
- Genes upregulated in non-T cell-inflamed tumors analyzed for pathway enrichment

| Upstream Regulator	Molecule Characteristics	Predicted State	Activation z-score	*P*-value of overlap	Target molecules in dataset
PPARG *peroxisome proliferator activator-γ*	• Ligand-dependent nuclear receptor • Regulates energy metabolism • Linked to "luminal" UBC molecular subtype	Activated	3.661	3.44E-03	ACADL, AQP3, BDH1, CYP4B1, DGAT2, GATA2, GDF15, GPT, GSTA1, HMGCS2, IGFBP3, IHH, KRT19, KRT20, LIPE, MYH14, OCLN, PLIN5, PPARG, SCNN1G, SNCG, UGT1A9
CTNNB1 *β-catenin*	• Transcription regulator • Activated by Wnt ligand binding • Drives T-cell exclusion in melanoma	Activated	3.654	2.58E-03	BMP7, CYB5A, CYP4F12, EMX2, EPCAM, ERBB3, FOXQ1, GAD1, GATA2, GATA3, GPX2, HAPLN1, HSD17B2, ID4, IHH, KLF5, KRT7, ME, COM, MSX2, NOX1, POU5F1, SCN5A, SEMA5A, SIM2, SMAD6, TFF1, TH, TSPAN8, WNT7B
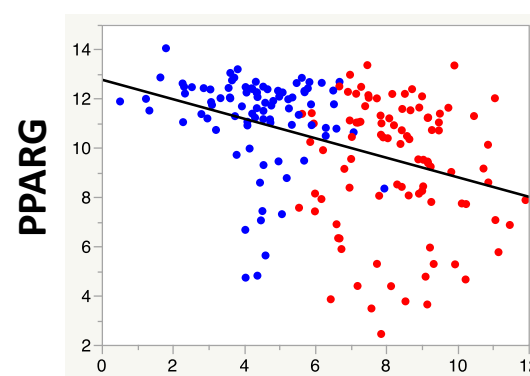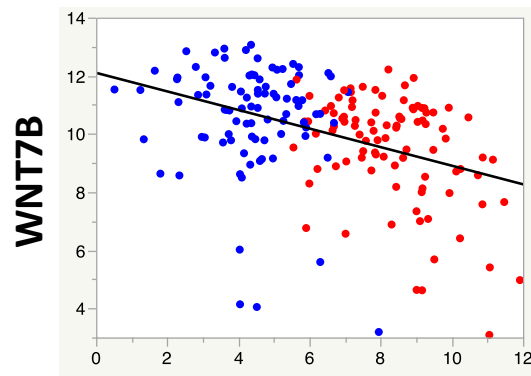
Pearson Corr.	Coefficient	*P*-value
CD8A – WNT7B	-0.4019	<.0001
CD8A – PPARG	-0.3869	<.0001

