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Immune profiling of the tumor microenvironment in classic Hodgkin's lymphoma using high-complexity mass cytometry

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Classic Hodgkin's Lymphoma (cHL)

- Cancer cells are Hodgkin Reed-Sternberg (HRS) cells
  - Abnormal B lymphocytes
  - Commonly overexpress PD-1 ligands
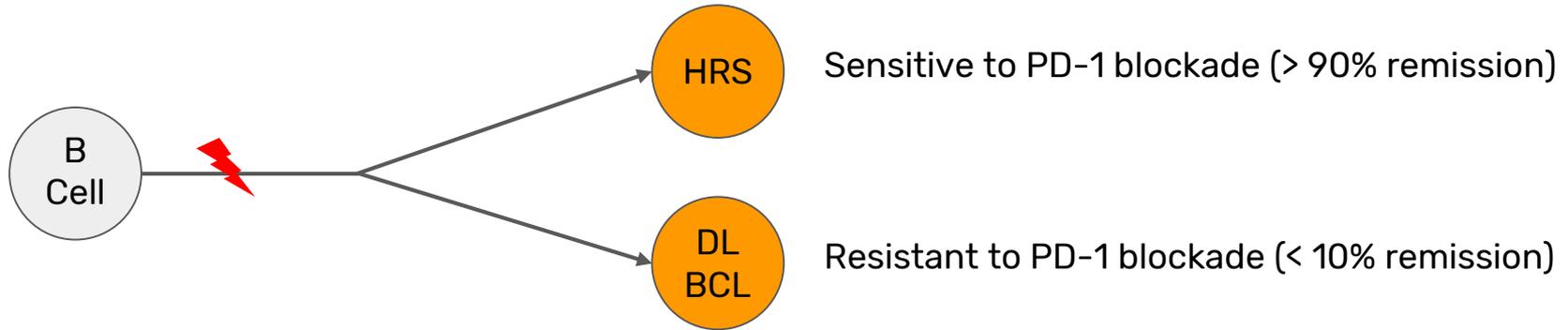
- Few malignant HRS cells surrounded by a <u>rich immune infiltrate</u>
- <u>High numbers of CD8+ T cells</u> is associated with better outcome

Understanding the immune system is <u>critical</u> for better treatment of cHL

- Biomarker with prognostic value
- Clinical significance as a therapeutic target

Immune Profiling

- Identifying and quantifying immune populations according to their phenotypic and functional features
- Complex and heterogeneous biological system
    - 50+ known immune subsets (just in circulating blood!)
    - Our knowledge of biological complexity of different populations continues to grow

Mass Cytometry (CyTOF)

TRENDS in Immunology
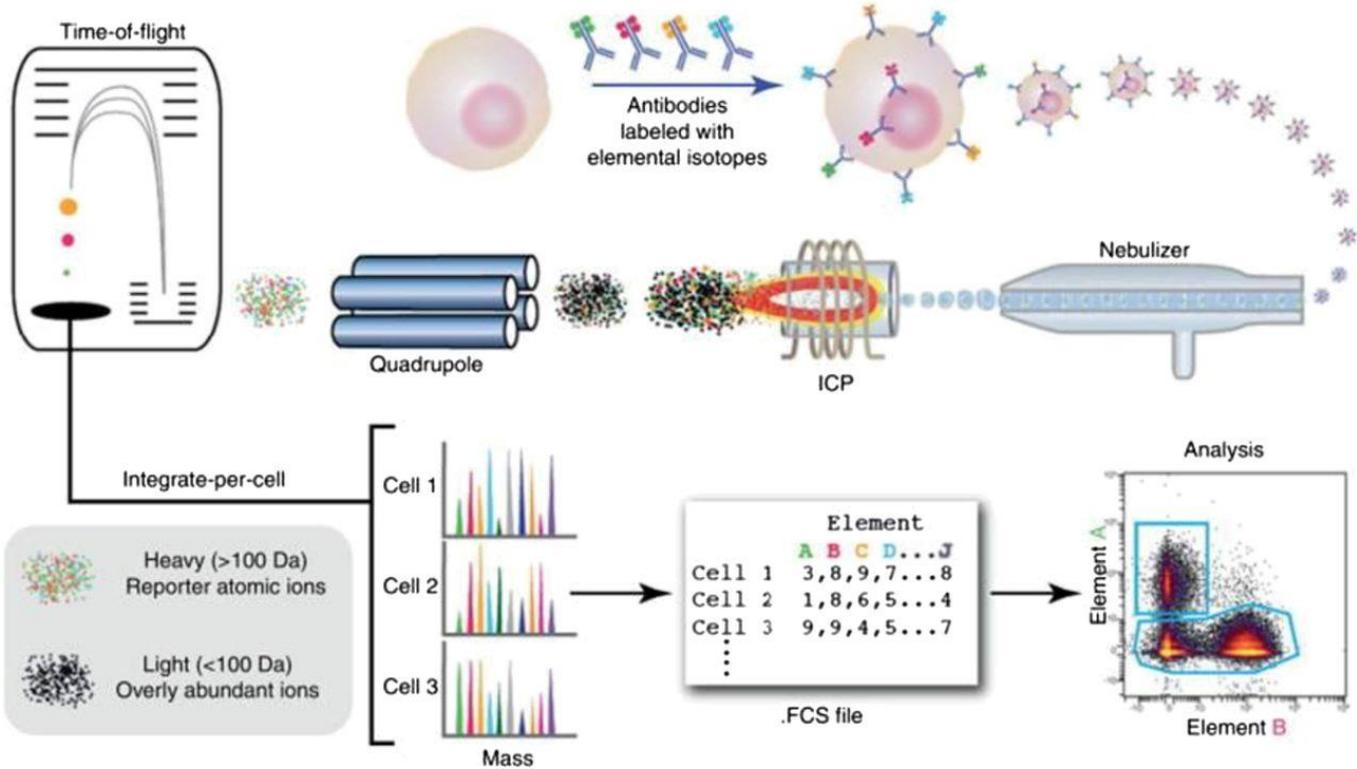
# Qualitative/Exploratory Experiment Design

