

Neoadjuvant Immunotherapy for glioblastoma

SITC 2019

A Tale of Two Brain Tumors: Primary versus Metastatic CNS
Tumors

November 9, 2019

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Disclosures

- Merck – Received research funding.
 - Consultant for biomarker development



Introduction

- Recurrent GBM is associated with a median survival of 6-9 months
- PD-1 blockade as monotherapy has demonstrated benefit in multiple cancer types
- GBM is generally considered not to be immunogenic
 - Relatively moderate mutational burden
- Only reported responses to immune checkpoint blockade in GBM have been case studies of individuals with hypermutated genotype and mismatch repair deficiency



Recent Developments in how the timing of checkpoint blockade can impact anti-tumor immune responses

nature
medicine

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

FOCUS | ARTICLES

<https://doi.org/10.1038/s41591-018-0337-7>

immunotherapy promotes
intratumoral and systemic
antitumor immune responses in
glioblastoma

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Patrick Y. Wen^{11,19} and Robert M. Prins^{2,3,5,17,19*}

Neoadjuvant nivolumab
impacts the immune microenvironment
in glioblastoma

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Angelo Porciuncula¹, Miguel A. Idoate
Sonia Tejada⁵, Pedro Berraondo⁸,
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Jose L. Perez-Gracia^{2,3,4,13} and Ignacio
Lacort Simpson¹, Elizabeth M. Porter
Shaojun Zhang⁸, Alexander J. Lazar¹⁴,
Alexandre Reuben³, Miles C. Anderson
James Allison⁵, Michael T.etzlaff^{9,11}

Neoadjuvant PD-1 Blockade in Resectable Lung Cancer

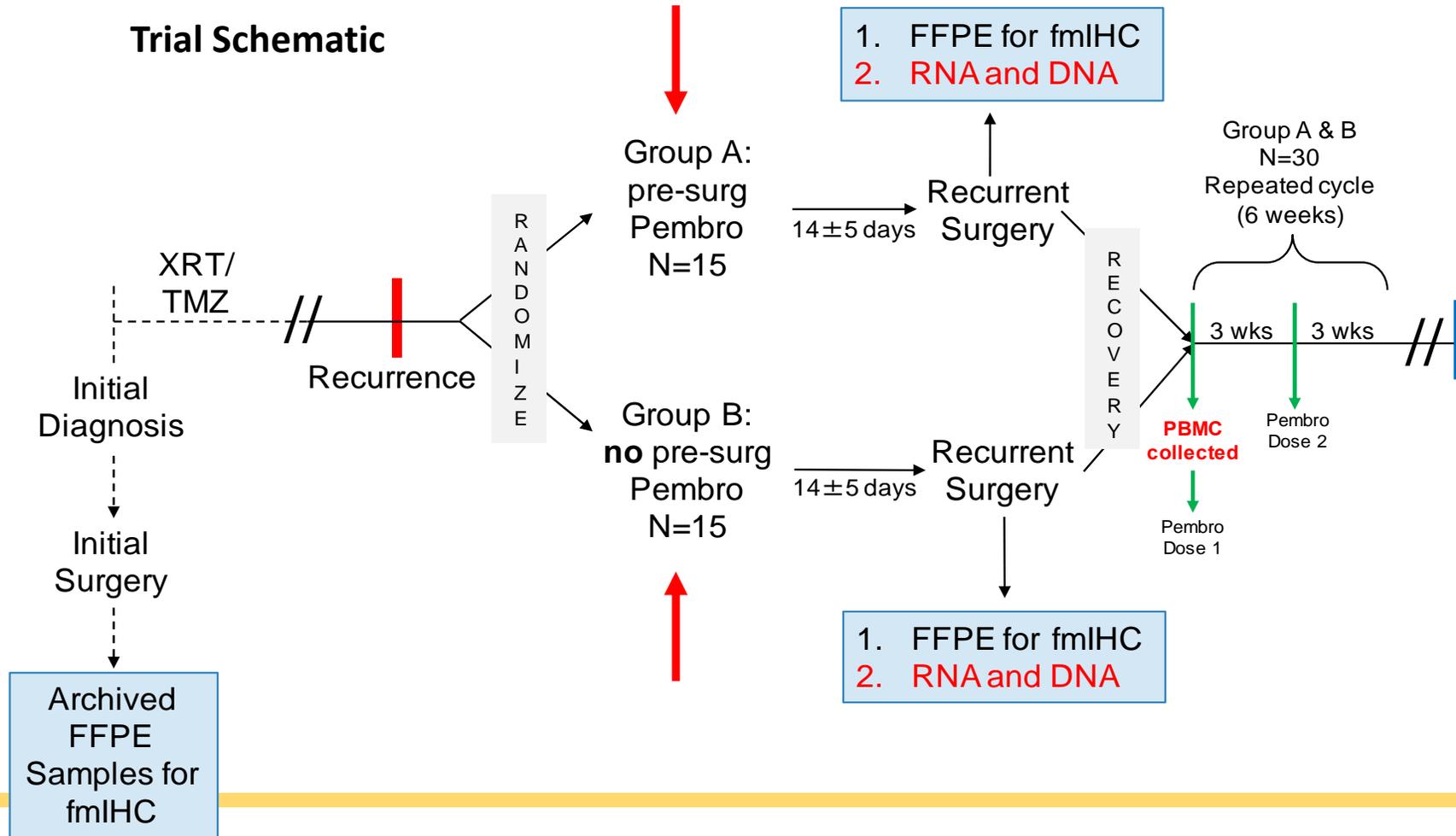
P.M. Forde, J.E. Chaft, K.N. Smith, V. Anagnostou, T.R. Cottrell, M.D. Hellmann,
M. Zahurak, S.C. Yang, D.R. Jones, S. Broderick, R.J. Battafarano, M.J. Velez,
N. Rekhtman, Z. Olah, J. Naidoo, K.A. Marrone, F. Verde, H. Guo, J. Zhang,
J.X. Caushi, H.Y. Chan, J.-W. Sidhom, R.B. Scharpf, J. White, E. Gabrielson,
H. Wang, G.L. Rosner, V. Rusch, J.D. Wolchok, T. Merghoub, J.M. Taube,
V.E. Velculescu, S.L. Topalian, J.R. Brahmer, and D.M. Pardoll



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Development of neoadjuvant surgery trial design to study the effects on the tumor microenvironment



Tim Cloughesy, MD
Patrick Wen, MD



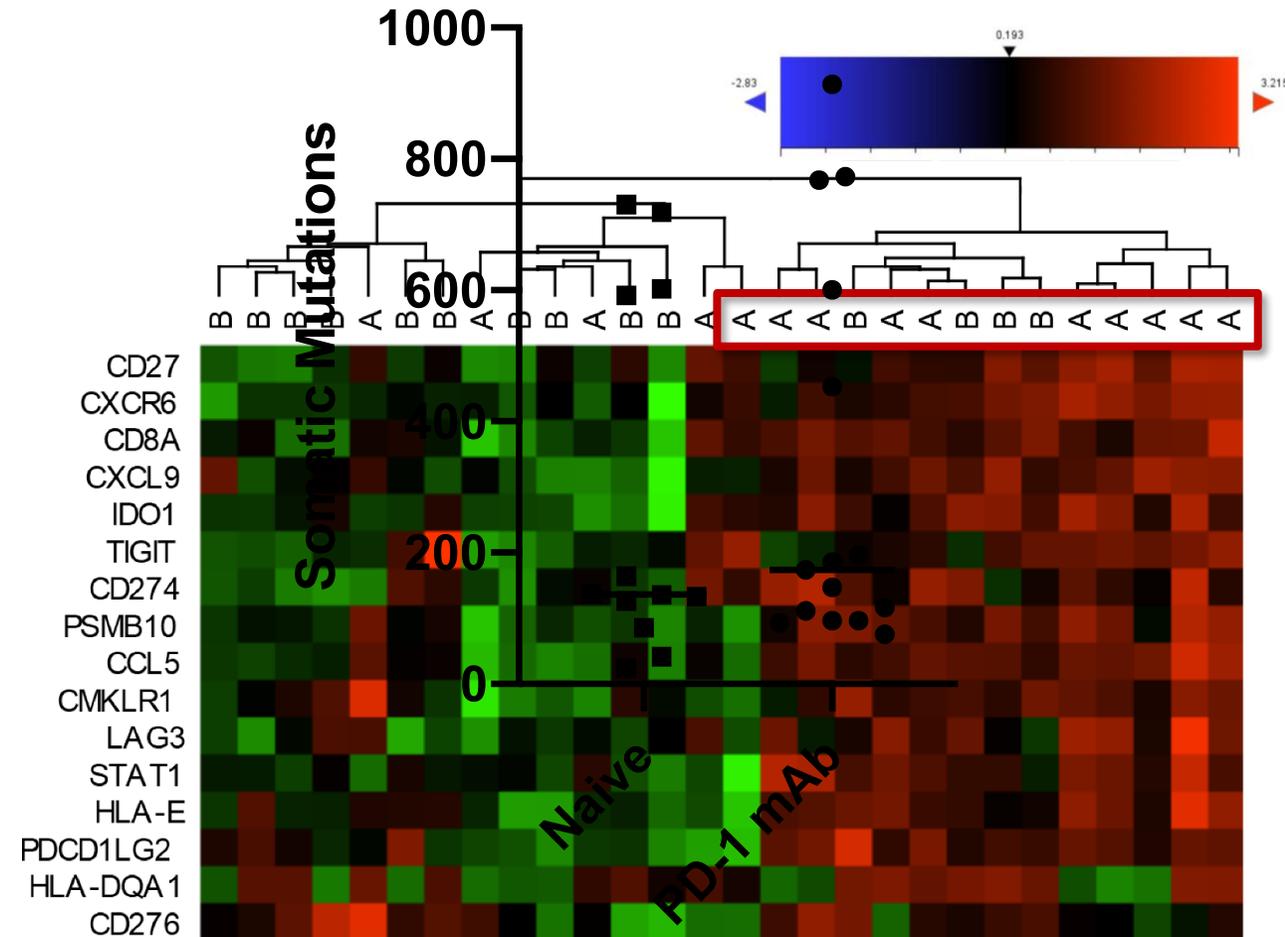
Exploratory Biomarkers to evaluate the influence of PD-1 mAb on the TME and peripheral blood

- **Gene expression profiling** (Nanostring PanCancer Immune Profiling Panel and RNA-seq). We will assess Gene Expression signature within tumors. *Performed/Analyzed at UCLA*
- **Quantitative T cell receptor Sequencing** (*Performed at Adaptive Biotech./Analyzed at UCLA*). We will assess estimated TIL count and T cell receptor diversity.
- **Multiplex Immunofluorescence**. We will quantitatively assess % CD8, %CD8+PD1+ TIL, %PD-L1+ cells (CD45 vs. GFAP). *Performed/Analyzed at UCLA.*
- **Mass Cytometry**. High dimensional analysis of immune cell phenotypes after treatment. *Performed at UCLA/Analyzed at UCLA with help from the Parker Institute.*