Sweis, et al., Cancer Immunol Res. 2016

Recently discovered oncogene-driven immune resistance pathways

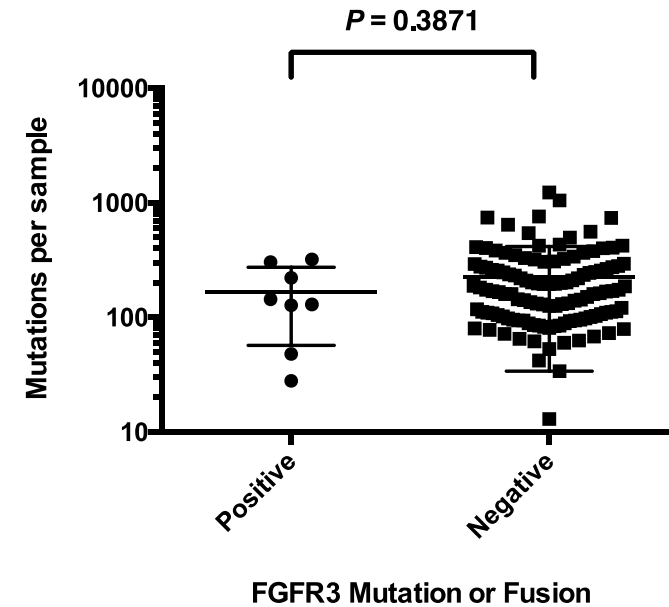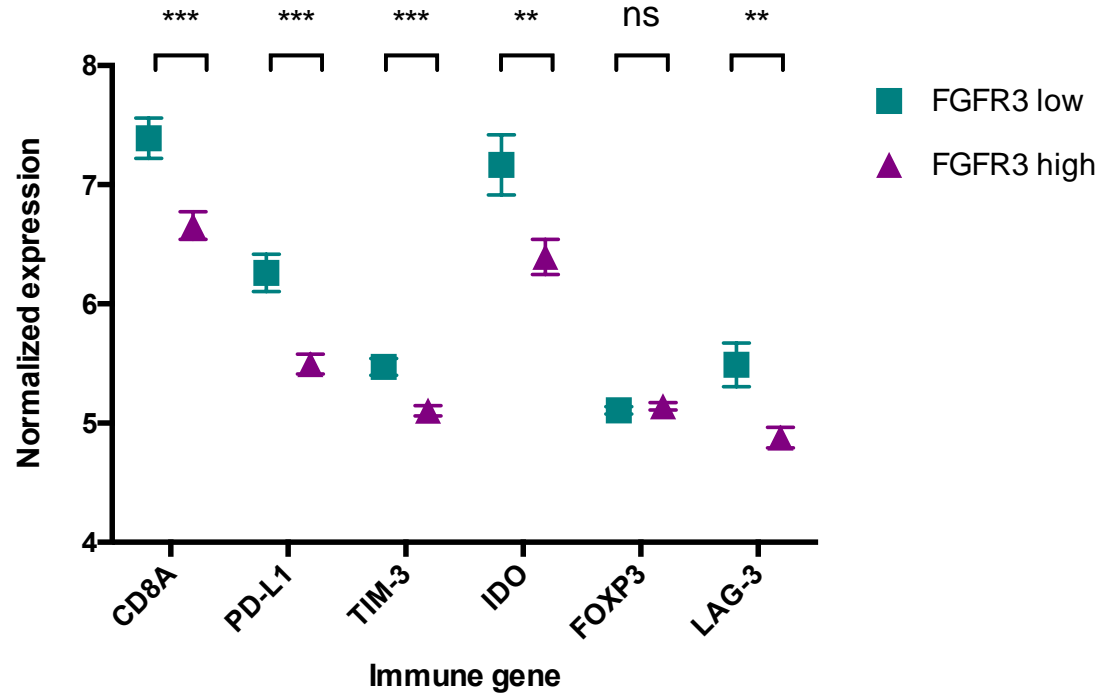| Oncogenic pathways	Cancer type
WNT/β-catenin activation	Melanoma (75)
	Bladder cancer (39)
PTEN loss/PI3K-AKT activation	Melanoma (41)
	Glioma (83)
	Sarcoma (84)
PPARγ/RXRα activation	Bladder cancer (39, 42)
Isocitrate dehydrogenase gain-of-function mutations (IDH1 and IDH2)	Lower grade gliomas (51)
FGFR3 activation	Bladder cancer (39)
MYC activation	Acute lymphoblastic leukemia, hepatocellular carcinoma, melanoma, NSCLC (43)
STAT3 oncogenic signaling	NSCLC (85)
AXL receptor tyrosine kinase expression	Breast cancer (86)
	Melanoma (87)
LKB1 (also known as STK11) loss of function	Endometrial cancer (49)
	NSCLC (50)
TP53 loss of function	Breast cancer (estrogen receptor-negative; ref. 45)

