T CELL FUNCTIONAL STATES: A DEEP DIVE IN CANCER IMMUNOTHERAPY TARGETS

Thursday, November 18, 2021

4:30 p.m. – 6:30 p.m. EDT

Targets for Cancer Immunotherapy: A Deep Dive Seminar Series is supported, in part, by grants from Alkermes, Inc., Genentech, a member of the Roche Group, Incyte Corporation, Merck & Co., Inc., and Regeneron Pharmaceuticals (*as of 10/05/2021*).

Webinar Agenda

4:30 – 4:35 p.m. ET: Welcome and Introductions

4:35 p.m. – 6:00 p.m. ET: Presentations and Q&A

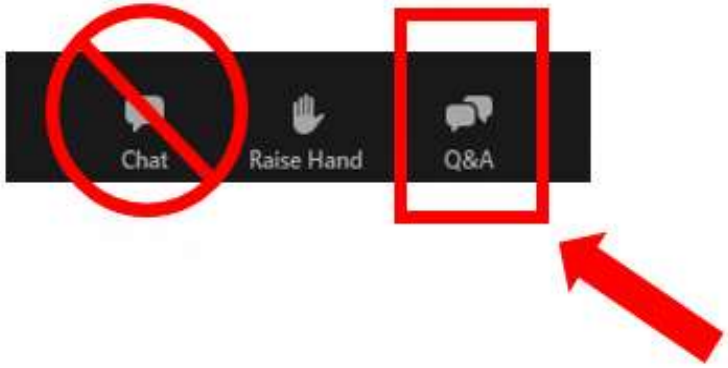
6:00 – 6:25 p.m. ET: Question and Answer Session

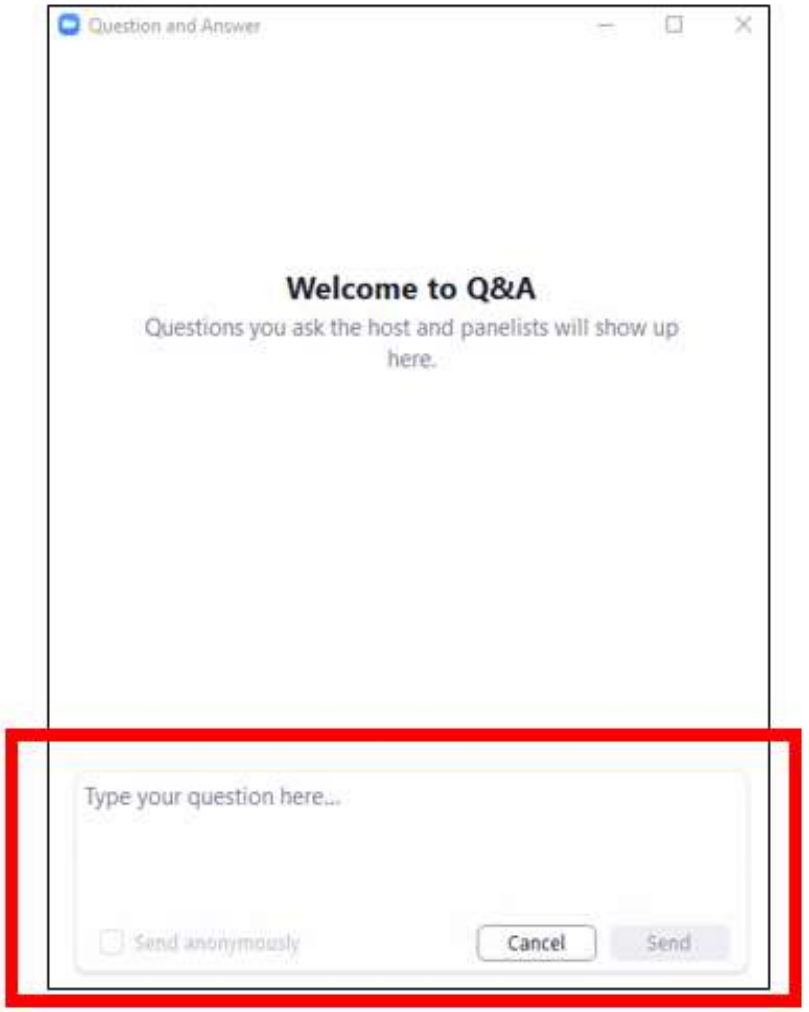
6:25 – 6:30 p.m. ET: Closing Remarks

How to Submit Questions

sitc
Society for Immunotherapy of Cancer

- Click the **"Q&A"** icon located on at the bottom of your Zoom control panel
- Type your question in the Q&A box, then click "Send"
- Questions will be answered:
  a. after each speaker's presentation
  b. in the Question & Answer session at the end of the seminar





Webinar Faculty

Moderators

John Wherry, PhD
University of Pennsylvania

Andrea Schietinger, PhD
Memorial Sloan Kettering Cancer Center

Speakers

Evan W. Newell, PhD
Fred Hutchinson Cancer Research Center

Ananda W. Goldrath, PhD
University of California, San Diego

Daniela S. Thommen, MD, PhD
The Netherlands Cancer Institute

Learning Objectives

- Describe the underlying biology and therapeutic mechanisms of T cell functional states in cancer immunotherapy
- Identify methods to address key scientific questions for T cell functional states in cancer
- Compare the strengths and weaknesses of various cancer immunotherapy approaches using T cell functional states

Webinar Outline

- Dr. Ananda W. Goldrath: Transcriptional side of T cell functional states; residency program; biomarkers
- Dr. Evan W. Newell: Cytometry; residency program (circulation and exhaustion)
- Dr. Daniela S. Thommen: Functional side; TLS (background, definition and function)

- Q&A: Dr. Wherry + Dr. Schietinger
  - Naming structures/terms/nomenclature
  - Translating t cell therapies into the clinic

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Transcriptional Programs Driving Differentiation of Tissue-resident Memory and Tumor Infiltrating CD8$^+$ T cells

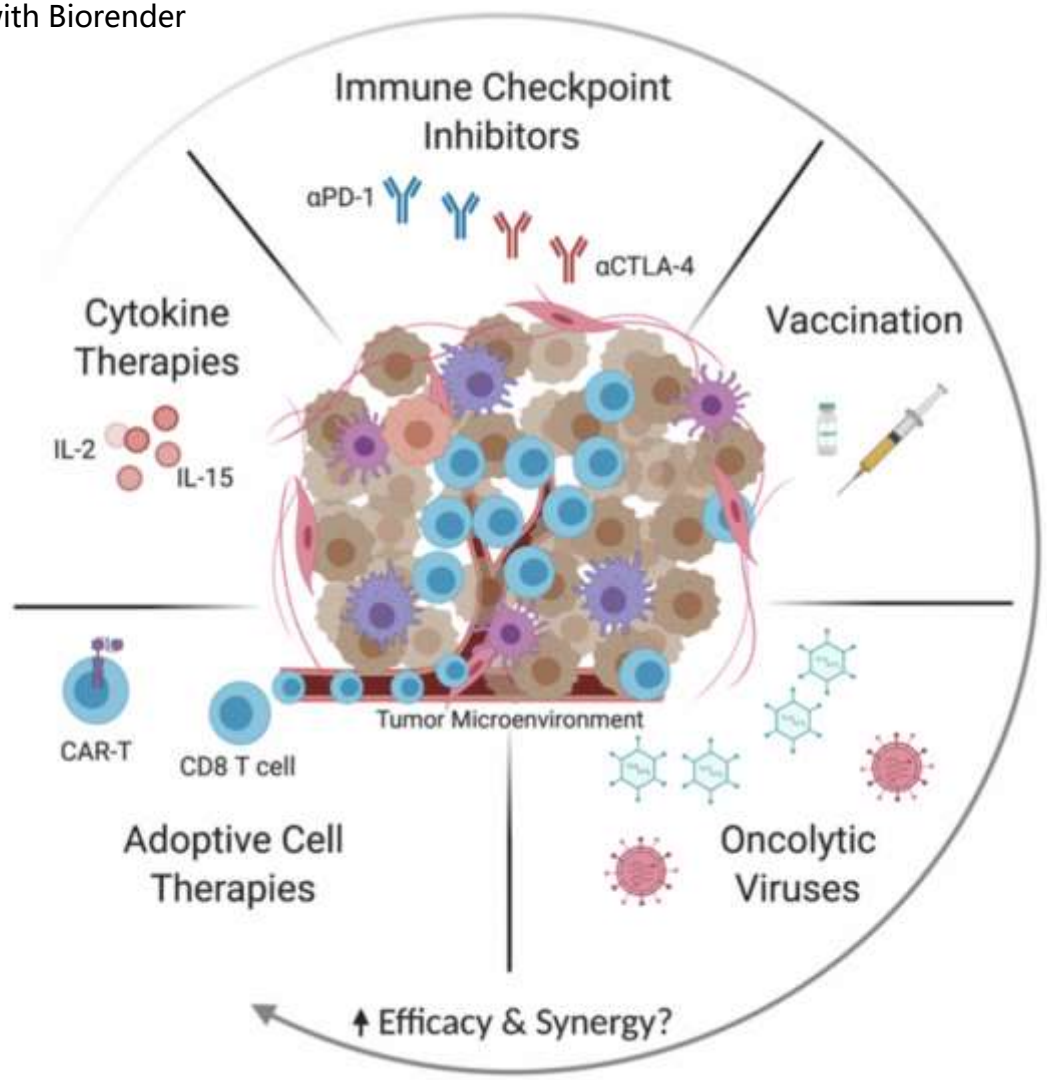
Collaboration with Matthew Pipkin (TSRI Florida) and Shane Crotty (LJI),
John Chang, Gene Yeo, Wei Wang UCSD
Max Krummel and Ken Hu UCSF

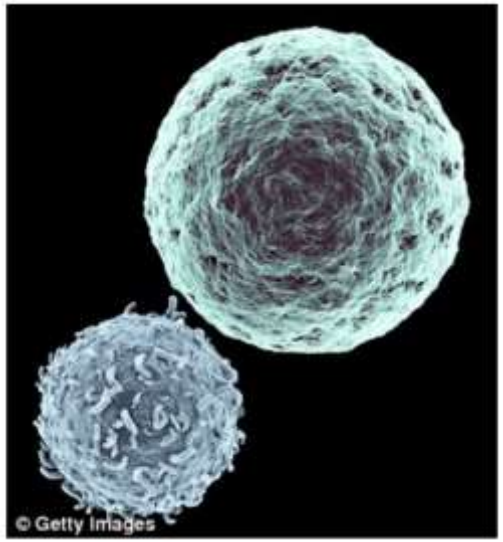
Disclosures: SAB of Pandion Therapeutics and ArsenalBio

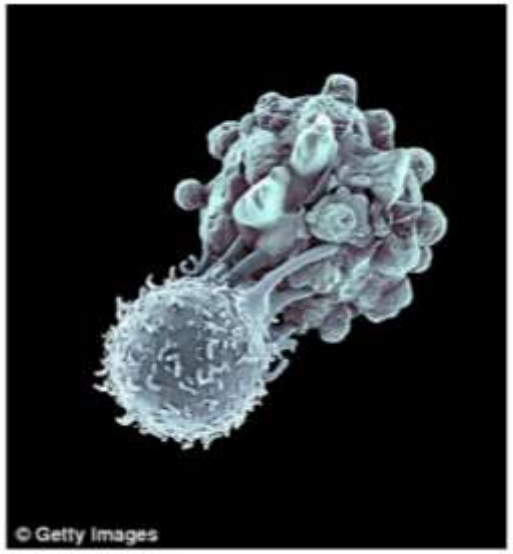
Biological Sciences
where discovery comes to life UC San Diego

CD8⁺ T cells are central players in anti-tumor immunotherapies

Image by Milner generated with Biorender

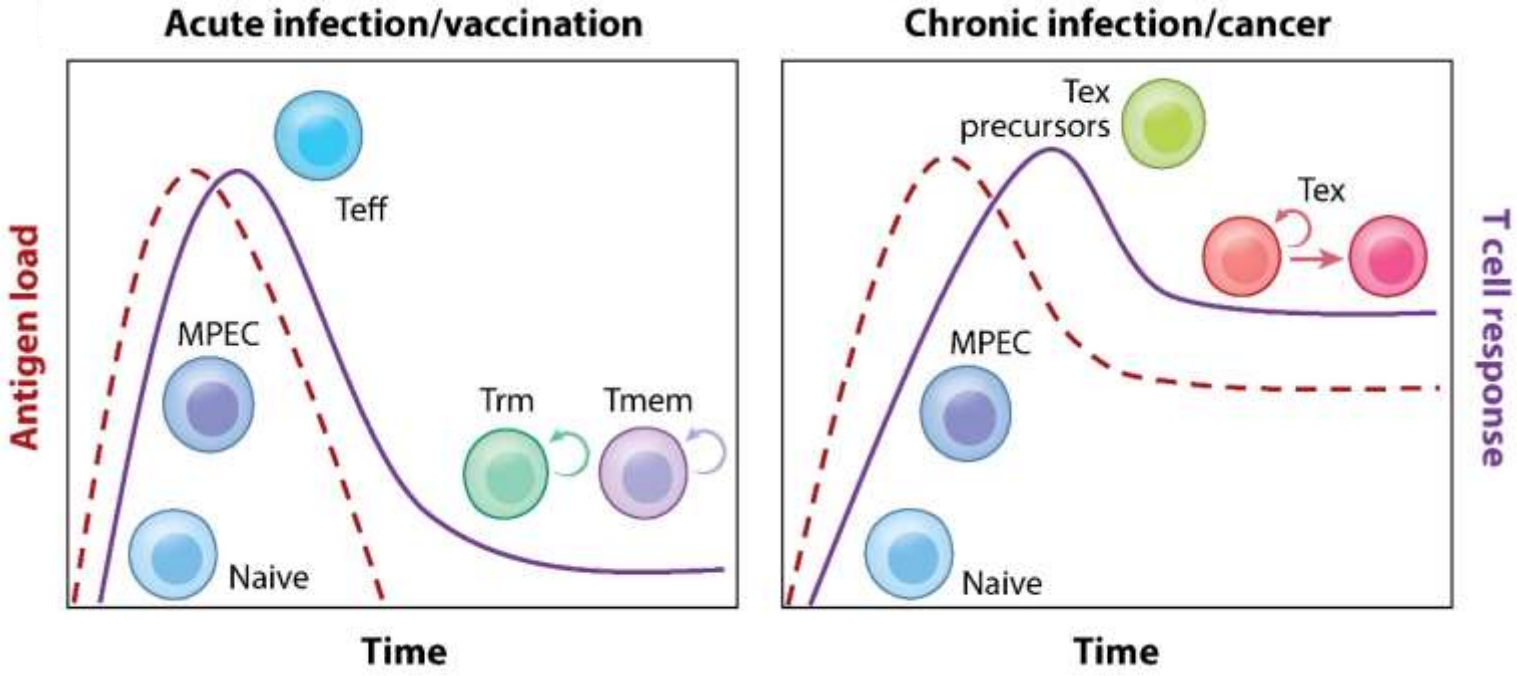


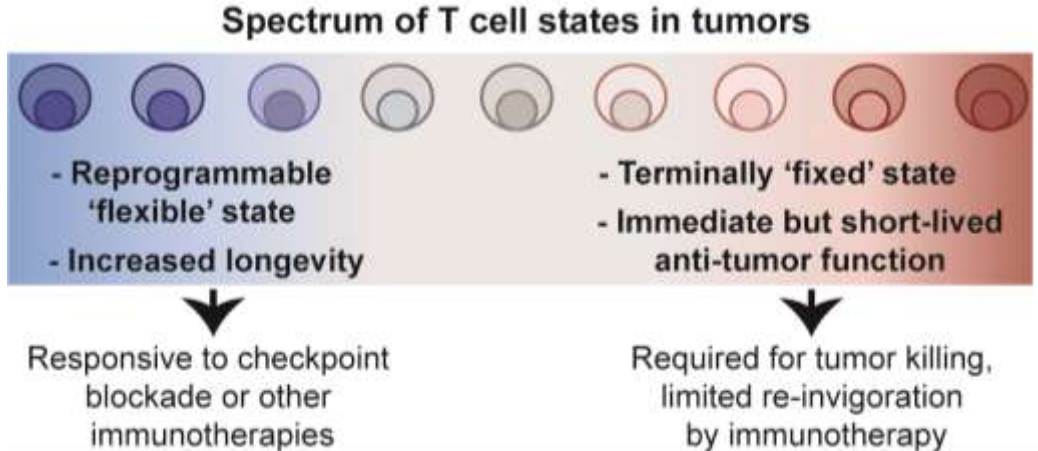




tiating adaptive immune responses to tumors can elicit long-lived protection in the form of immune memo

Differentiation of CD8⁺ T cells is dependent on clearance of the target

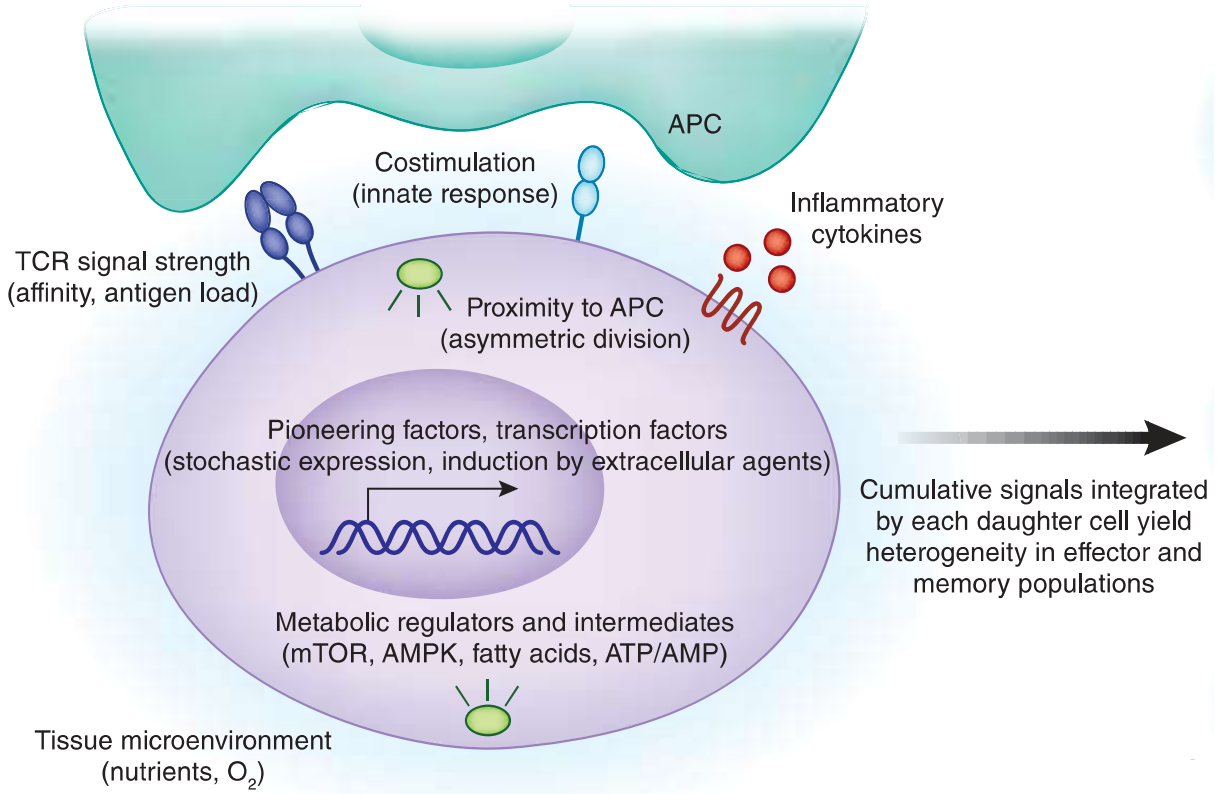




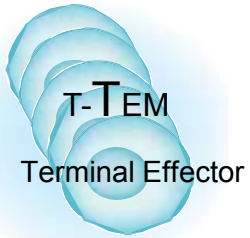
McLane et al (2019)

Naive CD8⁺ T cells integrate many signals to instruct heterogeneous memory cell differentiation

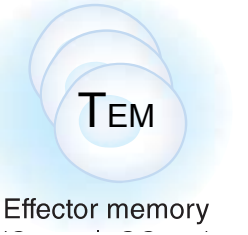
Chang, Wherry and Goldrath Nature Immunology 2014



Cumulative signals integrated by each daughter cell yield heterogeneity in effector and memory populations

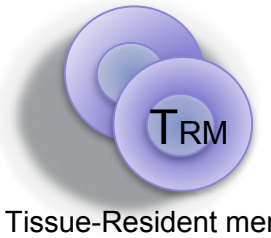


Rapid effector function, die at resolution of infection



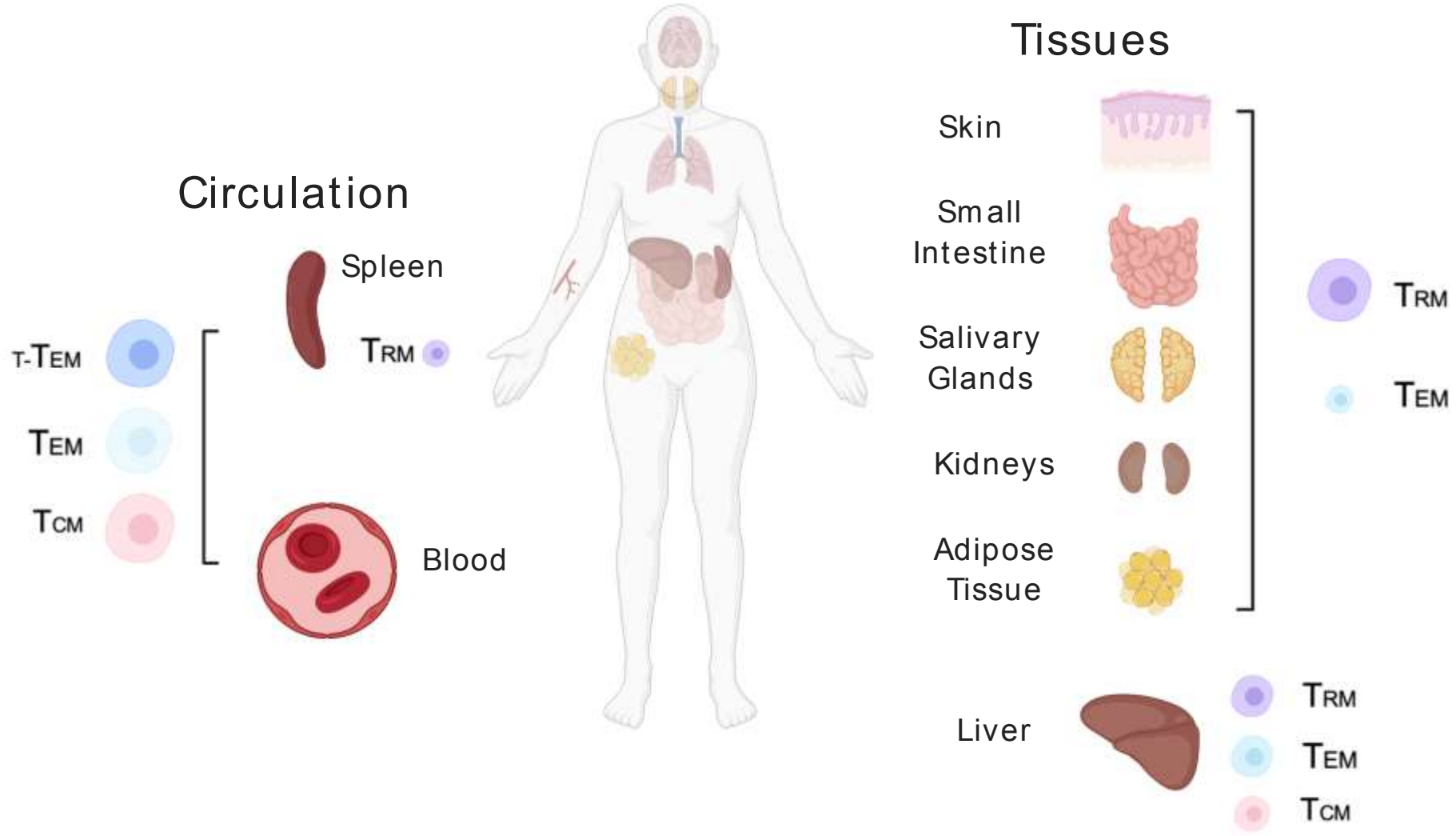
Rapid effector function low proliferation

Stem-like capacity for differentiation and self-renewal 2° memory, low effector function, long-term survival, high capacity for proliferation

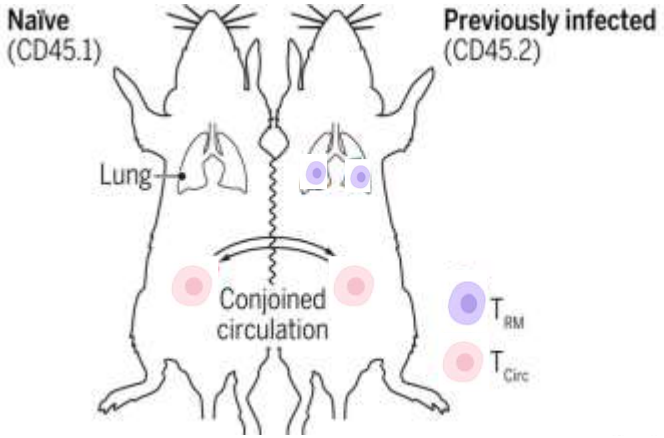


Rapid effector function, capacity for differentiation and self-renewal, long-term survival, capacity for proliferation

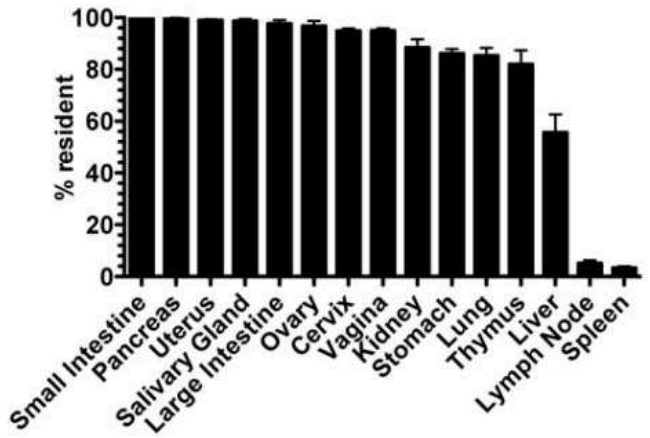
Tissue-specific memory T cell composition

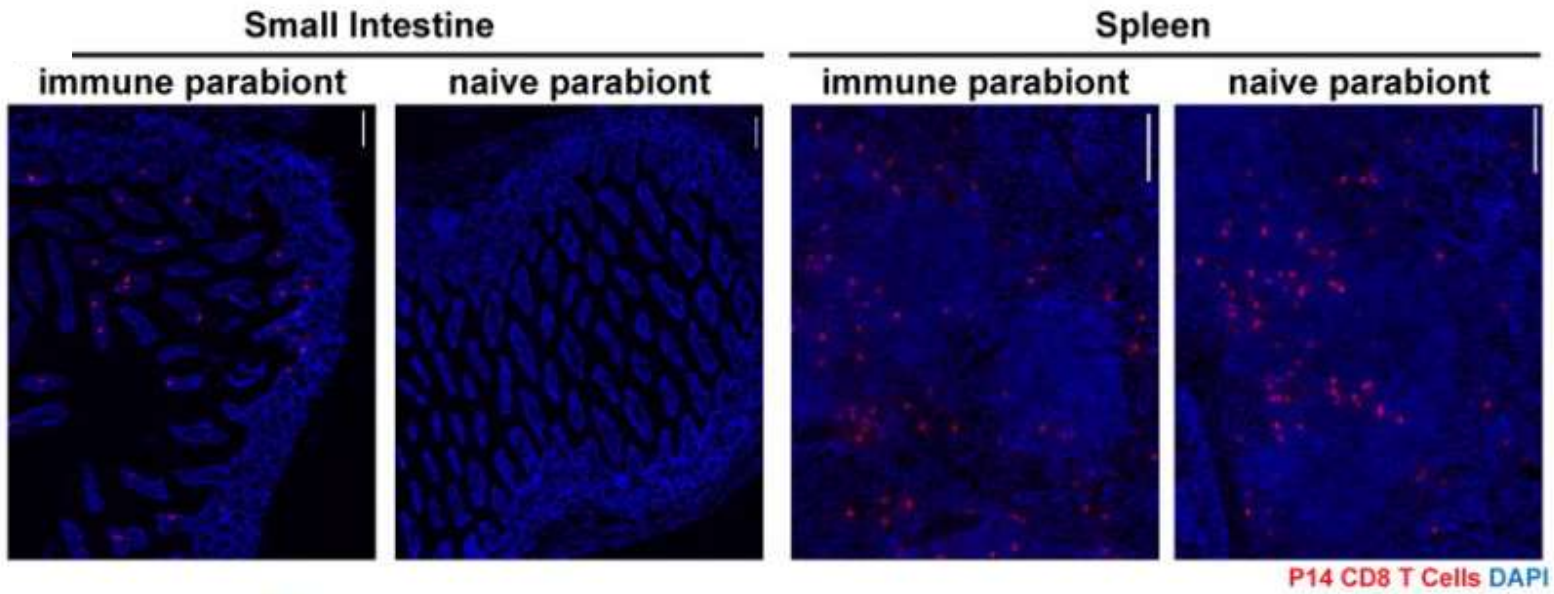


A large portion of T cells in tissues are non-recirculating, resident memory cells, T_{RM}



From Szabo Sci Imm 2019



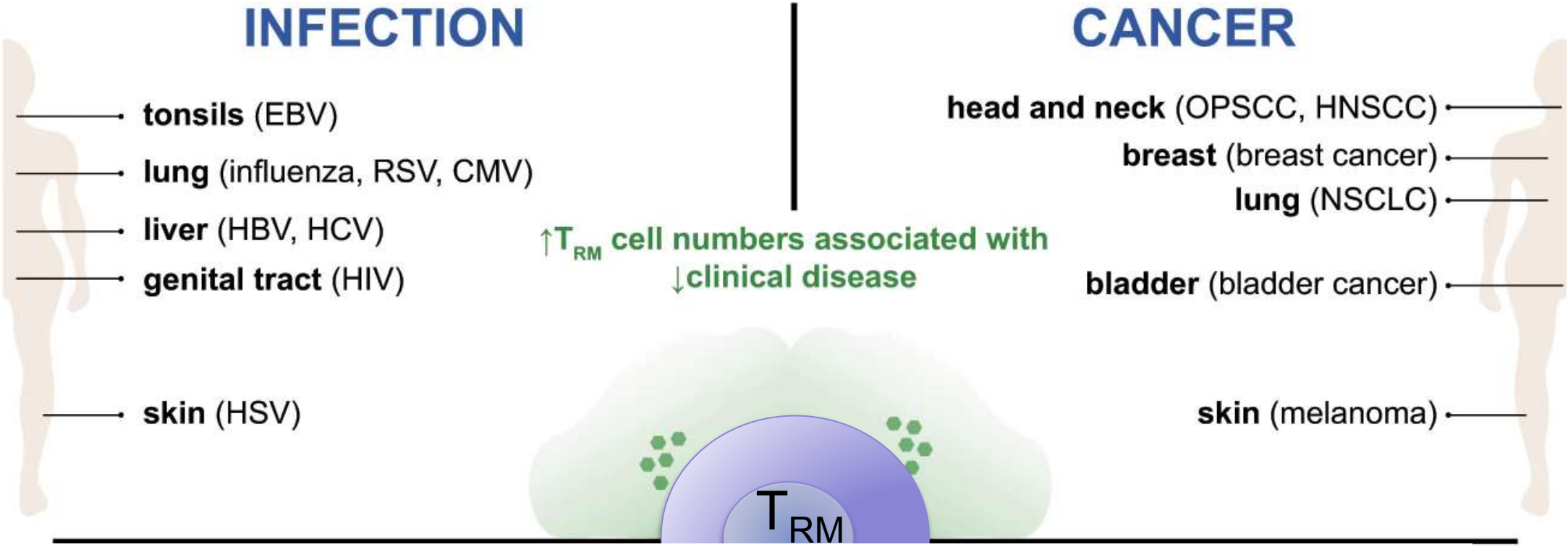


Steinert Cell 2015

- Provide early sentinel protection at body surfaces, within tissues
- Protection against pathogens and tumor growth
- Recruit innate and adaptive immune cells to the site of infection
- Many express CD103, most express CD69
- KLF2 and S1PR1, must be downregulated to prevent egress

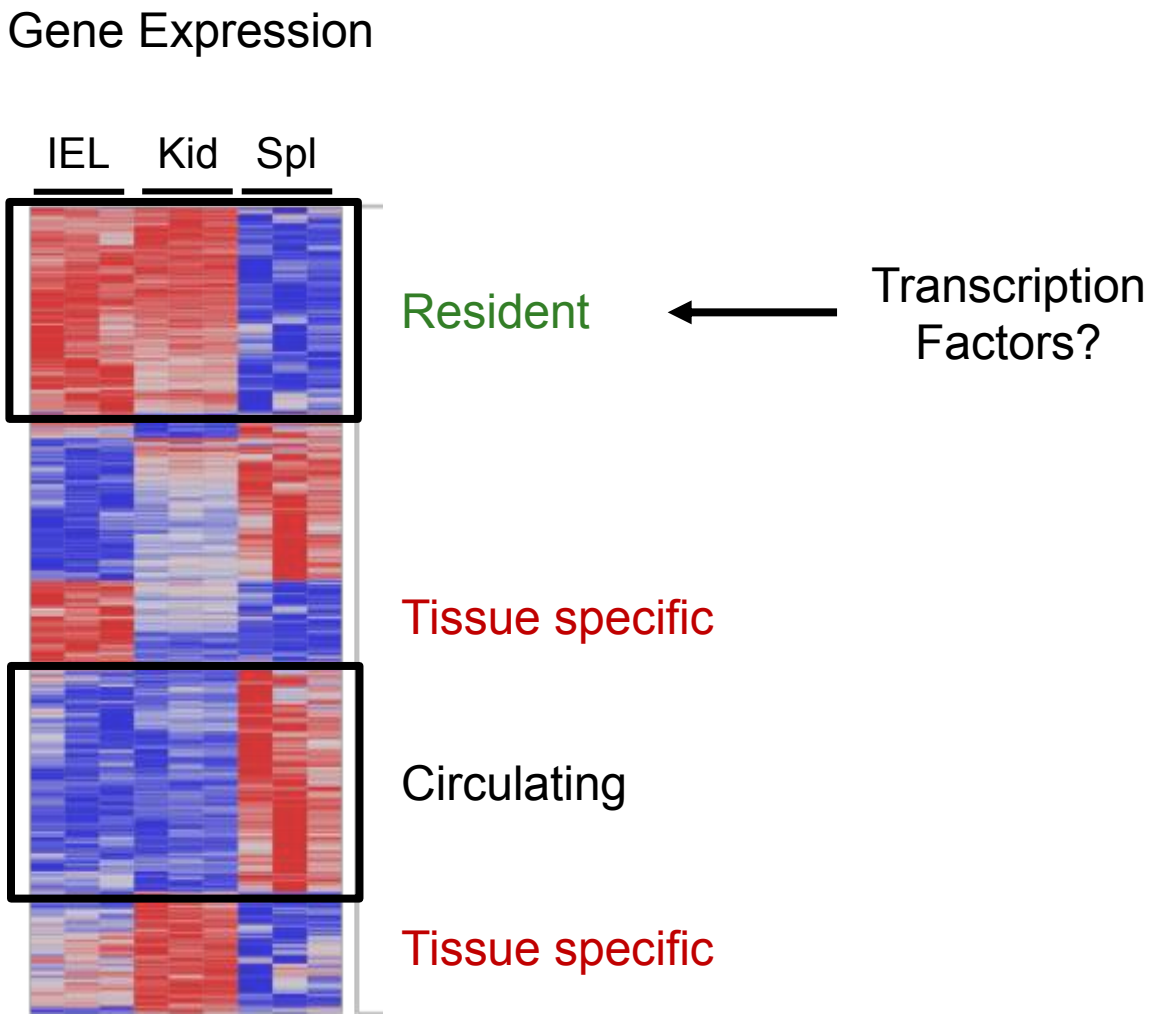
CD8$^+$ T Cell T_{RM} are associated with improved therapeutic outcomes in infection and cancer in humans

Sasson 2020

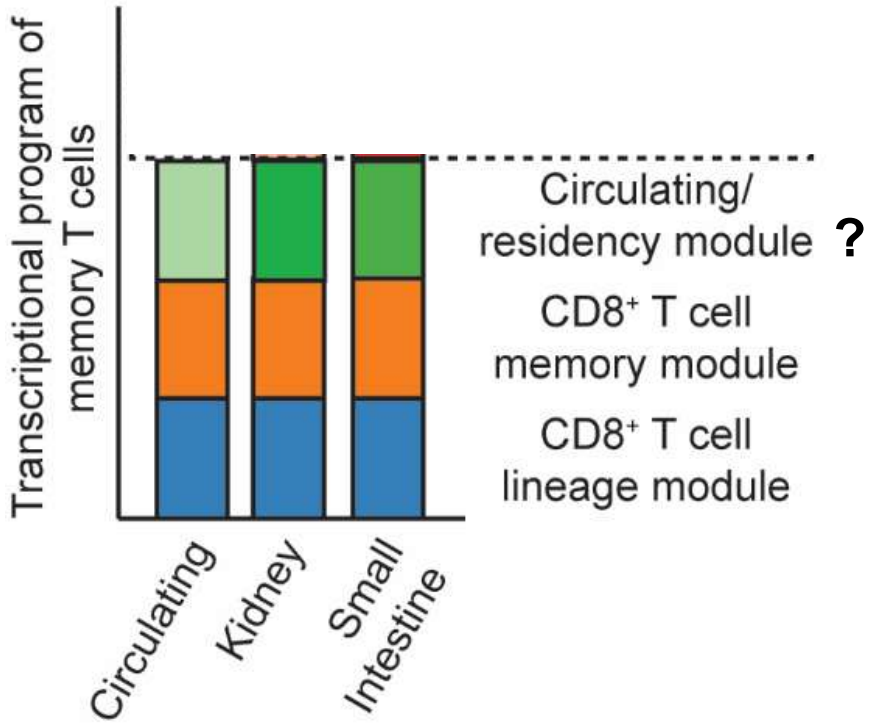


How are T_{RM} populations programmed in the context of known factors?
Is there T_{RM} specific transcriptional programming?
Does this inform programming of TIL for enhanced anti-tumor function?
How do cells adapt to distinct tissue microenvironments?