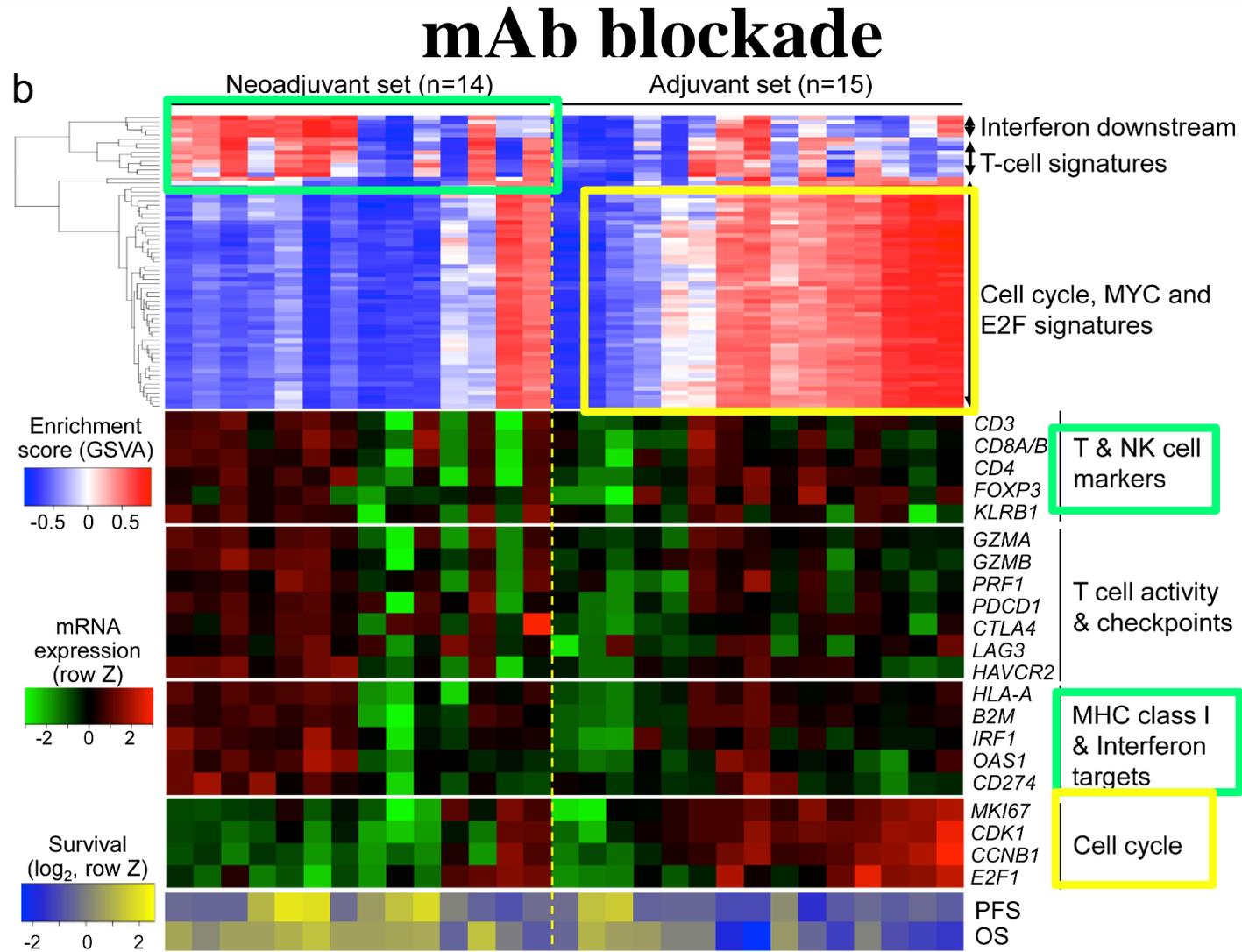


Clinical Responsiveness: Somatic mutations vs. IFN- γ Signature

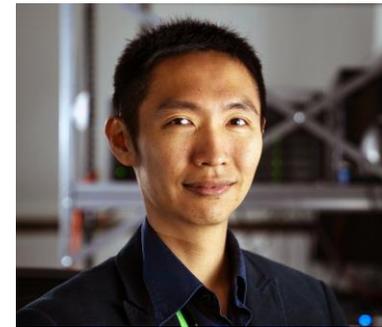
- No difference in somatic mutation burden between groups
- Nanostring PanCancer Immune Profiling Panel
- IFN- γ related Gene Expression Signature can predict “hot” and “cold” tumors



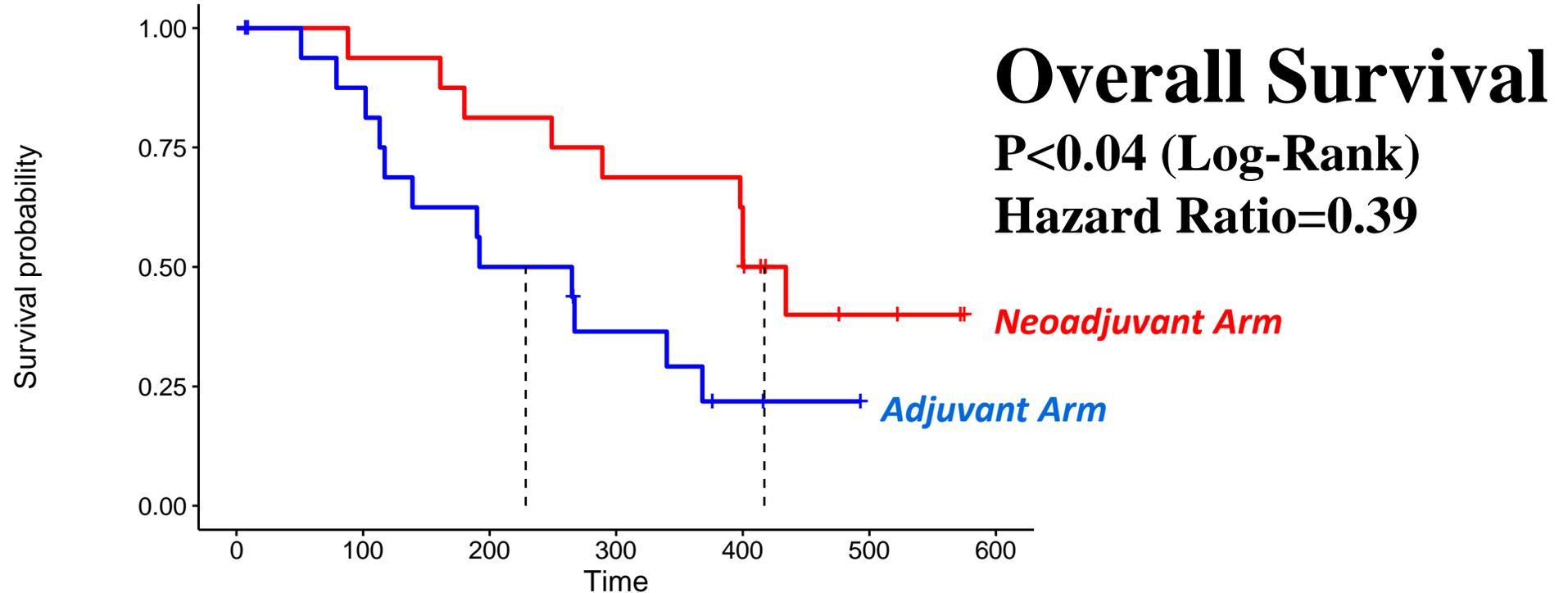
Cell Cycle signature downregulation following neoadjuvant PD-1 mAb blockade



Willy Hugo, Ph.D.



Extended Survival with neoadjuvant PD-1 mAb blockade



Number at risk

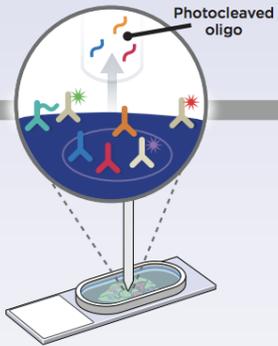
Neoadjuvant	16	15	13	11	10	3	0
Adjuvant	19	14	8	5	2	0	0

T Cloughesy and A Mochizuki, et.al.
Nature Medicine 2019

In-Depth Analysis of the TME: Digital Spatial Profiling

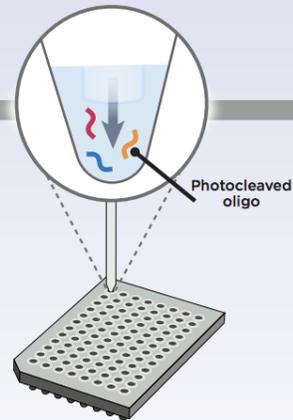
4. Aspirate Oligos

Decoupled oligonucleotides are rapidly aspirated using a microcapillary without touching the sample, thereby leaving the sample unaltered.



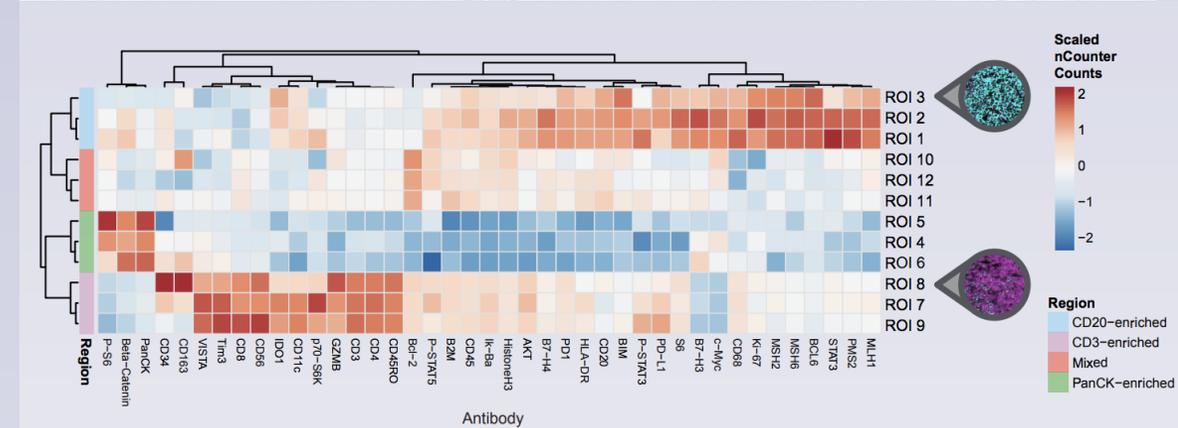
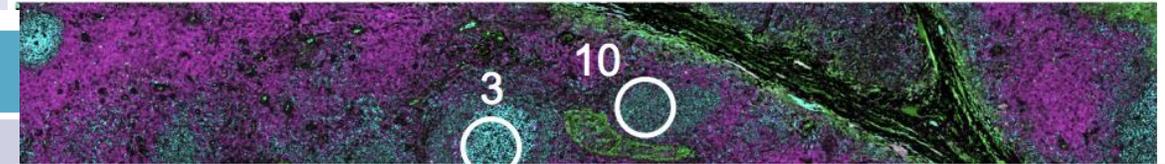
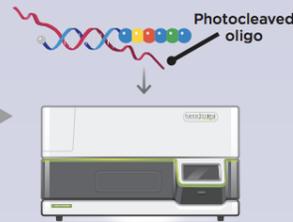
5. Dispense Oligos

Oligonucleotides are deposited into wells of a microtiter plate, and the information contained within each well is indexed to the ROI on the tissue.

