Evaluation of T cells by flow cytometry

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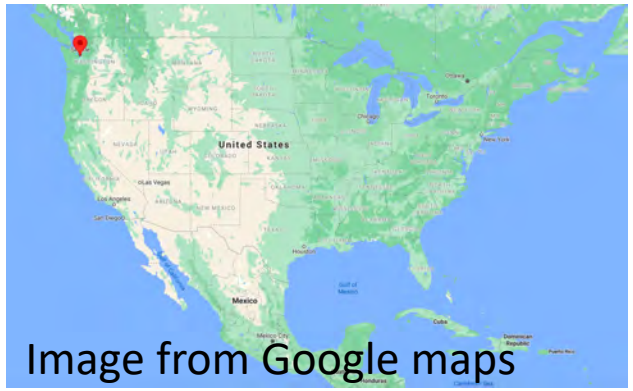

Image from Google maps

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*** I have no disclosures to report**

Flow cytometry: T cell neoplasms

- Flow cytometry is a useful tool to characterize T cell populations
- Identify and characterize normal T cells and subsets
- Distinguish abnormal from normal T cells
- Generate a differential diagnosis on the basis of the immunophenotype of an abnormal T cell population in conjunction with clinical and morphologic data

CD45 vs SSC gating
Pan T cell markers
CD4/CD8
NK cells

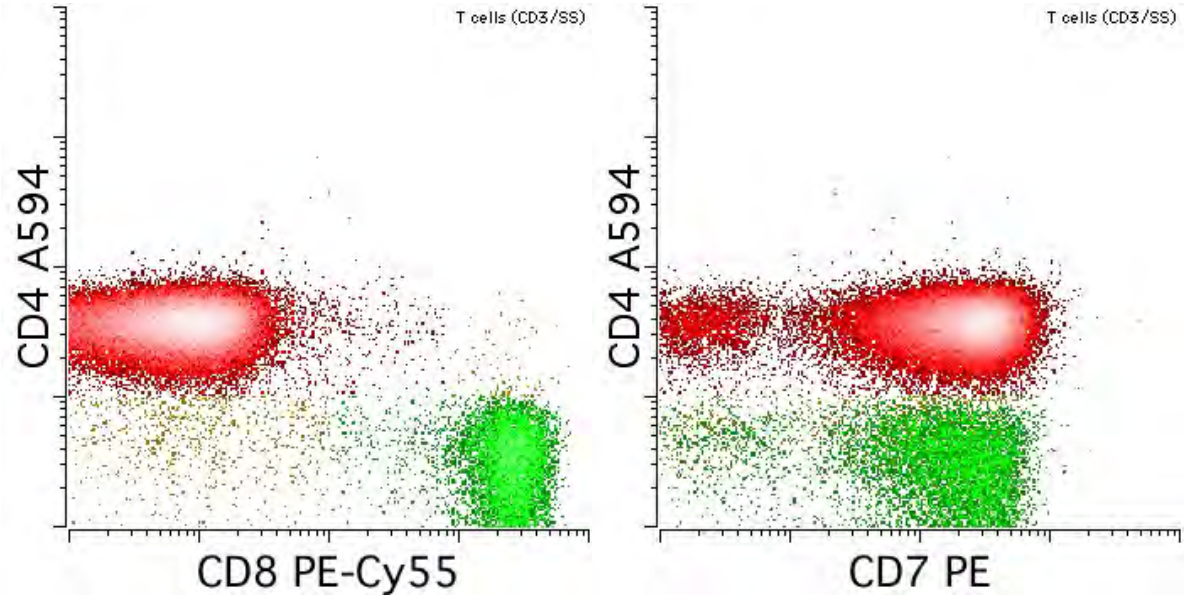
Antigen
CD8
CD2
CD5
CD34
CD56
CD3
CD4
CD7
CD30
CD45

In some cases we may add additional markers to:

1. **Further characterize cells**
   - Adult T cell leukemia/lymphoma: CD25
   - Sezary syndrome mycosis fungoides: CD26
   - T follicular cell: CD10, PD1
   - T cell prolymphocytic leukemia: TCL1
   - TCR $\alpha\beta$ or $\gamma\delta$
2. **Assess for clonality**
   - TCR v beta chain assessment or TRBC1 evaluation
3. **Characterize potential therapeutic targets**
   - CD25, CD30, CD52

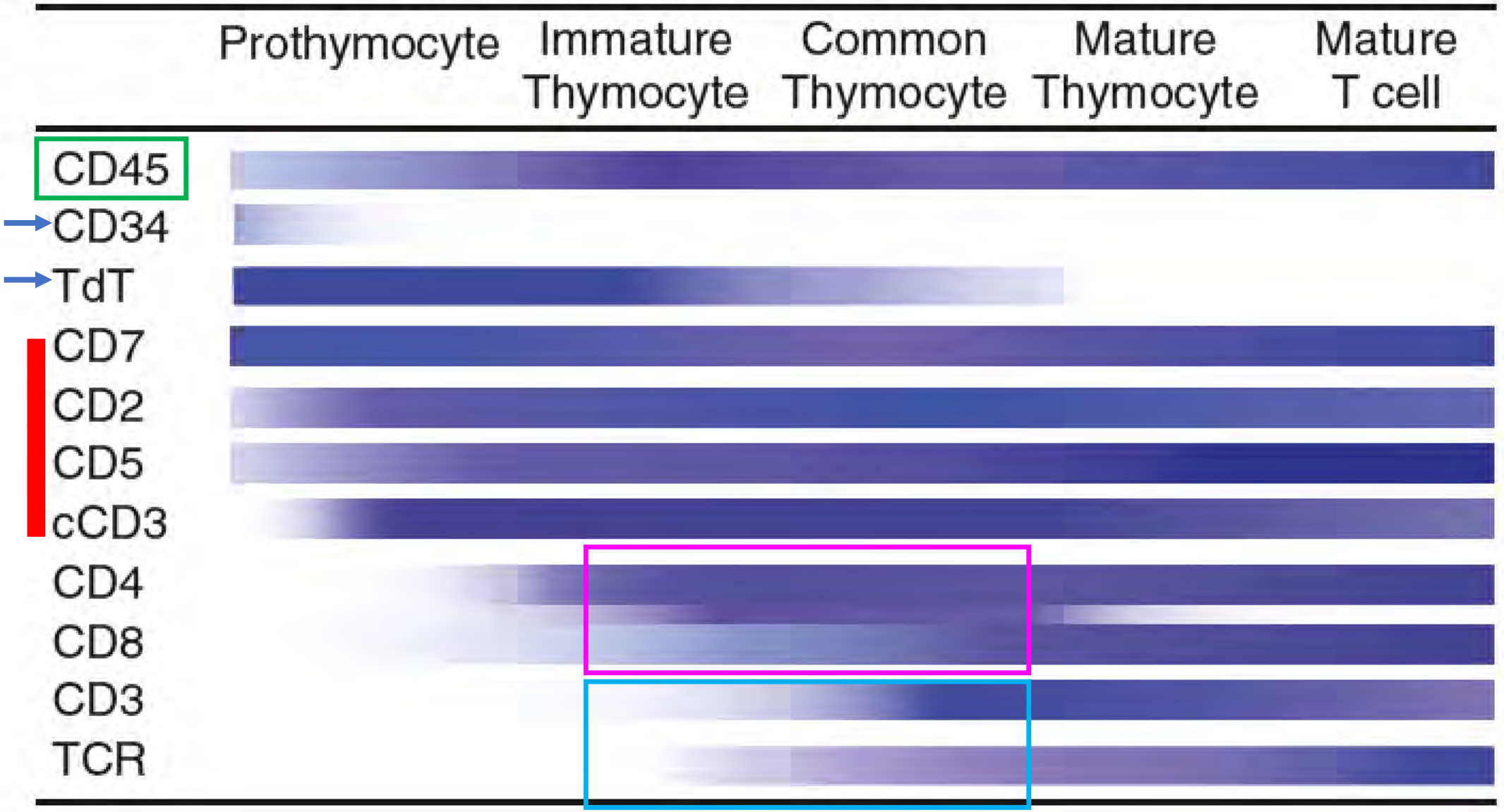
Normal T cells

- Express pan-T cell antigens
  - CD2
  - CD3
  - CD5
  - CD7
- CD4:CD8 ratio
  - Fluctuates with:
    - Anatomic sites
      - PB ~1-4
      - LN ~2-5
    - Reactive states



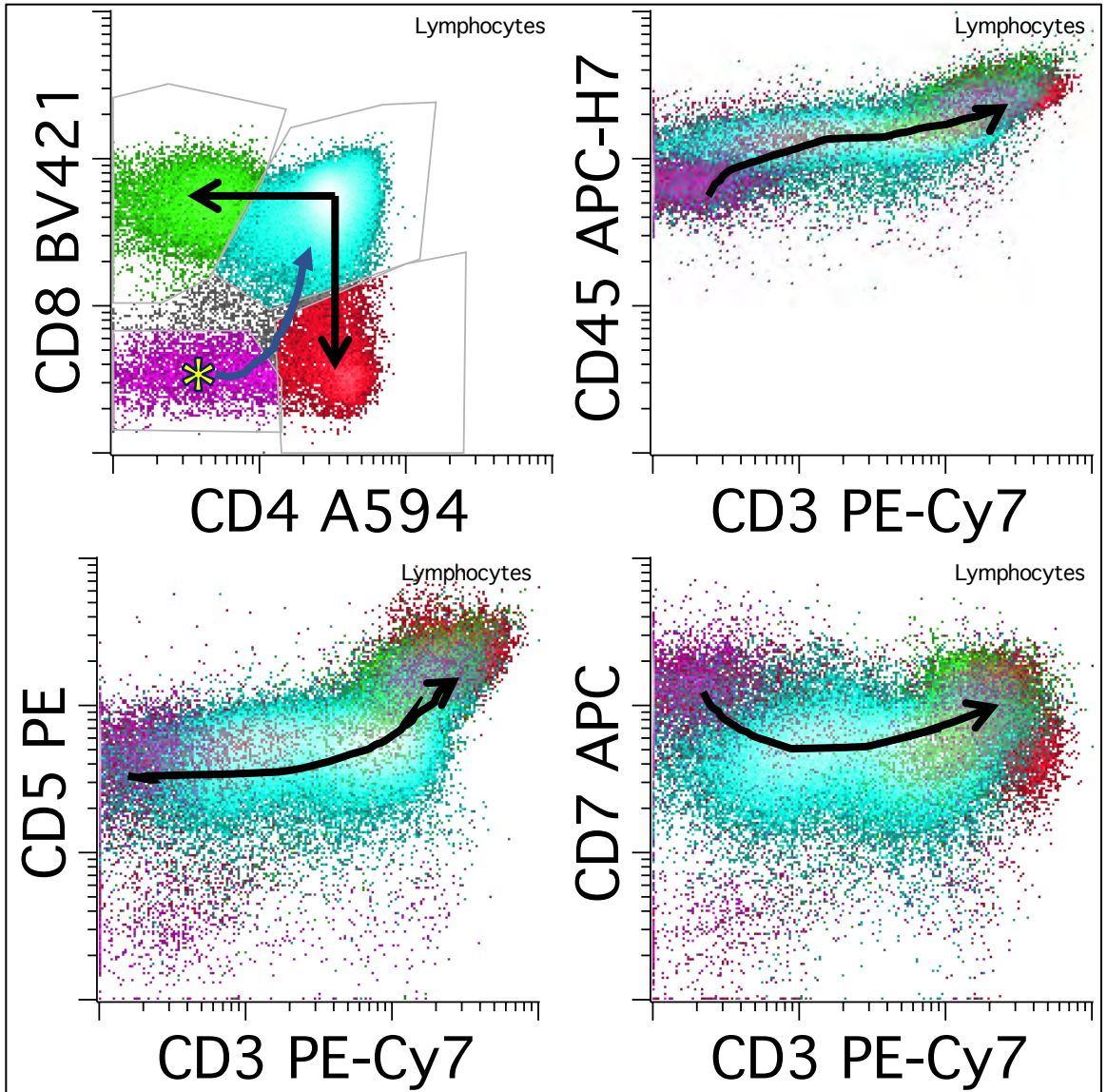
Reactive changes are often accompanied by antigenic shifts

Let's go back to the beginning…



Wood, Cherian, and Borowitz (2017) Henry's Laboratory Methods
Blom et al. Blood 1999;93(9):3033-3043.

