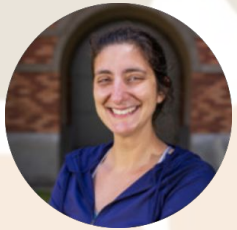
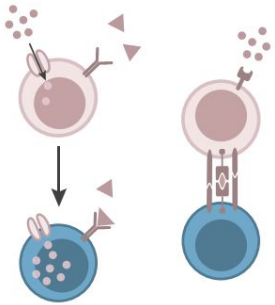
In-depth characterization of cell identity in the Tumor Microenvironment through Multimodal Single Cell Analysis

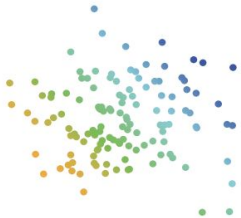


presented by Ana Ferreira
June 10th, 2020

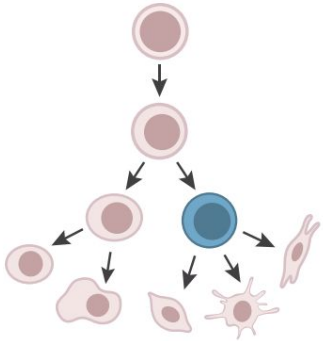
What shapes cell identity?

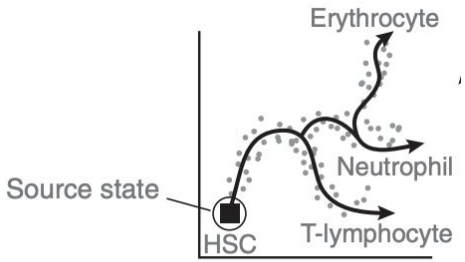
Environmental stimuli





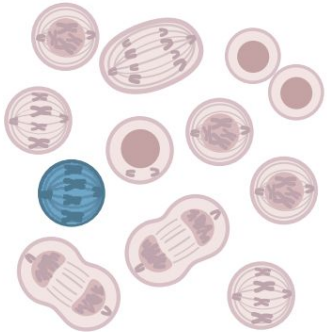
Cell development

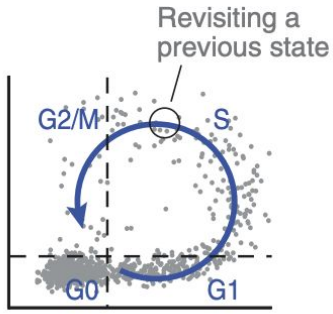




Unidirectional temporal progression

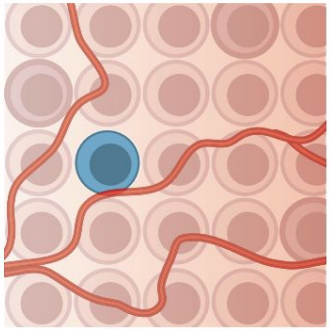
Cell cycle

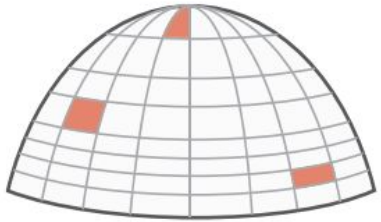




State vacillation

Spatial context





Spatial position

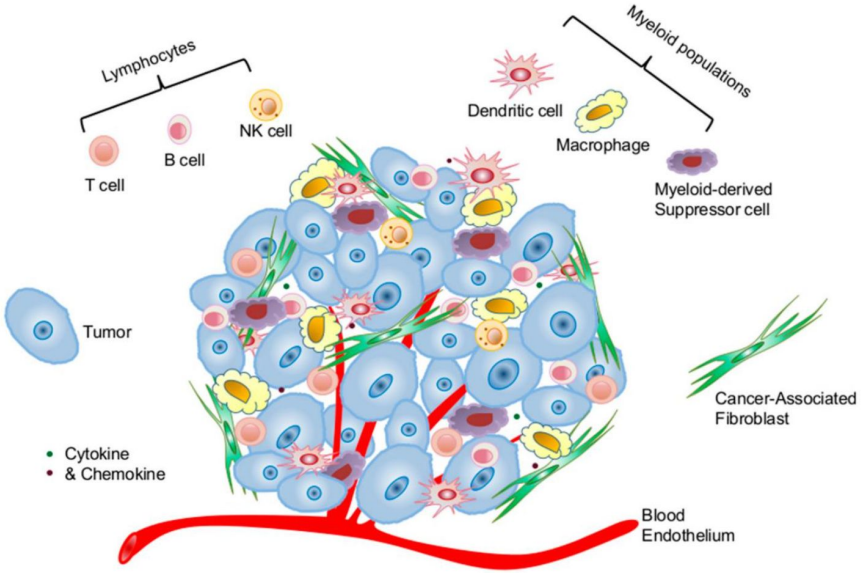
Wagner et al (”Revealing the vectors of cellular identity with single-cell genomics”. *Nature Biotechnology*, 2016. Vol 34 (11):1145-1160)

Why focus on the TME?

Complex and dynamic system that requires a **systems biology** approach

It is a unique system that has a major impact on the **efficacy of therapy**: hypoxia, T cell exhaustion, activation, and differentiation

It is comprised of multiple cellular components that play different interdependent roles in the immune response



Hirata and Shai, Cold Spring Harb Perspect Med. 2017; Wang *et al.*, J Cancer 2017.

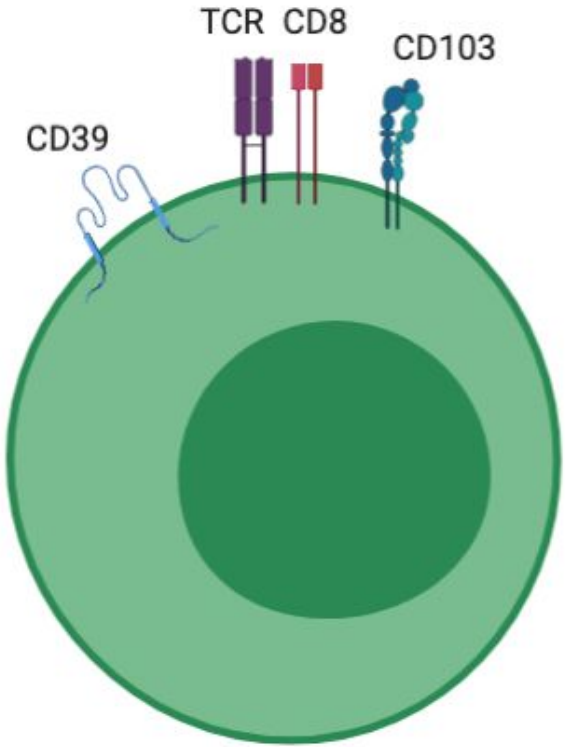
Trm cells: modulators of checkpoint blockade?