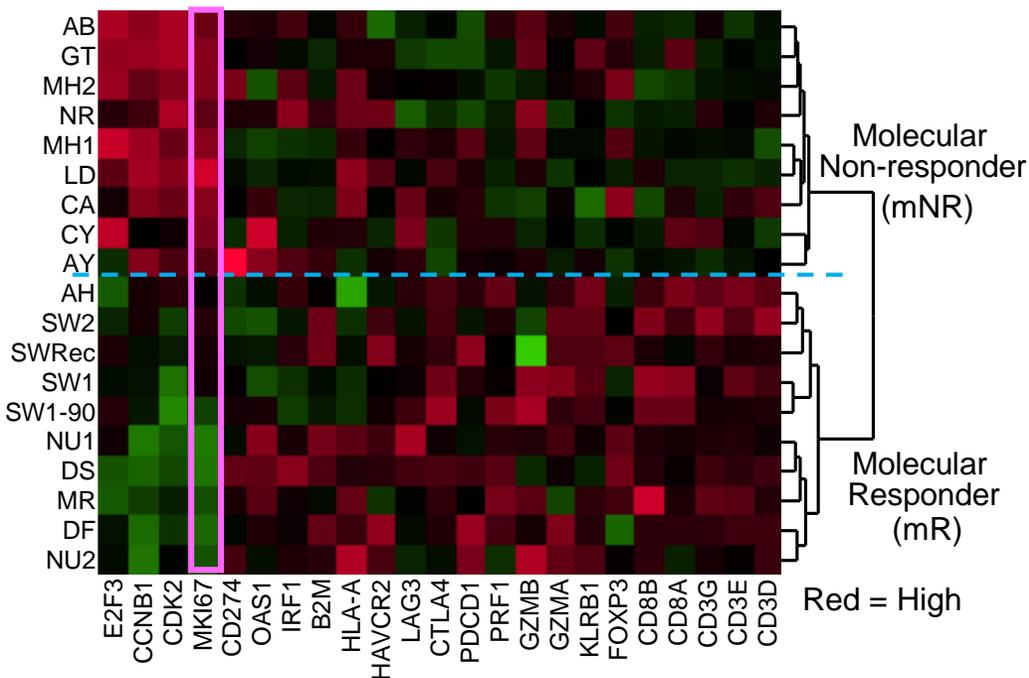


6. Count Barcodes

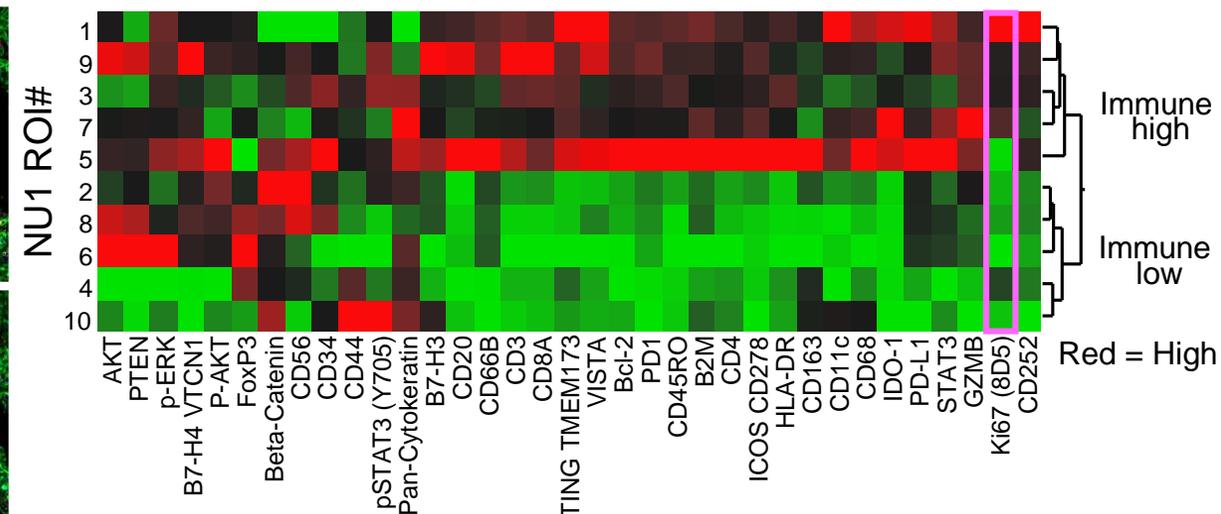
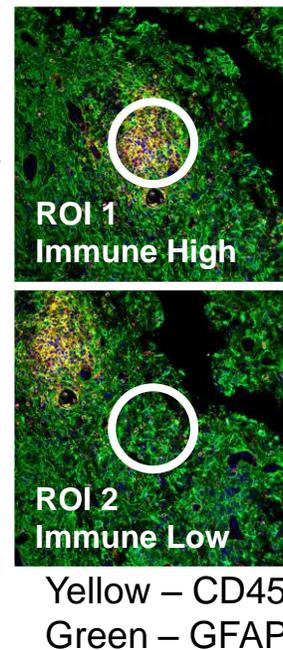
The oligonucleotides are hybridized to NanoString® barcodes and quantitated on the nCounter platform.



Bulk RNA Analysis in follow-on patients



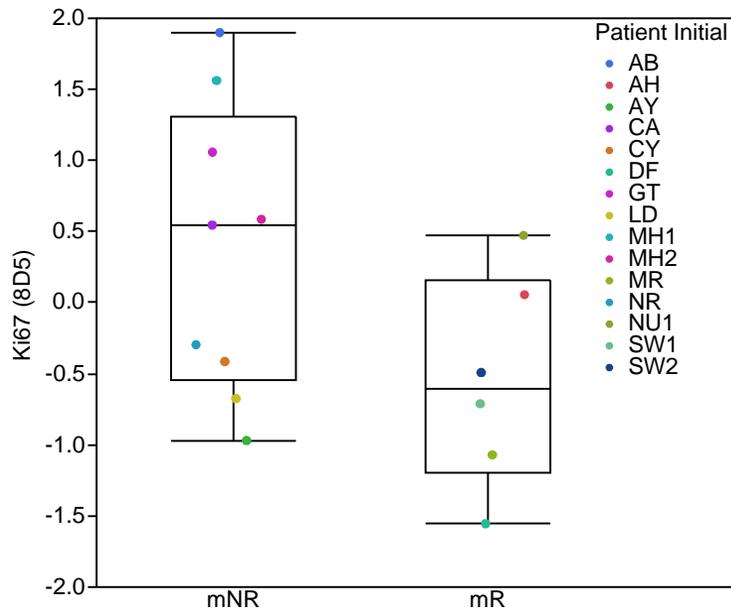
Digital spatial profiling (DSP) Example – Patient NU1, mR



40-plex protein panel analyzed in each ROI

DSP Dataset: 180 ROIs from 15 GBMs (9 mNR and 6 mR)

ROIs with <25% immune cells

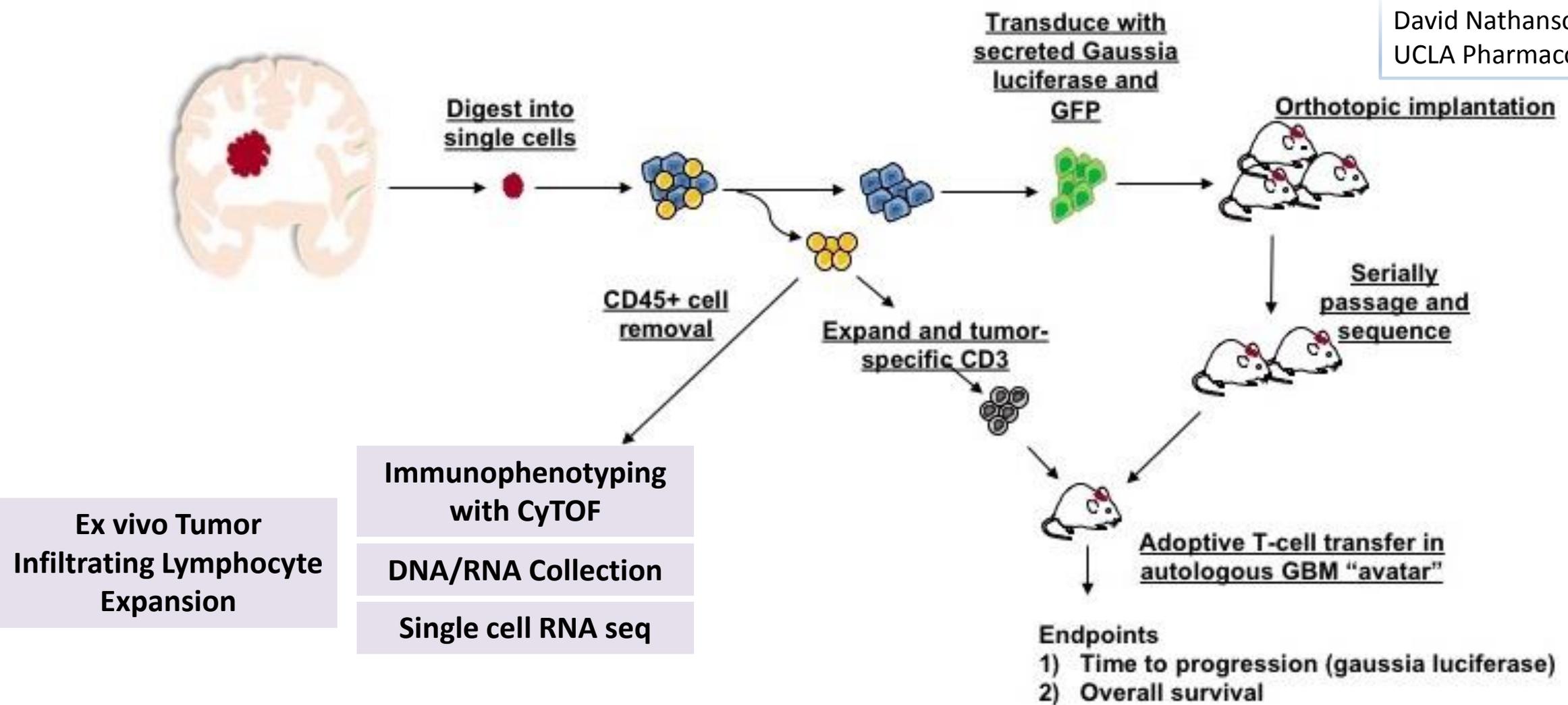


- Ki67 discriminates therapy response from non-response in bulk mRNA
- Microscopically, Ki67 is only a marker for response in immune cell poor regions
- A deeper analysis revealed that proteins such as B7-H3, PTEN, and STING TME173 determine the level of Ki67 in immune cell poor regions

(Unpublished with Jim Heath and Alphonsus Ng)