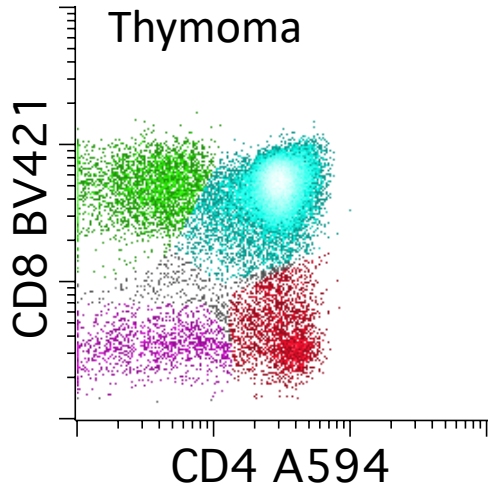
T cell maturation: Thymic tissue

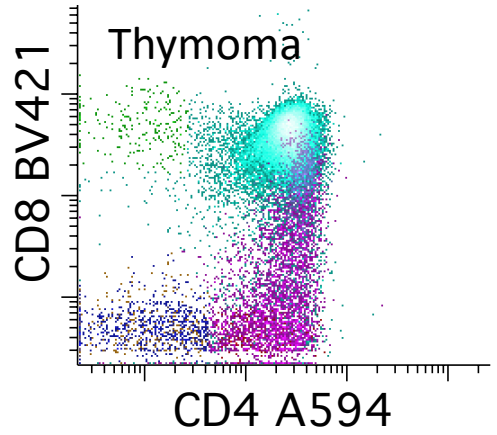


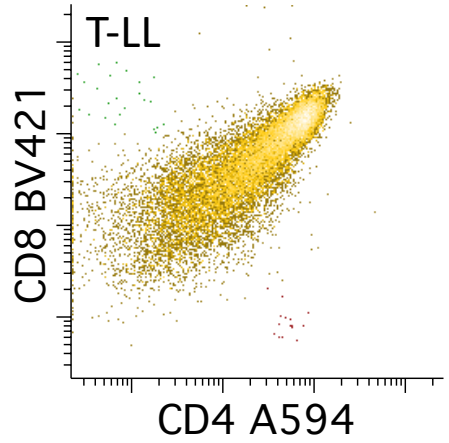
- Thymus, thymic hyperplasia, thymoma
  - With thymoma, the dual positive population often predominates, though single positive and double negative populations are seen.
- Ectopic thymus
- Indolent T lymphoblastic proliferations
  - Fromm et al. Clinical Cytometry 2020;98(3):282-87.
  - Occasionally immature T cell populations can be seen in reactive lymph nodes or tissues
    - Castleman's disease
    - Reactive lymphoid hyperplasia

Thymic tissue versus T-LL/L

- Thymoma typically show some evidence of 4 distinct populations (double negative, double positive, single positive) which form a continuum (smear)
  - Correlation with morphology is helpful in establishing a diagnosis (cytokeratin stain)
- T-lymphoblastic leukemia/lymphoma (T LL/L) by contrast forms a tight cluster of cells without discrete populations or a smearing pattern
  - Additional helpful features that can characterize T-LL/L
    - Aberrant antigen loss
    - Aberrant uniform CD34 expression



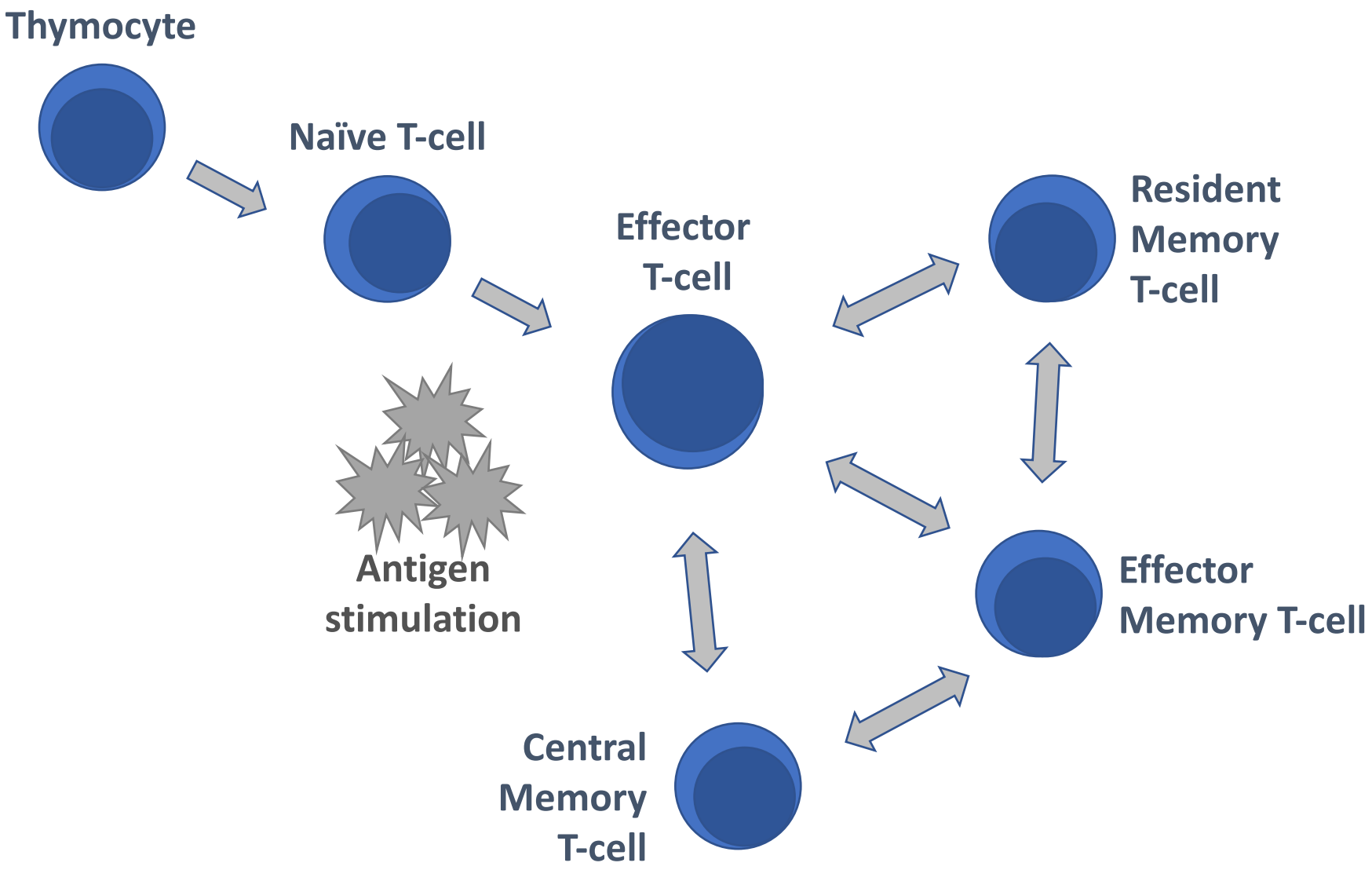




Li et al. AJCP 2004;121:268-74.