Interrogating bladder cancer immune phenotypes
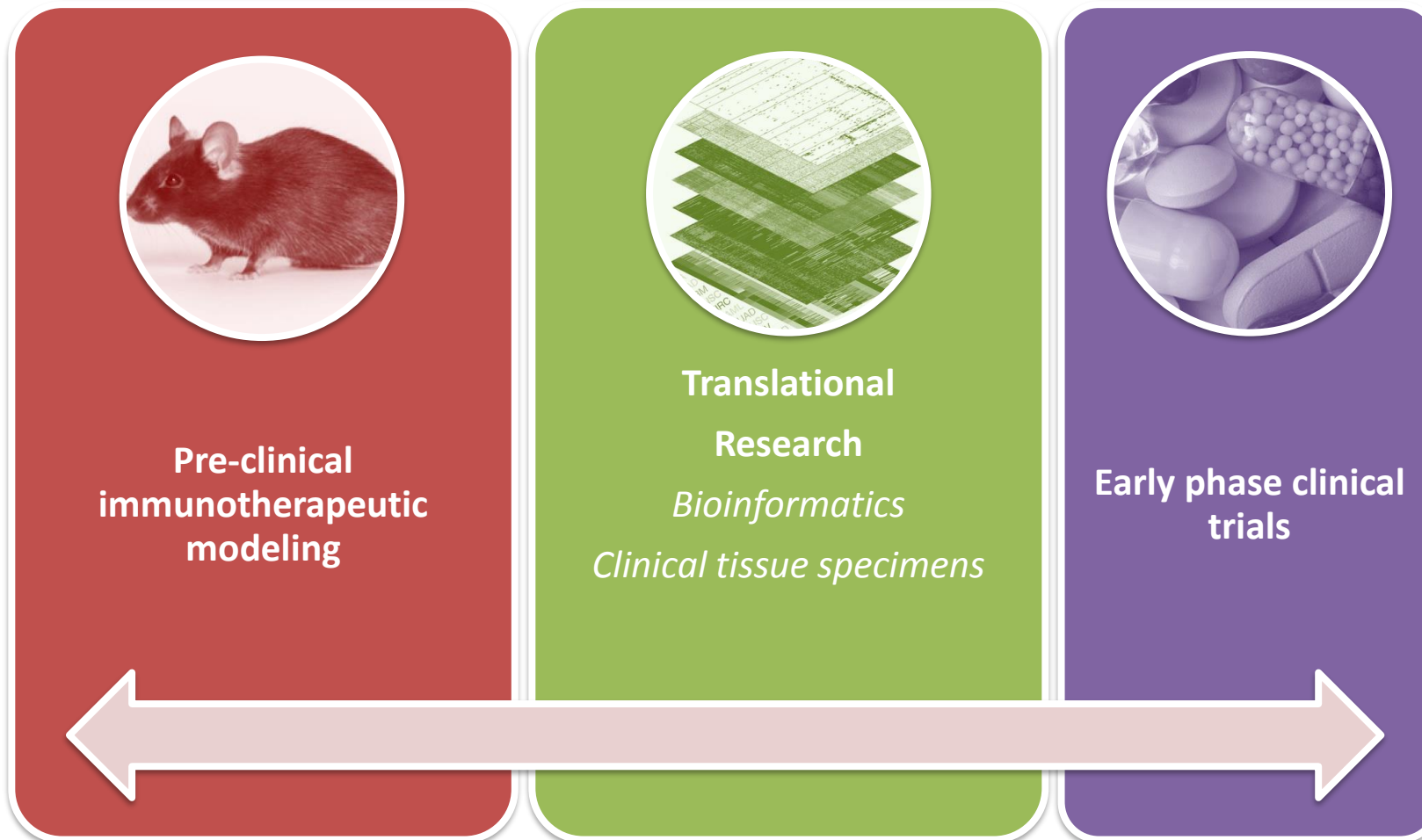
FGFR3 alterations are unique to non-T cell-inflamed tumors & expression correlates with lack of T cells
