Tumor-infiltrating lymphocytes in solid tumors

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OECI Oncology Days
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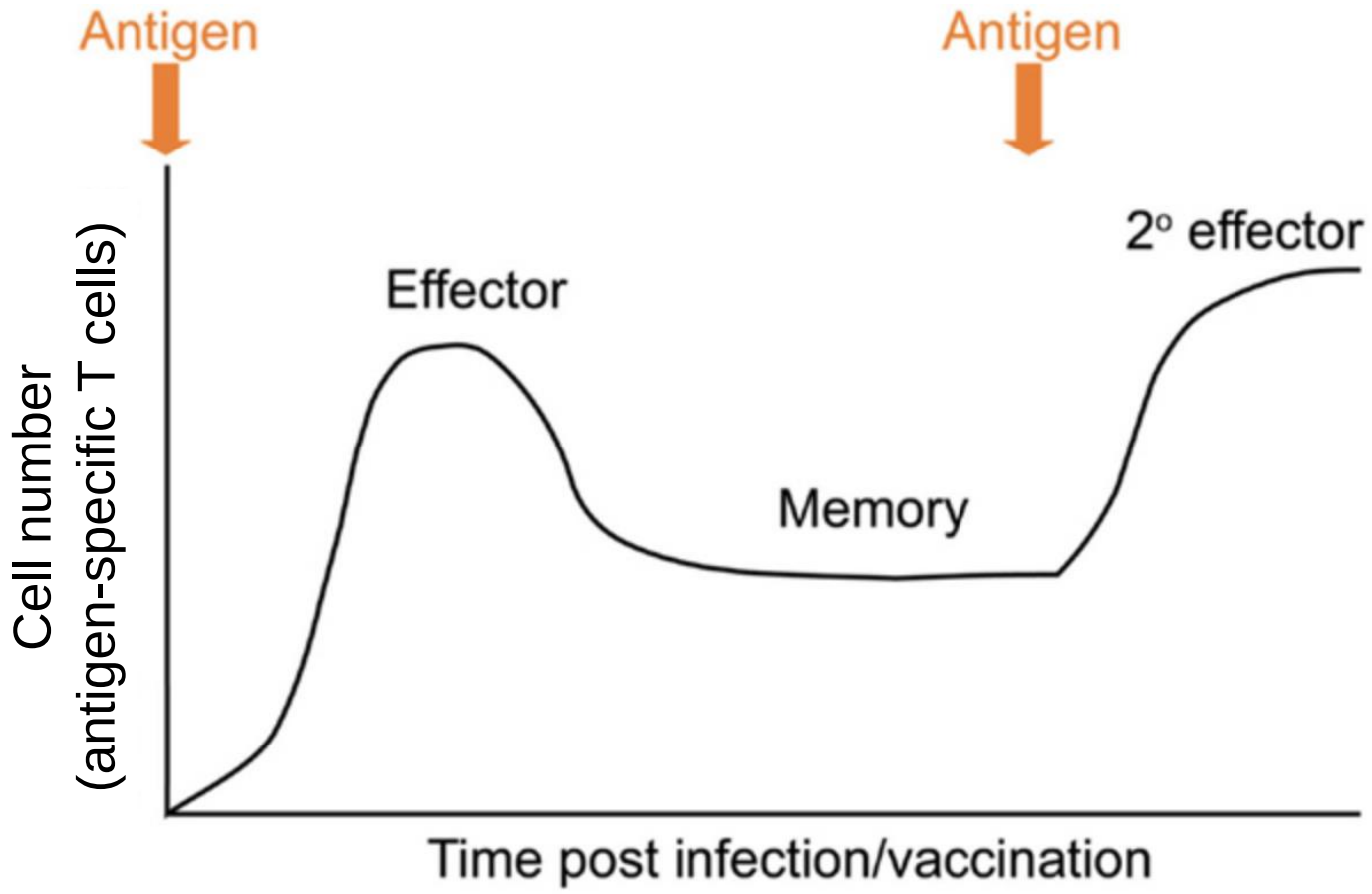
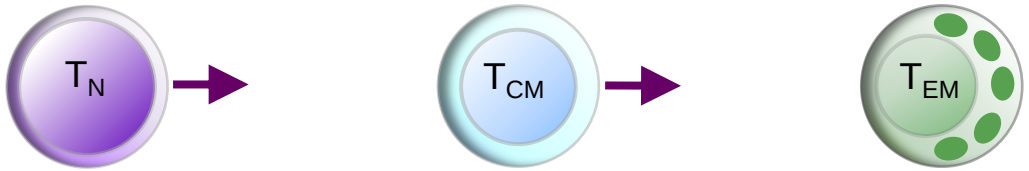
Memory CD8^{+} T cell differentiation



Hale and Ahmed, Front. Immunol. ***2015***

A “simplified” view of memory differentiation



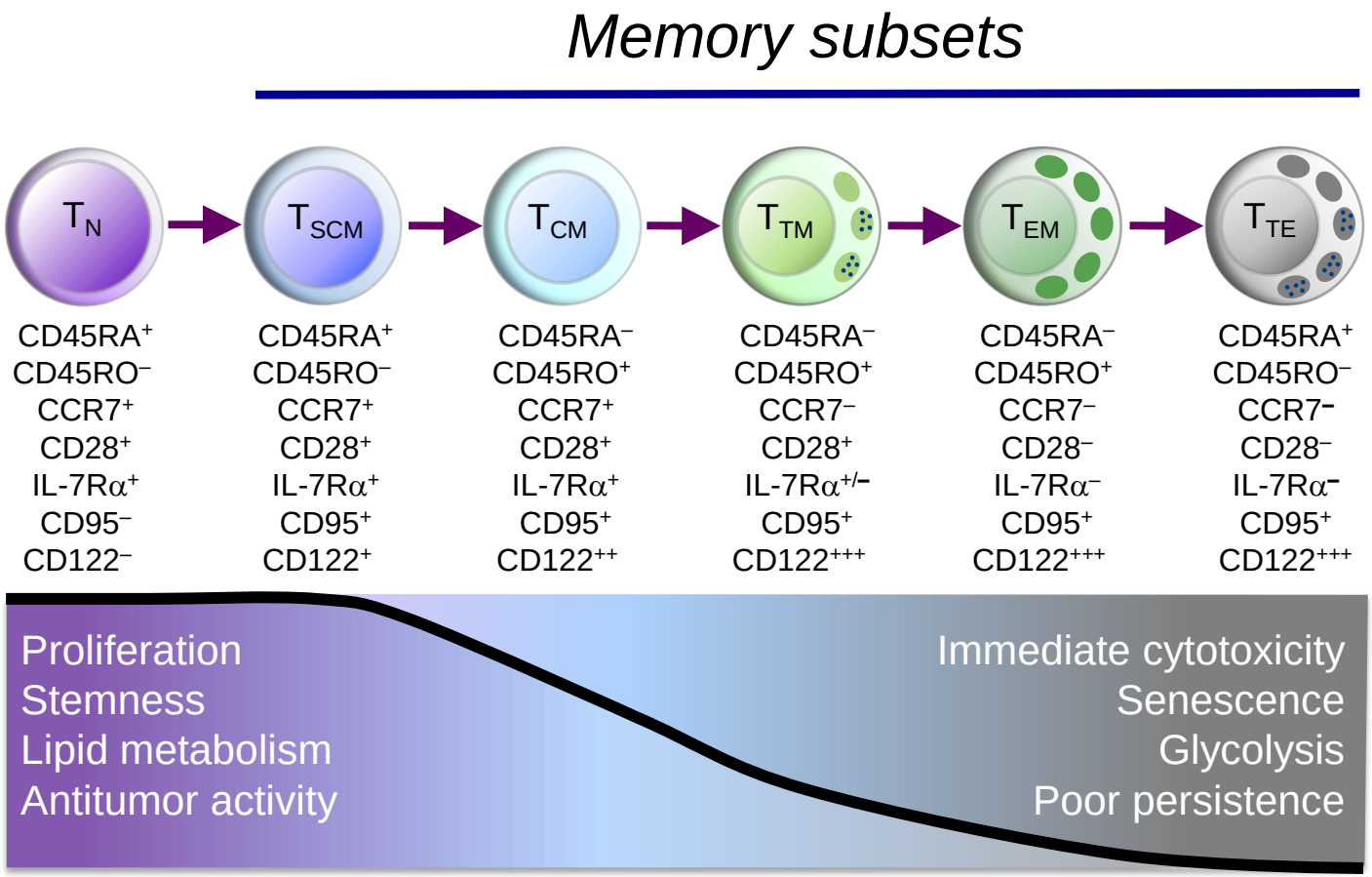
T_N	T_{CM}	T_{EM}
$CD45RA^+$	$CD45RA^-$	$CD45RA^-$
$CD45RO^-$	$CD45RO^+$	$CD45RO^+$
$CCR7^+$	$CCR7^+$	$CCR7^-$
$CD62L^+$	$CD62L^+$	$CD62L^-$

(mostly) lymphoid tissues
Proliferative potential
Long-term persistence
Precursors

(mostly) peripheral tissues
Immediate effector potential
Short-term persistence
Progeny

*Picker, J Immunol, **1993**; Hamann, J Exp Med, **1997**, Sallusto, Nature, **1999**; Masopust, Science, **2001**; Wherry, Nat Immunol, **2003***

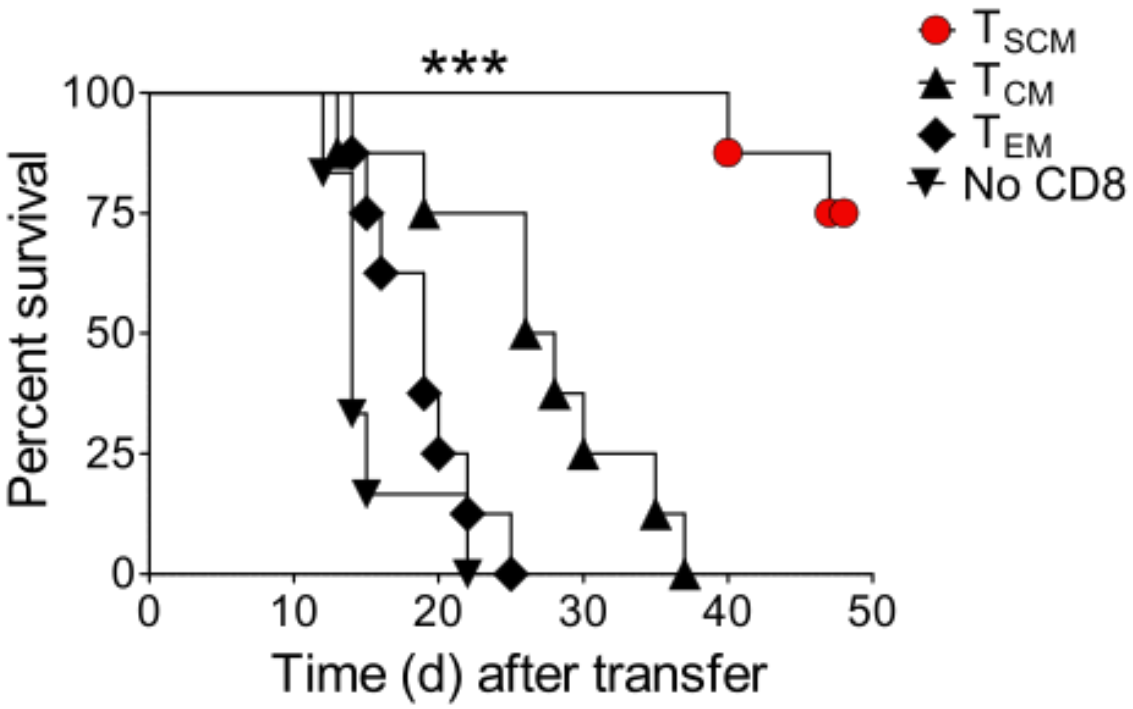
Heterogeneity of the $CD8^+$ T cell compartment