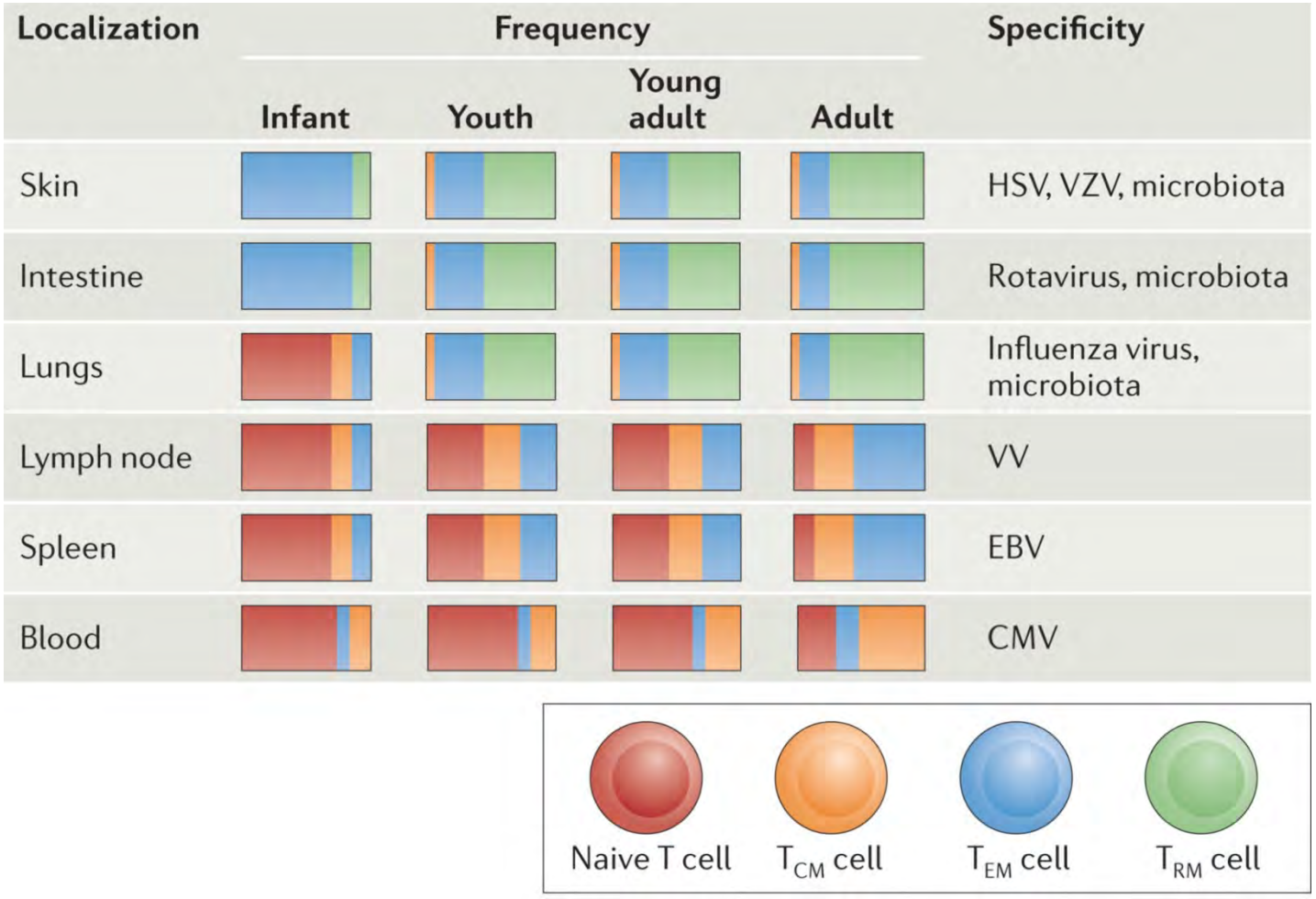
Slide Courtesy of Fiona Craig

T-cell differentiation and activation



Slide Courtesy of Fiona Craig

T-cell differentiation



Farber DL et al., *Nat Rev Immunol* 14(1): 24–35; 2014

Slide Courtesy of Fiona Craig

T-cell differentiation

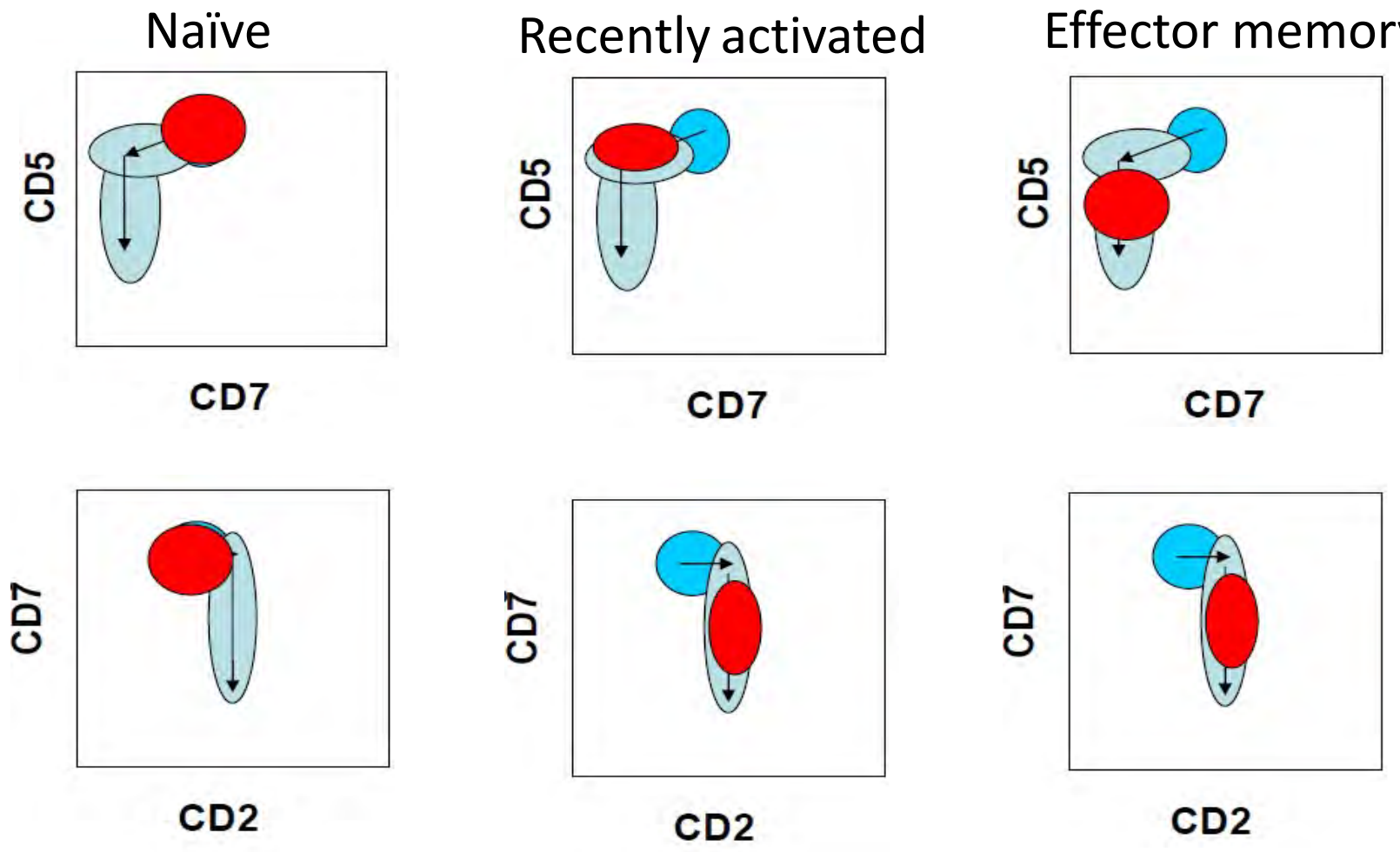
Antigen	Naive	Central Memory	Effector (Peripheral) Memory			Effector
			Early	Intermediate (Transitional)	Late (EMRA)	
CD45RA	+	-	-	-/+	+	+
CD45RO	-	+	+	+/-	-	-
CCR7	+	+	-	-	-	-
CD27	+	+	+	+/-	-	-
CD28	+	+	+	-/+	-	-

lymphoid lung, liver, gastrointestinal

Effector memory also divided by expression of cytokines (TH1 or TH2)

Slide Courtesy of Fiona Craig and Paula Fernandez

What happens with expression of pan T-cell markers with differentiation and activation?



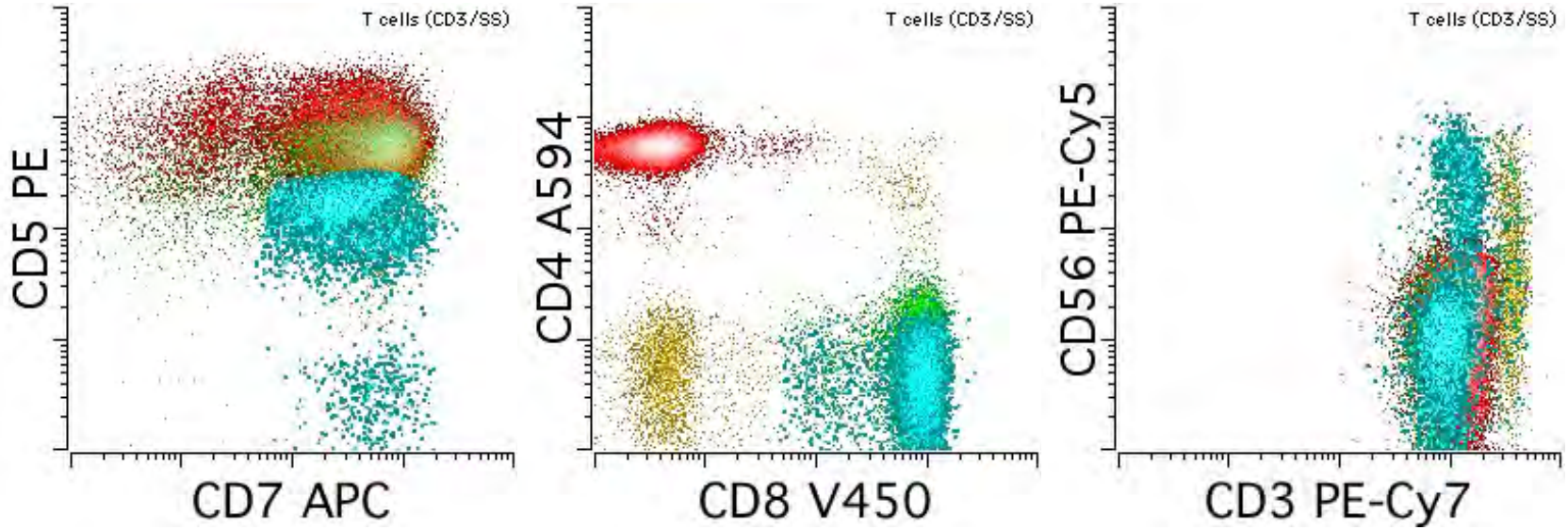
Why care about T-cell differentiation and activation?

- **Activation and differentiation are associated differences in expression of pan T-cell antigens**
- **In order to recognize immunophenotypic aberrancy that characterizes neoplastic cells, one must understand the spectrum of normal antigen expression**
- Resemblance of mature T-cell neoplasms to normal subsets can help with classification

Some notable T cell subsets will be described in the next few slides

Reactive T cell subset: Large Granular T Lymphocyte

- CD3+ T cells expressing CD8 with decreased CD5
- CD57+, CD56+/-
- Reactive LGLs may in increased in the blood in inflammatory or infections settings and in lymph nodes or tissues infiltrated by tumor



DDX:
T cell LGL lymphoproliferative disorders

Reactive T cell subset: Memory T cells

- CD4+ T cell subset with decreased CD7
- Frequent in the skin, effusion specimens (eg. pleural fluid), can be increased in inflammatory settings
- May show increased CD2 expression

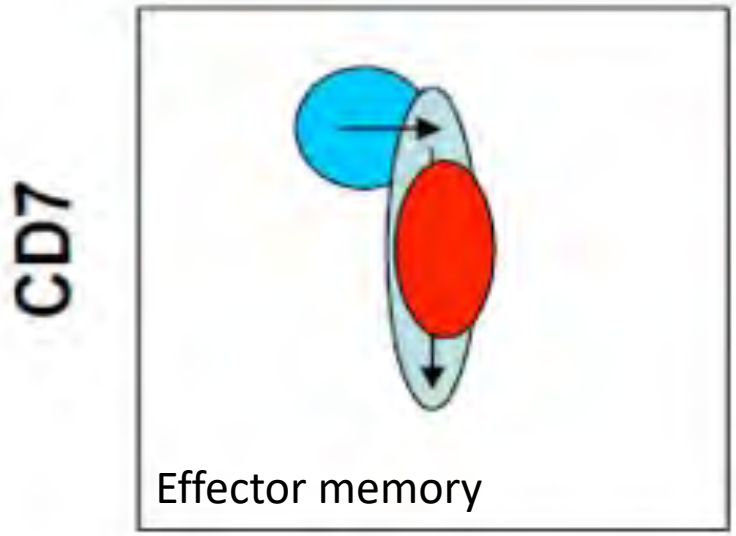
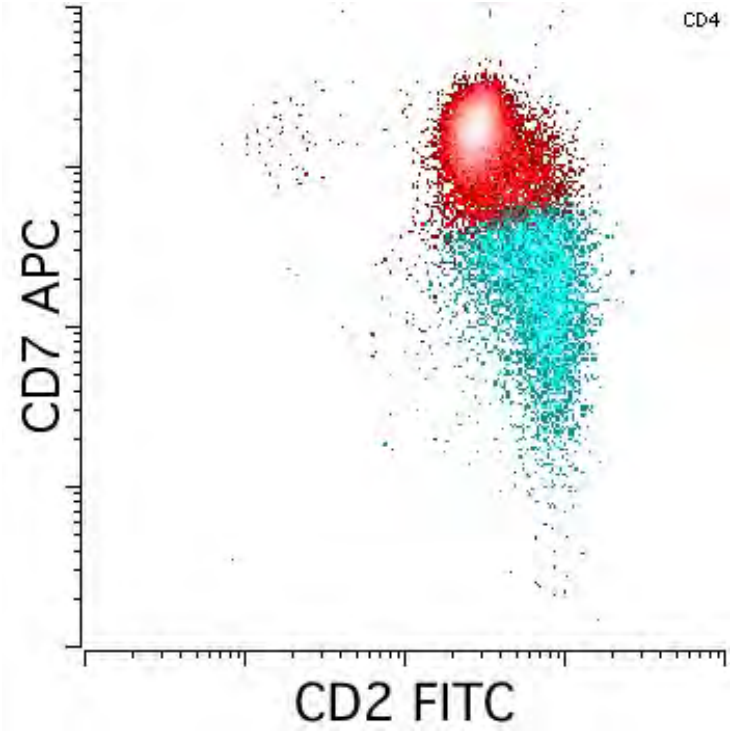


Image Courtesy
Paula Fernandez

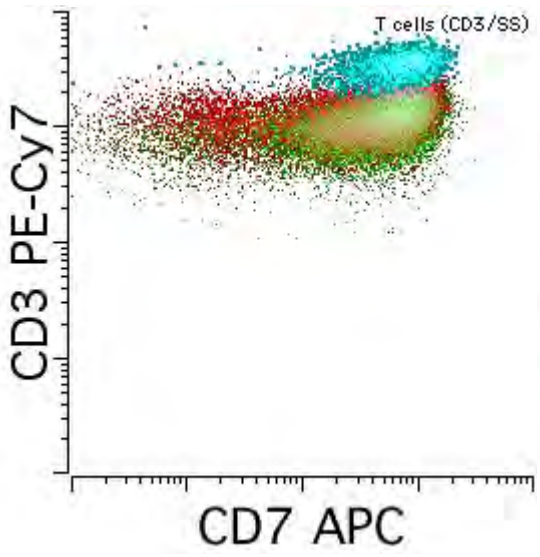


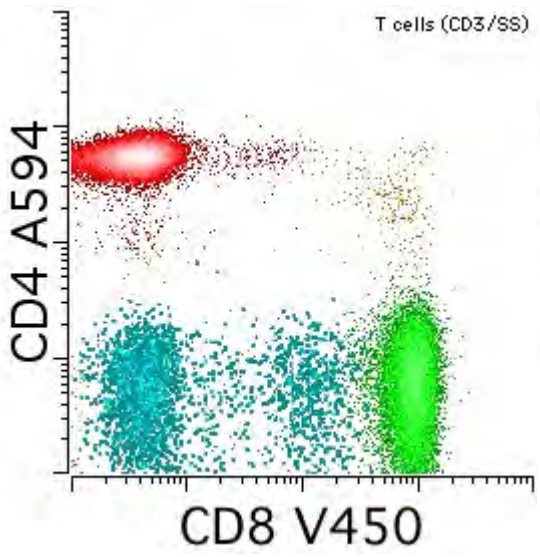
DDX:
T cell neoplasms that characteristically express CD4 without CD7:
SS/MF and ATLL

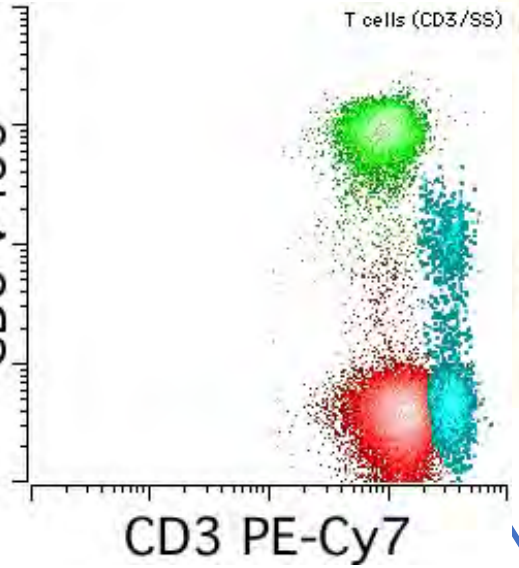
Sanders et al. The Journal of Immunology. 1988140(5):1401-7