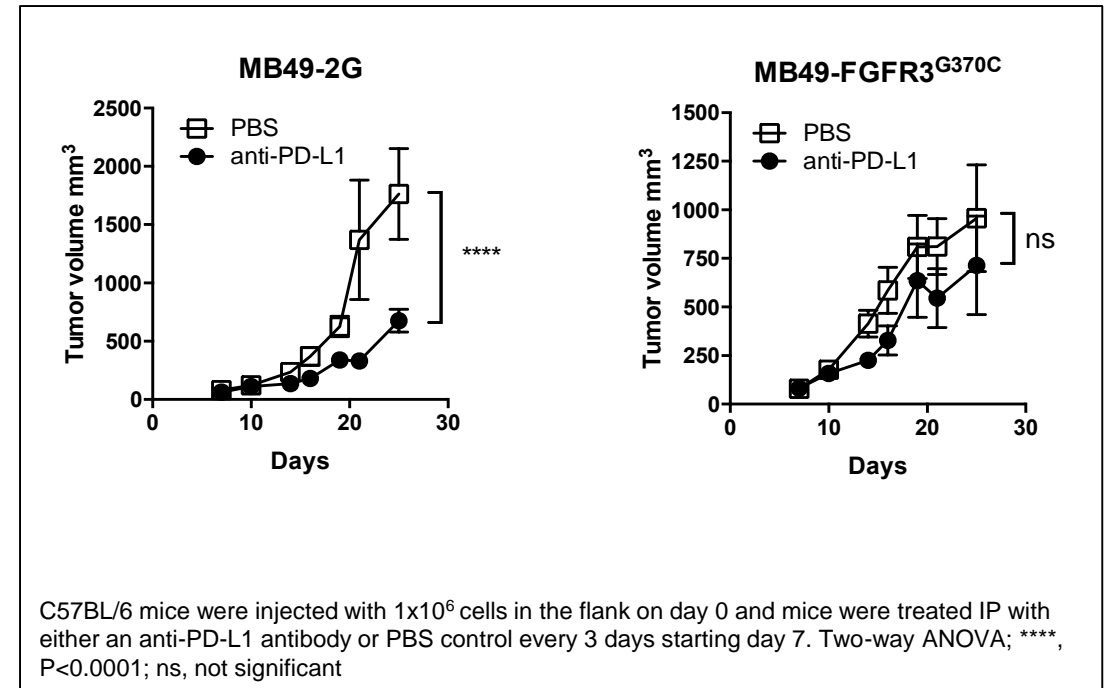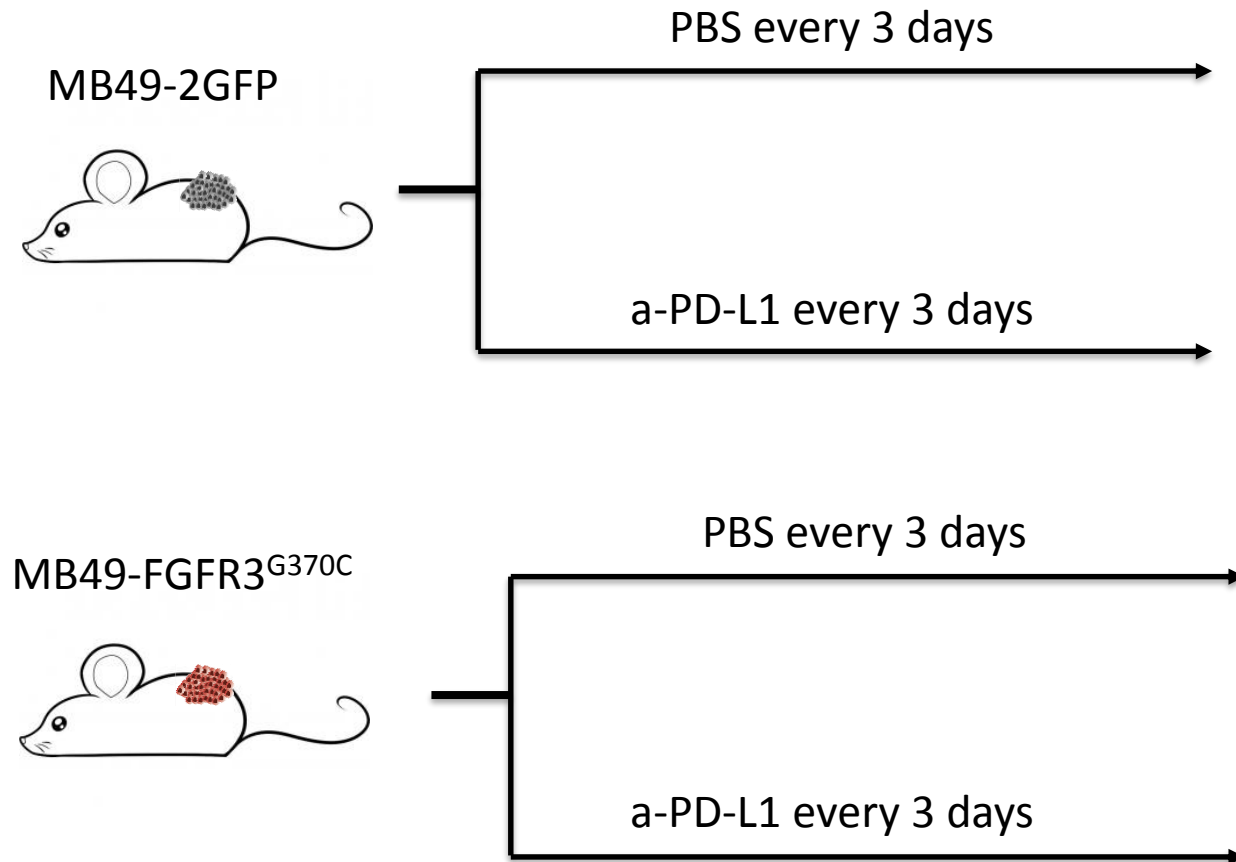
Gene Mutation or Fusion	Non-T cell-inflamed (n = 76)		T cell-inflamed (n = 85)	
	Samples	Variants	Samples	Variants
FGFR3	11	14	0	0
FGFR3-TACC3	3	-	0	-

