

ECBME4060 Final Project

Insights from Public Single-cell RNA Data to Identify Biomarkers in Mycosis Fungoides

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Neema Nkонтchou (nn2381), Sararose Suhl (scs2256)

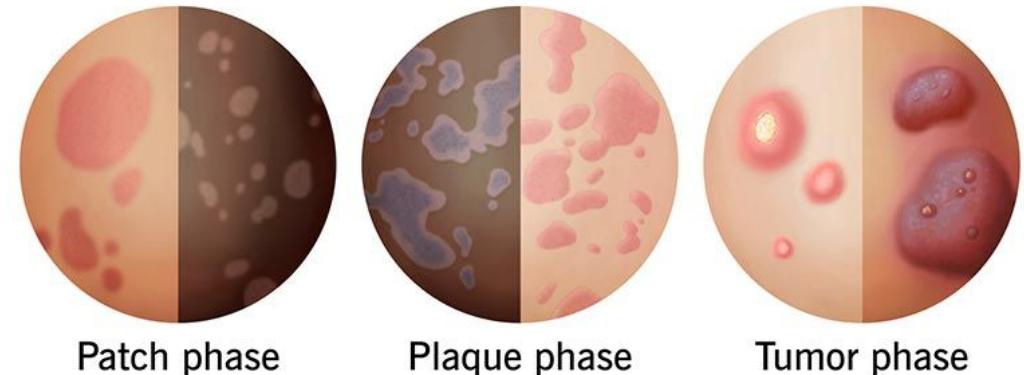
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What is Mycosis Fungoides?

- Mycosis Fungoides (MF) is the most common subtype of Cutaneous T-Cell Lymphoma (CTCL)
- Histopathology is characterised by infiltrates of malignant resident memory T-cells (TRMs)
- Clinical stages (patch, plaque, tumour) correlate with the progressive density of malignant T-cells.
- Unknown etiology, no cure

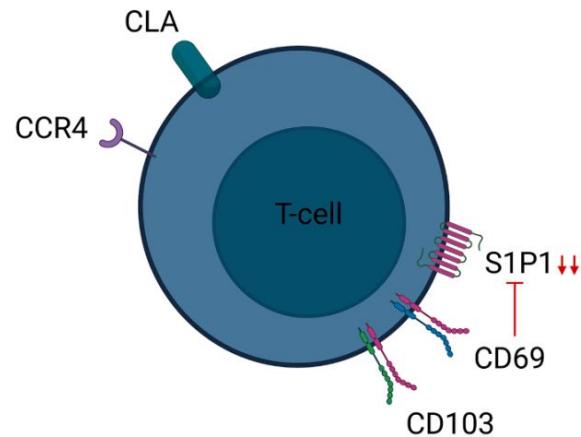
Mycosis Fungoides
(A type of cutaneous T-cell lymphoma)



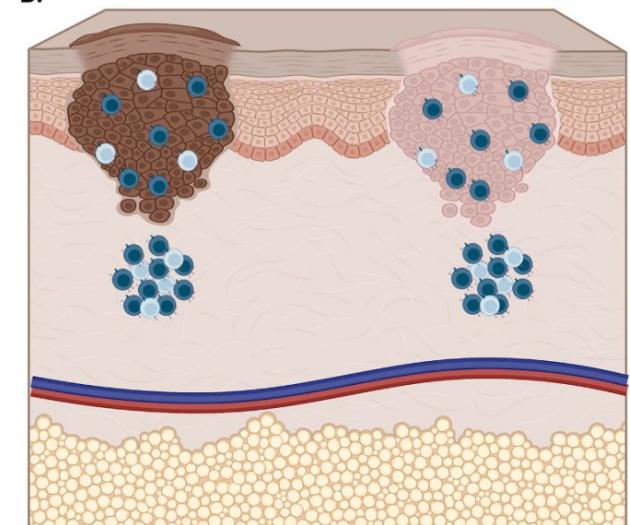
Cleveland Clinic ©2023

<https://my.clevelandclinic.org/health/diseases/21827-mycosis-fungoides>

A.



B.



(Desse et al., 2023)

Why Focus on Mycosis Fungoides (MF)?

Problem Statement

- MF exhibits a wide range of clinical presentations and can be associated with significant morbidity and mortality
- MF's similar appearance to benign inflammatory dermatoses (i.e. psoriasis and atopic dermatitis) lead to misdiagnosis and/or delayed diagnoses
- Some treatments for BID have been associated with worse outcomes in CTCL, making proper diagnosis essential
- **Lack of specific biomarkers challenges diagnosis, prognostication, and treatment.**

Psoriasis



Mycosis Fungoides



Atopic Dermatitis

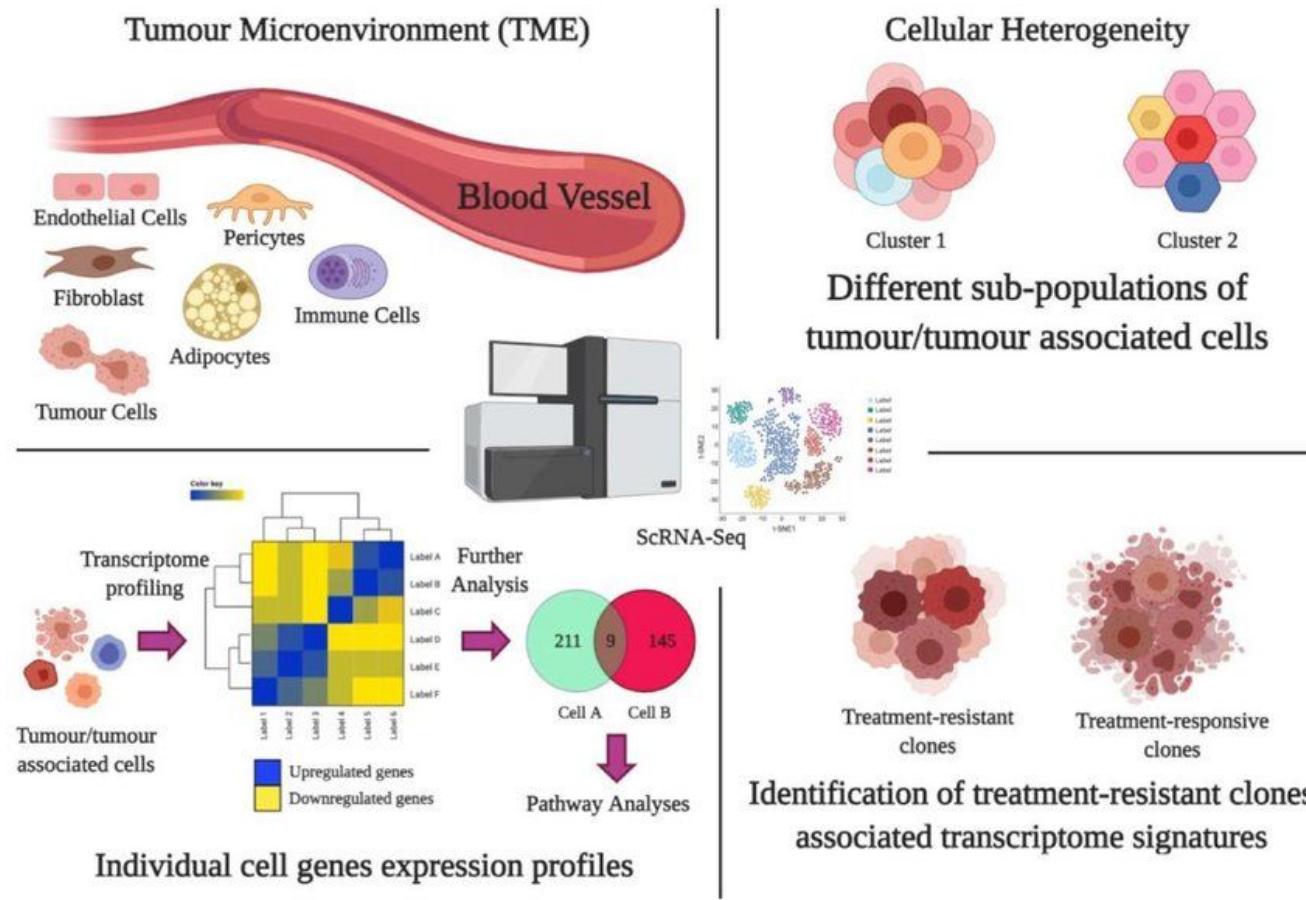


Mycosis Fungoides



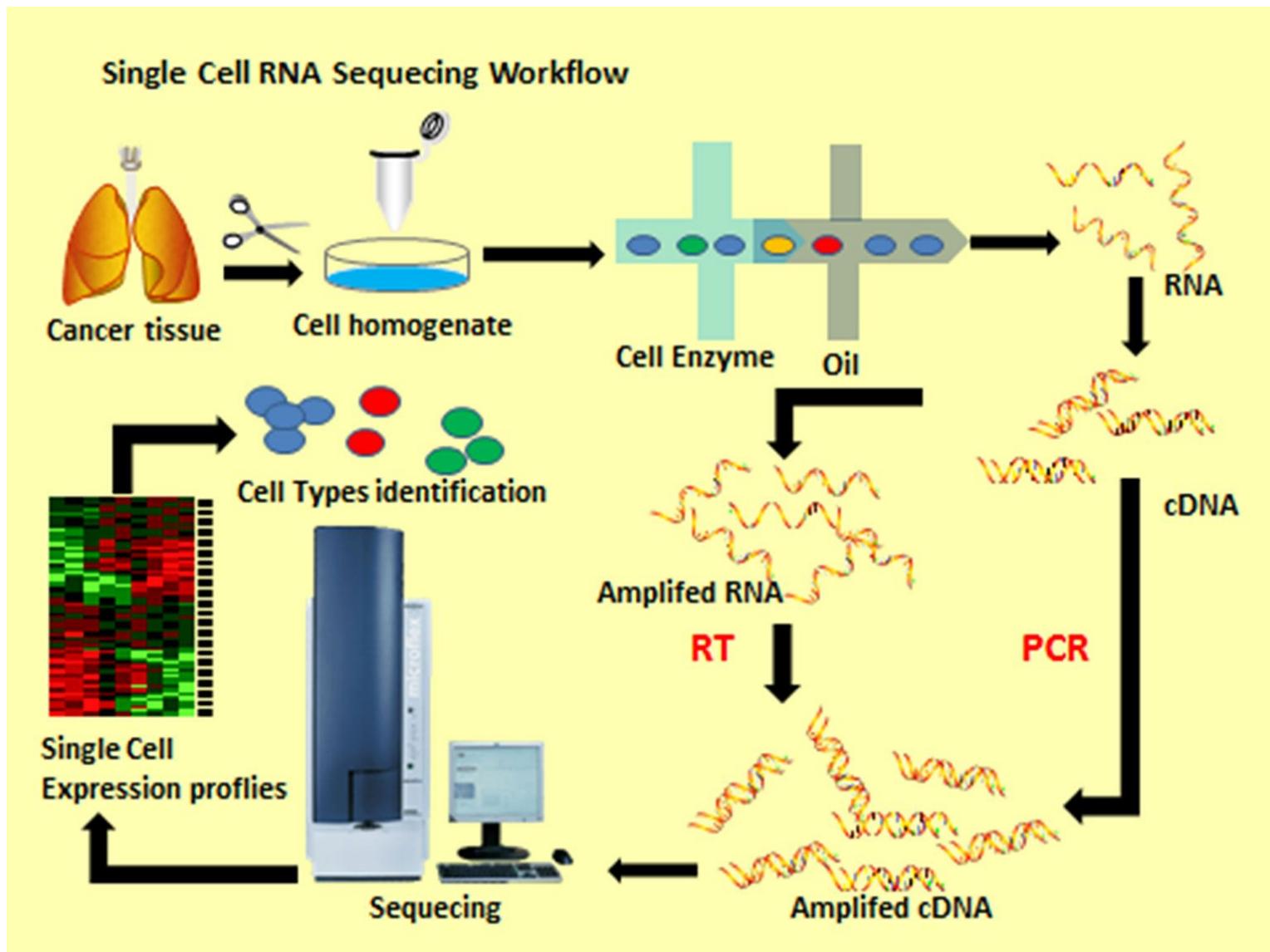
Proposed Solution

Use **scRNA-seq** to uncover molecular biomarkers for early detection, treatment targeting, and monitoring.