Gattinoni and Lugli*, Nat Med, **2011**; Lugli, J Clin Invest, **2013**, Lugli, Nat Protoc, **2013**; Cieri, Blood, **2013**; Roberto, Blood, **2015**; Oliveira, Science TM, **2015**; Gattinoni, Nat Med, **2017**, Abdelsamed, JEM, **2017**; Akondy, Nature, **2017;** Masopust and Jameson, Immunity, **2018***

Superior anti-tumor function of T_{SCM} upon ACT

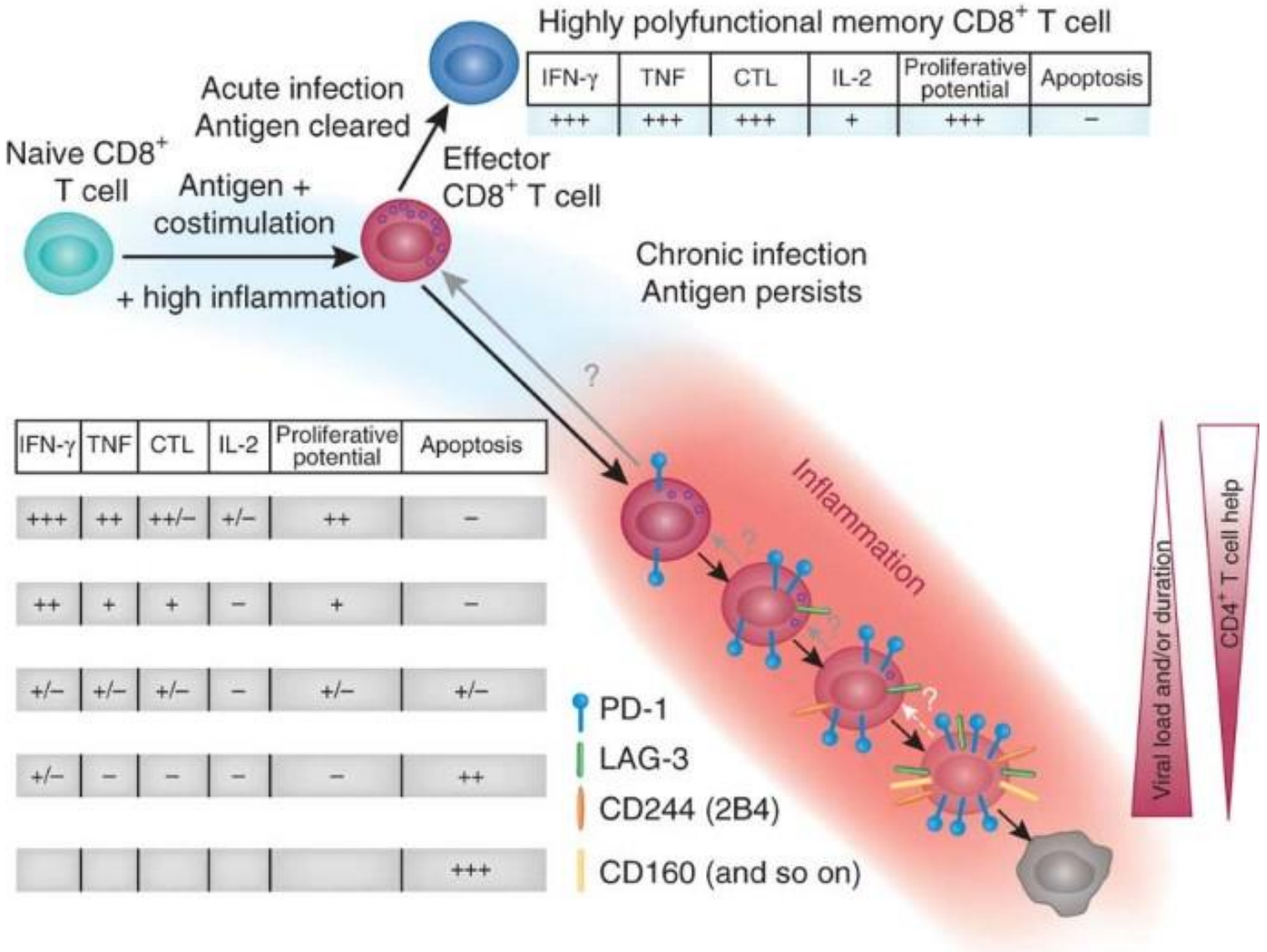
- T cells subsets redirected with a mesothelin-specific CAR
- Adoptive transfer in xenogeneic NSG model of mesothelioma



Gattinoni* and Lugli*, et al. Nat Med, 2011

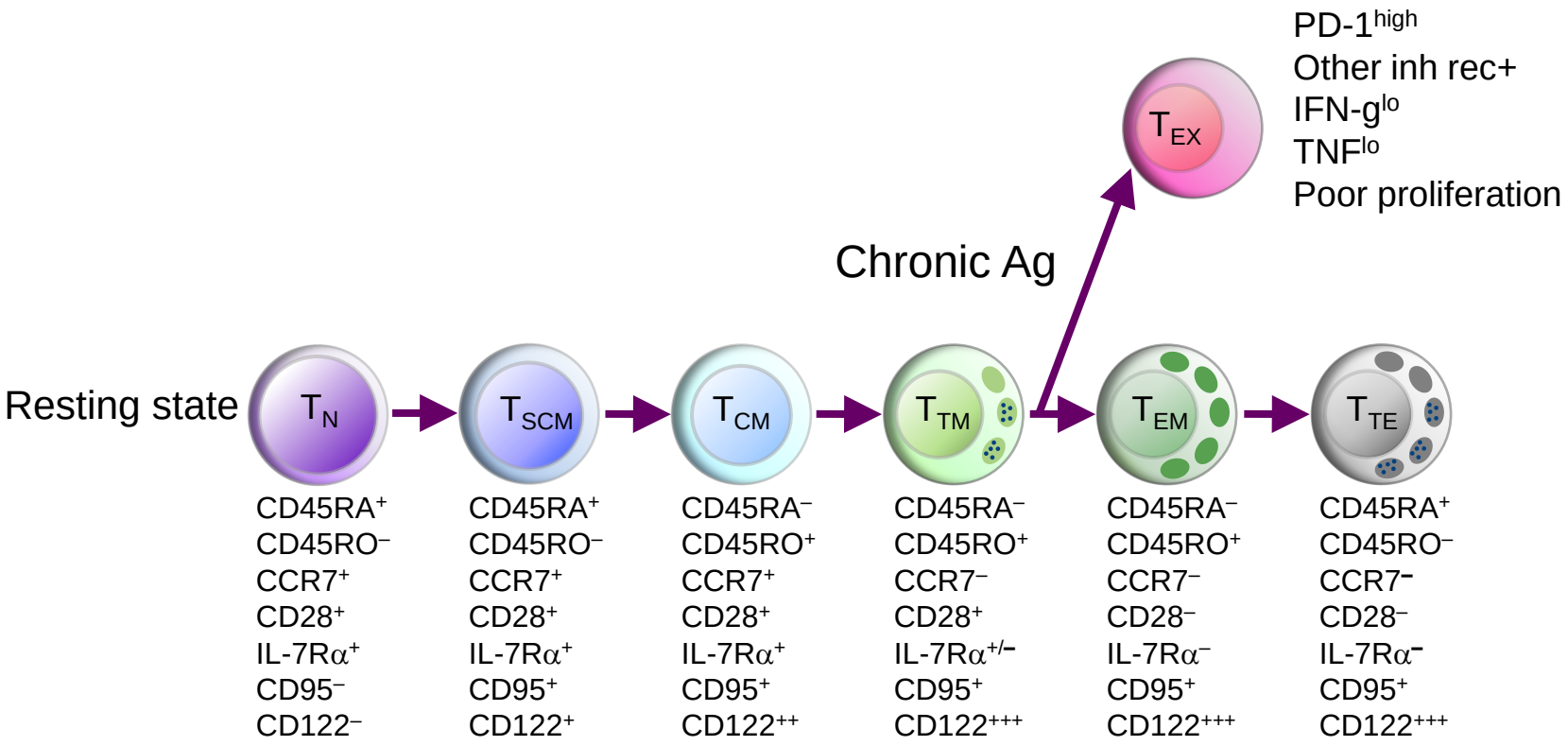
Then, exhaustion…

Chronic TCR stimulation induces T cell dysfunction



Wherry, Nat Immunol, 2011

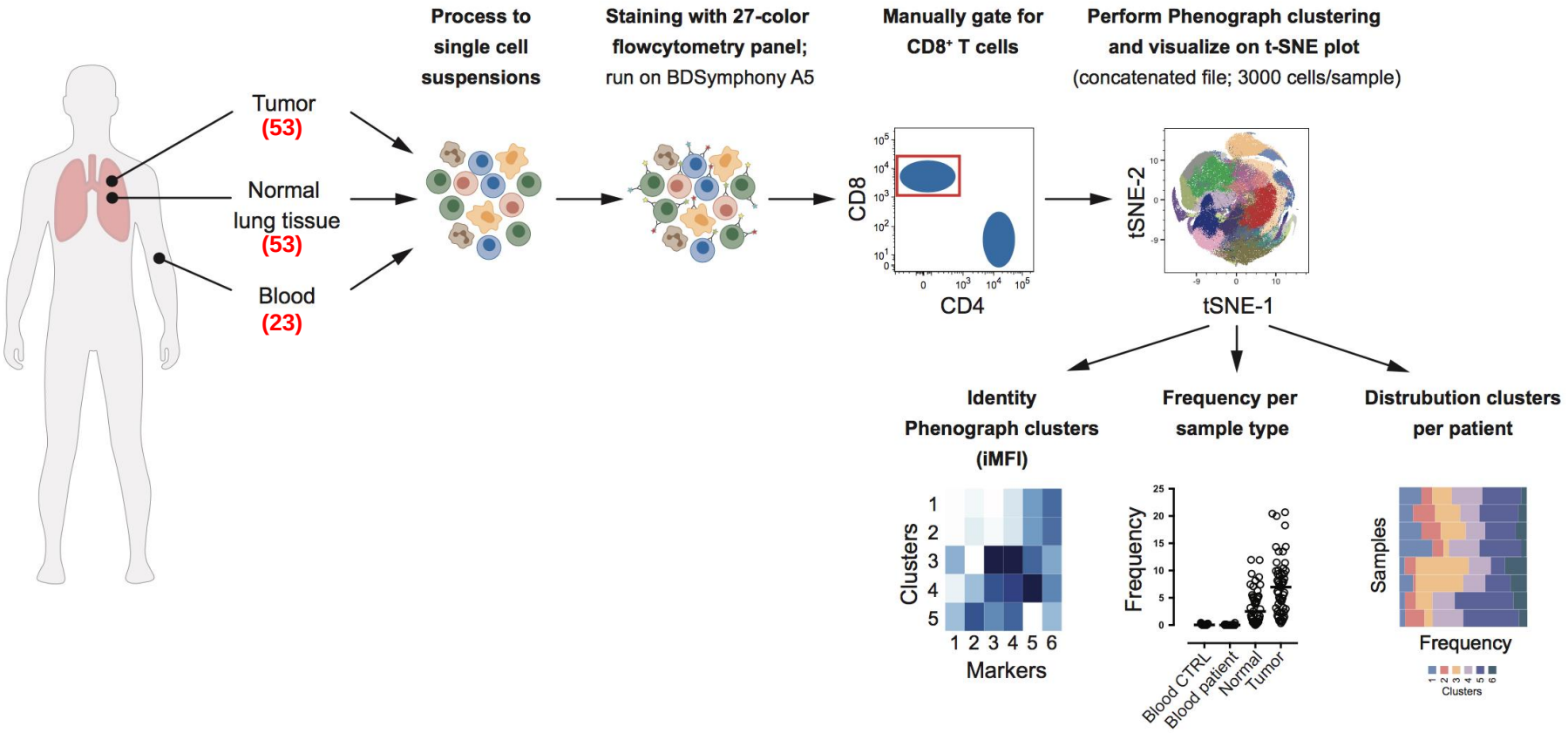
Chronic TCR stimulation induces T cell dysfunction



Can stem-like memory cells be identified in tumors and have a role in response?