- 8 patient LN samples (7 nodular sclerosing, 1 mixed cellularity)
- 7 donor samples (3 LNs, 4 tonsil)
- Commercial analysis solution (Astrolabe Cytometry Platform)

31 markers

CCR4	CD27	ICOS
CCR5	CD28	LAG3
CCR6	CD44	**PD1**
CCR7	CD45	TCRgd
CD3	CD45RA	TIM3
CD4	**CD45RO**	
CD5	**CD56**	
CD7	CD69	
CD8a	CD127	
CD11a	CD161	
CD16	CXCR3	
CD19	CXCR5	
CD25	**HLADR**	

15 major compartments

B Cell
CD4+ T Cell
CD8+ T Cell
NK Cell (CD56hi CD16–)
NK Cell (CD56mid CD16+)
NKT Cell
Lin– (Myeloids)

Within the T Cells
CD4+ T Cell: Treg (memory/naive),
Memory (Central/Effector), Naive,
EMRA
CD8+ T Cell: Memory (Central/Effector),
Naive, EMRA

Additional resolution

Unsupervised clustering identified 43 unique clusters

Manual exploration of PD1, ICOS, LAG3, and TIM3

CyTOF Identifies Canonical Subsets
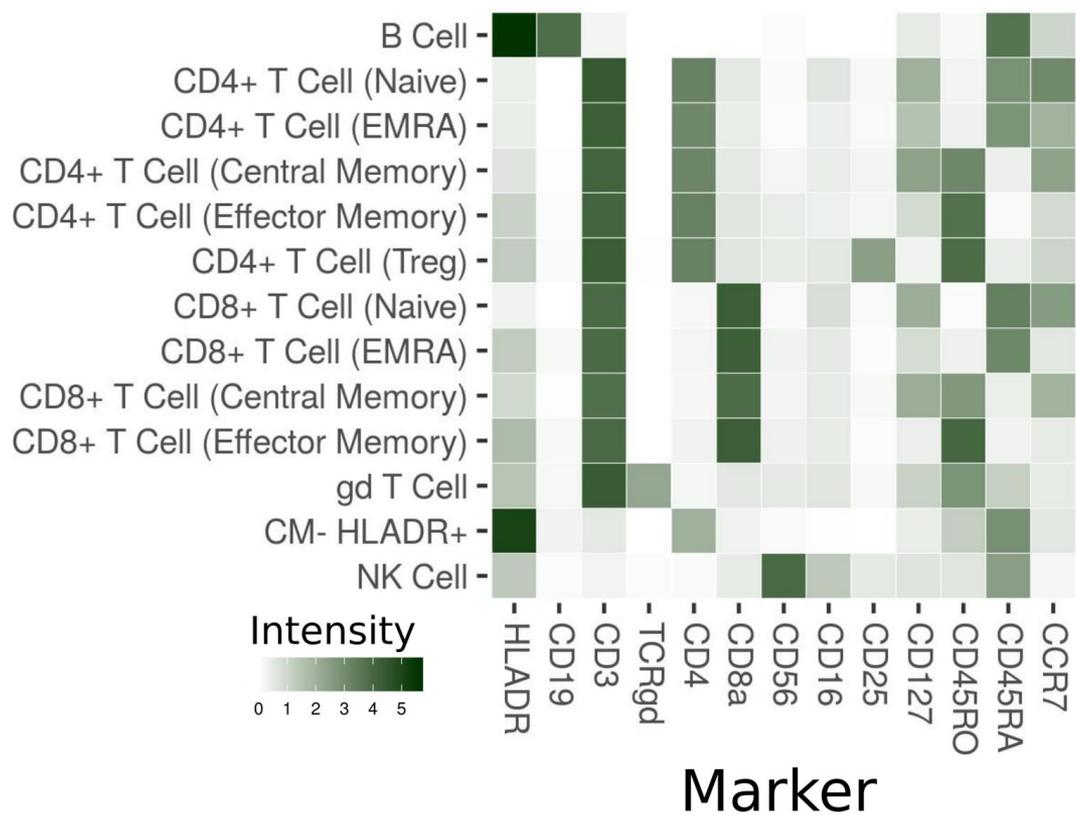
# Recapitulation of Known B Cell and T Cell Trends
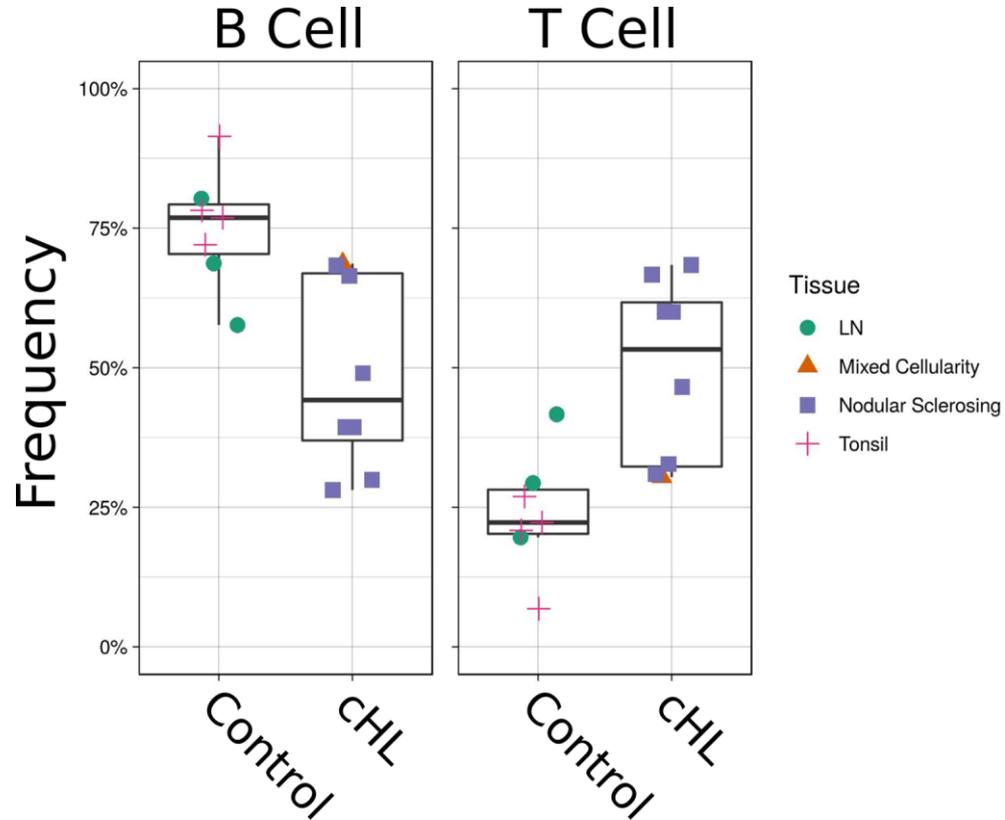
Dimensionality Reduction Reveals Further Structure