Single cell RNA Sequencing Workflow

1. Sample Preparation
2. Single-Cell Isolation
3. Cell Lysis and RNA Capture
4. Reverse Transcription and cDNA Synthesis
5. Library Preparation
6. High-Throughput Sequencing
7. Data Processing and Analysis



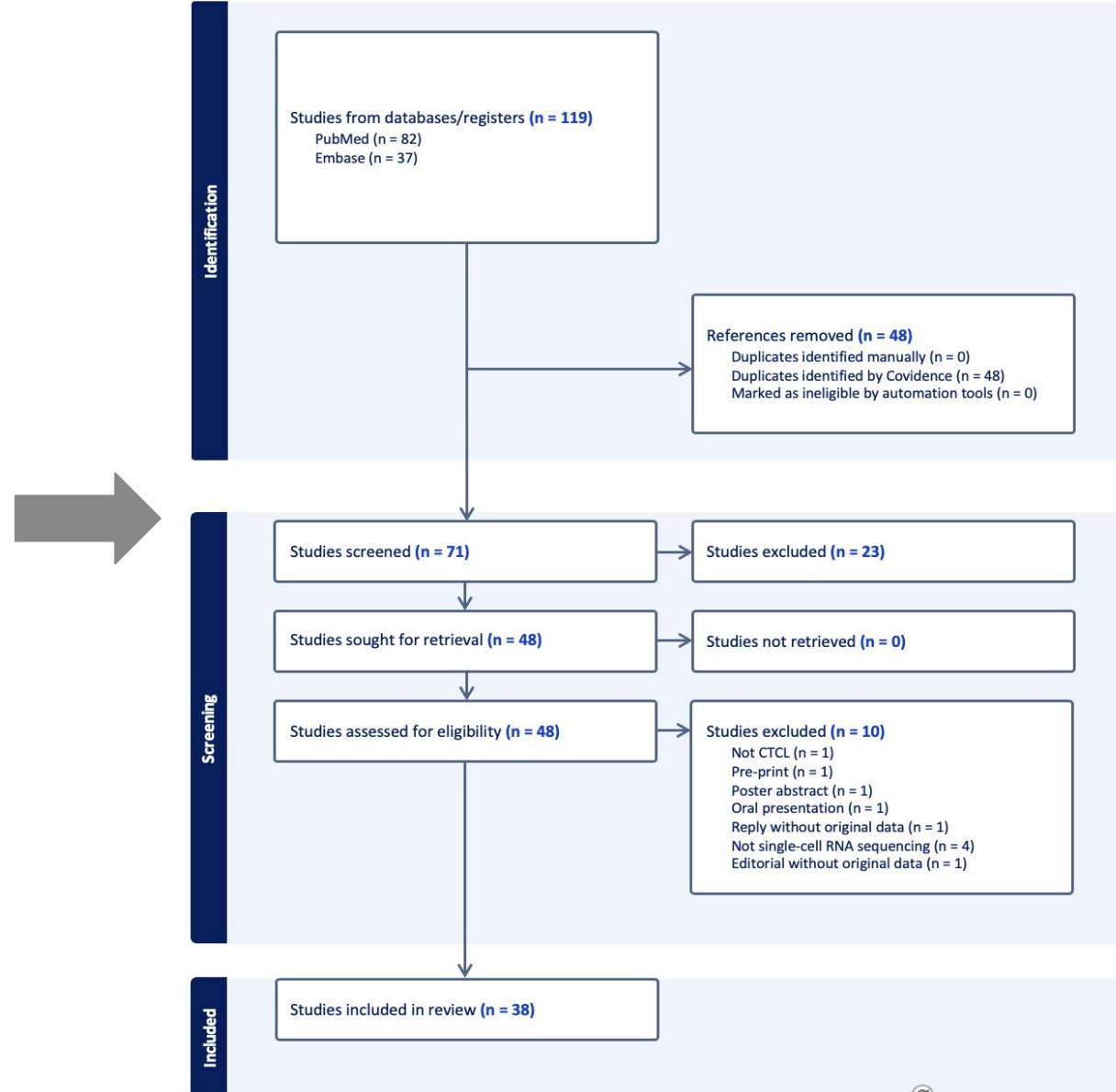
(Liu et al., 2021)

Dataset Acquisition

- Comprehensive search of PubMed and EMBASE databases for studies using single-cell RNA to study CTCL
- 38 studies were identified

Data Overview:

- 19 Studies of MF → 16 Unique Datasets → 13 Patients
- Datasets analyzed: GSE128531, GSE165623, ~~GSE182861~~
- Sample Types: Primarily skin biopsies, additional blood and lymph node biopsies



Overview of Datasets

Gaydosik et al., 2019

Single-Cell Lymphocyte Heterogeneity in Advanced Cutaneous T-cell Lymphoma Skin Tumors

Alyxzandria M Gaydosik ¹, Tracy Tabib ¹, Larisa J Geskin ², Claire-Audrey Bayan ²,
James F Conway ³, Robert Lafyatis ¹, Patrizia Fuschiotti ⁴

Affiliations + expand

PMID: 31010835 PMCID: [PMC6635080](#) DOI: [10.1158/1078-0432.CCR-19-0148](https://doi.org/10.1158/1078-0432.CCR-19-0148)

Rindler et al., 2021

Single-Cell RNA Sequencing Reveals Tissue Compartment-Specific Plasticity of Mycosis Fungoides Tumor Cells

	Katharina Rindler ^{1†}		Wolfgang M. Bauer ^{1†}		Constanze Jonak ¹
	Matthias Wielscher ¹		Lisa E. Shaw ¹		Thomas B. Rojahn ¹
	Felix M. Thaler ¹		Stefanie Porkert ¹		Ingrid Simonitsch-Klupp ²
	Wolfgang Weninger ¹		Marius E. Mayerhoefer ^{3,4}		Matthias Farlik ^{1†}
	Patrick M. Brunner ^{1†}				

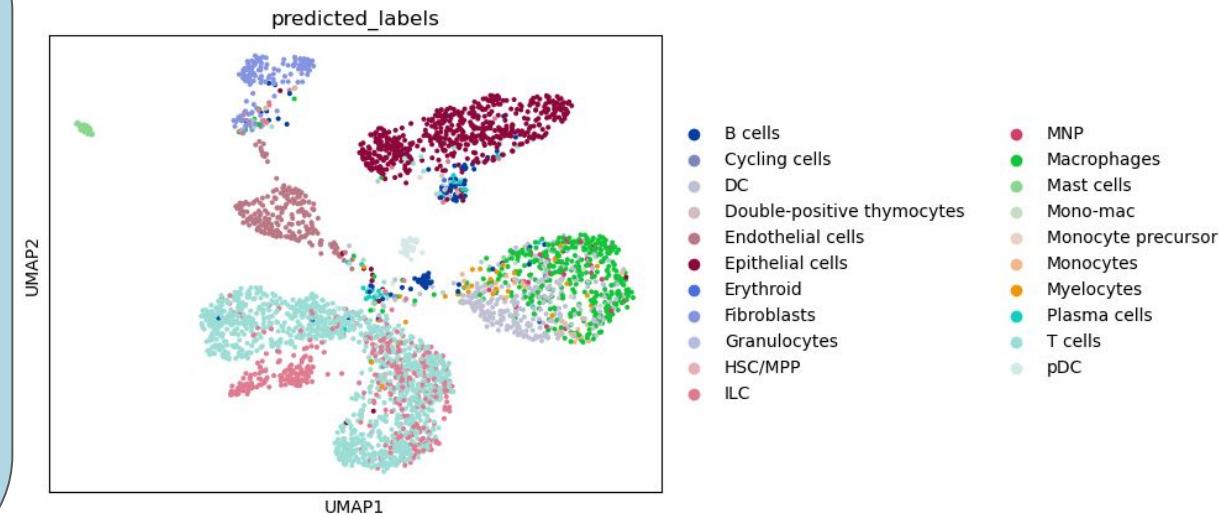
Single-
Transcri-
Lympho-

Alyxzandria M
Patrizia Fuschiotti
Affiliations +
PMID: 3542137

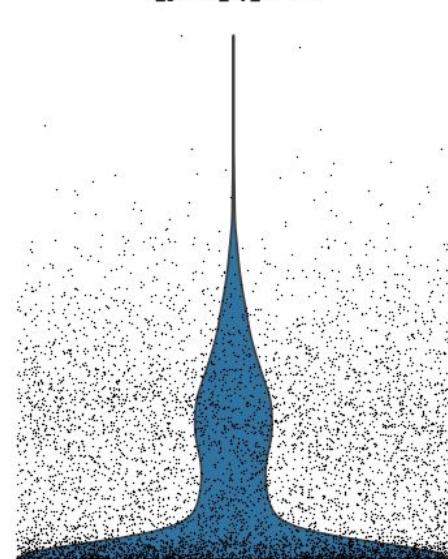


Filtering of data

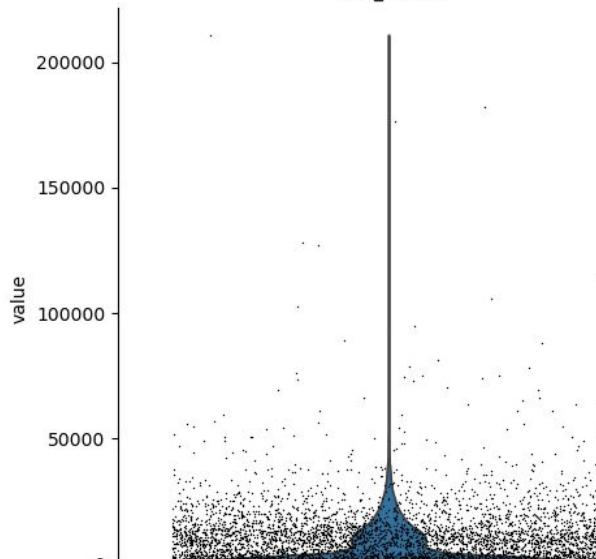
- Selection of patients with advanced (plaque or tumor-stage) disease
- Identify low-quality cells based on:
 - Library size and sequencing depth
 - Mitochondrial RNA
- Doublet detection
- Preliminary UMAP visualization
- CellTypist labels



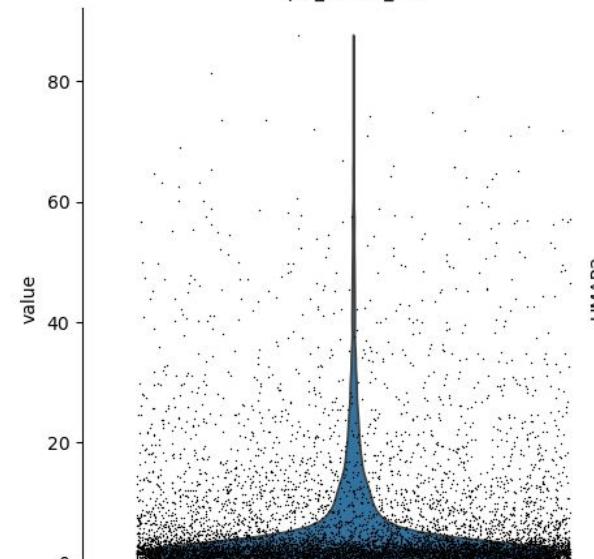
n_genes_by_counts



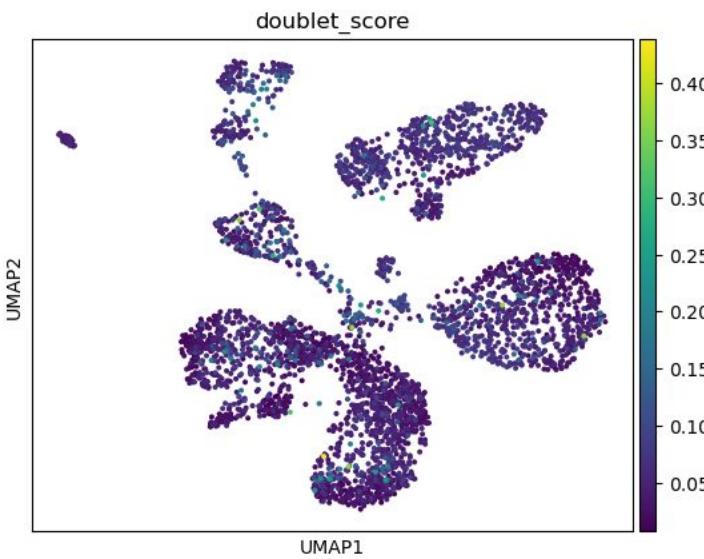
total_counts



pct_counts_mt



doublet_score



Overview of Workflow

Gaydosik et al., 2019

ScRNA data from skin biopsies

9 patients:

- 5 with Mycosis Fungoides
- 4 Healthy Controls

Rindler et al., 2021

ScRNA data from skin, blood,
lymph node

- 1 patient with Mycosis Fungoides

1. Filtering
2. Data processing
3. Cell UMAP for MF & HC samples
4. Aggregated cell UMAP for MF & HC datasets
5. Aggregated gene UMAP for MF & HC datasets
6. Dot plot for key cells
7. Correlation heatmap of top 20 features
8. Gene regulatory network

Dataset Overview

Single-Cell Lymphocyte Heterogeneity in Advanced Cutaneous T-cell Lymphoma Skin Tumors, Alyxzandria M. Gaydosik¹, Tracy Tabib¹, Larisa J. Geskin², Claire-Audrey Bayan², James F. Conway³, Robert Lafyatis¹, and Patrizia Fuschiotti

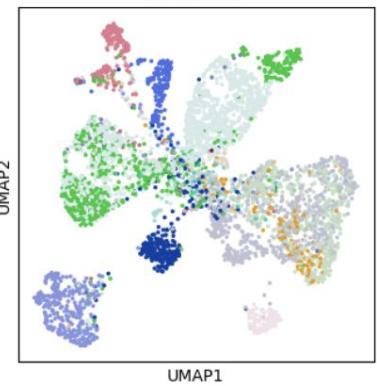
- Single-cell RNA-seq: 14,056 CD3+ lymphocytes from 5 CTCL patients and 4 healthy donors.
- **Large-scale analysis: 44,294 samples with 17,680 features each.**
- **Random Forest model identified top 20 potential biomarkers.**
- Paper validated protein expression using immunohistochemistry and confocal microscopy.

UMAP of Healthy Controls

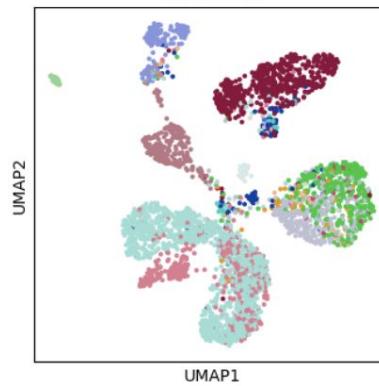
Single-Cell Lymphocyte Heterogeneity in Advanced Cutaneous T-cell Lymphoma Skin Tumors, Alyxzandria M. Gaydosik¹, Tracy Tabib¹, Larisa J. Geskin², Claire-Audrey Bayan², James F. Conway³, Robert Lafyatis¹, and Patrizia Fuschiotti

Healthy Controls

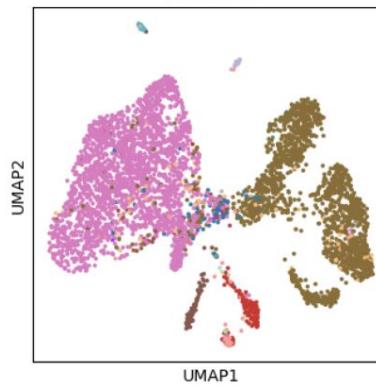
Dataset 1



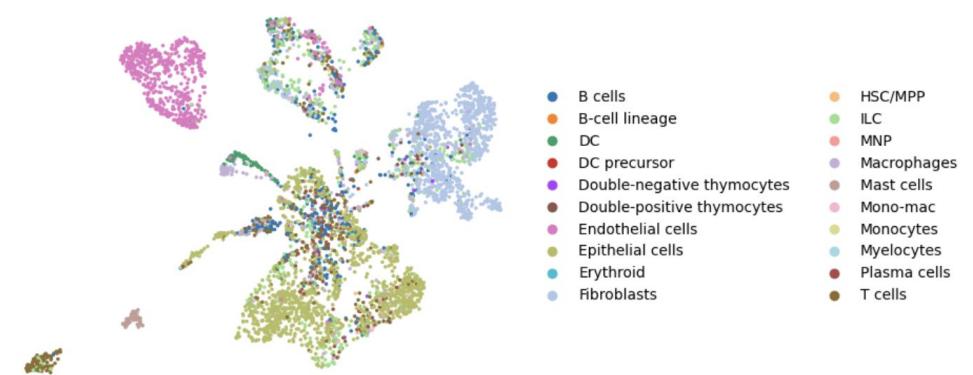
Dataset 2



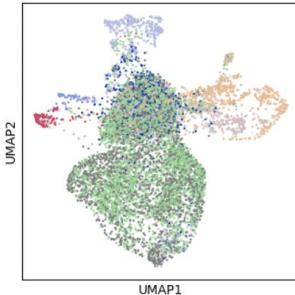
Dataset 3



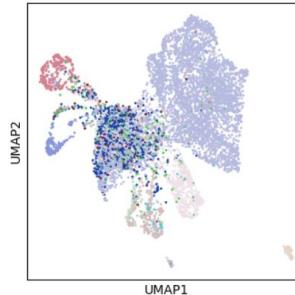
UMAP with Predicted Labels



Dataset 4



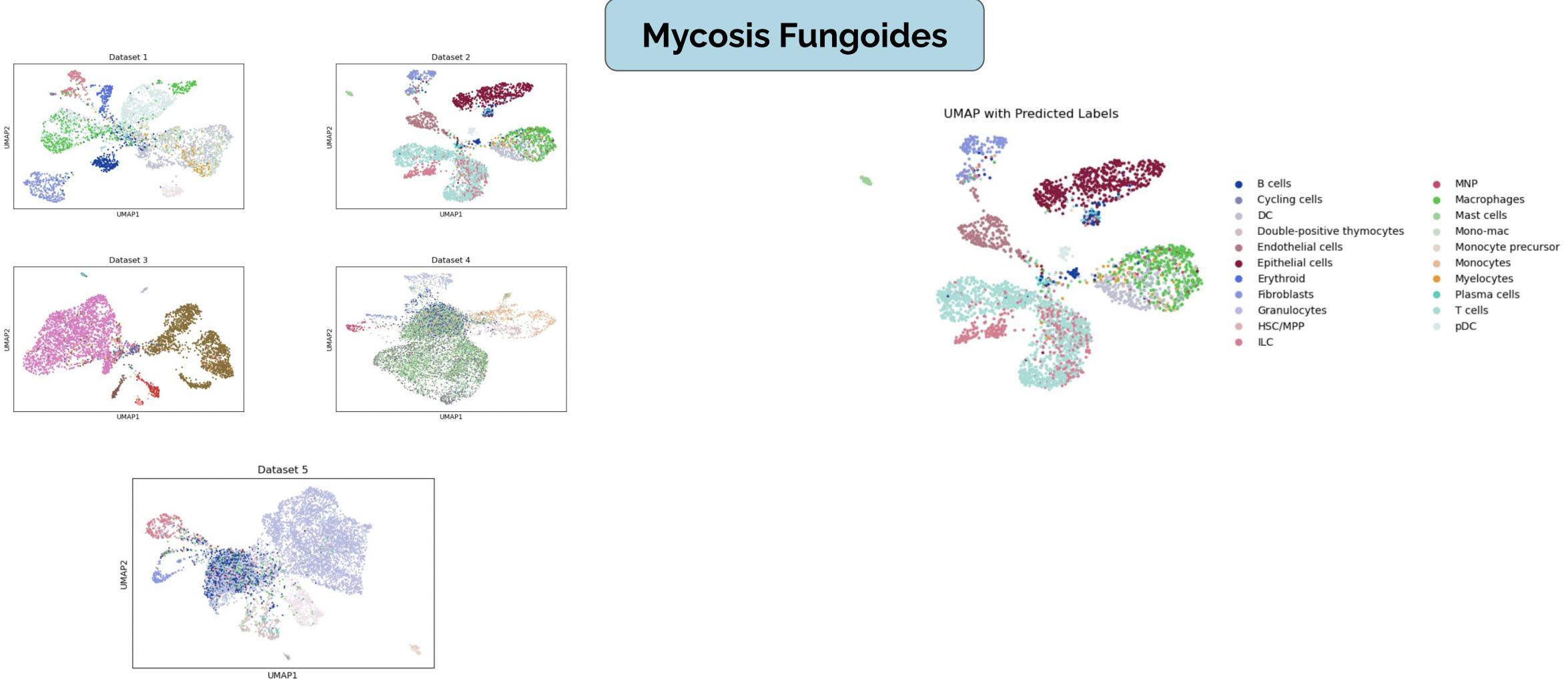
Dataset 5



- B cells
- B-cell lineage
- DC
- DC precursor
- Double-negative thymocytes
- Double-positive thymocytes
- Endothelial cells
- Epithelial cells
- Myelocytes
- Erythroid
- Fibroblasts
- HSC/MPP
- ILC
- MNP
- Macrophages
- Mast cells
- Mono-mac
- Monocytes
- T cells

UMAP of Mycosis Fungoides Patients

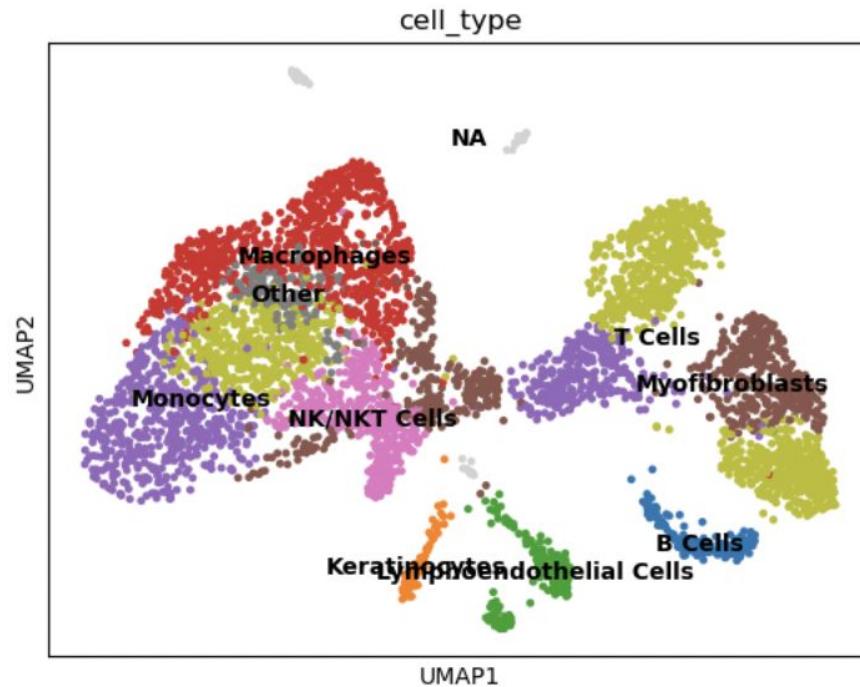
Single-Cell Lymphocyte Heterogeneity in Advanced Cutaneous T-cell Lymphoma Skin Tumors, Alyxzandria M. Gaydosik¹, Tracy Tabib¹, Larisa J. Geskin², Claire-Audrey Bayan², James F. Conway³, Robert Lafyatis¹, and Patrizia Fuschiotti