What is the hierarchy of the CD8+ response in tumors (if present)?

Application to human CD8+ TILs



Brummelman and Mazza et al., J Exp Med, 2018

Single cell analysis of the human immune system

Integration with scRNA-seq, high-dimensional population sorting and bulk RNA-seq, ATAC-seq ***AND FUNCTIONAL ASSAYS***

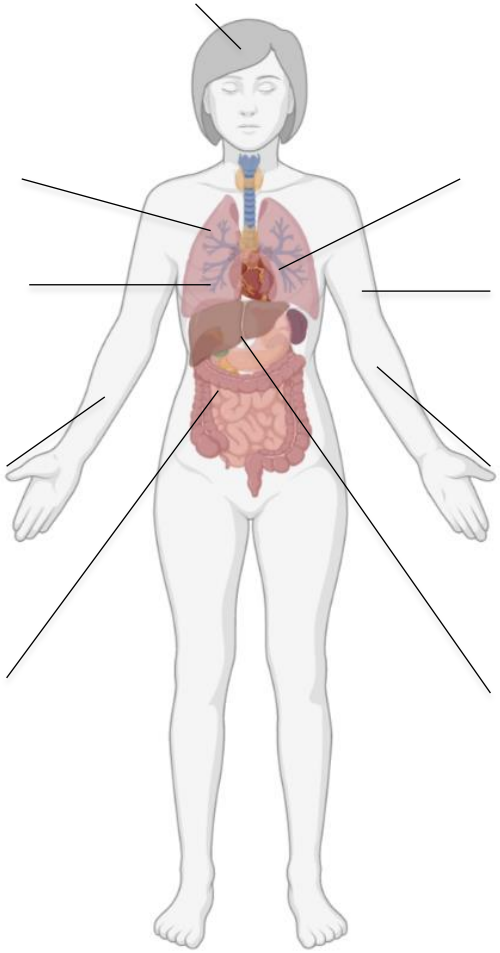
TILs in **brain cancer and mets**
Wischnewski, Nat Cancer, 2023

Stem-like CD8+ in **lung cancer**
Brummelman, Mazza
J Exp Med, 2018

Treg heterogeneity in **tumors**
Alvisi, Puccio
J Clin Invest, 2020

Progenitors of **exhausted**
Galletti, De Simone, Mazza
Nat Immunol, 2020

Type 1 regulatory CD4+ in **tumors**
Bonnal, Nat Immunol, 2021



CD39+ Trm in **breast cancer**
Losurdo, Scirgolea, Commun Biol, 2021

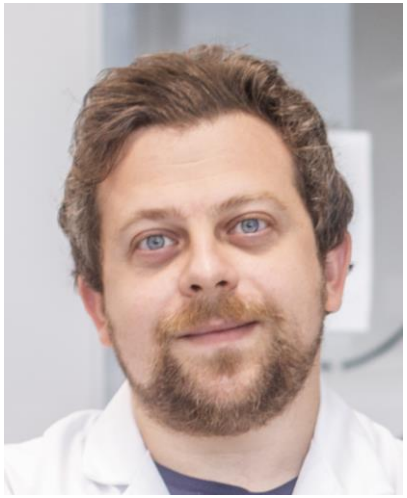
CD161 MAIT in melanoma
De Biasi, Nat Commun, 2021

CMV-specific T cell dynamics in **haplo-HSCT**
Van Beek, Haematologica, 2021

Treg activation
in **cholangiocarcinoma**
Alvisi, J Hepatol, 2022

- How do progenitor exhausted CD8+ T cells (Tpex) compare to long-lived memory cells?
- What is the origin and lineage relationships of dysfunctional CD8+ T cells?

Giovanni Galletti



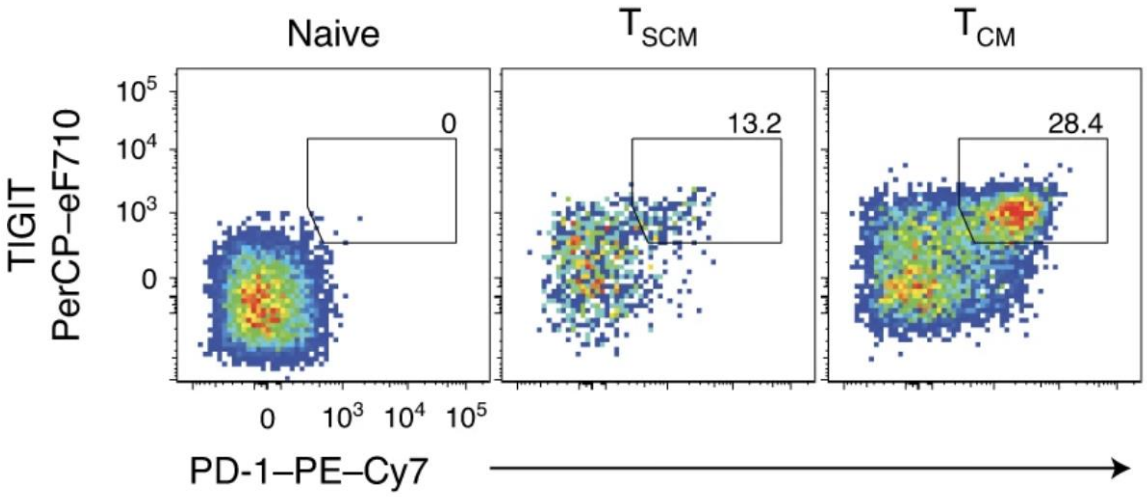
Gabriele De Simone

Emilia Mazza

Galletti, De Simone, Mazza et al., Nat Immunol, 2020

Redefining memory CD8$^+$ T cell differentiation

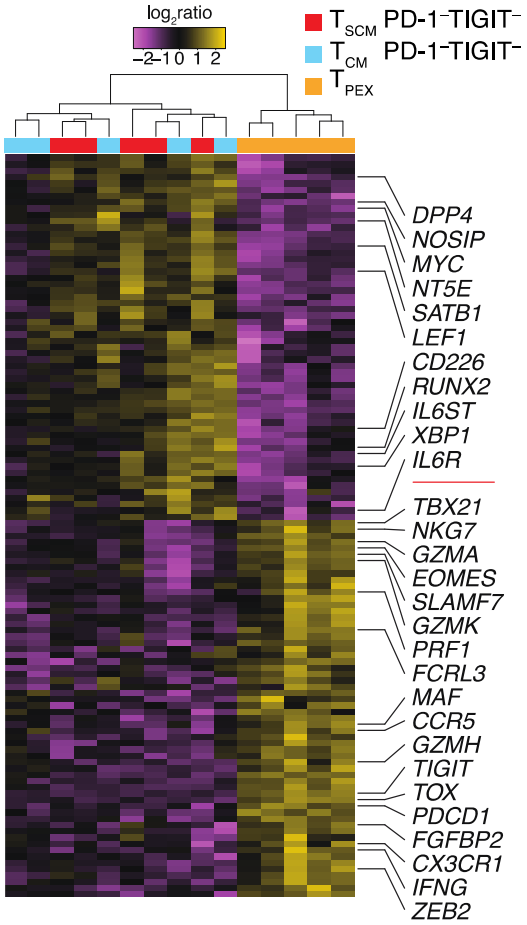
Gates for early differentiated memory cells



Galletti, De Simone, Mazza et al., Nat Immunol, 2020

PD-1^{+}TIGIT^{+} T_{PEX} are transcriptionally distinct

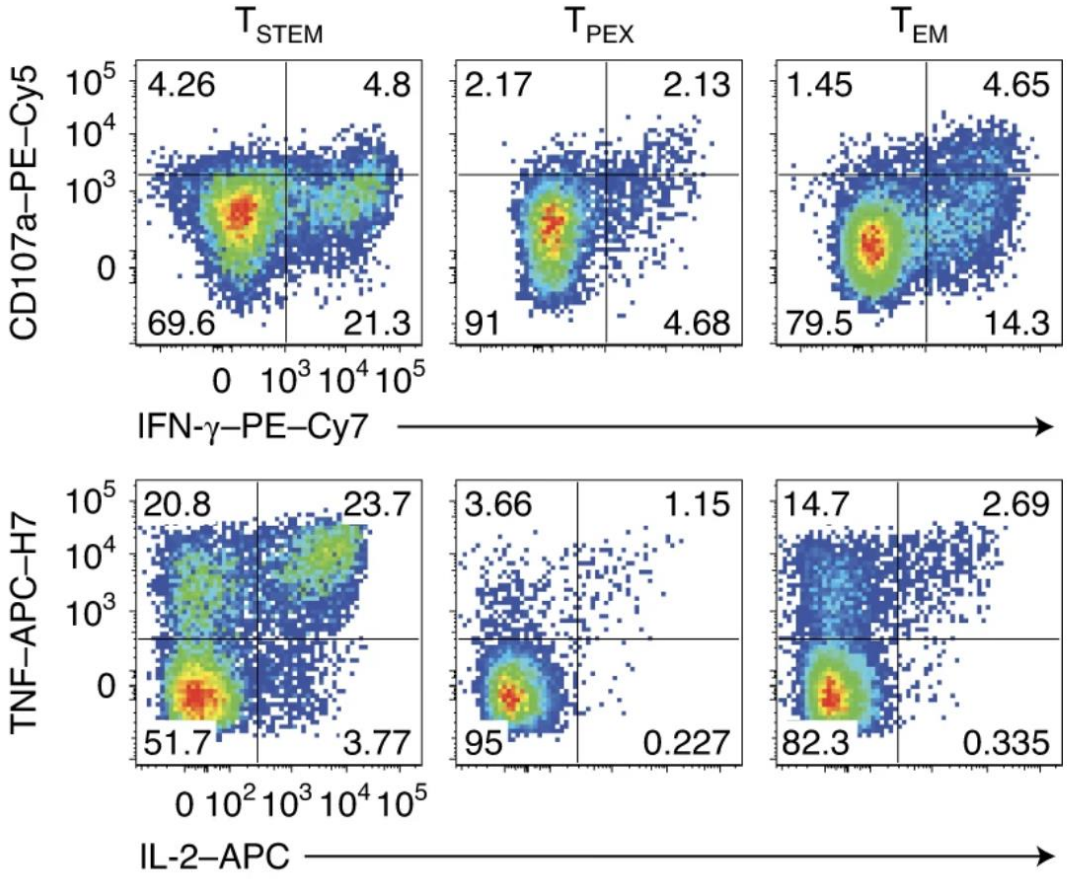
- T cell subset sorting by FACS from 5 healthy donors
- Ex vivo RNA-seq



Galletti, De Simone, Mazza et al., Nat Immunol, 2020

PD-1^{+}TIGIT^{+} T_{PEX} are functionally inefficient*

- T cell subset sorting by FACS
- In vitro cytokine production in response to anti-CD3/28