$CD8^+$ Trm cells express a high level of ICB and effector proteins: key targets of modulation by the immune checkpoint inhibition [1]

CD103 (subunit of αEβ7 integrin) binds to E-cadherine on epithelial cells [2]

CD39 is expressed by activated T cells; immunosuppressive environment; marker for exhausted phenotype in viral infections [3][4]

Tumor reactive ($CD39^+CD103^+$) CD8 T cells are associated with better outcome and survival in HN and lung patients; have a distinct TCR repertoire and efficiently kill in a MHC-I dependent manner [5]

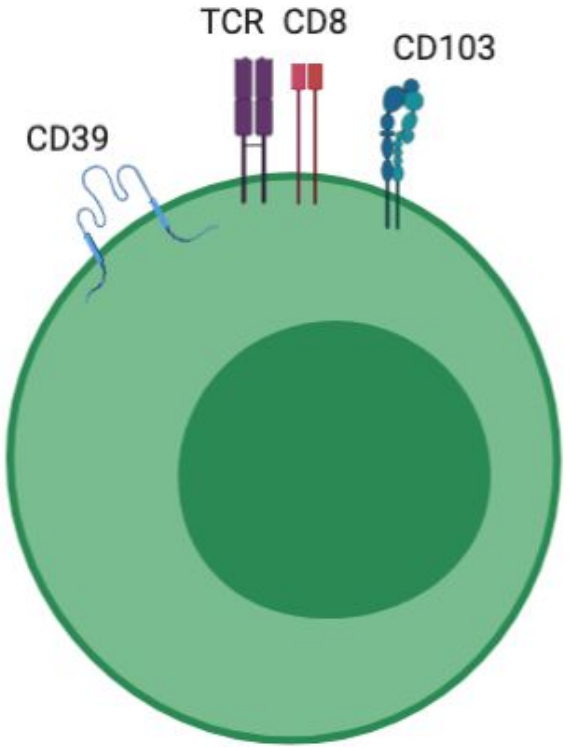


Trm cells: modulators of checkpoint blockade?

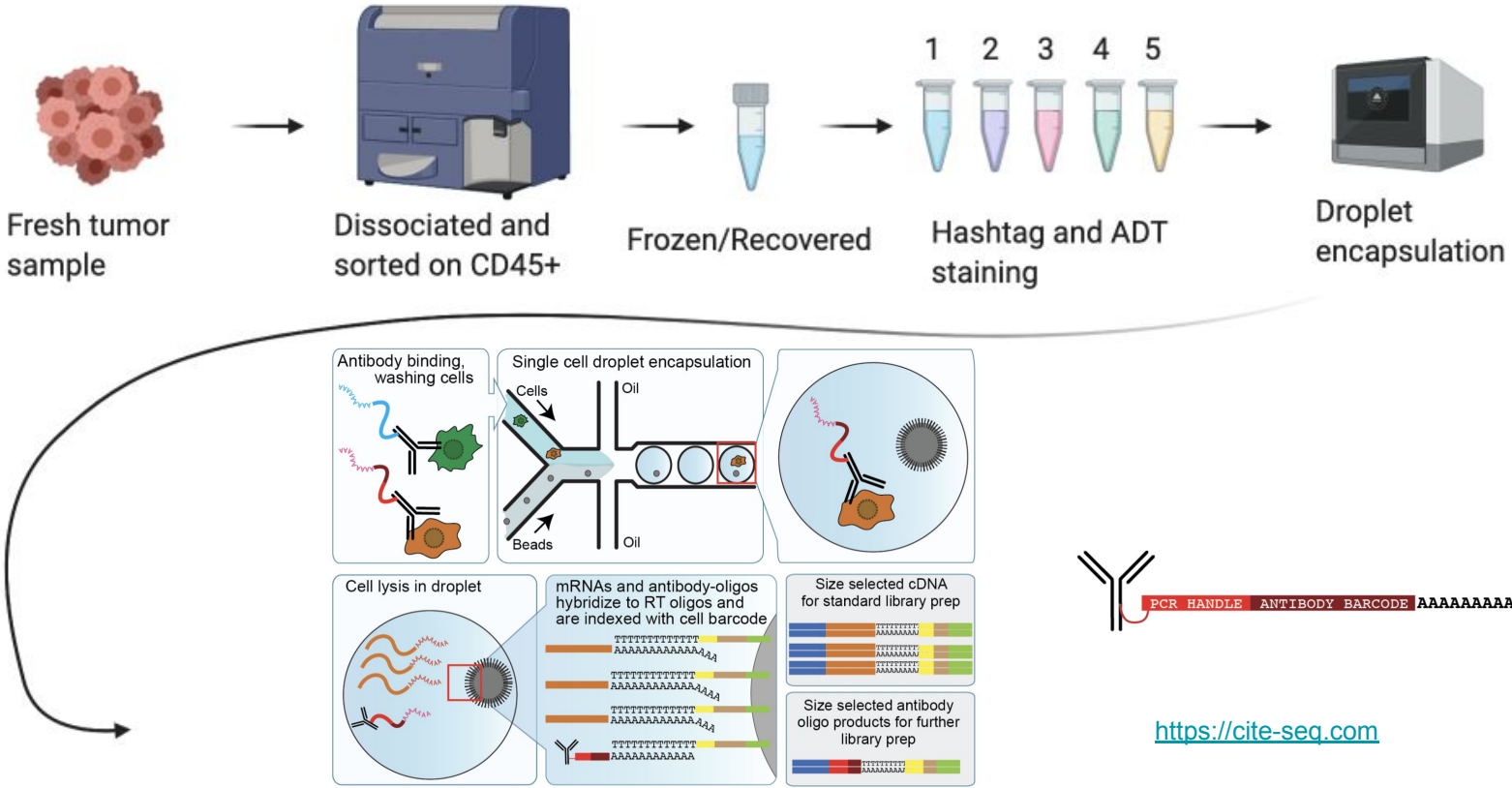
Main goal of this project is to fully characterize “double positive” cell populations in the TME

Question for this experiment: What is the identity of the T cell population with highest clonotype in the TME of lung patients?