A common gene-expression pattern for resident populations across tissues and circulating memory CD8⁺ T cells



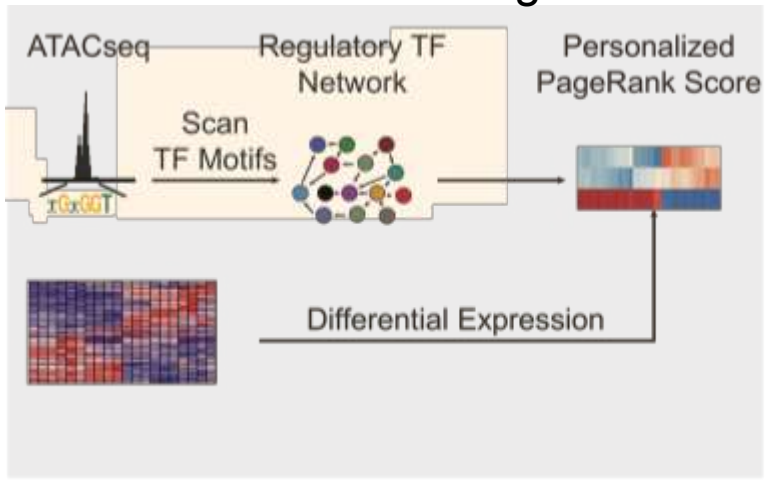
Milner et al, Nature 2017



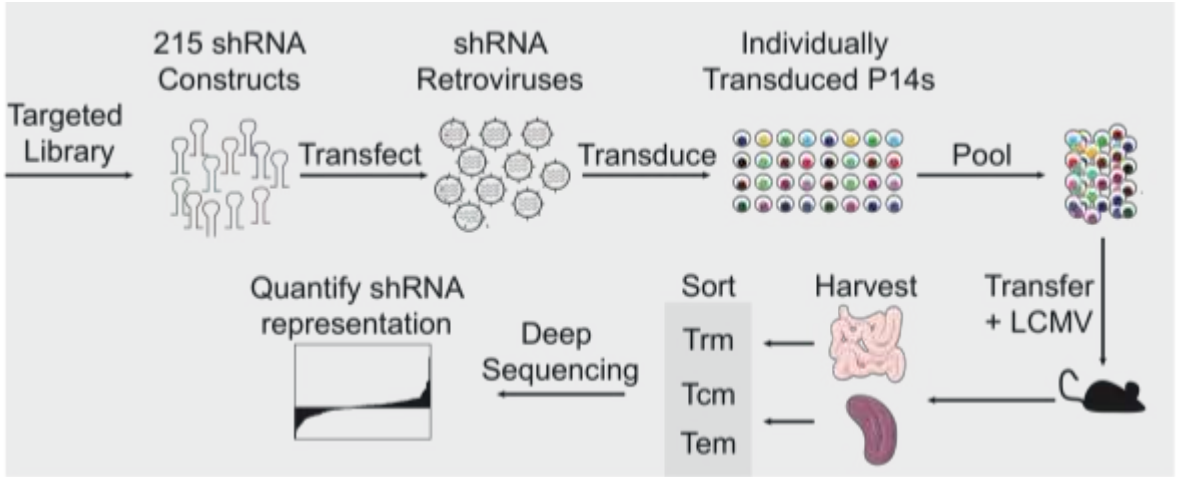
Modified from: Okabe & Medzhitov, 2015

Computational and functional screens to identify transcriptional regulators of CD8$^+$ T_{RM}

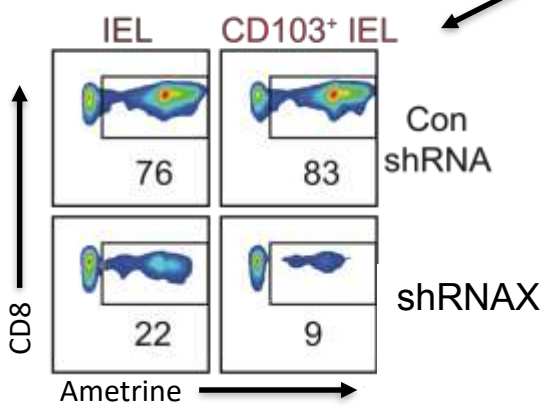
Predict TF that regulate TRM



in vivo knock-down of TF



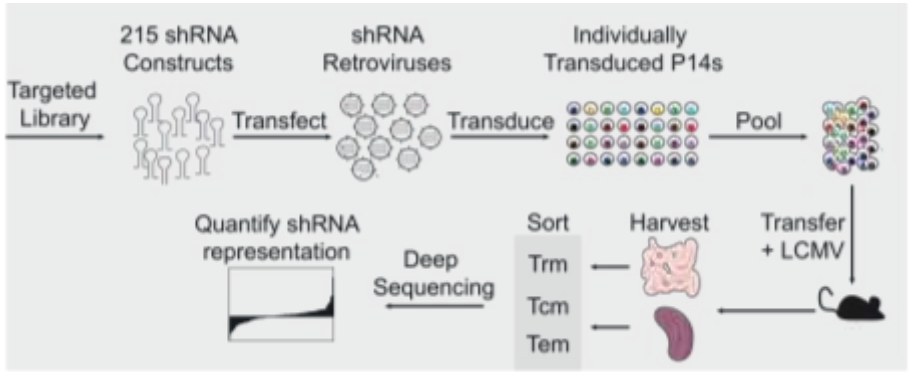
Justin Milner, Clara Toma and Bingfei Yu Milner et al. Nature 2017

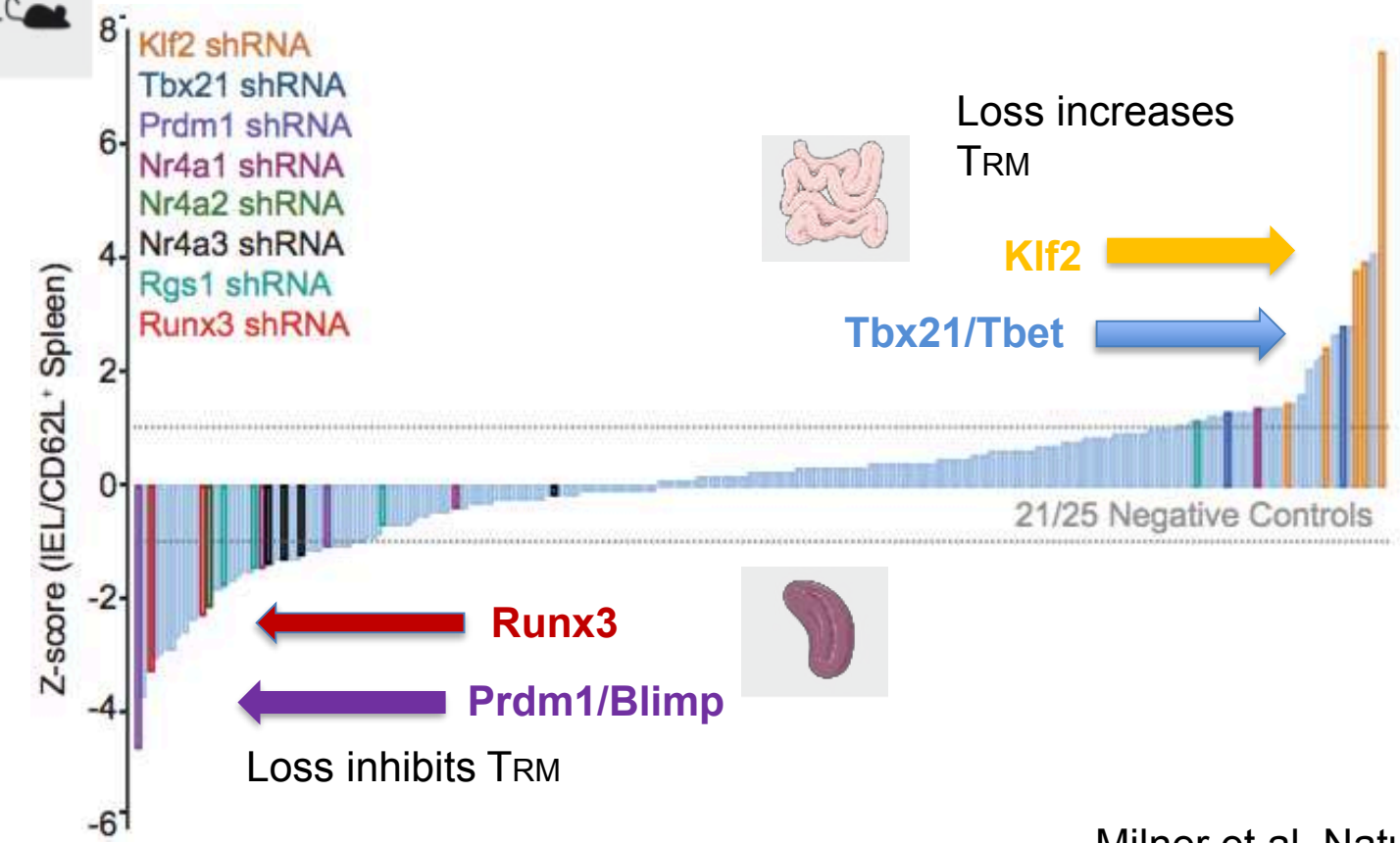


Test individual constructs, study impact of deletion, induced deletion affects on TRM generation, homeostasis and function.

Compile transcriptional network governing TRM differentiation.

In vivo RNAi screen for regulators of TRM

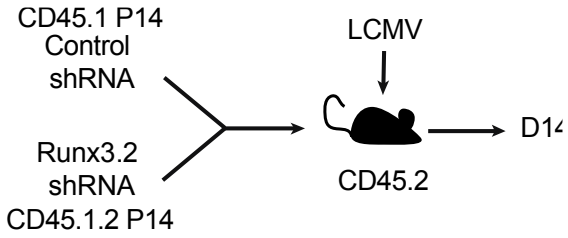


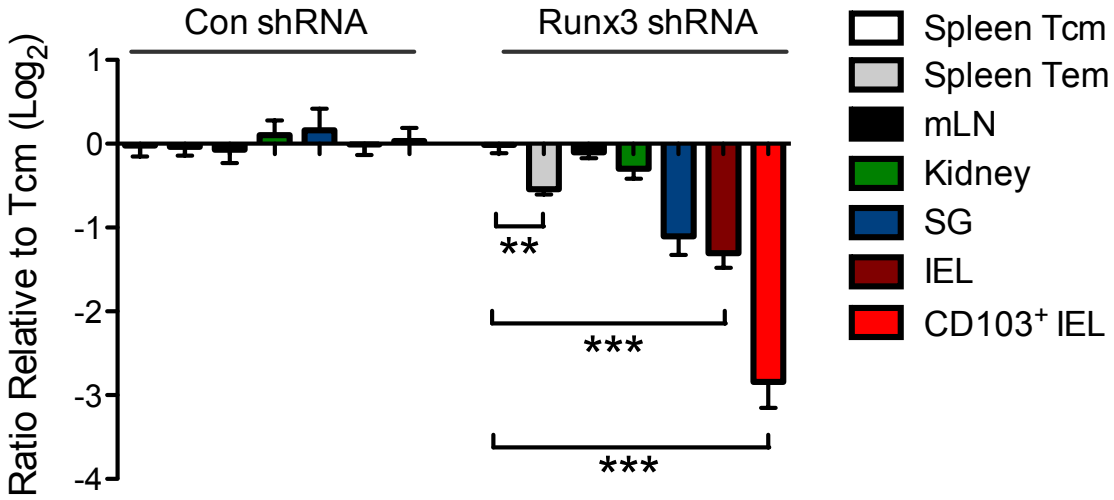


Milner et al. Nature 2017

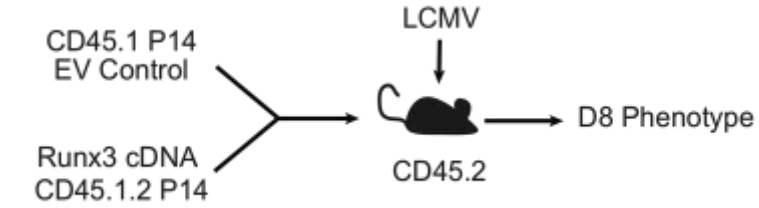
Runx3 regulates the T_{RM} transcriptional program

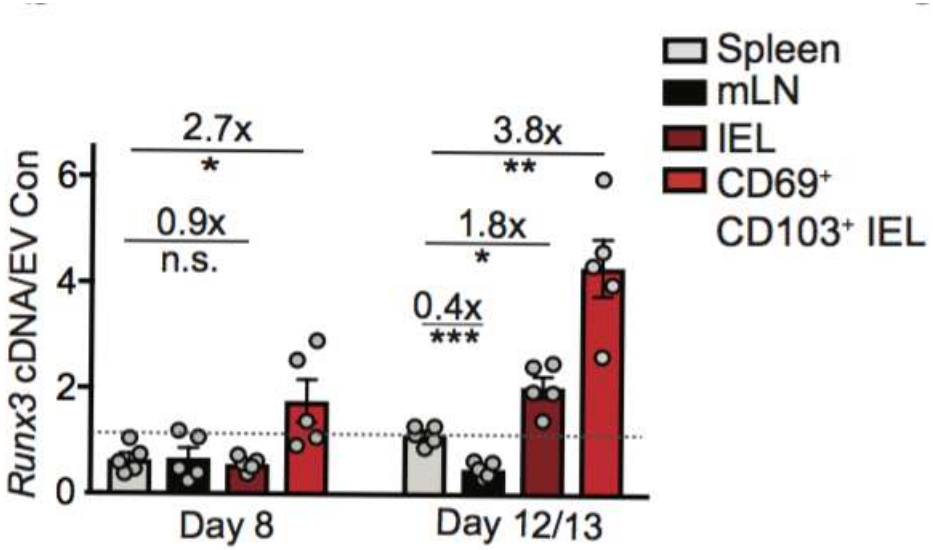
Loss of Runx3 impairs T_{RM}





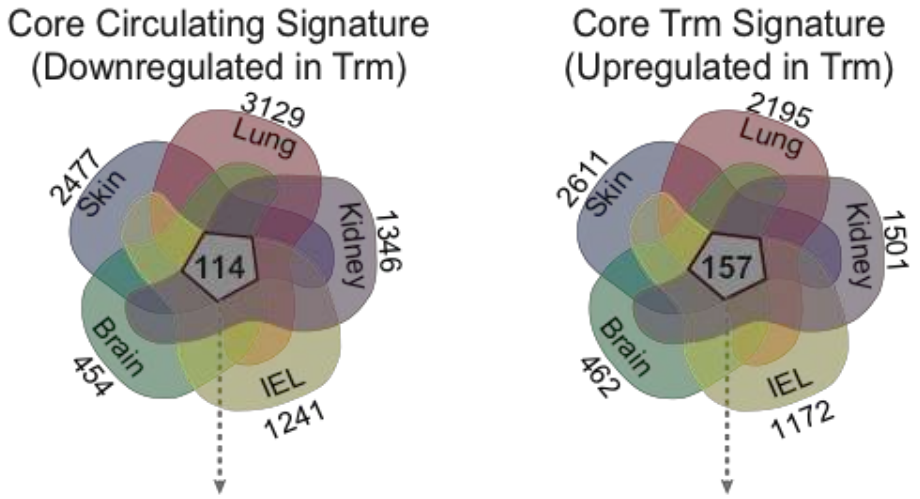
Overexpression of Runx3 accelerates and enhances

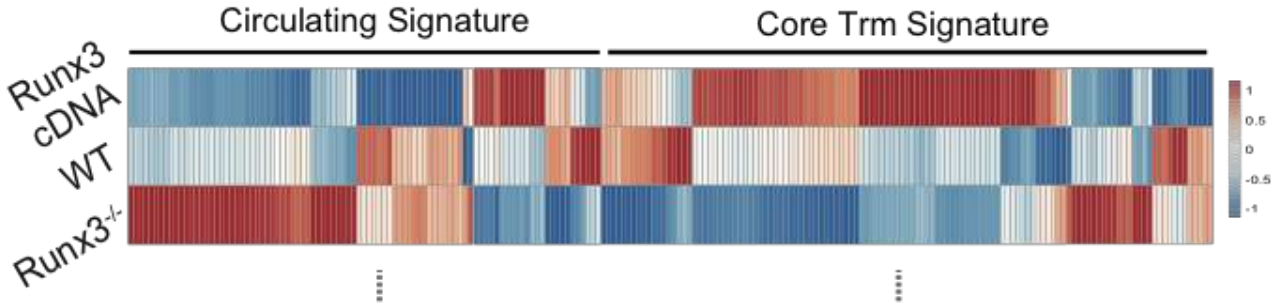


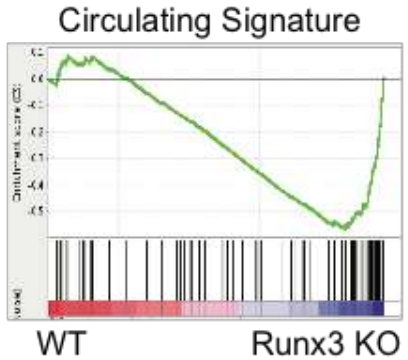


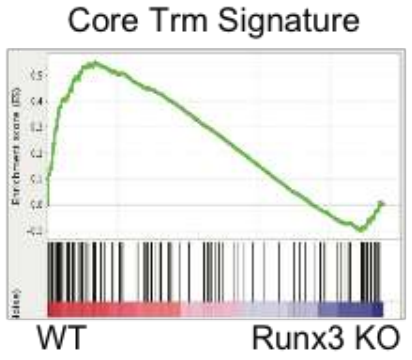
Milner et al. Nature 2017

Runx3 controls the core T_{RM} transcriptional program

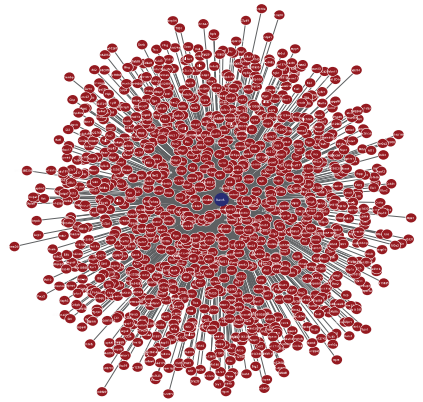




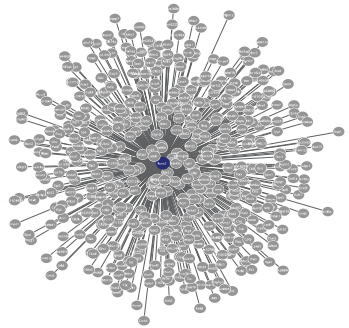




Runx3 network in IEL



Runx3 network in Spleen

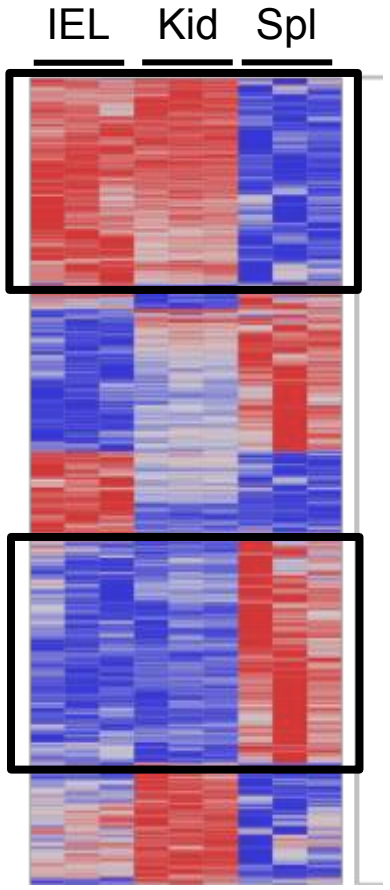


- Runx3 activity is tissue-dependent
- Runx3 appears to be upstream of Blimp1 and repression of KLF2/S1pr1 and T-bet/Eomes which is also key for T_{RM} differentiation

Milner et al. Nature 2017

Are CD8⁺ TIL programmed by common adaptations to tissue residency?

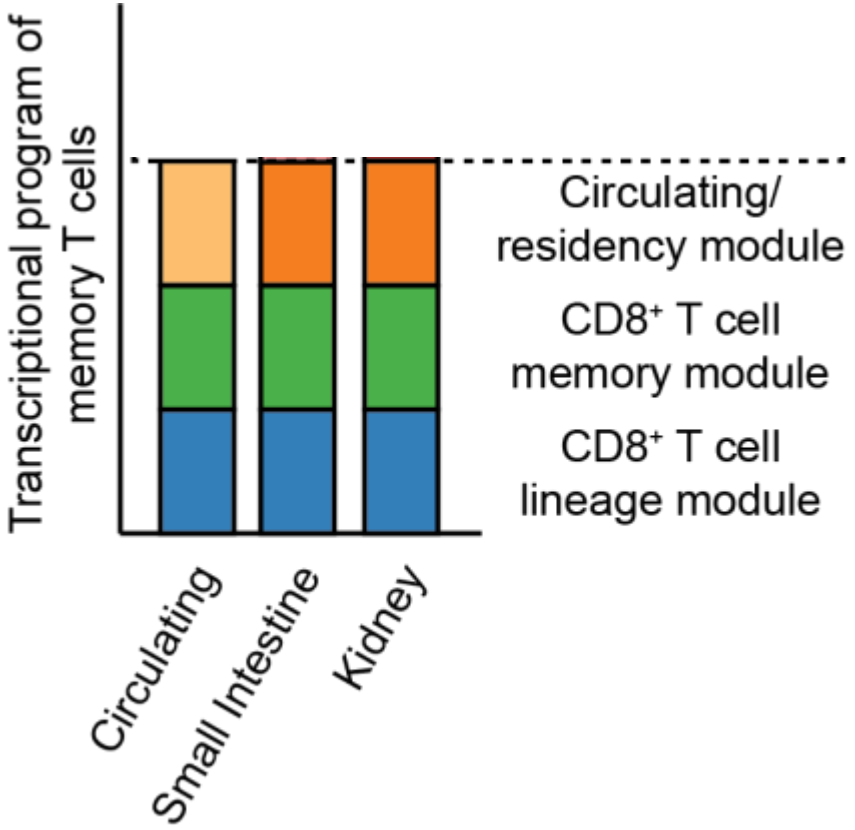
Gene Expression



Milner et al, Nature 2017

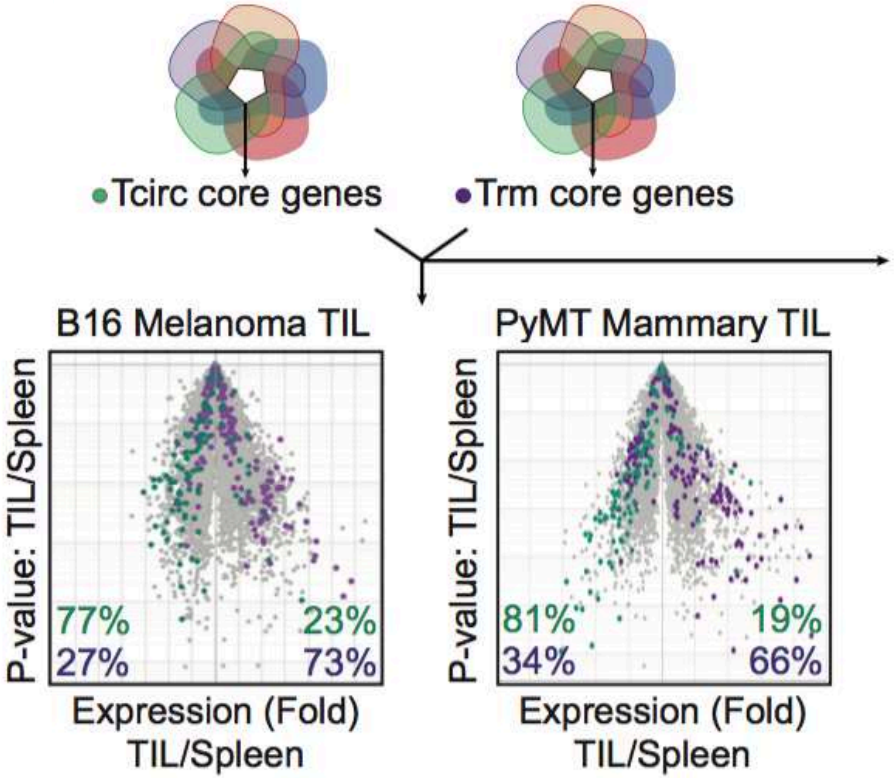
Transcription Factors

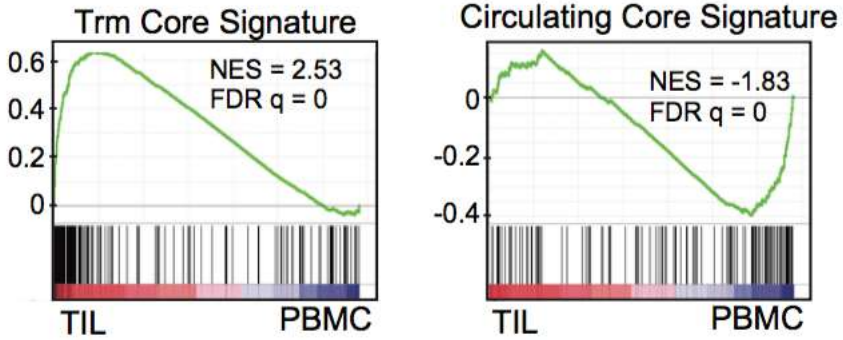
Runx3
Blimp1
Nr4a1
…



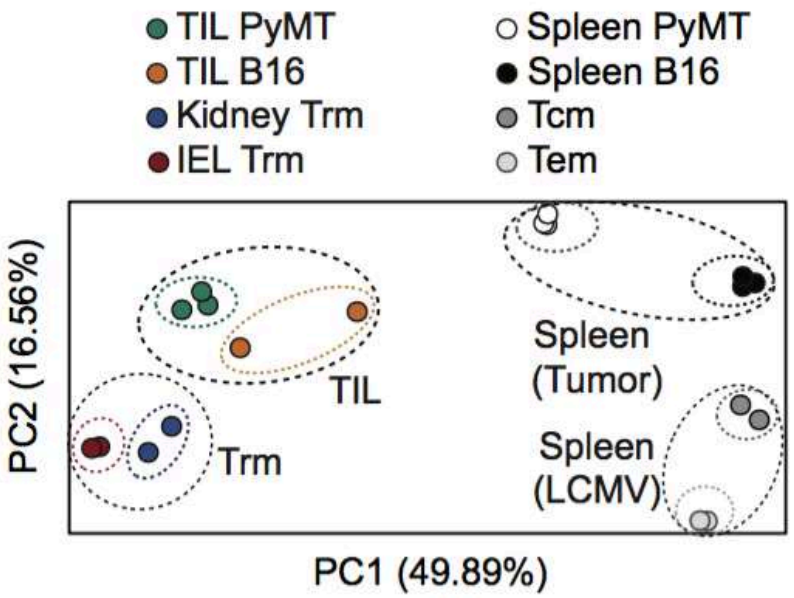
Tumors are tissue, too!!

TIL and T_{RM} share a "residency" gene-expression signature



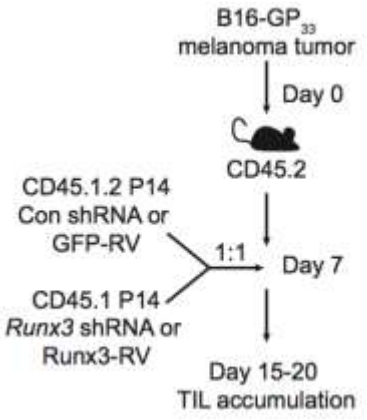


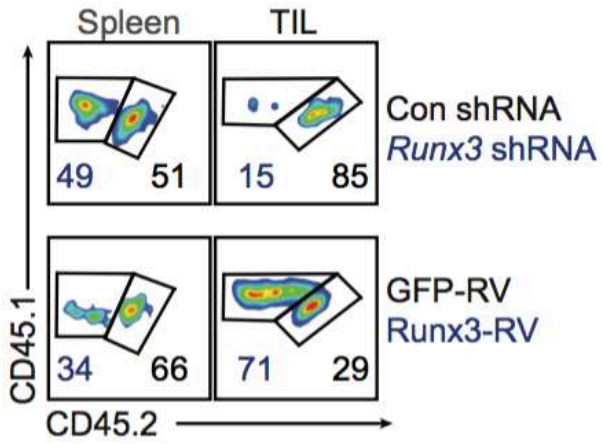
Milner et al. Nature 2017

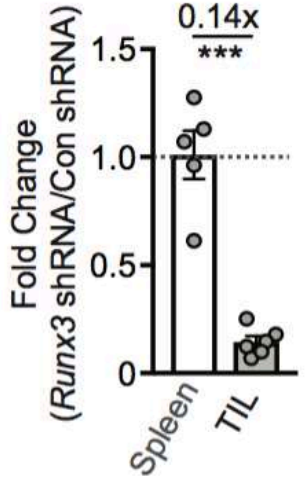


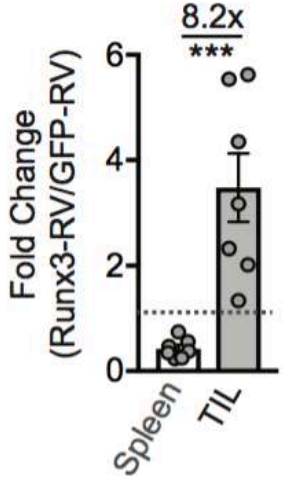
Growing number of examples of a significant positive correlation between T_{RM}-like TIL and survival/positive outcome in numerous malignancies.

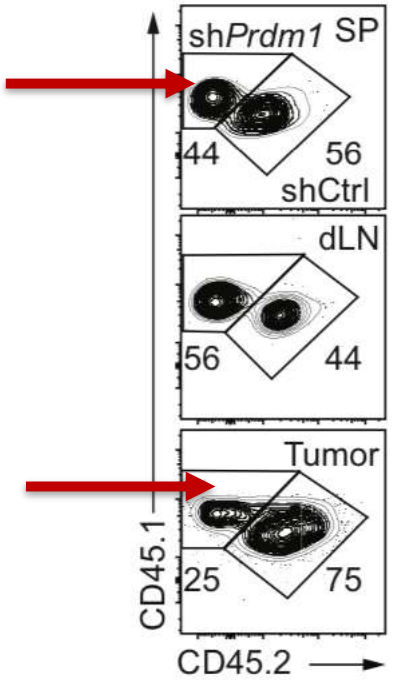
Pro-T_{RM} Runx3 and Blimp1 drive tissue residency by anti-tumor T cells

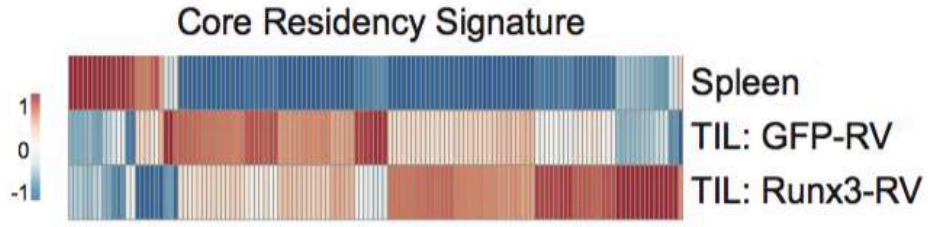


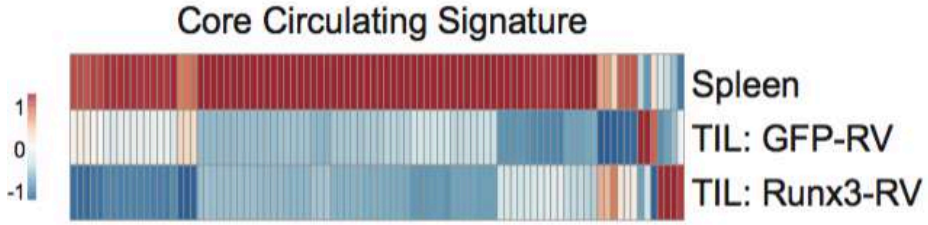


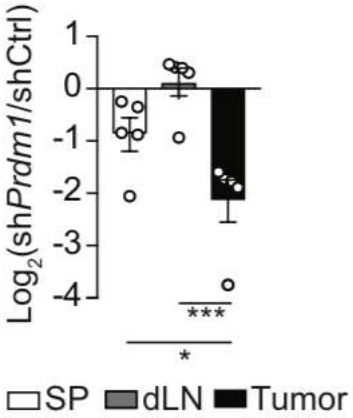






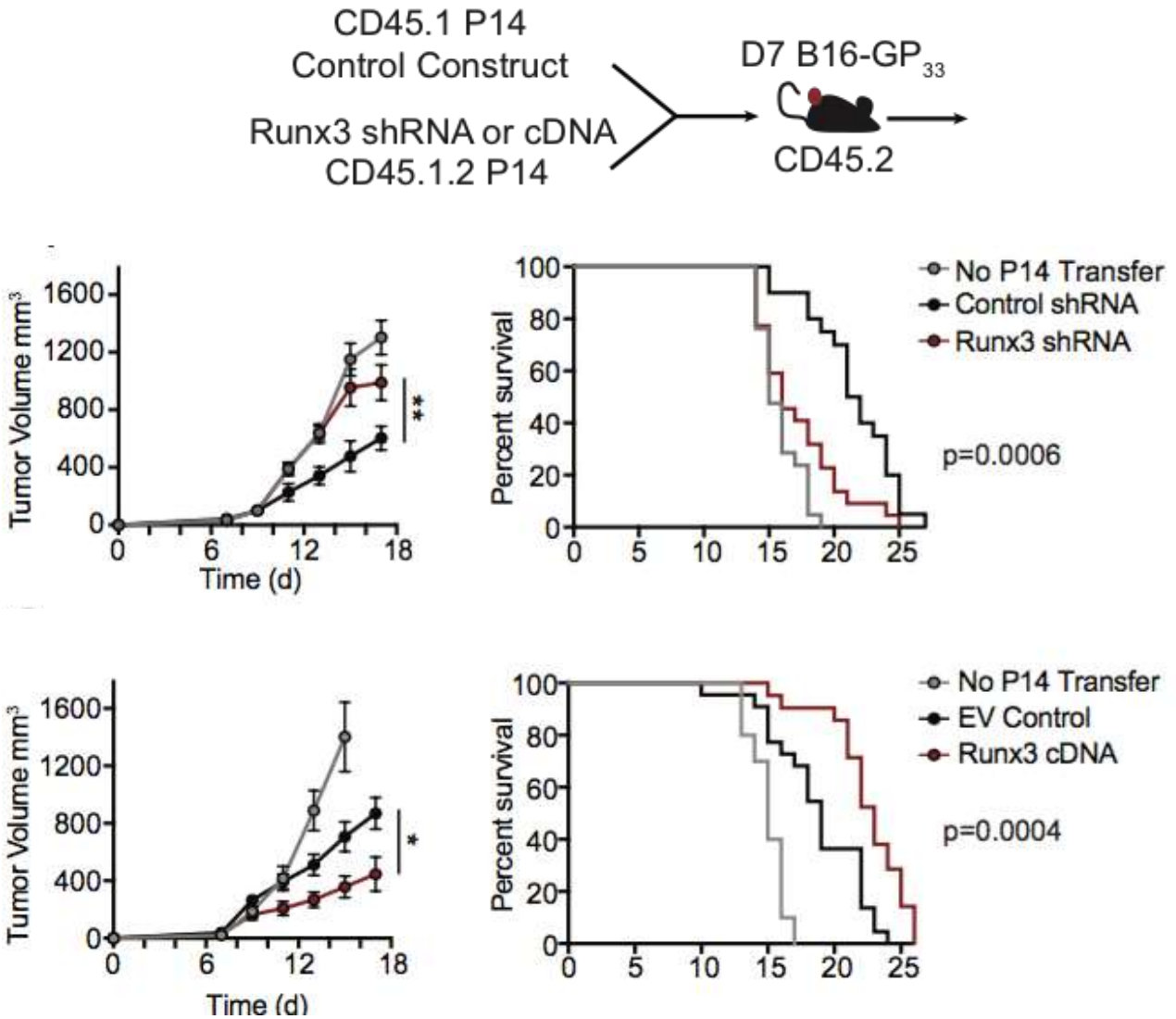






Milner et al. Nature 2017

Runx3 promotes anti-tumor activity by CD8^{+} T cells in ACT



Milner et al. Nature 2017

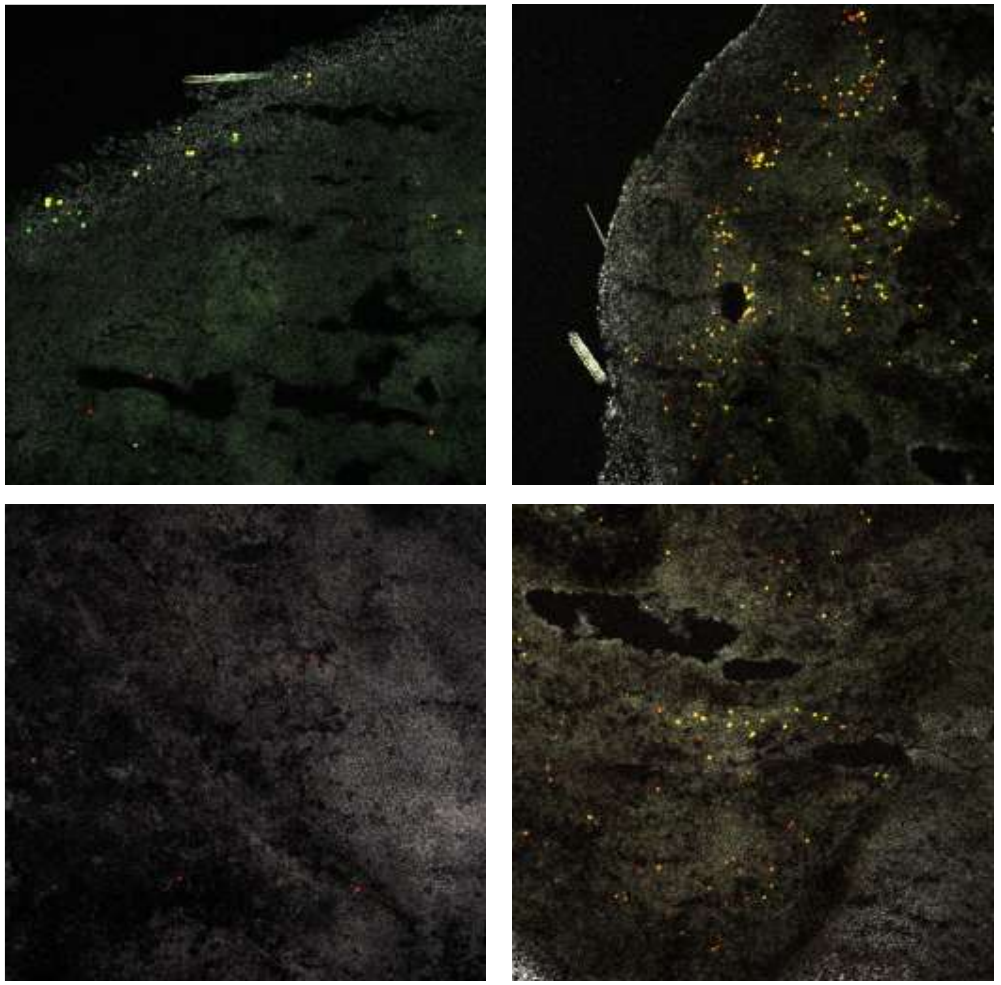
Runx3 over-expression leads to enhanced tumor infiltration

PMIG

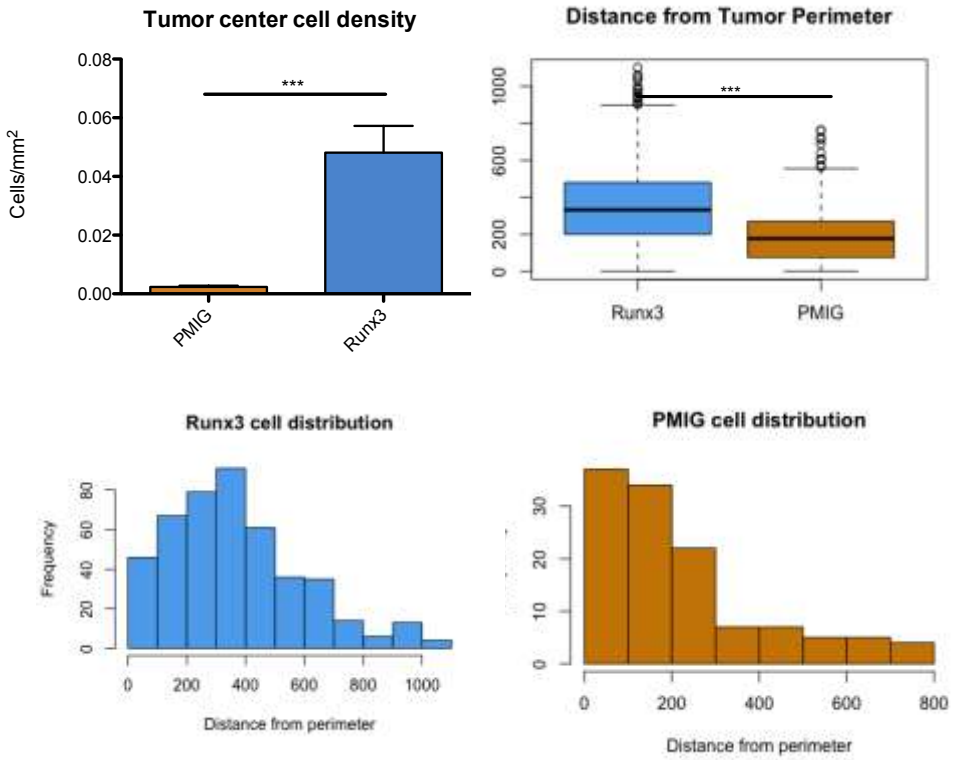
Runx3

Periphery

Center



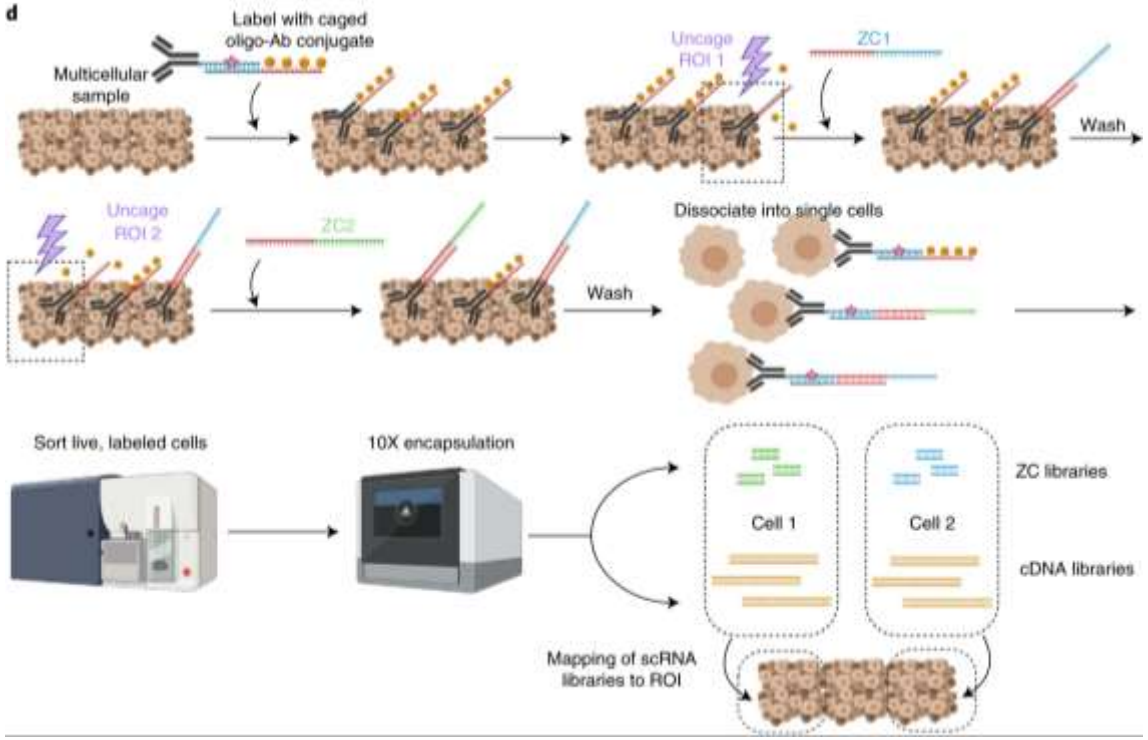
CD45.1 GFP CD45.1/GFP DAPI



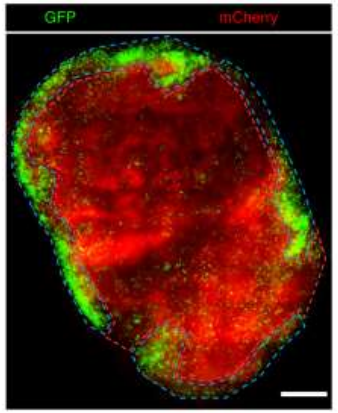
Ben Kedl and Justin Milner unpublished

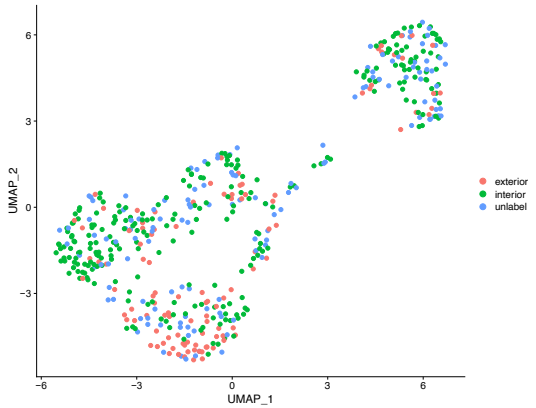
ZipSeq: barcoding for spatial mapping of single cell transcriptomes