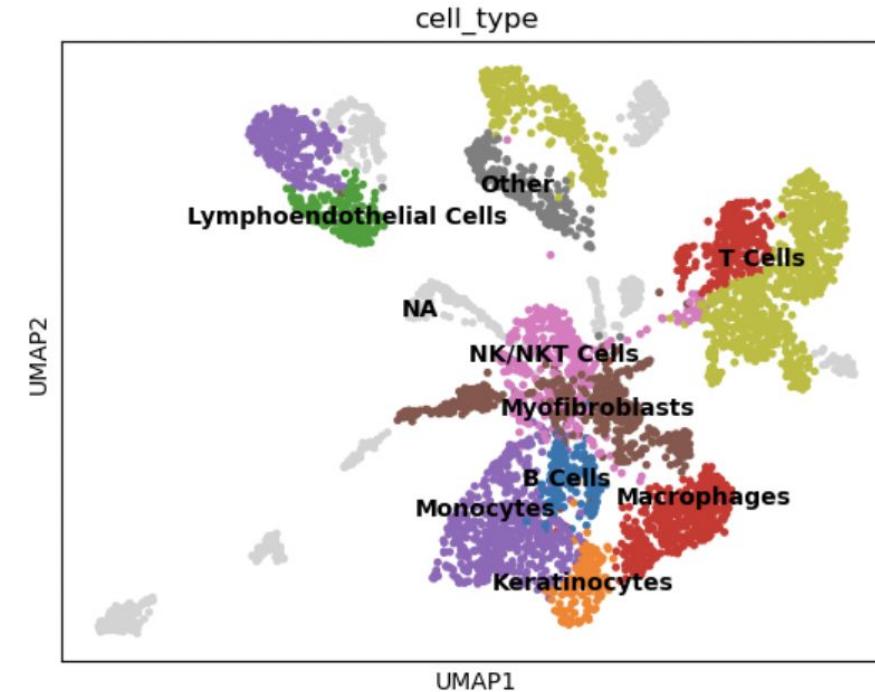
Aggregated UMAP of HC & MF Samples

Gaydosik et al., 2019

Mycosis Fungoides



Healthy Controls



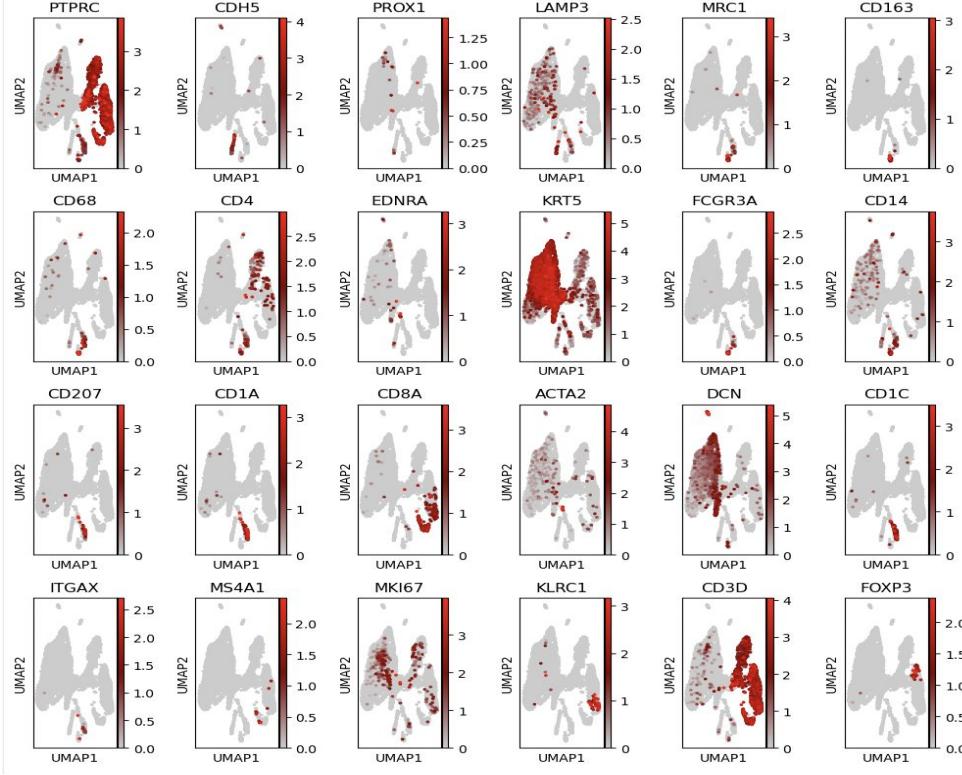
MF patient skin biopsies exhibit a higher proportion of

- T cells
- Macrophages
- Myofibroblasts

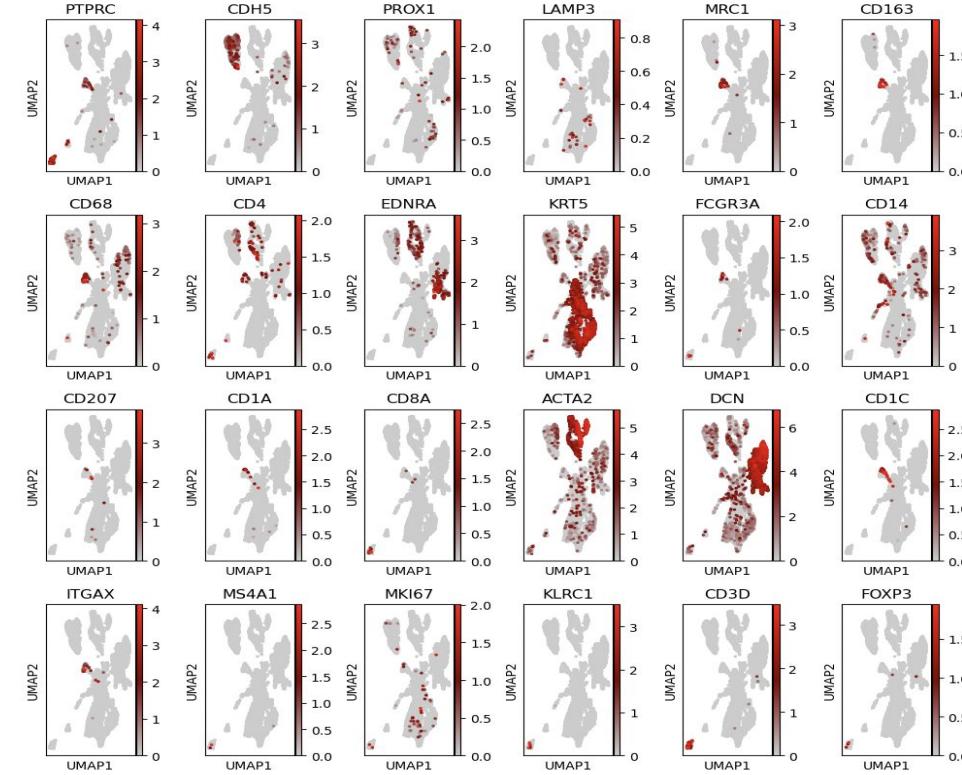
Gene UMAPs for HC & MF Patients

Gaydosik et al., 2019

Mycosis Fungoides



Healthy Controls



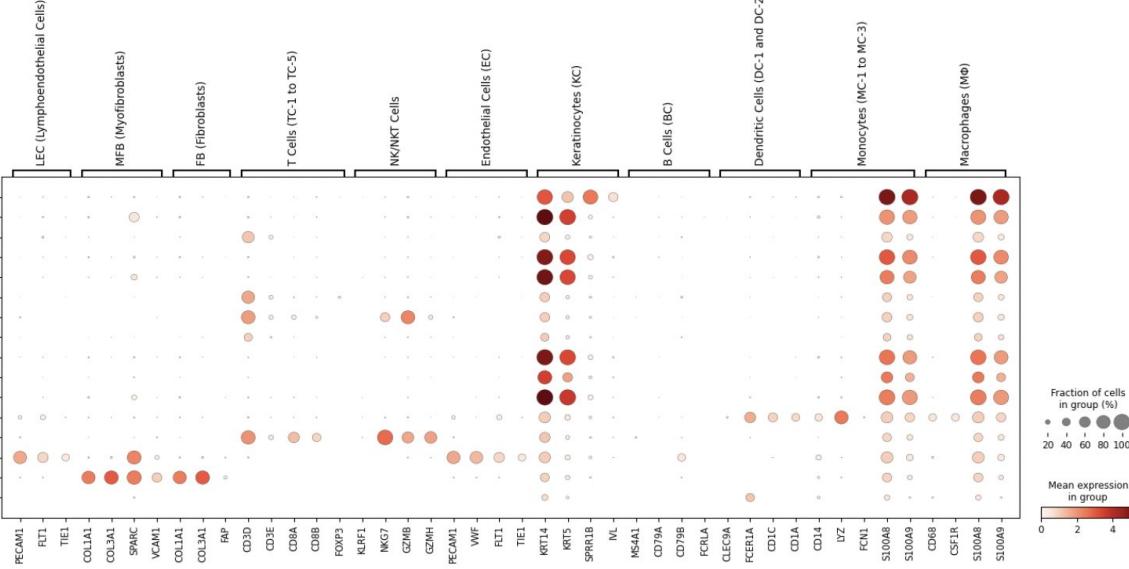
MF patient skin biopsies exhibit a higher expression of genes:

- **PTPRC** → highly expressed on **malignant CD4+ T-cells** and **infiltrating leukocyte⁷**
- **LAMP3** → highly expressed in **langerhans cells and dendritic cells** under tumor-associated conditions.⁷
- **MK167** → linked to **active proliferation of malignant T-cells** in MF⁸
- **CD3D** → **malignant CD4+ T-cells** in MF exhibit high expression of T-cell markers like **CD3D⁸**