Proof-of-concept: will this multimodal approach answer our initial questions and beyond?

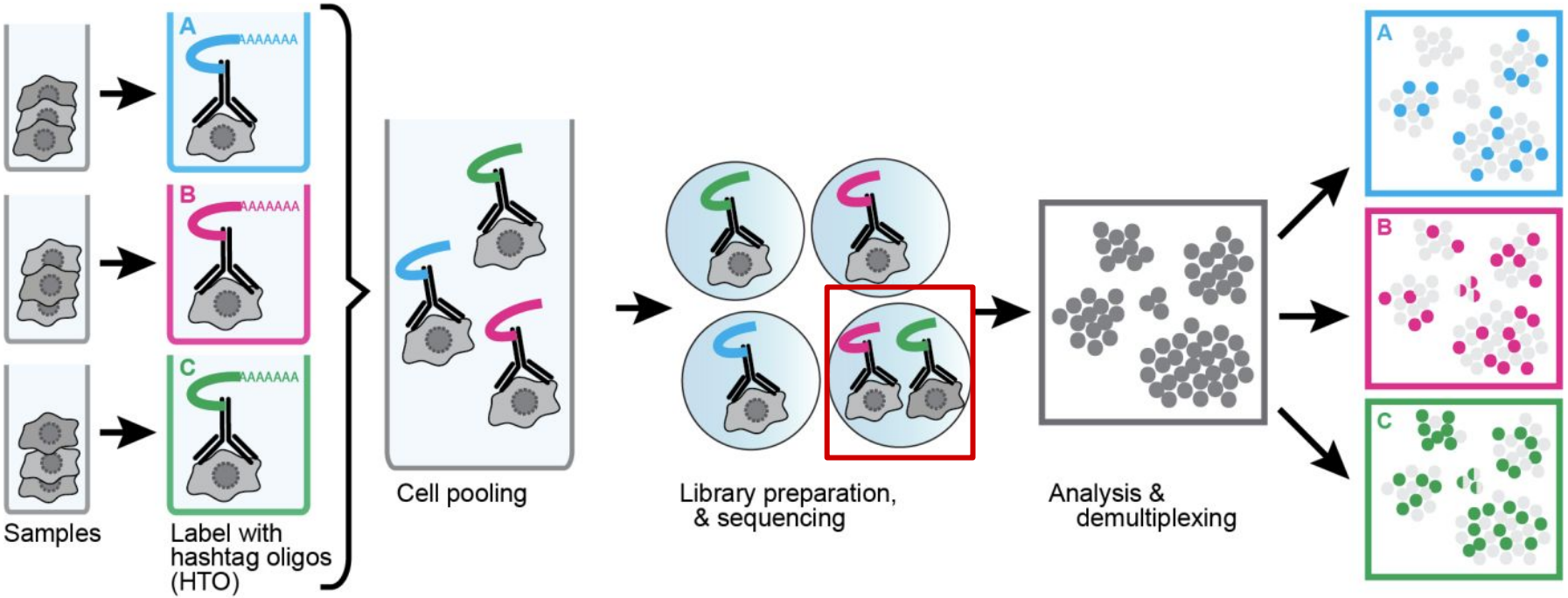


Droplet based capture



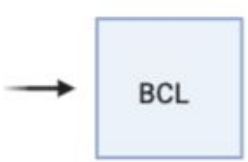
https://cite-seq.com

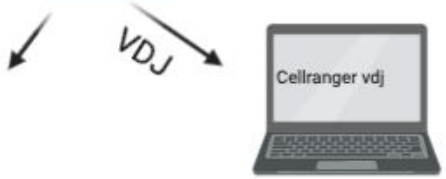
Sample multiplexing schematic with hashtag

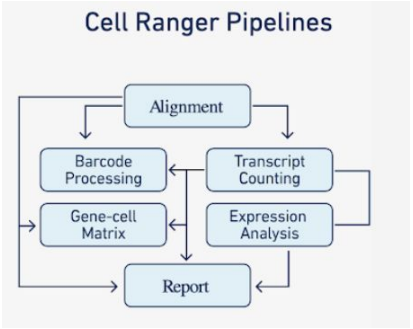


https://cite-seq.com/cell-hashing/

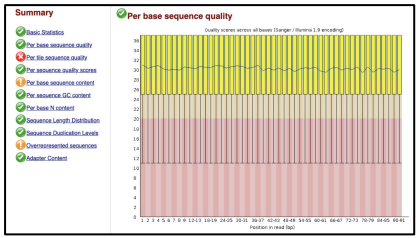
Cellranger pre-processing workflow and QC