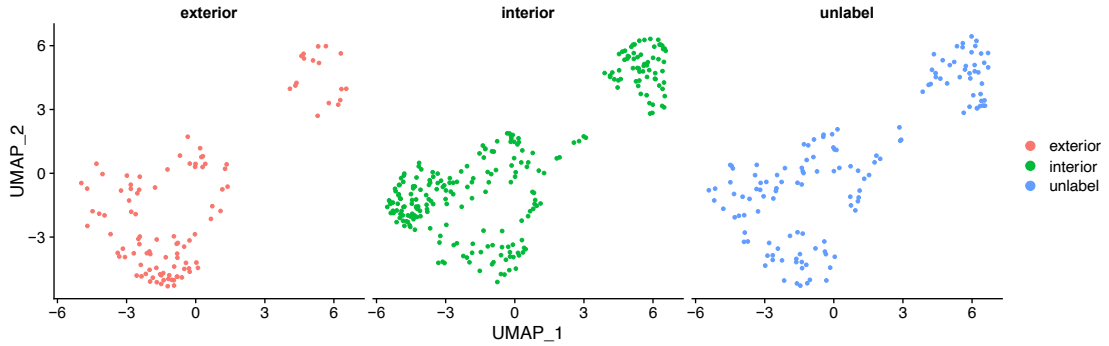
ZipSeq: barcoding for real-time mapping of single cell transcriptomes



PyMT - OT-I TIL



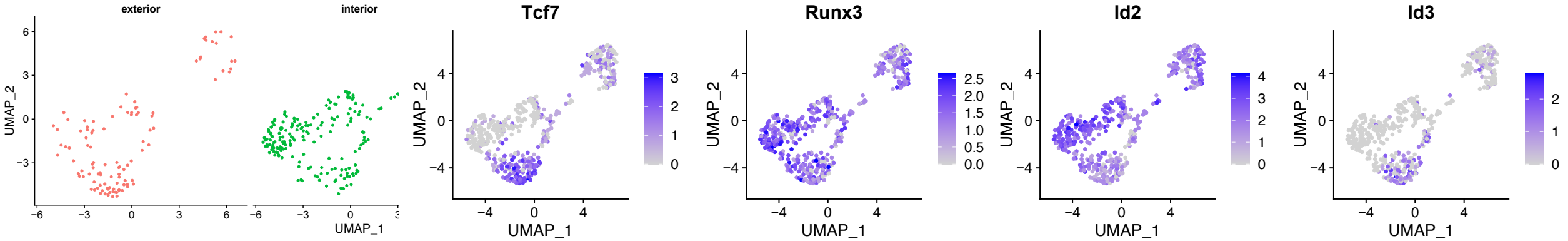




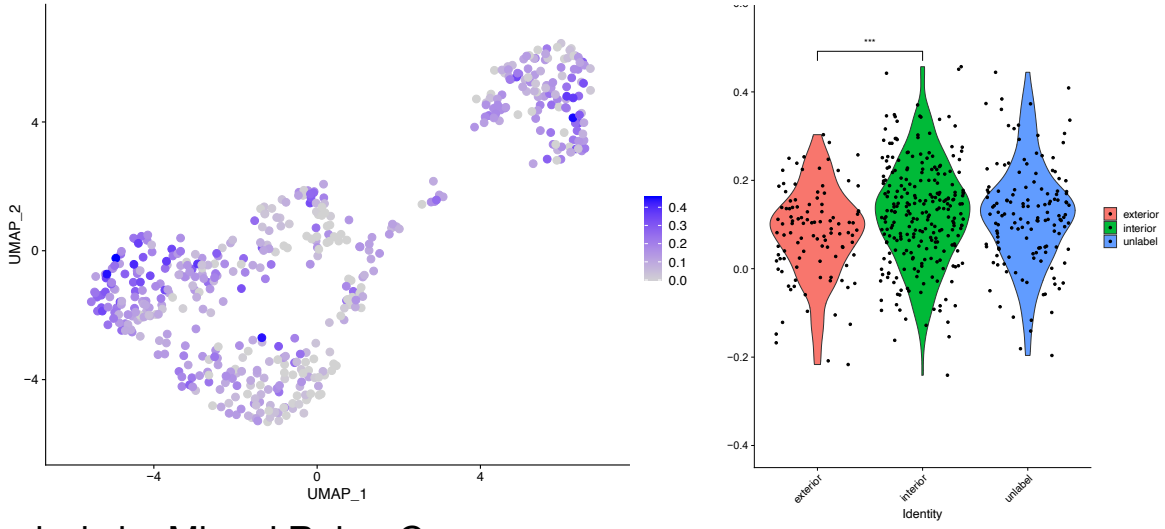
Krummel lab UCSF
Ken Hu et al. Nature Methods 2020

Zip-seq reveals T_{RM} signature associated with deeper infiltration of tumor

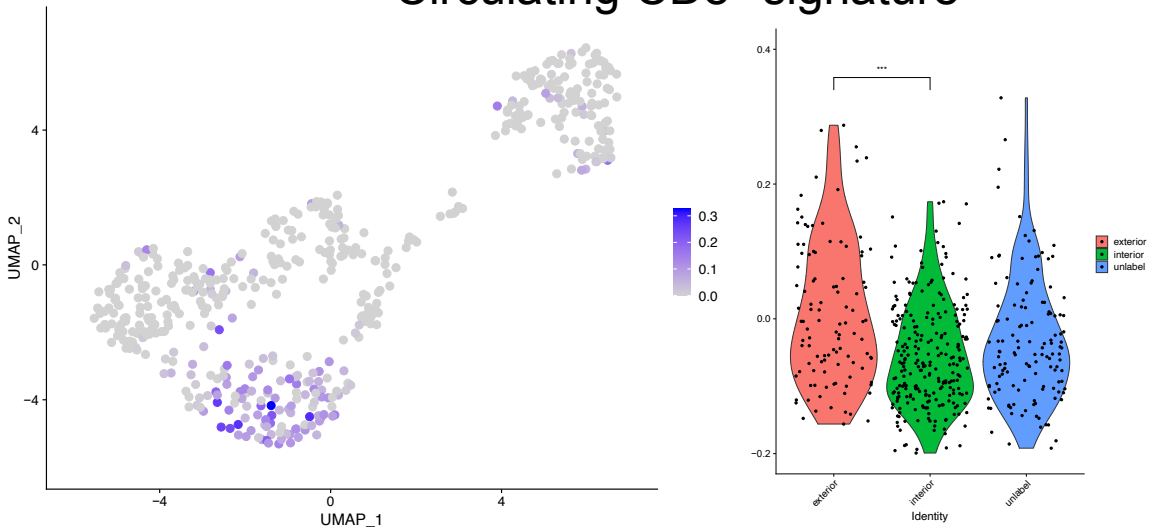
PyMT - OT-I TIL



CD8⁺ tissue-residency signature

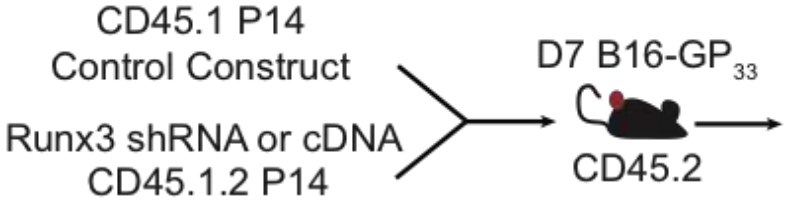


Circulating CD8⁺ signature



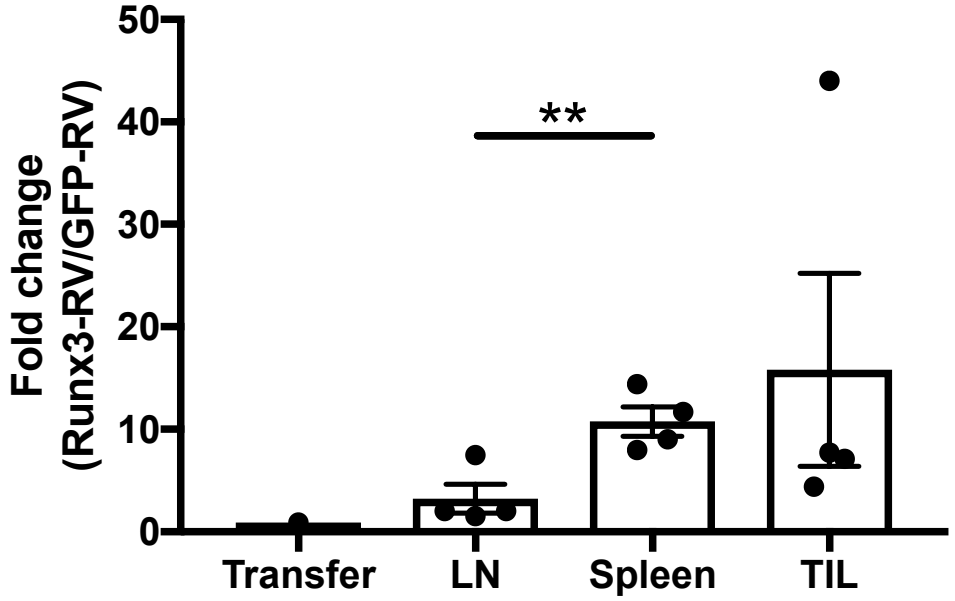
Analysis by Miguel Reina-Campos
Hu et al. Nature Methods 2020

Expression of Runx3 promotes CAR-T accumulation in solid tumors

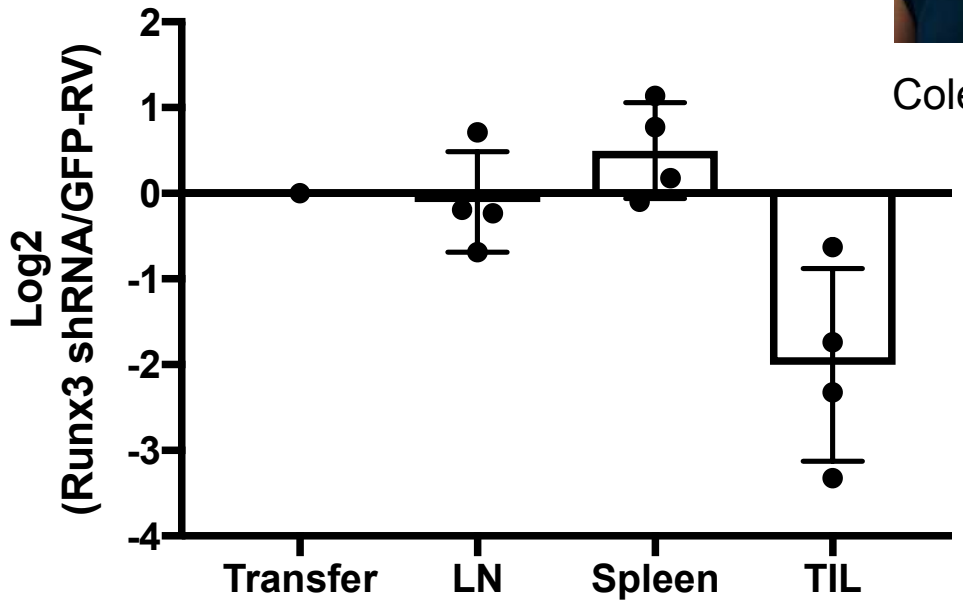


Colette Lauhan

Runx3 over-expression

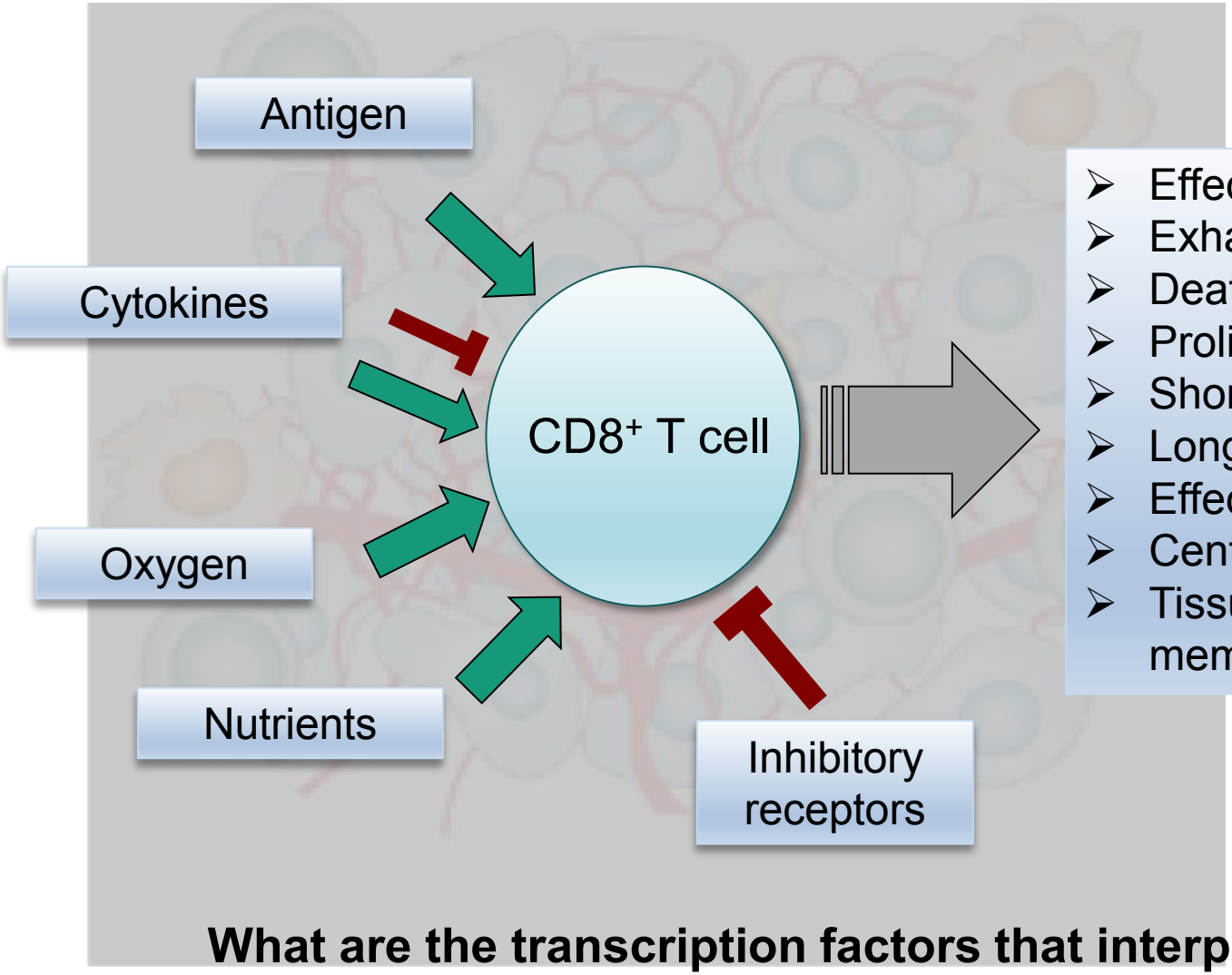


Runx3 shRNA log 2



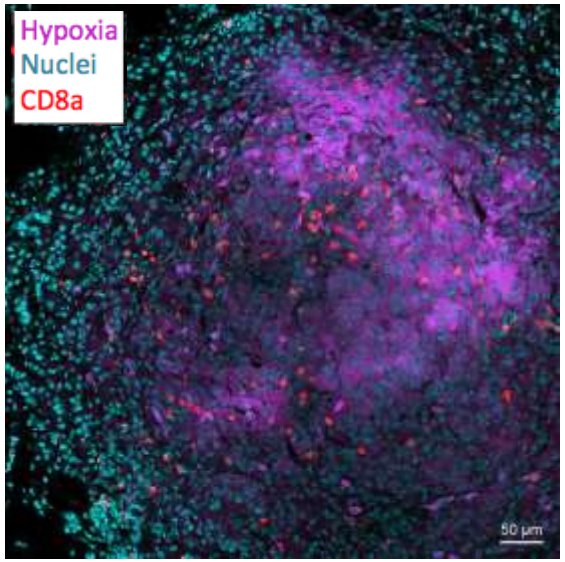
unpublished

Signals that drive optimal anti-tumor differentiation programs, for functional activity in the tumor microenvironment



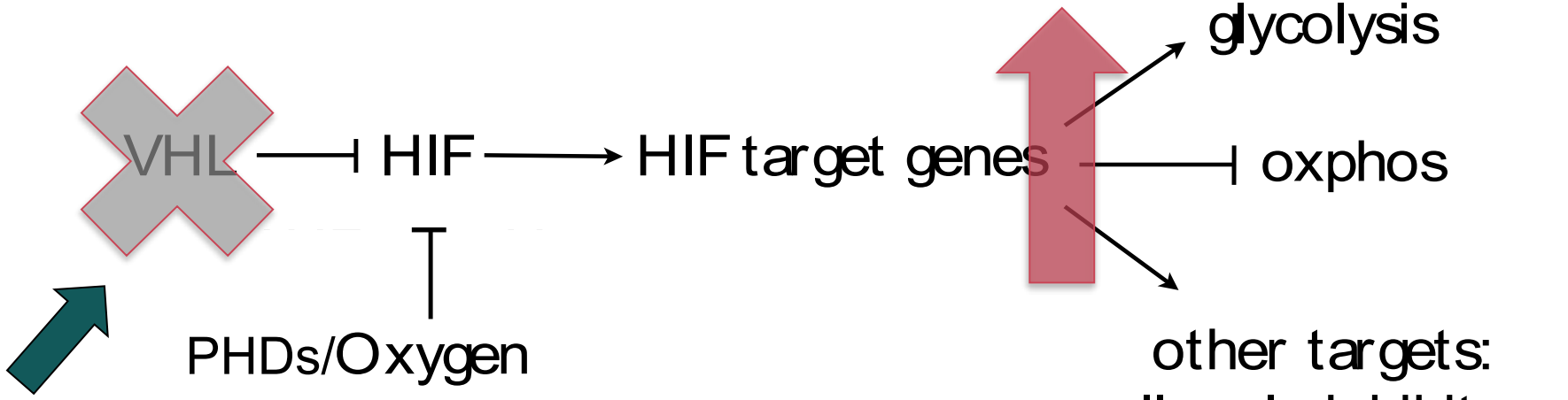
- Effector
- Exhausted effector
- Death
- Proliferate
- Short-lived memory
- Long-lived memory
- Effector memory
- Central memory
- Tissues resident memory

Melanoma



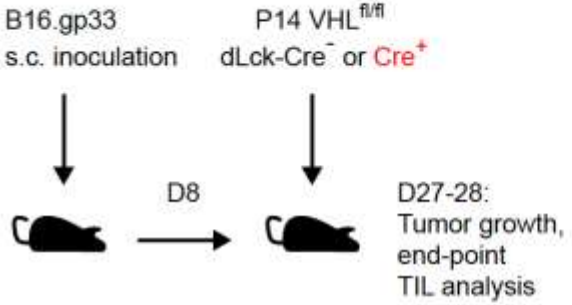
What are the transcription factors that interpret microenvironmental signals?

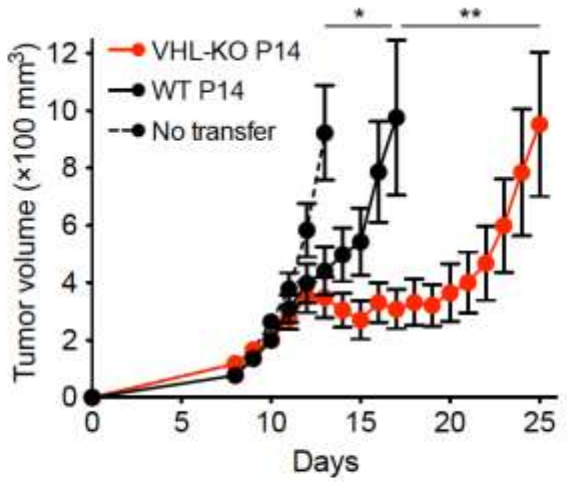
VHL is a negative regulator of the transcriptional response to hypoxia—Hypoxia Inducible Factor (HIF) activity

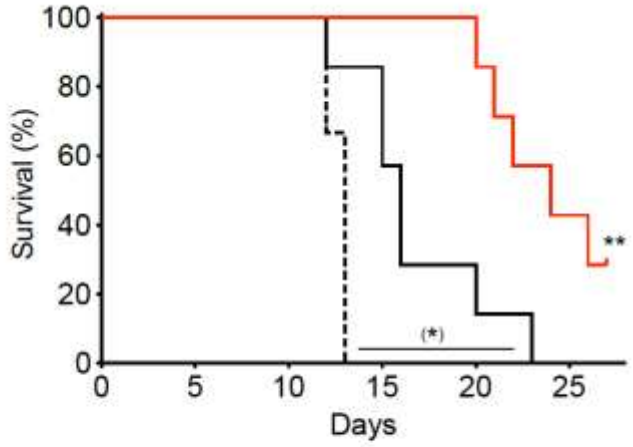


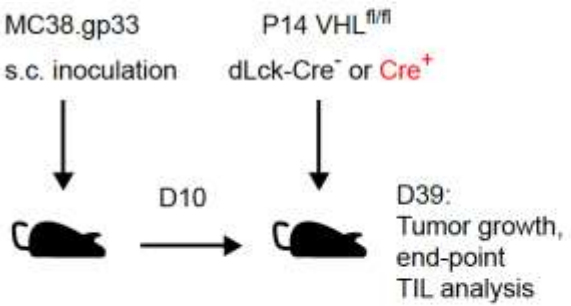
Mutations in VHL:
VHL syndrome: dominantly inherited predisposition to cancers
Chuvash Polycythemia: recessive inherited mutations in VHL leading to elevated hematocrit

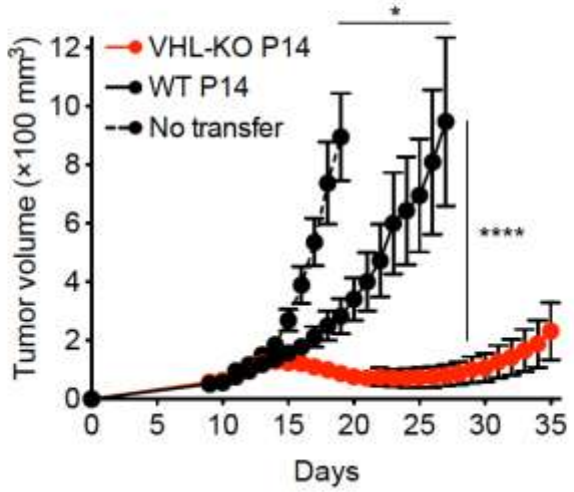
Enhanced Hypoxia Inducible Factor (HIF) activity in T cells due to VHL deficiency promotes anti-tumor activity by CD8+ T cells

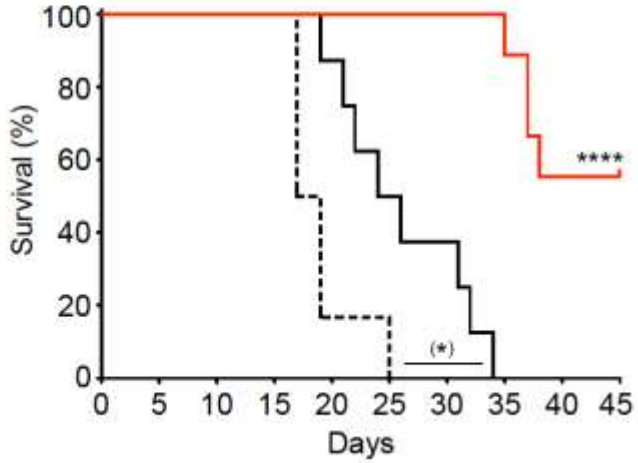












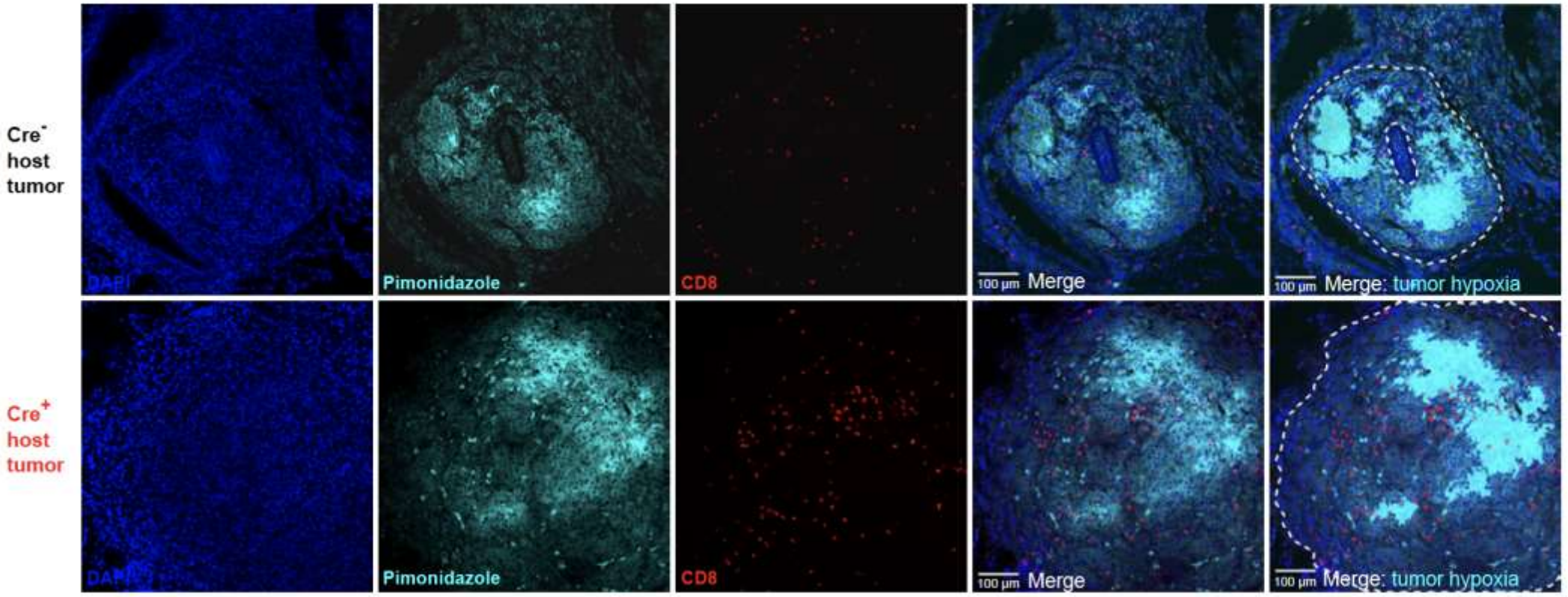
Ilkka Liikanen
Colette Lauhan
Deborah Witherden

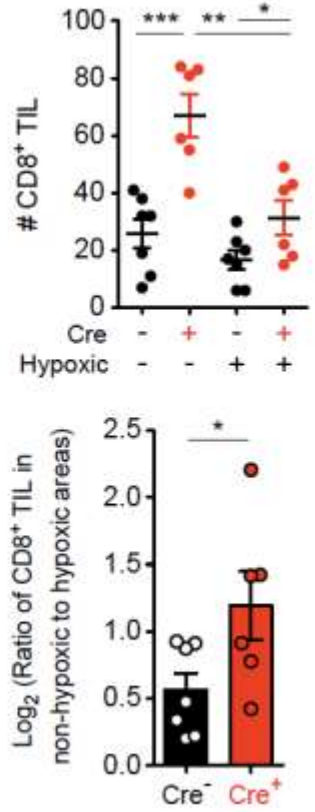
Doedens et al. Nature Immunology 2014

- Loss of VHL also rescues survival and functional exhaustion in LCMC clone 13 infection
- Enhanced glycolytic activity, enhanced function in spite of high levels of inhibitory receptors

Liikanen JCI 2021

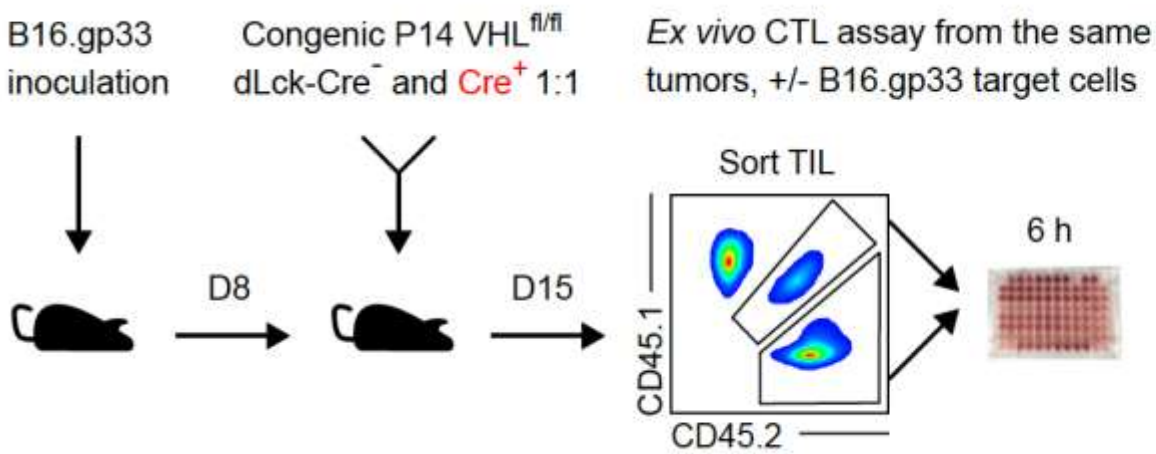
Enhanced Hypoxia Inducible Factor (HIF) activity in T cells due to VHL deficiency promotes CD8⁺ T cells accumulation in tumors

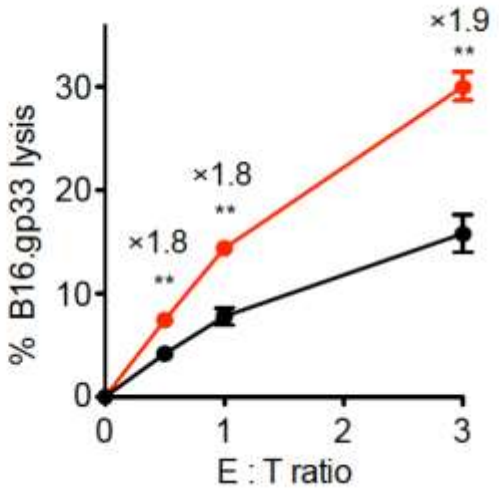


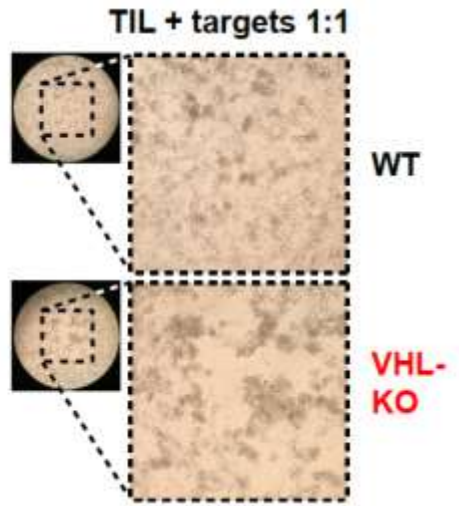


Liikanen JCI 2021

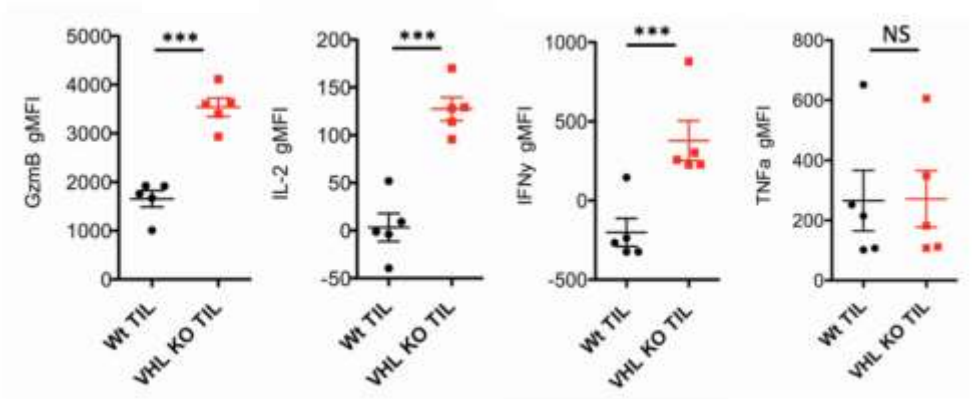
Enhanced HIF promotes TIL effector function

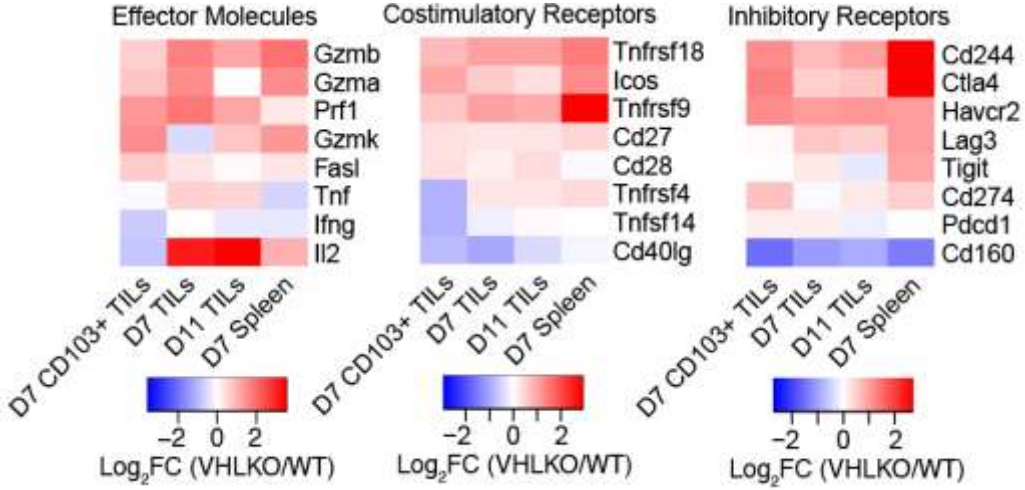






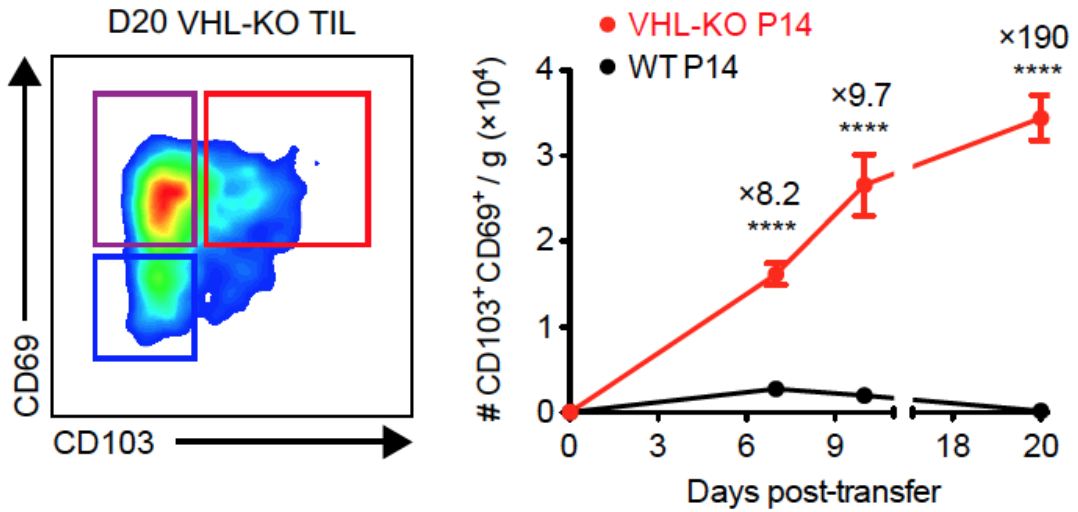
GzmB IL-2 IFNγ TNFα

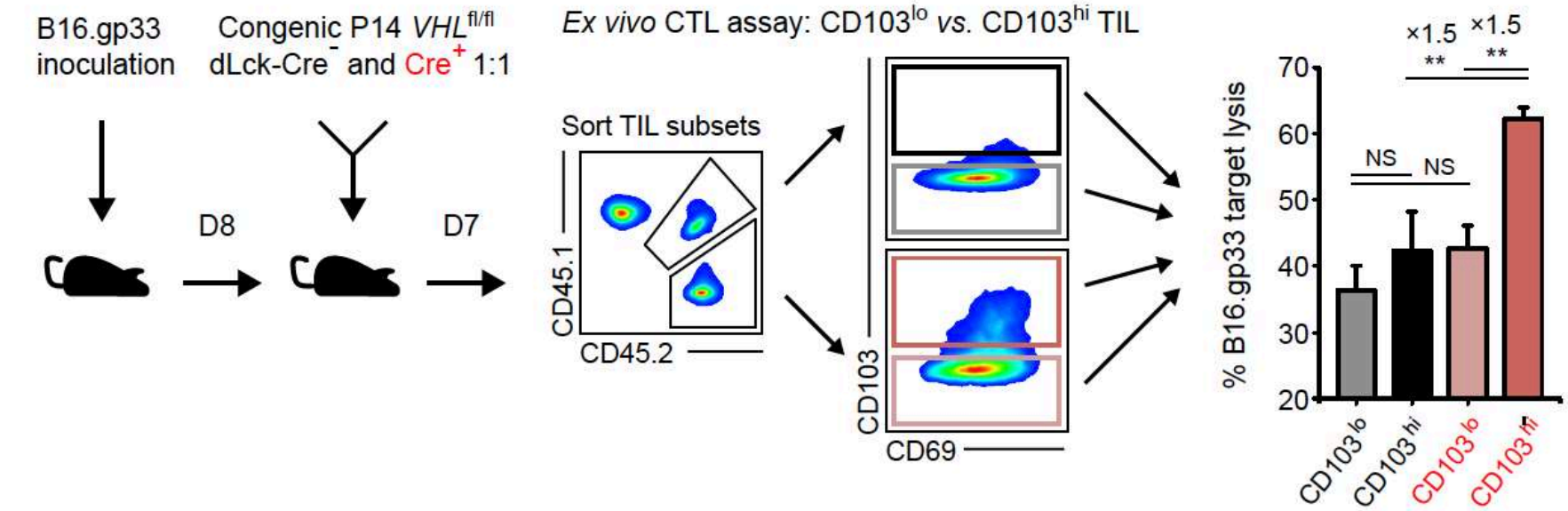




Liikanen JCI 2021

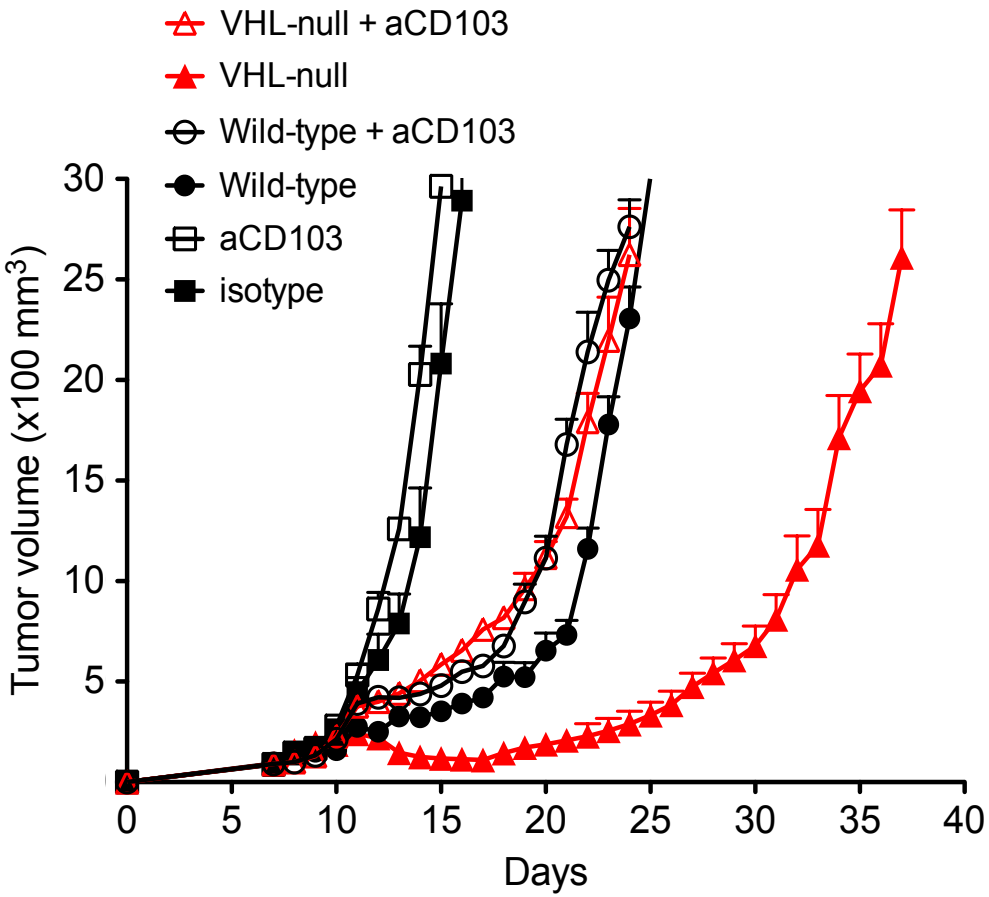
Elevated HIF leads to accumulation of CD103^{+}CD69^{+} T_{RM}-like TIL with enhanced killing activity





Liikanen JCI 2021

Enhanced anti-tumor function by with enhanced HIF is dependent on CD103



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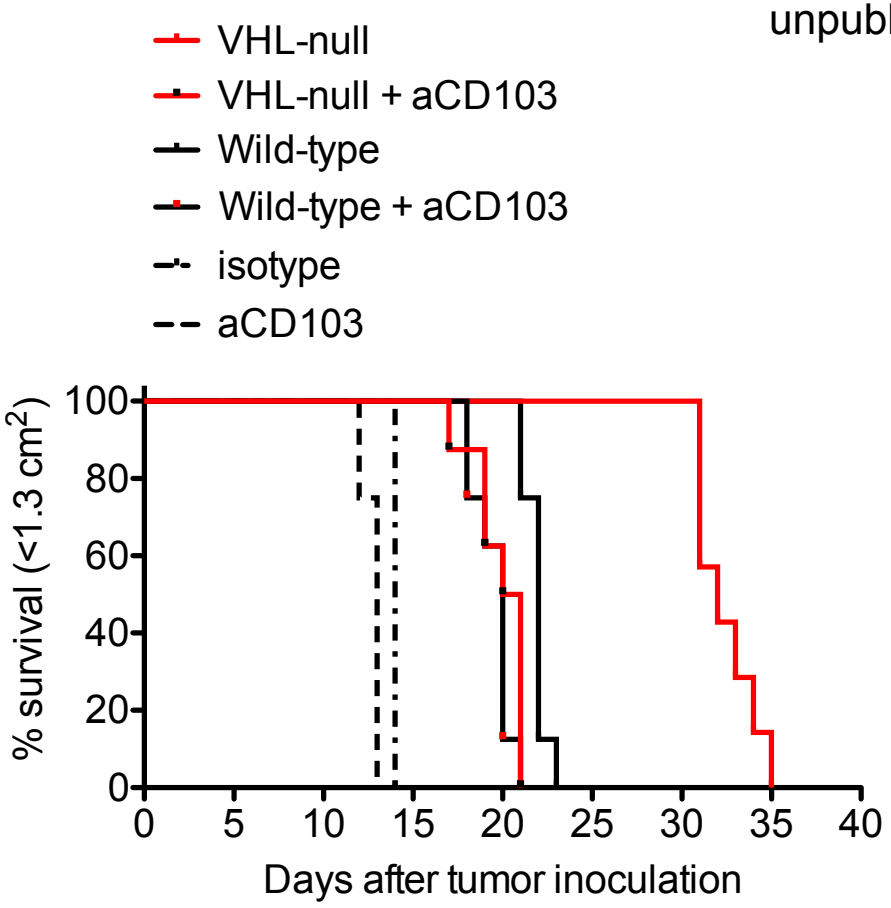
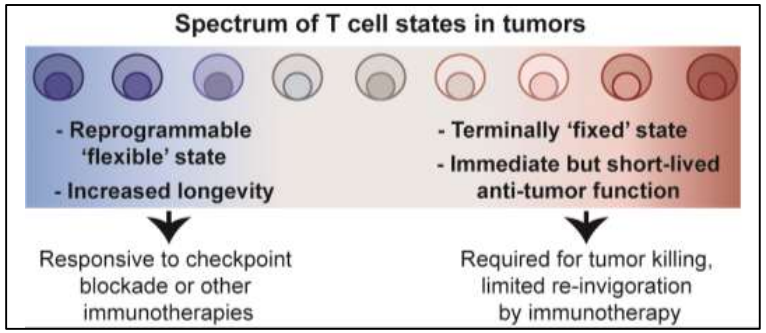


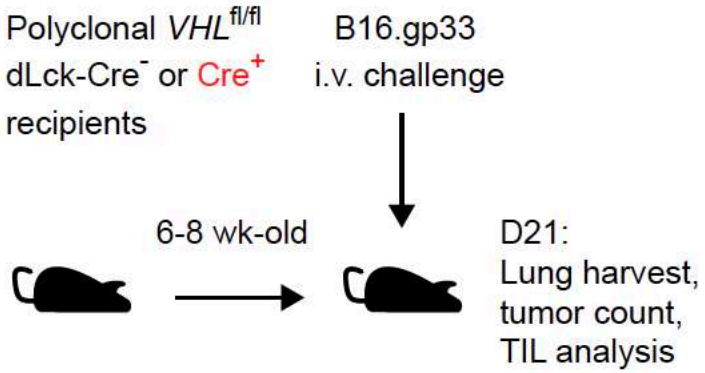
CD103 associates with integrin beta7 and is expressed intraepithelial lymphocytes, a small subset of peripheral lymphocytes, dendritic epidermal T cells and TRM. Binds E-cadherin mediates homing of lymphocytes to the intestinal epithelium.