Immune Cell Isolation protocol from tumors

David Nathanson, Ph.D.
UCLA Pharmacology



Ex vivo Tumor
Infiltrating Lymphocyte
Expansion

Immunophenotyping
with CyTOF

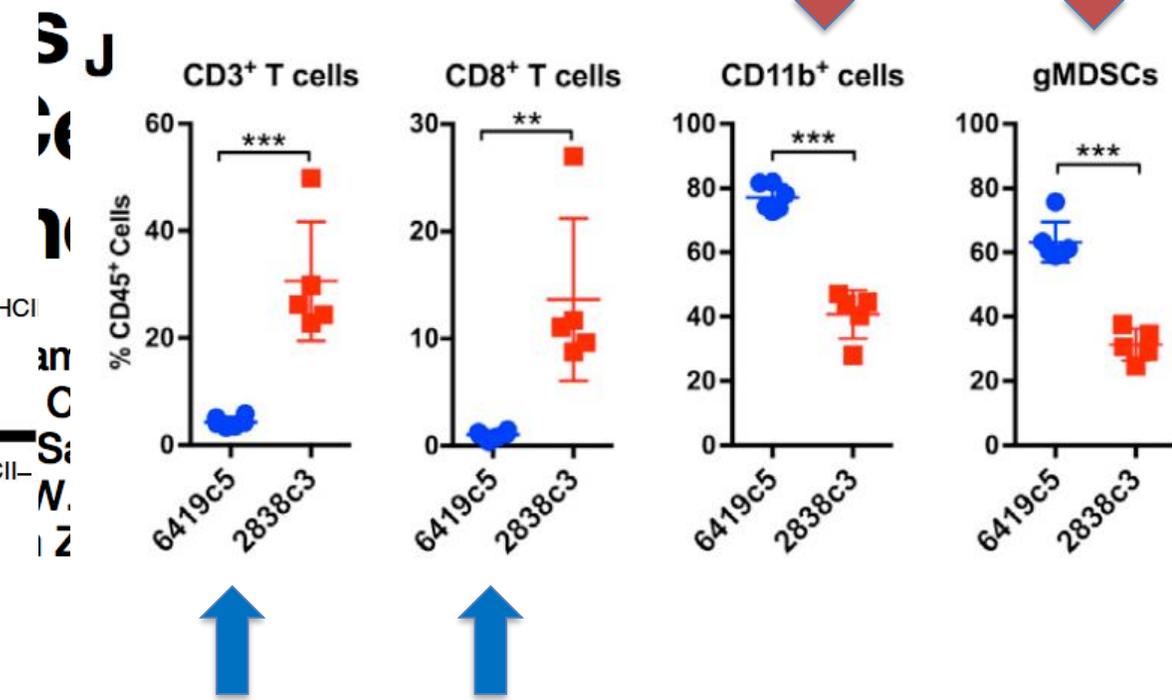
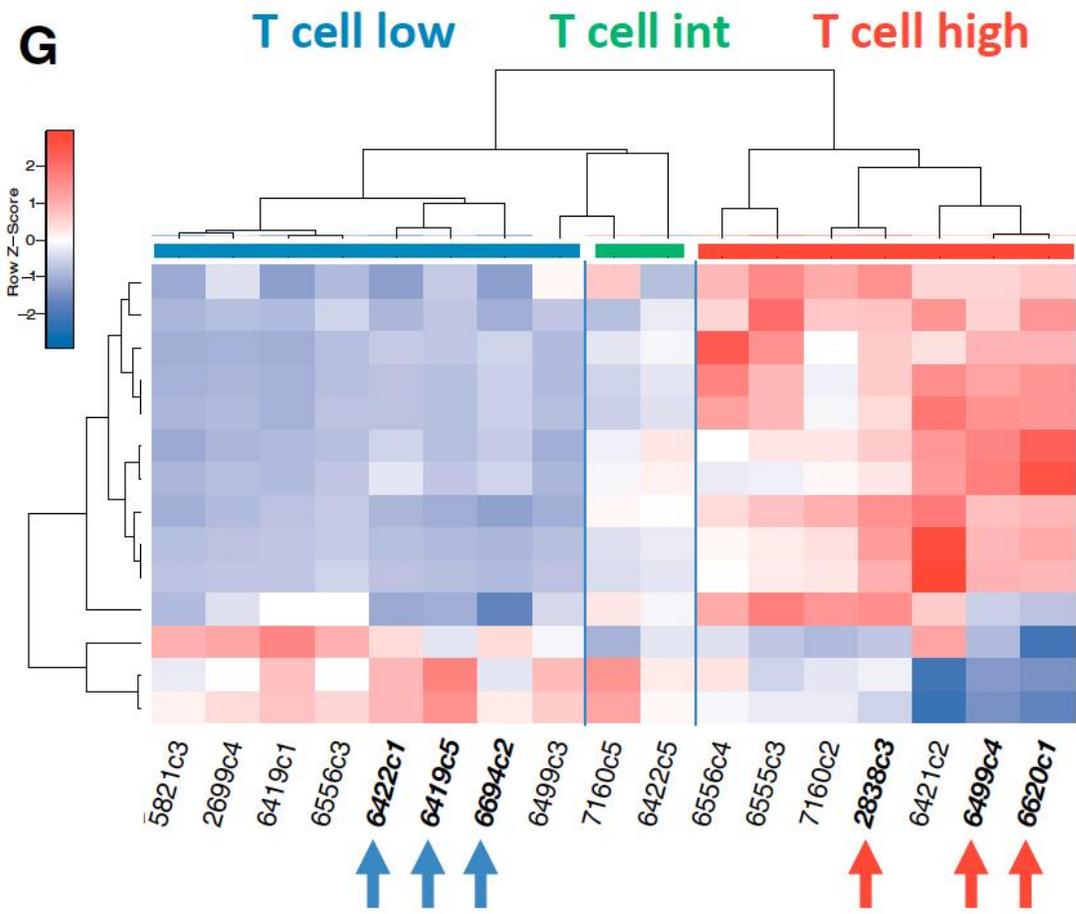
DNA/RNA Collection

Single cell RNA seq



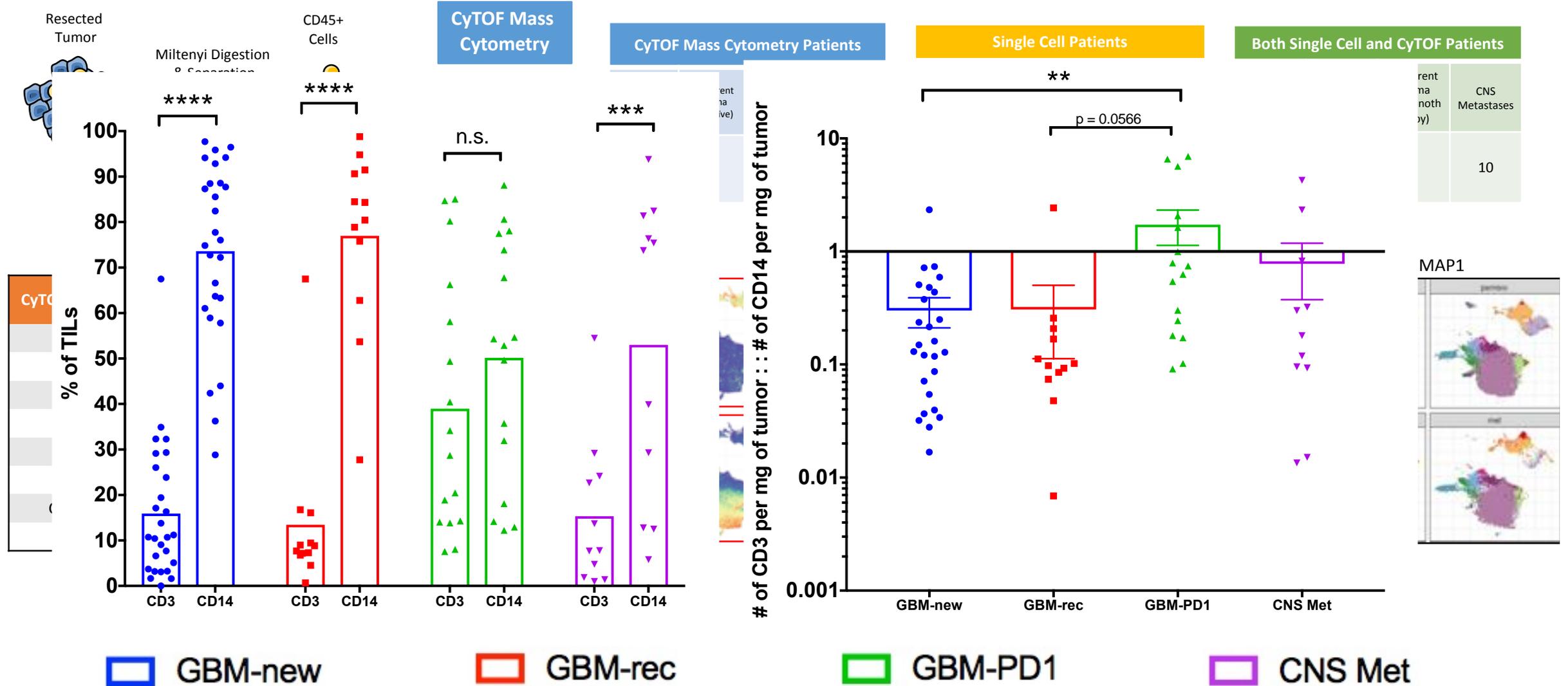
David Geffen
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UCLA Health System

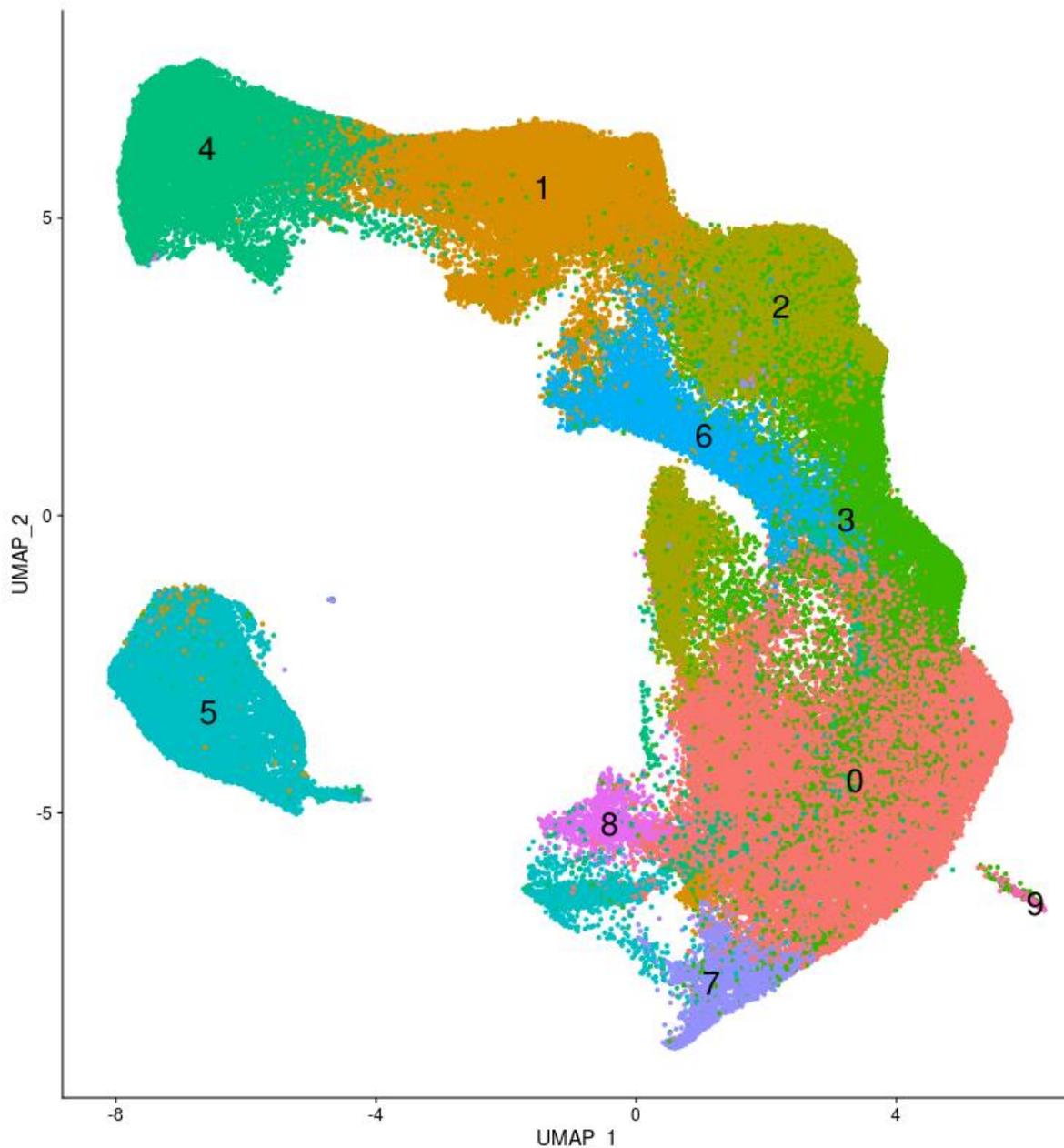


Mass Cytometry of tumor infiltrating immune cells

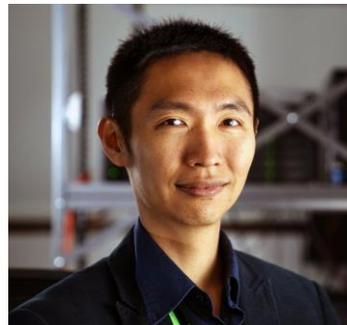
PD-1 Blockade alters the Myeloid cell:TIL Ratio



Single cell RNA Seq of tumor infiltrating immune cells



Willy Hugo, Ph.D.