Gene expression by RNAseq
Anti-PD-1/L1 treatment

FGFR3

FGFR3 gene

Mutations

ig   I-set   I-set   Pkinase_Tyr

0   200   400   600   806 aa

Extracellular   Transmembrane   Kinase domain

FGFR3

Non-responder
Responder

Number of Patients

15
10
5
0

FGFR3 +   FGFR3 -

FGFR3 by IHC

FGFR3 (+)   FGFR3 (-)

Sweis, et al. Cancer Immunol Res. 2016
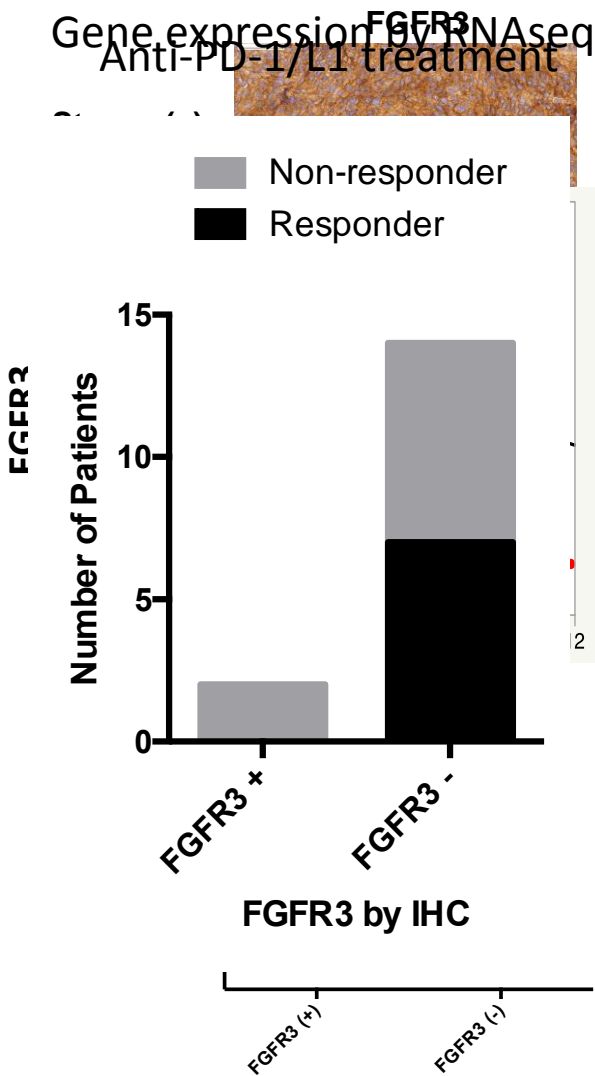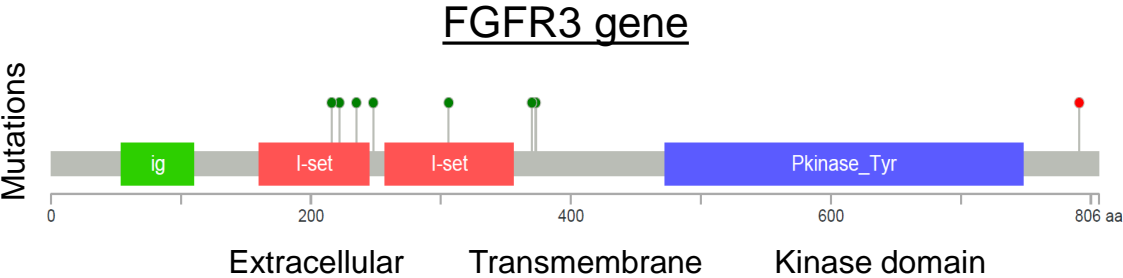
FGFR3+ tumors have lower expression of immune inhibitory genes not explained by mutational burden

FGFR3 activation leads to resistance to anti-PD-L1 tx

MB49-2GFP

PBS every 3 days

a-PD-L1 every 3 days

MB49-FGFR3^{G370C}

PBS every 3 days

a-PD-L1 every 3 days

C57BL/6 mice were injected with 1x10^6 cells in the flank on day 0 and mice were treated IP with either an anti-PD-L1 antibody or PBS control every 3 days starting day 7. Two-way ANOVA; ****, P<0.0001; ns, not significant