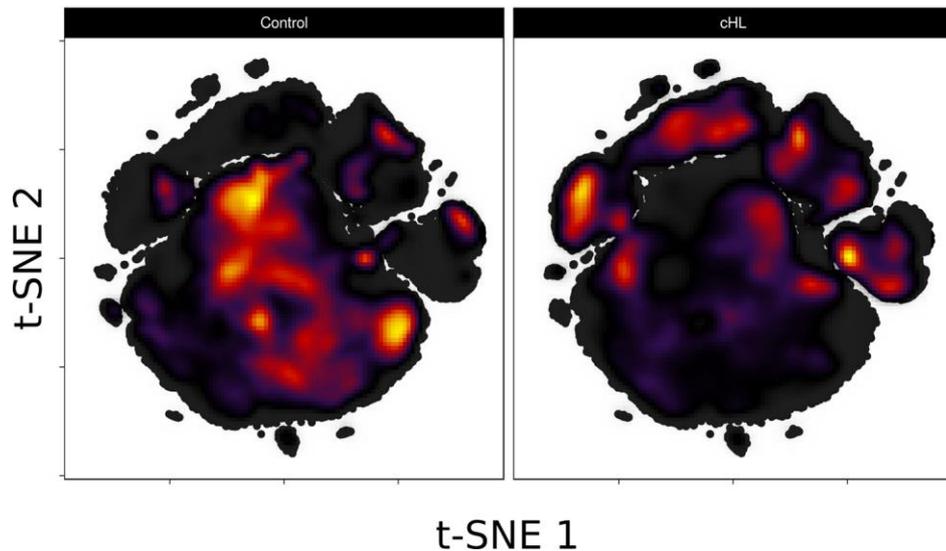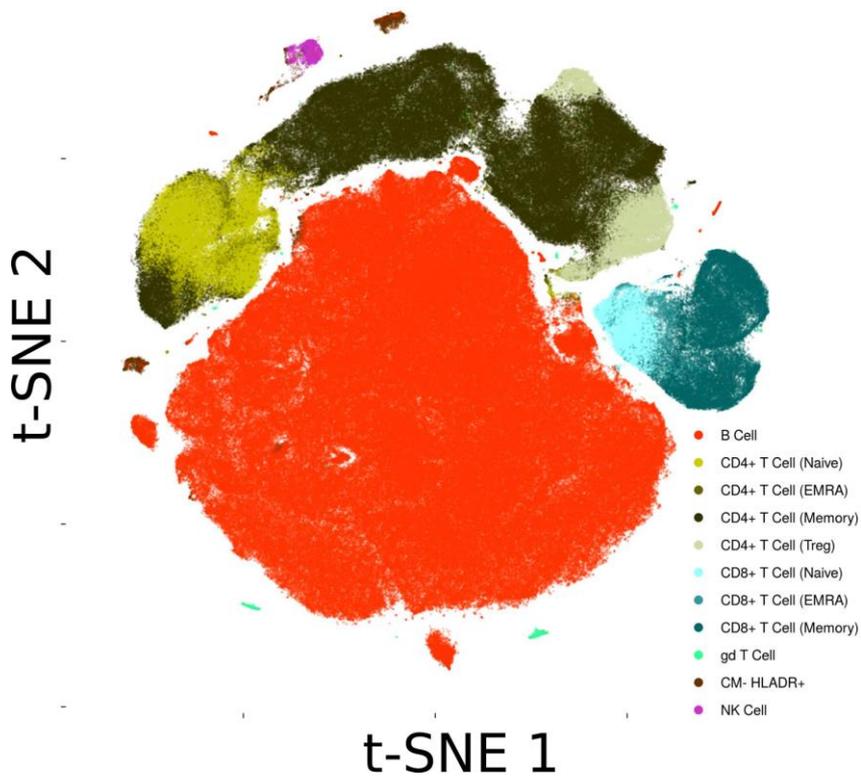
Immunophenotyping of T Cell Compartment
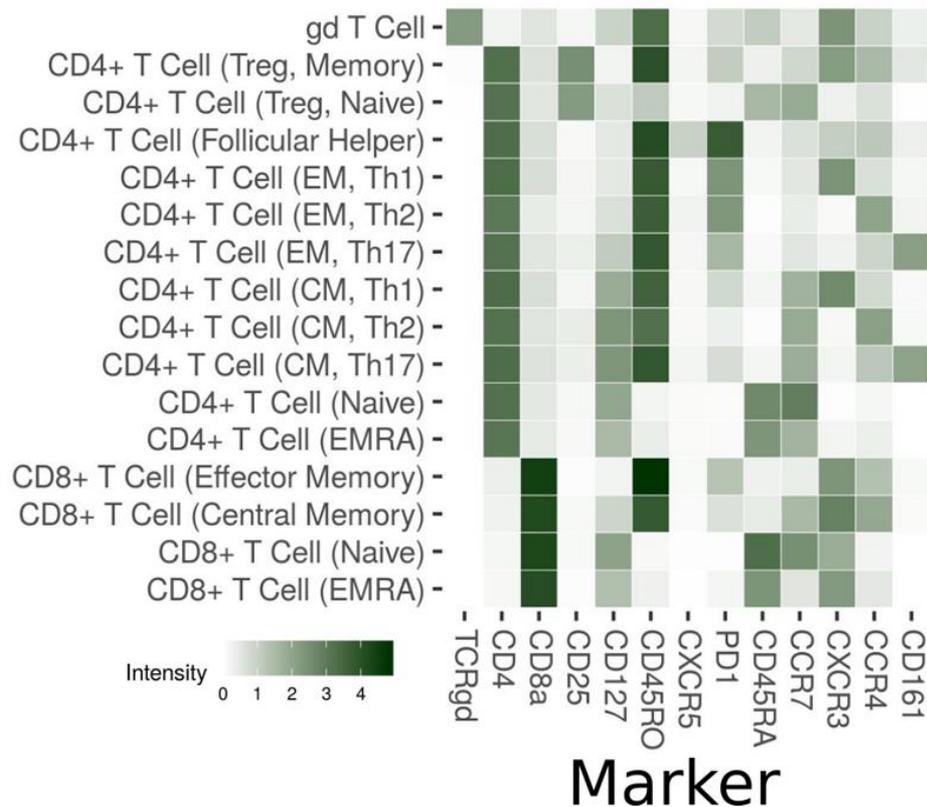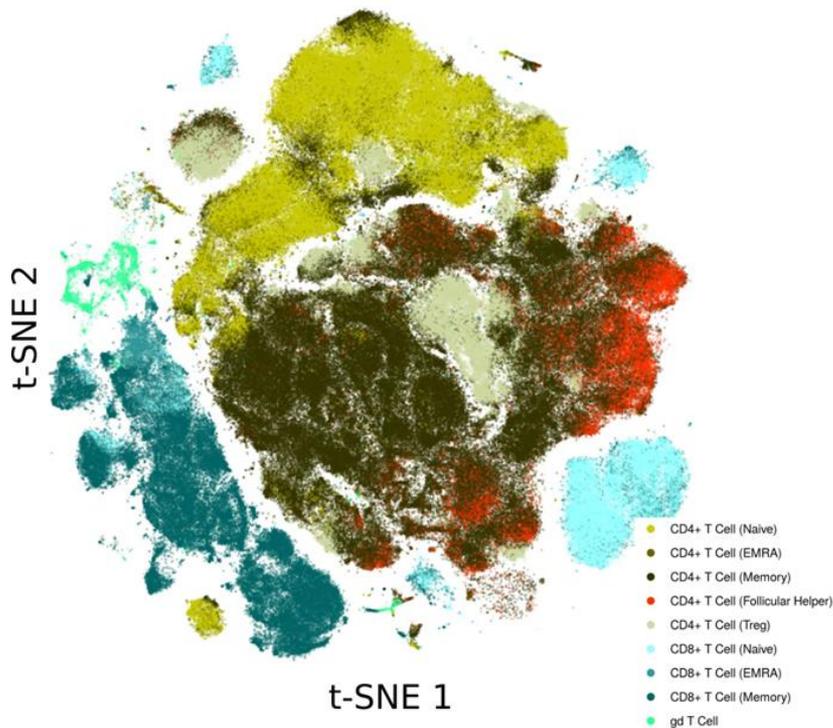
T$_h$ Frequencies Vary Between Patients
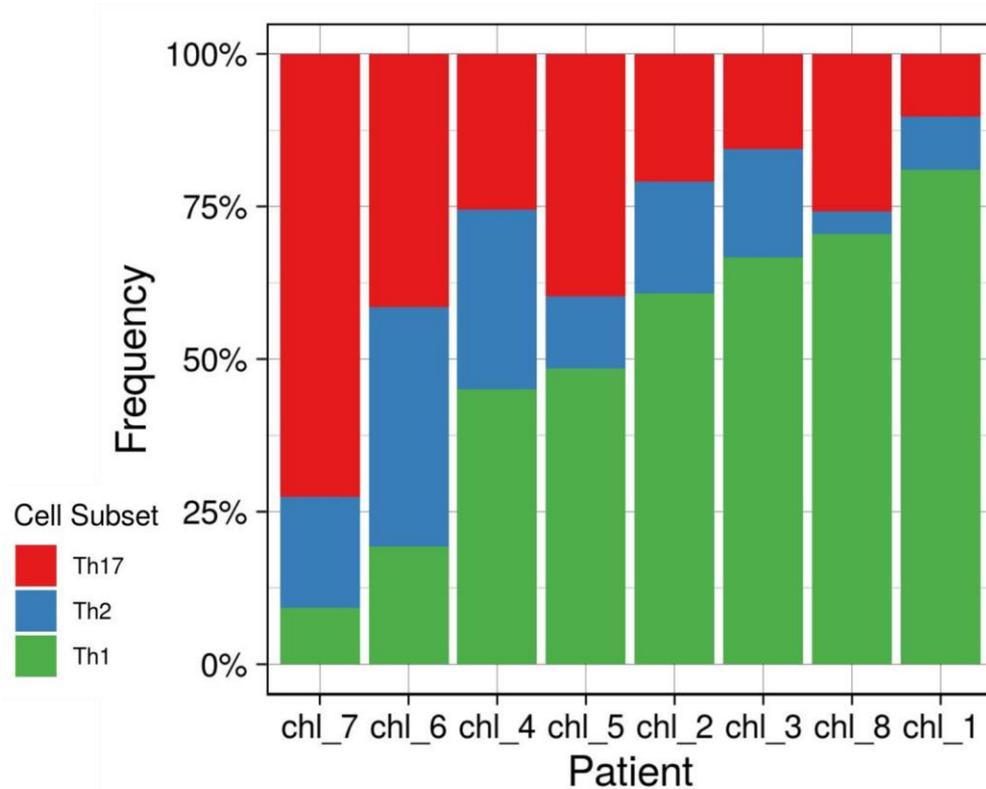
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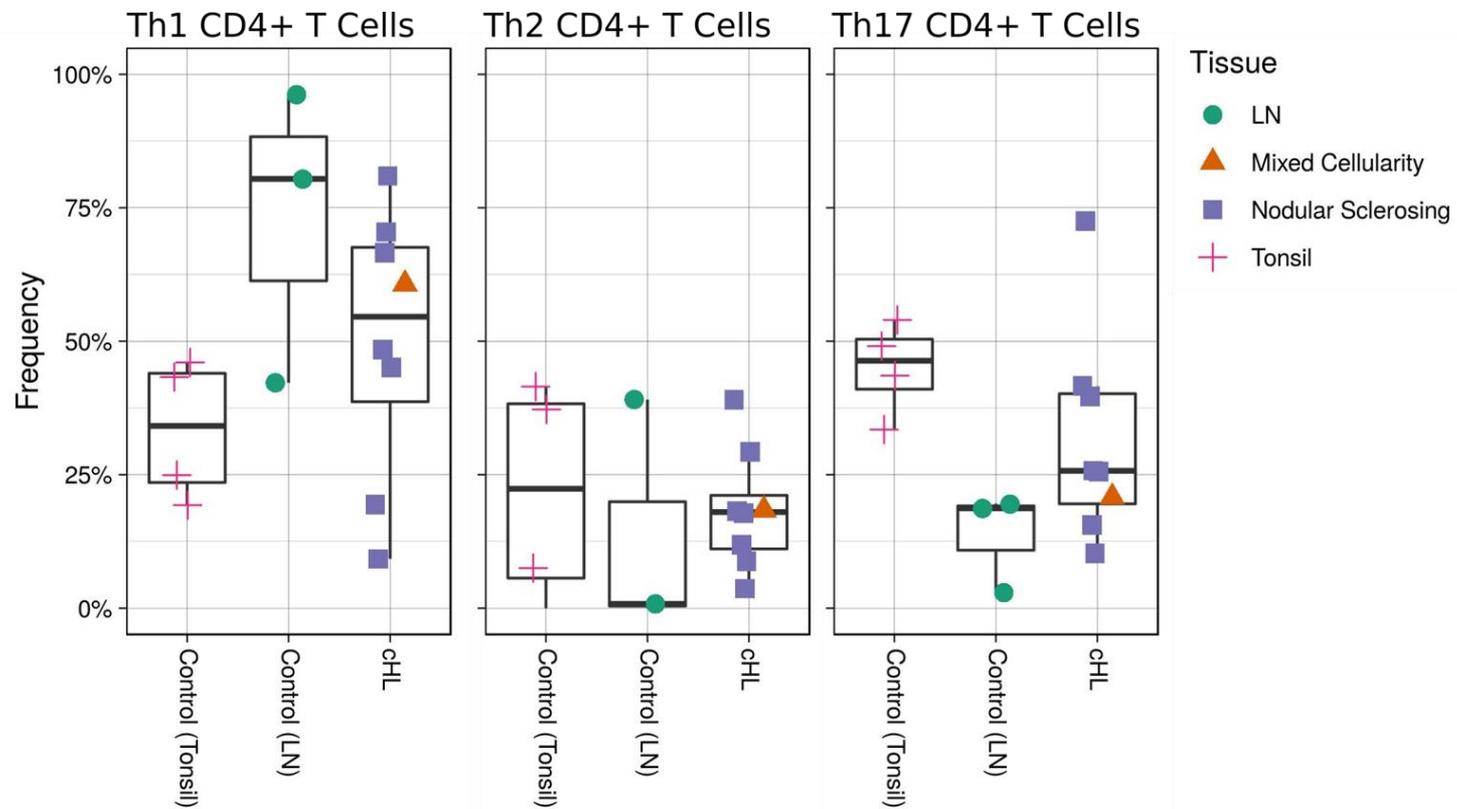
# CD8+ T Cell PD-1 Level is Heterogeneous Between Patients
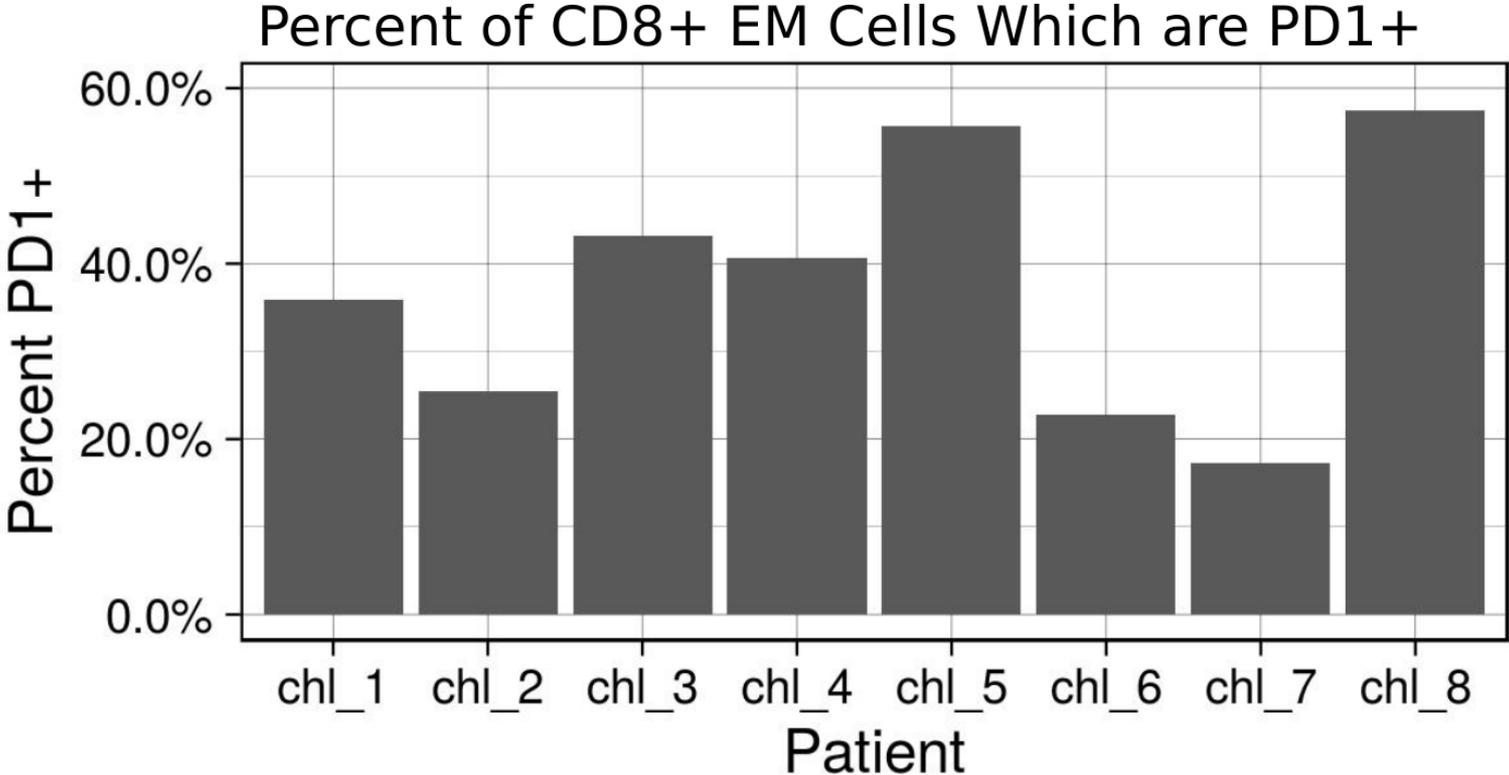
CD4+ T Cell PD-1 Level is Also Heterogeneous