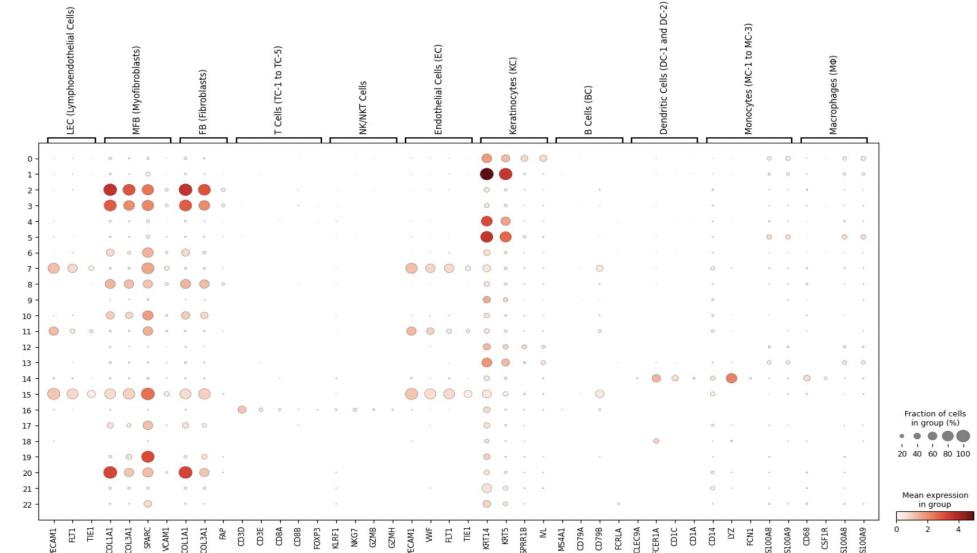
Dot Plots of HC & MF Patient Cells

Gaydosik et al., 2019

Mycosis Fungoides



Healthy Controls



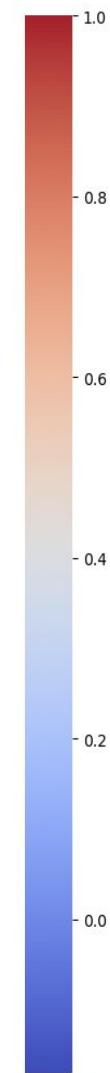
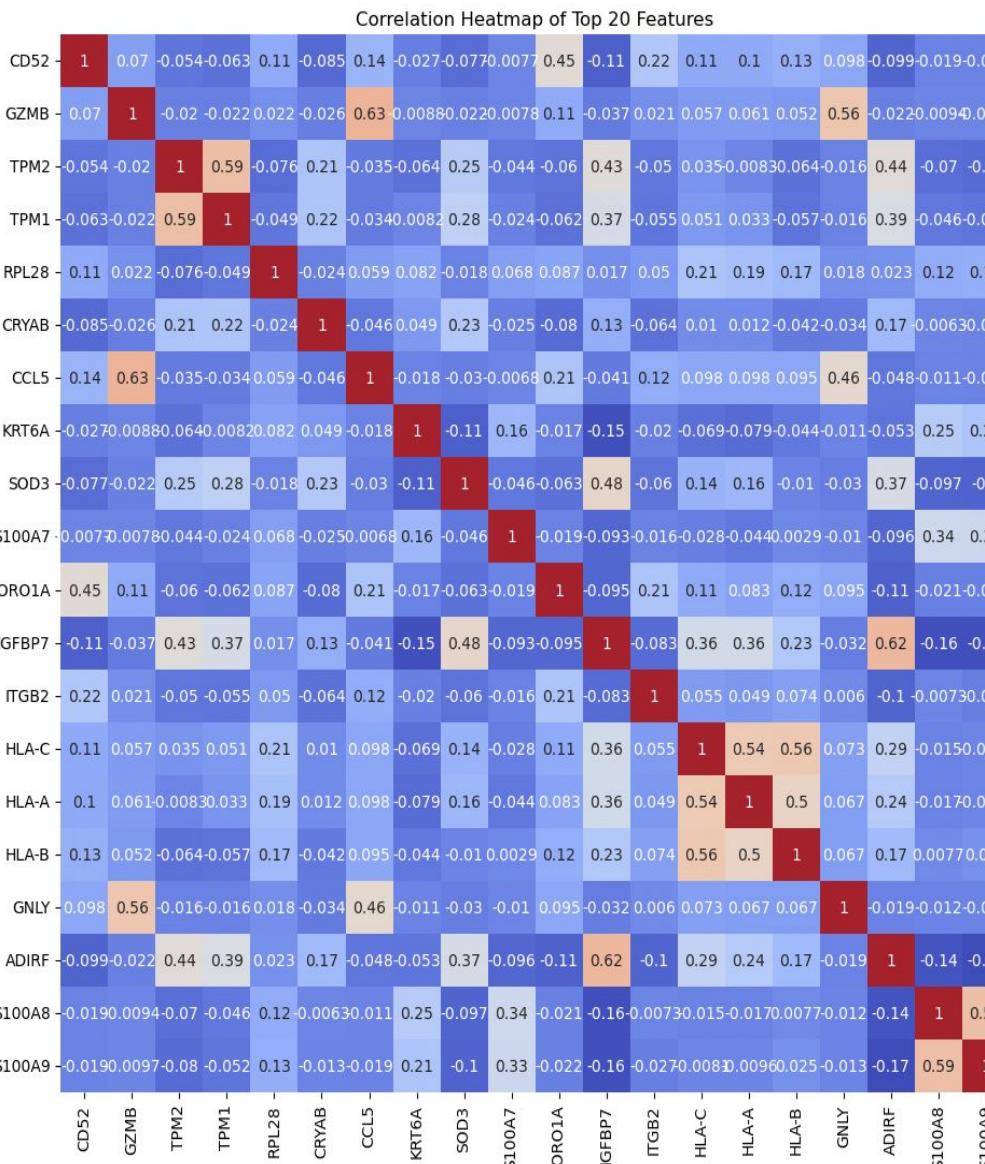
MF patient skin biopsy cells exhibit a **higher expression** of genes:

- **Keratinocytes: KRT14, KRT5** → Chronic inflammation & malignant T-cells infiltration triggers **keratinocyte hyperproliferation⁵**
- **Monocytes & Macrophages: S10018, S10019** → encode calcium-binding proteins which mediate **pro-inflammatory signaling and recruitment of immune cells⁶**

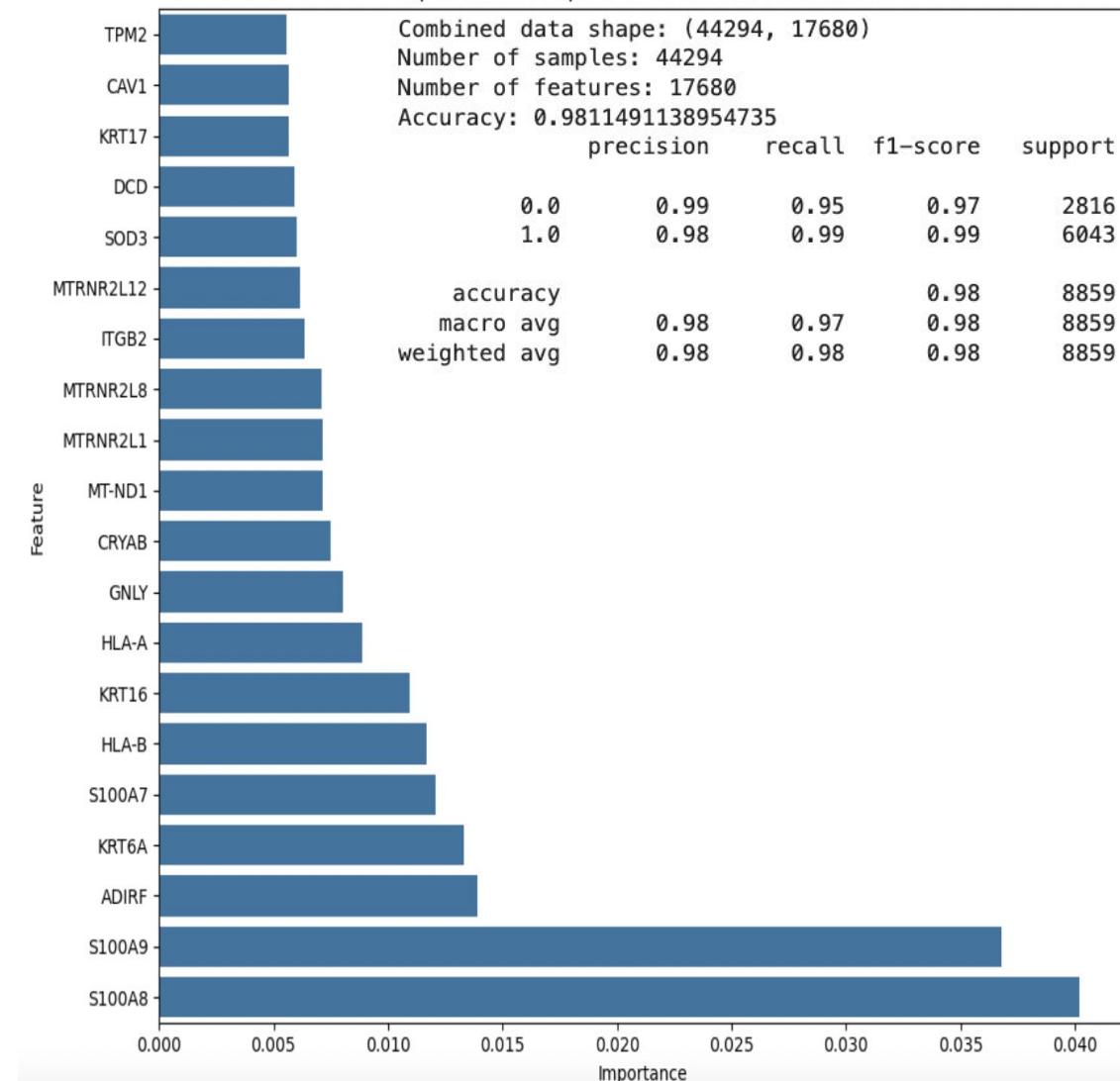
MF patient skin biopsy cells exhibit a **lower expression** of genes:

- **Myofibroblasts & Fibroblasts: COL1A1, COL3A1** → chronic inflammation and malignant cell infiltration disrupt the ECM, **reducing fibroblast activity and collagen synthesis⁶**