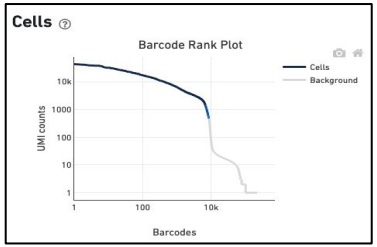




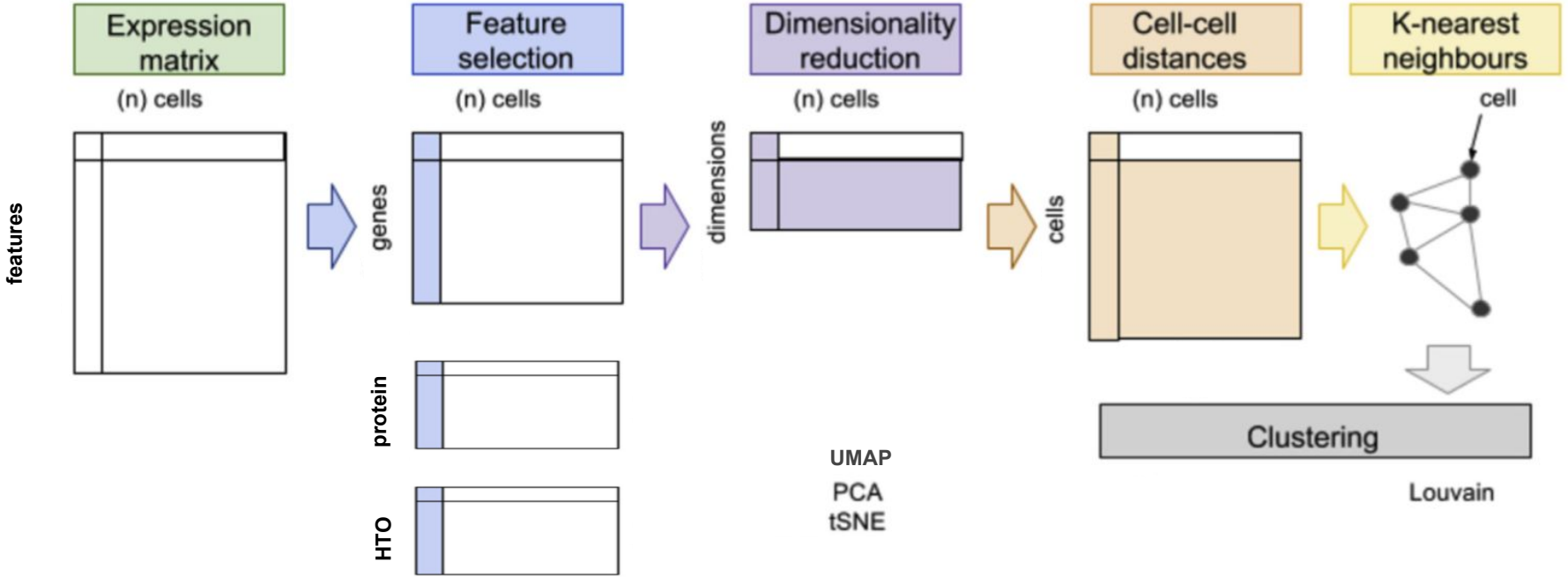


QC steps





Overall design of single cell analysis pipelines

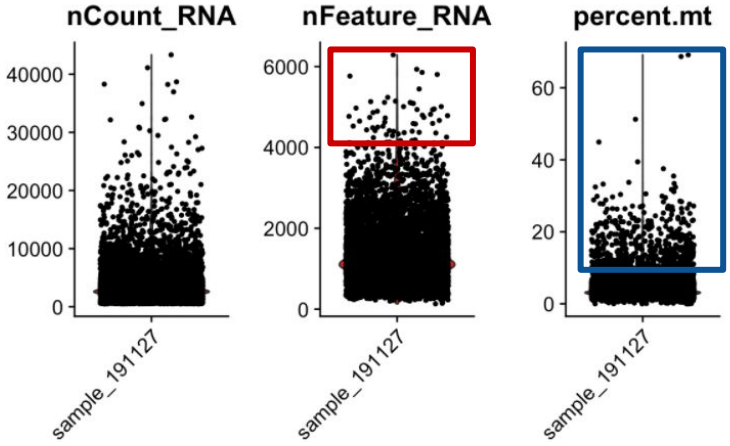


Adapted from Tallulah & Hemberg (”Identifying Cell Populations with scRNA-seq”. *Molecular Aspects of Medicine, 59 (February 1, 2018): 114-22.*

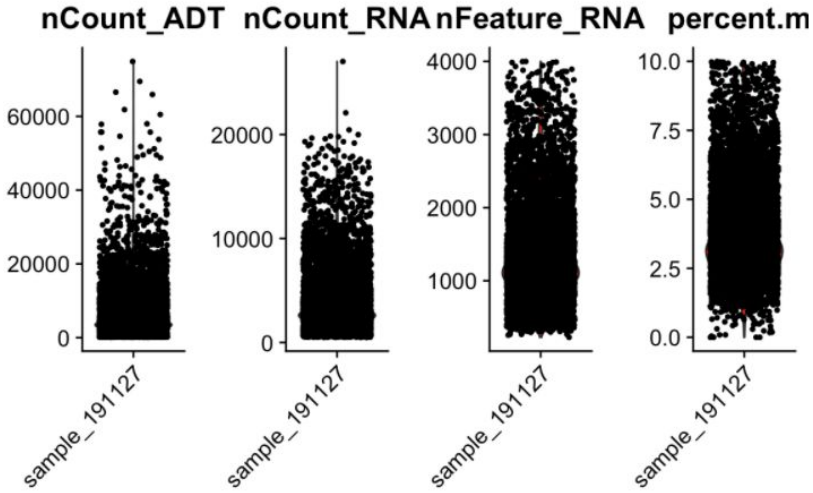
Analysing the data with Seurat: viability and integrity