Type	Cell #	Tumor #
AA.TIL	1286	2
GBM.new.TIL	41138	10
GBM.rec.TIL	24145	5
GBM.pembro.TIL	33325	9
met.pembro.TIL	27569	3
met.TIL	27355	7
OG.TIL	5249	1

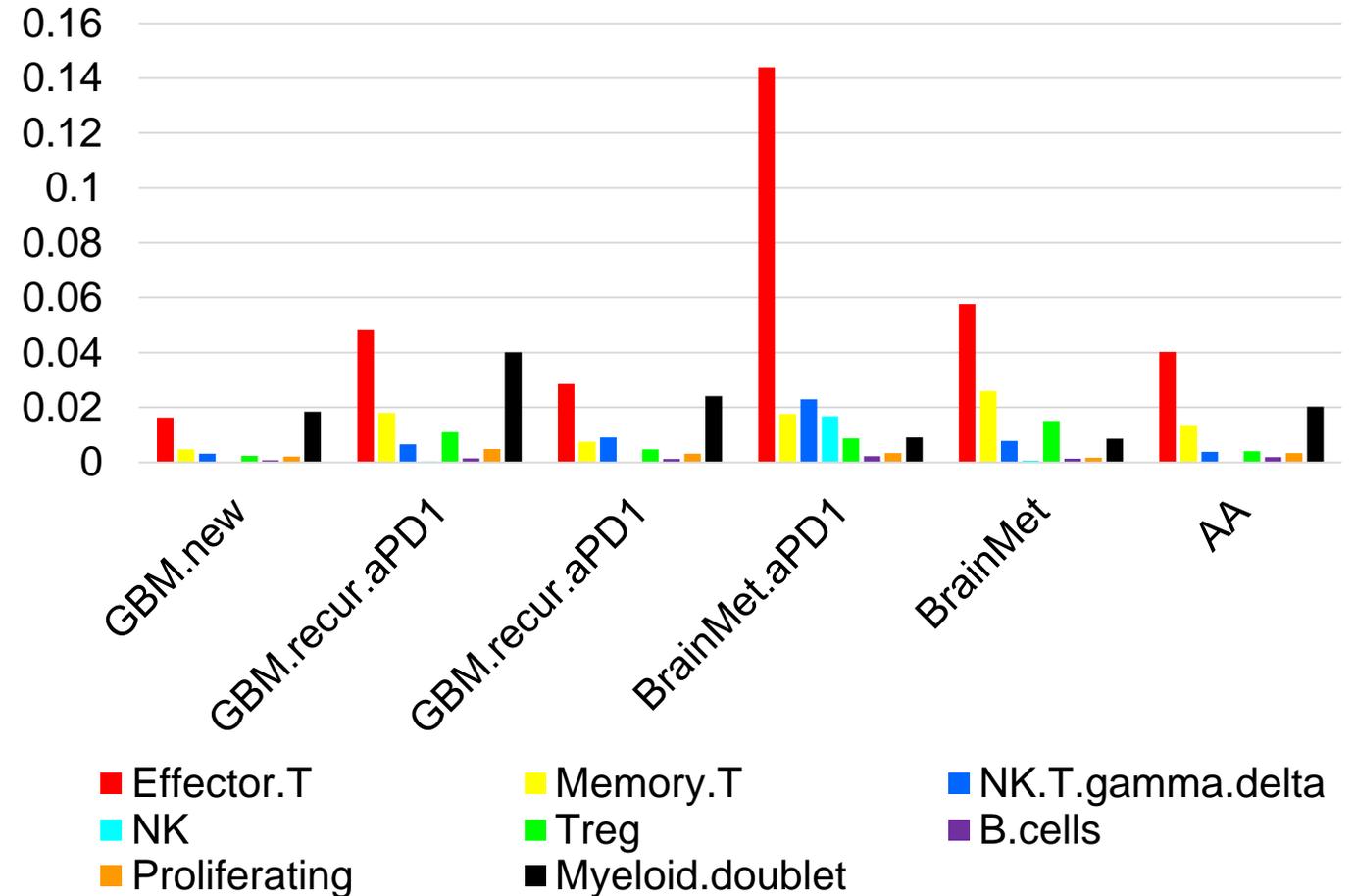
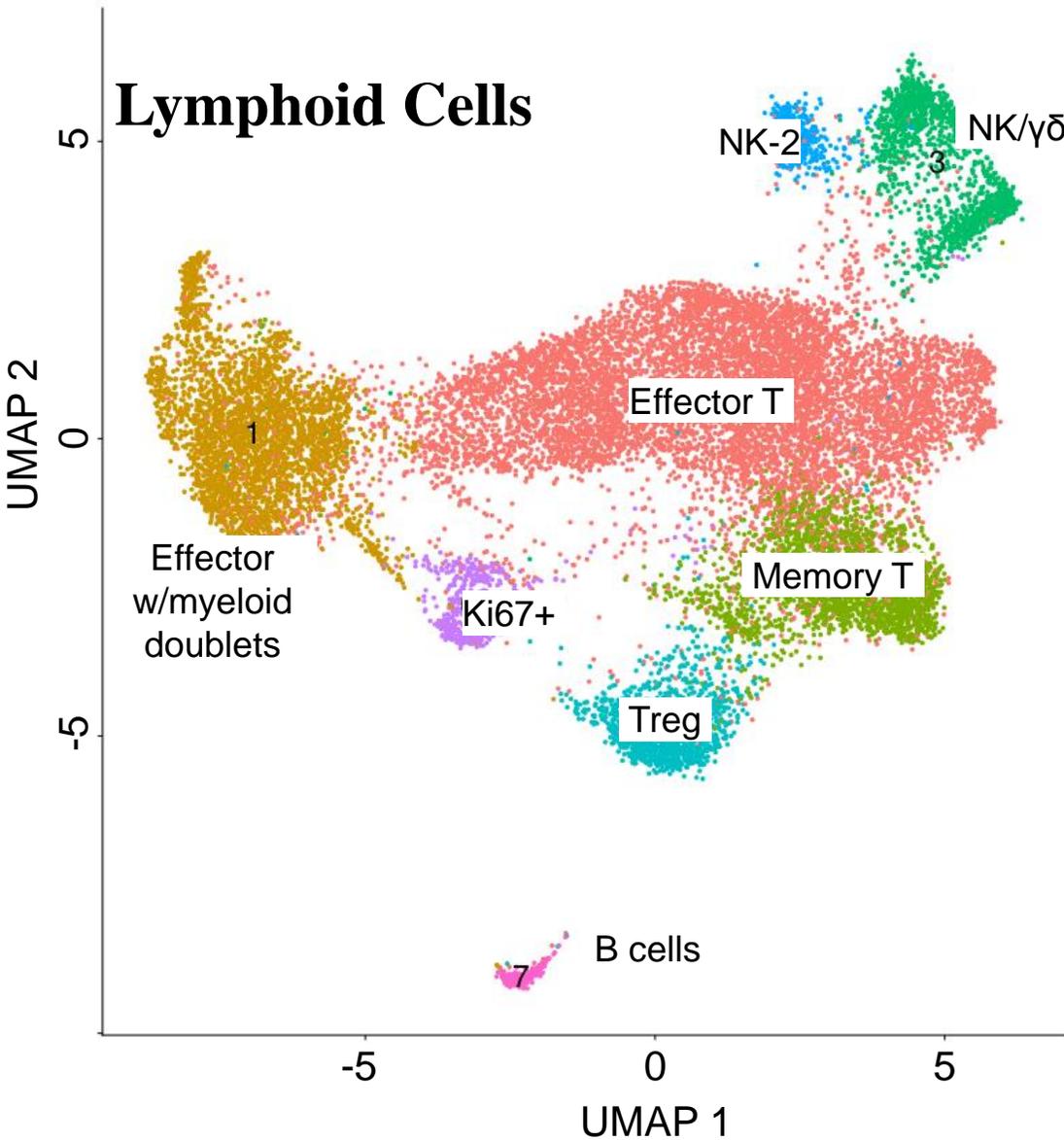
Parameters:

- 20% cutoff of mitochondria, giving a total of 160,067 cells
- Used the GBM.pembro.TIL (n=9) as reference to speed up computation. Memory required was ~ 100GB.
- The remaining mitochondrial percentage and the cell cycle score difference were regressed out.
- The clustering was done using PCA with 30 dimensions.
- UMAP was run using n.neighbors=10 and min.dist=0.1 and seed.use=22. The clustering was done using resolution = 0.2.