Unpublished data

MB49 cells can be instilled via catheter to generate orthotopic bladder cancers
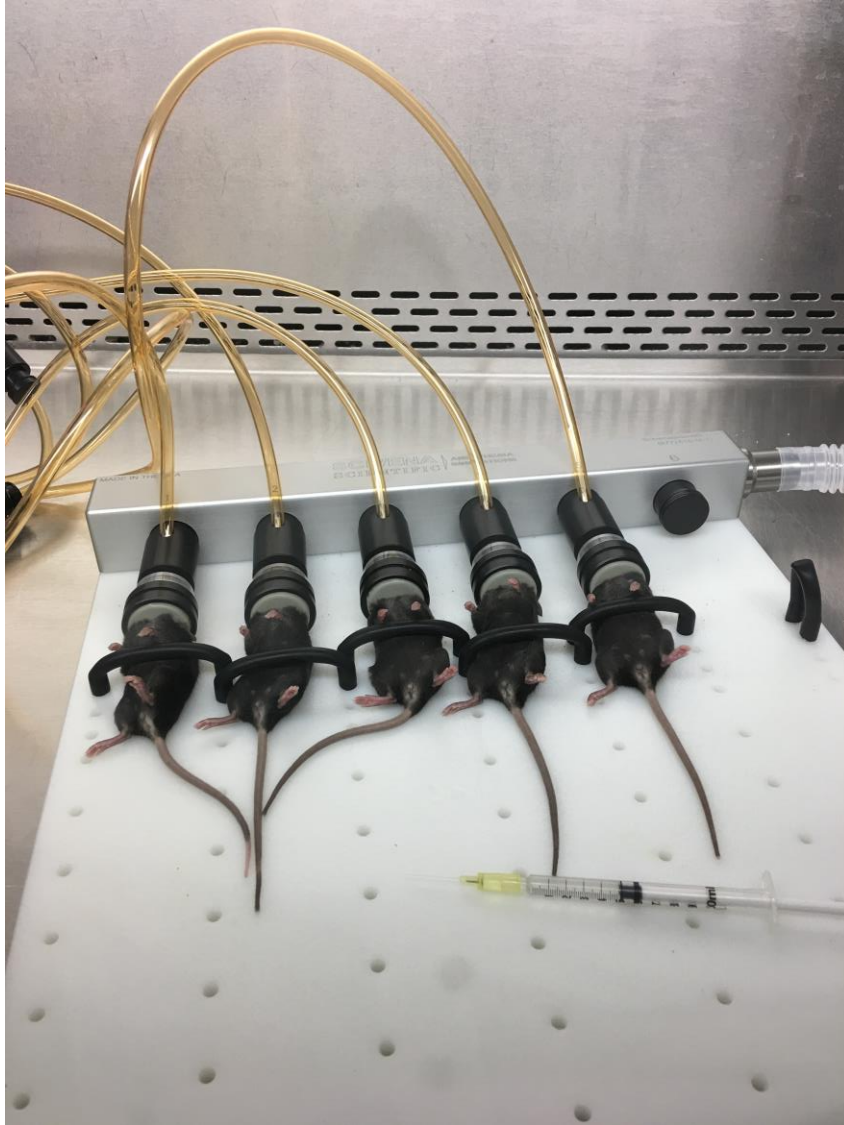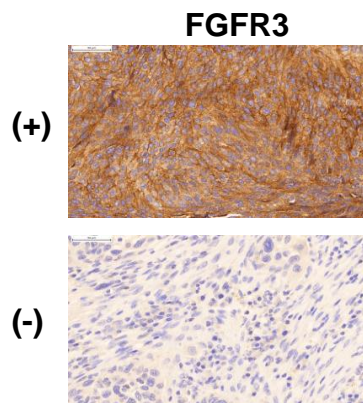
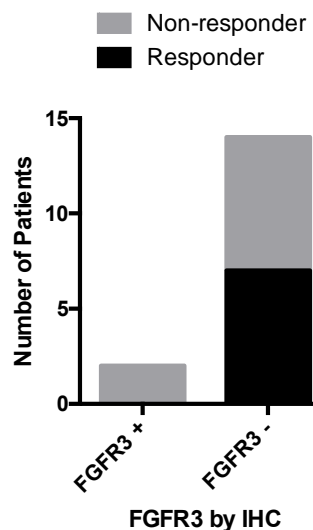# FGFR3 activated bladder tumors have fewer infiltrating antigen specific T cells
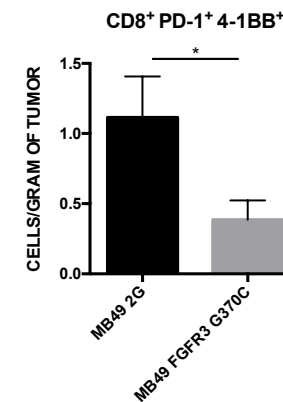