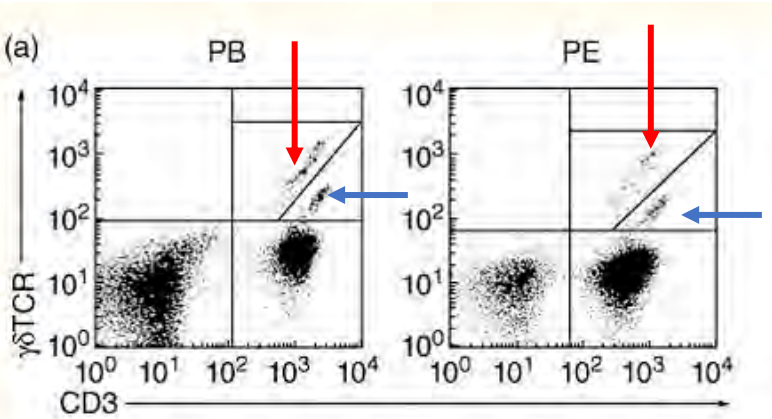
Reactive T cell subset: Gamma Delta T cells

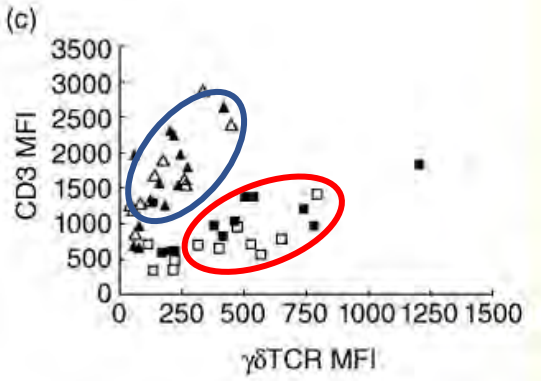
- Major subset is T cell population bright for CD3+
- Double negative for CD4/CD8 or express low CD8
- Some variation in CD3 may be seen related to gamma delta expression







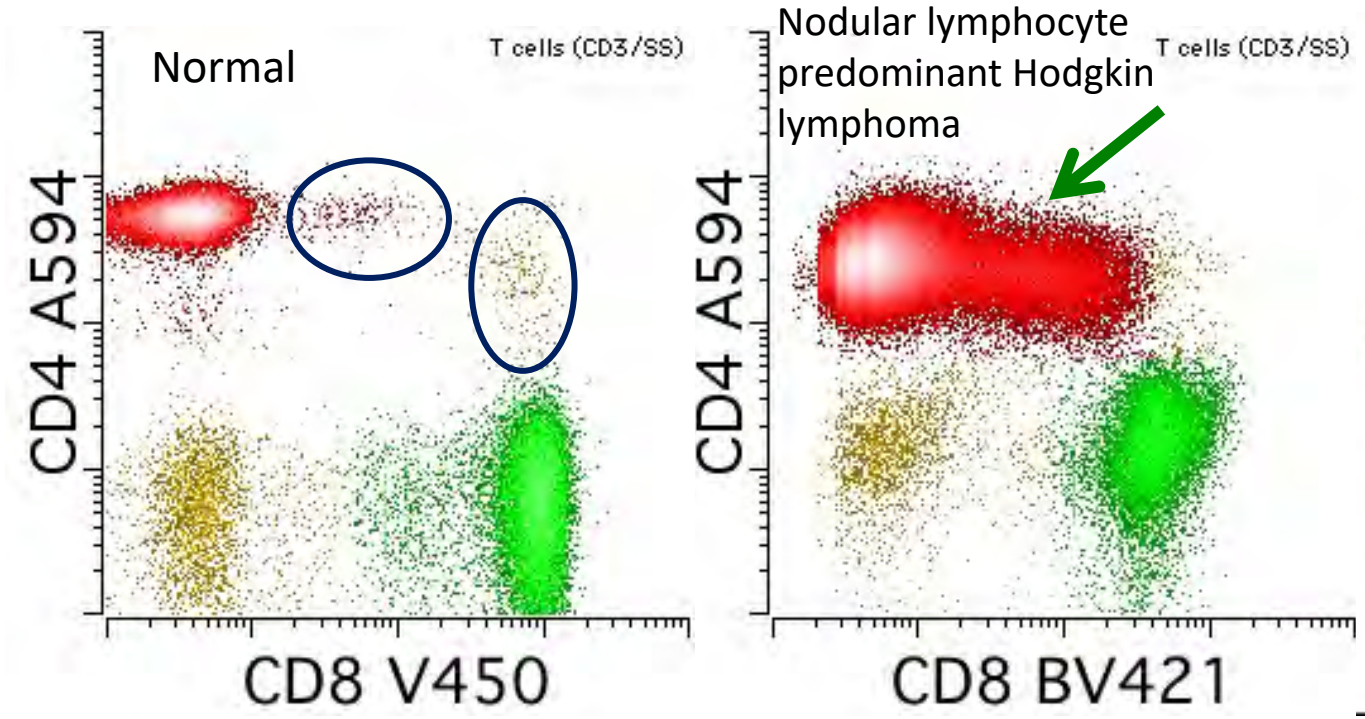


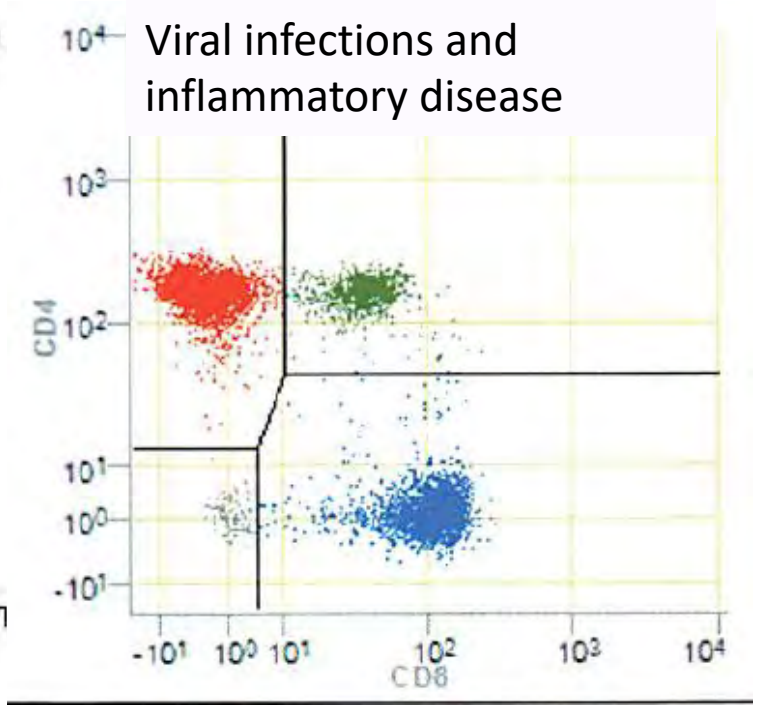


DDX:
Neoplasms of γδ T cells.

Clin Exp Immunol. 2009 Sep; 157(3): 385–394.
doi: 10.1111/j.1365-2249.2009.03974.x

Some mature reactive T cell populations may be CD4 and CD8 double positive





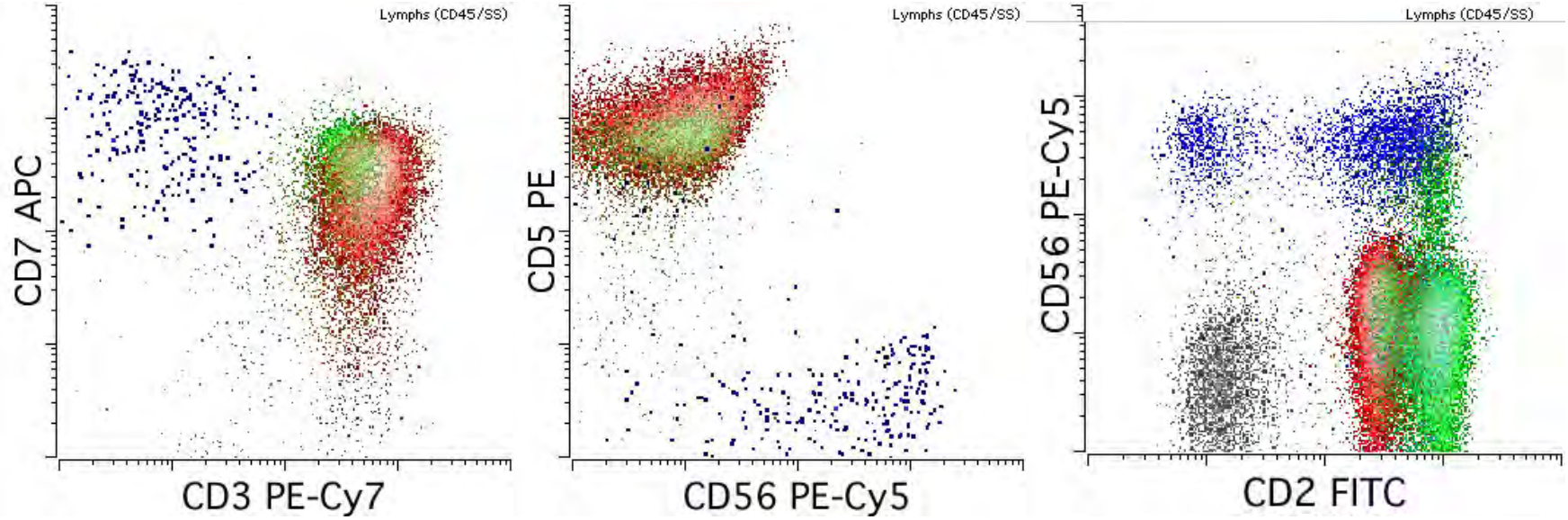
HIV+ long term non-progresser
Described in chronic CMV infection

Parel and Chizzolini. Autoimmunity reviews 3. 2004:215-220.
Wu et al Clinical Cytometry 2016;90(5):424-432

DDX:
Neoplasms composed of T cells co-expressing CD4 and CD8

NK cells

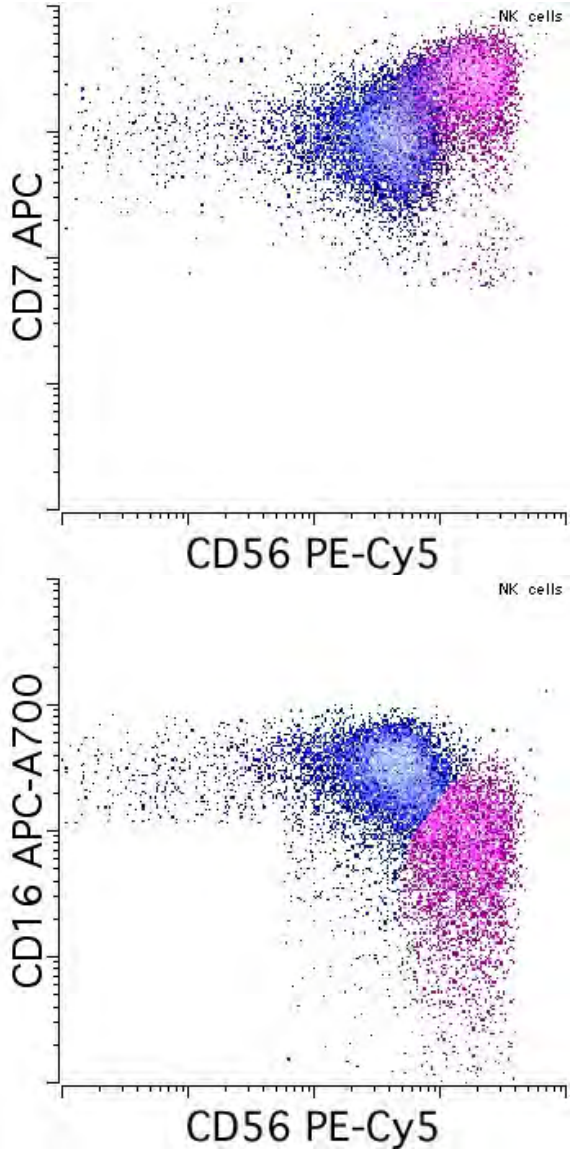
- Blood ~5-30%; lymph nodes <5%
- NK cells typically express CD2 and CD7 without surface CD3 or CD5
- A subset may lack CD2
- NK cells express CD56 and CD16
  - Intensity of these antigens is typically reciprocal



NK cells highlighted in blue, 1.2% of all lymphocytes

CD16 and CD56 on NK cells

- CD56 bright NK cells have low CD16 (bright CD7)
  - Minority of PB NK cells (~10%)
  - Predominate in reactive lymph nodes (~90%)
    - Lack perforin and KIRs although these (as well as CD16) can be up-regulated with activation
- CD16 bright T cells have lower CD56
  - Majority in PB (90-95%) and spleen (~85%)
  - Express perforin and KIRs



Fehniger et al. Blood. 2003;101(8):3052-7
Ferlazzo et al. The Journal of Immunology. 2004;172: 1455-62

Reactive changes can give rise to immunophenotypic shifts resulting in a variety of T and NK cell subsets

These reactive changes may overlap with the immunophenotype of some lymphomas

Knowledge of the variation in normal is required to avoid over interpreting a reactive T or NK cell subset

When assessing T cells, clinical and morphologic correlation is critical!