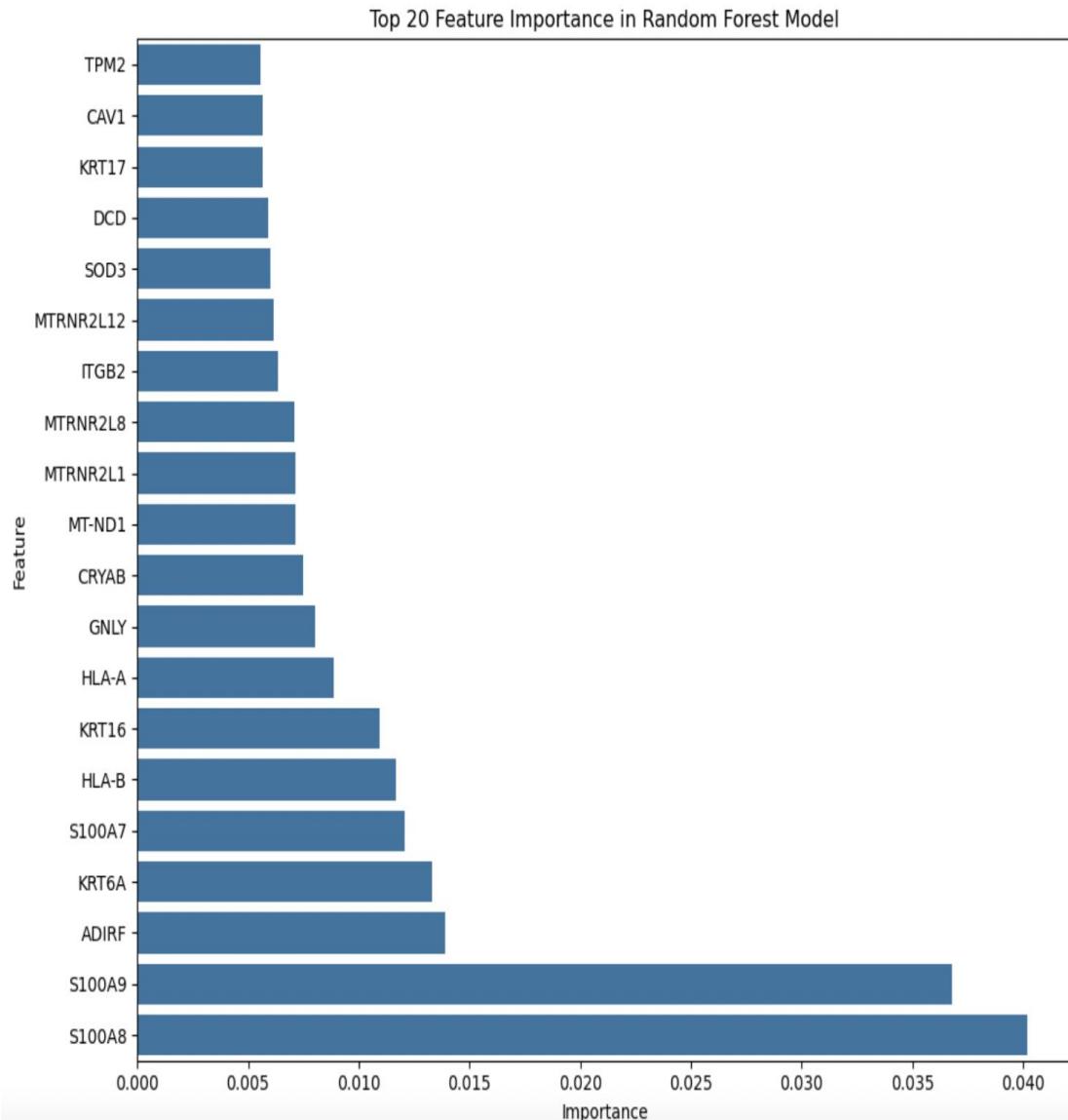
Random Forest Heatmap of Top Genes



Top 20 Feature Importance in Random Forest Model



Key Insights from Publicly Available Data

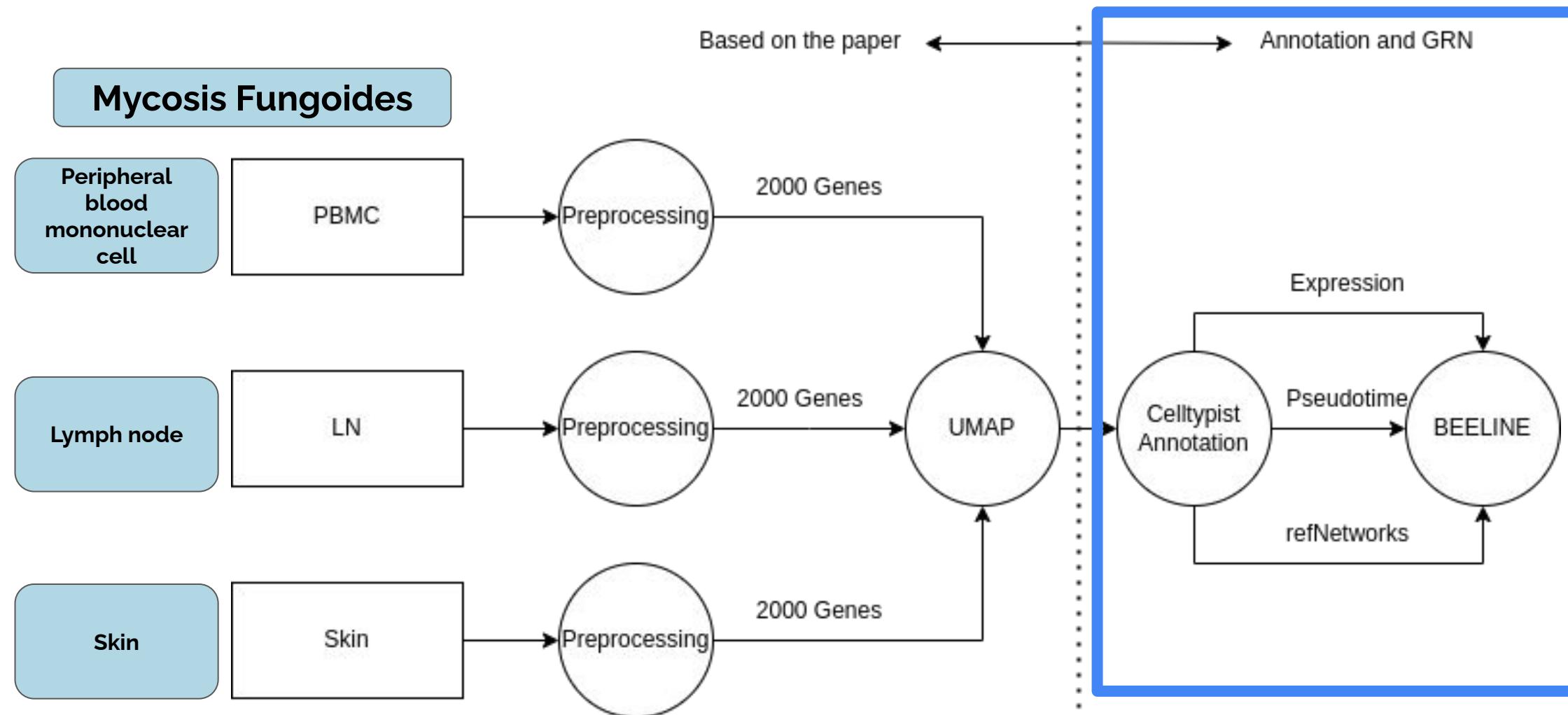


- **S100A8/A9:** (Zhou, 2023)
 - Calcium binding protein
 - Involved in the regulation of a number of cellular processes such as cell cycle progression and differentiation
 - Biomarker of cancer, inflammation
- **ADIRF:** adipogenesis regulatory factor
- **KRT6A/16:** keratin
 - Association with hyperkeratotic ("scaly") lesions?
- **HLA-B/A:** Upregulated in inflammation
- **GONLY:** protein released by killer lymphocytes in cytotoxic granules

Key Insights from Publicly Available Data

Single-Cell RNA Sequencing Reveals Tissue Compartment-Specific Plasticity of Mycosis Fungoides Tumor Cells

Katharina Rindler, Wolfgang M. Bauer, Constanze Jonak, Matthias Wielscher, Lisa E. Shaw, Thomas B. Rojahn, Felix M. Thaler, Stefanie Porkert, Ingrid Simonitsch, Wolfgang Weninger, Marius E. Mayerhoefer, Matthias Farlik

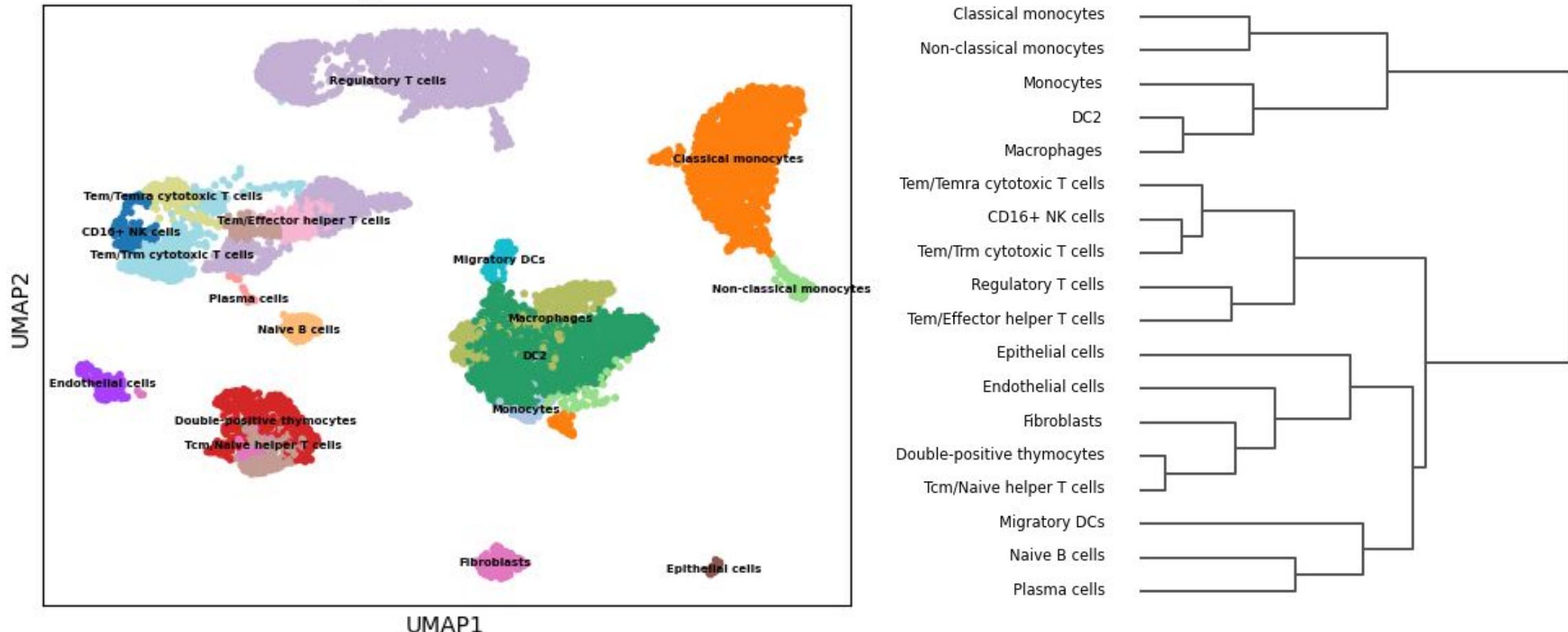


Key Insights from Publicly Available Data

Rindler et al., 2021

Mycosis Fungoides

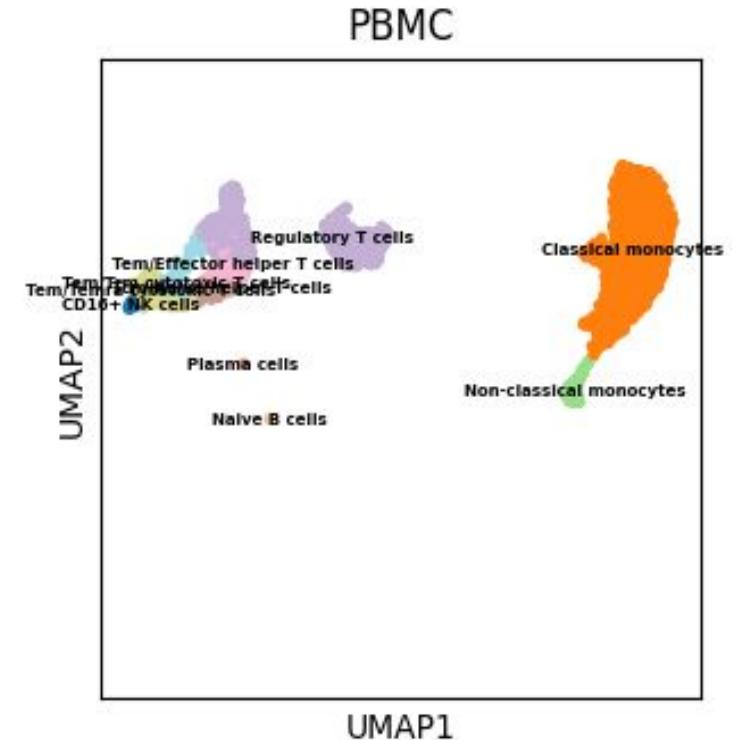
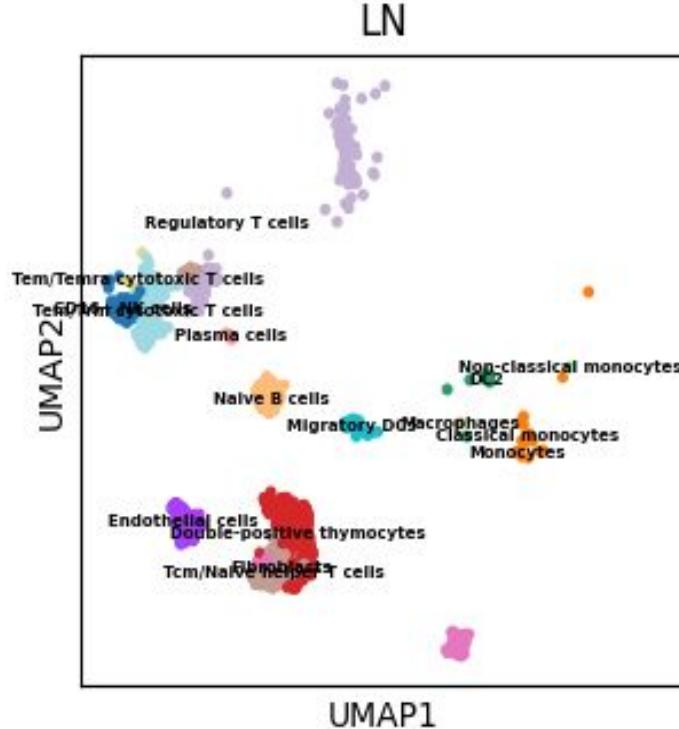
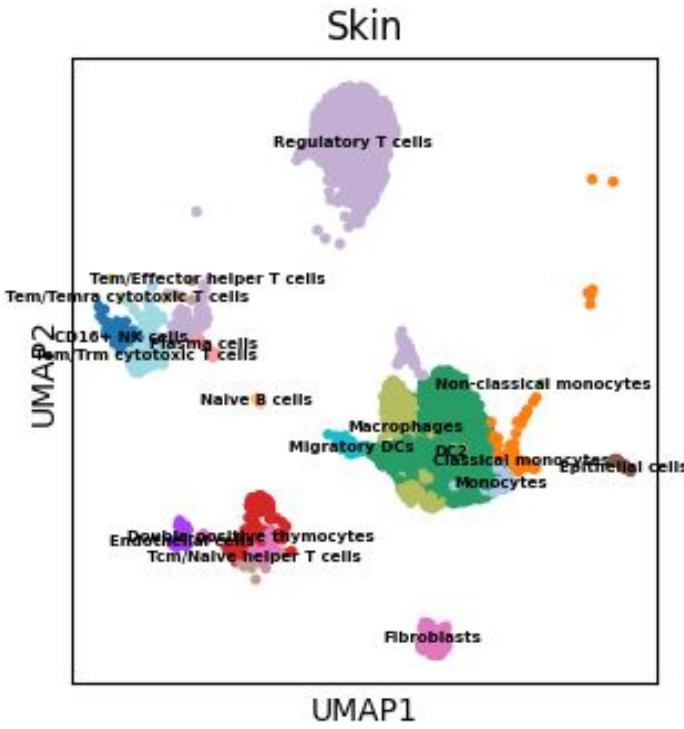
CellTypist Annotations