Percent of CD4+ EM Cells Which are PD1+
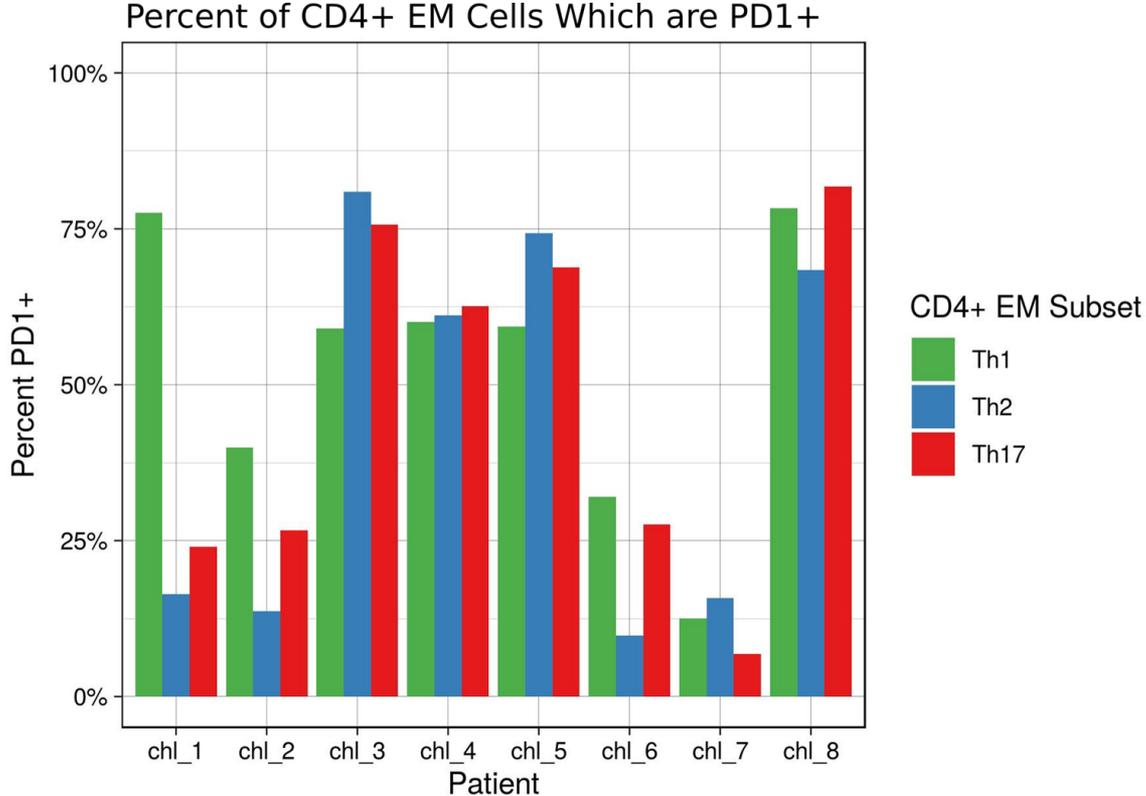
CXCR3-Dependent Differences Between Tregs