Leuk Lymphoma. 2015 Mar;56(3):639-44.

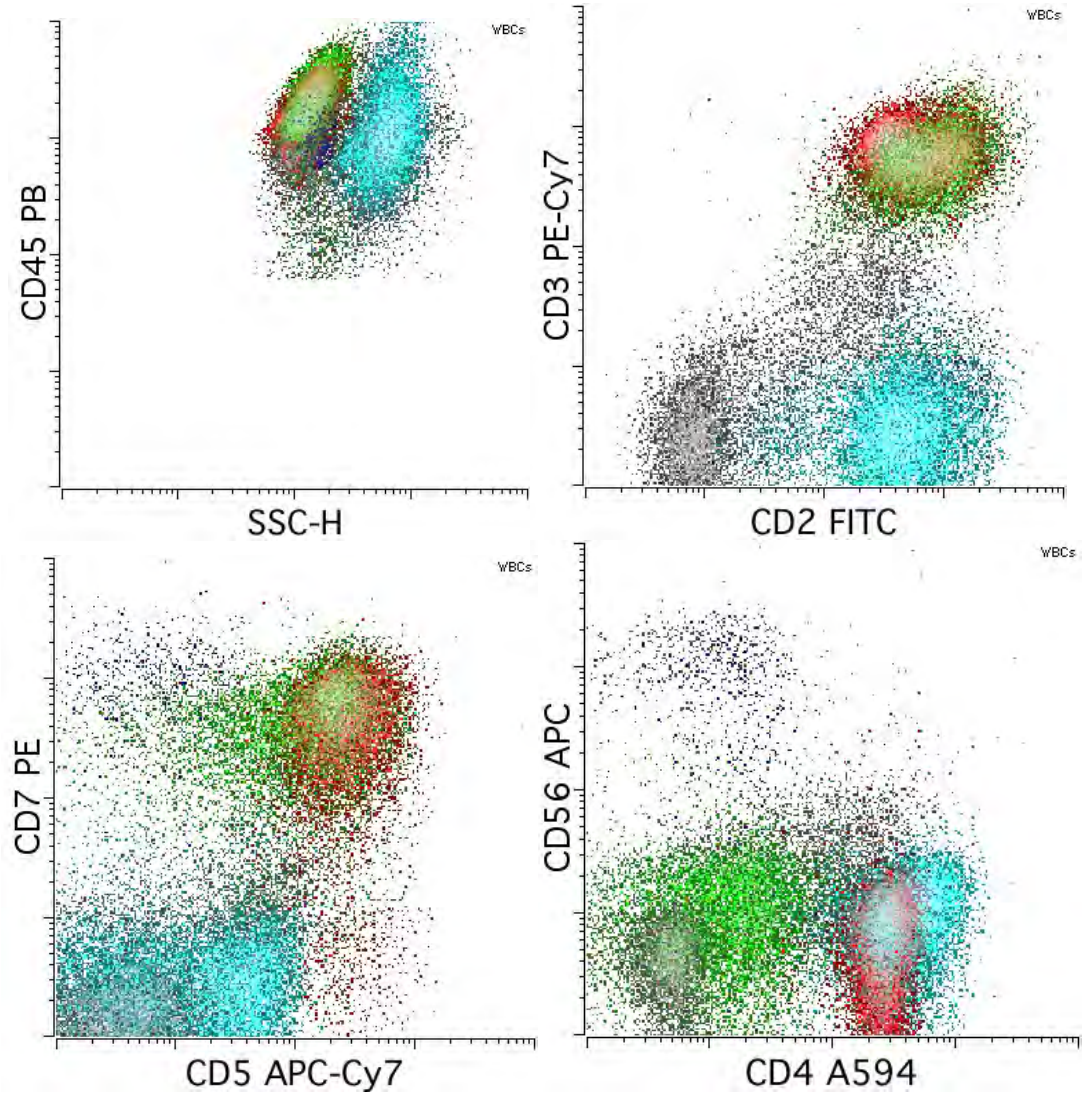
The following features should raise your suspicion for T cell lymphoma…

- Loss of or markedly decreased expression of CD45
- Complete loss of one or more pan-T cell antigens
- Decreased expression of more than 2 pan T cell antigens in conjunction with altered light scatter properties
- CD4/CD8 dual positive or dual negative populations
  - Exclusions
    - Thymus
    - Autoimmune lymphoproliferative syndrome-ALPS
    - Some reactive T cell subsets (either dual positive or dual negative)

Gorczyca et al Clinical Cytometry 2002 50;177-190

Is this T cell lymphoma?

Lymph node biopsy from a 72 year old male with B symptoms



- Increased scatter properties
- Loss of more than one antigen
  - CD7
  - Surface CD3
- Aberrant expression of other antigens
  - Decreased CD5
  - Increased CD4

No one pattern of antigen expression is completely specific for T cell lymphoma

Abnormal T cell populations identified by flow cytometry must be interpreted in the appropriate clinical and morphologic context

In this case morphology showed PTCL, NOS

Steps for assessing T cell populations

- Define the population you think is abnormal and ask the following questions:
  - What aberrant antigen expression do you see?
  - Are there normal counterparts that could have this immunophenotype?
  - Abnormal scatter characteristics?
  - CD4 vs CD8 expression?
  - Do you need clonality studies?
  - What clinical, morphologic, or laboratory data will you need to definitively establish your suspected diagnosis?
  - Potential targets for therapy:
    - CD25, CD30, CD52

Slide Courtesy of Fiona Craig

CD4 positive & CD8 negative

- Sezary syndrome / CTCL:
  - CD7-, CD25 variable, CD26-; central memory-like; leukemic and/or characteristic skin findings
- Adult T-cell leukemia / lymphoma (ATLL):
  - CD7-, CD25++, HLA-DR+; FOXP3; T-reg-like; HTLV-1 associated
- T-cell prolymphocytic leukemia (PLL):
  - Retention CD7, CD5 and CD2; may lack CD45 or CD3; naïve or central memory-like, TCL-1+; cytogenetics show TCL1 rearrangement
- Angioimmunoblastic T-cell lymphoma (AITL):
  - Follicular T-helper-like (CD10, CD279); may be sCD3 negative; characteristic morphology
- Anaplastic large cell lymphoma (ALCL):
  - Usually very large cells; may be sCD3 negative, CD30+; activated cytotoxic-like
  - May be positive or negative for ALK1
- Peripheral T-cell lymphoma (PTCL), NOS: varied

❖ ***List is not exhaustive***

❖ ***Exceptions occur***

Slide Courtesy of Fiona Craig

CD4 negative & CD8 positive *OR* negative

- T-cell large granular lymphocyte leukemia (LGL):
  - sCD3+, CD56-/+, CD57+; usually decreased CD5 & CD7
  - Can be alpha beta (more common) or gamma delta positive
- Enteropathy associated T-cell lymphoma (EATL):
  - Usually CD103+
- Peripheral T-cell lymphoma (PTCL), NOS: varied
- T-cell prolymphocytic leukemia (PLL):
  - CD8 expression is less common but can be seen.
  - Retention CD7, CD5 and CD2; may lack CD45 or CD3; naïve or central memory-like, TCL-1+; cytogenetics show TCL1 rearrangement

❖ ***List is not exhaustive***

❖ ***Exceptions occur***

NK cell neoplasms

- Chronic lymphoproliferative disorder (CLPD) NK-cells or gamma delta T cells
- Nasal NK / T-cell lymphoma, aggressive NK-cell leukemia
  - Cytotoxic; EBV positive

Slide Courtesy of Fiona Craig

CD4 positive & CD8 positive

- T-cell prolymphocytic leukemia (PLL):
  - Retention CD7, CD5 and CD2; may lack CD45 or CD3; naïve or central memory-like, TCL-1+; cytogenetics

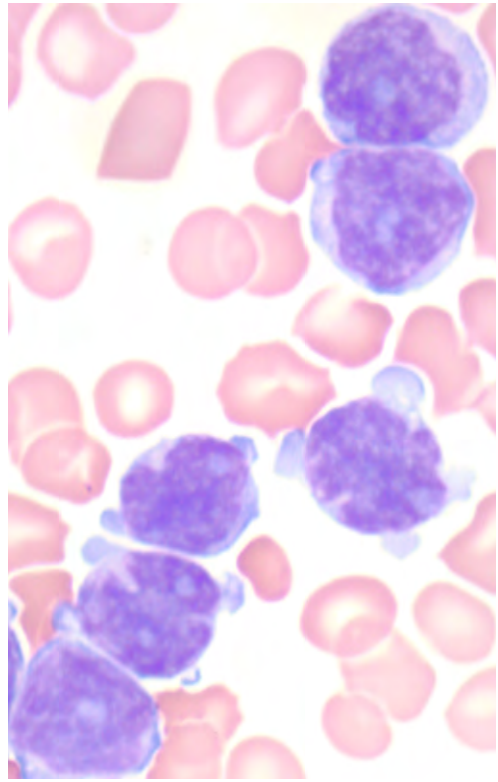
❖ ***List is not exhaustive***

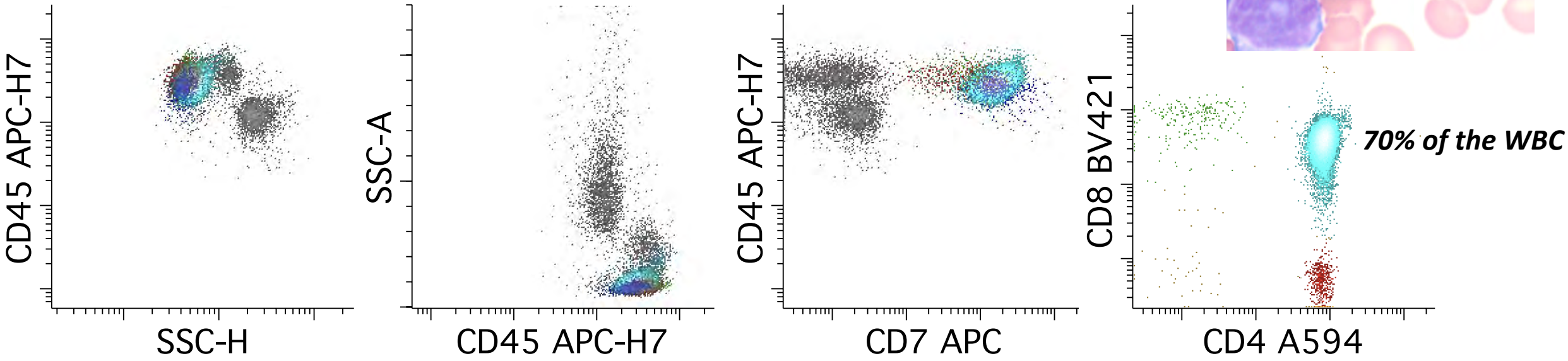
❖ ***Exceptions occur***

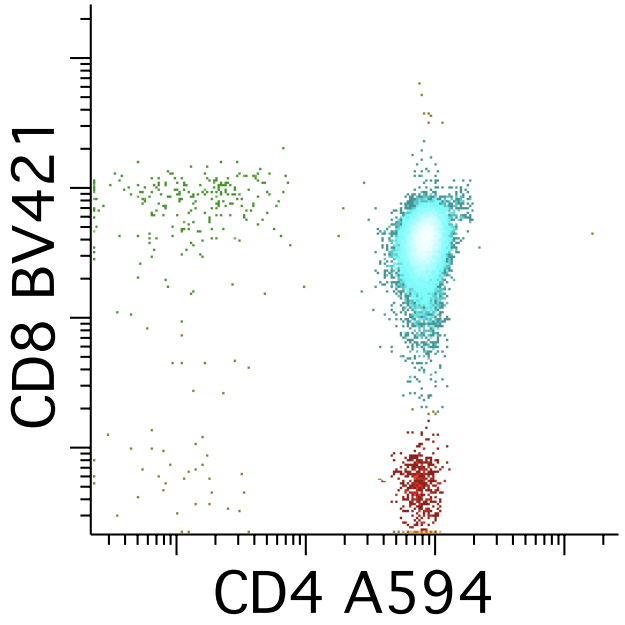
❖ ***If a T cell neoplasm is either double positive or double negative for CD4 and CD8, Make sure it is mature (consider TdT, cD1a, CD34)***