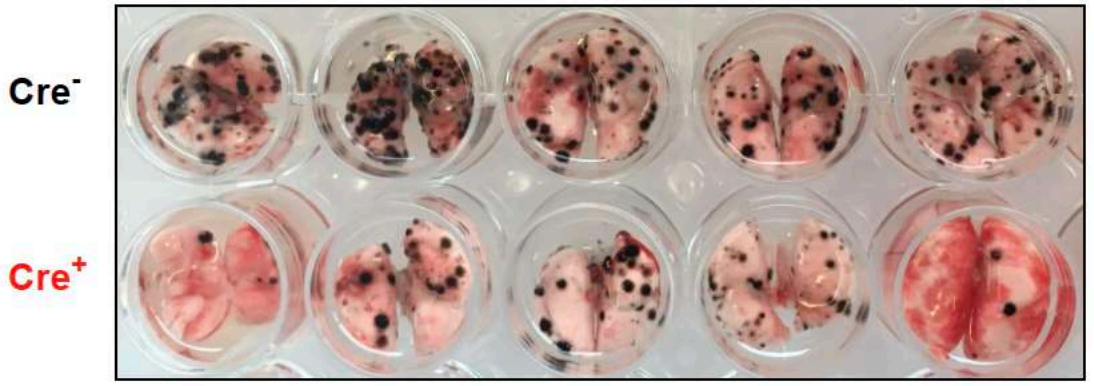
Liikanen JCI 2021

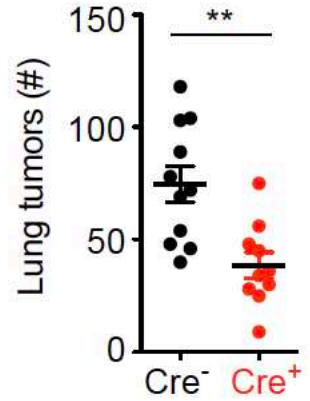
Summary:



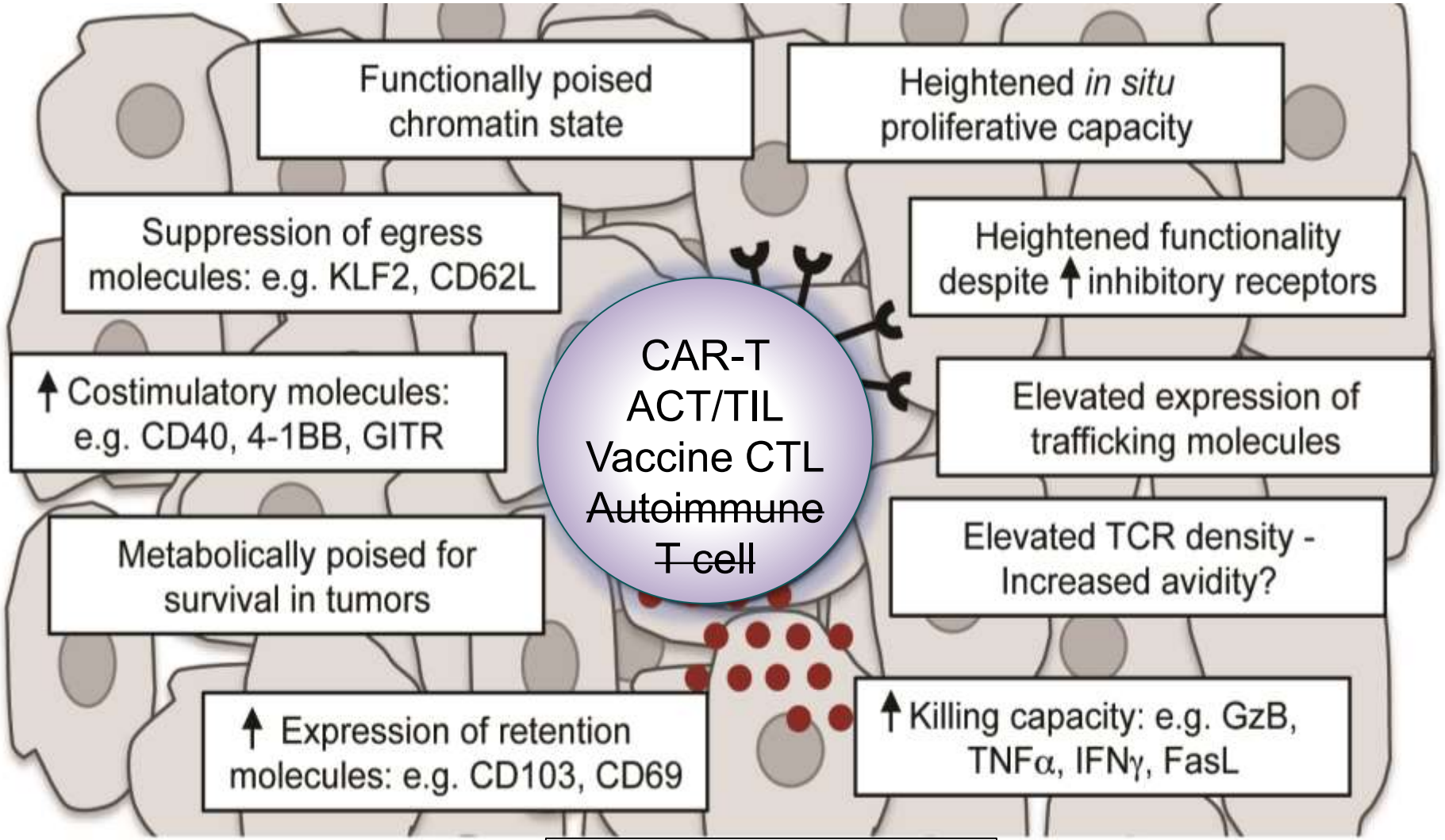
- Enhanced HIF activity promotes survival and accumulation of TIL that restrain tumor growth
- VHL deletion promotes differentiation of T_{RM}-like TIL with heightened tumor killing and cytokine production
- Elevated HIF promotes TIL accumulation by CAR T and restrains metastatic tumor growth with ACT







Understanding T_{RM} programming informs T cell therapies



↑ Function in hypoxia

Goldrath Lab:

Ty Crowl
Justin Milner
Kyla Omilusik
Quynh Nguyen
Tianda Deng
Hong Nguyen
Sara Quon
Clara Toma
Colette Lauhan
Debbie Whitherdon
Miguel Reina Campos
Nicole Scharping
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Collaborators:

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Wei Wang

Disclosures:

SAB of Arsenal Bio

National Institute of Allergy and Infectious Diseases

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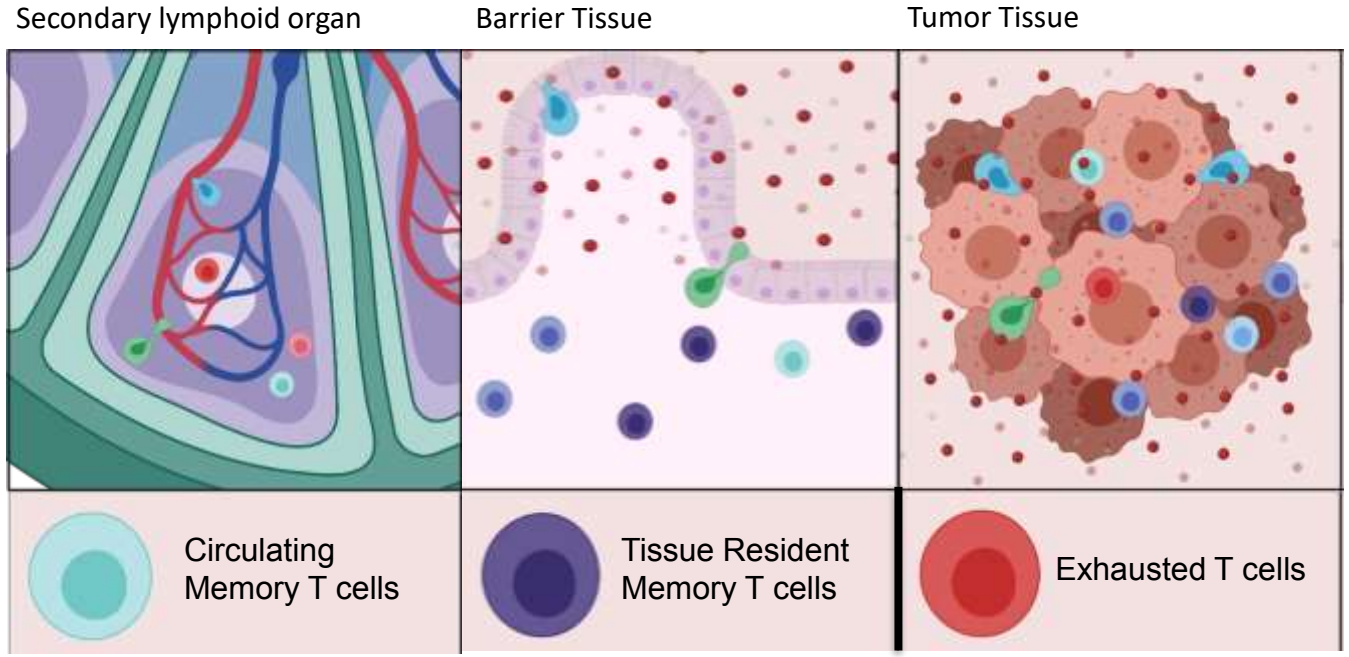
PEW

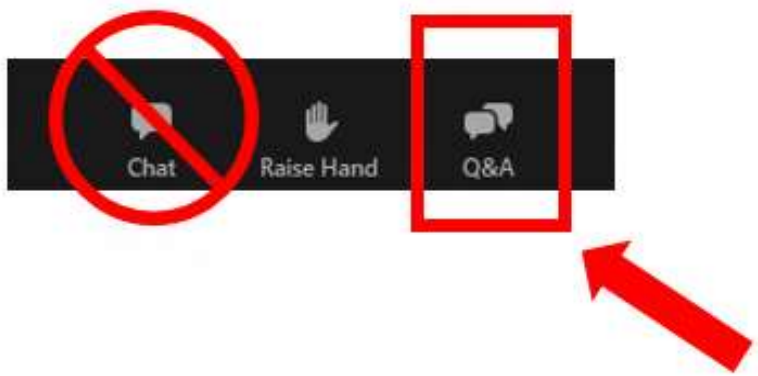

Secondary lymphoid organ
Barrier Tissue
Tumor Tissue
Circulating
Memory T cells
Tissue Resident
Memory T cells
Exhausted T cells

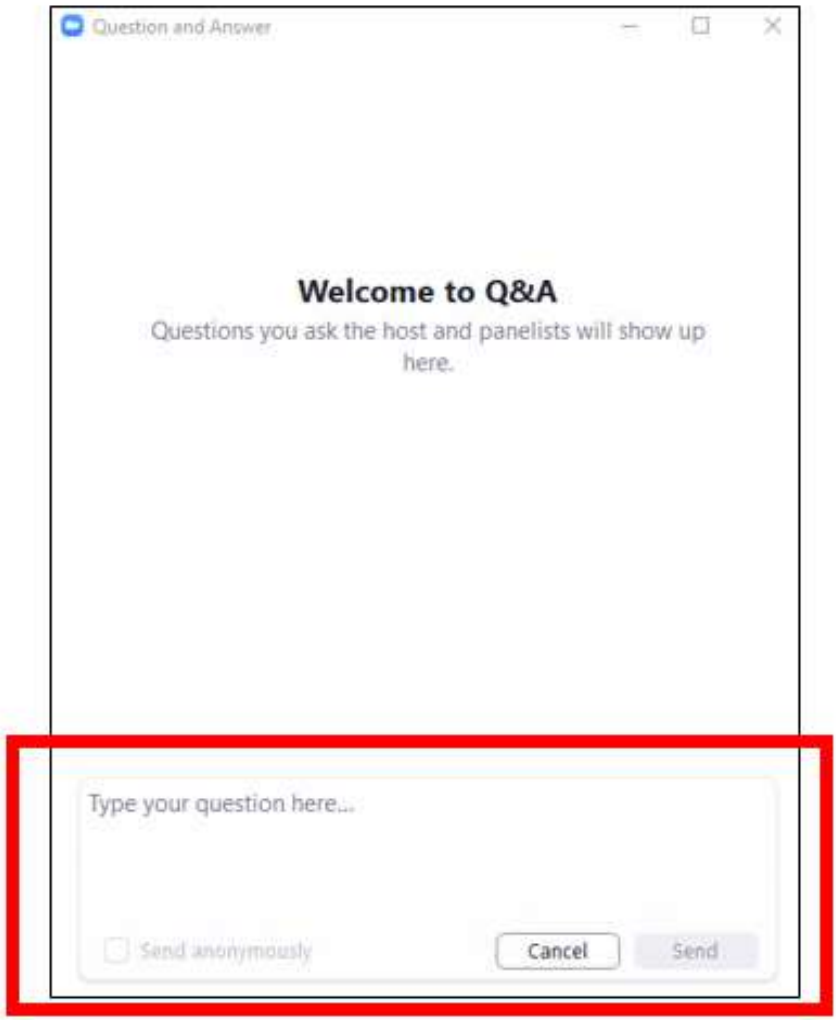
BioRender

How to Submit Questions

Society for Immunotherapy of Cancer

- Click the **"Q&A"** icon located on at the bottom of your Zoom control panel
- Type your question in the Q&A box, then click "Send"
- Questions will be answered:
  a. after each speaker's presentation
  b. in the Question & Answer session at the end of the seminar





Webinar Outline

- Dr. Ananda W. Goldrath: Transcriptional side of T cell functional states; residency program; biomarkers
- Dr. Evan W. Newell: Cytometry; residency program (circulation and exhaustion)
- Dr. Daniela S. Thommen: Functional side; TLS (background, definition and function)

- Q&A: Dr. Wherry + Dr. Schietinger
  - Naming structures/terms/nomenclature
  - Translating t cell therapies into the clinic

Decoding the diversity of T-cell responses in cancer through the analysis of antigen specificity

Evan W. Newell, PhD
Fred Hutchinson Cancer Research Center, Seattle, WA
enewell@fredhutch.org

@EvNewell1

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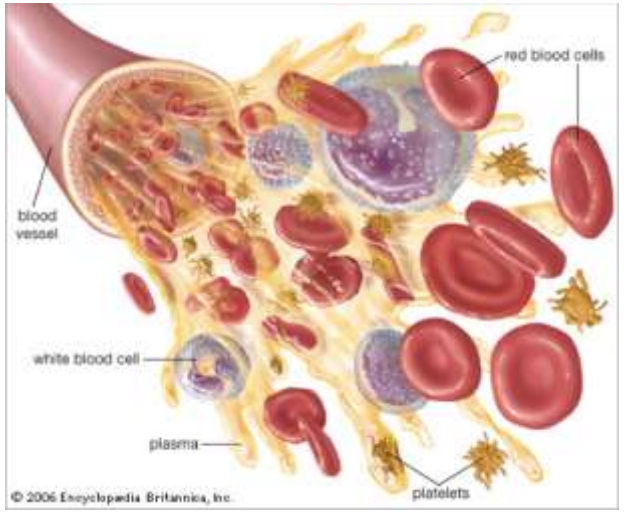
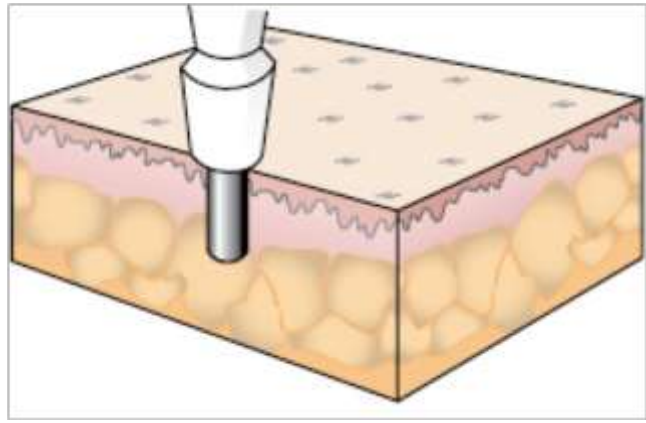
Studying the disease-specific immune cell response in humans

- Highly diverse immune cell phenotypes in blood and in tissue
- Which are involved in disease?
- What can their profiles tell us in blood vs. tissue?
- Benefits of tracking disease-specific immune responses in blood for immunotherapy trials/studies

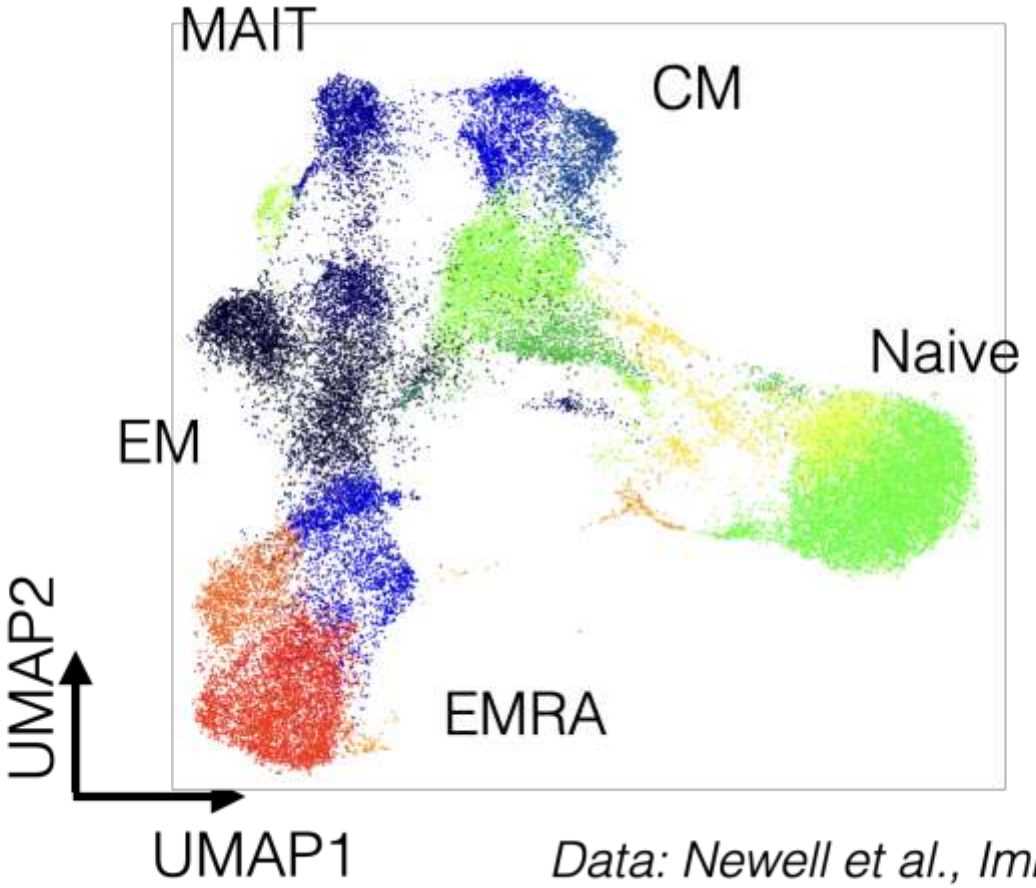




Vision: Decoding T cells to track immunity in human health and disease

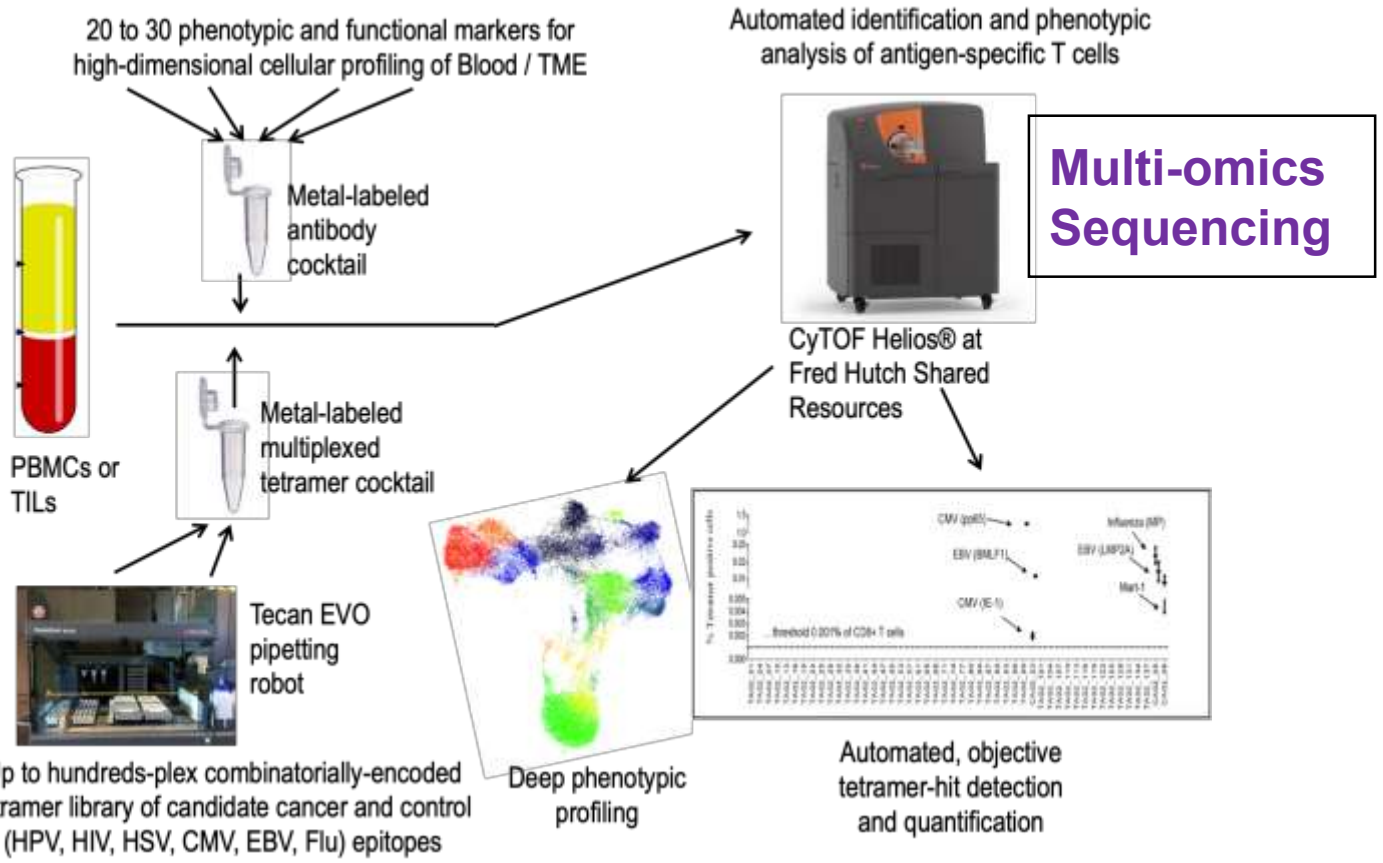
T cell receptor

peptide-MHC

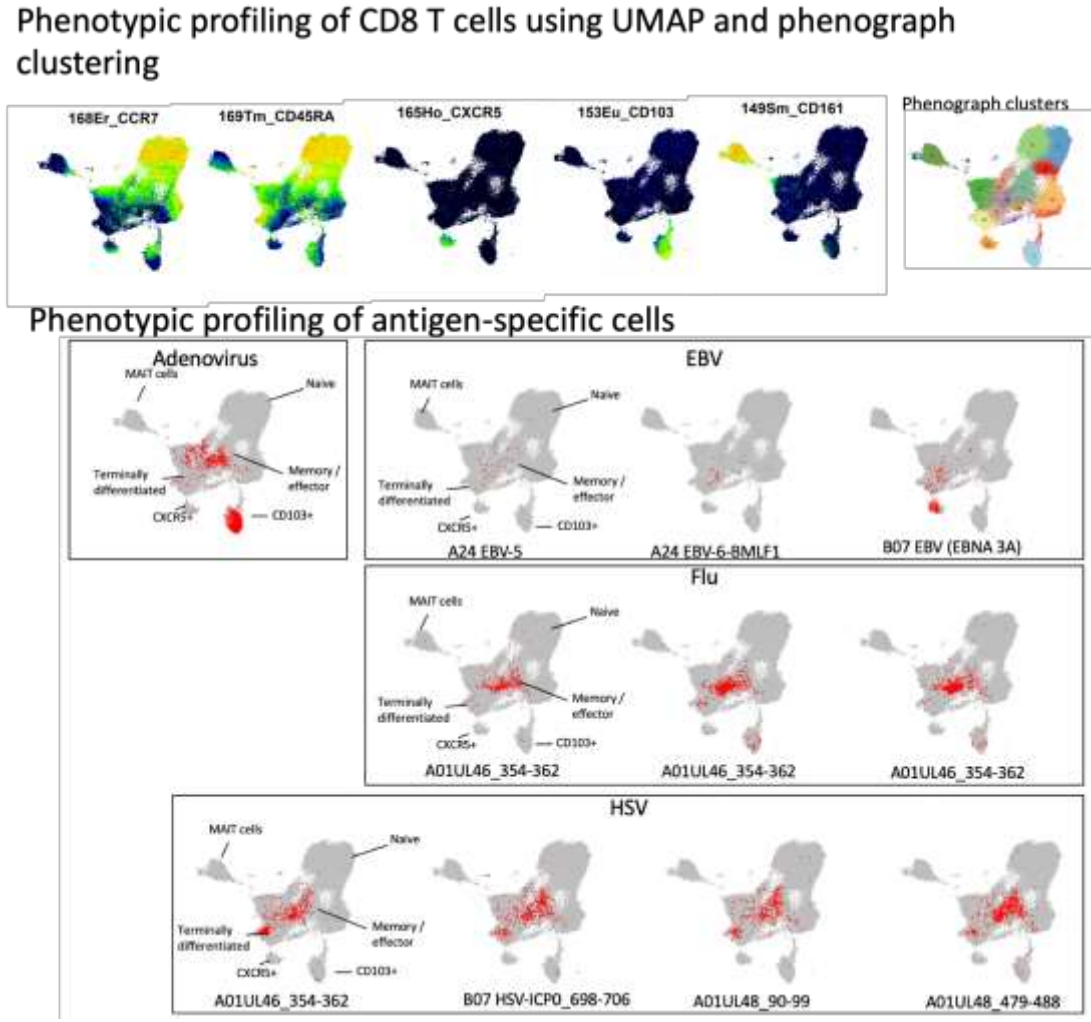


Data: Newell et al., Immunity 2012
Phenograph clusters

Overall approach

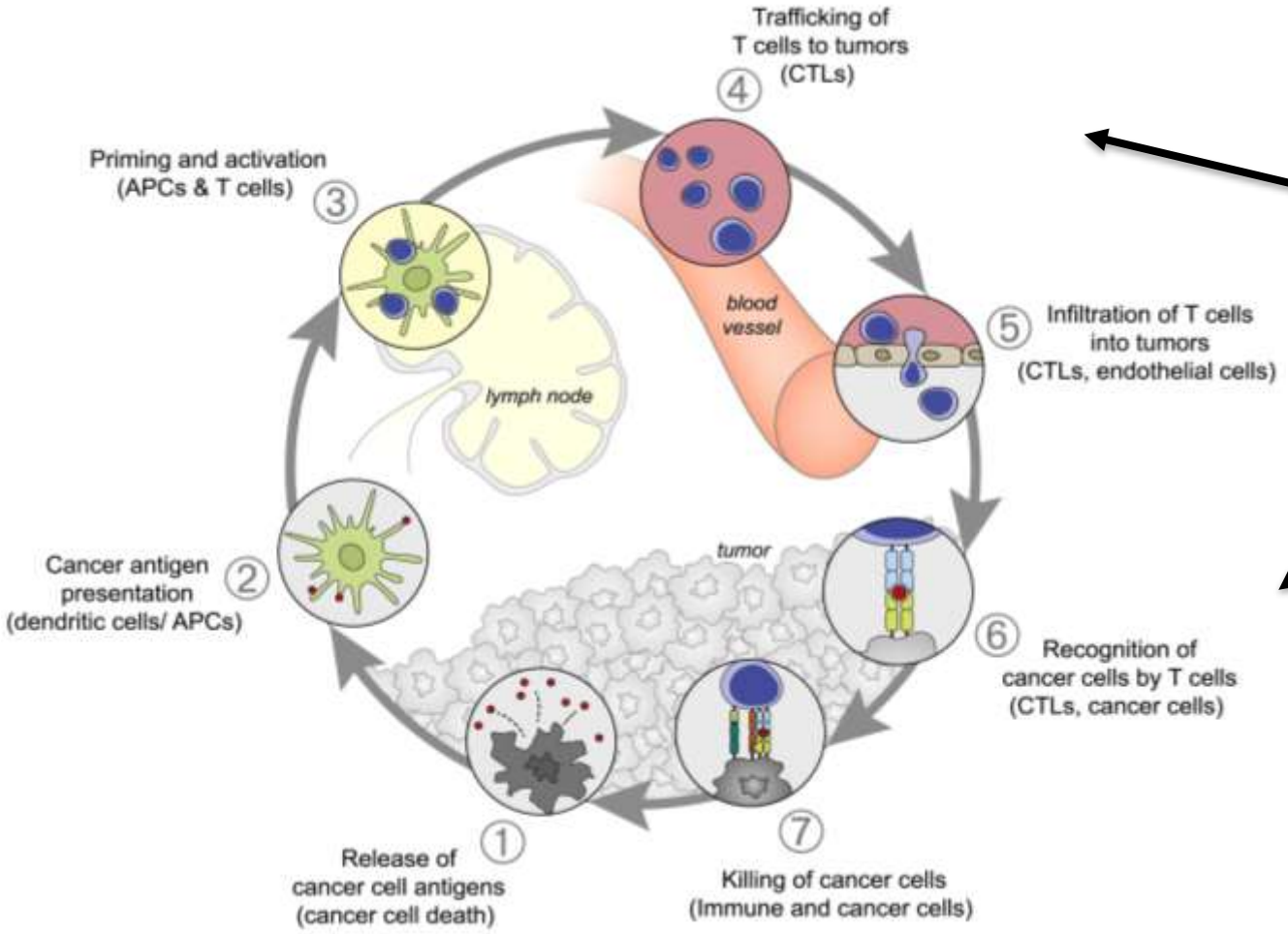


Newell et al. Nat. Methods 2009, Nat. Biotech 2013



Laura Islas, Timothy Bi

Antigen specificity in the Cancer Immunity Cycle



Which are tumor specific and what can we learn from them?

Goals:

- More accurate biomarkers for immunotherapy
- Easier access to tumor-specific T cells / TCR / targetable antigens

Questions

- Tumor-infiltrating T cell diversity
  - What can we learn from cells that we know are specific vs. not specific for cancer?
  - Can focusing on tumor-specific T cells make it easier to identify more accurate biomarkers?