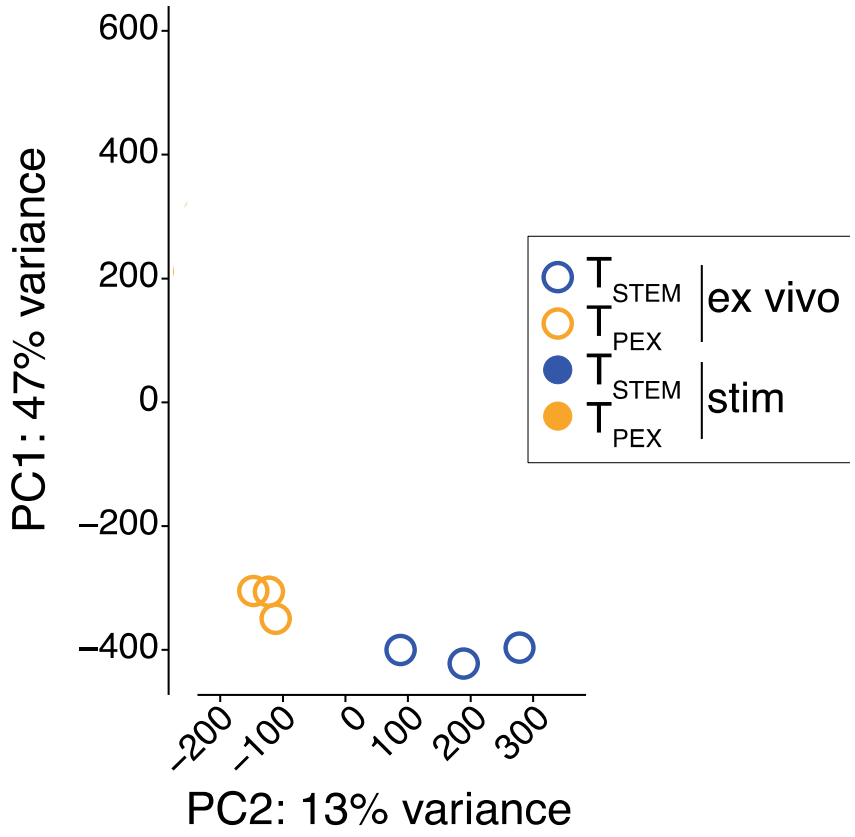


*not predicted by scRNA-seq

T_{PEX} are “hard-wired” to their transcriptional and epigenetic state

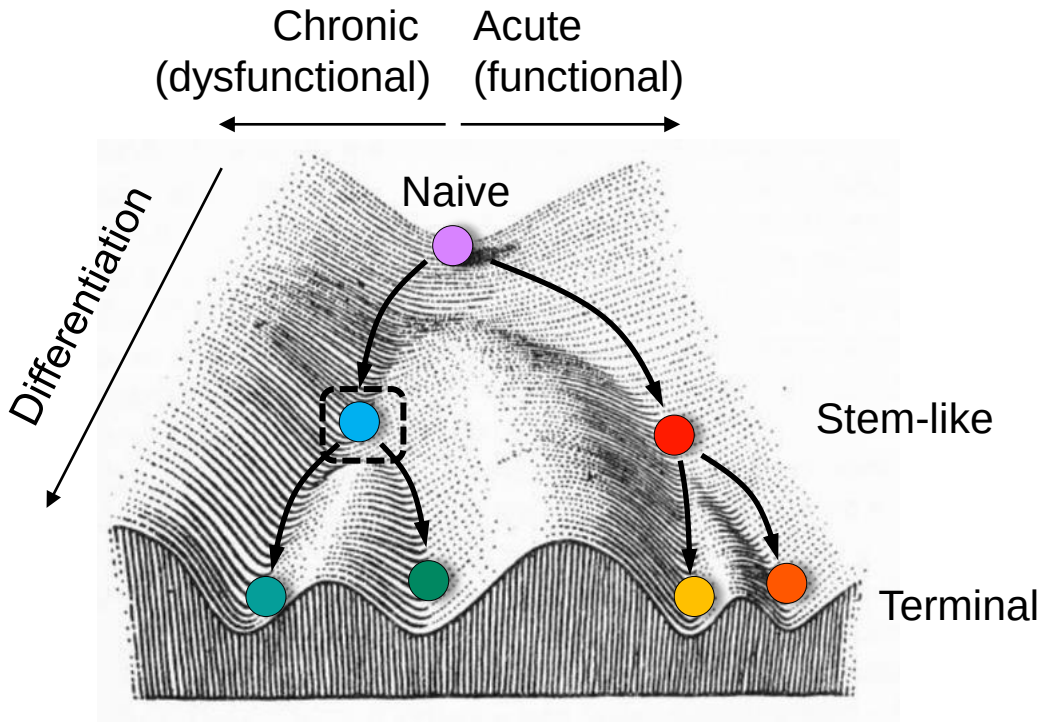
- T cell subset sorting by FACS, stimulation with aCD3/28+IL2 and IL-12
- ATAC-seq/RNA-seq

ATAC-seq



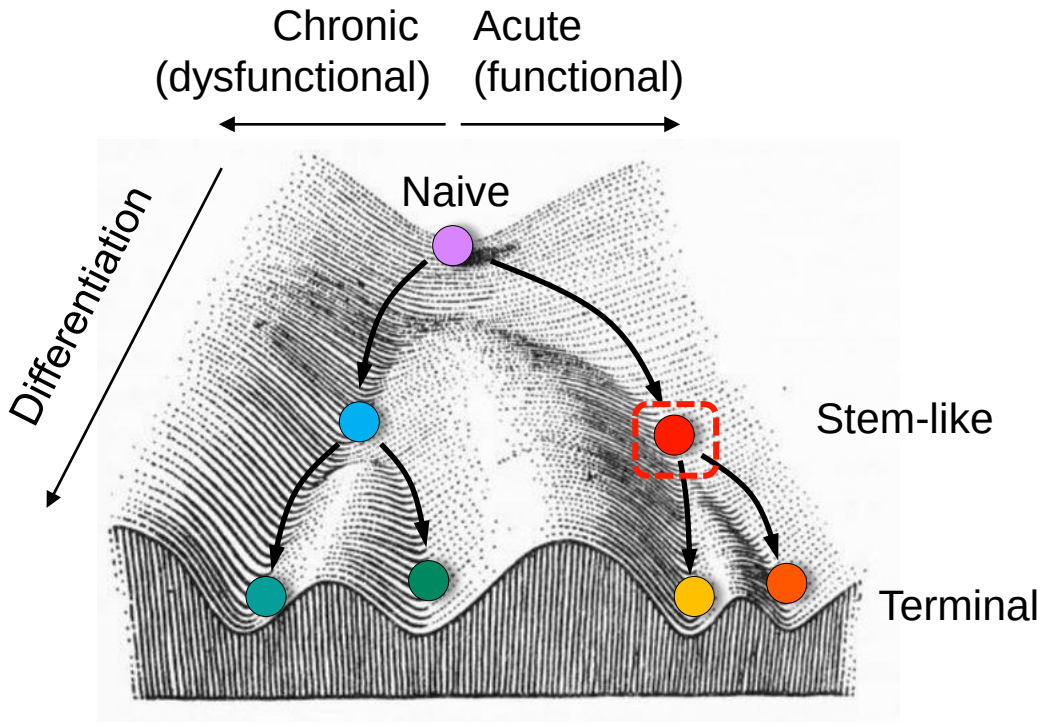
Galletti, De Simone, Mazza et al., Nat Immunol, 2020

Trajectories of memory T cell differentiation



Response to checkpoint blockade
Hard-wired to dysfunction

Trajectories of memory T cell differentiation

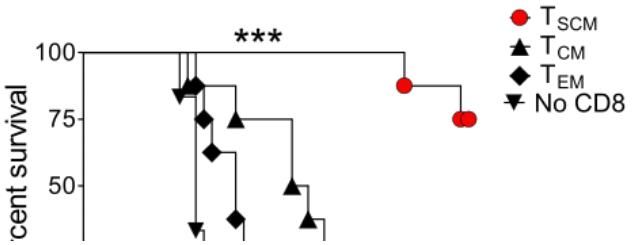


Exhaustion “resistant”
Improved functionality upon ACT

To be or not to be STEM?

That is the question

Superior clinical response with adoptive transfer of stem-like CAR T cells



CAR T cell manufacturing from naive/ste
T lymphocytes enhances antitumor responses while
curtailing cytokine release syndrome

Silvia Arcangeli,[1] Camilla Bove,[1] Claudia Mezzanotte,[1] Barbara Camisa,[1,2] Laura Falcone,[1] Francesco Manfredi,[2] Eugenia Bezzecchi,[3] Rita El Khoury,[1] Rossana Norata,[4] Francesca Sanvito,[5] Maurilio Ponzoni,[3,6] Beatrice Greco,[1] Marta Angiola Moresco,[1] Matteo G. Carrabba,[7] Fabio Ciceri,[6,7] Chiara Bonini,[2,6] Attilio Bondanza,[1] and Monica Casucci[1]

RESEARCH ARTICLE

IMMUNOTHERAPY

Article

Cancer Cell

Depletion of BATF in CAR-T cells enhances antitumor activity by inducing resistance against exhaustion and formation of central memory cells

Antioxidant metabolism regulates CD8
T memory stem cell formation and
antitumor immunity

Cell Reports
Medicine

Tissue-resident mem
characteristics displa
solid and liquid tumor

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Antitu
ESO-1 c259T Cells in Synovial Sarcoma

Article

blood

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

CANCER

T_{STEM}-like CAR-T cells exhibit improved persistence and tumor control compared with conventional CAR-T cells in preclinical models

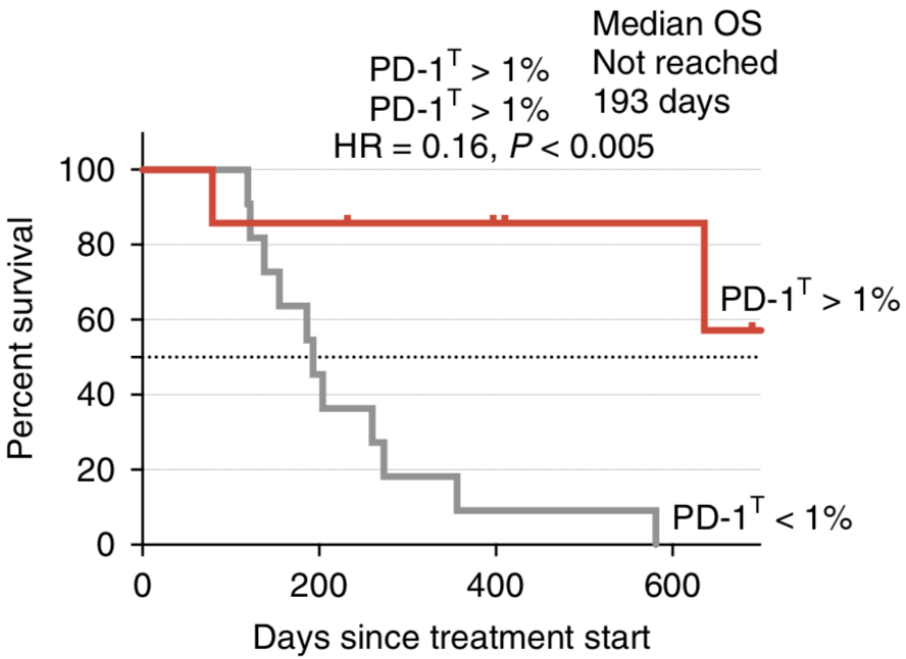
CANCER IMMUNOTHERAPY

Stem-like CD8 T cells mediate response
cell immunotherapy against human cancer

Sri Krishna[1]*, Frank J. Lowery[1]*, Amy R. Copeland[1], Erol Bahadiroglu[2], Ratnadeep Mukherjee[2], Li Jia[3], James T. Anibal[2], Abraham Sachs[1], Serifat O. Adebola[2], Devikala Gurusamy[1], Zhiya Yu[1], Victoria Hill[1], Jared J. Gartner[1], Yong F. Li[1], Maria Parkhurst[1], Biman Paria[1], Pia Kvistborg[4], Michael C. Kelly[5], Stephanie L. Goff[1], Grégoire Altan-Bonnet[2], Paul F. Robbins[1]†, Steven A. Rosenberg[1]†

Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia

Infiltration of PD-1^{high} T cells and response to anti-PD1 in non-small cell lung cancer