Key Insights from Publicly Available Data

Rindler et al., 2021

Mycosis Fungoides



- Heterogeneity in tumor microenvironment across tissue types
- Notable population of regulatory T-cells across tissue types
- Significant immune infiltration of skin

Key Insights from Publicly Available Data

Rindler et al., 2021

Upstream:

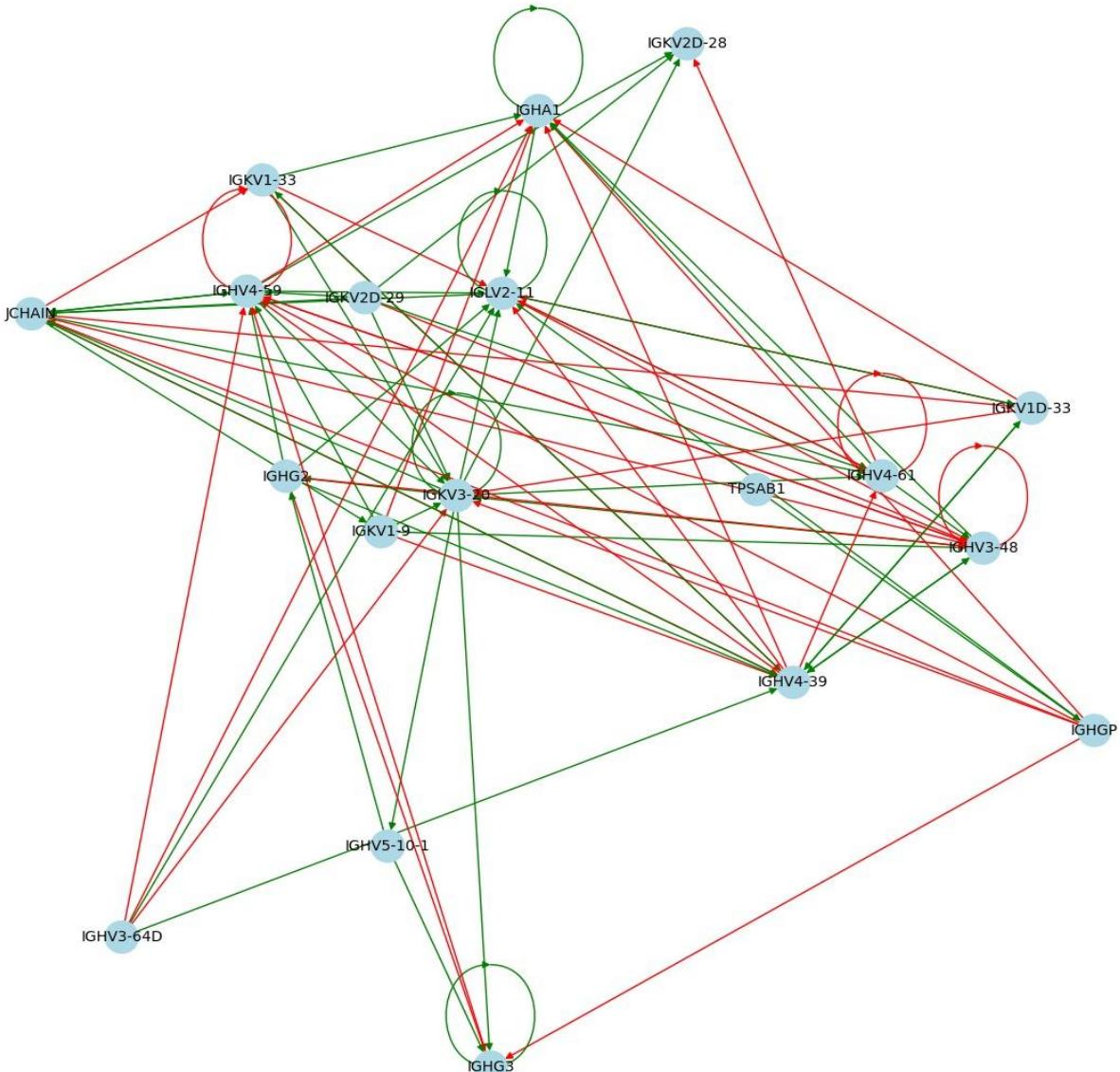
- JCHAIN
- IGHV3-64D
- IGHGP

Downstream:

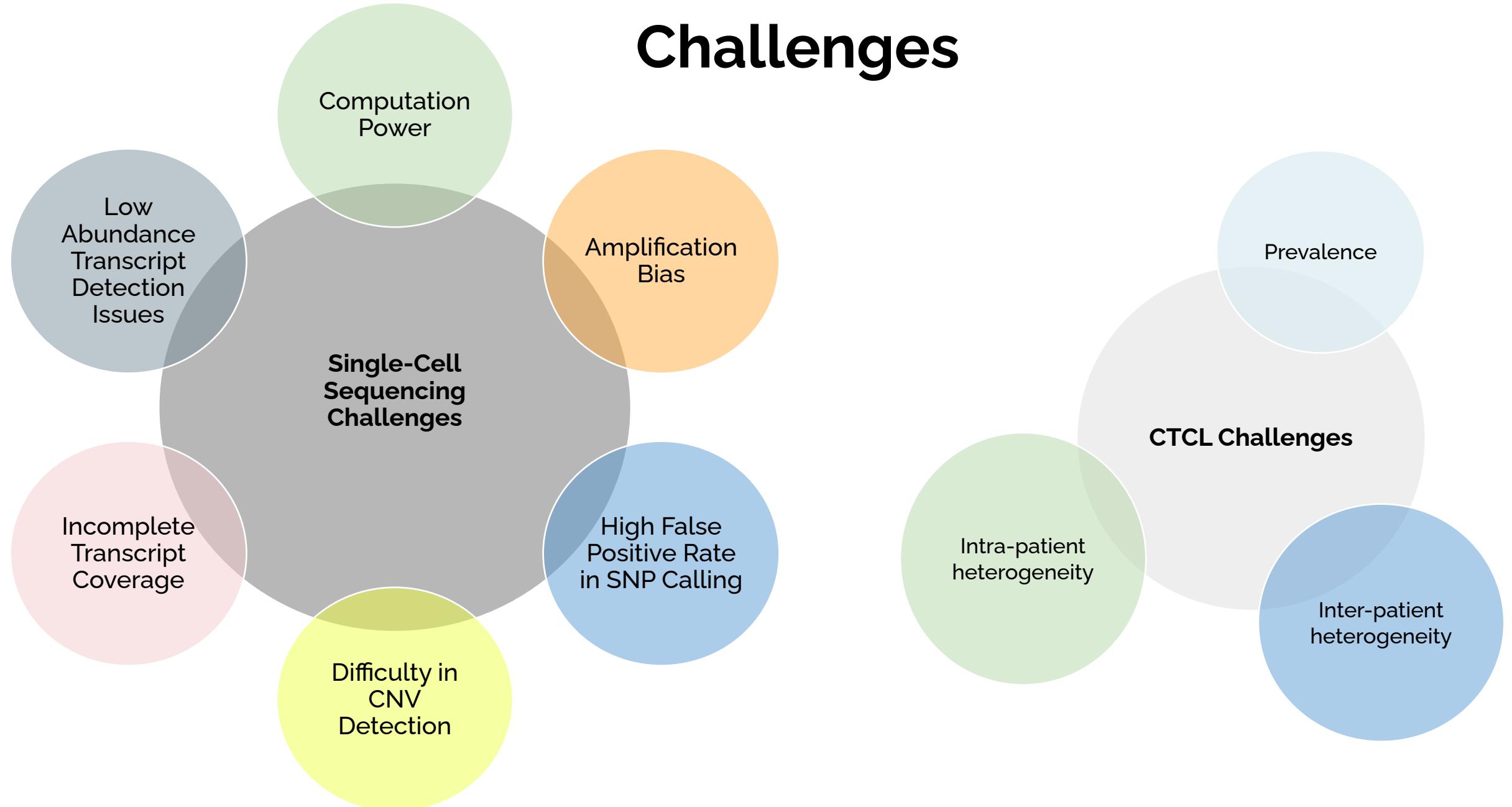
- IGLV2-11
- IGKV3-20
- IGHV4-39
- IGHV4-59

Innate immune response

Gene regulatory network from BEELINE output



Challenges



Why Invest in Our Team ?



Innovation

- Using scRNA-seq to bridge the gap in MF biomarker identification.
- Combining insights from multiple datasets for robust and novel conclusions



Impact

- Early detection → Improved patient outcomes.
- Biomarker-based prognostication and therapy → Personalized medicine.



Feasibility

- Leveraging publicly available data
=Cost-effective and efficient.

Conclusions

Re-analysis of two publicly available single-cell RNA datasets revealed

- Significant tumor heterogeneity across tissue types
- Upregulation of known tumor markers, inflammatory markers, and evidence of issue deregulation

Looking Ahead

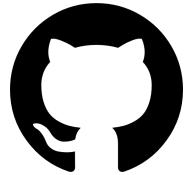
- Expand analysis to include additional datasets with deeper analysis to refine MF-specific biomarkers. (~6 months)
- Explore therapeutic targets based on identified gene expression patterns.
- Collaborate with clinical researchers for validation with novel samples

SOOOOO...