Unsupervised Clustering

CD4+ T Cell (Treg, Memory) CD69hi CXCR3hi CD161lo, −log10(FDR) = 0.102
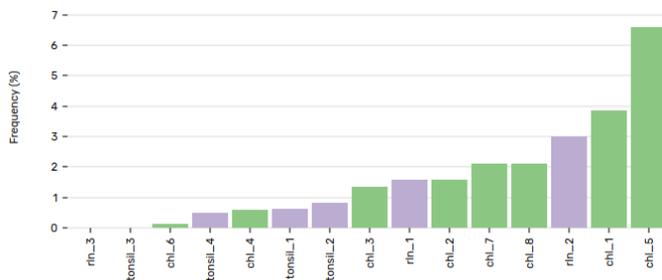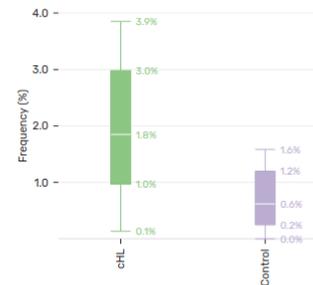
3x fold **increase** in cHL

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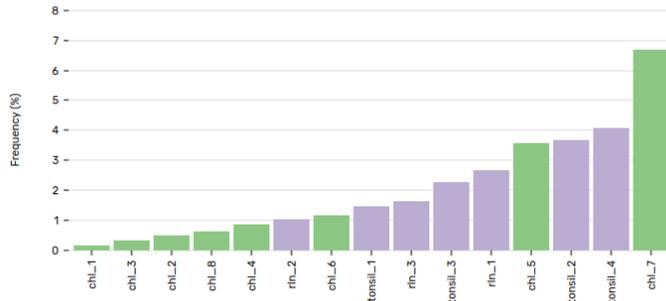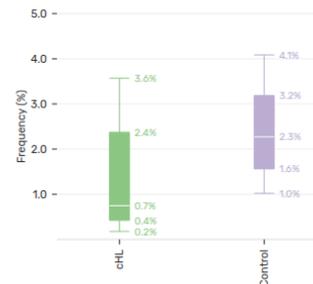
3x fold **decrease** in cHL