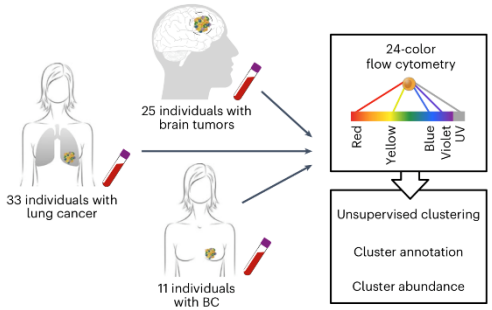
PD-1^{high} cells:

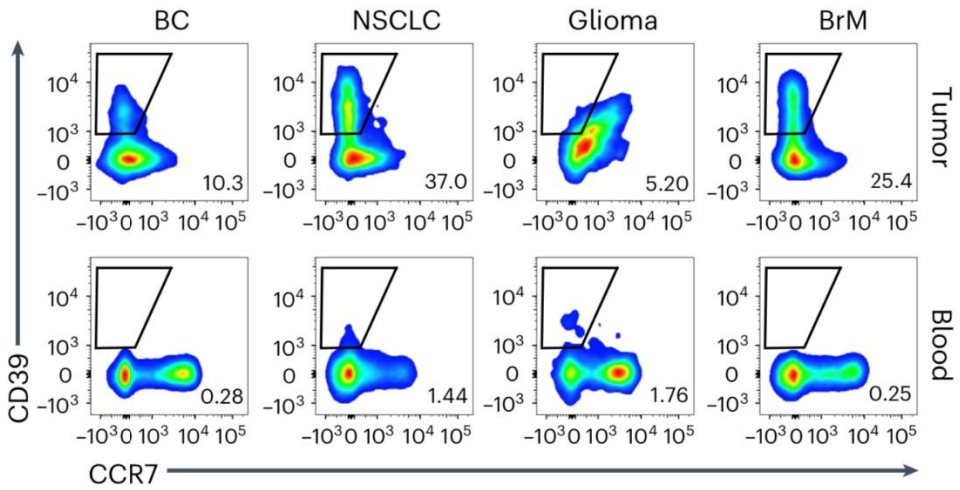
- express several inhibitory receptors (PD-1, TIM3) and are largely dysfunctional
- Are induced by TGF-β, an immunosuppressive cytokine
- Have tissue-resident memory characteristics (CD103, CD69), generally correlating with favorable prognosis
- Tumor-specific T cells mainly reside in the PD-1^{high} fraction and are CD39+

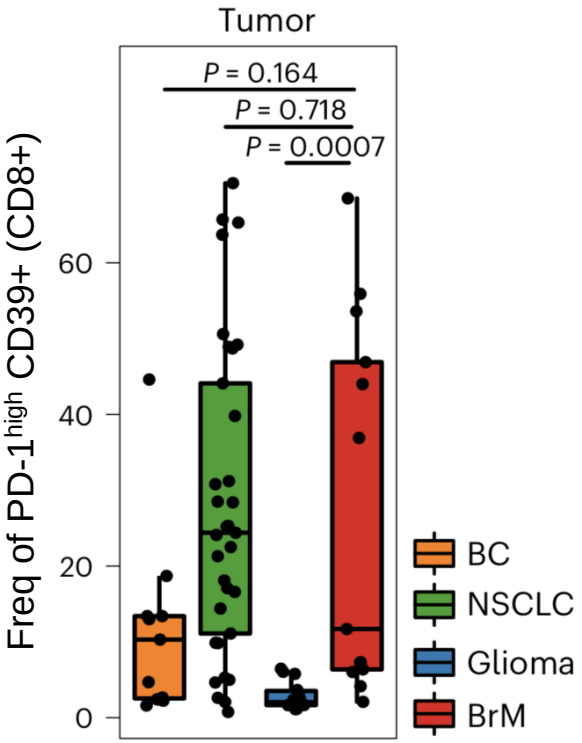
Thommen, Nat Med, 2018; Duhen, Nat Comms, 2018; Savas, Nat Med, 2018; Simoni, Nature, 2019; Li, Cell, 2019; Zhang, Cancer Cell, 2021; Liu, Nat Cancer, 2022; Wischnewski, Nat Cancer, 2023

Brain metastatic lesions harbor PD-1^{high} CD39^{+} T cells

- Brain metastases (BrM) are partially responsive to checkpoint inhibition while primary gliomas are not
- What are the differences in CD8+ T cell qualities in their microenvironment?

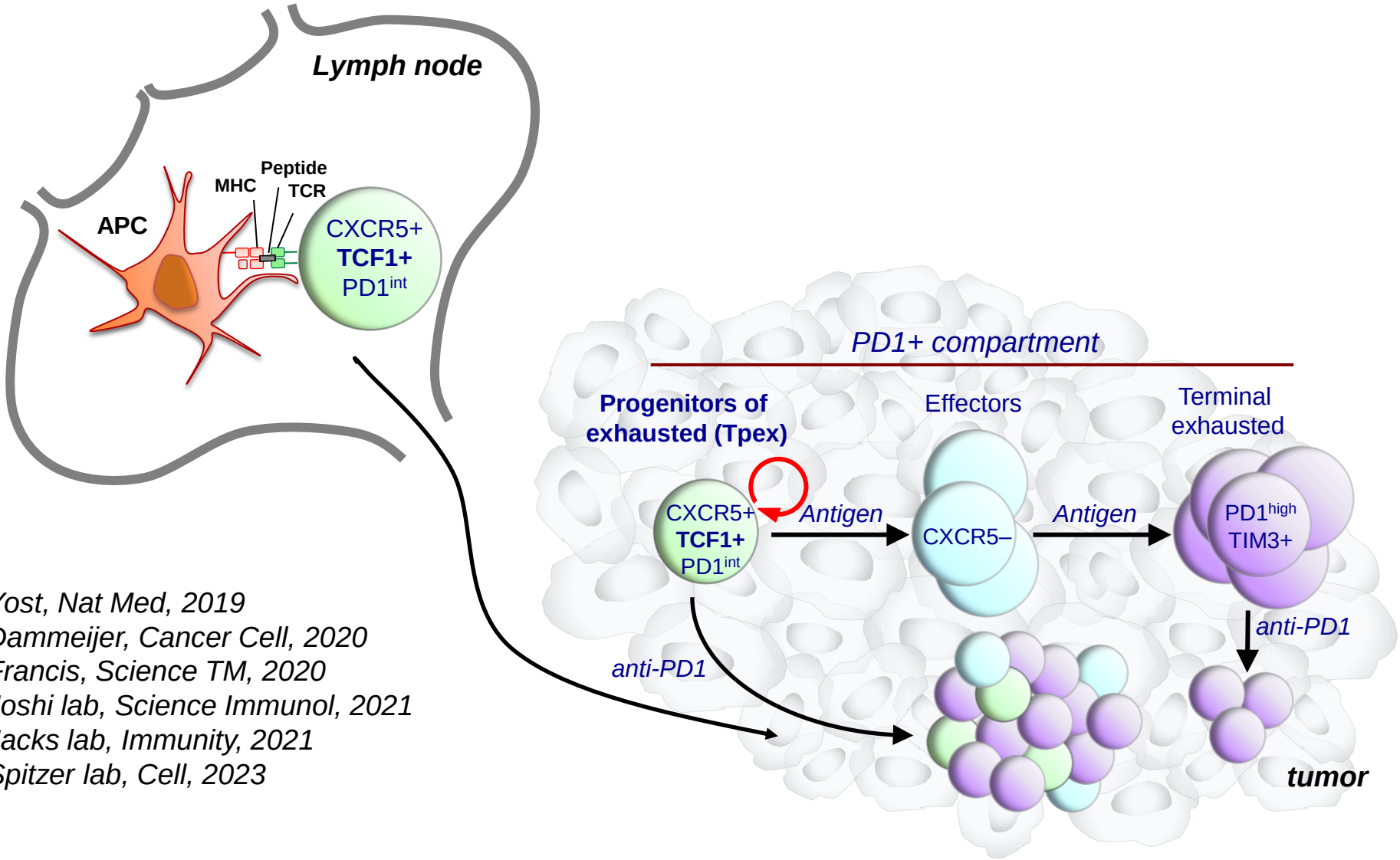






*Wischnewski et al, Nat Cancer, 2023; * results also confirmed by scRNA-seq*

Hierarchy of differentiation in PD-1^{+} CD8^{+} T cells

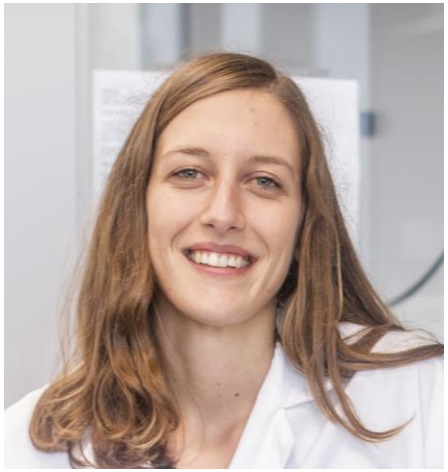


Yost, Nat Med, 2019
Dammeijer, Cancer Cell, 2020
Francis, Science TM, 2020
Joshi lab, Science Immunol, 2021
Jacks lab, Immunity, 2021
Spitzer lab, Cell, 2023

Brummelman and Mazza, et al., J Exp Med, 2018

Sade-Feldman, Cell, 2018; Kurtulus, Immunity, 2019; Siddiqui, Immunity, 2019; Miller, Nat Immunol, 2019; TCF-1^{+} provide long-term response to anti-PD1

What about the surrounding tumor microenvironment?