ANY QUESTIONS?

memes.com

Appendix



github.com/fefoglino/MF_CTCL_ECBM4060



<https://drive.google.com/drive/folders/1K4mewvCDDzQ0CbK3cuPJpYOnV0pVjtzC?usp=sharing>

Who Did What

Team Contributions

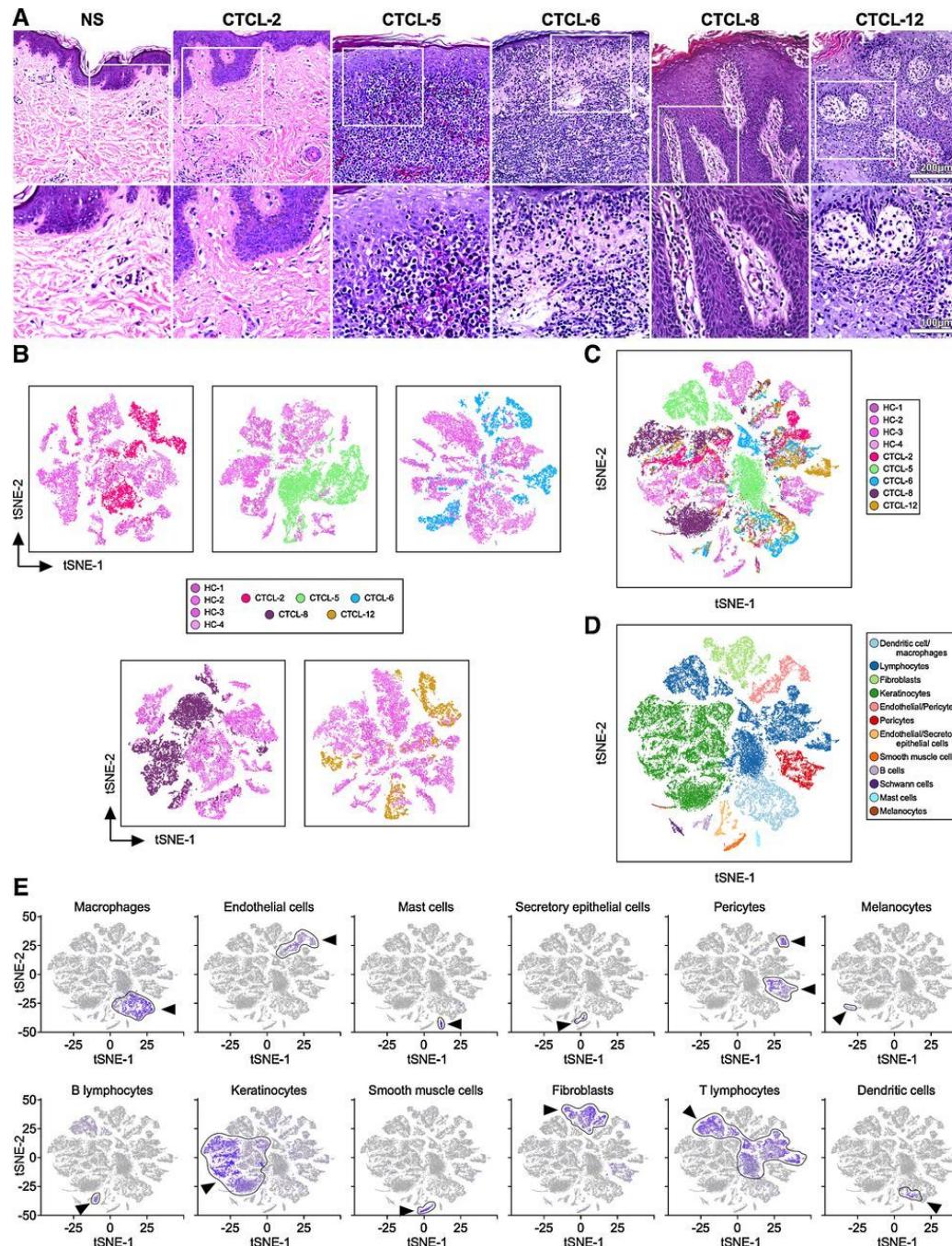
- **Abdulaziz Alharmoodi (aaa2479)**: Peer Review, Data analysis, scRNA-seq workflows, template creation.
- **Arsalan Firooz (af3410)**: Literature review, scRNA-seq workflows.
- **Francesca Foglino (fef2116)**: Literature review, data curation and filtering, scRNA-seq workflows.
- **Neema Nkонтчou (nn2381)**: Interpretation and integration of immune pathways, statistical analysis.
- **Sararose Suhl (scs2256)**: Literature review, dataset identification, interpretation of immune pathways, final report synthesis.

References

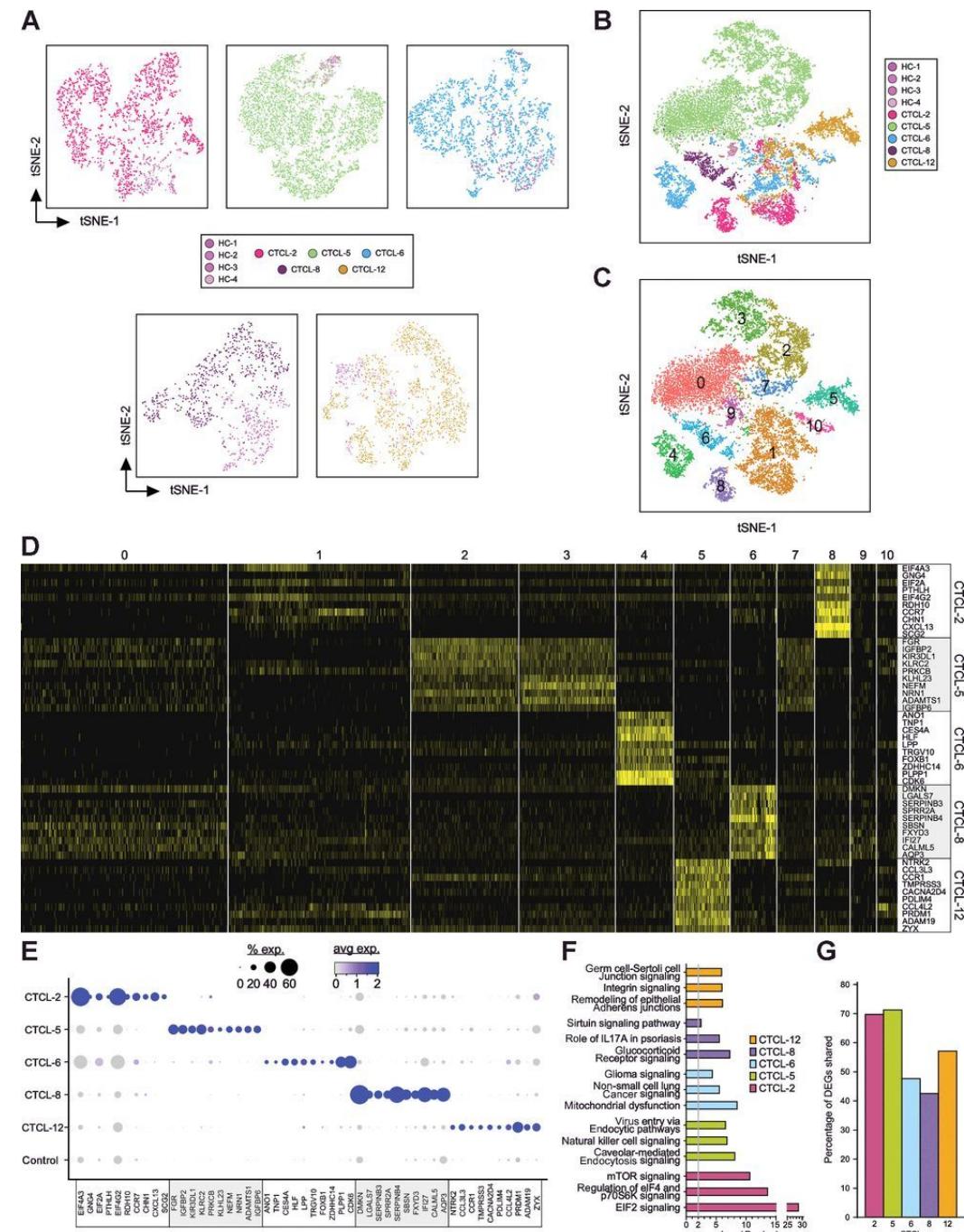
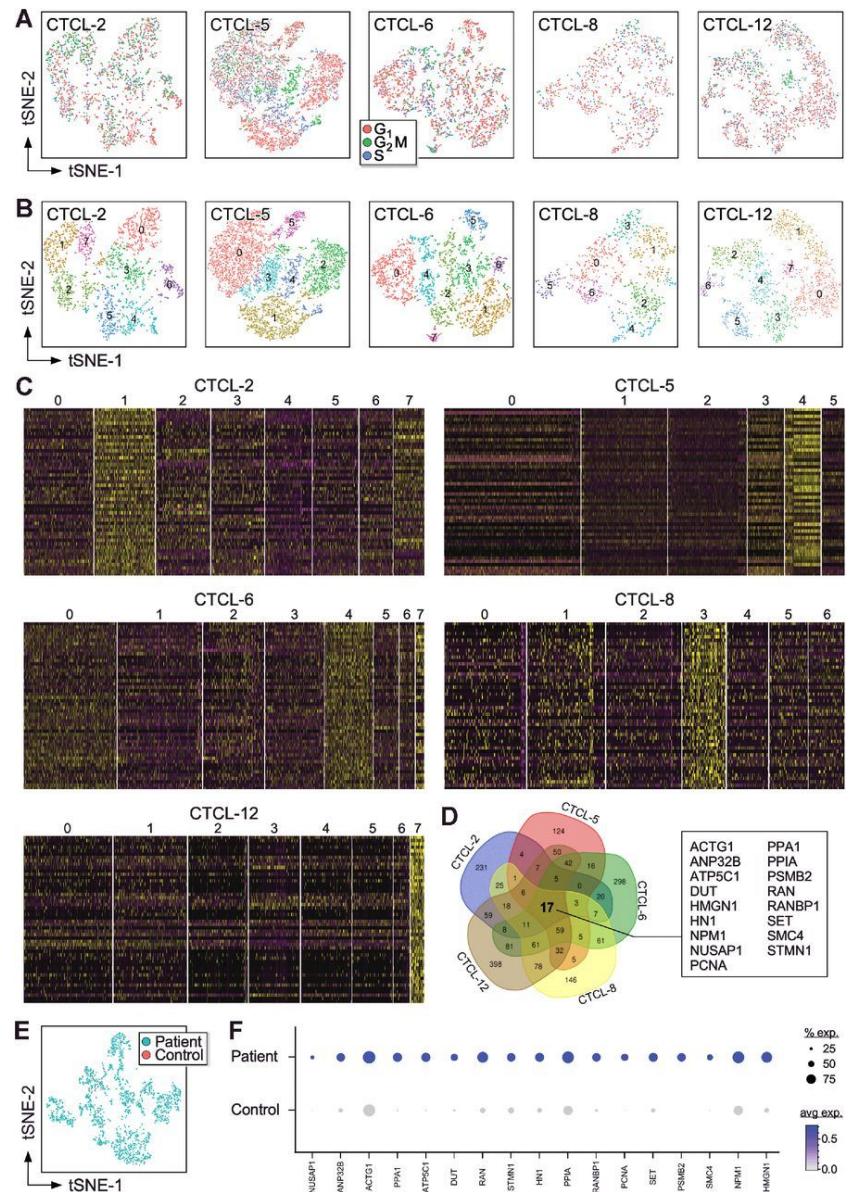
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2. Rindler K, Bauer WM, Jonak C, et al. Single-Cell RNA Sequencing Reveals Tissue Compartment-Specific Plasticity of Mycosis Fungoides Tumor Cells. *Frontiers in Immunology*. 2021;12. doi:10.3389/fimmu.2021.666935
3. Zhou H, Zhao C, Shao R, Xu Y, Zhao W. The functions and regulatory pathways of S100A8/A9 and its receptors in cancers. *Front Pharmacol.* 2023;14:1187741. Published 2023 Aug 28. doi:10.3389/fphar.2023.1187741
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6. Deng, M., Odhiambo, W.O., Qin, M., To, T.T., Brewer, G.M., Kheshvadjian, A.R., Cheng, C. and Agak, G.W. (2024). Analysis of intracellular communication reveals consistent gene changes associated with early-stage acne skin. *Cell Communication and Signaling*, 22(1). doi:<https://doi.org/10.1186/s12964-024-01725-4>.
7. Sumanth Chennareddy, Rindler, K., Ruggiero, J.R., Alkon, N., Cohenour, E.R., Tran, S., Weninger, W., Griss, J., Jonak, C. and Brunner, P.M. (2024). Single-cell RNA sequencing comparison of CD4+, CD8+ and TCR- $\gamma\delta$ + cutaneous T-cell lymphomas reveals subset-specific molecular phenotypes. *British Journal of Dermatology*. doi:<https://doi.org/10.1093/bjd/ljae313>.
8. Motamedi, M., Xiao, M.Z.X., Iyer, A. and Gniadecki, R. (2021). Patterns of Gene Expression in Cutaneous T-Cell Lymphoma: Systematic Review of Transcriptomic Studies in Mycosis Fungoides. *Cells*, 10(6), pp.1409–1409. doi:<https://doi.org/10.3390/cells10061409>.
- 9.

Thank you!

Gaydosik et al.



Gaydosik et al.



Gaydosik et al.