doublets

Non-viable cells

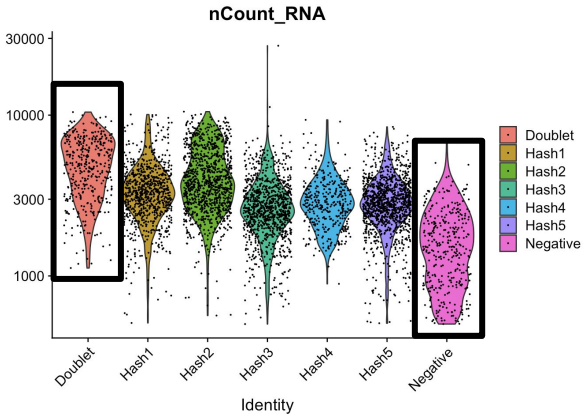


Before filtering

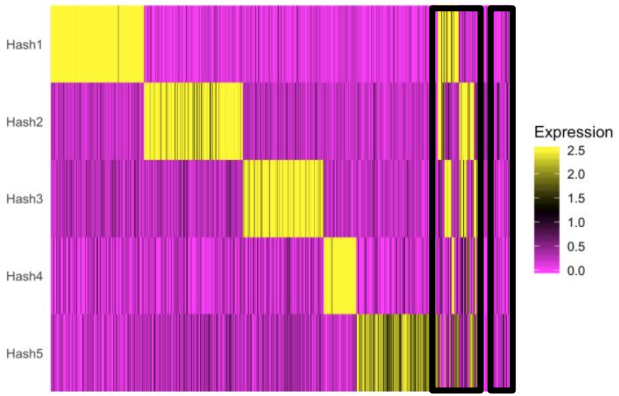


After filtering

Analysing the data with Seurat: doublet removal



Barcode distribution based on HTO



Prior to doublet removal

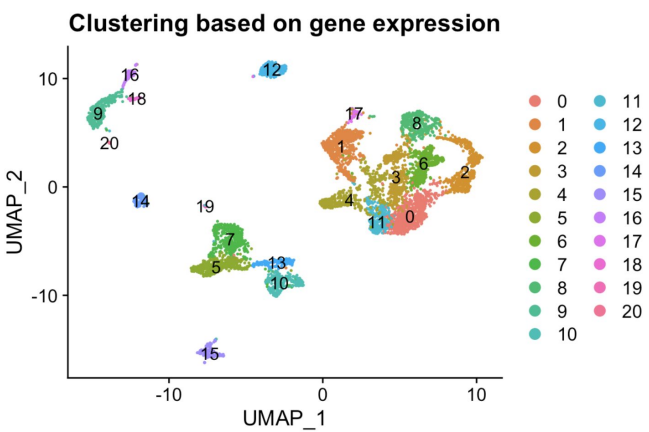
After doublet removal

Analyzing the data with Seurat: clustering

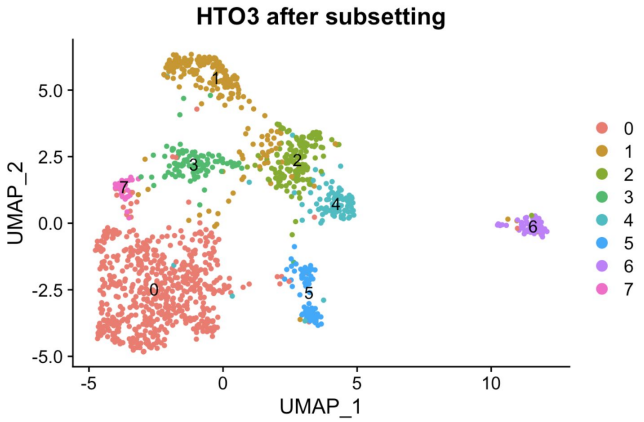
Apply **graph-based clustering** by building a shared nearest-neighbors graph (KNN)

Cluster using a **modularity optimizer** (Louvain algorithm)