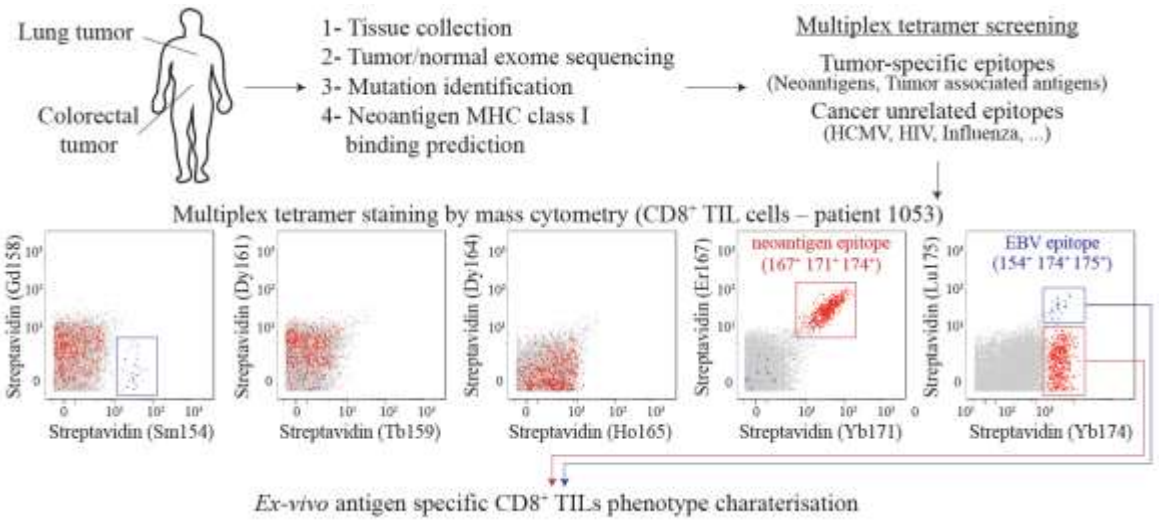
- Tumor-specific T cells from the blood to assess the status of response in tumors?
  - Therapeutic biomarkers
  - Accessible tumor-specific TCRs

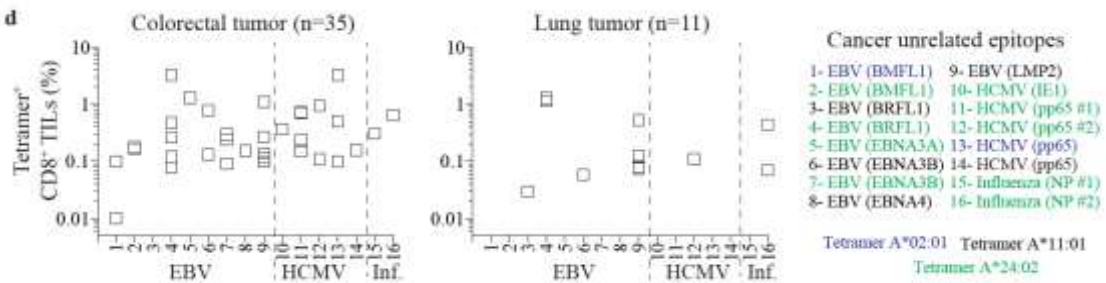
LETTER

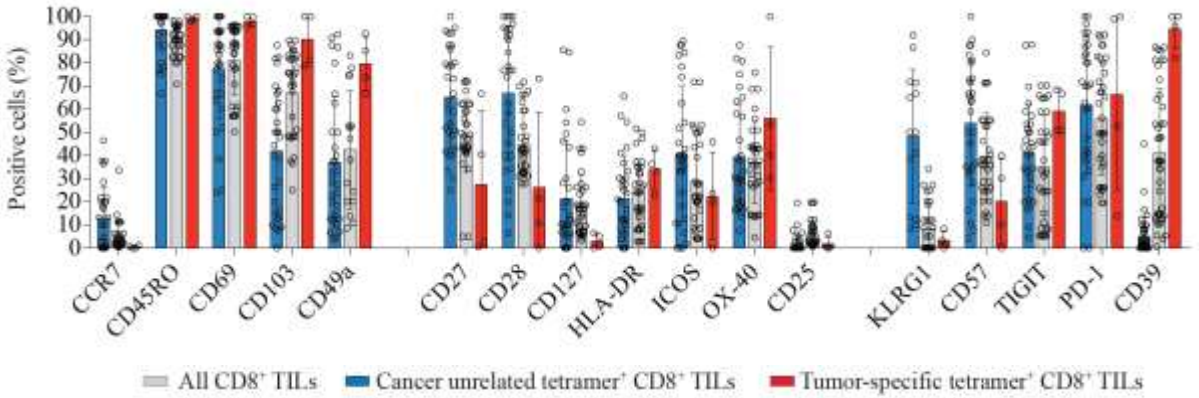
https://doi.org/10.1038/s41586-018-0130-2

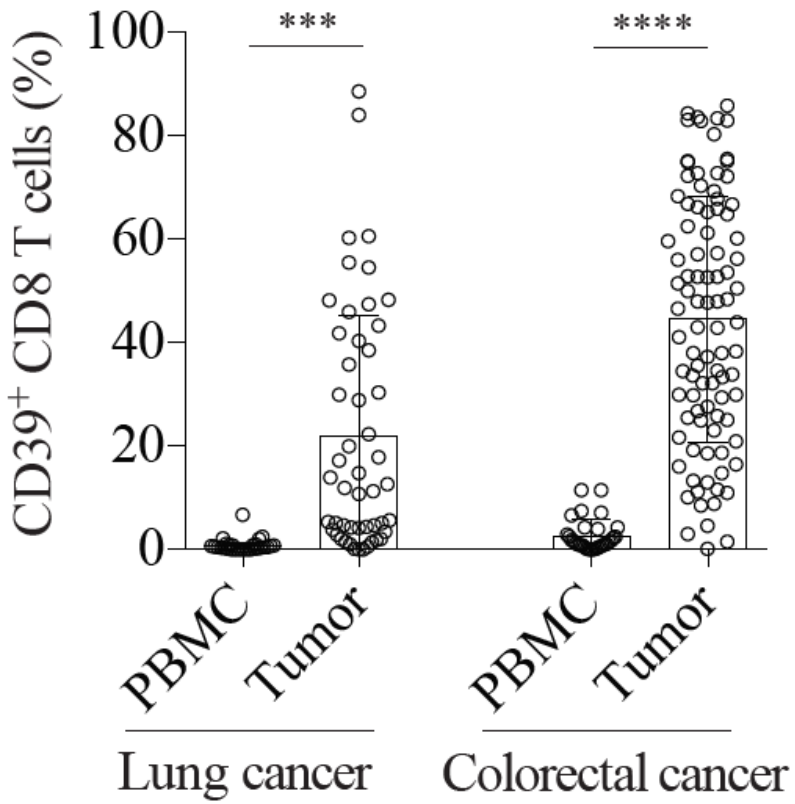
Bystander CD8$^+$ T cells are abundant and phenotypically distinct in human tumour infiltrates

Yannick Simoni[1]*, Etienne Becht[1], Michael Fehlings[1,2], Chiew Yee Loh[1], Si-Lin Koo[3], Karen Wei Weng Teng[1], Joe Poh Sheng Yeong[1,4], Rahul Nahar[5], Tong Zhang[5], Hassen Kared[1], Kaibo Duan[1], Nicholas Ang[1], Michael Poidinger[1], Yin Yeng Lee[5], Anis Larbi[1], Alexis J. Khng[5], Emile Tan[6], Cherylin Fu[6], Ronnie Mathew[6], Melissa Teo[7], Wan Teck Lim[3], Chee Keong Toh[3], Boon-Hean Ong[8], Tina Koh[7], Axel M. Hillmer[5], Angela Takano[4], Tony Kiat Hon Lim[4,5,9], Eng Huat Tan[3], Weiwei Zhai[5], Daniel S. W. Tan[3,5], Iain Beehuat Tan[3,5,9] & Evan W. Newell[1]*





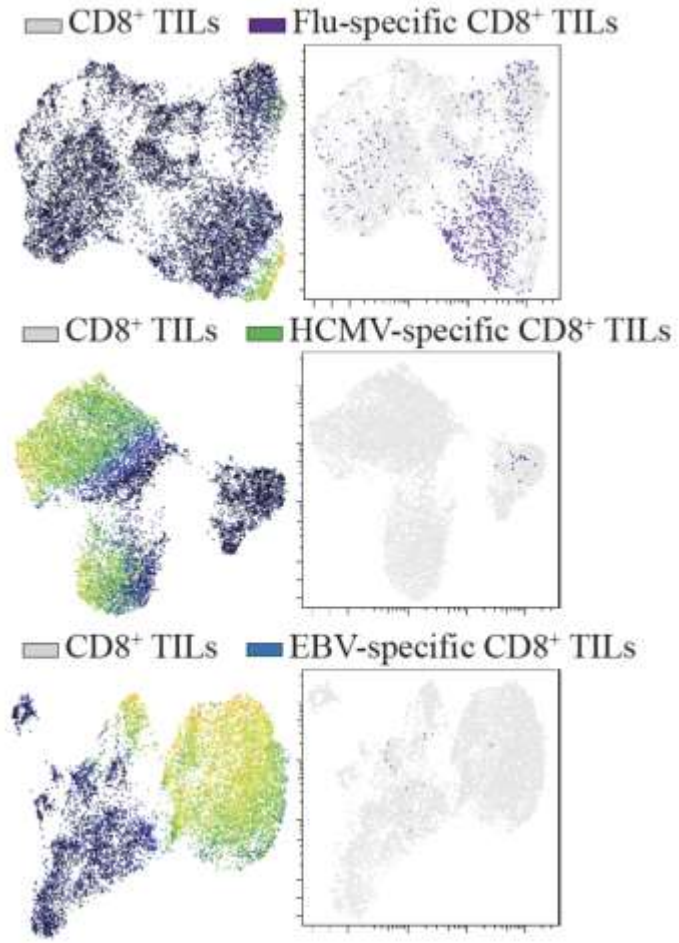




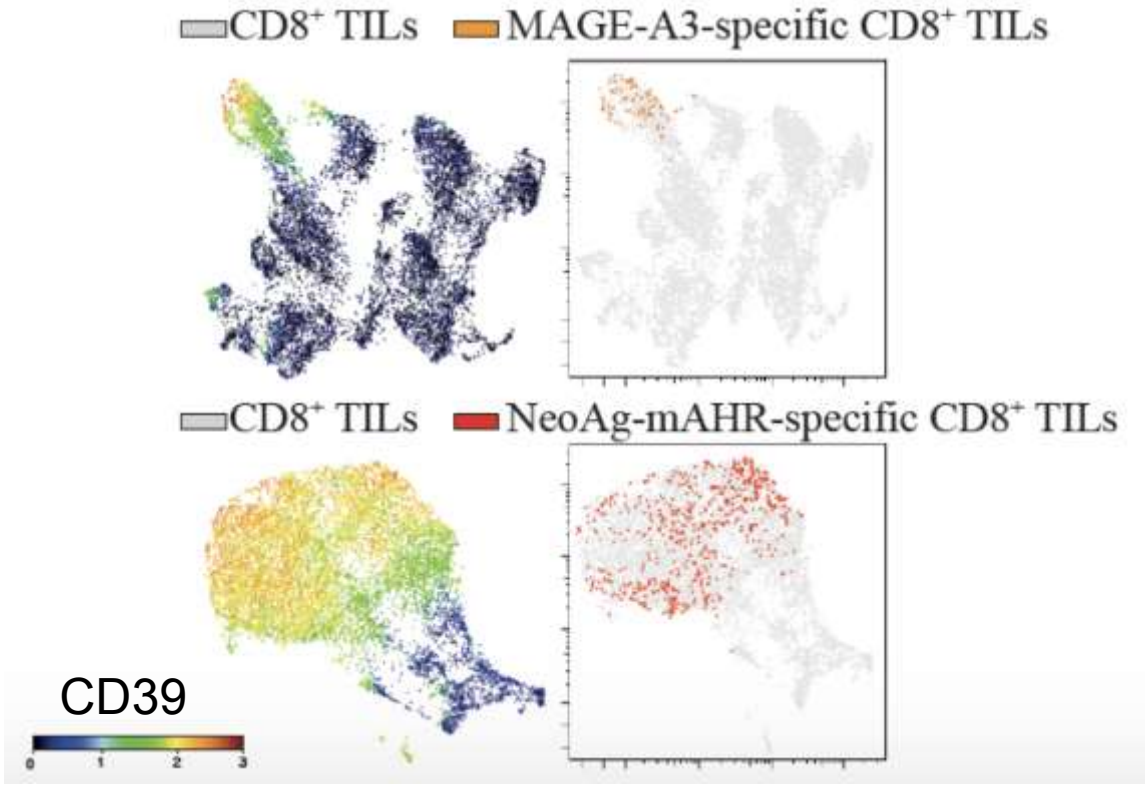
Yannick Simoni, et al. Nature, 2018

Bystander T cells are diverse but CD39-negative

Bystander



Tumor-specific



Yannick Simoni

Other evidence for prevalence of tumor infiltrating bystander T cells

ARTICLE

DOI: 10.1038/s41467-018-05072-0 OPEN

Co-expression of CD39 and CD103 identifies tumor-reactive CD8 T cells in human solid tumors

Thomas Duhen[1], Rebekka Duhen[2], Ryan Montler[1], Jake Moses[1], Tarsem Moudgil[2], Noel F. de Miranda[3], Cheri P. Goodall[2], Tiffany C. Blair[1], Bernard A. Fox[2], Jason E. McDermott[4], Shu-Ching Chang[5], Gary Grunkemeier[5], Rom Leidner[2], Richard Bryan Bell[2] & Andrew D. Weinberg[1,2]

ARTICLE

https://doi.org/10.1038/s41467-019-08534-1 OPEN

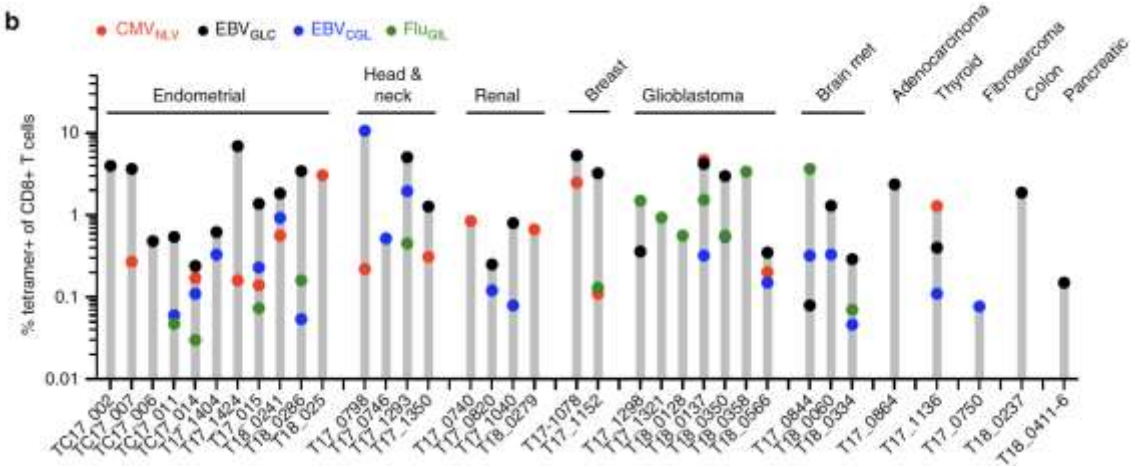
Virus-specific memory T cells populate tumors and can be repurposed for tumor immunotherapy

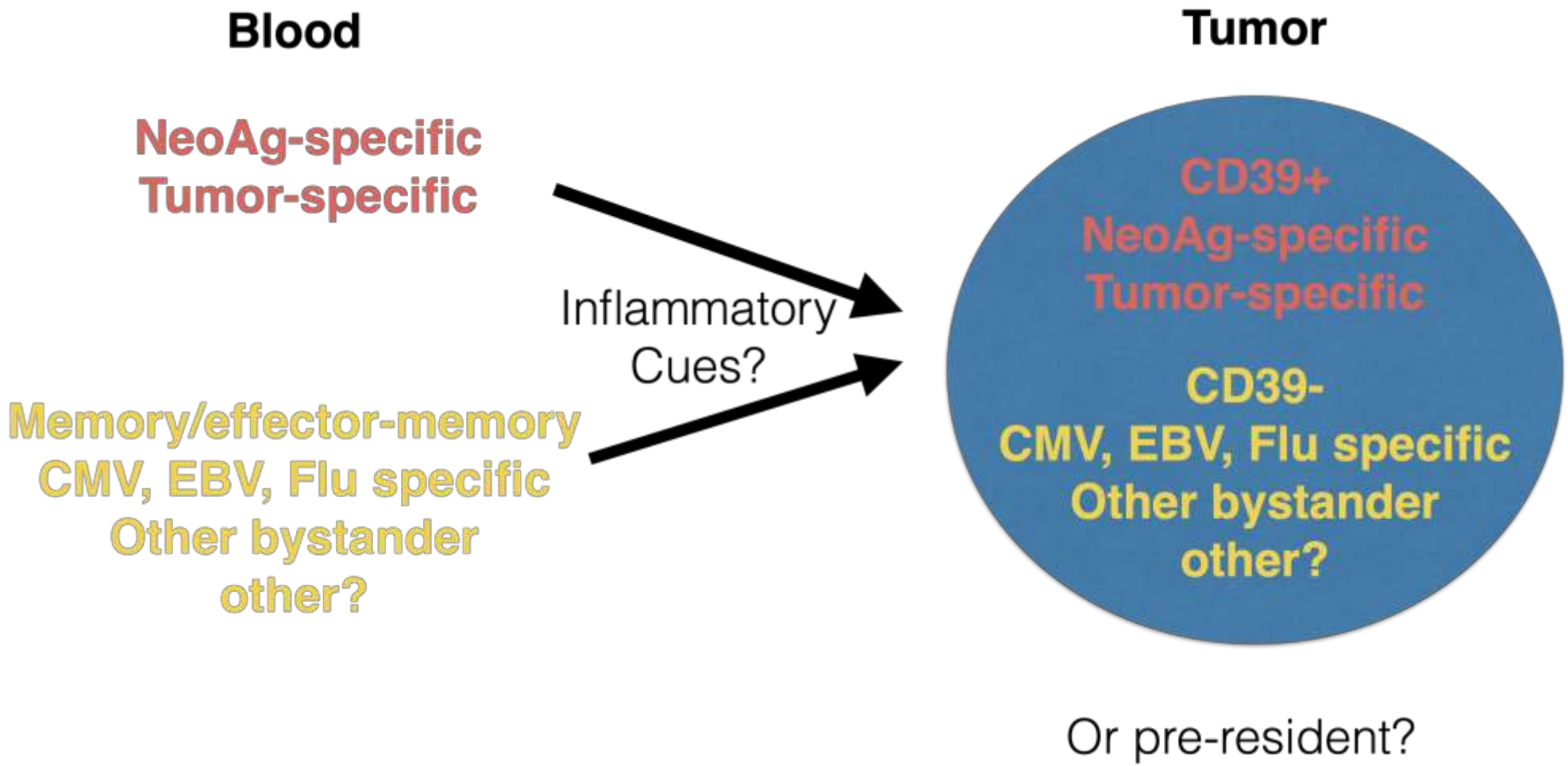
Pamela C. Rosato[1], Sathi Wijeyesinghe[1], J. Michael Stolley[1], Christine E. Nelson[1], Rachel L. Davis[1], Luke S. Manlove[1], Christopher A. Pennell[2], Bruce R. Blazar[3], Clark C. Chen[4], Melissa A. Geller[5], Vaiva Vezys[1] & David Masopust[1]

nature medicine LETTERS https://doi.org/10.1038/s41591-018-0266-5

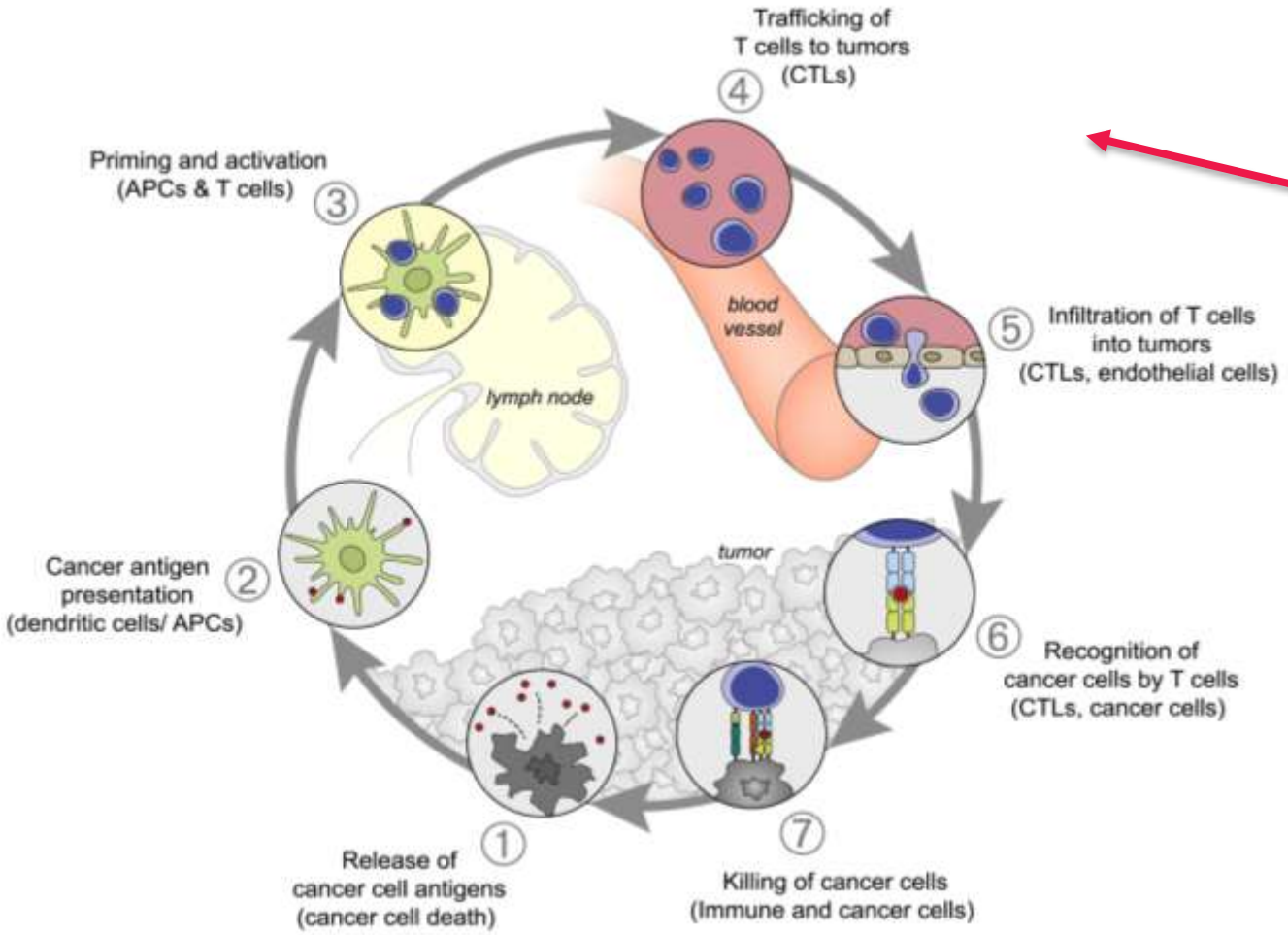
Low and variable tumor reactivity of the intratumoral TCR repertoire in human cancers

Wouter Scheper[1,10], Sander Kelderman[2,10], Lorenzo F. Fanchi[1], Carsten Linnemann[2], Gavin Bendle[2], Marije A. J. de Rooij[2], Christian Hirt[3], Riccardo Mezzadra[1], Maarten Slagter[1,4], Krijn Dijkstra[2], Roelof J. C. Kluin[5], Petur Snaebjornsson[6], Katy Milne[7], Brad H. Nelson[7], Henry Zijlmans[8], Gemma Kenter[8], Emile E. Voest[2,9], John B. A. G. Haanen[2,9] and Ton N. Schumacher[1*]



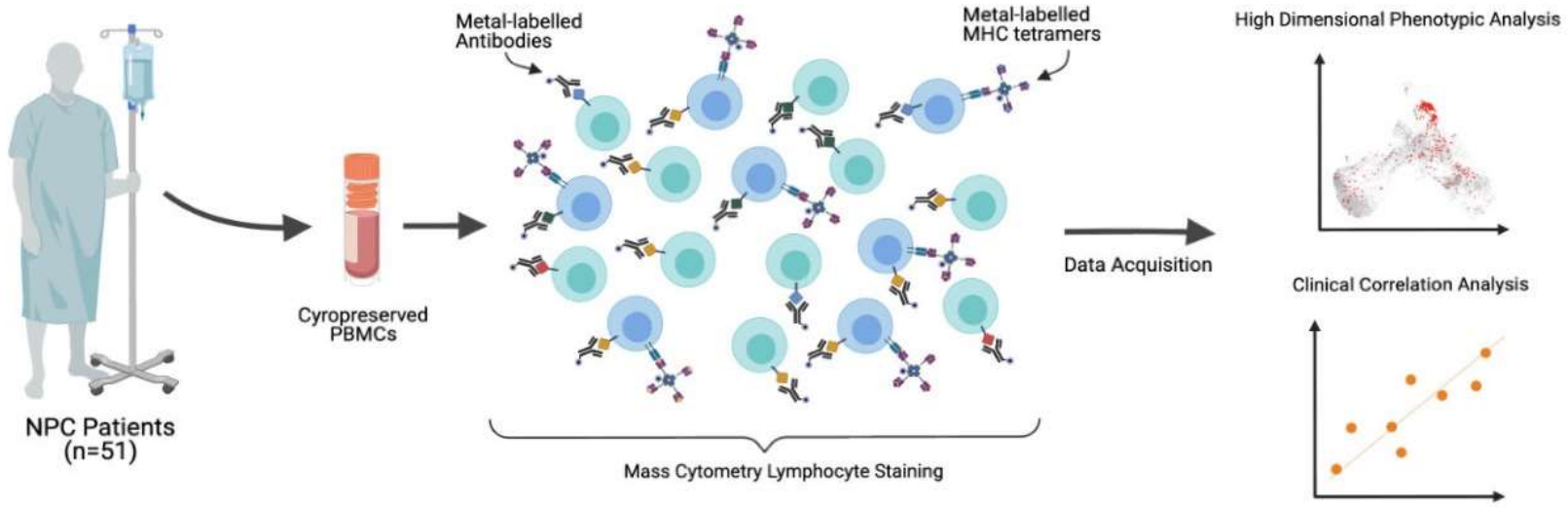

Blood
NeoAg-specific
Tumor-specific
Memory/effector-memory
CMV, EBV, Flu specific
Other bystander
other?
Inflammatory
Cues?
Tumor
CD39+
NeoAg-specific
Tumor-specific
CD39-
CMV, EBV, Flu specific
Other bystander
other?
Or pre-resident?

Antigen specificity in the Cancer Immunity Cycle



Which are tumor specific?

A Nasopharyngeal Carcinoma (NPC) cohort



35 phenotypic markers

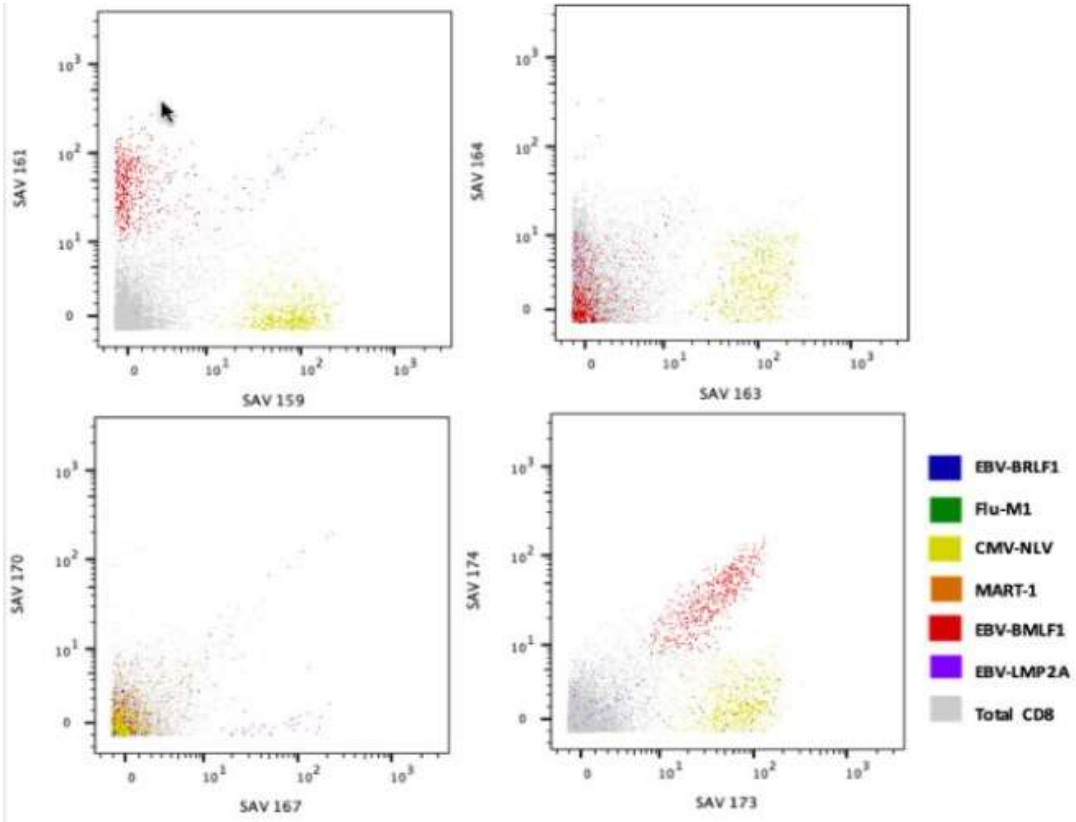
- Lymphocyte lineage
- Activation
- Exhaustion
- Migration
- Memory/naive
- Senescence

56 unique MHC tetramers

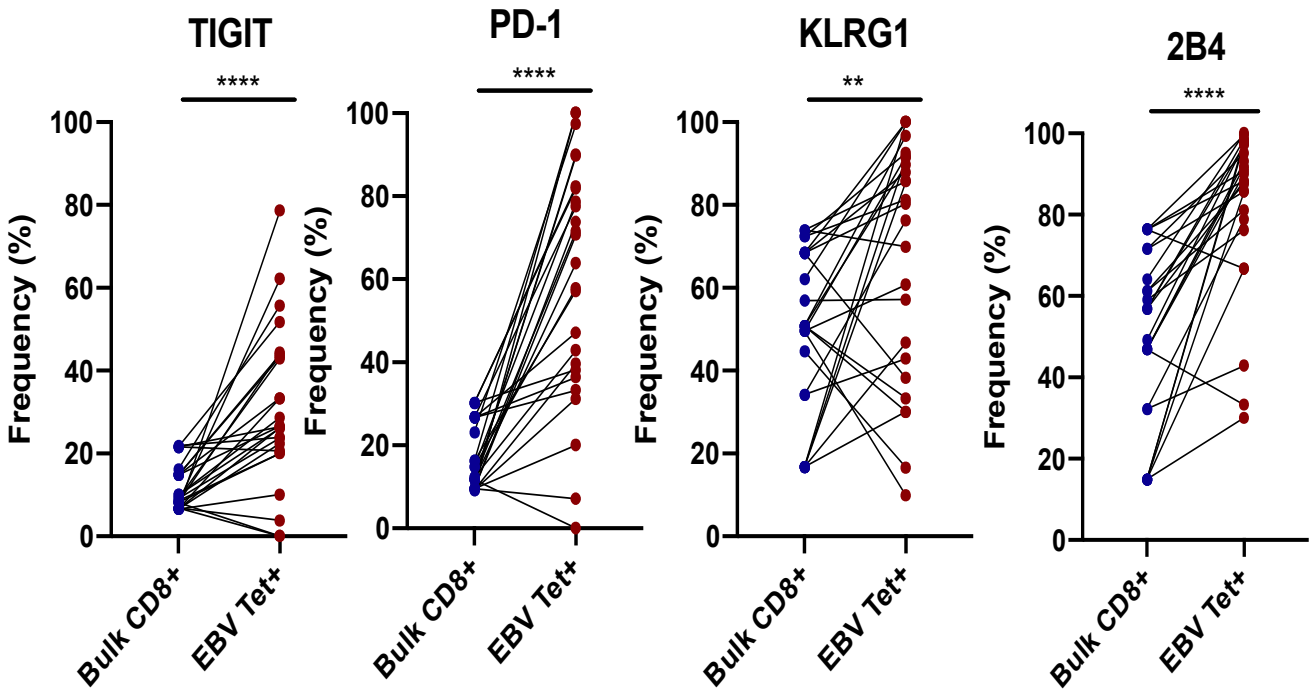
- 21 EBV epitopes
  - Latent and Lytic epitopes
- 7 TAA epitopes
- 28 bystander viral epitopes

Nandita Kumar, Amit Jain

EBV-specific T cells detectable in most patient samples



Multiplex-tetramer staining
NPC patient PBMC



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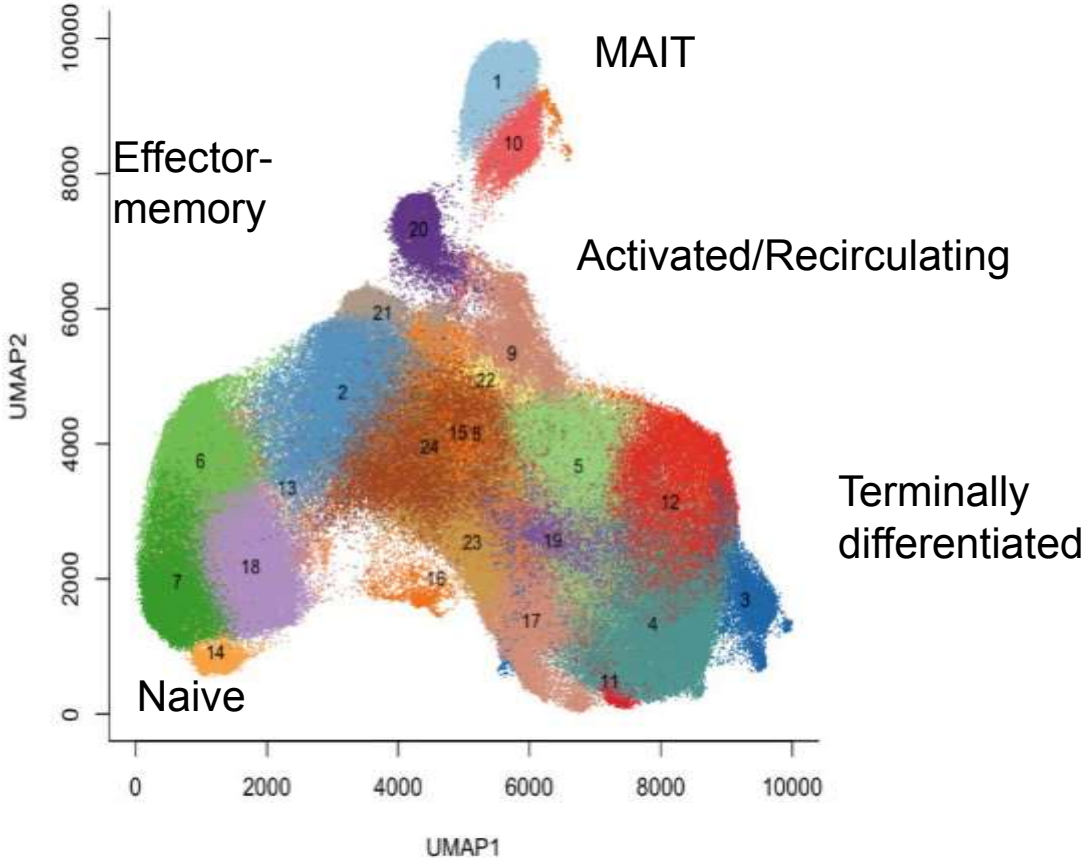
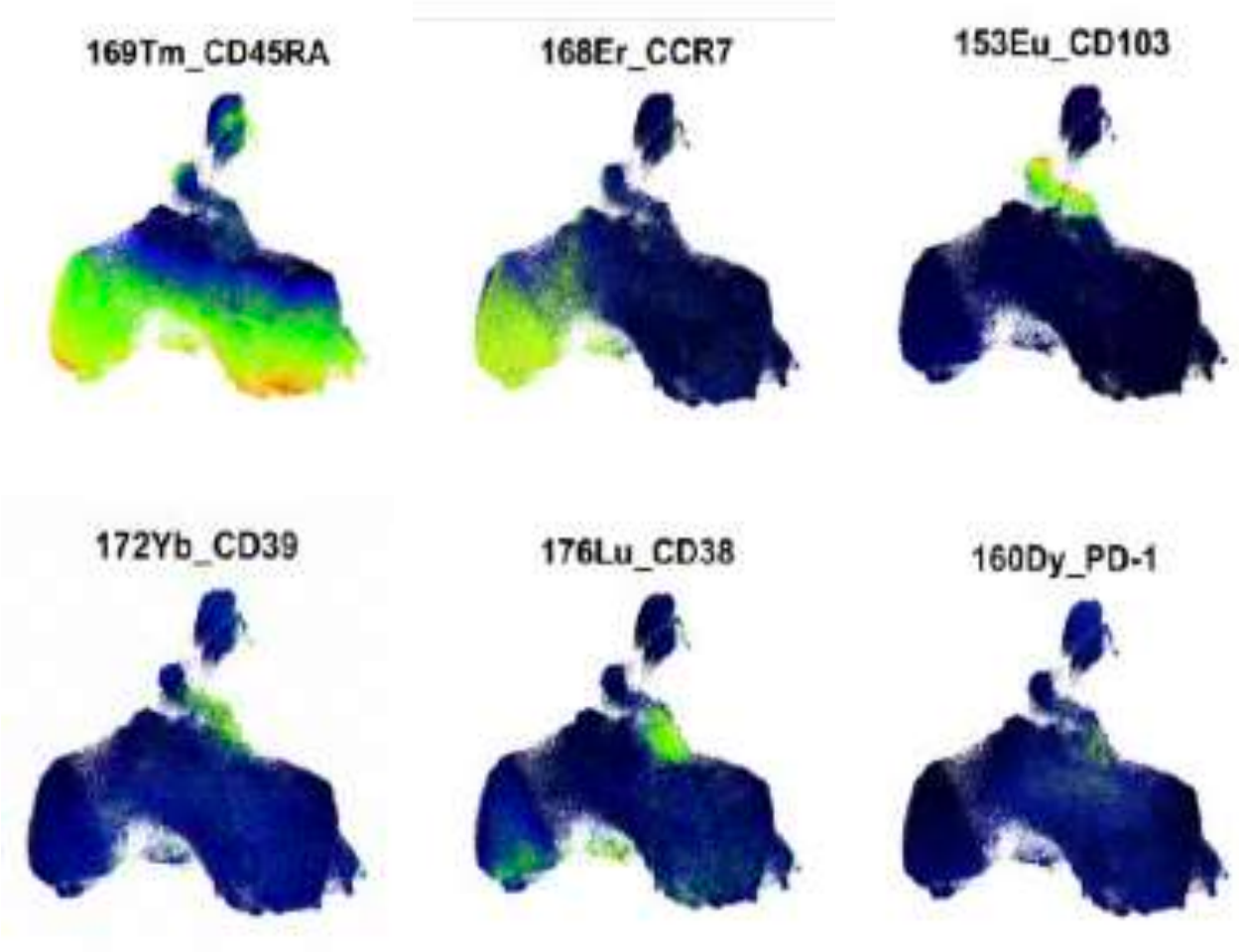
CD8 profiles in NPC cohort





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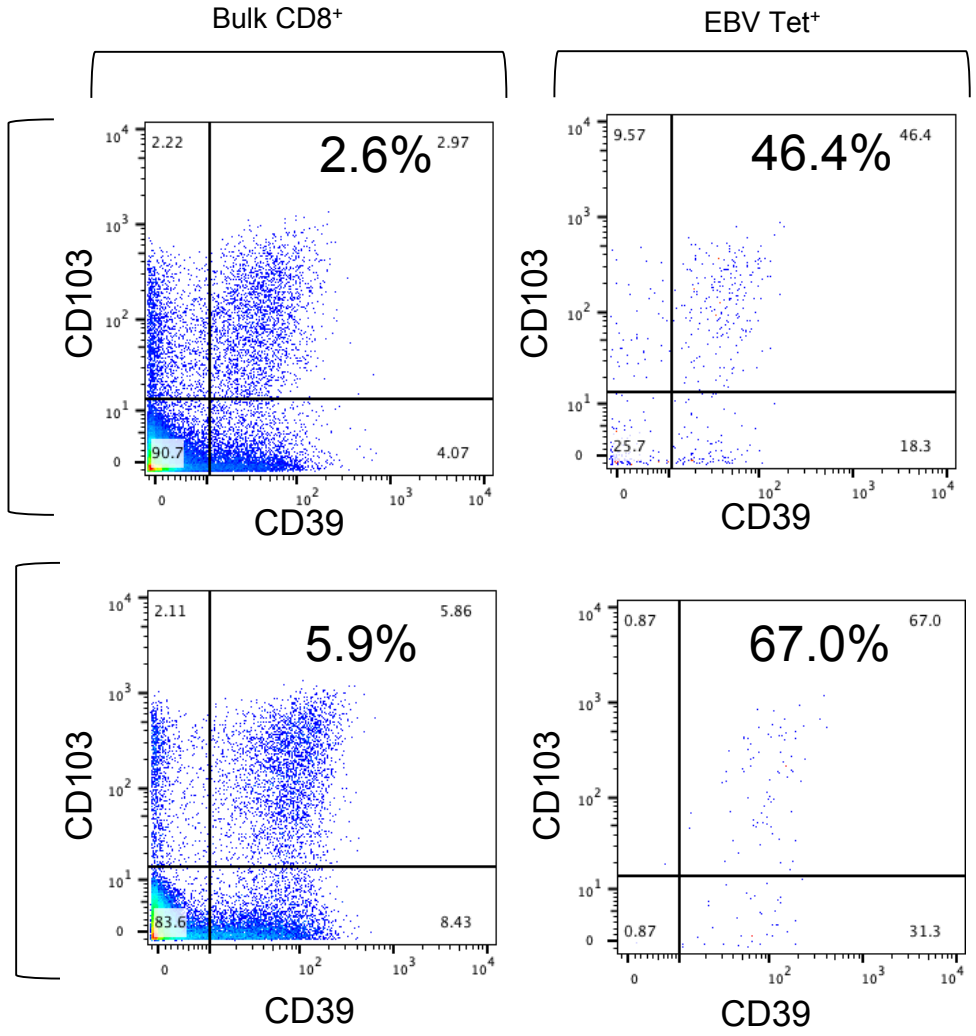
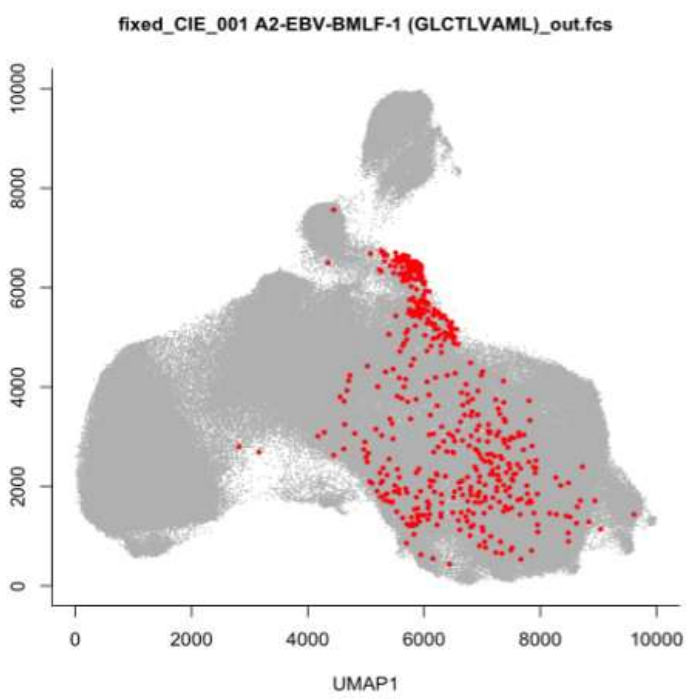
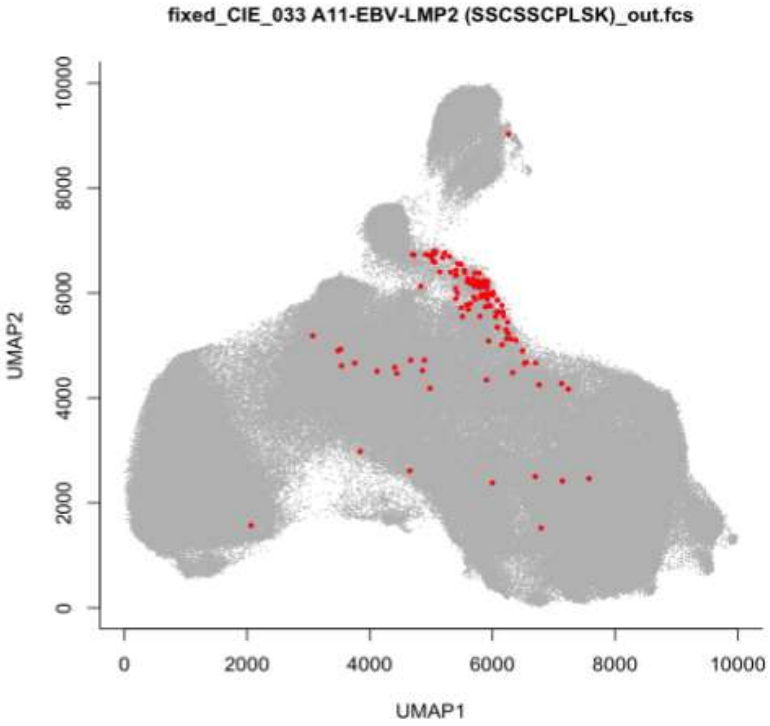
Peripheral blood CD39⁺CD103⁺ EBV-specific cells in NPC