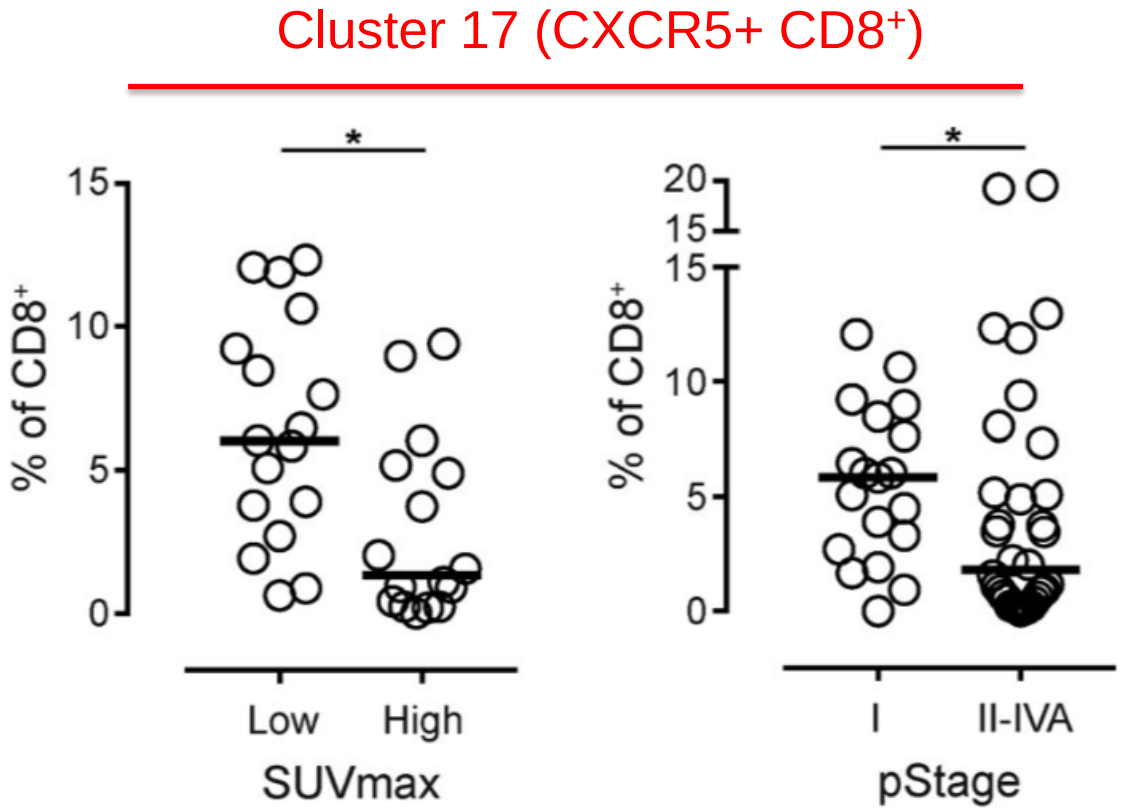


Giorgia Alvisi

Jolanda Brummelman

Simone Puccio

Stem-like CD8$^+$ TILs are lost with NSCLC progression

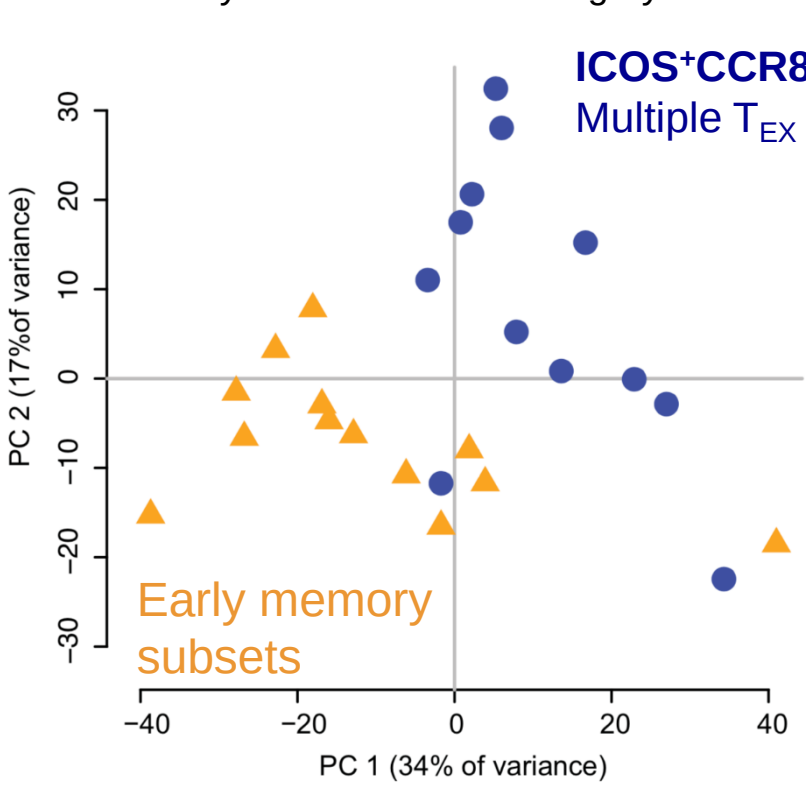


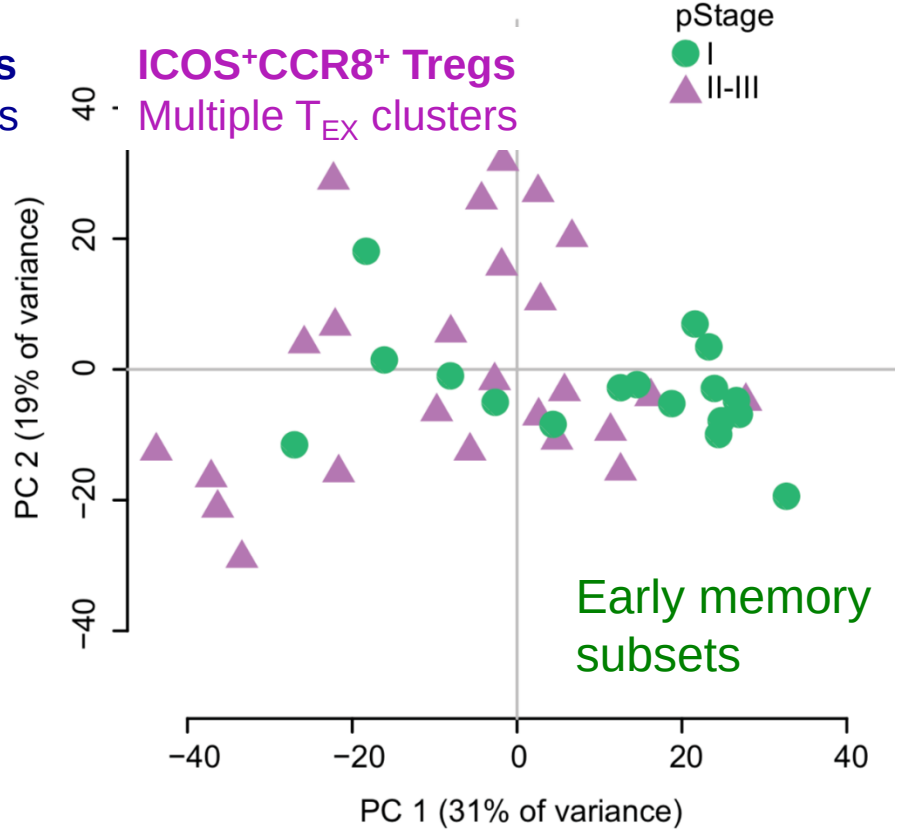
Brummelman, J Exp Med, 2018; Kissick, Nature, 2019 in RCC

Landscape of T cell phenotypes and disease progression

Integrated landscape of CD4+ and CD8+ T cell phenotypes (high-dimensional single cell clusters)

SUVmax: indicator of tumor glycolysis as obtained by PET scan before surgery



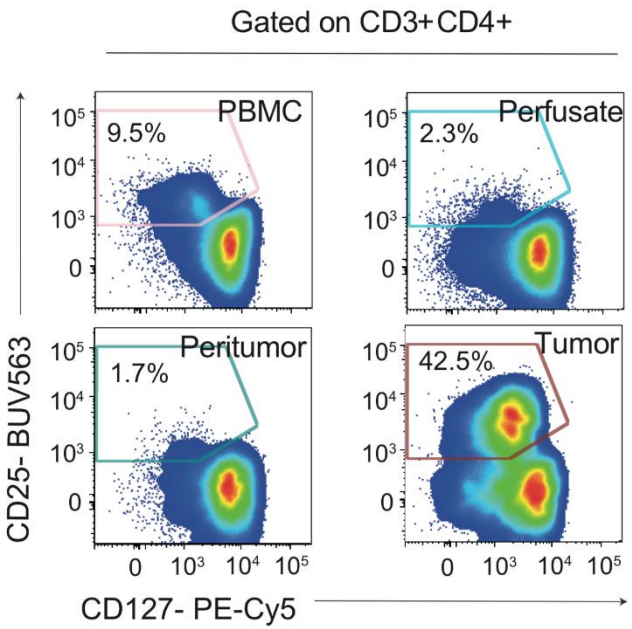


Alvisi et al., J Clin Invest, 2020

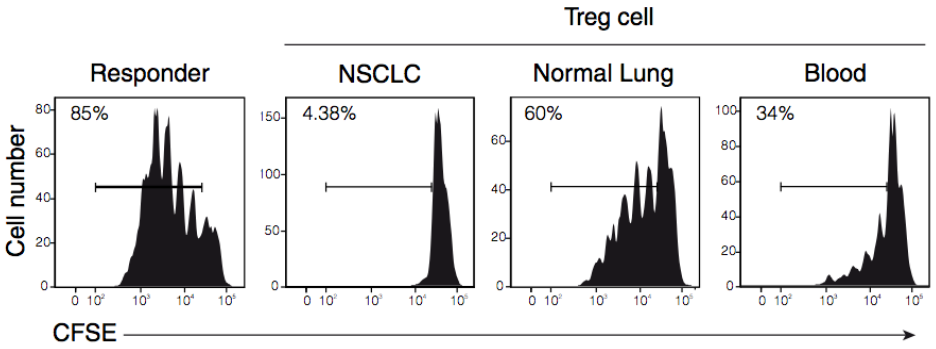
CD4+ regulatory T cells (Tregs) in cancer

- Maintain **immune homeostasis** by suppressing inflammation and are controlled by the transcription factor **FOXP3**
- Are preferentially recruited in tumors, where they become **highly activated**
- Are **detrimental** in cancer because limiting anti-tumor immune responses by several mechanisms
- Their abundance correlates with **worse prognosis** in virtually all cancers
- Treg depletion results in potent anti-tumor immunity but also induces autoimmunity