Generate UMAP plots for HTO and gene expression



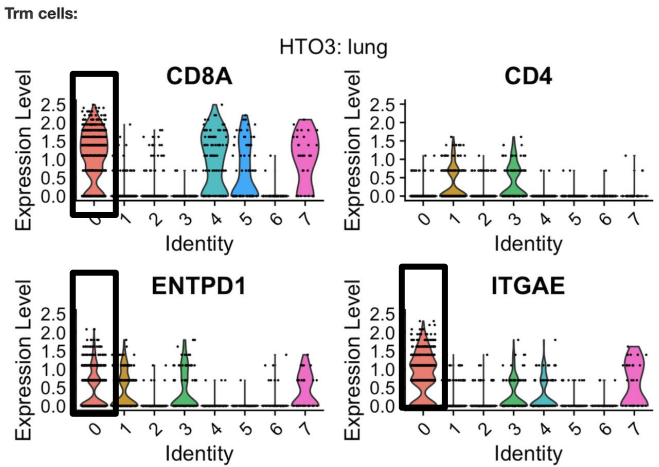
All samples



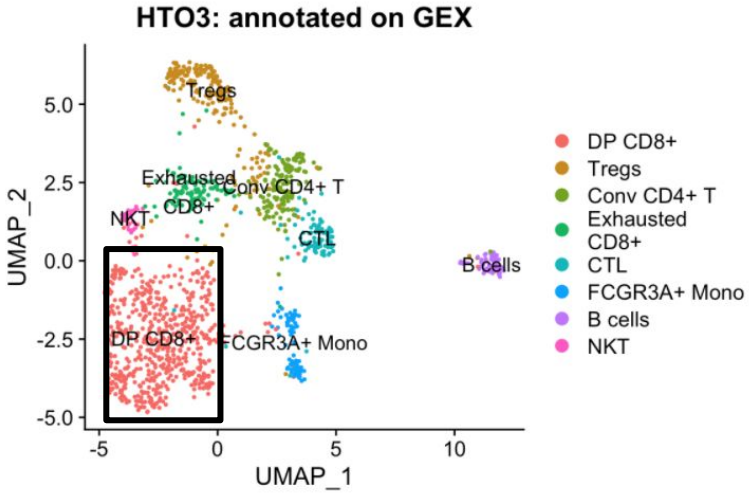
HTO3 only: lung

Analysing the data with Seurat: gene expression

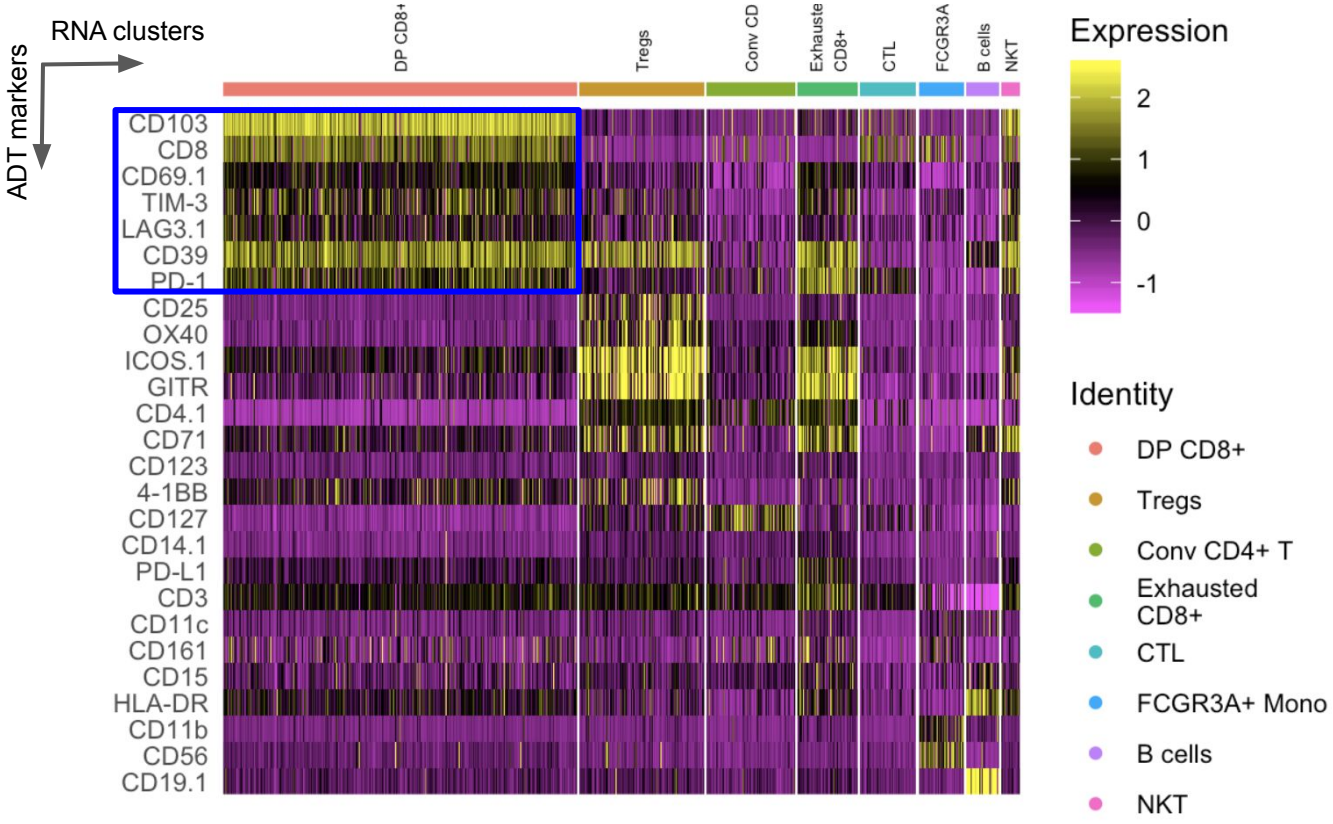
Manual annotation of clusters based on gene signatures: **one-vs-all marker** genes

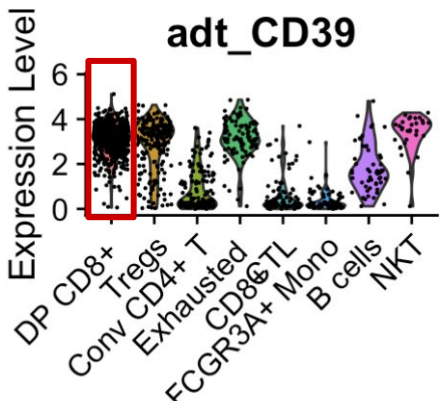


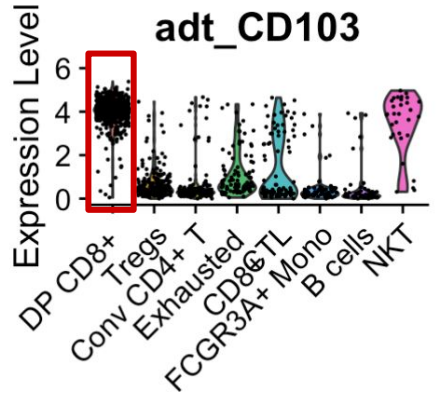
CD39 CD103



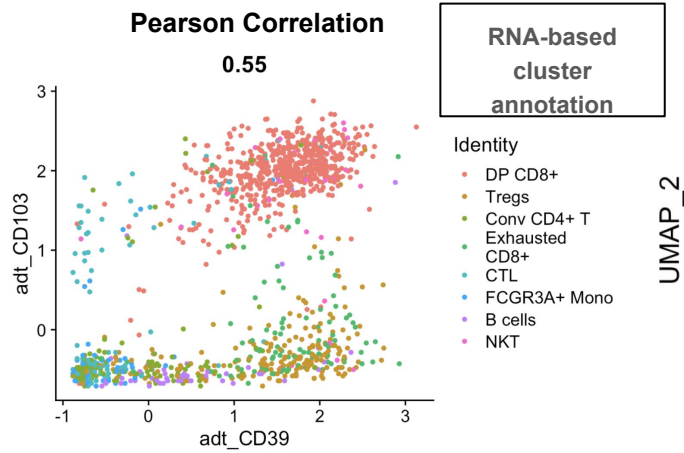
Analysing the data with Seurat: protein expression

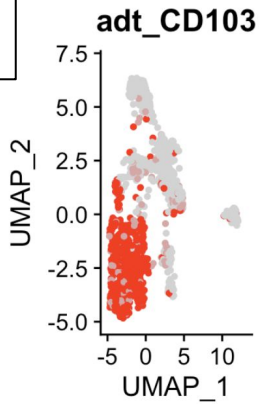


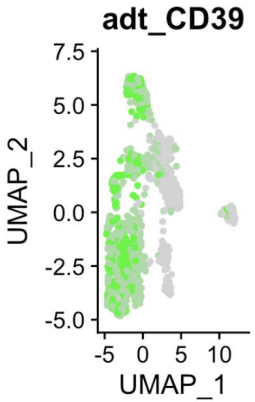


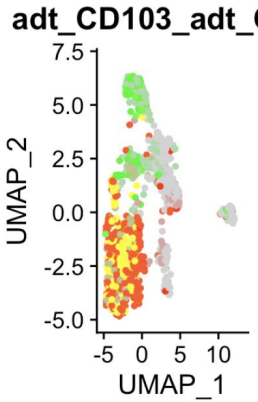


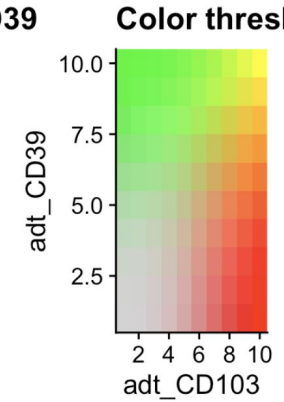
Analysing the data with Seurat: protein expression