EBV/BMLF-1

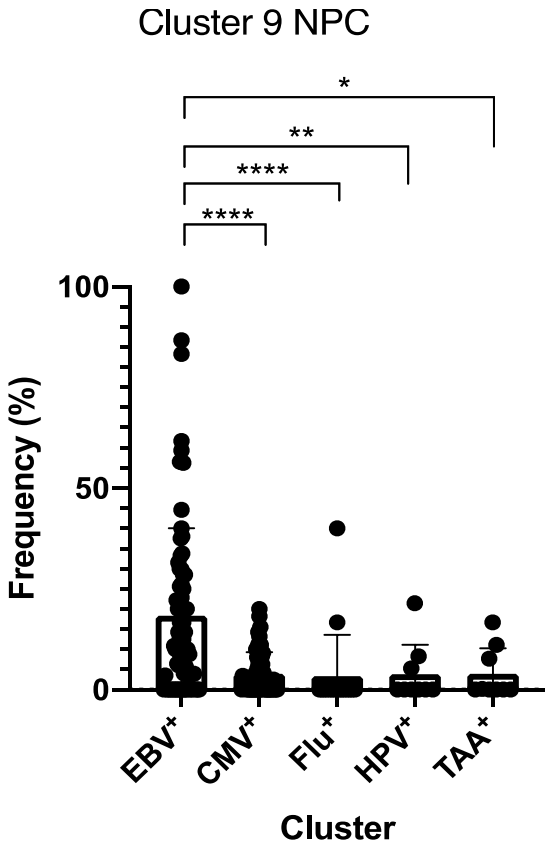


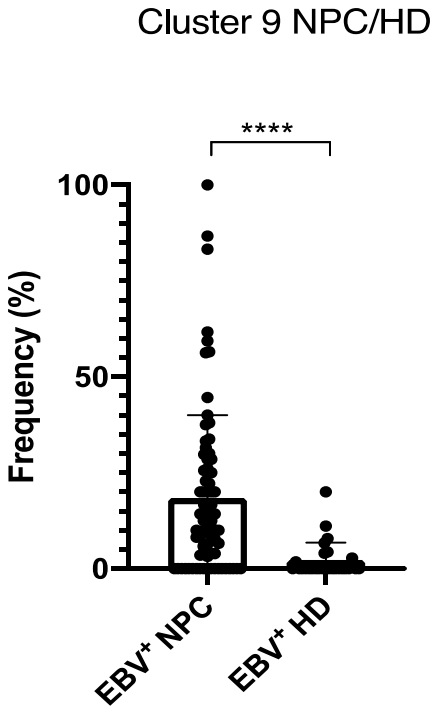
EBV/LMP-2



Nandita Kumar

Peripheral blood CD39^{+}CD103^{+} EBV-specific cells in NPC





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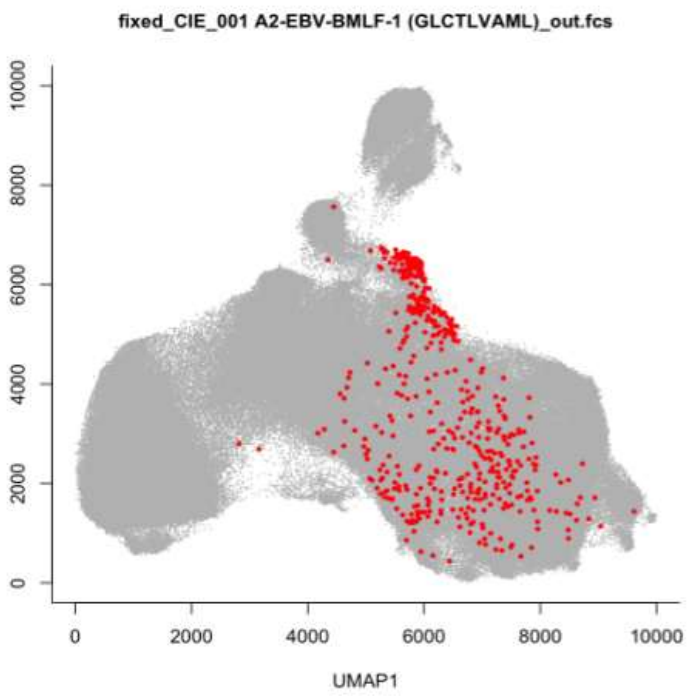
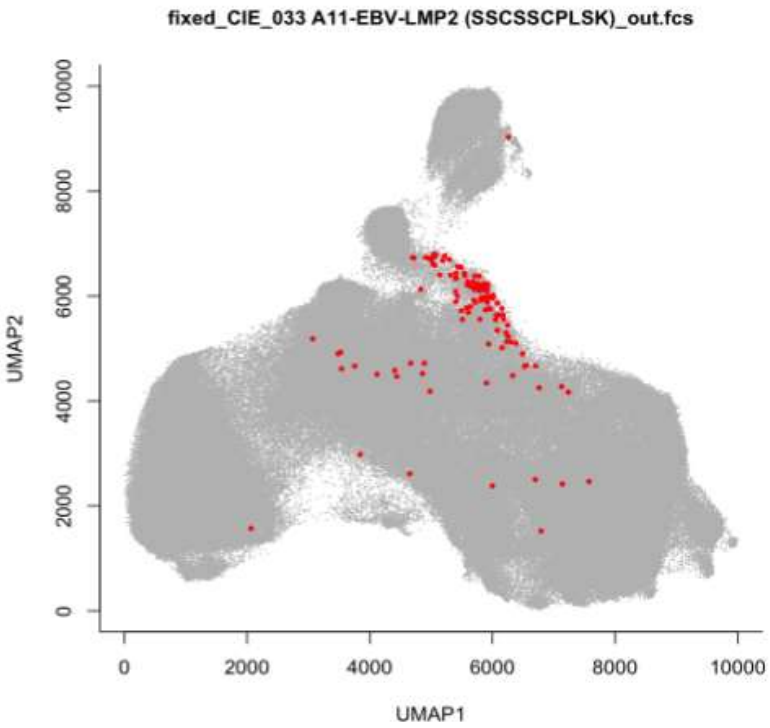
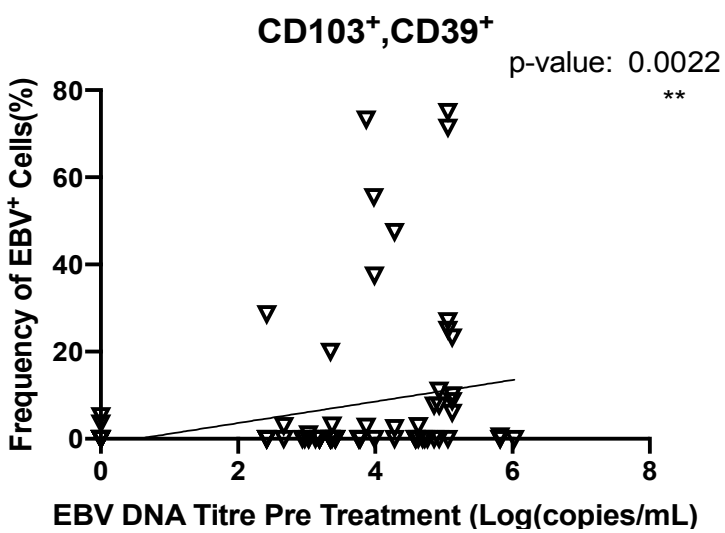


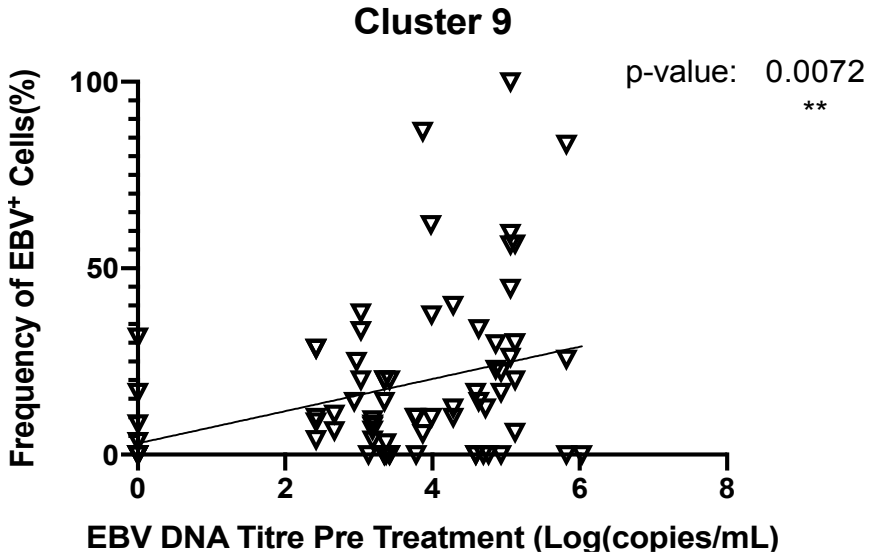
EBV/LMP-2

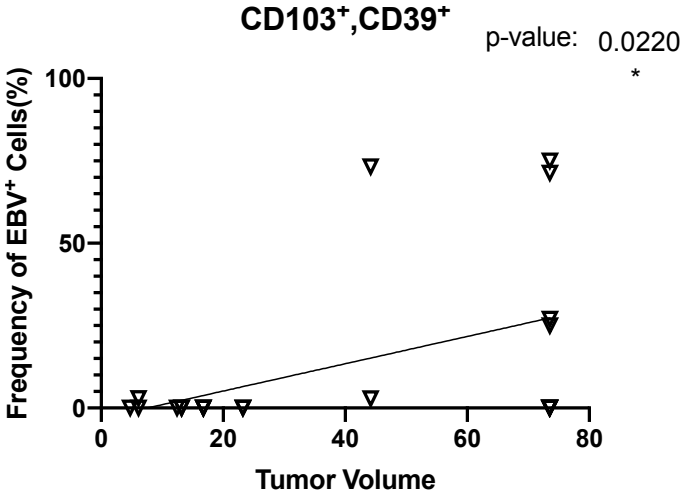


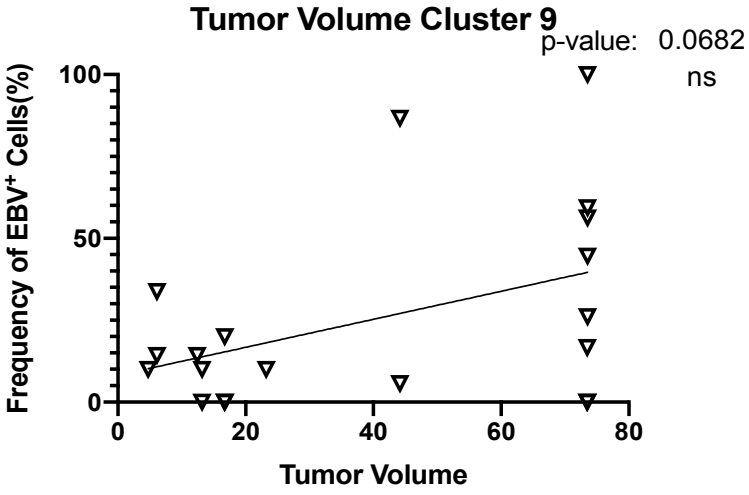
Nandita Kumar

Activated and exhaustion within EBV-specific cells correlates with disease burden



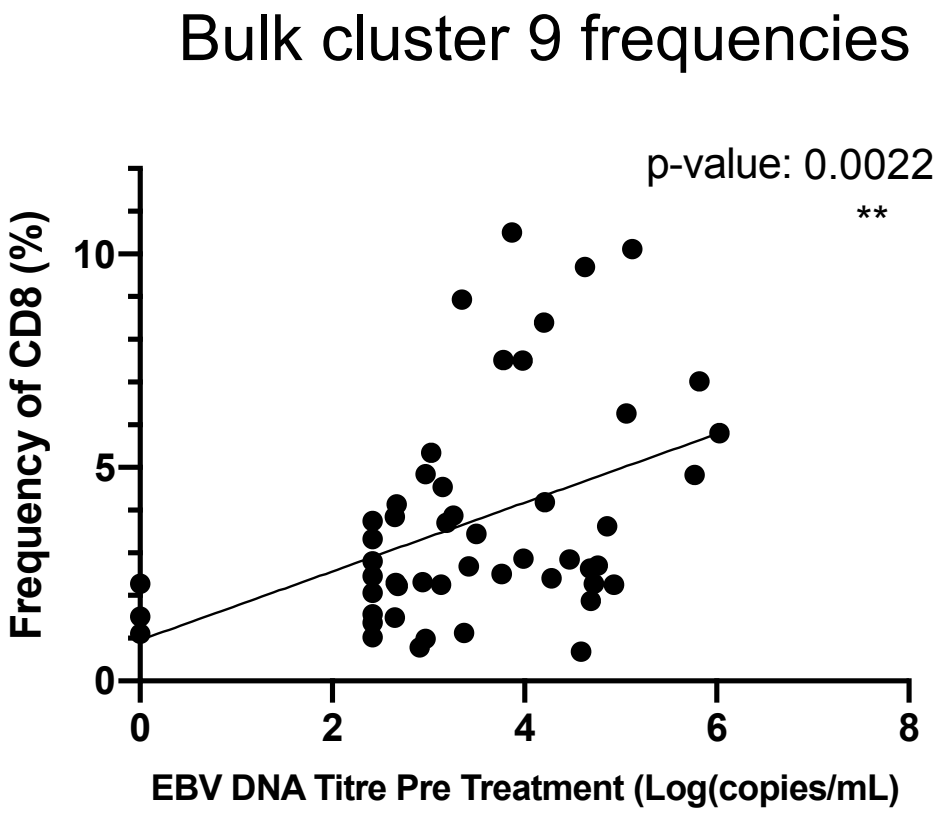


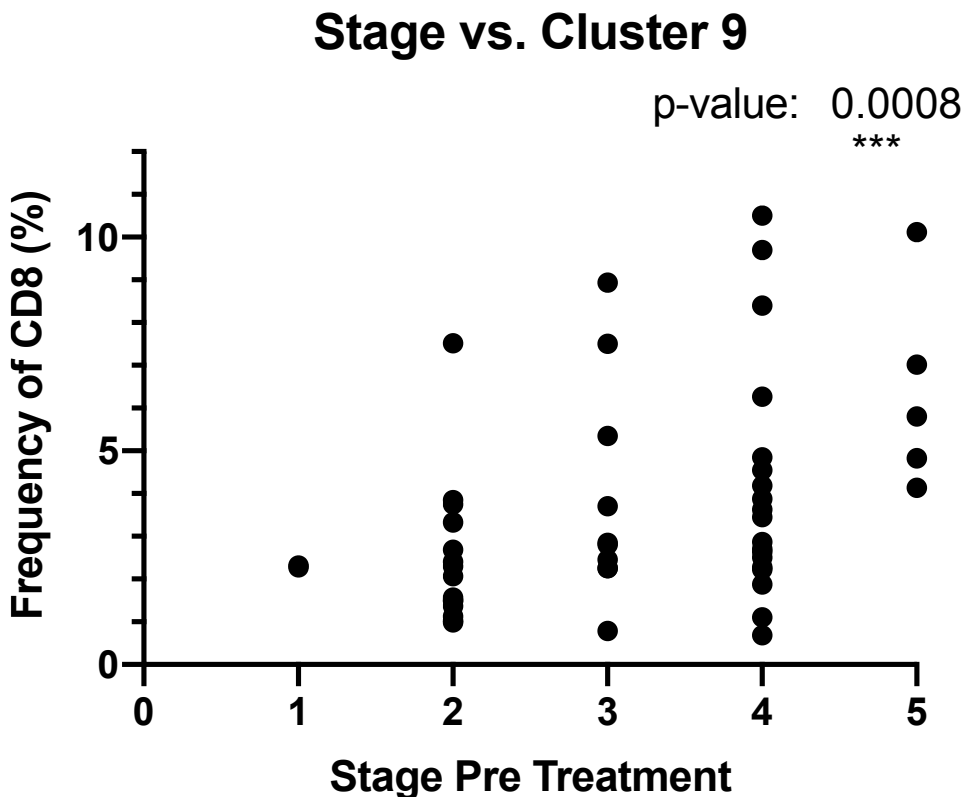




Nandita Kumar

Activated and exhaustion within EBV-specific cells correlates with disease burden





Nandita Kumar

Are CD39$^+$CD103$^+$ recirculating terminally exhausted cells?

CD39

CD39 Expression Identifies Terminally Exhausted CD8$^+$ T Cells

Prakash K. Gupta, Jernej Godec, David Wolski, Emily Adland, Kathleen Yates, Kristen E. Pauken, Cormac Cosgrove, Carola Ledderose, Wolfgang G. Junger, Simon C. Robson, E. John Wherry, Galit Alter, Philip J. R. Goulder, [...], W. Nicholas Haining [view all]

Published: October 20, 2015 • https://doi.org/10.1371/journal.ppat.1005177

Bystander CD8$^+$ T cells are abundant and phenotypically distinct in human tumour infiltrates

Yannick Simoni[1*], Etienne Becht[1], Michael Fehlings[1,2], Chiew Yee Loh[1], Si-Lin Koo[3], Karen Wei Weng Teng[1], Joe Poh Sheng Yeong[1,4], Rahul Nahar[3], Tong Zhang[3], Hassen Kared[1], Kaibo Duan[1], Nicholas Ang[1], Michael Poidinger[1], Yin Yeng Lee[3], Anis Larbi[1], Alexis J. Khng[5], Emile Tan[6], Cherylin Fu[6], Ronnie Mathew[6], Melissa Teo[7], Wan Teck Lim[3], Chee Keong Toh[3], Boon-Hean Ong[8], Tina Koh[7], Axel M. Hillmer[5], Angela Takano[4], Tony Kiat Hon Lim[4,5,9], Eng Huat Tan[3], Weiwei Zhai[5], Daniel S. W. Tan[3,5], Iain Beehuat Tan[3,5,9] & Evan W. Newell[1*]

ARTICLE

DOI: 10.1038/s41467-018-05072-0 OPEN

Co-expression of CD39 and CD103 identifies tumor-reactive CD8 T cells in human solid tumors

Thomas Duhen[1], Rebekka Duhen[2], Ryan Montler[1], Jake Moses[1], Tarsem Moudgil[2], Noel F. de Miranda[3], Cheri P. Goodall[2], Tiffany C. Blair[1], Bernard A. Fox[2], Jason E. McDermott[4], Shu-Ching Chang[5], Gary Grunkemeier[5], Rom Leidner[2], Richard Bryan Bell[2] & Andrew D. Weinberg[1,2]

CD103

Dietary gluten triggers concomitant activation of CD4$^+$ and CD8$^+$ αβ T cells and γδ T cells in celiac disease

Arnold Han[a,b], Evan W. Newell[b,c], Jacob Glanville[d], Nielsen Fernandez-Becker[a], Chaitan Khosla[e,f], Yueh-hsiu Chien[b,d], and Mark M. Davis[b,d,g,1]

SCIENCE IMMUNOLOGY | RESEARCH ARTICLE

T CELLS

Human CD4$^+$CD103$^+$ cutaneous resident memory T cells are found in the circulation of healthy individuals

Maria M. Klicznik[1*], Peter A. Morawski[2*], Barbara Höllbacher[1,2], Suraj R. Varkhande[1], Samantha J. Motley[2], Leticia Kuri-Cervantes[3], Eileen Goodwin[3], Michael D. Rosenblum[4], S. Alice Long[2], Gabriele Brachtl[5], Thomas Duhen[2], Michael R. Betts[3], Daniel J. Campbell[2,6†‡], Iris K. Gratz[1,2,7†‡]

Developmental plasticity allows outside-in immune responses by resident memory T cells

Raissa Fonseca[1,5,8], Lalit K. Beura[1,6,8], Clare F. Quarnstrom[1,8], Hazem E. Ghoneim[2,7], Yiping Fan[3], Caitlin C. Zebley[2], Milcah C. Scott[1], Nancy J. Fares-Frederickson[1], Sathi Wijeyesinghe[1], Emily A. Thompson[1], Henrique Borges da Silva[4], Vaiva Vezys[1], Benjamin Youngblood[2] and David Masopust[1]

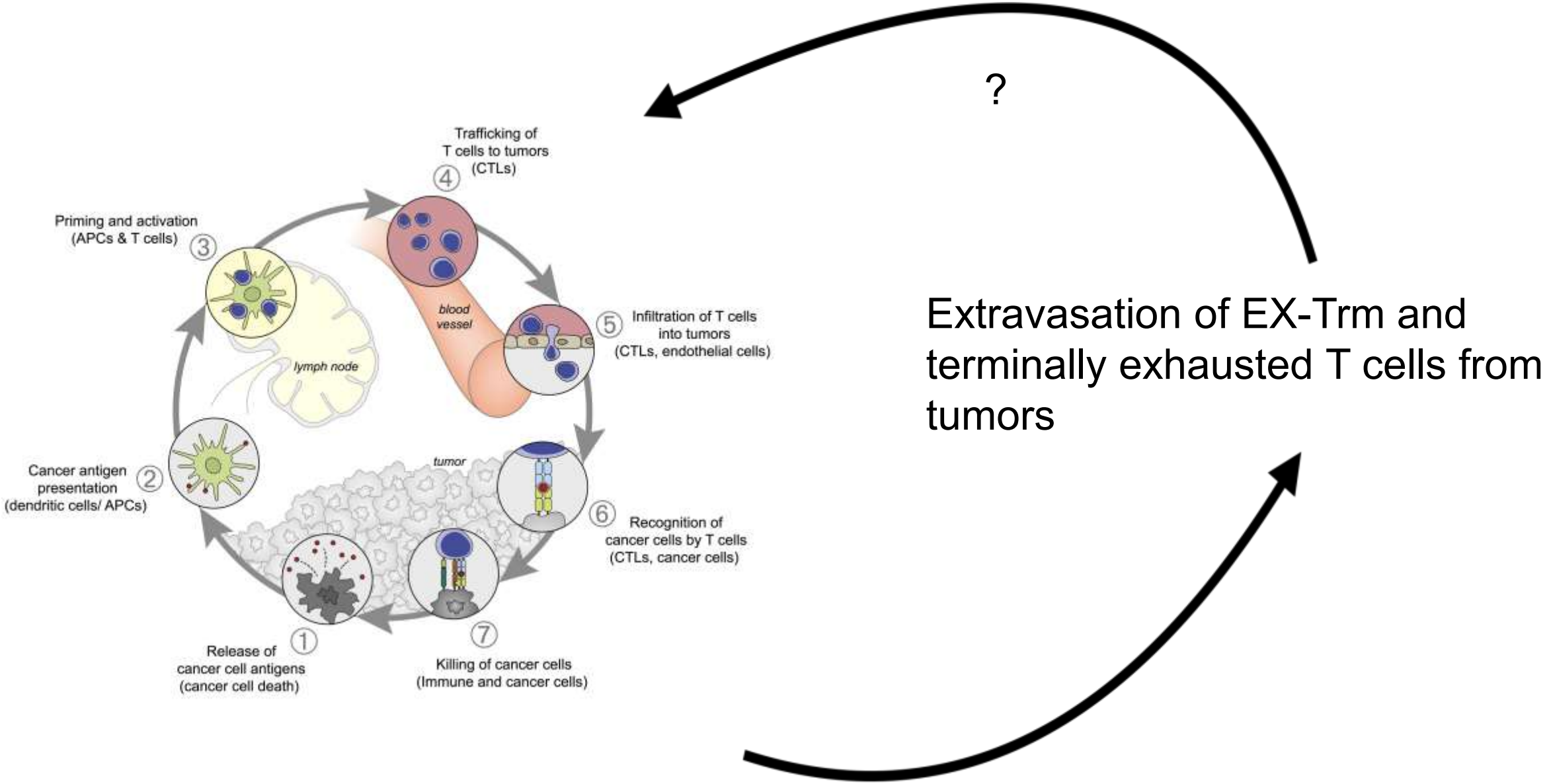
Article

Expansible residence decentralizes immune homeostasis

https://doi.org/10.1038/s41586-021-03351-3

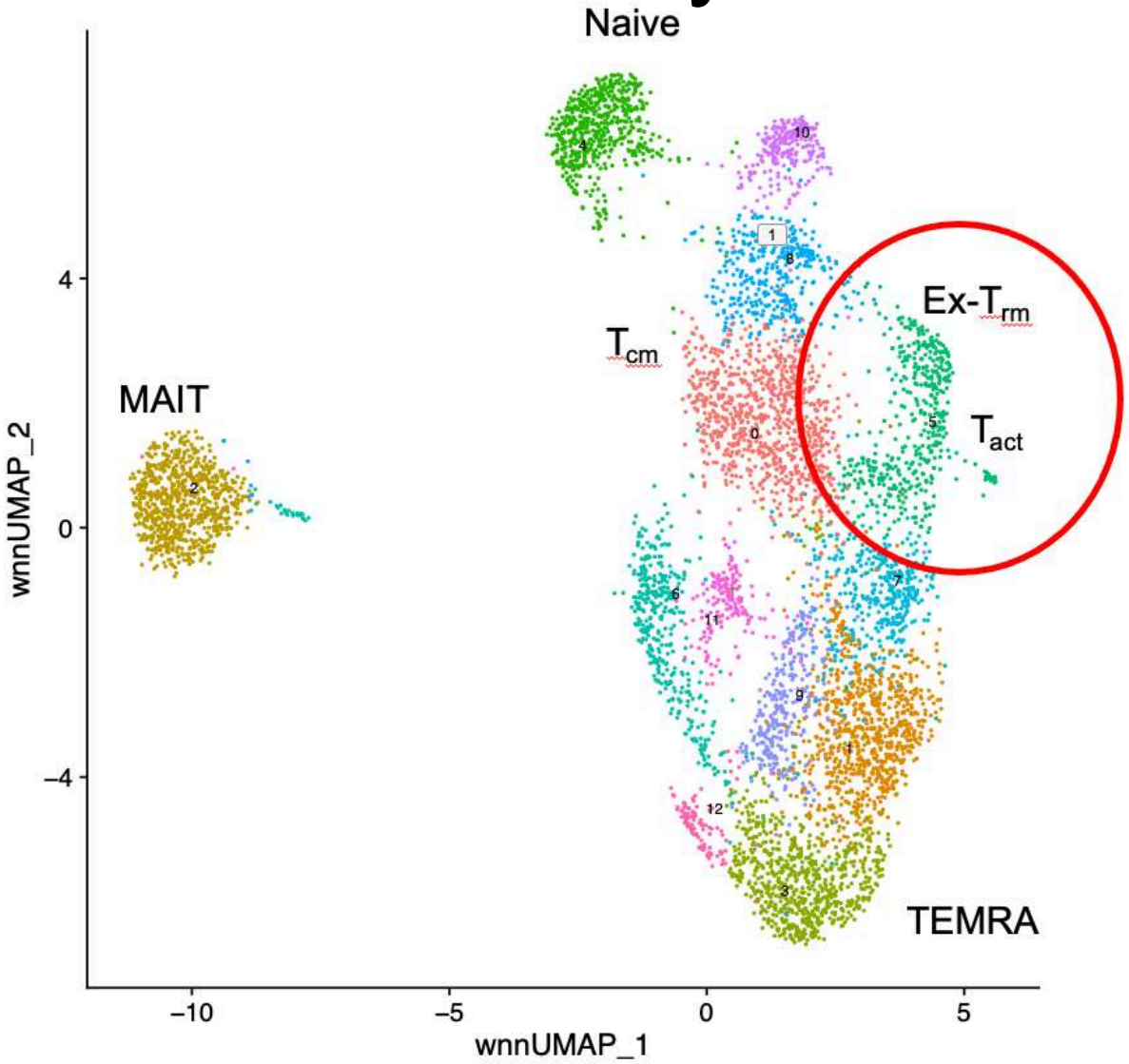
Received: 19 May 2020

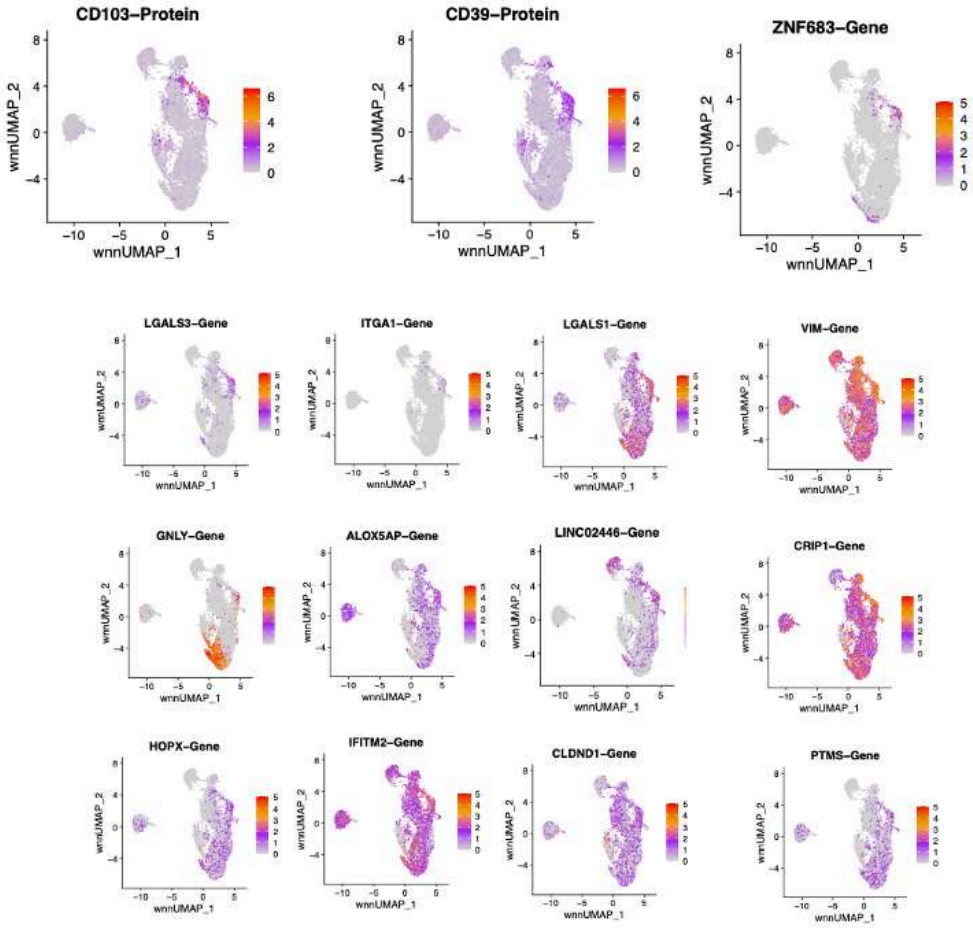
Sathi Wijeyesinghe[1], Lalit K. Beura[1,3], Mark J. Pierson[1], J. Michael Stolley[1], Omar A. Adam[1], Roland Ruscher[2,4], Elizabeth M. Steinert[1], Pamela C. Rosato[1,5], Vaiva Vezys[1] & David Masopust[1]



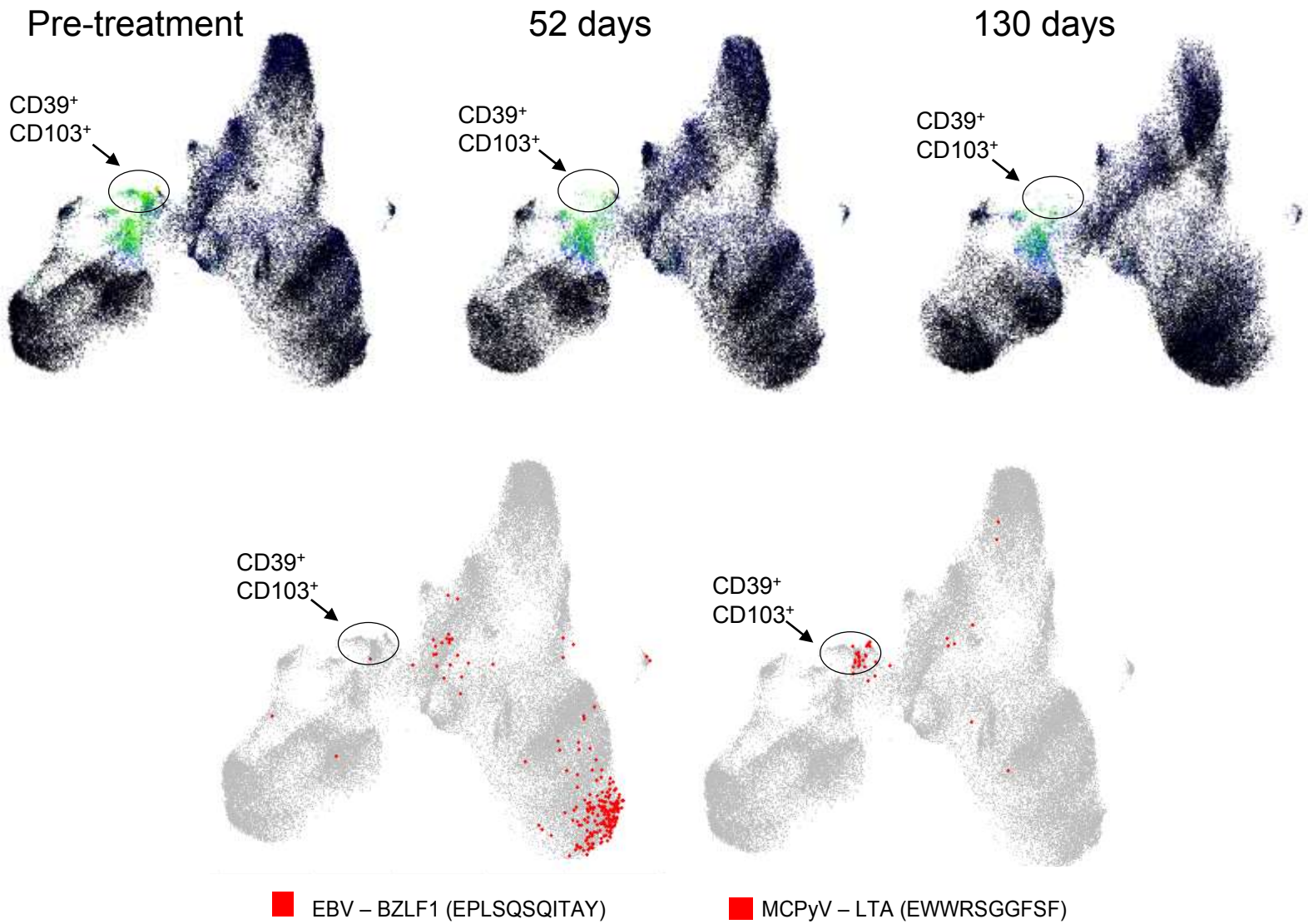
The Cancer Immunity Cycle
Chen and Melman

10x Data Analysis
Surface Antibody & Gene Expression UMAP





Similar for MCPyV-specific T cells in Merkel Cell Carcinoma? (complete responders to anti-PD-1)

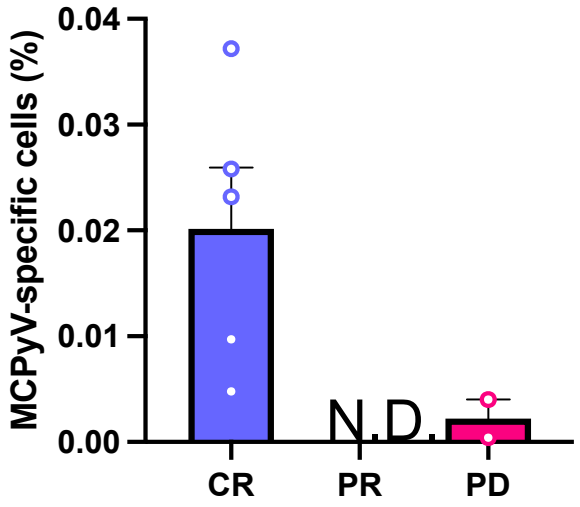


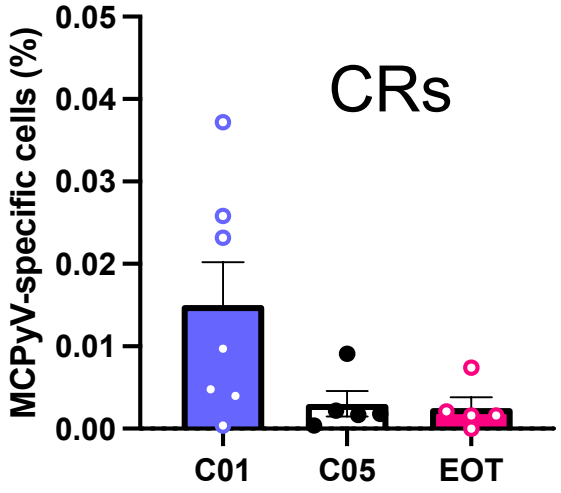
Paul Nghiem
Candice Church
Thomas Pulliam
Sine Hadrup
Ulla Hanen

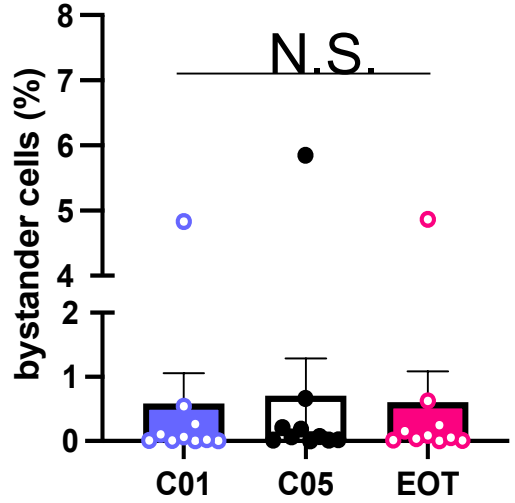
Heeju Ryu
Amy Codd
Korok Sarker
Timothy Bi

Preliminary correlations with response to anti-PD-1 therapy (CITN-09)

Frequencies of tetramer-positive cells

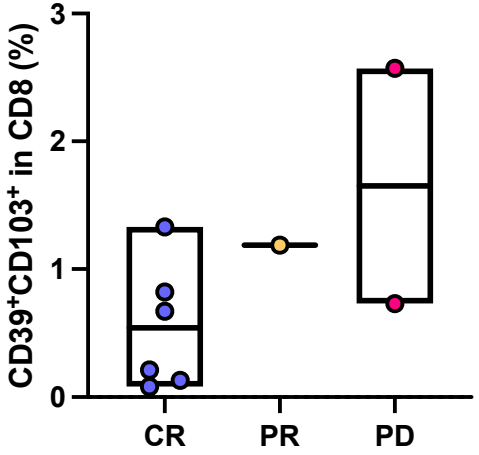




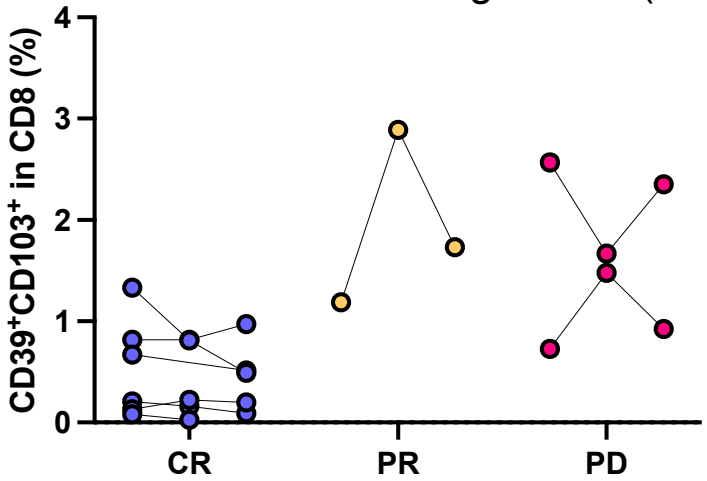


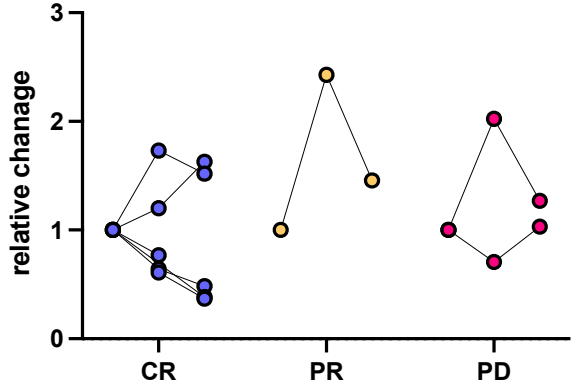
Analysis of CD39⁺CD103⁺ among total CD8

Baseline



Longitudinal (baseline, ~12 wks, EOT)





Overall summary

- Goal of using T cells as biomarkers for health and disease outcomes
- Application to vaccines and infectious diseases (Dengue, Covid, etc.)
- Insights from profiling unrelated antigen-specific T cells in tumor-infiltrates
  - Prevalence of CD39-negative bystander T cells in tumors
- Study of virally driven cancer to study peripheral profiles of tumor-specific T cells
  - Relevance of the $CD39^{+}CD103^{+}$ phenotype of blood CD8 T cells
  - Exhausted recirculating (ex-Trm) tumor-specific cells?
    - Biomarker of tumor burden * T cell exhaustion?
    - Source of tumor-specific cells / TCRs?
  - Ongoing efforts to test this hypothesis using paired PBMC and tumor tissue samples (NPC, Lung, Kidney)

Acknowledgments

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Yannick Simoni
Shamin Li
Nandita Kumar
Timothy Bi
Heeju Ryu
Rachael Parks

Laura Islas
Anthony Cessna
Nicholas Bradley
Tony Chour
Hugh MacMillan
Amy Codd
David Glass
Korok Sarker

Julie McElrath
Kristen Cohen
Steve De Rosa
Andrew Fiore-Gartland
Koshlan Mayer-Blackwell
Maria Lemos
Zoe Moodie
Lemar Fleming