CXCR3-Dependent Differences Between Tregs
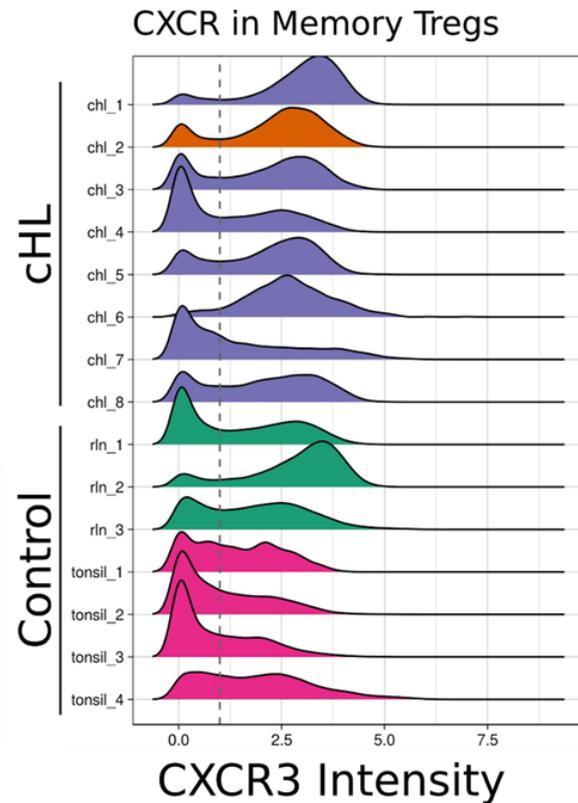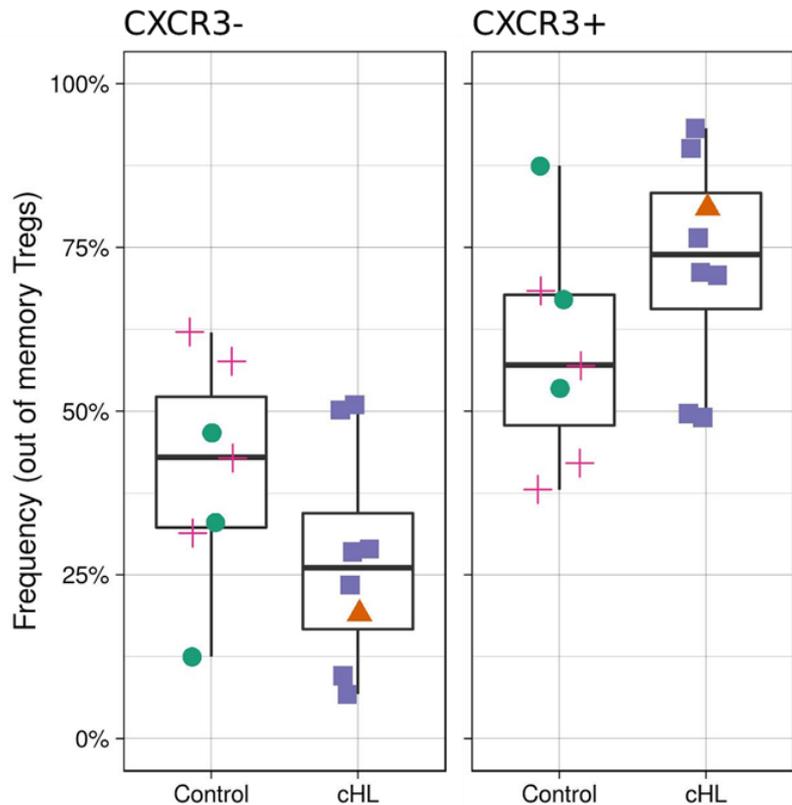
Detection of HRS Cells using CyTOF + Semi-Supervised DR