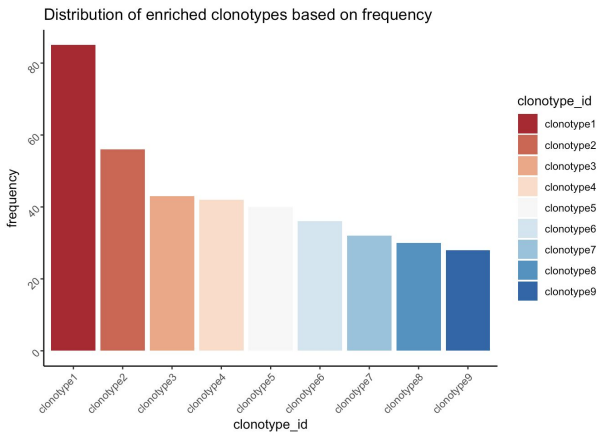


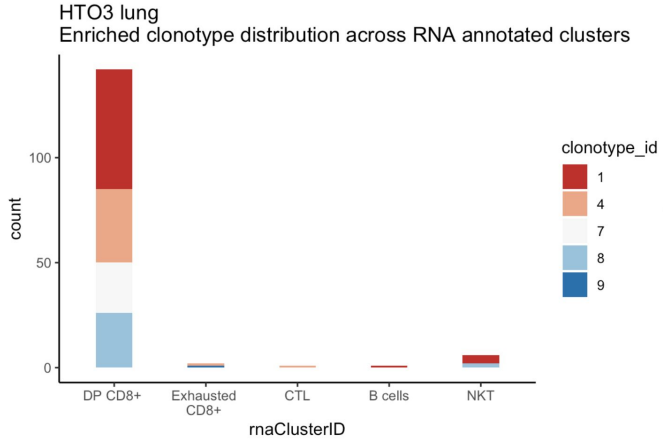


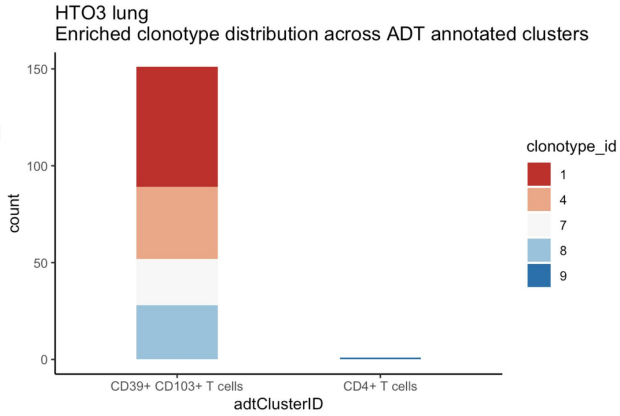


Overlap expression

Analysing the data with Seurat: clonotype

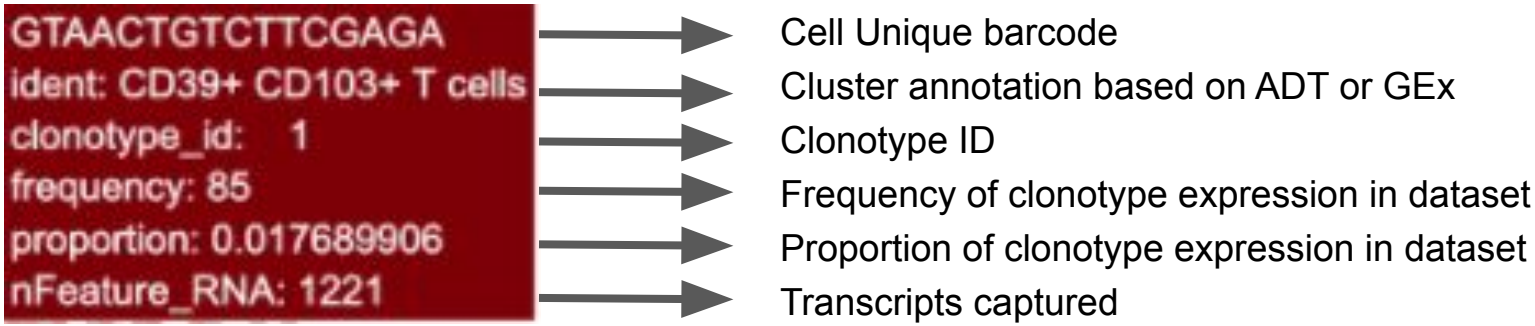




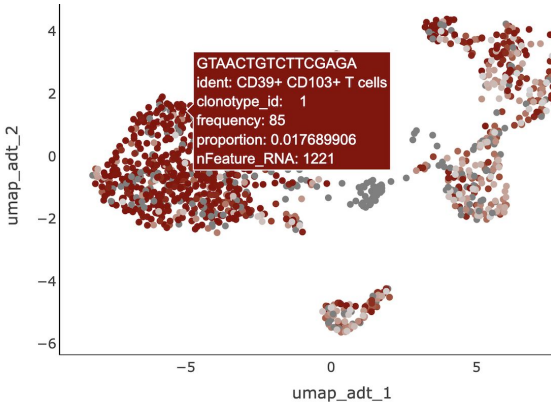


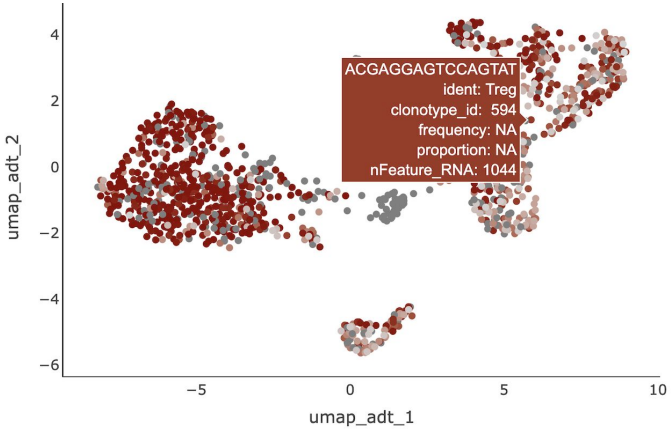
(CD39^{+}CD103^{+}) CD8 T cells

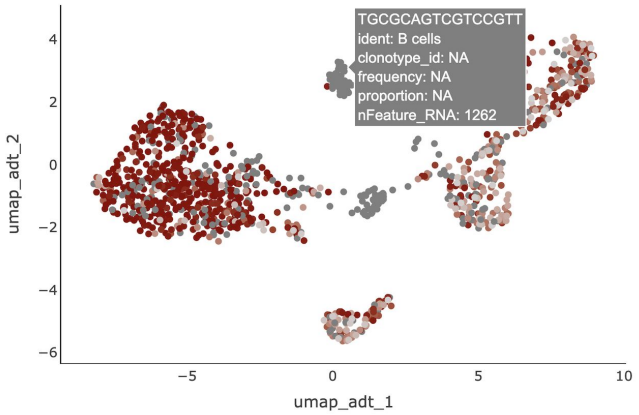
Measuring multiple modalities from same cell



Generating and “Identity card” for cells







(CD39^{+}CD103^{+}) CD8 T cells

Treg cells

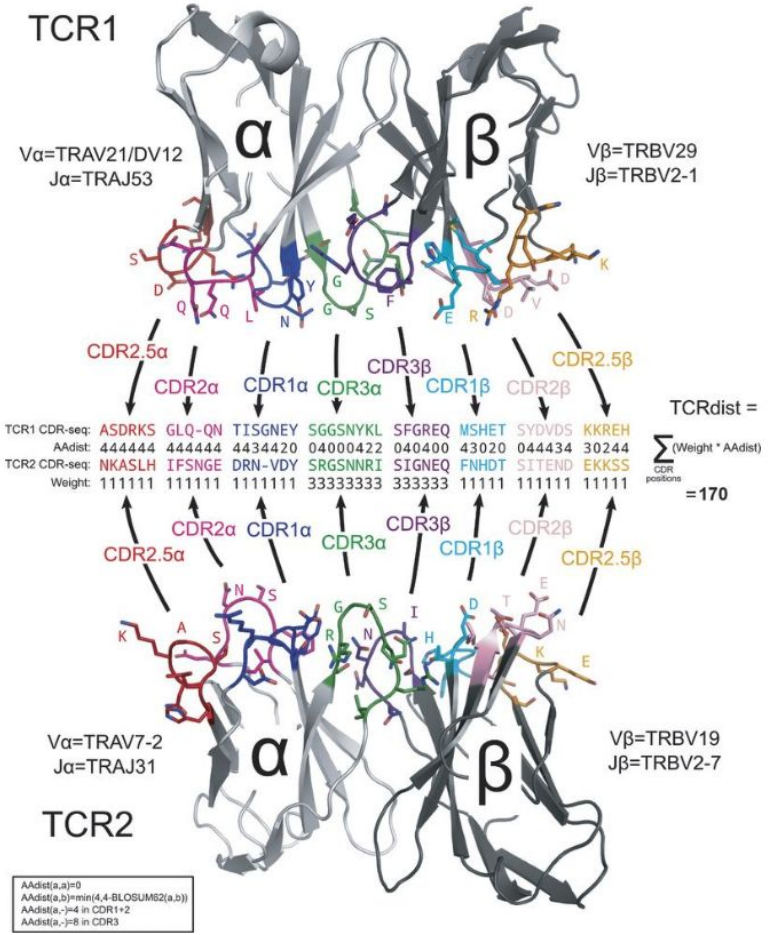
B cells

Next steps

Continuous iteration and future directions for this single cell project:

Perform Differential Expression analysis, cell trajectory and TCR clonotype studies to investigate **how these subsets arise**

Applications: predicting antigen-specificity based on TCR-seq for adoptive T cell therapy; assessing what gives rise to persisting T cells upon transfer or functioning T cells upon immunotherapy treatment



https://tcrdist2.readthedocs.io/en/latest/#

Acknowdlegments

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Immune Monitoring lab

Yoshi Yoshinobu
Tanisha Christie
William Miller

Redmond lab

William Redmond

Citations