CD4+ Tregs are abundant in the TME and are highly immunosuppressive

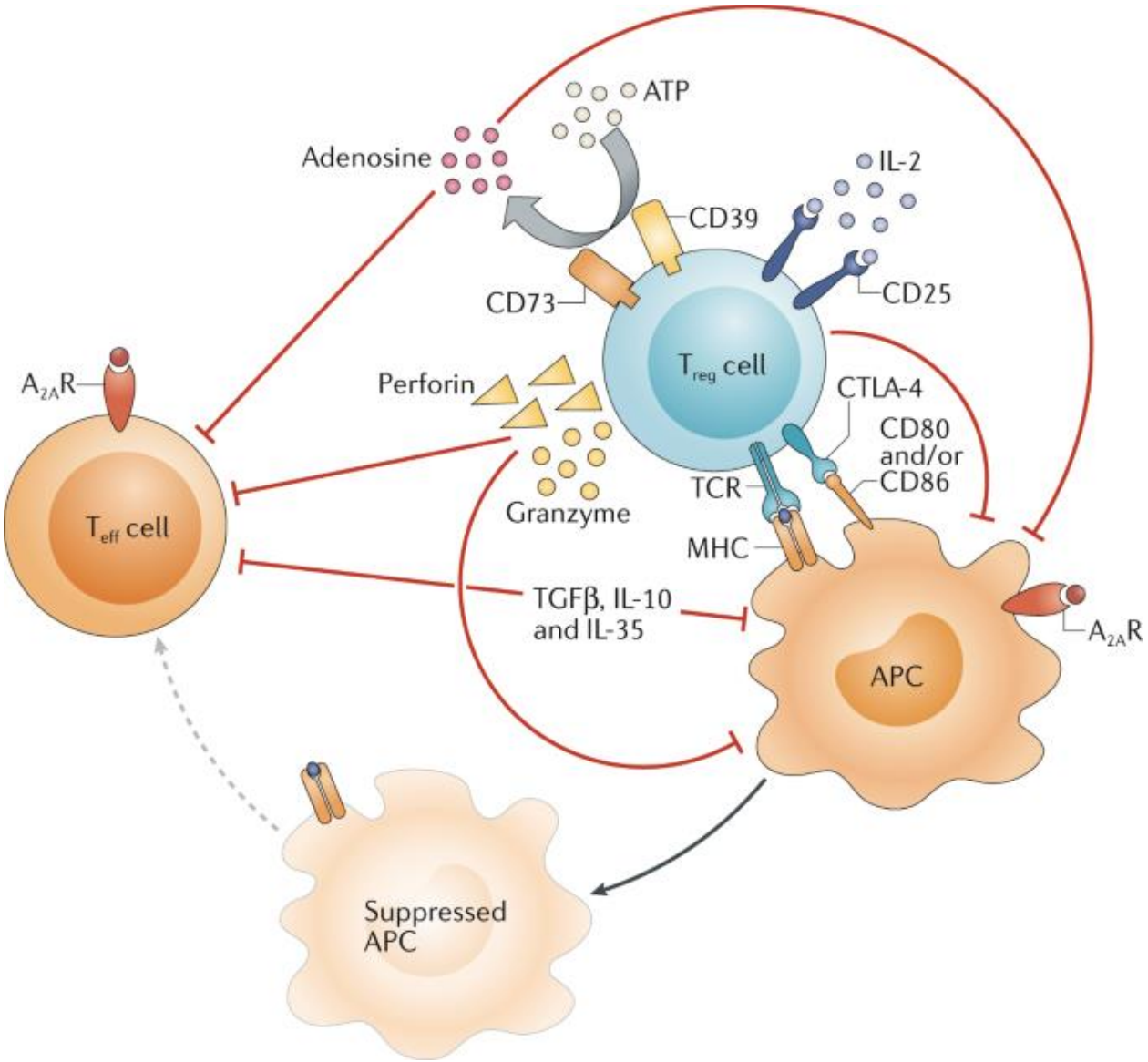


Alvisi et al., J Hepatol, 2022

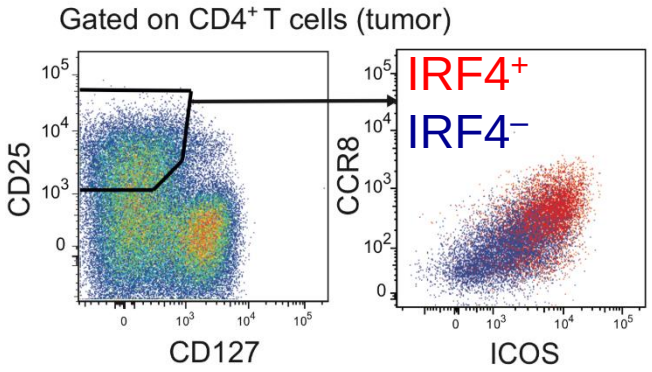


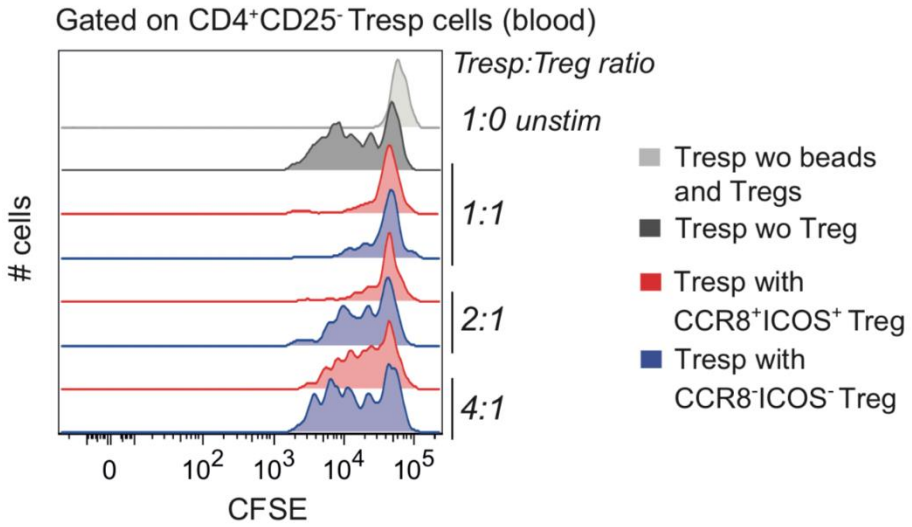
De Simone, Immunity, 2016

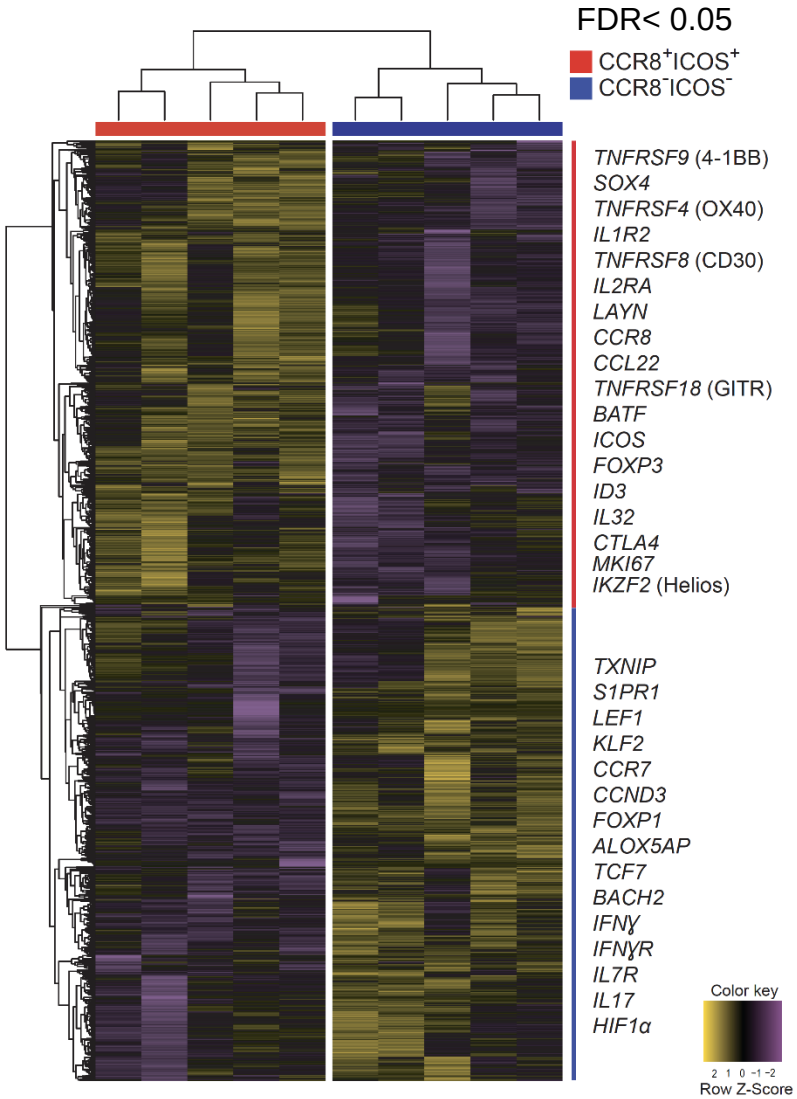
$CD4^+$ Tregs are abundant in tumors and suppress immunity by several mechanisms



Enhanced suppressive nature of IRF4^{+} Tregs

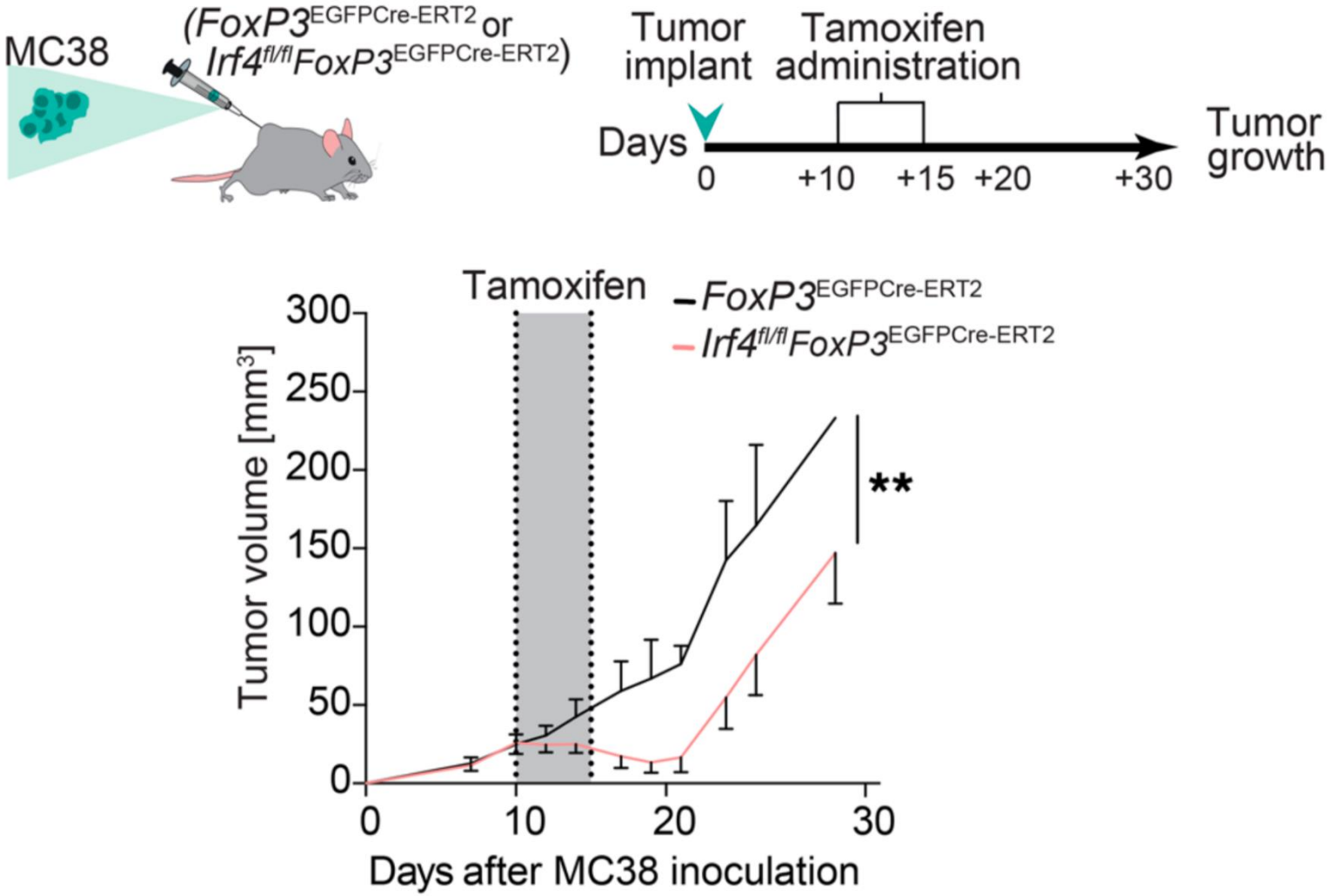






Alvisi et al., J Clin Invest, 2020

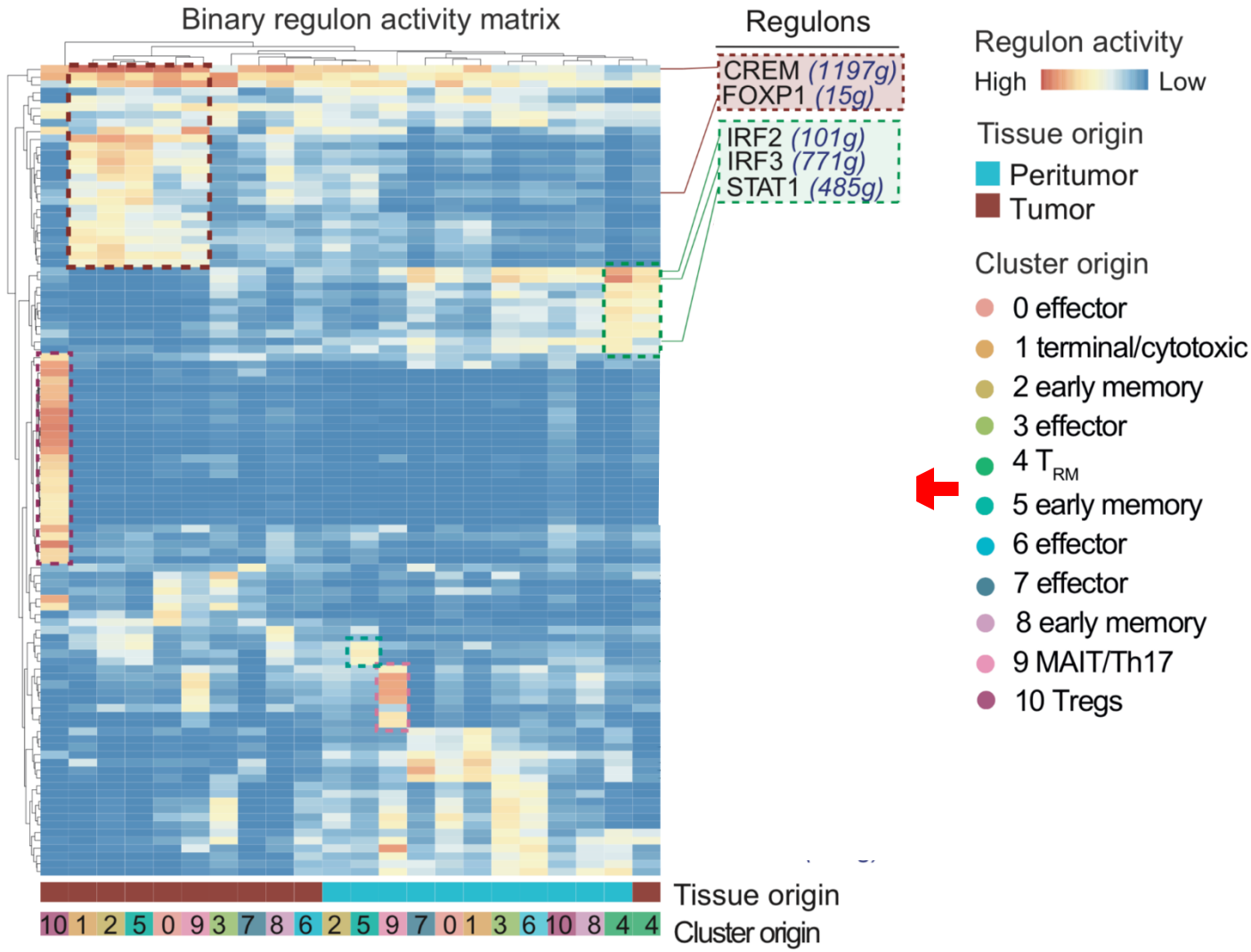
IRF4^{+} Tregs dampen anti-tumor T cell responses