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CD19	CXCR5	
CD25	**HLADR**	

... and CD30, PD-L1, and PD-L2

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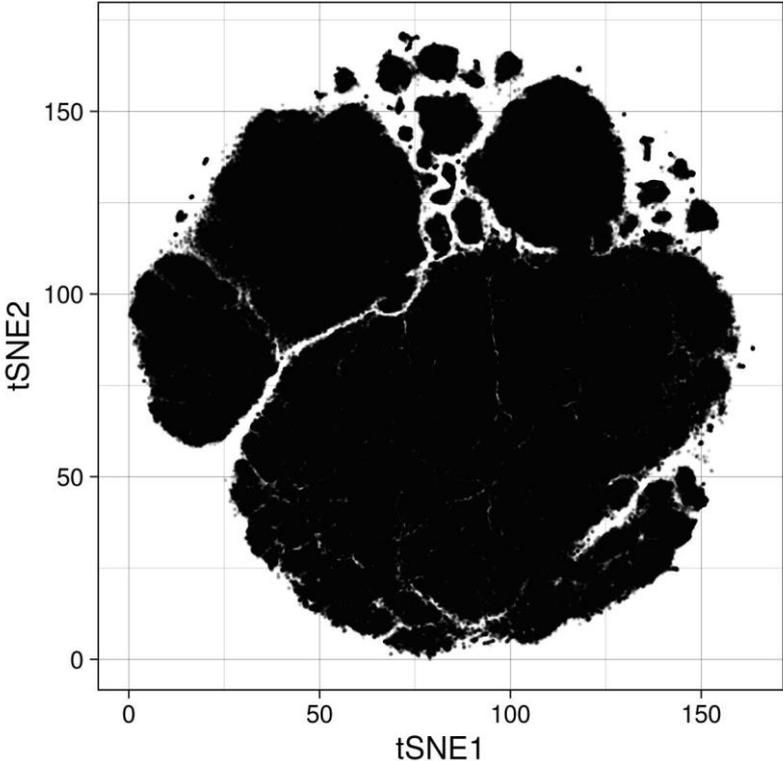
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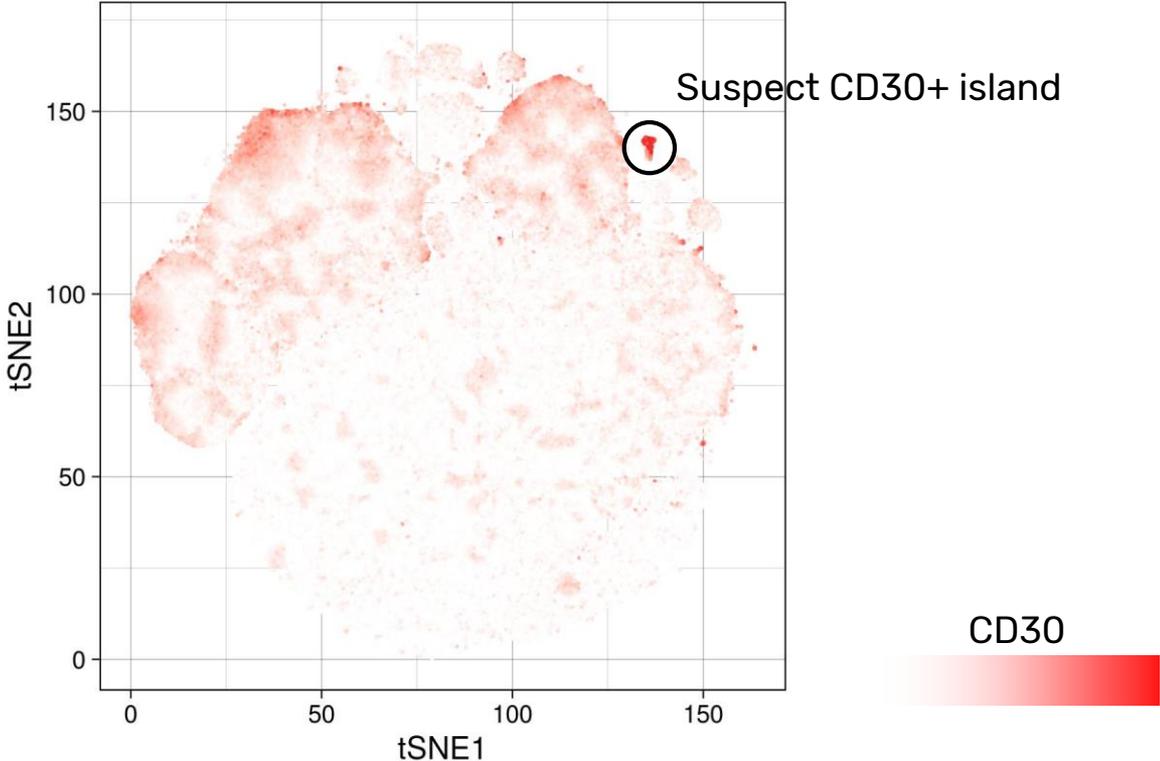
Further Investigation Confirms HRS Phenotype
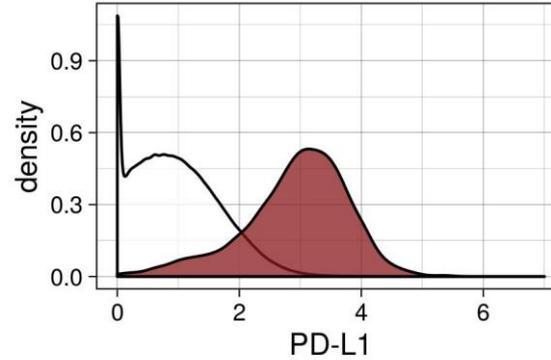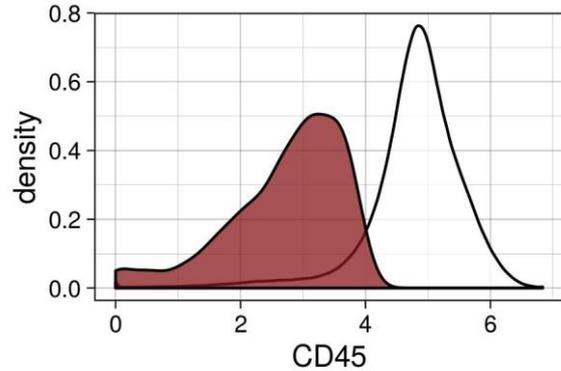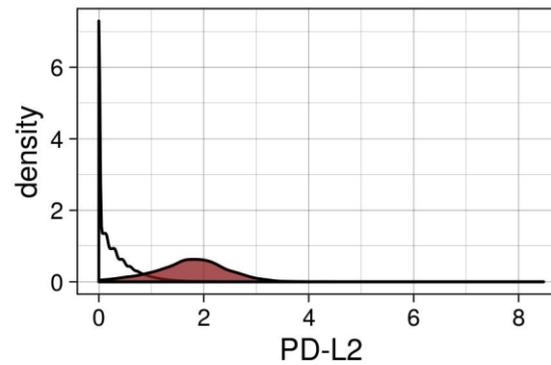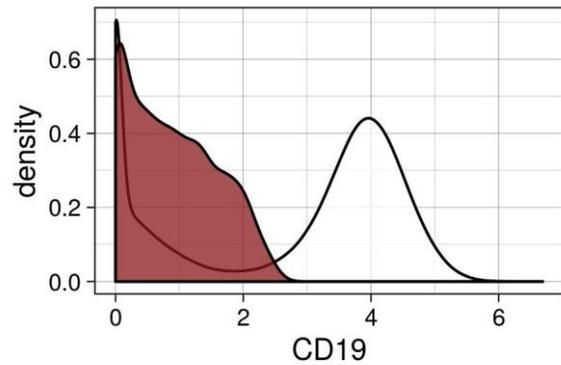
Summary

- The combination of high-complexity cytometry and sophisticated analytics enables immune profiling of cHL
- PD-1 expression on cytotoxic T cells might be only part of the story, other subsets are potentially involved
- Semi-supervised analysis can identify HRS cells

Acknowledgements

The patients and their families