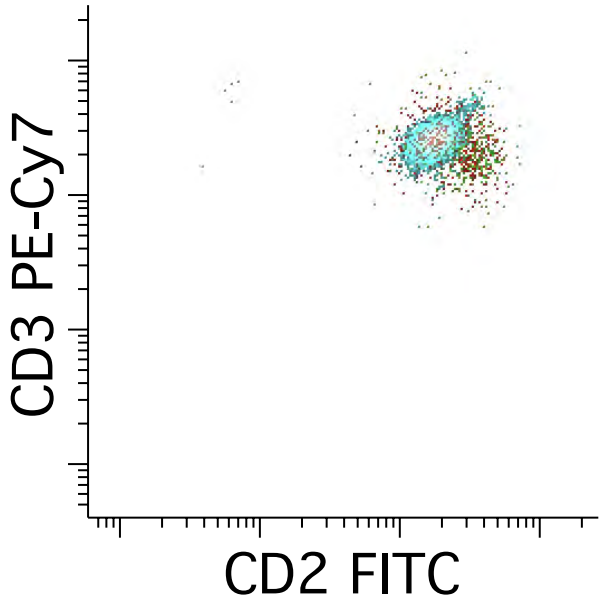
Case 1

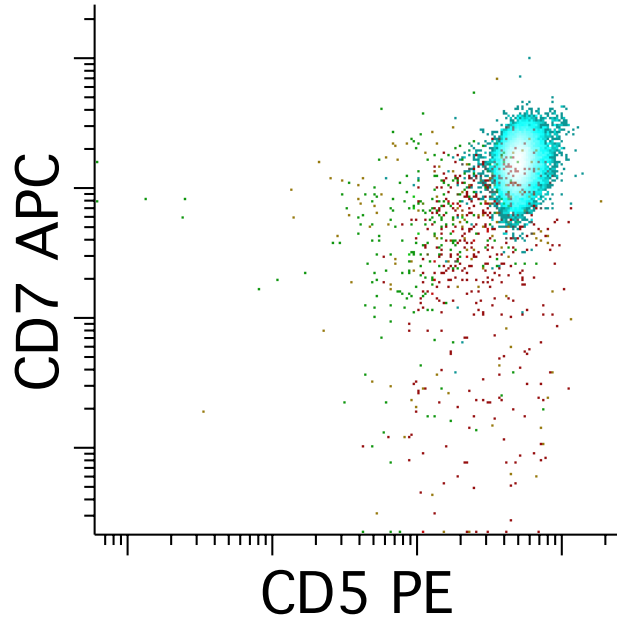
- 51 year old male with complicated prior oncologic history presents with an absolute leukocytosis with a white blood cell count of 30 thousand cells per microliter
  - ➢The patient has a personal history of colon cancer, renal cell carcinoma, and desmoid fibromatoses
  - ➢His family history is notable for colon cancer

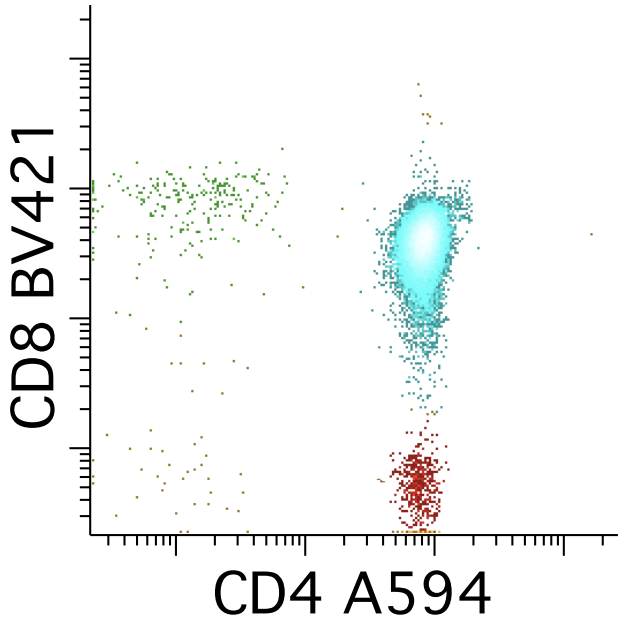












Negative:

CD1a, CD34, TdT

CD57

What is your next step?

Is it time to make a diagnosis or do you need more information?

Additional data and diagnosis

- Concurrent cytogenetics showed a very complex karyotype including a rearrangement of the TCL-1 gene on chromosome 14 as well as gains and losses of several chromosomes
- ***Diagnosis: T cell prolymphocytic leukemia***

- T cell prolymphocytic leukemia is a rare aggressive mature T cell neoplasm characterized by lymphocytosis (which can be pronounced), hepatosplenomegaly, lymphadenopathy, skin lesions, and serous effusions
- Association with ataxia-telangectasia
  - Younger age of onset

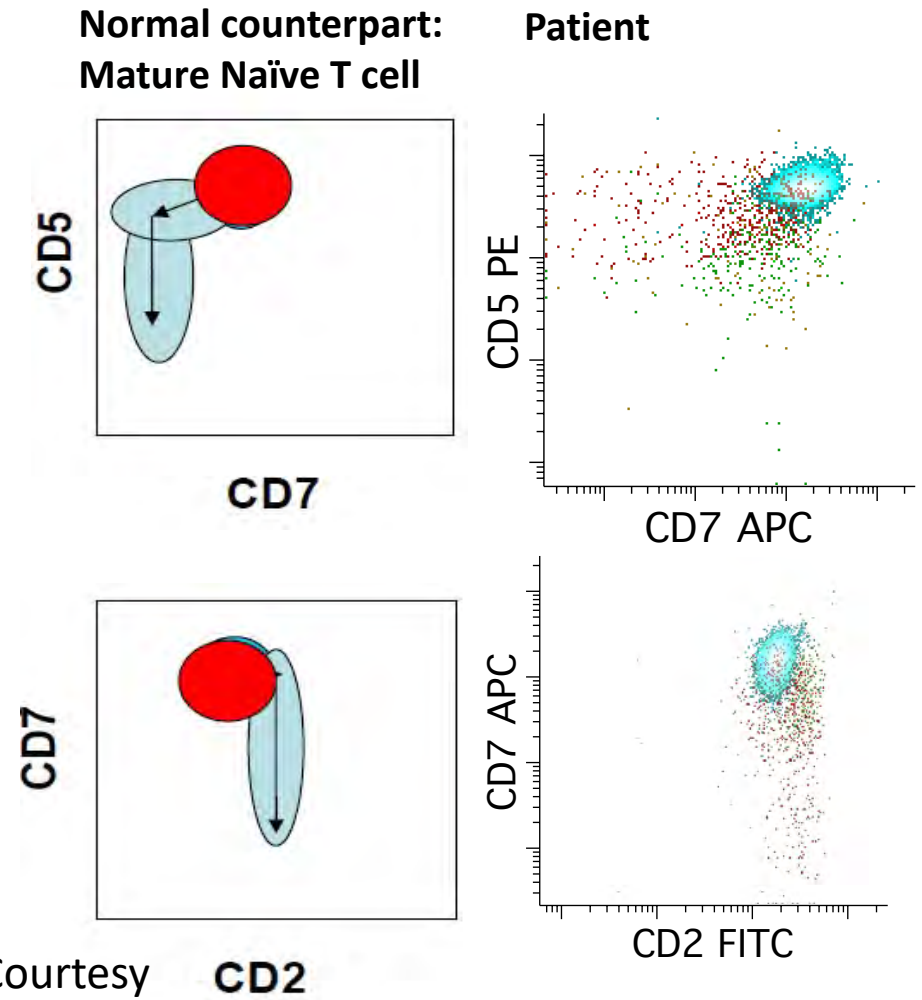
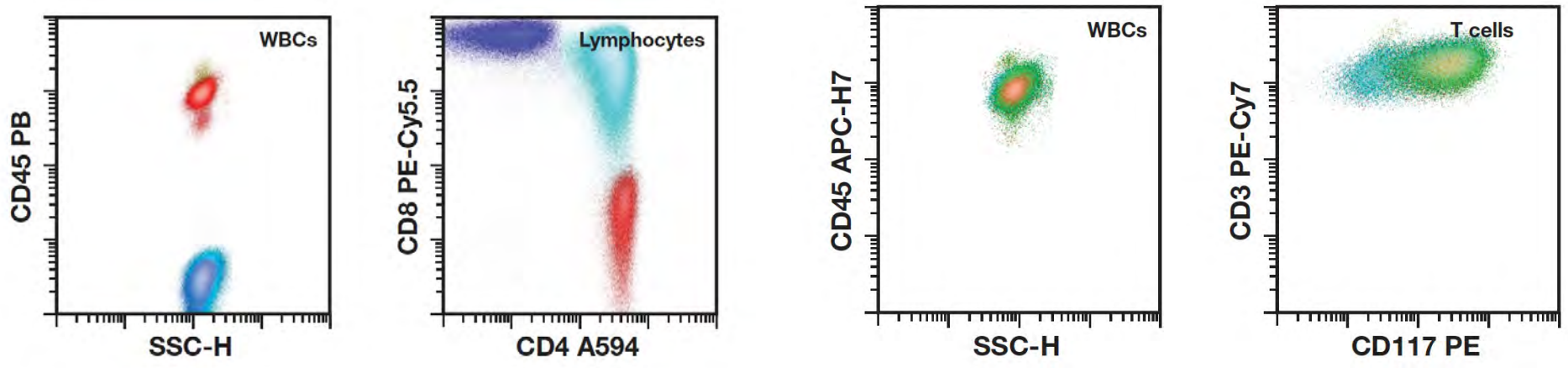


Image Courtesy
Paula Fernandez

Learning points

- T cell PLL may have differential expression with regard to CD4 and CD8
  [CD4+ (38%); CD4+/CD8+ (41%); CD8+ (17%)]
- One may see a homogeneous population or distinct subsets
- Some features may raise the question of immaturity
  - Decreased surface CD3 or CD45, co-expression of CD4/CD8, CD117 expression
  - Importantly , T-PLL is uniformly negative for CD1a, CD34 and TdT



Genetics
TCL1 rearrangement helps to confirm a suspected diagnosis

Chen and Cherian AJCP 2013;140(5):727-735.

Case 2

- 76 year old female with an erythematous pruritic rash
- Leukocytosis with WBC count o 16 cells/uL.
- A peripheral blood is sent for flow cytometry and a skin biopsy is performed.