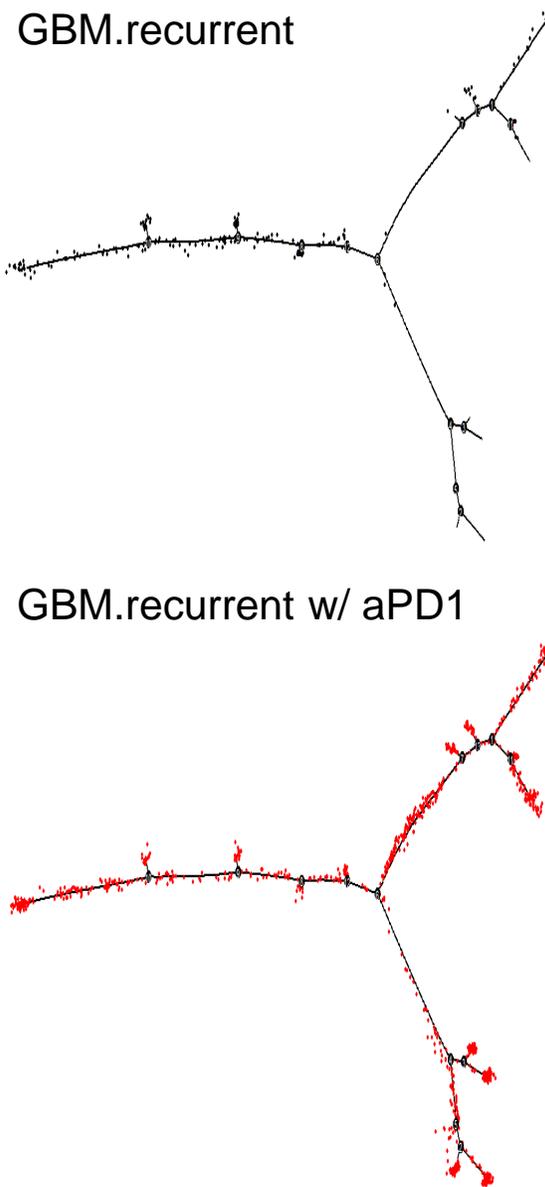
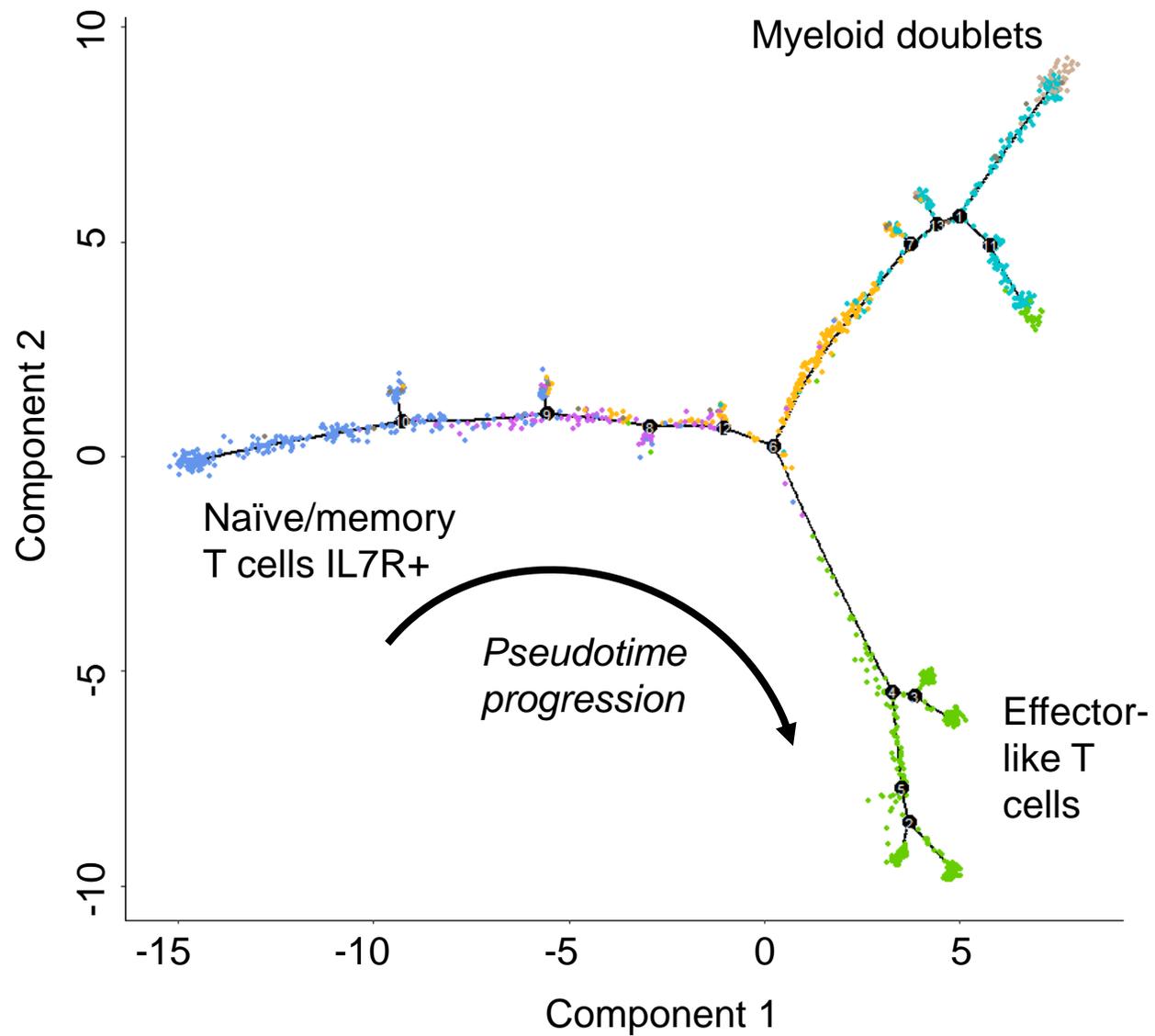
Unpublished

Single Cell RNA seq of tumor infiltrating immune cells

- C0 = Activated Effector CD8 (CD27, IFNG, CCL5, GZMK, PDCD1)
- C1 = Myeloid doublet (CD14, MHC II+, complement factors, APOE)
- C2 = Memory (IL7R, CD40LG, CCR7)
- C3 = NK and T gamma delta (CD16, TRDC, GNLY)
- C4 = Treg (FOXP3, IL2RA, CTLA4, TNFRSF4)
- C5 = FGFBP2+ NK cell
- C6 = Proliferating (MKI67+)
- C7 = B cells (CD79A/B, MS4A1, immunoglobulin, MHC II)



CD8 T cells' monocle2 trajectory (GBM recurrent without vs. with aPD1)

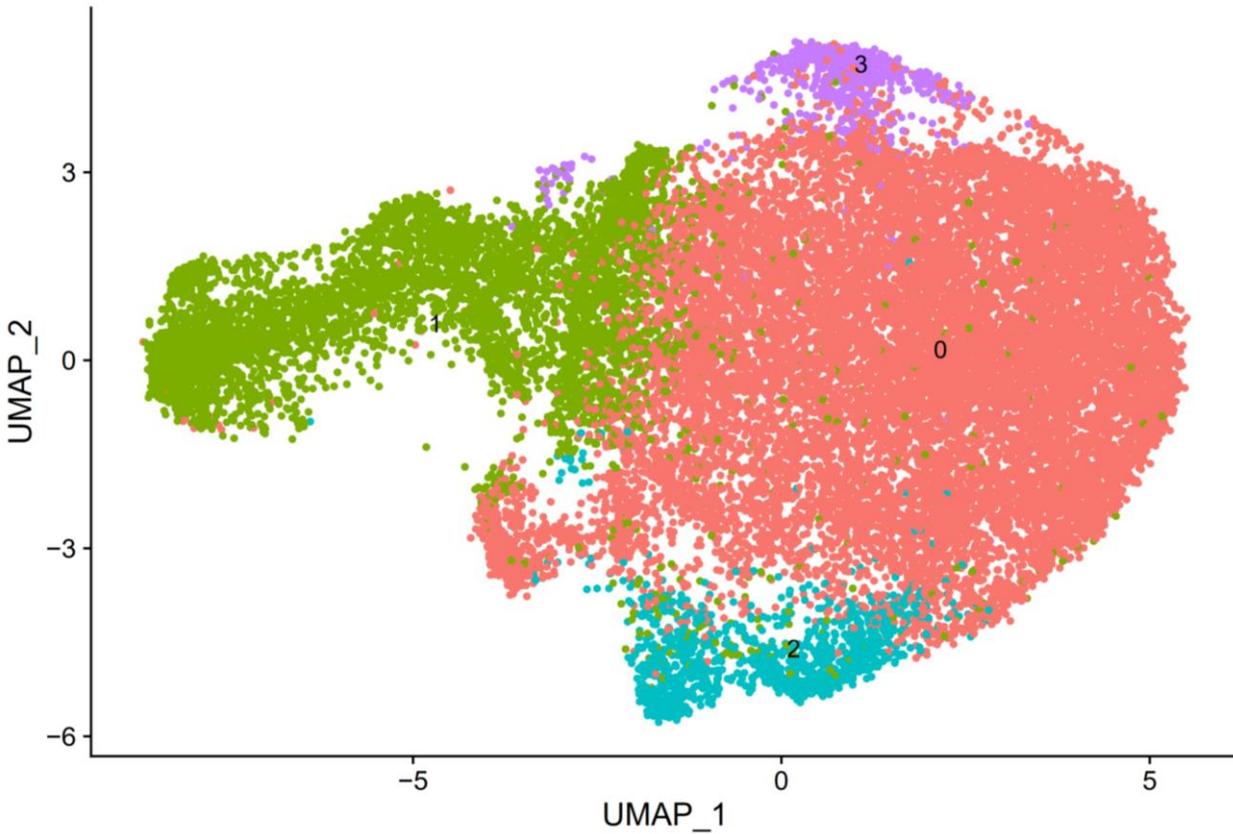


Unpublished



Aaron Mochizuki, D.O.
Pediatric Neuro-Oncology Fellow

Myeloid Cells



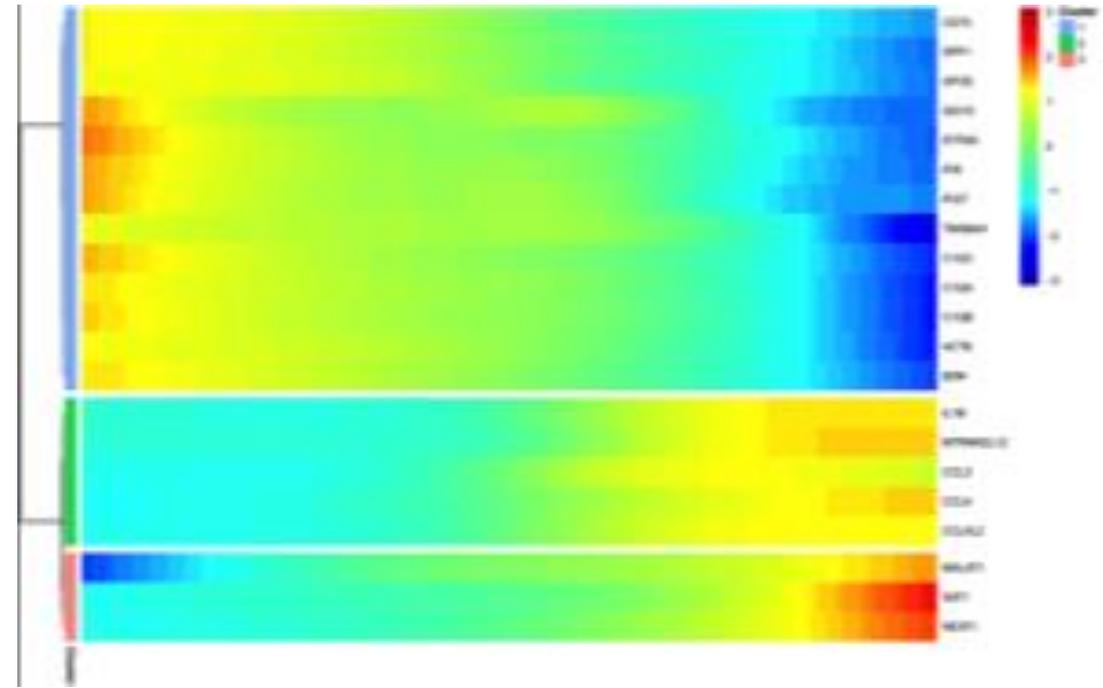
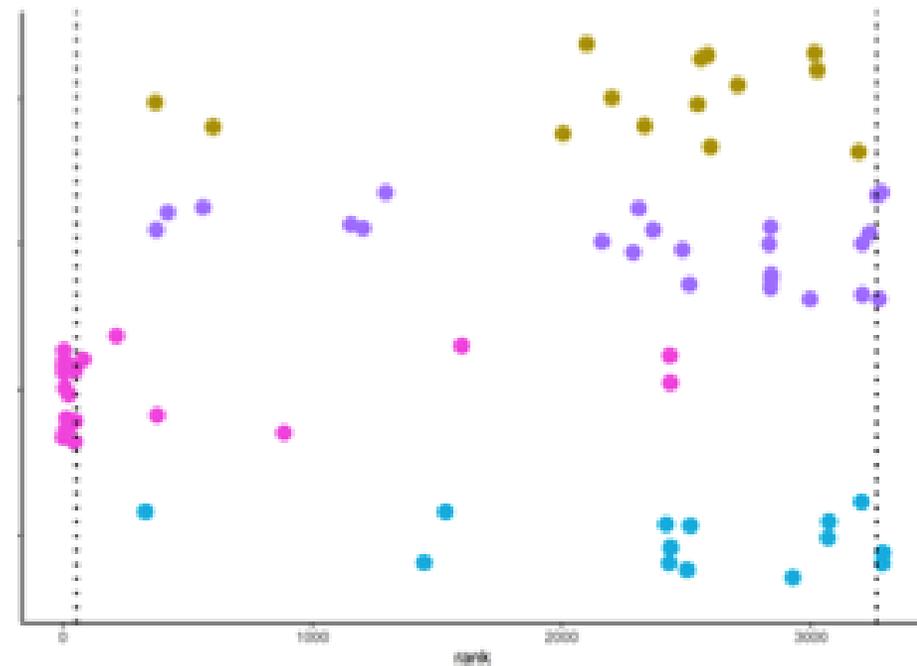
- 0
- 1
- 2
- 3