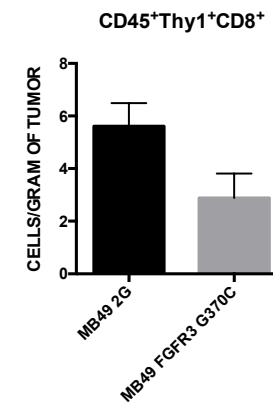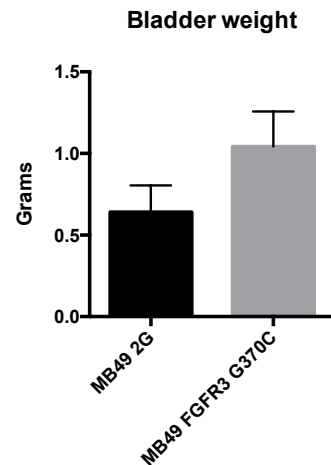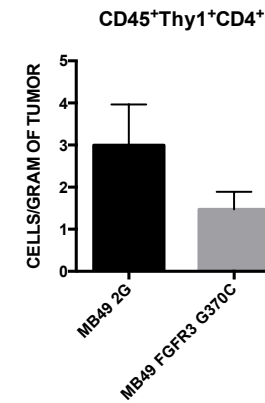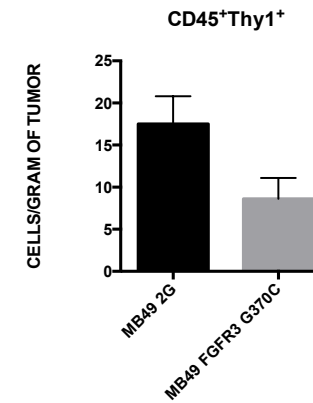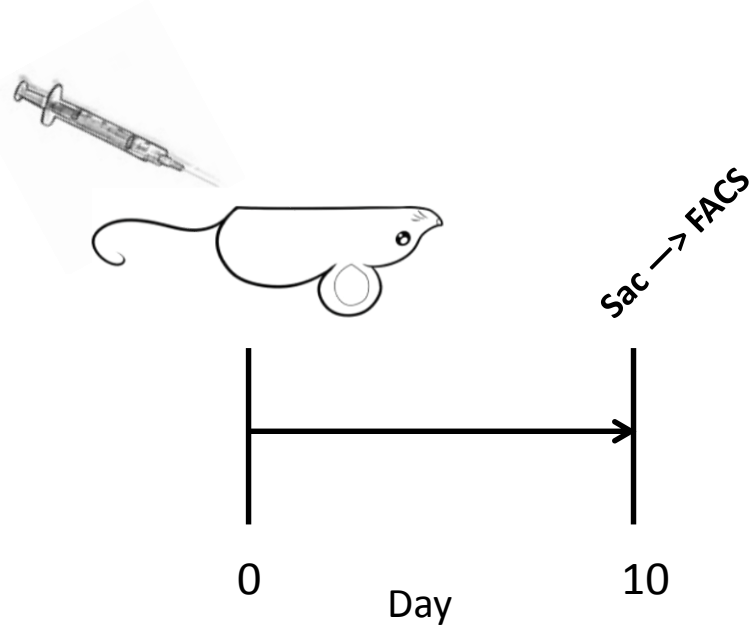
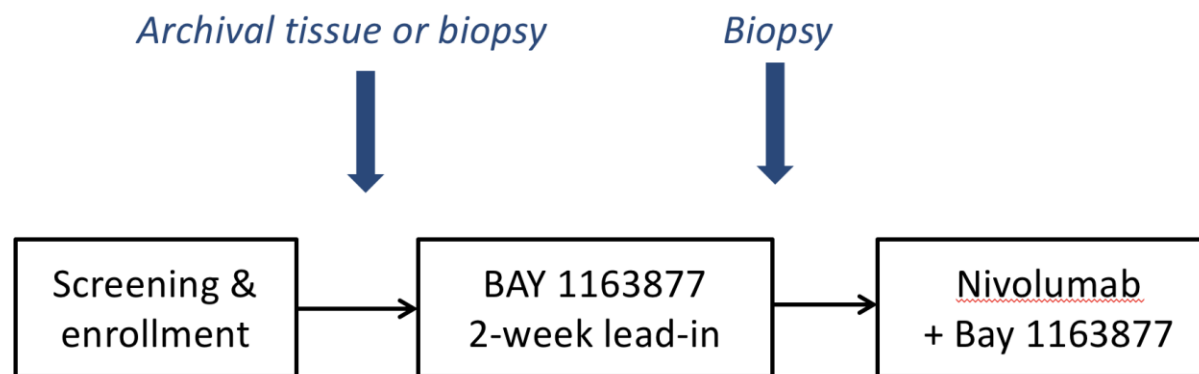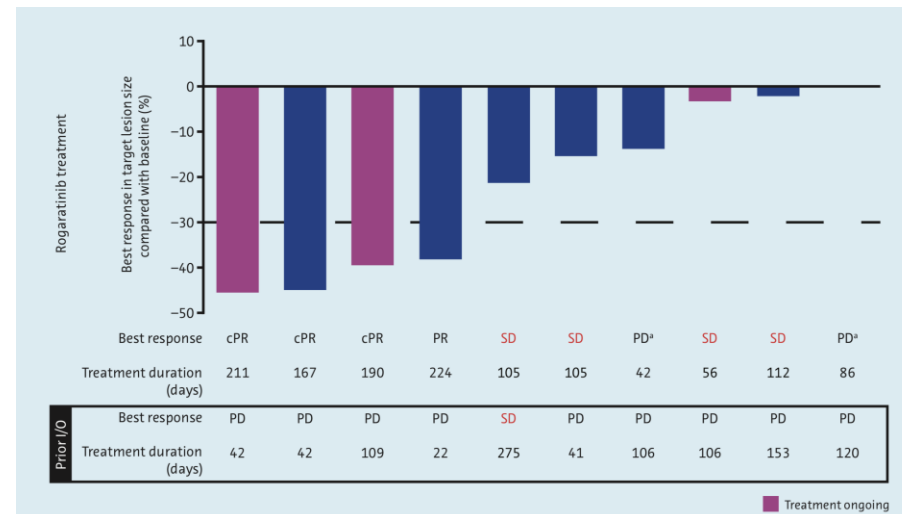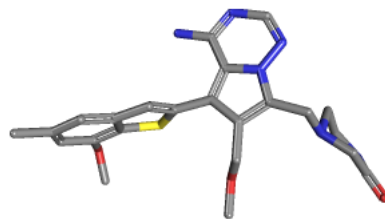# Clinical translation – FGFR inhibitor (rogaratinib)