[1] Peter Savas, Balaji Virassamy, Chengzhong Ye, Agus Salim, Christopher P. Mintoff, Franco Caramia, et al. “Single-Cell Profiling of Breast Cancer T Cells Reveals a Tissue-Resident Memory Subset Associated with Improved Prognosis.” *Nature Medicine* 24, no. 7 (July 2018): 986–93. https://doi.org/10.1038/s41591-018-0078-7.

[2] Webb, J. R., K. Milne, P. Watson, R. J. deLeeuw, and B. H. Nelson. “Tumor-Infiltrating Lymphocytes Expressing the Tissue Resident Memory Marker CD103 Are Associated with Increased Survival in High-Grade Serous Ovarian Cancer.” *Clinical Cancer Research* 20, no. 2 (January 15, 2014): 434–44. https://doi.org/10.1158/1078-0432.CCR-13-1877.

[3] Bastid, J, A Cottalorda-Regairaz, G Alberici, N Bonnefoy, J-F Eliaou, and A Bensussan. “ENTPD1/CD39 Is a Promising Therapeutic Target in Oncology.” *Oncogene* 32, no. 14 (April 2013): 1743–51. https://doi.org/10.1038/onc.2012.269.

[4] Allard, David, Bertrand Allard, and John Stagg. “On the Mechanism of Anti-CD39 Immune Checkpoint Therapy.” *Journal for ImmunoTherapy of Cancer* 8, no. 1 (February 2020): e000186. https://doi.org/10.1136/jitc-2019-000186.

[5] Duhen, Thomas, Rebekka Duhen, Ryan Montler, Jake Moses, Tarsem Moudgil, Noel F. de Miranda, Cheri P. Goodall, et al. “Co-Expression of CD39 and CD103 Identifies Tumor-Reactive CD8 T Cells in Human Solid Tumors.” *Nature Communications* 9, no. 1 (December 2018): 2724. https://doi.org/10.1038/s41467-018-05072-0.