Dendritic cell

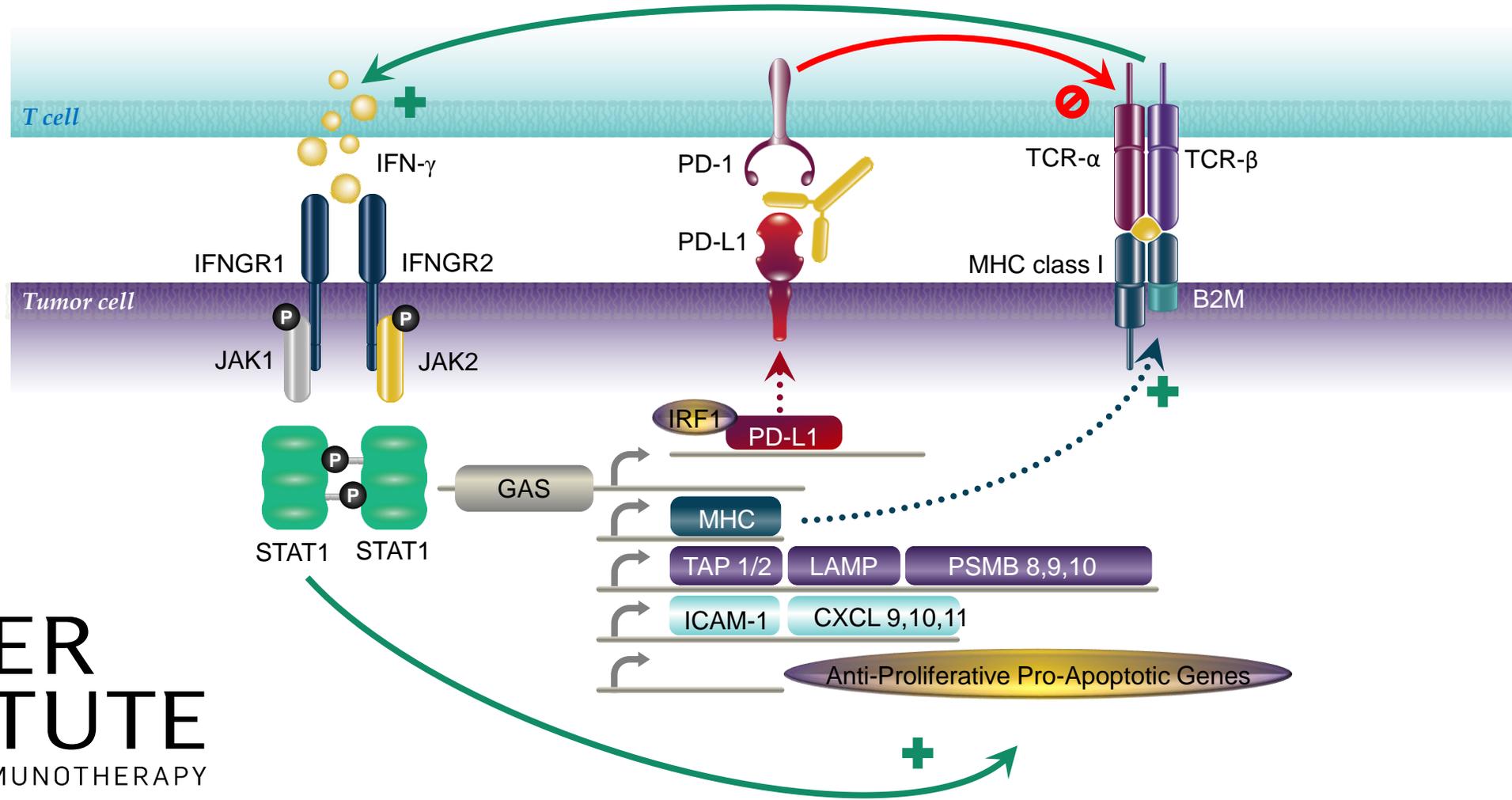
TNF

Type 1
Interferon

NFκB



Role of PD-1 Blockade, Interferon-gamma Receptor signaling, and Cell Cycle Activity



**PARKER
INSTITUTE**
for CANCER IMMUNOTHERAPY



David Geffen
School of Medicine

UCLA Health System

Conclusions

- Neoadjuvant PD-1 mAb blockade is associated significant survival benefit over adjuvant therapy alone
- Benefit appears to be driven by systemic expansion of tumor-specific T-cells with high interferon- γ signaling and downregulation of the cell cycle signature
- See coordinated T cell responses only in the neoadjuvant treatment arm



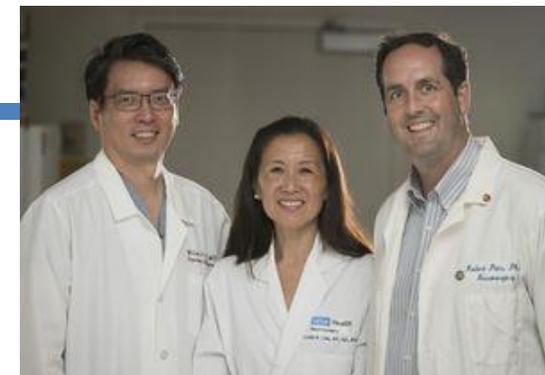
Funding Sources

- Merck & Co., Inc. Investigator Studies Program
- Adaptive Biotechnologies, Inc.
- NIH SPORE in Brain Cancer (P50CA211015)
- Parker Institute for Cancer Immunotherapy
- Ben and Catherine Ivy Foundation
- Defeat GBM Program of the NBTS
- The Cancer Research Institute
- Uncle Kory Foundation
- Ziering Family Foundation





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- Willy Hugo PhD
- Alexander Lee, BS
- Frances Chow, MD
- Mildred Galvez, BS
- Richard Everson, MD



- **Harvard-Dana Farber Cancer Inst**

- Patrick Wen, MD
- David Reardon, MD

- **Ivy Foundation Early Phase Clinical Trials Consortium**





The most robust, quantitative way to sequence millions of T- and B-cell receptors, period.

T Cell Receptor Sequencing – immunoSEQ platform (Adaptive Biotechnologies)

Cohort & Sample Overview

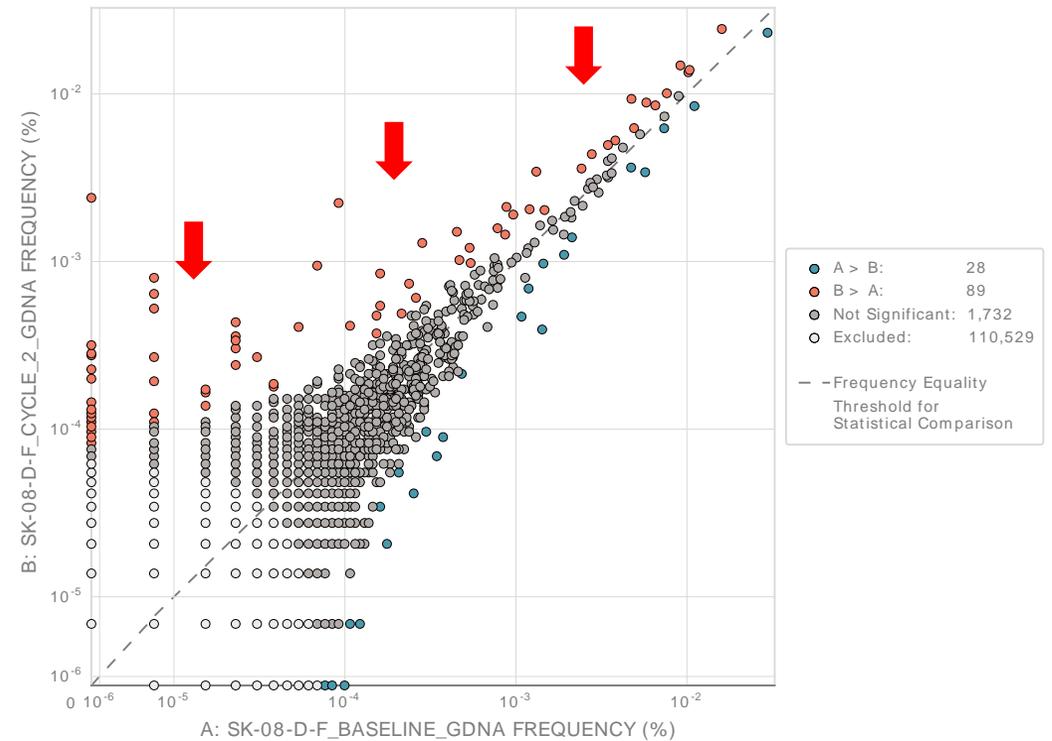
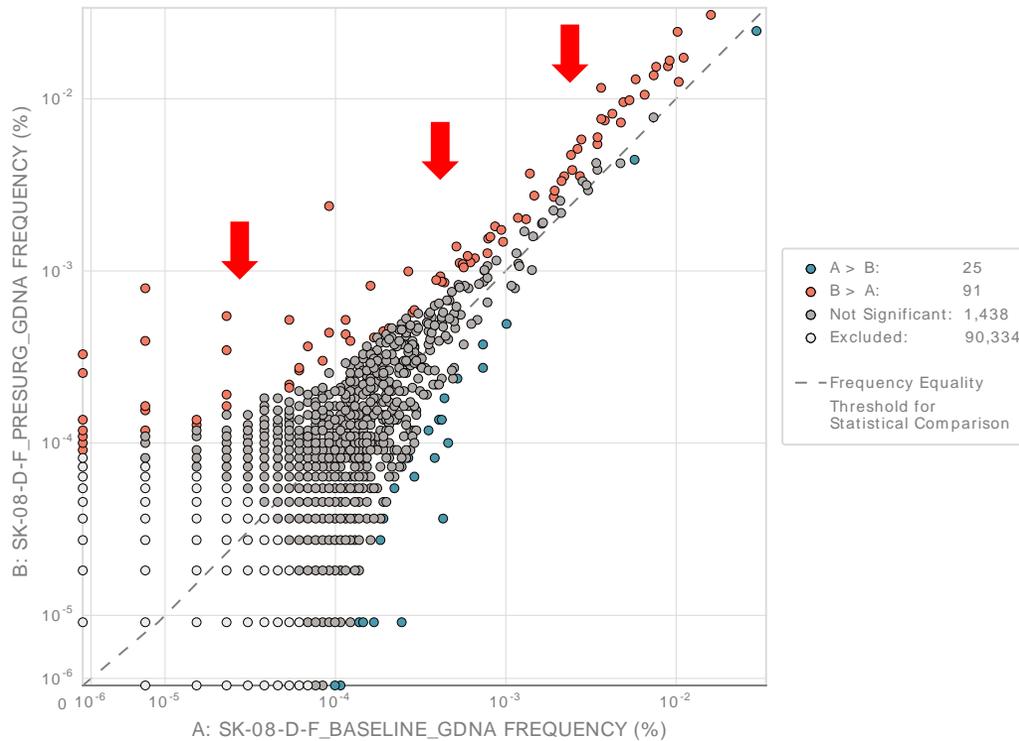
- 29 subjects have 1 tumor sample taken at time of surgery + 3 blood samples taken at baseline (pre-treatment), pre-surgery, and at Cycle 2 post-treatment