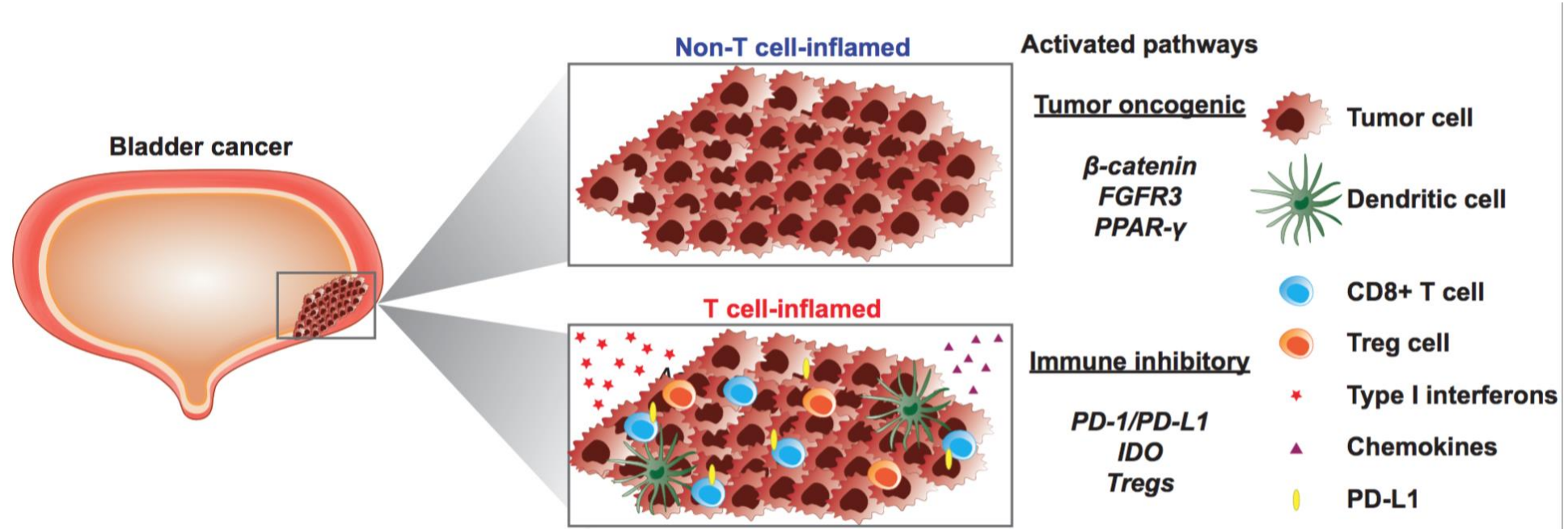UChicago bladder cancer patients tx w/ anti-PD-1

FGFR3

(+)

(-)

n=16

Rogaratinib

Investigator-initiated trial approved - opening 10/2019

Conclusions

- Inhibitory molecules are overexpressed in T cell-inflamed bladder cancers

- Mutational density is similar between immune subtypes

- BATF3 DCs are important for developing a T cell-inflamed phenotype

- FGFR3 activating mutations are exclusive to non-T cell-inflamed tumors

Sweis and Galsky, Urologic Oncology 2016

Patients and families

Sweis Lab
Jeffrey Bloodworth
Lomax Pass
Aubrianna Ramsdale
Ken Hatogai
Ciro Andolfi
Abby Mishory
Jenna Nimer

Immune Monitoring Core
Yuanyuan Zha

Thomas Gajewski
Jessica Fessler
Shyuin Li
Vyara Matson
Michael Leung

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