National Cancer Centre Singapore
SingHealth

NPC
Amit Jain
Darren Lim
Joe Yeong

A*STAR Singapore Immunology Network

Katja Fink

All past lab members
Yang Cheng

UW Medicine

RCC
nwbiospecimen

MCC
Paul Nghiem
Candice Church

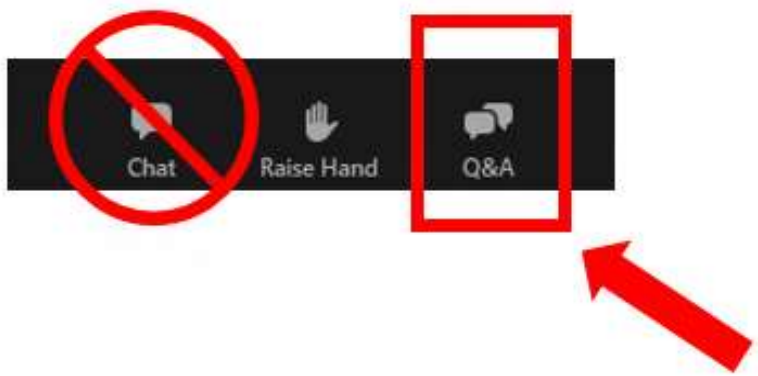
DTU

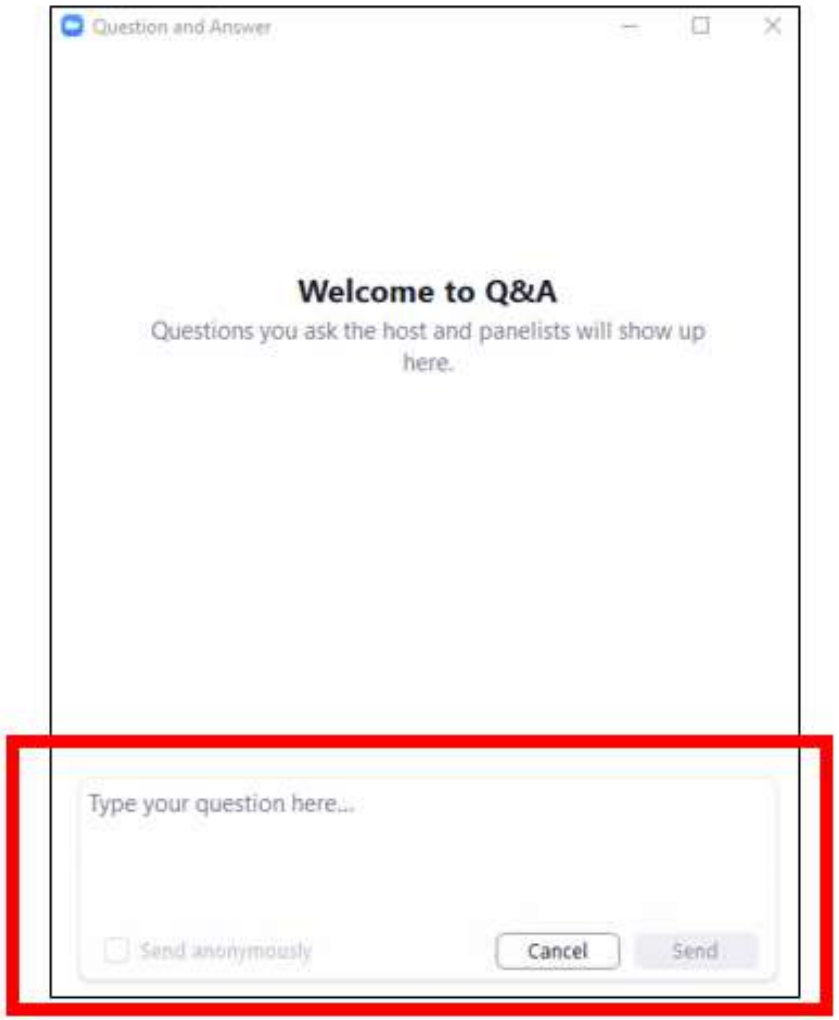
Sine Reker Hadrup
Ulla Kring Hanen

How to Submit Questions

Society for Immunotherapy of Cancer

- Click the **"Q&A"** icon located on at the bottom of your Zoom control panel
- Type your question in the Q&A box, then click "Send"
- Questions will be answered:
  a. after each speaker's presentation
  b. in the Question & Answer session at the end of the seminar





Webinar Outline

- Dr. Ananda W. Goldrath: Transcriptional side of T cell functional states; residency program; biomarkers
- Dr. Evan W. Newell: Cytometry; residency program (circulation and exhaustion)
- Dr. Daniela S. Thommen: Functional side; TLS (background, definition and function)

- Q&A: Dr. Wherry + Dr. Schietinger
  - Naming structures/terms/nomenclature
  - Translating t cell therapies into the clinic

Dissecting and modulating T cell function in human cancer

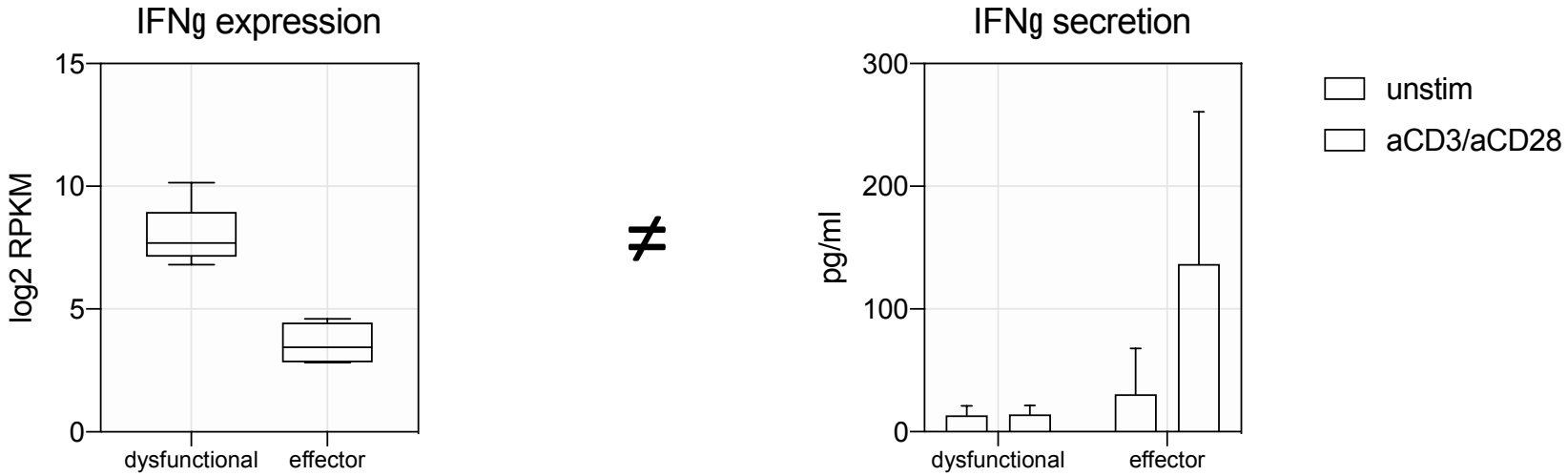
Daniela Thommen

The Netherlands Cancer Institute, Amsterdam

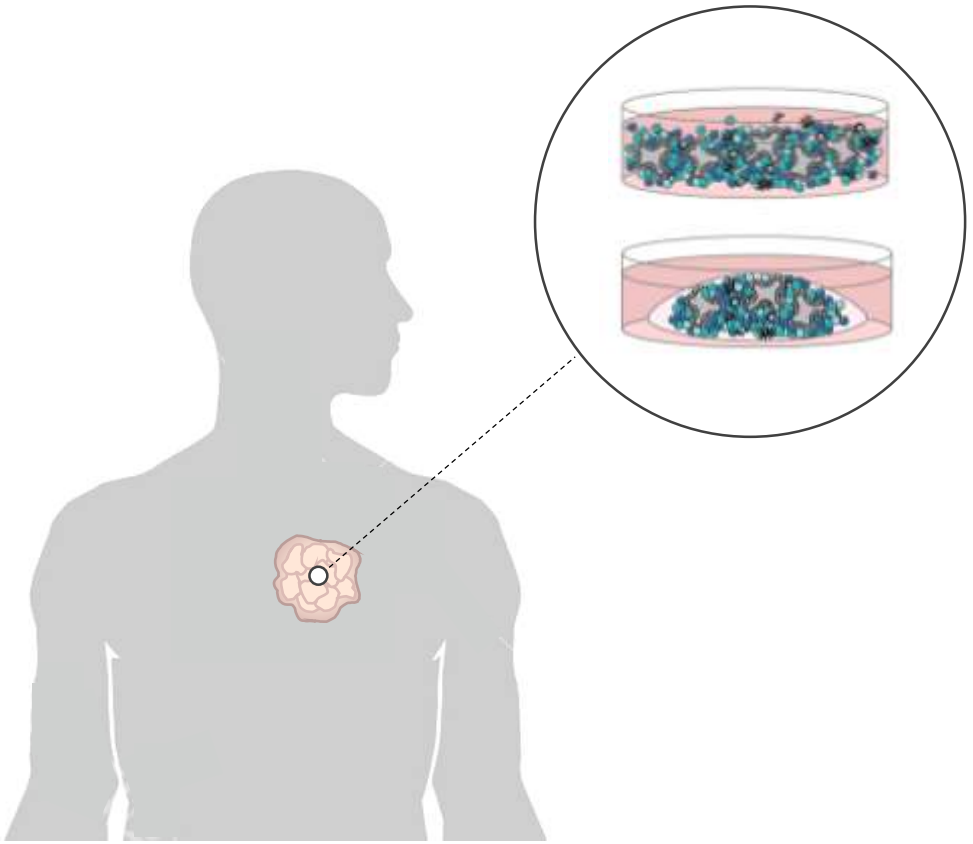
Content

- (Dys-)Function of T cells in the human tumor microenvironment
- Reactivation of T cell function by immune checkpoint blockade
- Impact of location and context on T cell function

Why study T cell function?



Gene expression and function do not always correlate



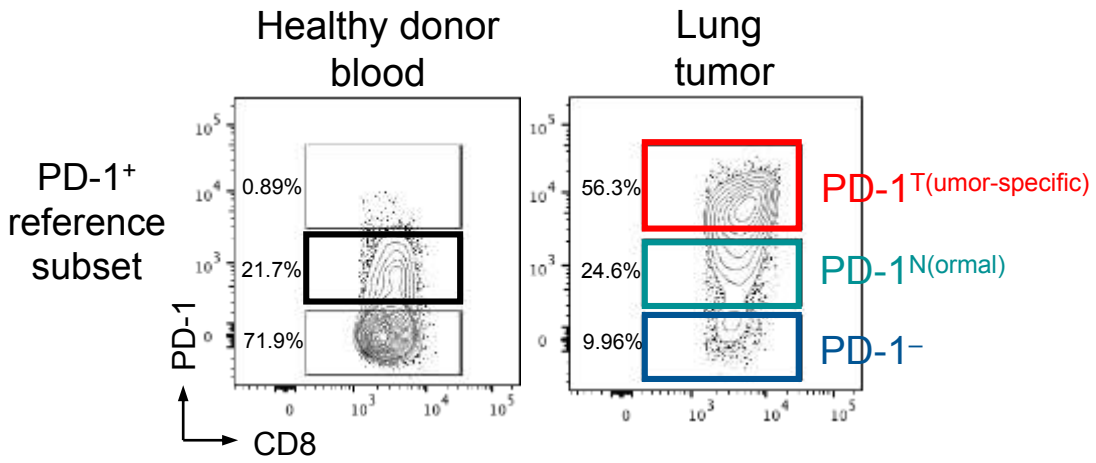
Human ex vivo models

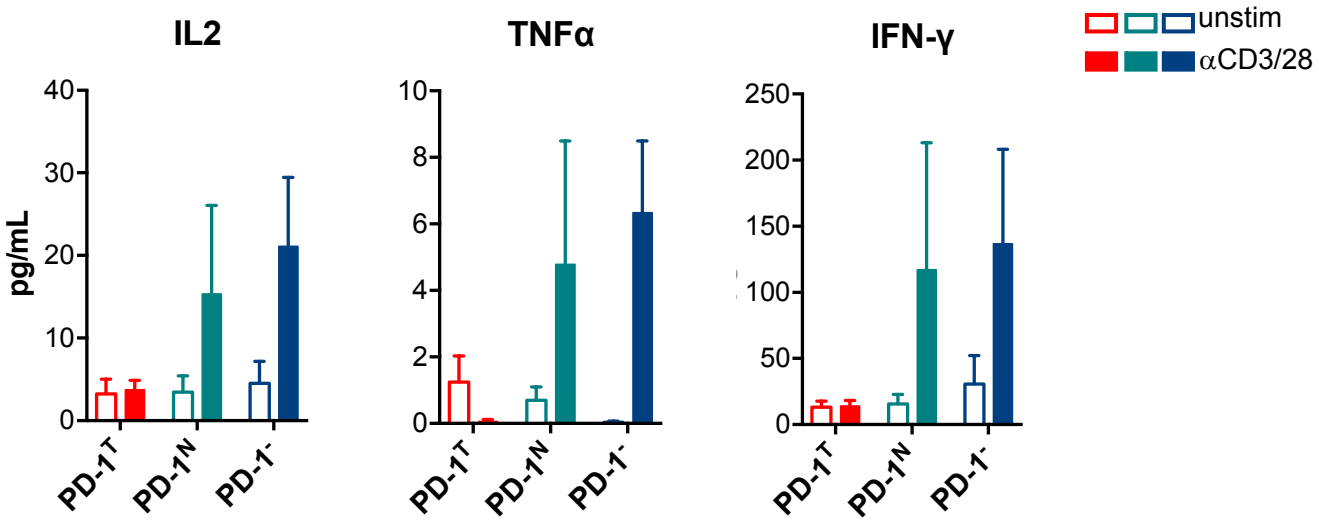
- Based on patient-derived material
- Possibility to perturb cell function

Challenges for studying T cell (dys)function in human cancer

- Intratumoral T cell pool is heterogenous
- Specificities of tumor-reactive T cells are often unknown
- Separation of tumor-specific and bystander T cells is difficult

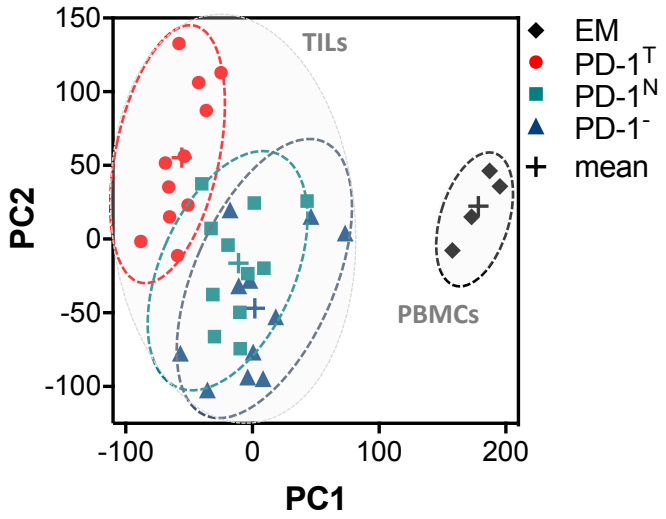
PD-1^{T} TILs are impaired in classical effector cytokine secretion

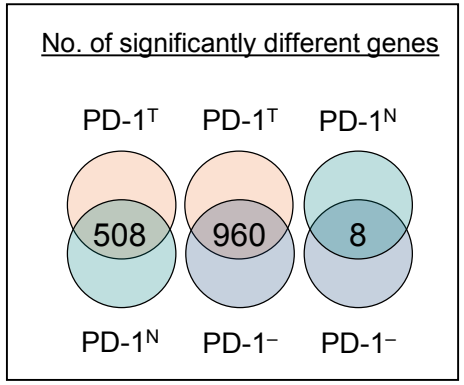


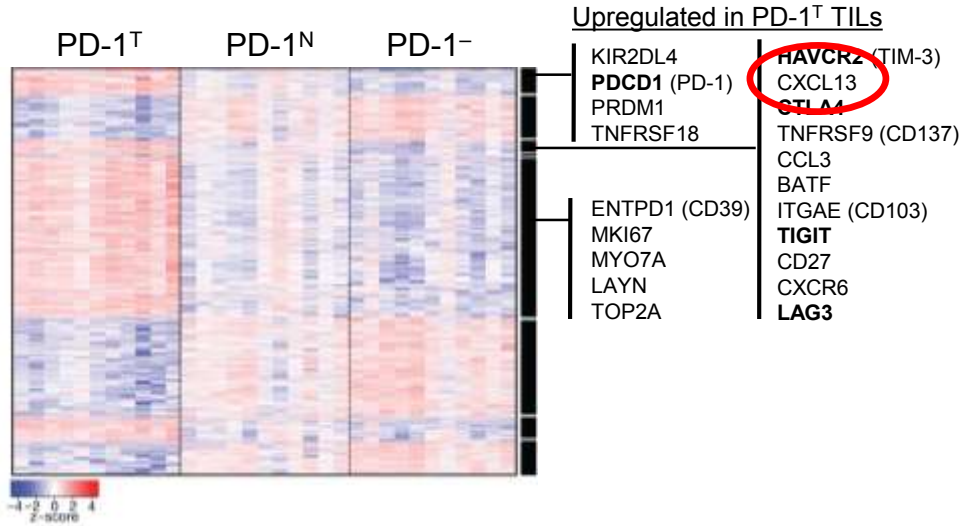


Thommen et al, Nat Med, 2018

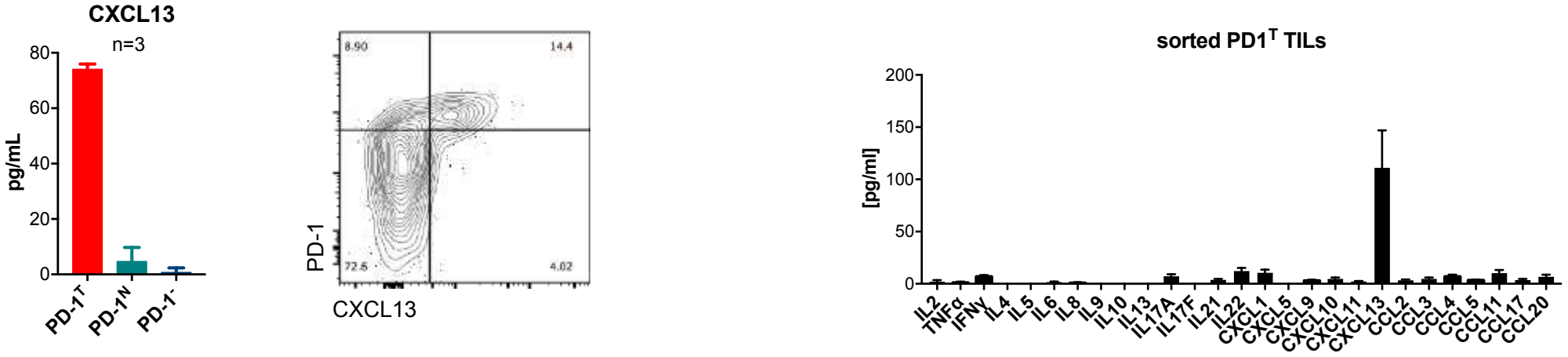
PD-1^{T} TILs are transcriptionally distinct from other TIL subsets







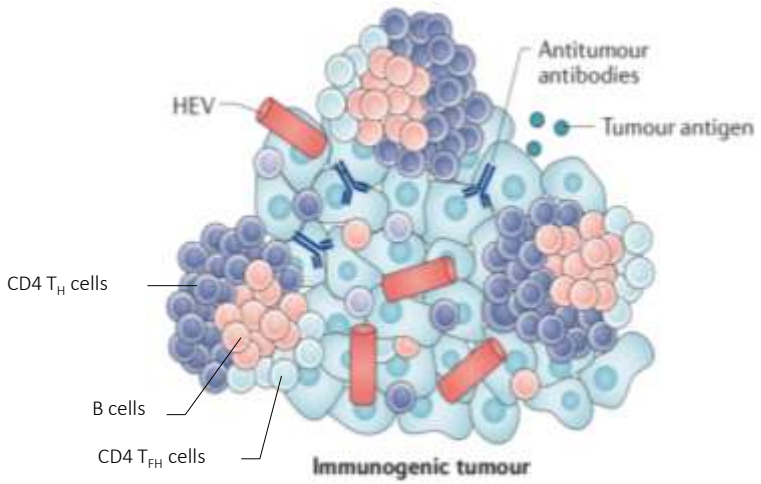
PD-1^{T} TILs acquire a new function in the tumor microenvironment



PD-1^{T} TILs are not functionless, but display an **altered function** in the TME

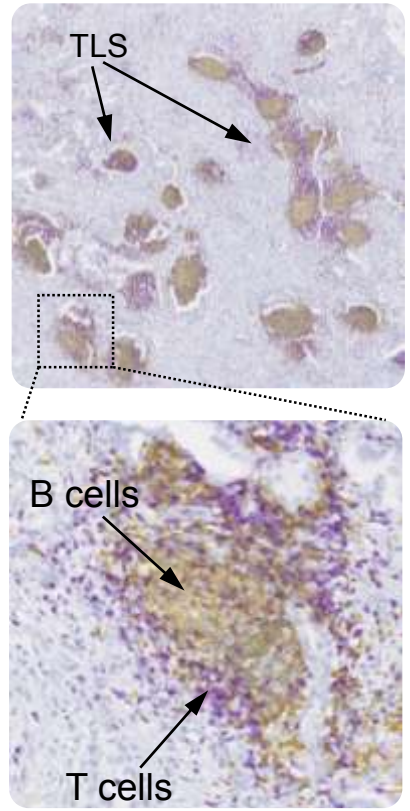
Tertiary lymphoid structures are immune hotspots in chronically inflamed tissues

CXCL13 is crucial for the formation and maintenance of lymph follicles



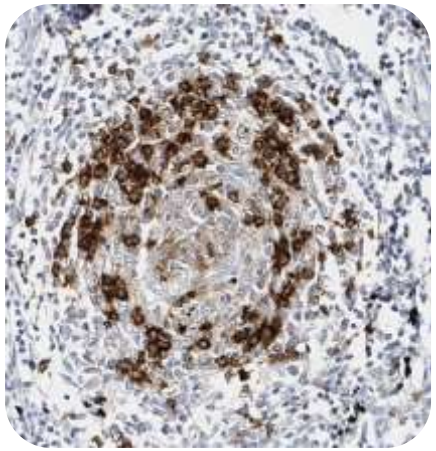
Tertiary lymphoid structures (TLS):

- Lymphoid aggregates at the tumor site
- Promote immune cell recruitment and local immune cell activation *(Sautes-Fridman, Nat Rev Cancer, 2019)*
- Associated with response to immunotherapy in melanoma, renal cell carcinoma, sarcoma *(Helmink, Nature, 2020; Cabrita, Nature, 2020; Petitprez, Nature, 2020)*

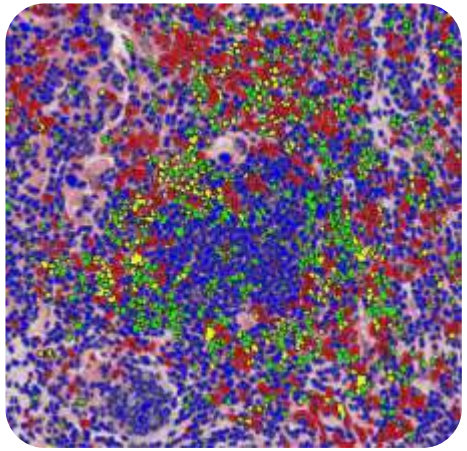


CD20 CD3

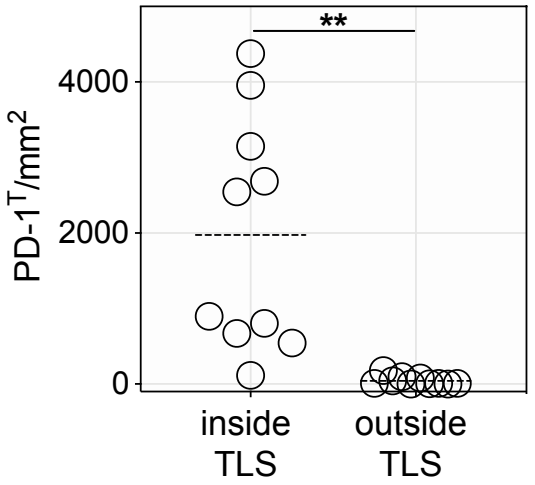
PD-1^{T} TILs predominantly localize within tertiary lymphoid structures



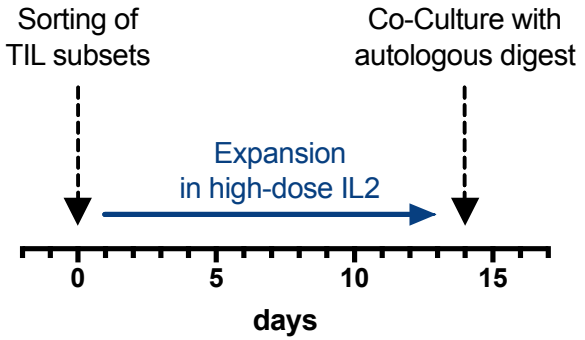
stained for PD-1

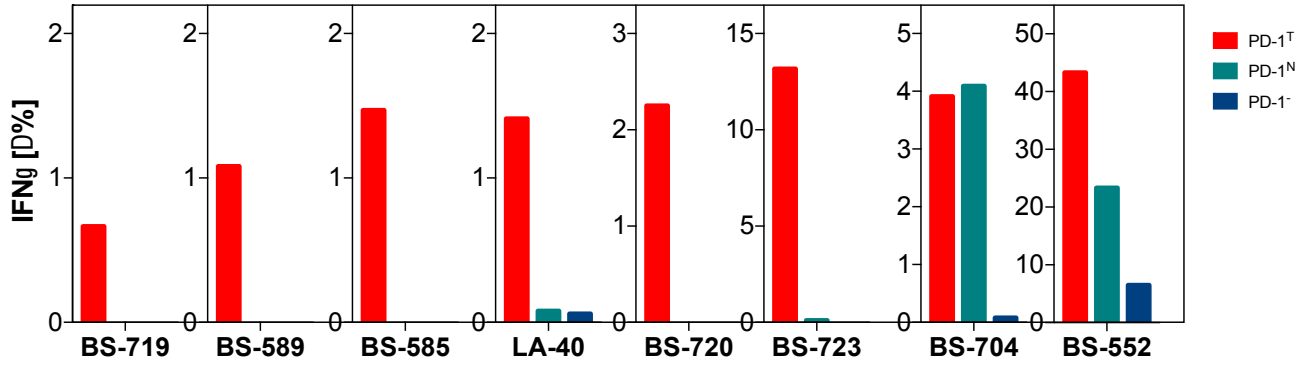


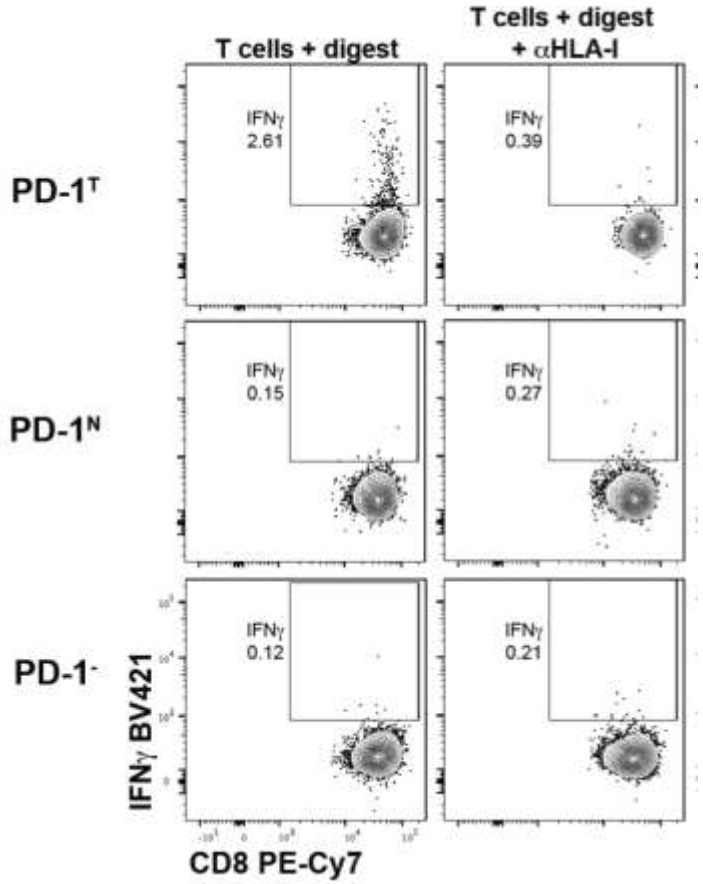
CD8 PD-1^{T} CD8/PD-1^{T} neg



PD-1^{T} TILs have an increased capacity for tumor recognition

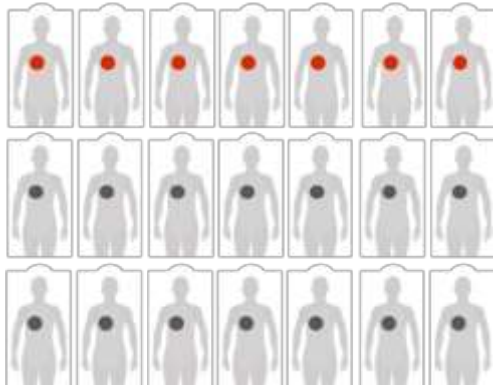




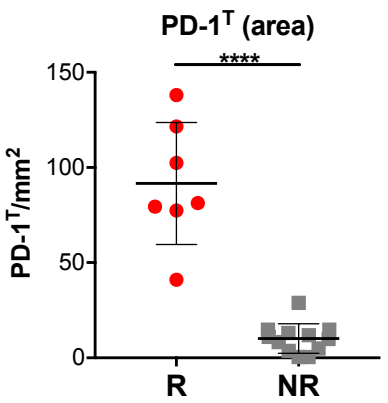


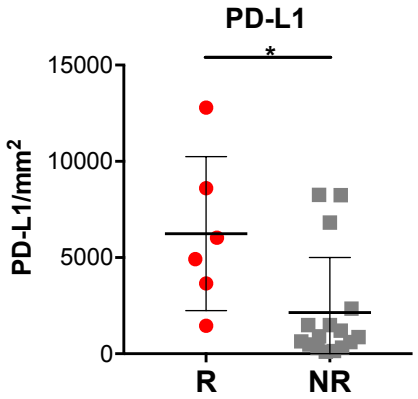
PD-1^{T} TILs correlate with response to PD-1 blockade

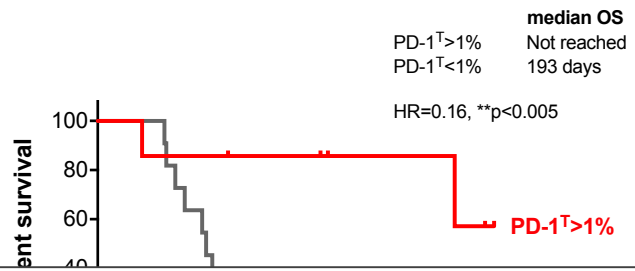
NSCLC pilot cohort



- 21 patients treated with anti-PD-1 therapy
- Stage IV NSCLC
- No
- Pat





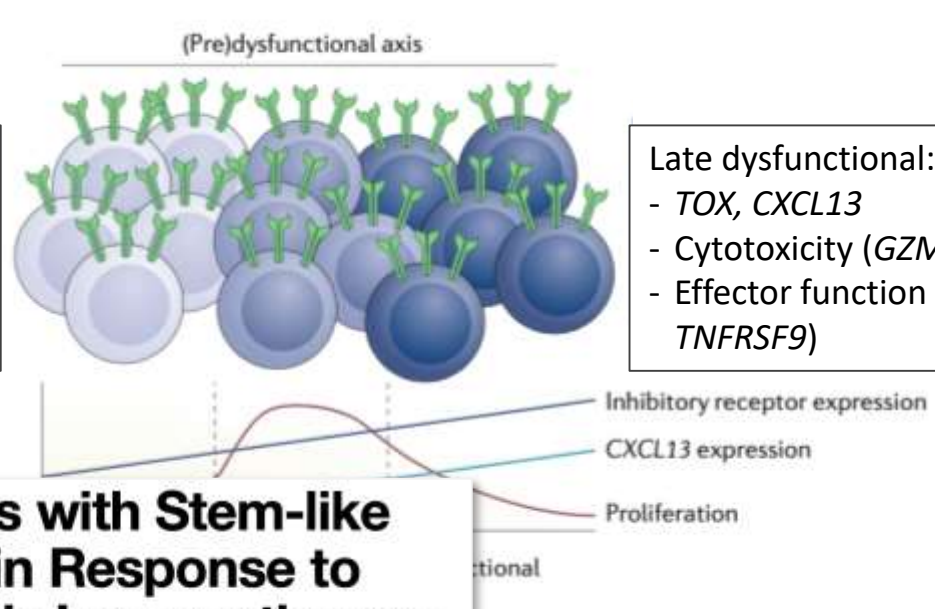


It is unclear whether PD-1^{T} TILs can be reactivated by PD-1 blockade.
However, they might be an indicator for the presence of a tumor-specific T cell pool.

Thommen et al, Nat Med, 2018

Which T cell subsets are relevant for anti-tumor immunity?

Pre-/early dysfunctional:
- *TCF7, GZMK*
- Proliferation (Cell cycle genes, *MKI67)*
- Stem-like properties



Late dysfunctional:
- *TOX, CXCL13*
- Cytotoxicity (*GZMB)*
- Effector function (*IFNG, TNFRSF9*)

Intratumoral Tcf1$^+$PD-1$^+$CD8$^+$ T Cells with Stem-like Properties Promote Tumor Control in Response to Vaccination and Checkpoint Blockade Immunotherapy

Imran Siddiqui,[1] Karin Schaeuble,[1] Vijaykumar Chennupati,[1,6] Silvia A. Fuertes Marraco,[1] Sandra Calderon-Copete,[2] Daniela Pais Ferreira,[1] Santiago J. Carmona,[1,3,4] Leonardo Scarpellino,[5] David Gfeller,[1,3,4] Sylvain Pradervand,[2] Sanjiv A. Luther,[5] Daniel E. Speiser,[1] and Werner Held[1,7,*]

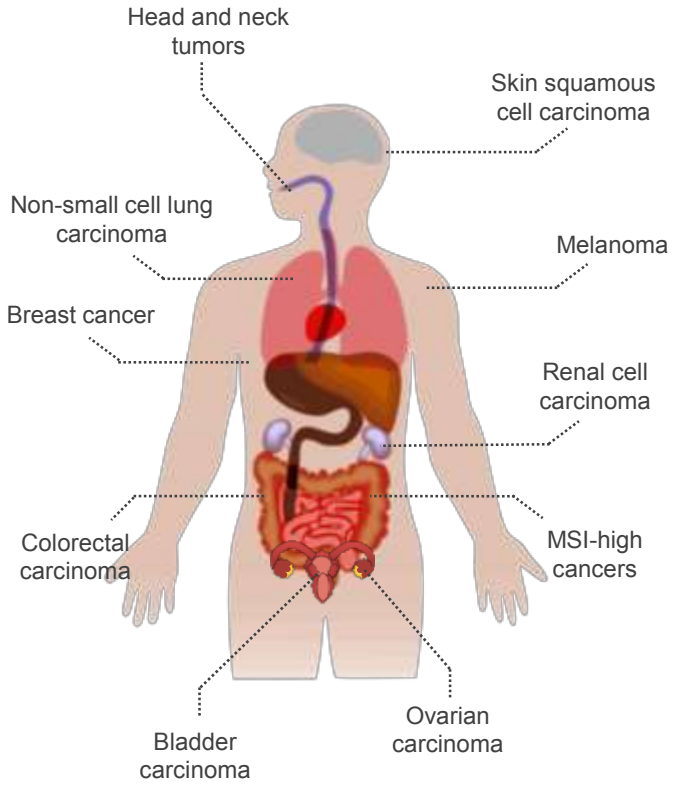
Siddiqui et al, Immunity, 2019; Kurtulus et al, Immunity, 2019; Miller et al, Nat Immunol, 2019

Adapted from van der Leun, NRC, 2020

Patient-derived tumor fragment platform

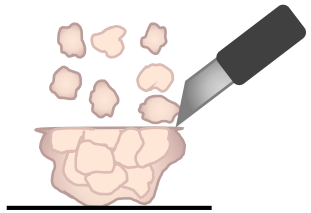
Society for Immunotherapy of Cancer

Tumor tissue collection

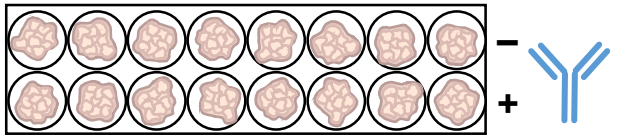


Tumor samples n=425, Fragments n~70,000

Dissection into Tumor fragments (PDTFs)



- 1x1x1mm tissue fragments
- Preservation of cellular composition & architecture



- Comparison of multiple treatments in the same tumor
- Possibility to perturb

Ex vivo immunotherapy treatment

Tumor microenvironment characteristics

Cellular composition
Flow cytometry

Tumor architecture
IHC, Digital pathology

T cell phenotype
Flow cytometry

Soluble mediators
Cytokine/chemokine analysis

Tumor reactivity
Functional assays

Immune cell activation
Flow cytometry

Soluble mediators
Multiplex cytokine/chemokine analysis

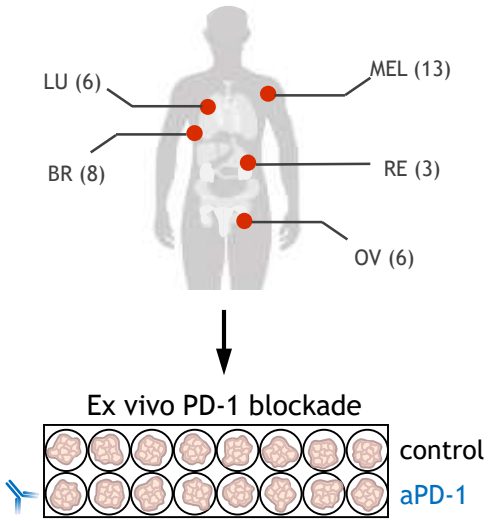
Cytotoxicity
Analysis of soluble cytotoxic markers

Single cell kinetics
Single cell RNA sequencing

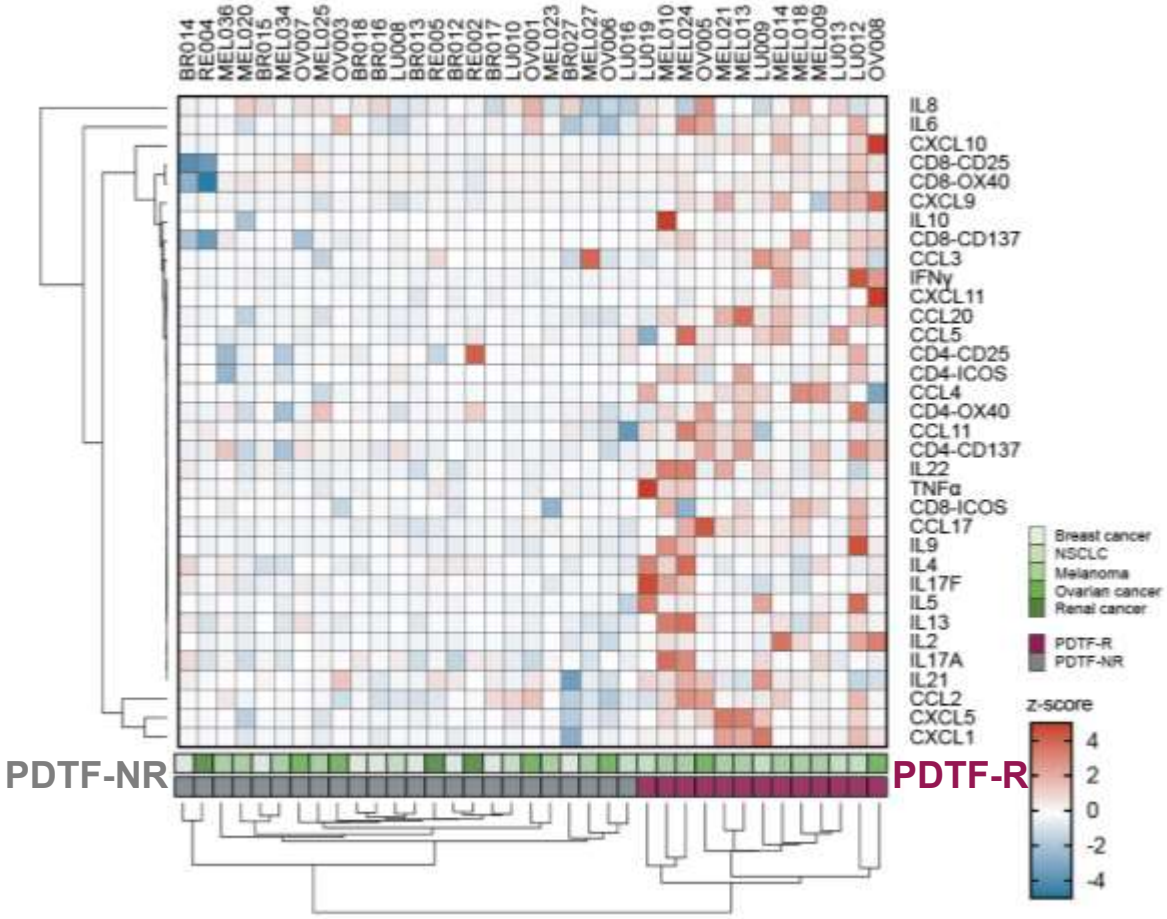
Spatio-temporal changes
Multiplex imaging

Visualization of treatment-induced changes

Immunological response to PD-1 blockade in PDTFs

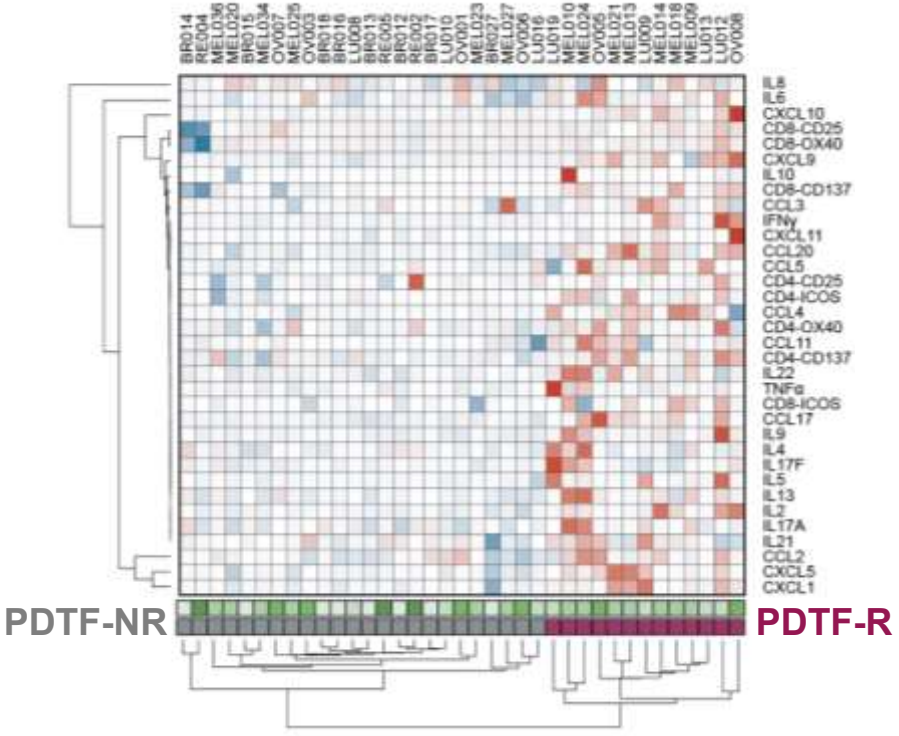


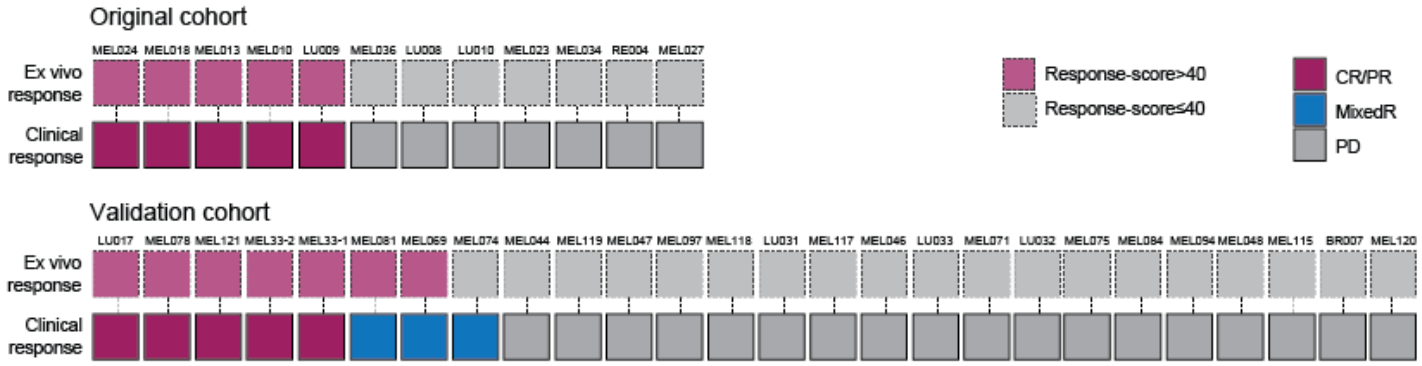
Readouts (after 48 hrs):
- 4 T cell activation markers
- 27 cytokines and chemokines



Voabil, de Bruijn, Roelofsen, et al, Nat Med, 2021

Can the capacity of intratumoral immune cells to be reactivated by PD-1 blockade predict the capacity for clinical response?