Differential Abundance of TCR Clones

Patient SK-08 (OS= 416 Days)

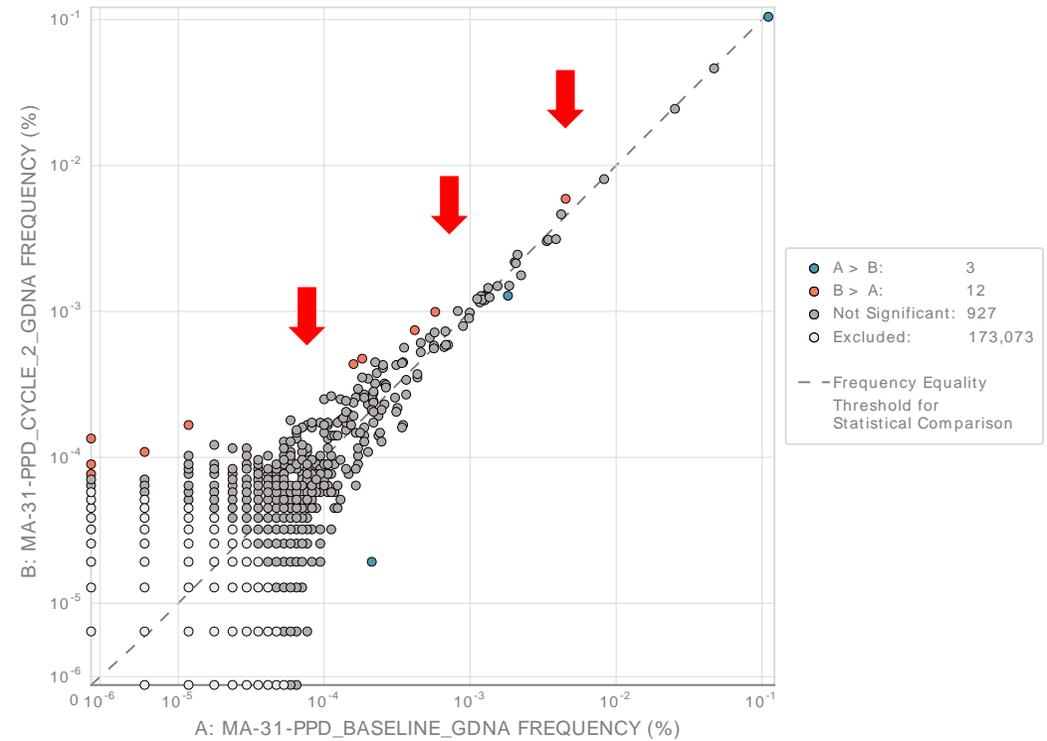
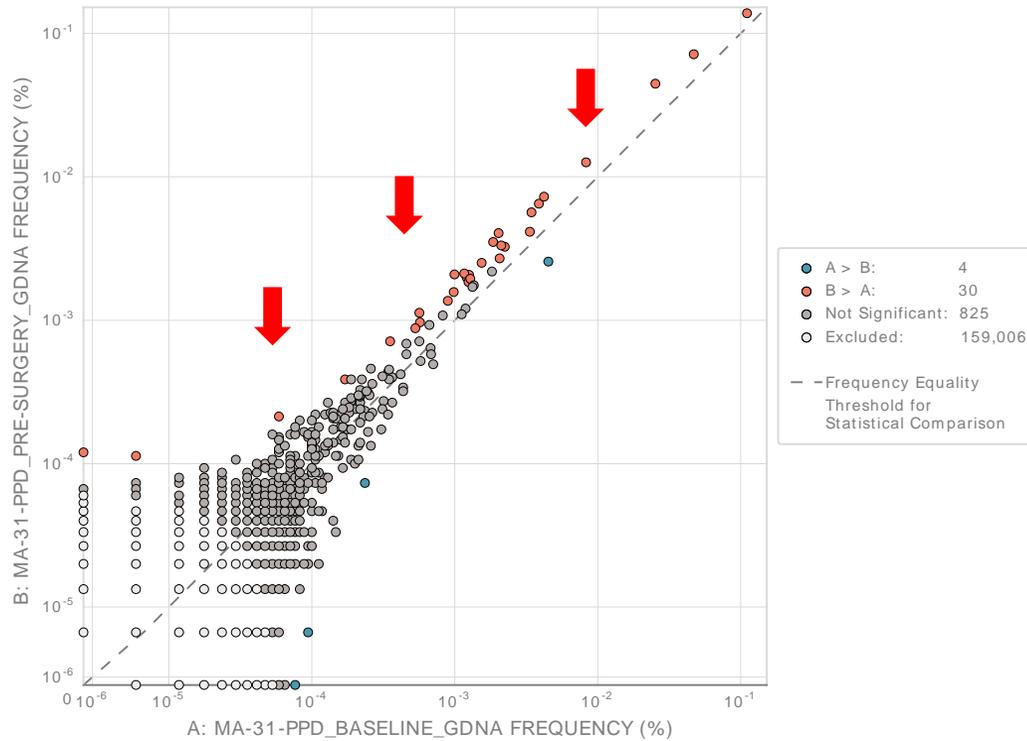
Tx Group A – Neoadjuvant+Adjuvant PD-1 mAb Blockade



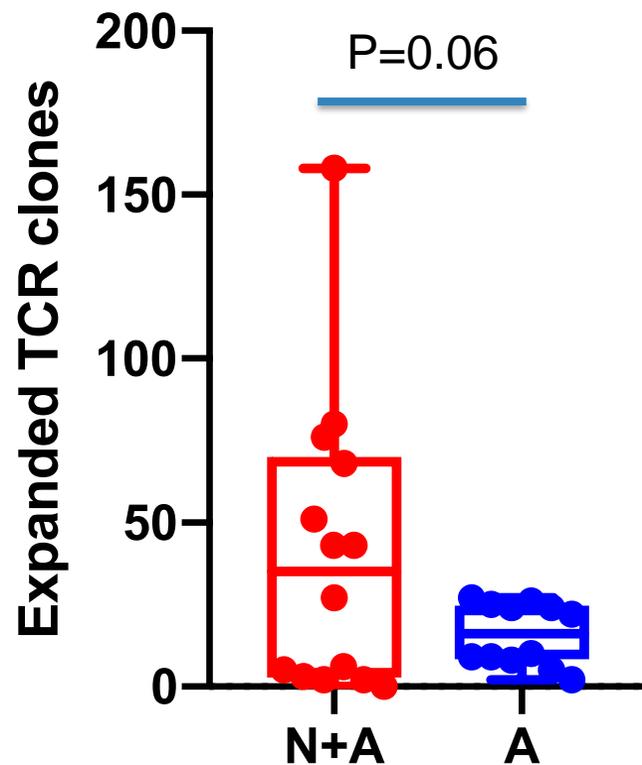
Differential Abundance of TCR Clones

Patient MA-31 (OS=58 days)

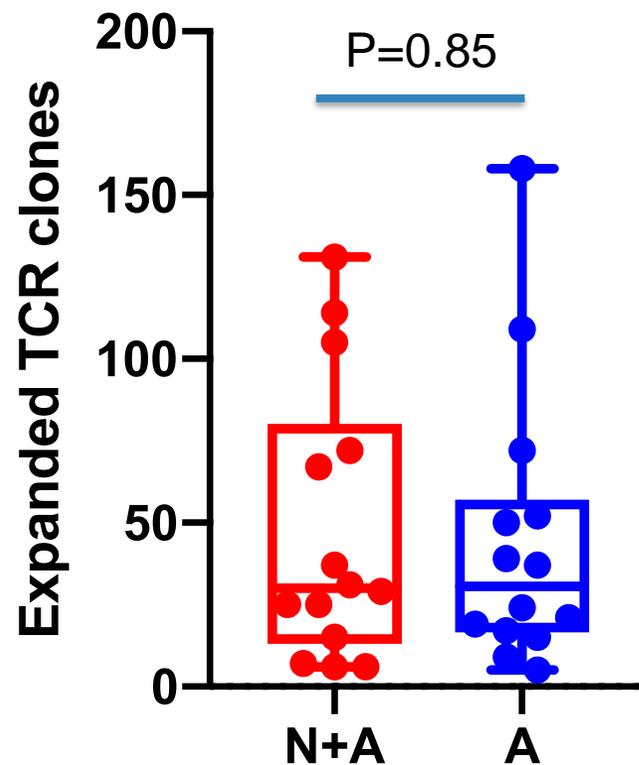
Tx Group B – Adjuvant only PD-1 mAb Blockade



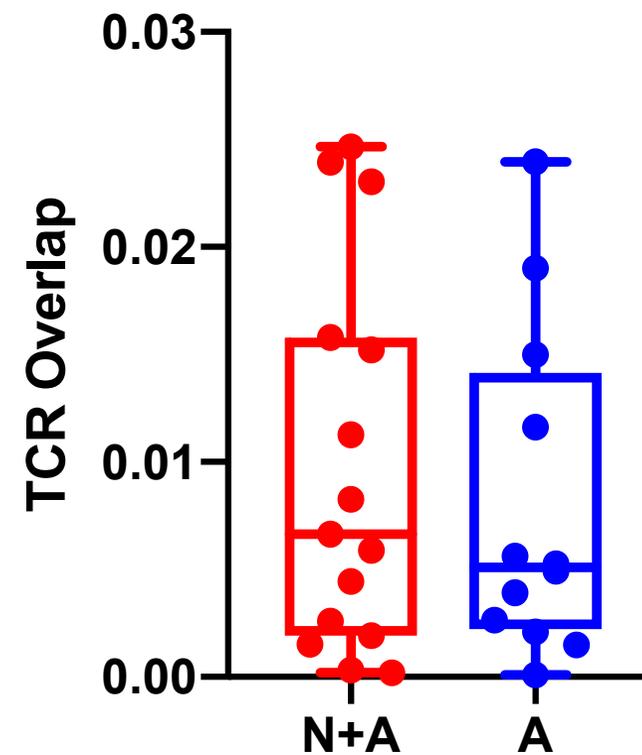
Clonal expansion (relative to Baseline) in the peripheral repertoire: At surgery vs on-Tx



Baseline to Surgery



Surgery to On-Treatment



At Surgery

Influence of PD-1 Blockade on the Tumor Microenvironment