with Jonas Blume and Axel Kallies, Univ of Melbourne

TF landscape of cholangiocarcinoma-infiltrating T cells

SCENIC computational algorithm predicts TF activity from analysis of promoters of expressed genes



Alvisi et al., J Hepatol, 2022

Mesenchyme homeobox-1 (MEOX1, MOX1, KFS2)

- Mesodermal transcription factor
- Regulates somitogenesis; **sclerotome development**
- **Cell cycle arrest** and endothelial cell senescence
- Mitotic transition and **proliferation** in cardiac fibroblasts

MEOX-1 mutations cause **Klippel-Feil Syndrome**

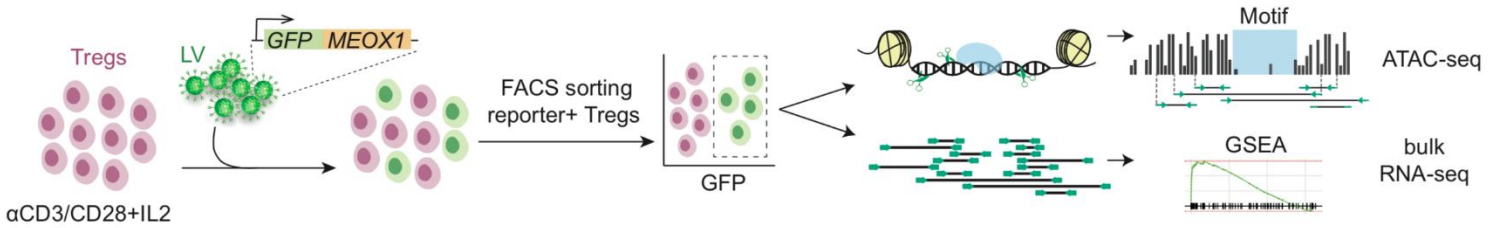
congenital fusion of cervical vertebrae

failure of normal segmentation of cervical somites

Function in the immune system is unknown

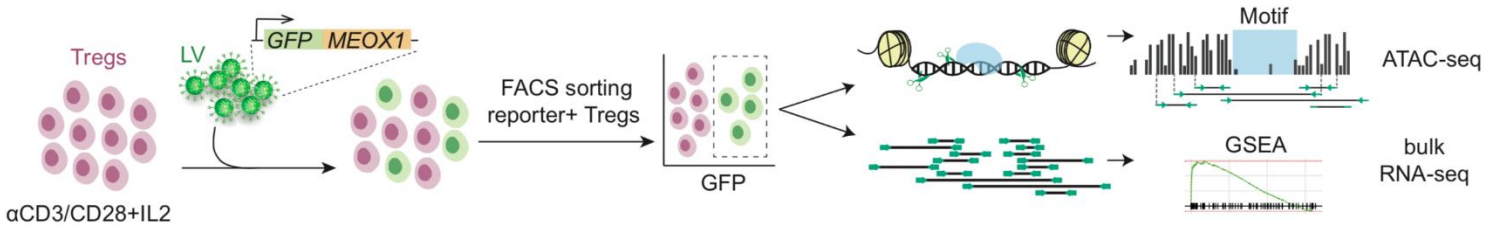
Toyoshima et al., Ped Neurol, 2006

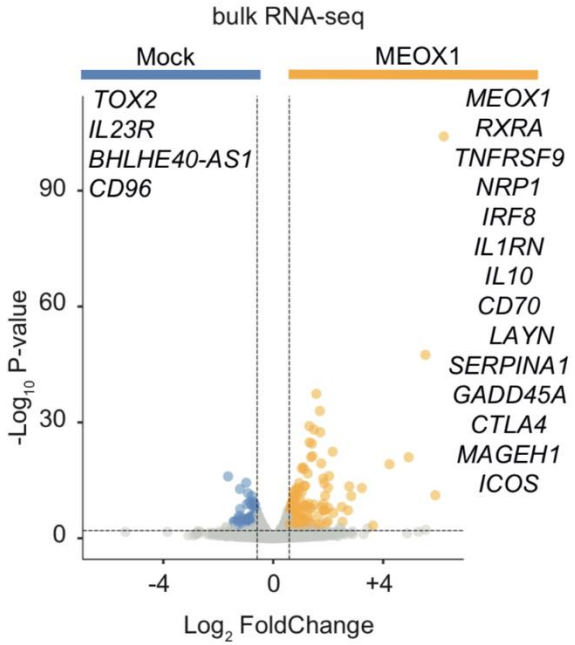
Mechanistic evaluation of MEOX-1 function



Alvisi et al., J Hepatol, 2022

MEOX1 OE favors accessibility to AP-1, BATF and IRF4



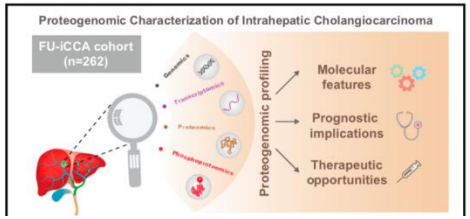


Alvisi et al., J Hepatol, 2022

MEOX1 gene program and iCCA prognosis

Proteogenomic characterization identifies clinically relevant subgroups of intrahepatic cholangiocarcinoma

Graphical abstract



Authors

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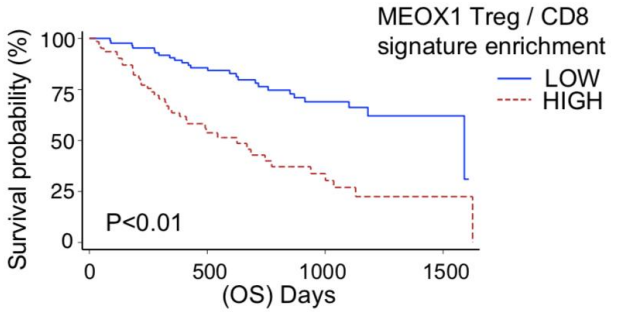
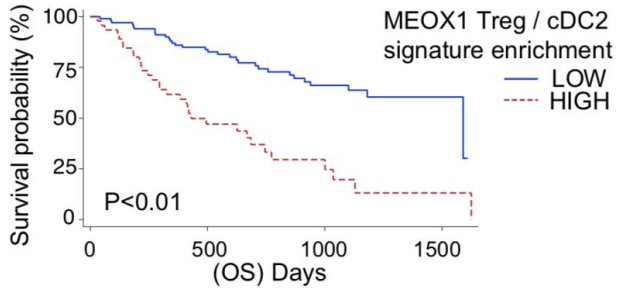
n=147 patients suitable for analysis





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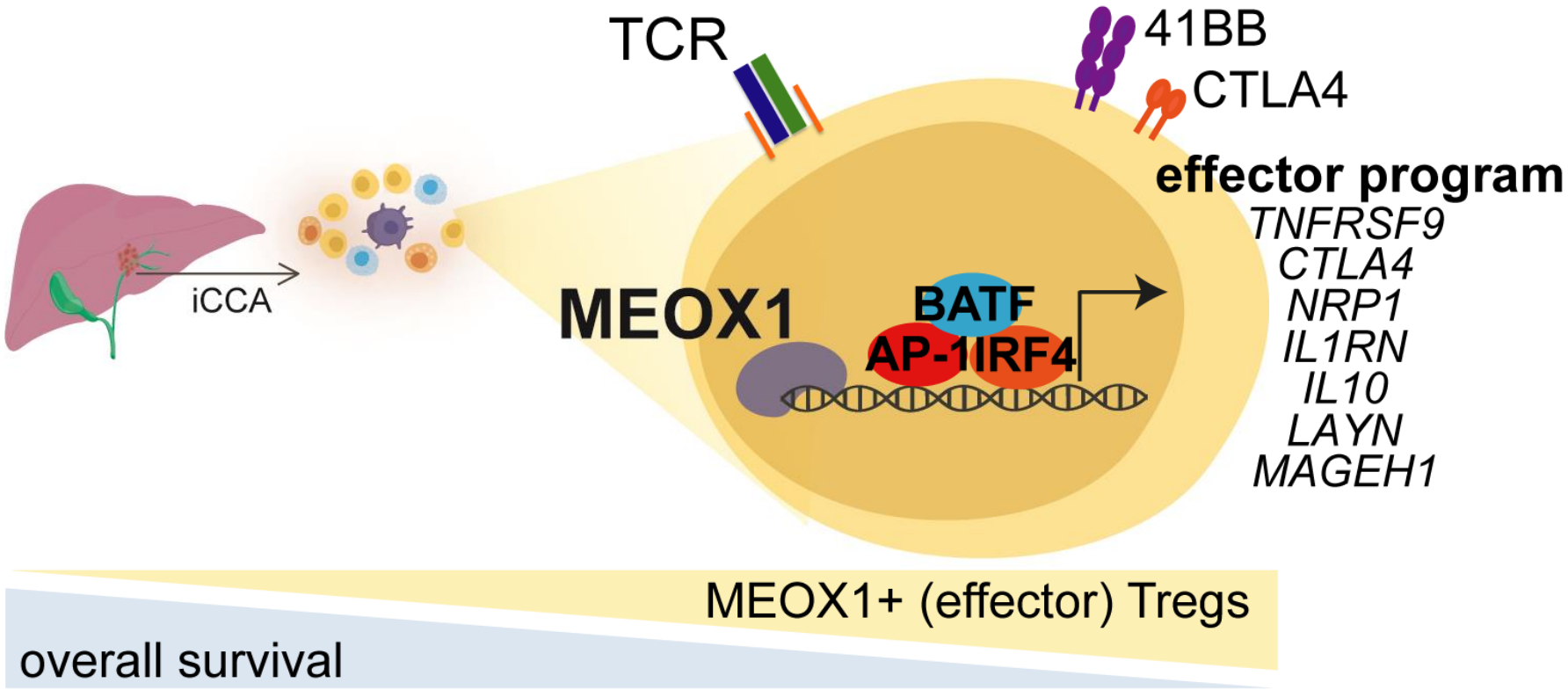
Immune landscape and tumor progression



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