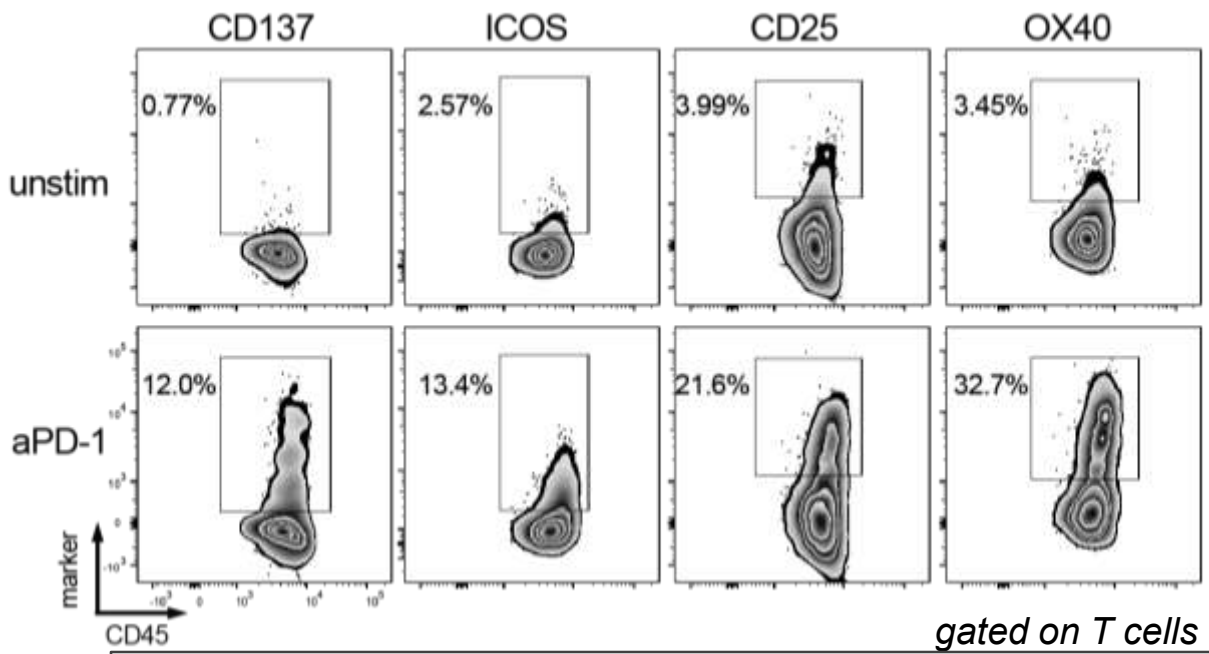




Voabil, de Bruijn, Roelofsen, et al, Nat Med, 2021

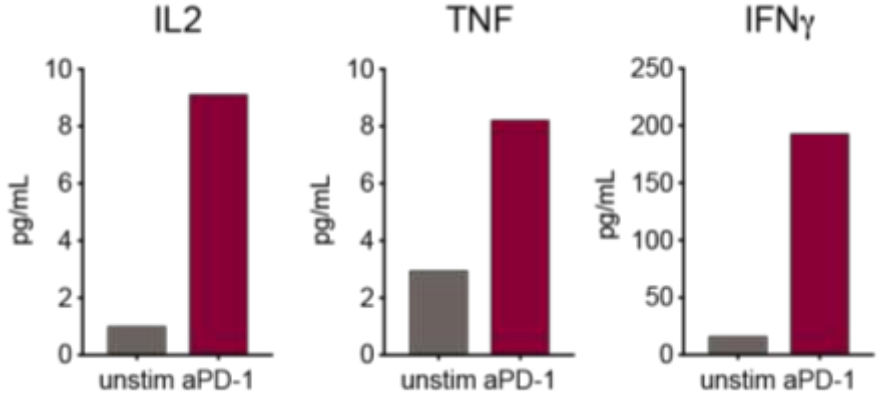
Activation of intratumoral T cells by PD-1 blockade

T cell activation

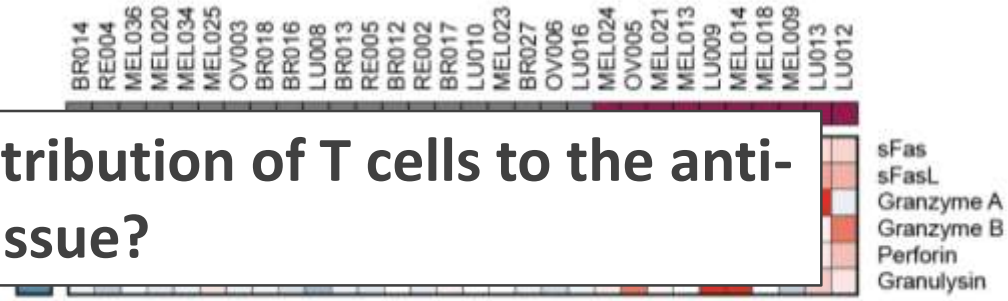


gated on T cells

Effector cytokine secretion

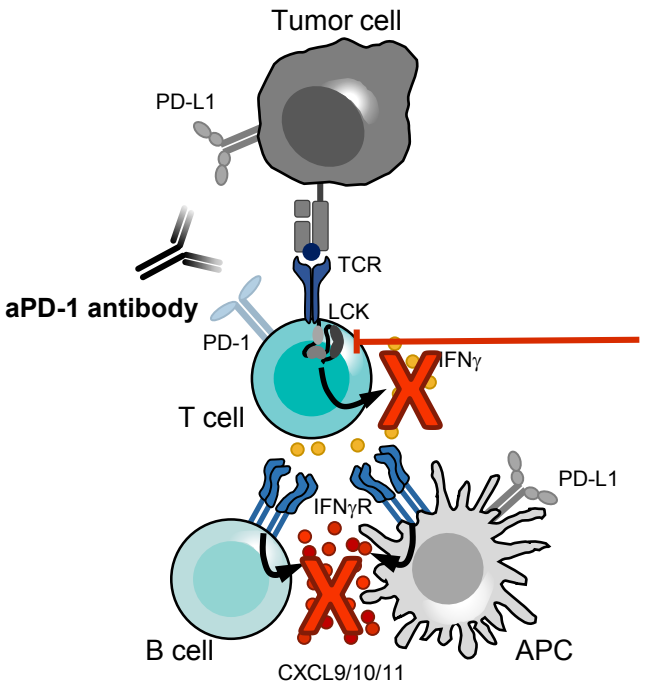


Cytotoxicity



Can we perturb the system to directly test the contribution of T cells to the anti-PD-1 response in cancer tissue?

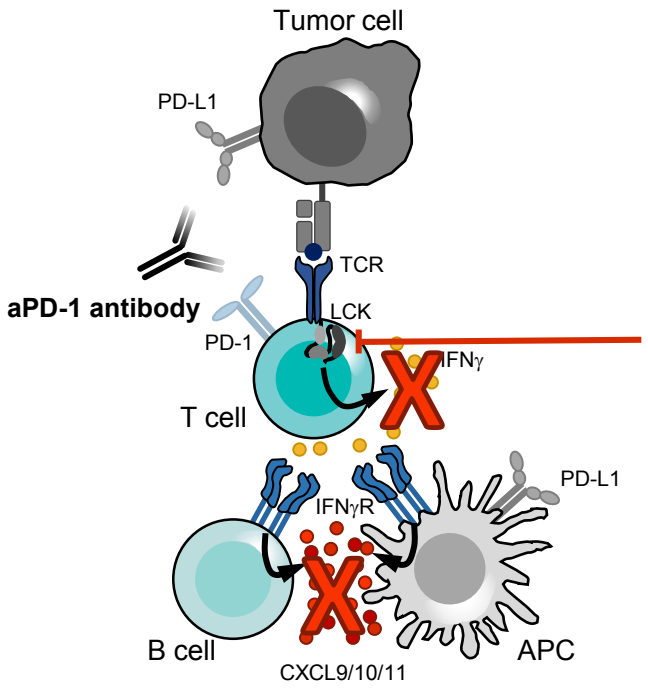
Contribution of intratumoral T cells to the immunological response upon aPD-1



Inhibition of TCR signaling

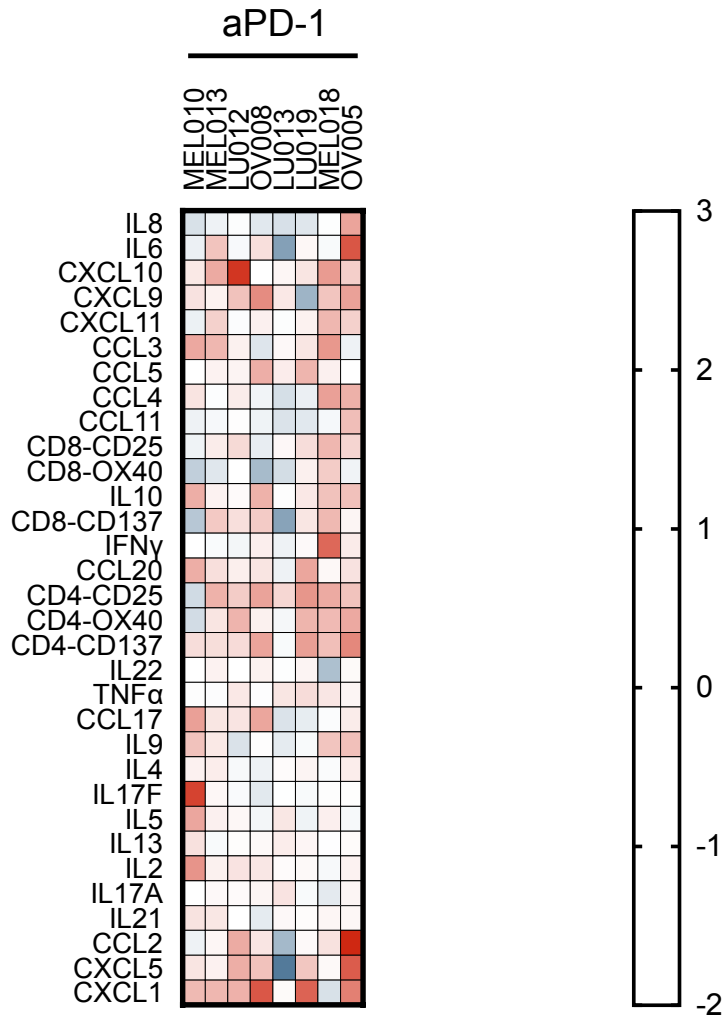
LCK inhibitor

Contribution of intratumoral T cells to the immunological response upon aPD-1

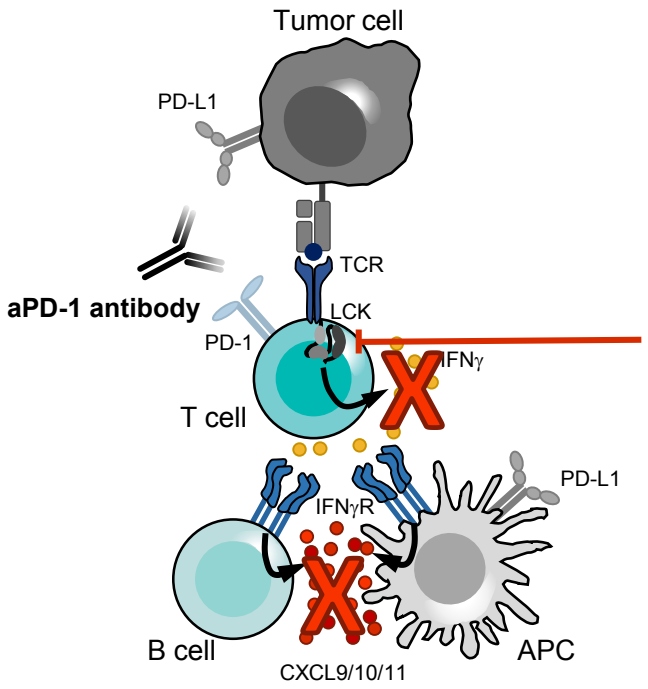


Inhibition of TCR signaling

LCK inhibitor

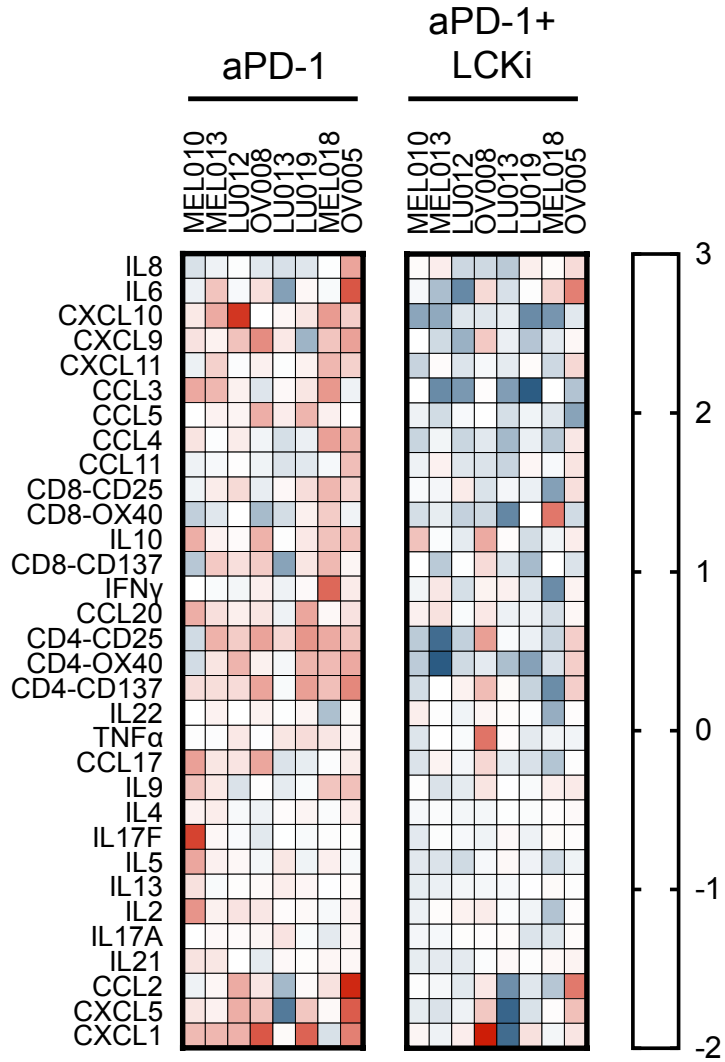


Contribution of intratumoral T cells to the immunological response upon aPD-1

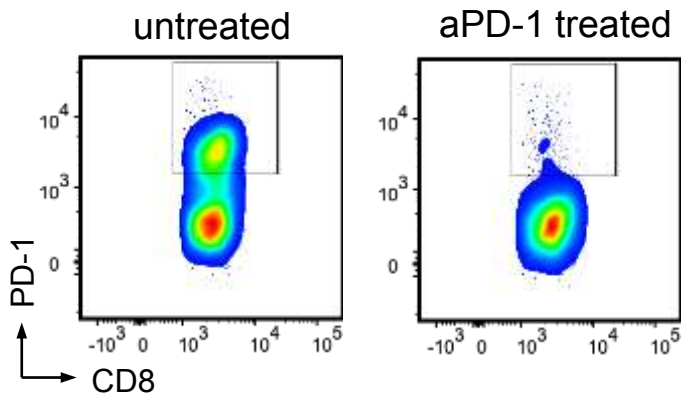


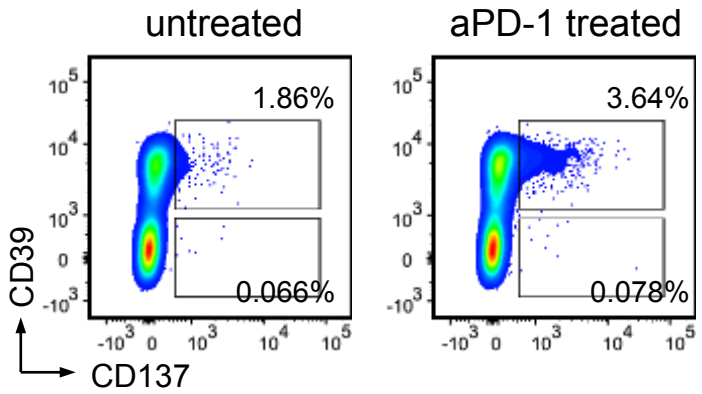
Inhibition of TCR signaling

LCK inhibitor

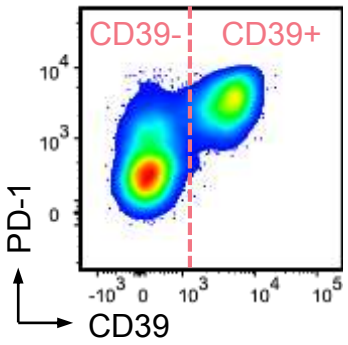


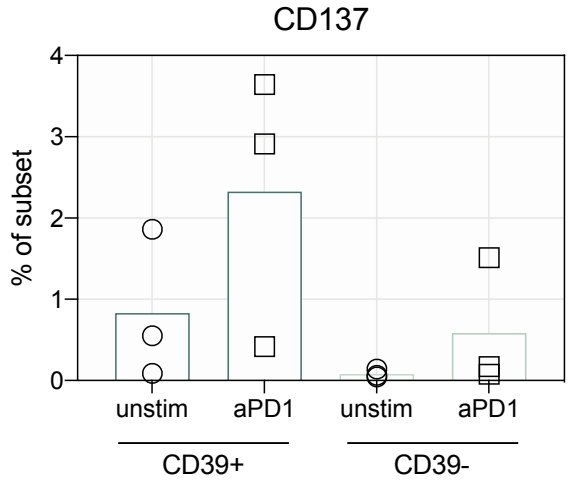
PD-1 blockade reactivates late-dysfunctional cells at the tumor site

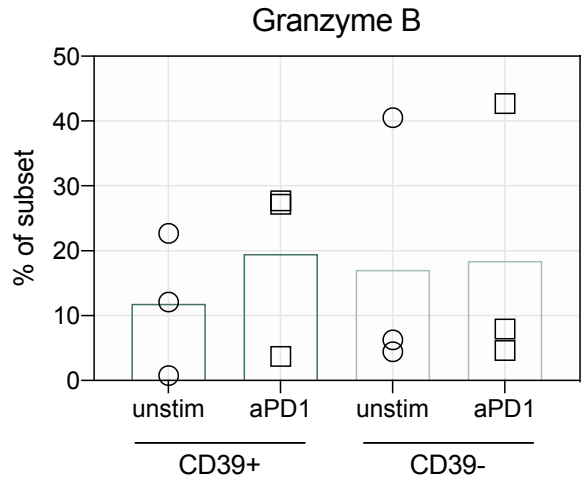




≈Late-dysfunctional T cells



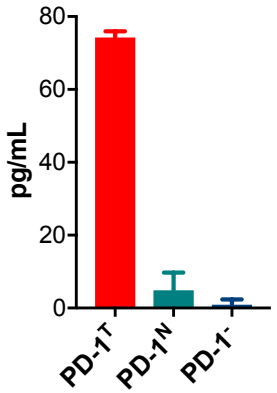




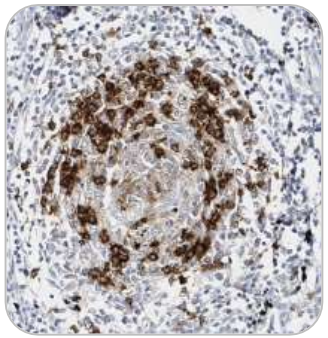
unpublished data

Does the localization and context of T cells influence their state and capacity for reactivation?

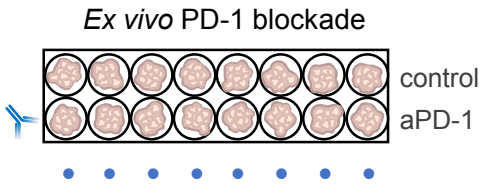
Production of CXCL13 by PD-1^{T} TILs



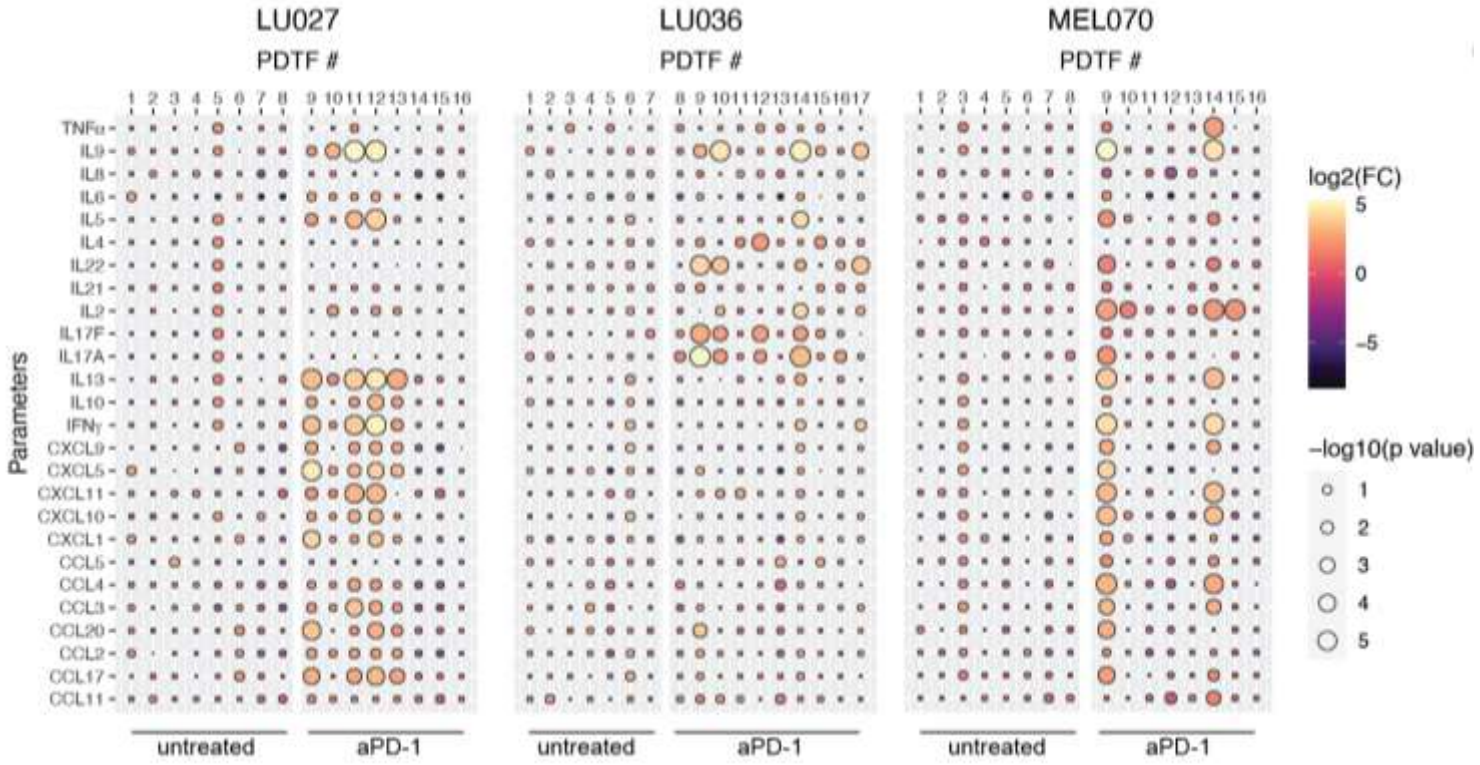
Location of PD-1^{T} TILs in TLS



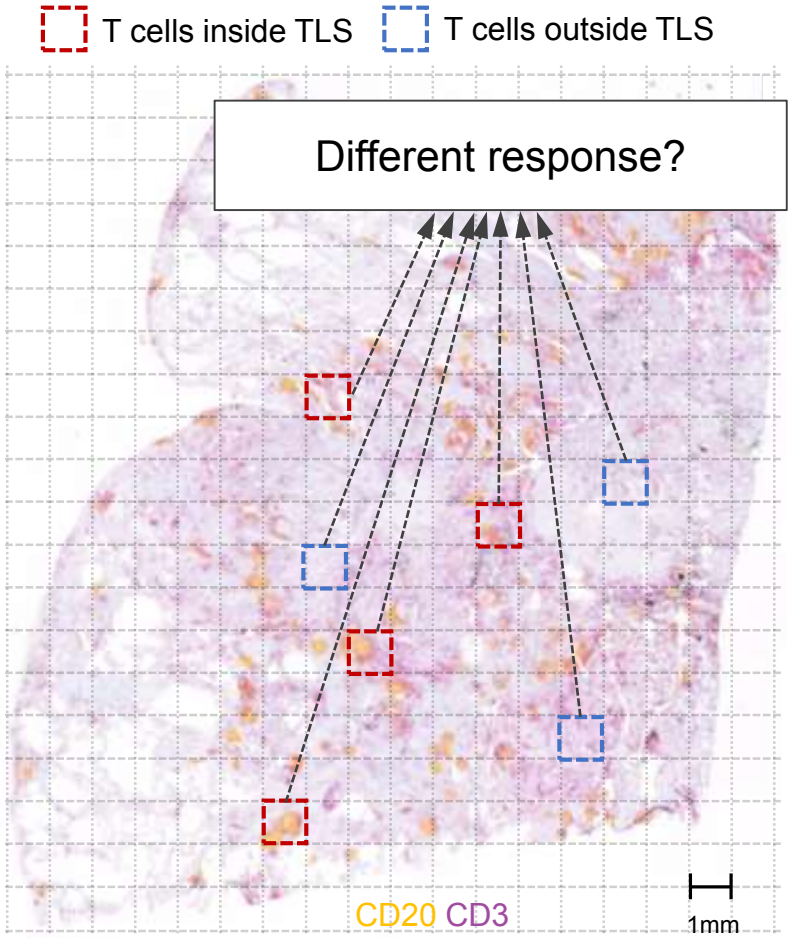
Immune reactivation upon PD-1 blockade shows spatial and patient-specific heterogeneity

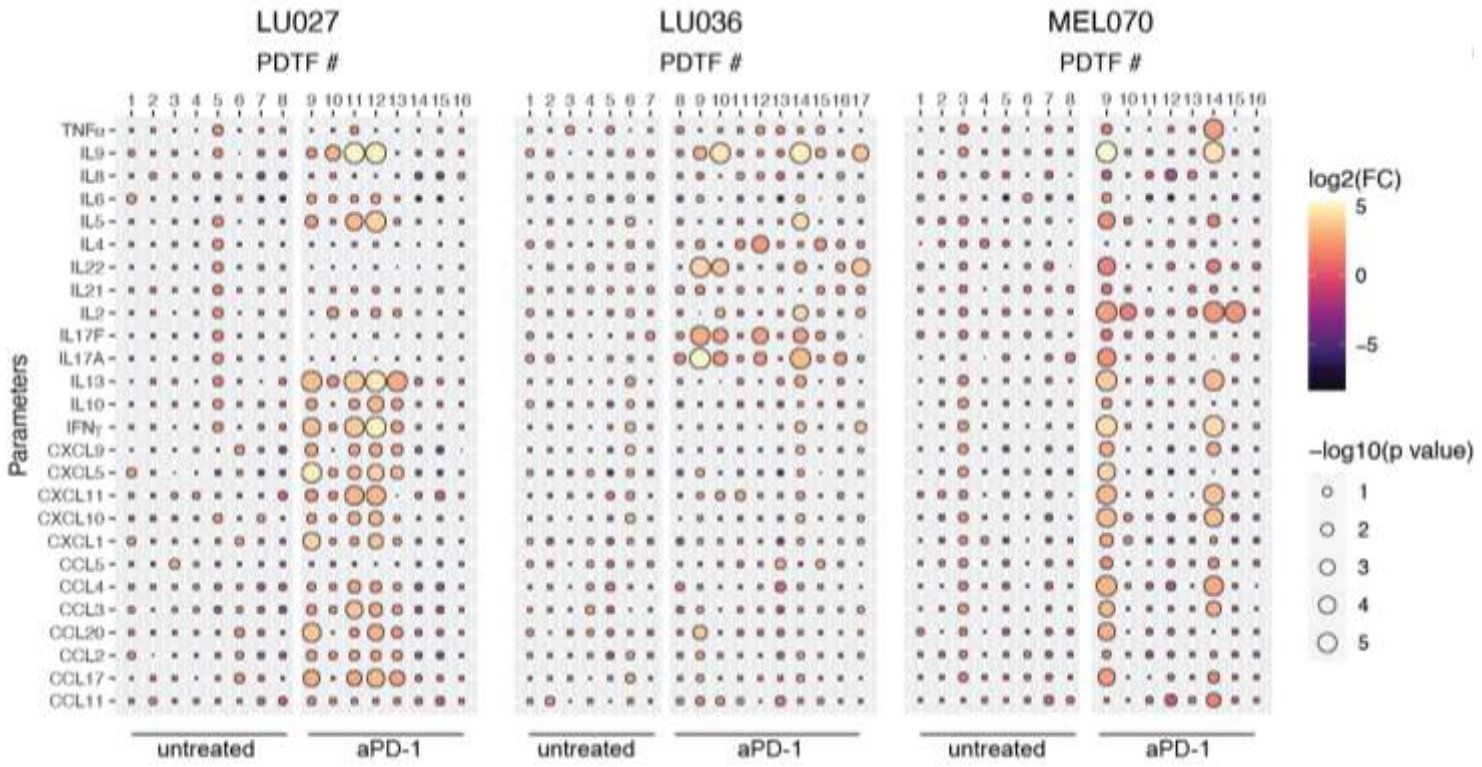


- Separate testing of individual tumor fragments



Is the spatial heterogeneity in response caused by TLS?





Summary

- Exhausted/dysfunctional T cells are not functionless, but display an altered function in the TME
- (Late-dysfunctional) PD-1^{T} TILs are predictive for response to PD-1 blockade
- Potential link between T cell state/function and context/location in the tumor (TLS)
- Spatial and interpatient heterogeneity in T cell reactivation upon PD-1 blockade

Acknowledgments

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Christian Blank

Department of Pathology, NKI
Kim Monkhorst
Karlijn Hummelink
Joyce Sanders

Department of Surgery, NKI
Koen Hartemink

All patients and their families

NKI Facilities
Core Facility Molecular Pathology and Biobanking
Flow cytometry Facility
Genomic Core Facility

Department of Biomedicine Basel
Alfred Zippelius
Petra Herzig

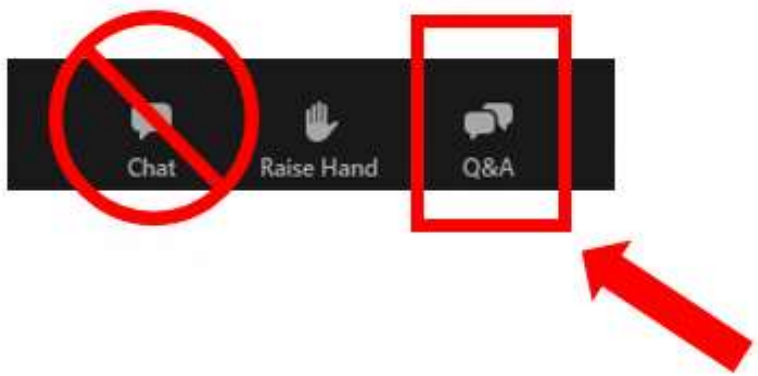
Cantonal Hospital Baselland
Kirsten Mertz

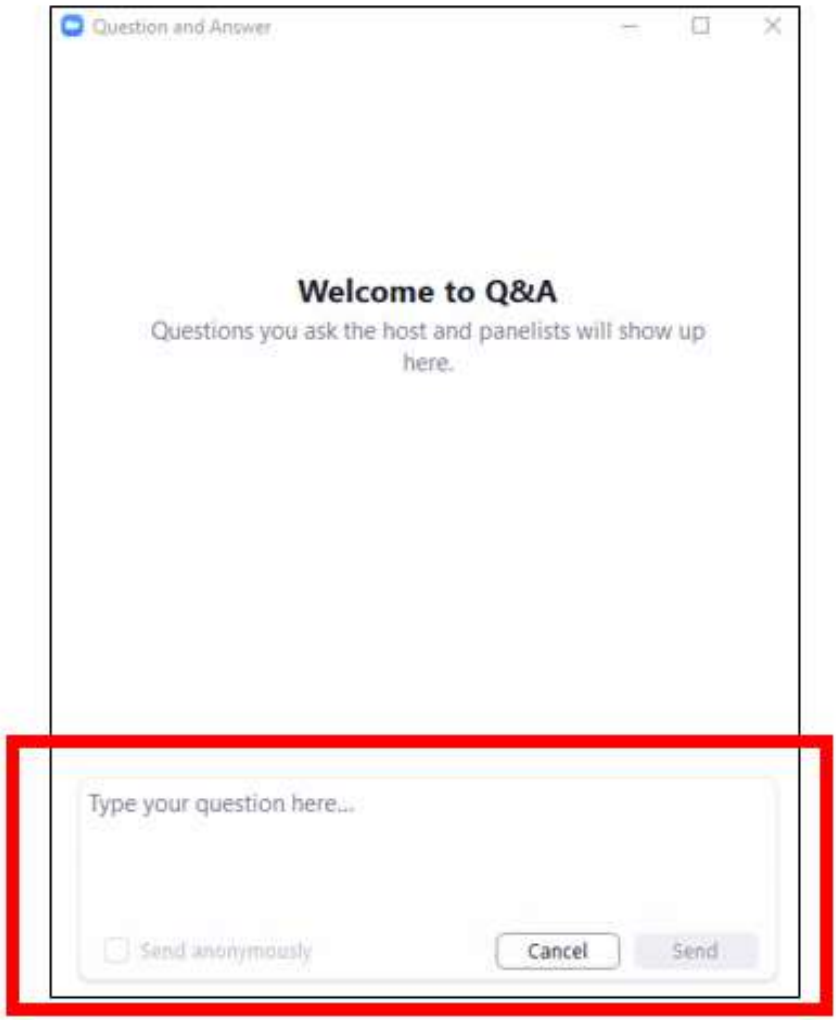
University Hospital Zurich
Viktor Koelzer

How to Submit Questions

Society for Immunotherapy of Cancer

- Click the **"Q&A"** icon located on at the bottom of your Zoom control panel
- Type your question in the Q&A box, then click "Send"
- Questions will be answered:
  a. after each speaker's presentation
  b. in the Question & Answer session at the end of the seminar





Webinar Outline

- Dr. Ananda W. Goldrath: Transcriptional side of T cell functional states; residency program; biomarkers
- Dr. Evan W. Newell: Cytometry; residency program (circulation and exhaustion)
- Dr. Daniela S. Thommen: Functional side; TLS (background, definition and function)

- Q&A: Dr. Wherry + Dr. Schietinger
  - Naming structures/terms/nomenclature
  - Translating t cell therapies into the clinic

Attend the next Deep Dive Seminar

Society for Immunotherapy of Cancer

T cell Selection: A Deep Dive in Cancer Immunotherapy Targets

Tuesday, January 25 from 11:30 a.m. – 1:30 p.m. EDT

Co-chairs and Moderators:

Cara Haymaker, PhD – *The University of Texas MD Anderson Cancer Center*
Cassian Yee, MD – *The University of Texas MD Anderson Cancer Center*

Speakers:

Marcela V. Maus, MD, PhD – *Massachusetts General Hospital*
Chrystal M. Paulos, PhD – *Winship Cancer Institute at Emory University*
Stanley R. Riddell, MD – *Fred Hutchinson Cancer Research Center*

sitcancer.org/education/deepdive

Targets for Cancer Immunotherapy: A Deep Dive Seminar Series is supported, in part, by grants from Alkermes, Inc., Genentech, a member of the Roche Group, Incyte Corporation, Merck & Co., Inc., and Regeneron Pharmaceuticals (*as of 10/05/2021*).

Other SITC Online Seminars

Neo-antigen Discovery: Computational Science in Immuno-Oncology

Wednesday, December 17 from 2:30 p.m. – 3:30 p.m. EDT

Faculty:

Yi Xing, PhD – *University of Pennsylvania; NCI Cancer Moonshot IOTN*

Moderator:

Kellie N. Smith, PhD – *John Hopkins School of Medicine*

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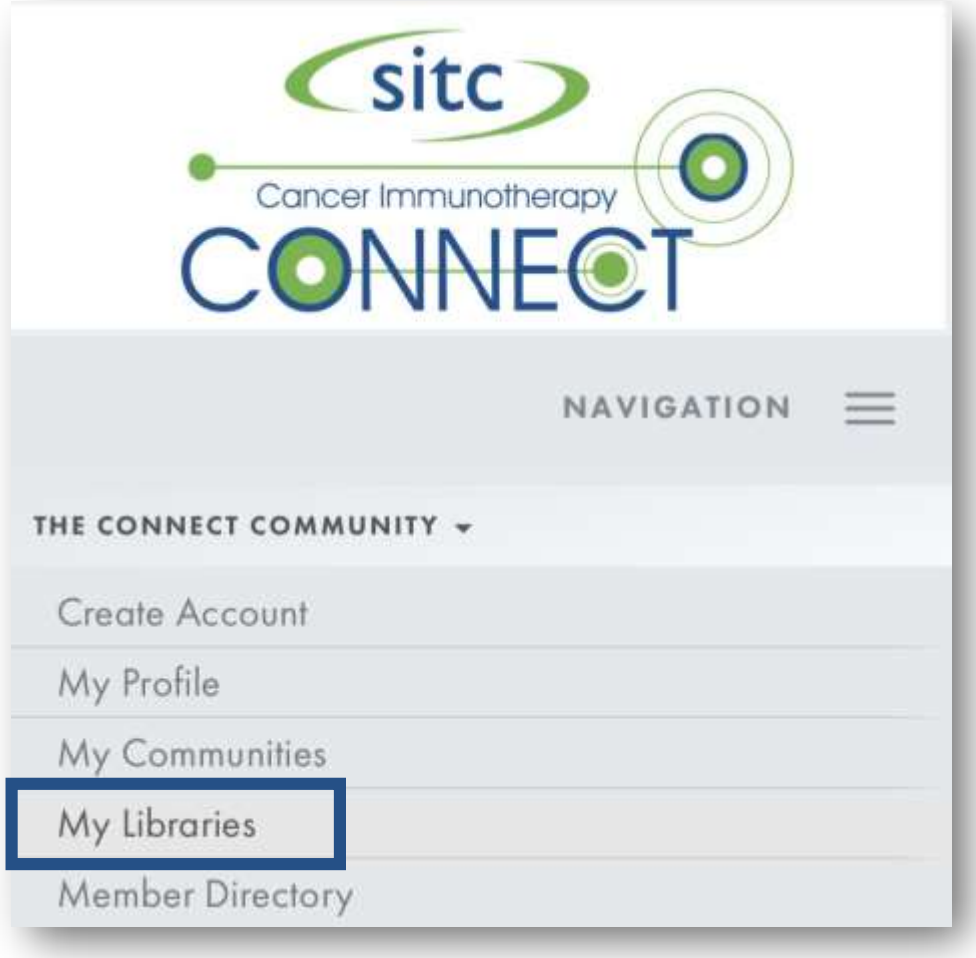

Questions and comments: connectED@sitcancer.org

Thank you for attending the seminar!

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