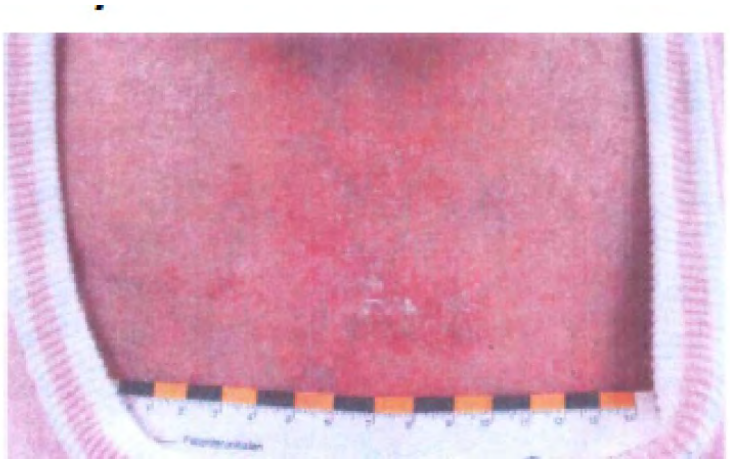
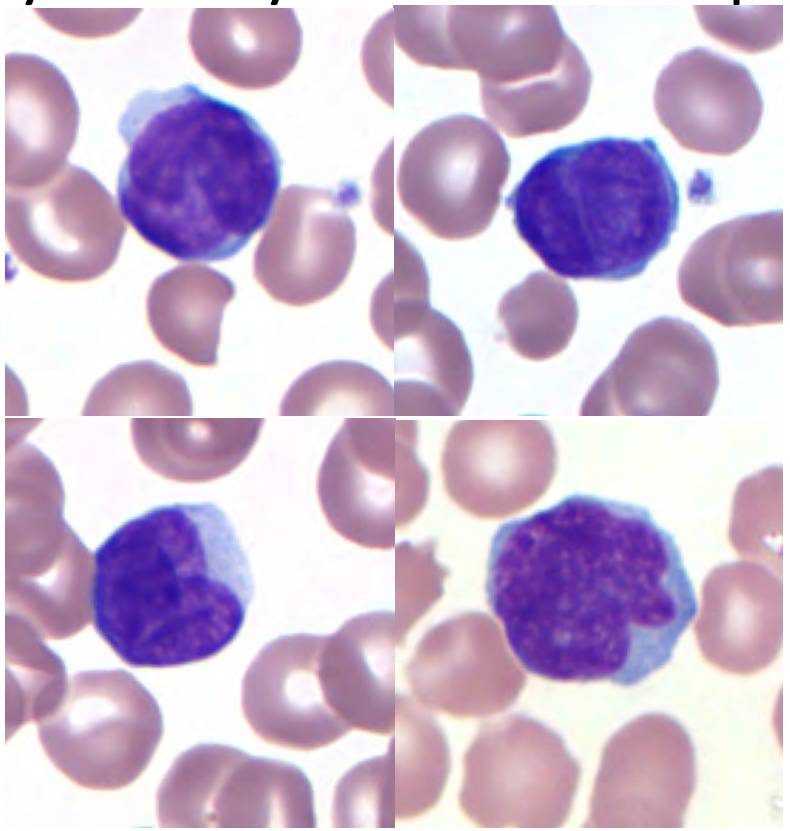
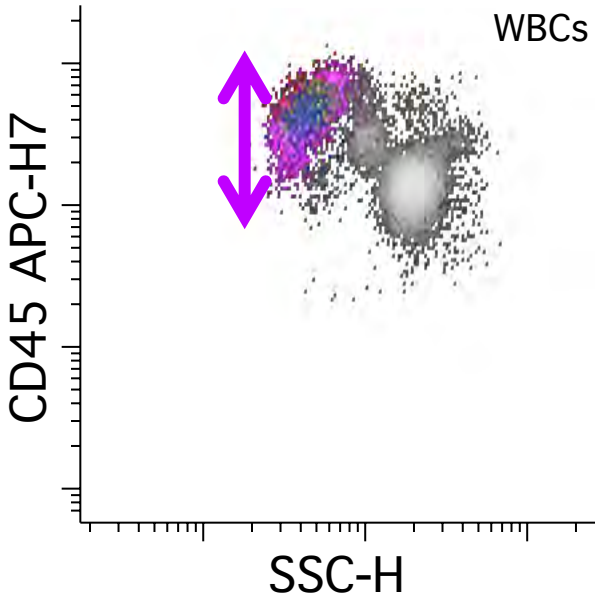
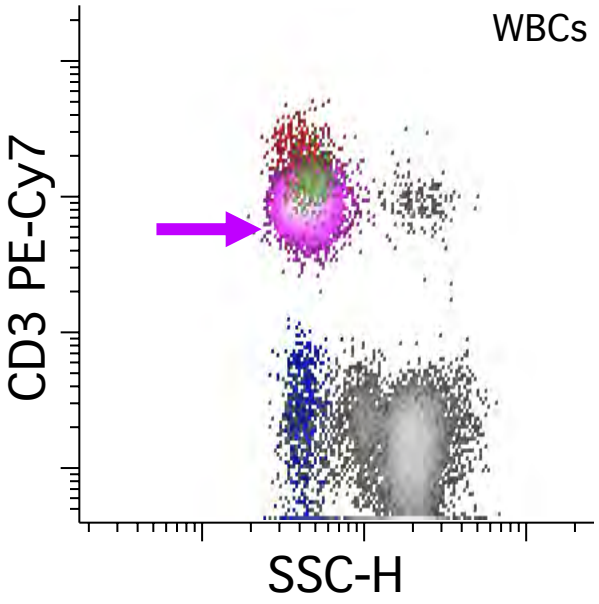


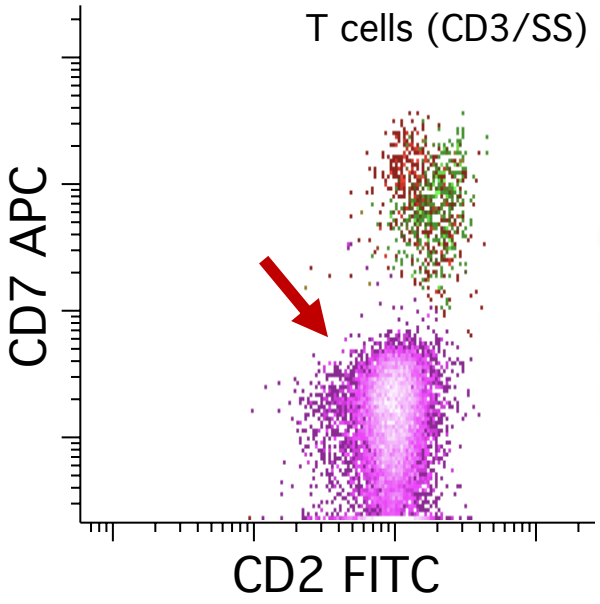
Image courtesy of Paula Fernandez

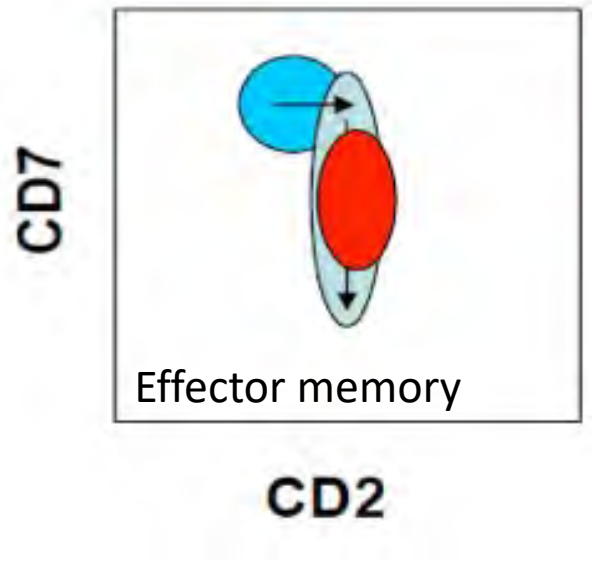


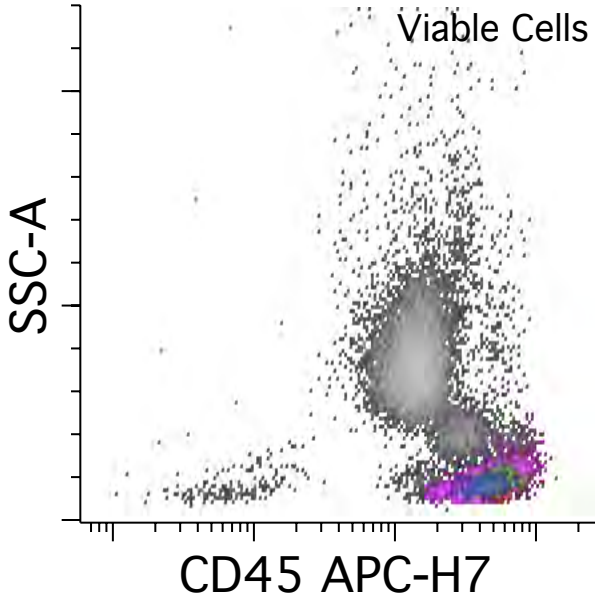
Abnormal population 45% of the WBC

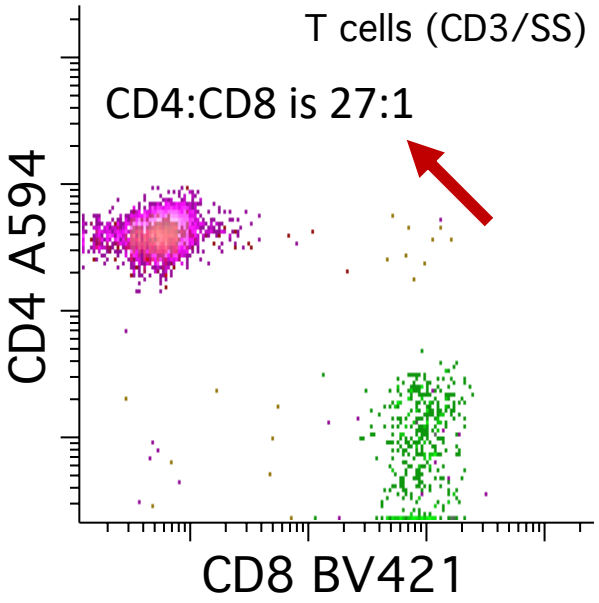


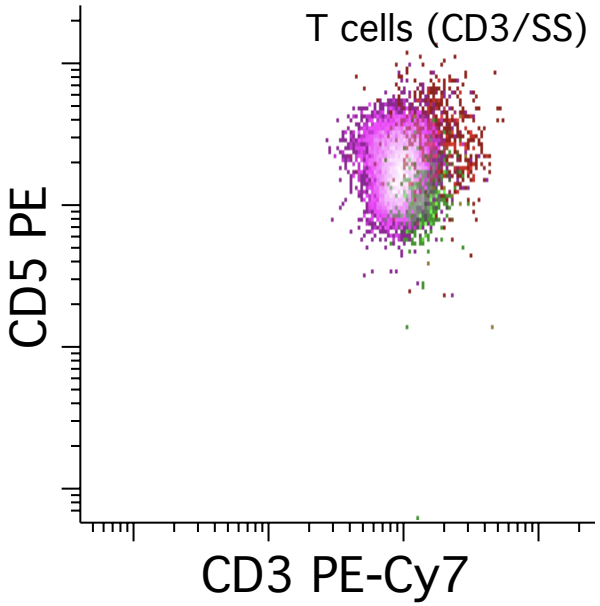


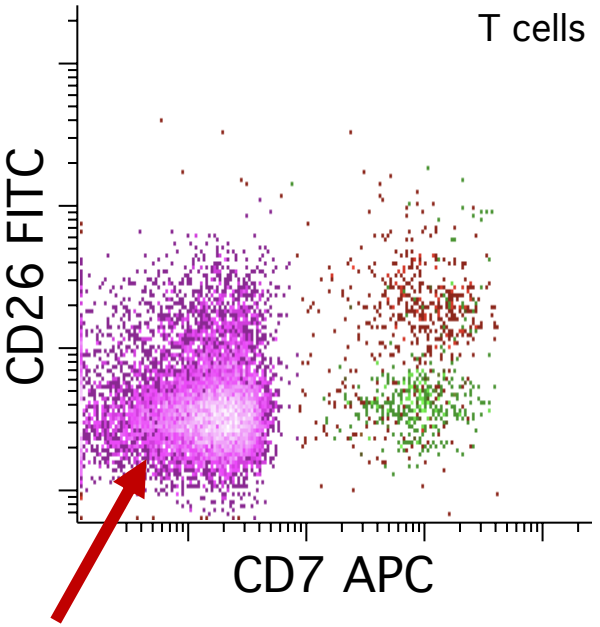












Diagnosis

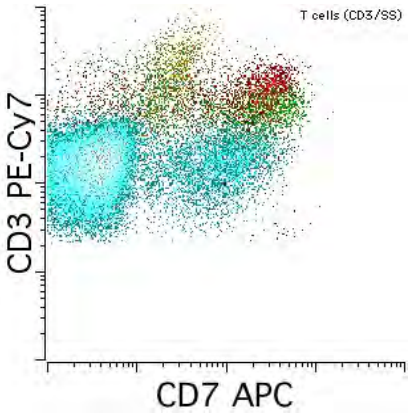
- In the context of the clinical and morphologic data the findings are diagnostic of ***Sezary syndrome***

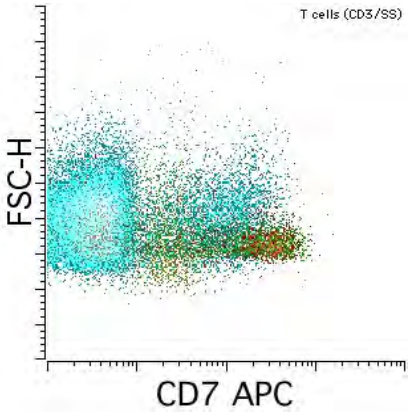
- Sezary syndrome is a subset of cutaneous T cell lymphoid neoplasms (CTCL) with peripheral blood involvement and erythroderma
  - Sezary syndrome and mycosis fungoides have an overlapping immunophenotype
- Lymphoid neoplasms of the skin are often of T cell lineage and can show some immunophenotypic overlap with reactive populations seen in benign dermatoses

T cells and the Skin

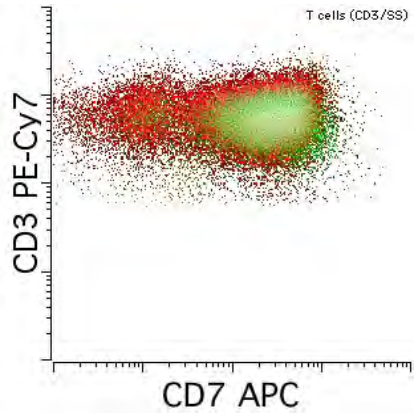
- Beware! Benign dermatoses can be associated with:
  - Increase memory T cells
    - CD3+ T cells that express CD4 without CD7
  - Increased CD4:CD8 ratio
  - Clonal T cell populations
- Malignant T cells populations in the skin are associated with:
  - Loss of T cell markers other than CD7 (CD2, CD3, CD5)
  - Aberrant expression of more than one antigen
  - High light scatter properties (side or forward scatter)

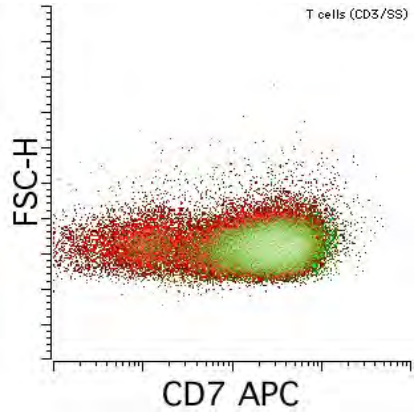
Neoplasm

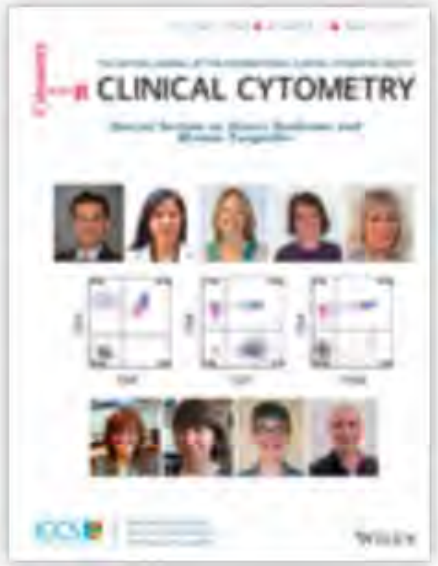




Reactive T cells in the skin







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International guidelines for the flow cytometric evaluation of peripheral blood for suspected Sézary syndrome or mycosis fungoides: Assay development/optimization, validation, and ongoing quality monitors

Andrea Illingworth, Ulrika Johansson, Shuguang Huang, Pedro Horna, Sa A. Wang, Julia Almeida, Kristy L. Wolniak, Katherina Psarra, Richard Torres, Fiona E. Craig

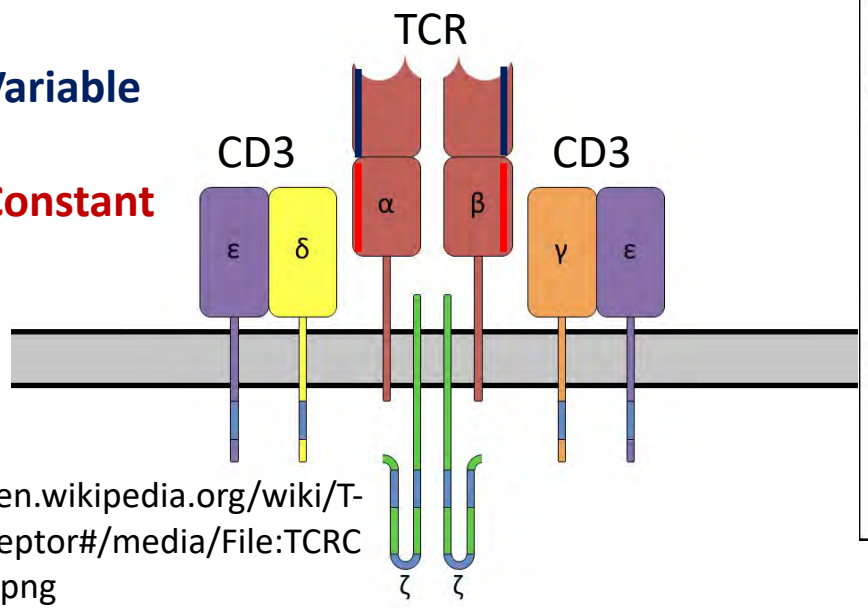
First published: 28 October 2020

https://doi.org/10.1002/cyto.b.21963

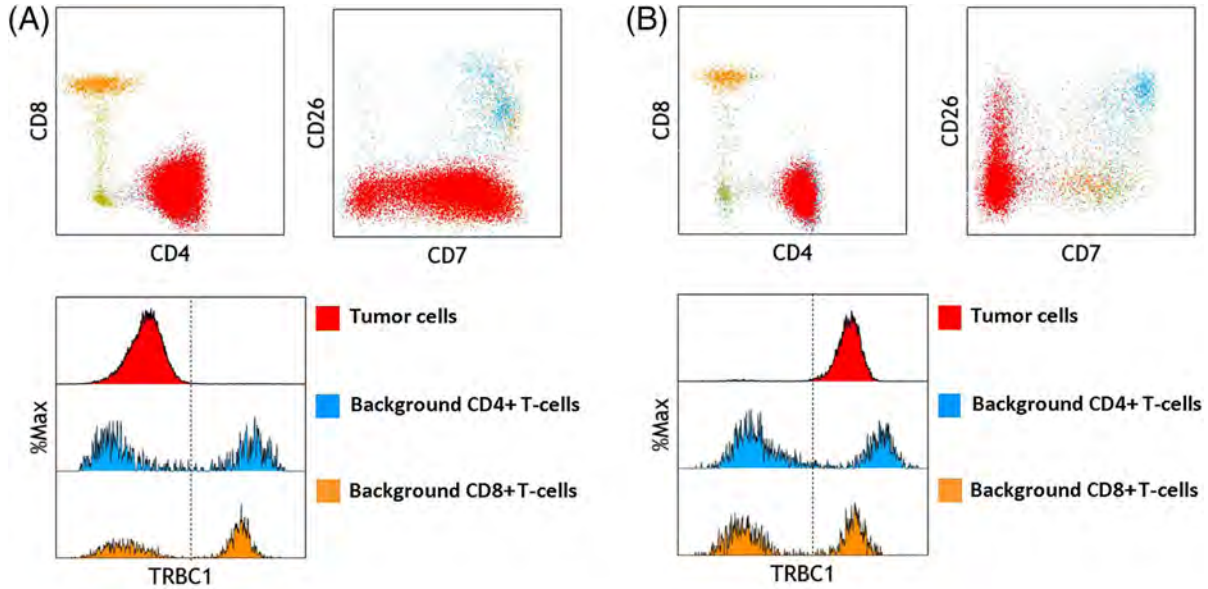
https://onlinelibrary.wiley.com/toc/15524957/2021/100/2

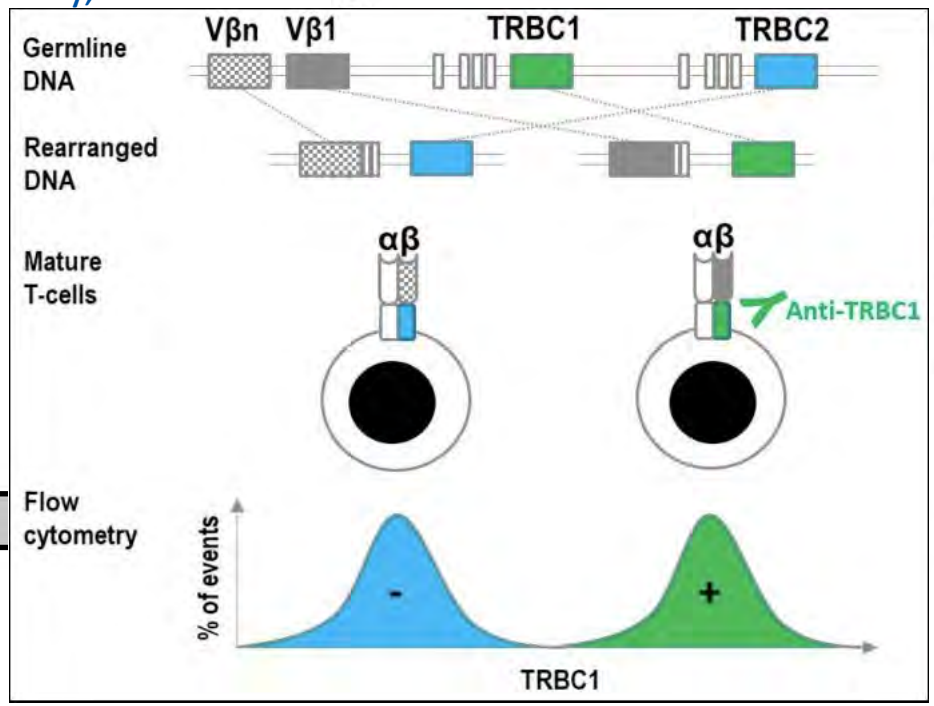
Clonality assessment for T cells by flow cytometry

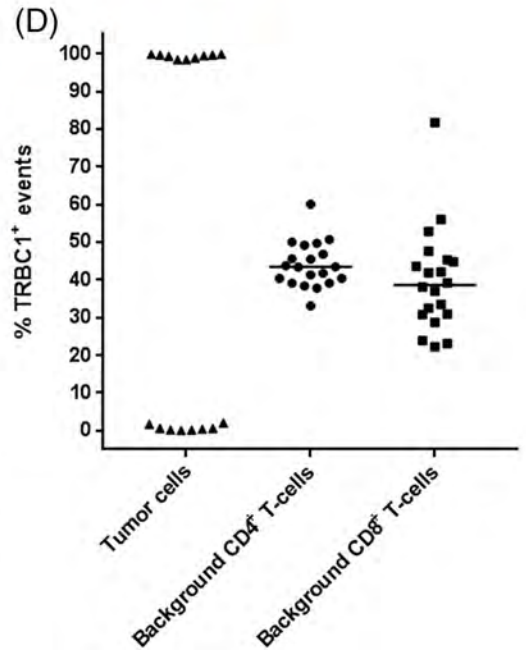
- **TCR V beta chain analysis**
  - Wu et al. AJCP. Volume 145, Issue 4, April 2016, Pages 467–485, https://doi.org/10.1093/ajcp/aqw015
- **TRBC1 analysis**
  - Hoena et al. Int J Mol Sci. 2021 Feb; 22(4): 1817.
  - Shi et al. Cytometry Part B: Clinical Cytometry, Volume: 98, Issue: 1, Pages: 99-107. DOI: (10.1002/cyto.b.21782)



https://en.wikipedia.org/wiki/T-cell_receptor#/media/File:TCRComplex.png







Shi et al. Cytometry Part B: Clinical Cytometry, Volume: 98, Issue: 1, Pages: 99-107, First published: 11 April 2019, DOI: (10.1002/cyto.b.21782)

**** Cautionary Tale: Clone ≠ Neoplasm***

Concluding remarks

- **Flow cytometry is a useful tool to characterize T cell populations**
  - Identify and characterize normal T cells and subsets
  - Distinguish abnormal populations from normal T cells
  - Generate a differential diagnosis on the basis of the immunophenotype of an abnormal T cell population in conjunction with clinical and morphologic data
  - Assess for clonality if needed
  - Identify potential therapeutic targets
- **No one immunophenotypic profile is completely specific for T cell lymphoma**
- **Abnormal T cell populations identified by flow cytometry must be interpreted in the appropriate clinical and morphologic context**

Thank you!

Thank you to my colleagues at the University of Washington, the patients whose samples we analyze, and to you for your attention! Questions?

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